

CLINICAL AND POPULATION STUDIES



# Diagnostic and Prognostic Significance of Serum Levels of SeP (Selenoprotein P) in Patients With Pulmonary Hypertension

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**OBJECTIVE:** Despite the recent progress in upfront combination therapy for pulmonary arterial hypertension (PAH), useful biomarkers for the disorder still remain to be developed. SeP (Selenoprotein P) is a glycoprotein secreted from various kinds of cells including pulmonary artery smooth muscle cells to maintain cellular metabolism. We have recently demonstrated that SeP production from pulmonary artery smooth muscle cells is upregulated and plays crucial roles in the pathogenesis of PAH. However, it remains to be elucidated whether serum SeP levels could be a useful biomarker for PAH.

**APPROACH AND RESULTS:** We measured serum SeP levels and evaluated their prognostic impacts in 65 consecutive patients with PAH and 20 controls during follow-up (mean, 1520 days; interquartile range, 1393–1804 days). Serum SeP levels were measured using a newly developed sol particle homogeneous immunoassay. The patients with PAH showed significantly higher serum SeP levels compared with controls. Higher SeP levels (cutoff point, 3.47 mg/L) were associated with the outcome (composite end point of all-cause death and lung transplantation) in patients with PAH (hazard ratio, 4.85 [1.42–16.6];  $P < 0.01$ ). Importantly, we found that the absolute change in SeP of patients with PAH ( $\Delta$ SeP) in response to the initiation of PAH-specific therapy significantly correlated with the absolute change in mean pulmonary artery pressure, pulmonary vascular resistance ( $\Delta$ PVR), and cardiac index ( $\Delta$ CI;  $R = 0.78, 0.76,$  and  $-0.71$  respectively, all  $P < 0.0001$ ). Moreover, increase in  $\Delta$ SeP during the follow-up predicted poor outcome of PAH.

**CONCLUSIONS:** Serum SeP is a novel biomarker for diagnosis and assessment of treatment efficacy and long-term prognosis in patients with PAH.

**VISUAL OVERVIEW:** An online [visual overview](#) is available for this article.

**Key Words:** biomarker ■ hypertension, pulmonary ■ prognosis ■ pulmonary circulation ■ selenoprotein P

Pulmonary arterial hypertension (PAH) is characterized by histological changes in the distal pulmonary arteries, such as intimal lesions, medial thickening, and perivascular inflammation.<sup>1</sup> Pulmonary vascular remodeling and progressive obliteration of the vessel lumen increase vascular resistance and pulmonary arterial pressure, resulting in right ventricular failure and premature death.<sup>2</sup> In addition to genetic considerations such as gene mutations in BMPR2 (bone morphogenetic protein receptor 2),<sup>3</sup> many environmental and hemodynamic

factors are involved in the development of PAH, including hypoxia,<sup>4</sup> infection,<sup>5</sup> smoking,<sup>6</sup> air pollution,<sup>7</sup> sex hormones,<sup>8,9</sup> daily diet,<sup>10</sup> and medications,<sup>11</sup> and volume overload due to congenital heart disease<sup>12</sup> and inflammation due to collagen disease.<sup>13,14</sup> All of these factors constitute complex interactions, affecting pulmonary vasculature in a multistage manner.<sup>2</sup>

Recent advances in the understanding of the pathogenesis of PAH have led to the identification of several potential biomarkers that enable us to noninvasively

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## Nonstandard Abbreviations and Acronyms

<b>AMPK</b>	5' AMP-activated protein kinase
<b>BMPR2</b>	bone morphogenetic protein receptor 2
<b>BNP</b>	brain natriuretic peptide
<b>CI</b>	cardiac index
<b>DM</b>	diabetes mellitus
<b>HDL</b>	high-density lipoprotein
<b>hsCRP</b>	high-sensitivity C-reactive protein
<b>IL-6</b>	interleukin-6
<b>LDL</b>	low-density lipoprotein
<b>mPAP</b>	mean pulmonary artery pressure
<b>NT-proBNP</b>	N-terminal pro-BNP
<b>PAH</b>	pulmonary arterial hypertension
<b>PVR</b>	pulmonary vascular resistance
<b>ROC</b>	receiver operating characteristic
<b>SeP</b>	selenoprotein P
<b>SPIA</b>	sol particle homogeneous immunoassay

assess the development of PAH and the severity of right heart failure.<sup>15–21</sup> These biomarkers include hsCRP (high-sensitivity C-reactive protein), BNP (brain natriuretic peptide), and NT-proBNP (N-terminal pro-BNP), endothelial peptides such as endothelin-1, and inflammatory cytokines such as IL-6 (interleukin-6).<sup>16–22</sup> However, these biomarkers could be influenced by variety of clinical conditions and thus reflect the consequences of inflammation and right heart failure. For example, although BNP and NT-proBNP have been adopted as prognostic biomarkers in many guidelines for PAH management, they have high variations and are not specific for pulmonary hypertension (PH) as they could be affected by left heart failure as well.<sup>18</sup> Thus, a useful novel biomarker for PH still remains to be developed.

SeP (Selenoprotein P, encoded by *SELENOP*) is a secreted protein mainly produced by hepatocytes, but also from many types of cells.<sup>22,23</sup> SeP contains 10 selenocysteine residues and transports the trace element of selenium to maintain cellular redox state and metabolism.<sup>24–27</sup> Recently, we have demonstrated that SeP production in pulmonary artery smooth muscle cells (PASCs) from patients with PAH is highly upregulated and promotes PASC proliferation and apoptosis resistance through dysregulation of redox status and mitochondrial dysfunction associated with activated hypoxia-inducible factor-1 $\alpha$  and dysregulated glutathione metabolism.<sup>28</sup> It has also been reported that SeP is upregulated in the liver of patients with type 2 diabetes mellitus (DM), downregulating the cellular metabolic switch molecule, AMPK (5' AMP-activated protein kinase).<sup>29</sup> Moreover, single nucleotide polymorphisms in

## Highlights

- Circulating serum SeP (Selenoprotein P) levels were significantly higher in patients with pulmonary arterial hypertension compared with controls.
- The rates of all-cause death and lung transplantation were significantly higher in patients with pulmonary arterial hypertension with higher serum SeP levels than in those with lower serum SeP levels.
- Absolute changes in serum SeP levels during the follow-up strongly correlated with those in several hemodynamic parameters.
- The increase in serum SeP levels in response to the initiation of pulmonary arterial hypertension-specific therapy predicted poor prognosis.
- Serum SeP levels are useful diagnostic and prognostic biomarker in patients with pulmonary arterial hypertension.

the *SELENOP* gene has been reported to be associated with abdominal aortic aneurysm formation and peripheral arterial disease development.<sup>30,31</sup> Taken together, these findings suggest that SeP promotes the development of vascular diseases and that serum SeP levels are associated with the development of PAH and the long-term prognosis of the patients.

However, to examine the possible importance of SeP in the pathogenesis of vascular diseases, several issues remain to be addressed. First, a recent study has shown that there are considerable variations in the determinants of SeP measurement in commercially available kits.<sup>32–34</sup> Details about the antibodies used in these kits were not reported, and it also remains unclear whether they detect full-length SeP and its fragments. Second, it has been reported that SeP has several variants of different molecular weight due to premature translational termination and posttranslational proteolysis.<sup>35,36</sup> Thus, we developed a new technique based on a sol particle homogeneous immunoassay (SPIA) system for full-length SeP measurement.<sup>37</sup>

In the present study, we thus examined whether serum levels of SeP could be a useful novel biomarker for early diagnosis and prediction of long-term prognosis in patients with PAH.

## MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

### Blood Samples From Patients

All protocols using human specimens were approved by the institutional review board of Tohoku University, Sendai, Japan (No. 2013-1-160). We enrolled a total of 65 patients with PAH who were diagnosed as having the disorder by right heart

catheter examination in our Tohoku University Hospital and collected their serum and plasma samples. As controls, we collected serum samples from 20 patients who underwent coronary angiography and should be diagnosed as having chest pain syndrome based on the absence of organic coronary stenosis or coronary vasospasm without any known comorbidities including DM. Baseline characteristics of the 65 patients with PAH and 20 controls in the present study are shown in Table 1. All patients provided written informed consent for the use of their serum and plasma samples for the present study. All venous

blood samples were obtained with or without ethylenediaminetetraacetic acid tubes from peripheral vein for plasma and serum samples respectively. Samples were then centrifuged at +4°C for 10 minutes at 2500 *g* 2×, and aliquots were stored in cryotubes at –80°C. Samples were sent in dry ice to the reference laboratory at Alfresa Pharma Corporation (Osaka, Japan) for SeP level determination. We used a SPIA system to determine the SeP concentration as previously described.<sup>37</sup>

**Table 1. Baseline Characteristics**

	Controls	PAH	P Value
	(n=20)	(n=65)	
Age, y	63.1±11.5	47.9±15.1*	<0.001
Female sex, no. (%)	11 (55)	47 (72)	0.17
Body mass index	22.0±3.5	21.0±3.3	0.27
Clinical status, no. (%)			
Idiopathic or heritable PAH	...	26 (40)	
Connective tissue disease	...	18 (28)	
Portal hypertension	...	3 (5)	
Congenital heart disease	...	17 (26)	
Pulmonary veno-occlusive disease	...	1 (2)	
Medication, no. (%)			
ACE inhibitor/ARB	...	9 (14)	
Ca channel blocker	...	15 (23)	
β-blocker	...	4 (6)	
Diuretic	...	33 (51)	
Aldosterone antagonist	...	23 (35)	
Endothelin-receptor antagonist	...	23 (35)	
Phosphodiesterase type 5 inhibitor	...	22 (34)	
Orally active prostacyclin analogue	...	20 (31)	
Intravenous epoprostenol	...	17 (26)	
Hemodynamic variables			
Mean arterial blood pressure, mmHg	91.1±12.1	84.3±17.6	0.06
Right atrial pressure, mmHg	...	5.9±3.8	
Mean pulmonary artery pressure, mmHg	...	41.7±16.4	
Pulmonary-capillary wedge pressure, mmHg	...	9.0±3.6	
Cardiac index, L/min per m <sup>2</sup>	...	2.5±0.5	
Pulmonary vascular resistance, dyne/s per cm <sup>-5</sup>	...	716.0±623.1	
BNP, pg/mL	25.3 (19.7–52.3)	55.8 (17.9–271.6)*	<0.05
NYHA class, no. (%)			
I	...	11 (17)	
II	...	33 (52)	
III	...	13 (21)	
IV	...	6 (10)	

Results are expressed as mean±SD or median. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BNP, brain natriuretic peptide; NYHA, New York heart association; and PAH, pulmonary arterial hypertension.

\**P*<0.05 vs control group.

## Medical History

In all patients, the medical history was recorded, including details of any cardiac and pulmonary dysfunctions, hypertension, previous stroke or transient ischemic attacks, type 2 DM, and any other comorbidities. Patients with hypertension were regarded as being at risk if their blood pressure was ≥140/90 mmHg or if they had a history of antihypertensive drug use. Patients with DM were regarded as being at risk if their fasting glucose level was ≥126 mg/dL, glycated hemoglobin (HbA1c) ≥6.5 %, or if they had a history of antidiabetic drugs or insulin use. Patients with dyslipidemia were regarded as being at risk if their LDL (low-density lipoprotein) cholesterol level was ≥140 mg/dL or their HDL (high-density lipoprotein) cholesterol level was ≤40 mg/dL, or if they were taking lipid-lowering drugs. Values of other laboratory parameters were obtained with an auto-analyzer at the Tohoku University Hospital.

## Follow-Up

Information on all-cause death and lung transplantation was collected annually using follow-up questionnaires, telephone interviews, and medical records. The end point was the composite of all-cause death and lung transplantation during the follow-up. The end points were analyzed based on the time to the first occurrence. Subgroup analyses were performed according to baseline clinical parameters.

## Measurement of Serum SeP Levels

Serum SeP levels were measured using a SPIA system that can be measured automatically using a Model 7180 Hitachi clinical analyzer, as previously reported.<sup>37</sup> We assessed serum levels of full-length SeP selectively by using 2 types of SeP monoclonal antibodies; one recognizing N-terminal domain of SeP and the other recognizing the C-terminal domain.<sup>37</sup>

## Measurement of Cardiac Function

We performed right heart catheterization using a 6-Fr Swan-Ganz catheter (Edwards Life Science, Irvine, CA) in supine position as previously described.<sup>38</sup> Hemodynamic parameters, including mean pulmonary arterial pressure (mPAP), pulmonary arterial wedge pressure, right atrial pressure, cardiac output, and pulmonary vascular resistance (PVR), were measured. The mPAP was calculated using average systolic and diastolic PAP of consecutive 10 beats. Cardiac output was determined by the thermodilution method and indirect Fick method and calculated cardiac index (CI) dividing them by body surface area.<sup>39</sup>

## Statistical Analysis

Categorical variables were presented as numerals and percentages. Continuous variables were presented as mean±SD

or median (interquartile range), as appropriate. Correlations among serum and plasma SeP levels, mPAP, pulmonary vascular resistance (mPVR), and CI were analyzed using the Spearman correlation coefficient. Differences in discrete variables were analyzed by Fisher exact test. Differences in continuous variables were compared by Welch *t* test or Wilcoxon rank-sum test, as appropriate. To identify the cutoff point of the serum SeP levels that was able to classify patients with PH for all-cause death and lung transplantation, we performed the classification and regression trees analysis.<sup>40</sup> The classification and regression trees analysis is an empirical, statistical technique based on recursive partitioning of the data space to predict the response. The models were obtained by binary splitting of the data by the value of predictors, and the split variable and split-point were automatically selected from possible candidate predictor values to achieve the best fit. Then, one or both child nodes were split into 2 regions recursively, and the process continued until some stopping rule was applied. Finally, the result of this process has been represented as a binary decision tree. Kaplan-Meier curves were plotted based on the cutoff points of SeP or absolute change in SeP for the composite end point of all-cause death and lung transplantation. The log-rank test was applied to compare the outcome between groups. Cox proportional hazards model was used for multivariable survival analyses. Logistic regression model was used to predict the probabilities of the risks for discrimination. All *P* were 2-tailed, and a *P*<0.05 was considered to be statistically significant. Statistical significance was evaluated with GraphPad Prism 7.05 (GraphPad Software, Inc, La Jolla, CA) or R version 3.3.2 (<http://www.R-project.org/>). The time-dependent data were analyzed by repeated-measures linear mixed-effect model with lmer 1.1-12 and lmerTest 2.0-33 packages of R.

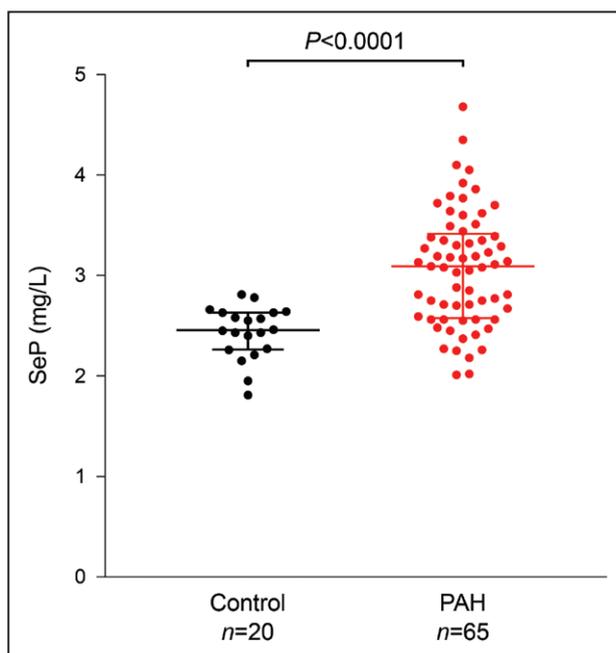
## RESULTS

### Patient Characteristics

Baseline characteristics of the 65 patients with PAH and 20 controls are shown in Table 1. As controls, we collected serum samples from patients who underwent coronary angiography and should be diagnosed as having chest pain syndrome based on the absence of organic coronary stenosis or coronary vasospasm without any known comorbidities including DM (*n*=20). The mean age of controls and patients with PAH were 63.1 and 47.9 years, and 55 % and 72 % of them were female, respectively. The median follow-up time was 1520 days (interquartile range, 1393–1804 days). Functional class of PAH was in New York heart association Class I (17.4 %), II (52.4 %), III (20.6 %), and IV (9.5 %). In the present study, 65 patients with PAH consisted of idiopathic or heritable PAH (*n*=26), connective tissue disease (*n*=18), portal hypertension (*n*=3), congenital heart disease (*n*=17), and pulmonary veno-occlusive disease (*n*=1). mPAP was 41.7 mmHg and mPVR was 716.0 dyne·sec·cm<sup>-5</sup>. At the time of enrollment, 39 patients with PAH received specific therapy, including endothelin-receptor antagonists, phosphodiesterase type 5 inhibitors, and prostacyclin analogues.

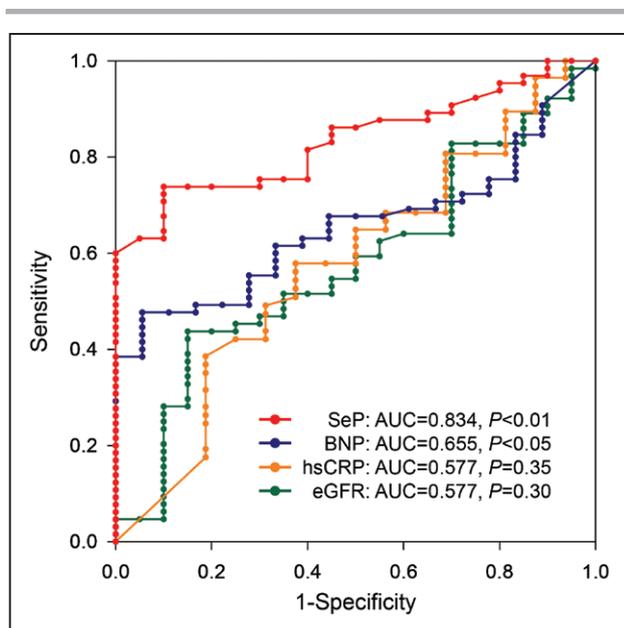
### Discriminating Capacity of Serum SeP Levels in Patients With PAH

Serum SeP levels were significantly higher in patients with PAH compared with controls, with and without adjustment for the other covariates including age ( $3.07\pm 0.57$  versus  $2.43\pm 0.25$  mg/L, *P*<0.0001; Figure 1A). Here, we also confirmed the variation of the results derived from the assay system (Figure I in the [online-only Data Supplement](#)). Comparison between serum levels of SeP and plasma levels of SeP using samples obtained from the identical patients at the same time showed significant and strong correlation (*R*=0.97, regression equation was  $y=0.98x+0.017$ , *P*<0.0001), suggesting the small variation and well reproducibility regardless of sampling tube in our SPIA system for measurement of SeP levels. There was no significant correlation in patients with PAH between serum SeP levels and the number of medications for PAH therapies (Figure II in the [online-only Data Supplement](#)). Analysis using a receiver operating characteristic curve revealed that the area under the curve of SeP was 0.834 to discriminate patients with PAH from controls with the cutoff point of 2.795 mg/L and that SeP was superior to BNP, hsCRP, and estimated glomerular filtration rate (area under the curve=0.655, 0.577, and 0.577, respectively; Figure 2). These results indicate that serum SeP levels can discriminate patients with PAH from controls with acceptable sensitivity and



**Figure 1. Comparison of the serum SeP (selenoprotein P) levels between control and patients with pulmonary arterial hypertension (PAH).**

Serum SeP levels were significantly higher in patients with pulmonary arterial hypertension (*n*=65) compared with controls (*n*=20). Baseline characteristics of samples can be found in Table 1. Comparisons of parameters were performed with the Welch *t* test.



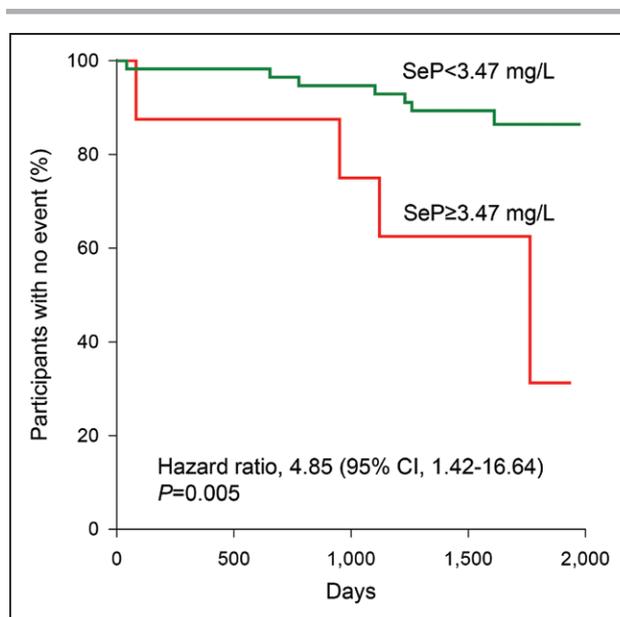
**Figure 2. Receiver operating characteristic (ROC) curves for SeP (Selenoprotein P) and other biomarkers to discriminate pulmonary arterial hypertension (PAH).**

ROC curves of SeP, BNP (brain natriuretic peptide), hsCRP (high-sensitivity C-reactive protein), and eGFR (estimated glomerular filtration rate) to discriminate PAH from controls ( $n=85$  for SeP, BNP, and eGFR, and  $n=73$  for hsCRP). AUC indicates area under the curve.

specificity regardless of the number of medications. Interestingly, patients with idiopathic pulmonary arterial hypertension and pulmonary arterial hypertension related to connective tissue disease (CTD-PAH), both of which are included in the same subgroup of PH (PAH, group 1 of World Health Organization PH classification), also showed significantly higher serum SeP levels compared with controls (Figure III in the [online-only Data Supplement](#)).

### Long-Term Outcomes Based on Serum SeP Levels

During the follow-up period, 11 patients (17 %) reached the composite end point of all-cause death and lung transplantation. Kaplan-Meier curve showed that the group with higher serum SeP levels had significantly poorer prognosis (composite end point) compared with the group with lower serum SeP levels (cutoff point, 3.47 mg/L; hazard ratio, 4.85 [1.42–16.64];  $P=0.005$ ; Figure 3). The cutoff point was determined using the classification and regression trees analysis.<sup>40</sup> Thus, higher serum SeP levels can predict poor outcome in patients with PAH. Next, we used a Cox proportional hazards model for multivariable analysis. The analysis showed that only serum SeP levels remained associated with all-cause death and lung transplantation in patients with PAH (Table 2). These results suggest that higher serum SeP levels can predict poor outcome in patients with PAH.



**Figure 3. Kaplan-Meier curves based on the serum SeP (Selenoprotein P) levels.**

Kaplan-Meier curve of the 2 groups of patients with pulmonary arterial hypertension according to the serum SeP levels (cutoff level, 3.47 mg/L). The outcome was composite of death from any cause and lung transplantation. Statistical significance was assessed using log-rank test.

### Treatment Response and Outcomes

In the present study, 34 patients with PAH underwent repeated catheterizations with sample collection before and after the initiation and increase of PAH-specific therapy. Here, we assessed the relationship between absolute changes in serum SeP levels ( $\Delta$ SeP) and those in mPAP ( $\Delta$ mPAP), pulmonary vascular resistance ( $\Delta$ PVR), and cardiac index ( $\Delta$ CI). We divided patients into 2 groups with increased or decreased SeP levels before and after the therapy. Baseline characteristics including pulmonary hemodynamics before the initiation and increase of PAH-specific therapy in the 2 groups are shown in Table 3. We found that there were significant positive correlations

**Table 2. Multivariable Predictors of Death or Lung Transplantation in Patients With PAH**

	Hazard ratio (95 % CI)	P Value
SeP	6.63 (1.18–37.3)	0.03
mRAP	1.09 (0.88–1.36)	0.44
mPAP	1.08 (0.94–1.24)	0.31
CI	0.87 (0.20–3.74)	0.86
PVR	1.00 (0.99–1.00)	0.39
BNP	1.00 (0.99–1.00)	0.34
hsCRP	2.94 (0.66–13.1)	0.16
eGFR	0.99 (0.94–1.03)	0.50

BNP indicates brain natriuretic peptide; CI, cardiac index; eGFR, estimate glomerular filtration rate; hsCRP, high-sensitivity C reactive protein; mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance; and SeP, selenoprotein P.

**Table 3. Baseline Characteristics of Patients With PAH in Figure 3**

	SeP- Decrease	SeP- Increase	P Value
	(n=18)	(n=16)	
Age, y	44.2±14.4	42.4±14.2	0.72
Female sex, no. (%)	13 (72)	11 (69)	>0.99
Clinical status, no. (%)			
Idiopathic or heritable PAH	10 (56)	9 (56)	>0.99
Connective tissue disease	6 (33)	2 (13)	0.23
Portal hypertension	1 (6)	2 (13)	0.59
Congenital heart disease	1 (6)	3 (19)	0.32
Coexisting conditions, no. (%)			
Hypertension	4 (22)	5 (31)	0.70
Diabetes mellitus	2 (11)	0 (0)	0.49
Dyslipidemia	3 (17)	3 (19)	>0.99
Connective tissue disease	6 (33)	2 (13)	0.23
Scleroderma	1 (6)	0 (0)	>0.99
Medication, no. (%)			
ACE inhibitor/ARB	3 (17)	2 (13)	>0.99
Ca channel blocker	4 (22)	1 (6)	0.34
β-blocker	0 (0)	0 (0)	>0.99
Diuretic	8 (44)	8 (50)	>0.99
Aldosterone antagonist	6 (33)	5 (31)	>0.99
Endothelin-receptor antagonist	8 (44)	9 (56)	0.73
Phosphodiesterase type 5 inhibitor	6 (33)	10 (63)	0.17
Orally active prostacyclin analogue	5 (28)	7 (44)	0.48
Intravenous epoprostenol	6 (33)	6 (38)	>0.99
Hemodynamic variables			
Mean arterial blood pressure, mmHg	76.1±12.1	73.3±25.6	0.68
Right atrial pressure, mmHg	5.0±2.4	6.7±3.3	0.09
Mean pulmonary artery pressure, mmHg	40.8±11.0	47.8±14.7	0.12
Pulmonary-capillary wedge pressure, mmHg	8.7±2.5	10.0±3.1	0.19
Cardiac index, L/min per m <sup>2</sup>	2.6±0.6	2.5±0.5	0.60
Pulmonary vascular resistance, dyne/s per cm <sup>-5</sup>	578.4±280.4	741.8±354.3	0.14
BNP, pg/mL	119.5±266.1	114.1±112.6	0.94

Results are expressed as mean±SD. SeP-decrease means the sample group which consists of patients who showed decrease in SeP in the follow-up. SeP-increase means the sample group which consists of patients who showed increase in SeP in the follow-up. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BNP, brain natriuretic peptide; and PAH, pulmonary arterial hypertension.

between  $\Delta$ SeP and  $\Delta$ mPAP or  $\Delta$ PVR ( $r=0.78$  and  $r=0.76$ , respectively, all  $P<0.0001$ ), and negative correlation between  $\Delta$ SeP and  $\Delta$ CI ( $r=-0.71$ ,  $P<0.0001$ ; Figure 4). Moreover, Kaplan-Meier curve showed that the group with increased SeP levels during follow-up showed significantly poorer prognosis (composite end point, all-cause death, and lung transplantation) compared with the group with decreased SeP levels (hazard ratio, 4.29

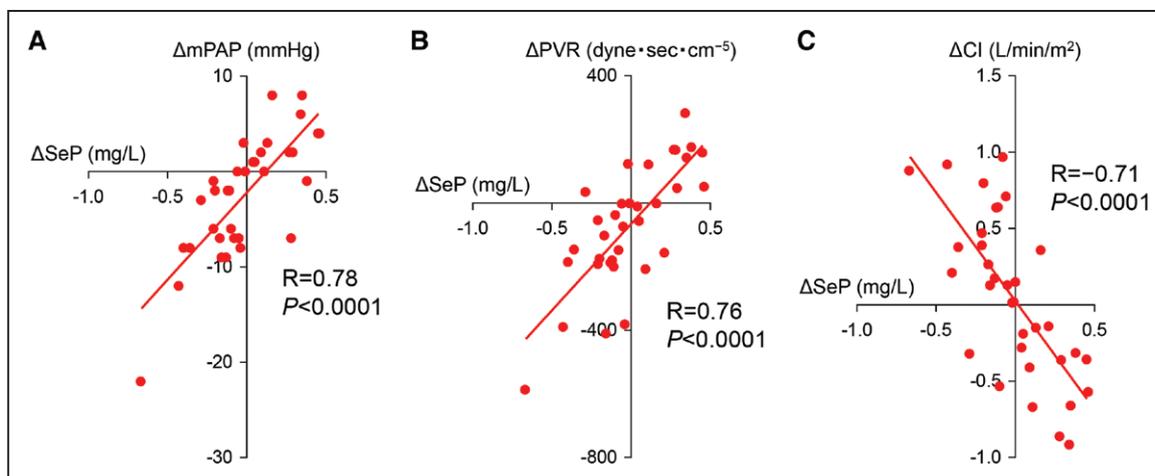
[1.27–14.4],  $P<0.05$ ; Figure 5). Regarding the changes in serum SeP levels, 8 of 16 patients reached the composite end point of all-cause death and lung transplantation in the group with increased SeP levels, while 3 of 18 reached the end point in the group with decreased SeP levels. These results indicate that the changes in serum SeP levels during follow-up can predict hemodynamic changes in response to medical therapy and long-term prognosis without any invasive tests.

## DISCUSSION

The present study highlights the usefulness of serum SeP levels in patients with PAH for diagnosis and assessment of long-term prognosis. Our findings demonstrate that (1) circulating serum SeP levels were significantly higher in patients with PAH compared with controls, (2) the rates of all-cause death and lung transplantation were significantly higher in patients with PAH with higher serum SeP levels than in those with lower serum SeP levels, (3) absolute changes in serum SeP levels during follow-up strongly correlated with those in several hemodynamic parameters, and (4) increase in serum SeP levels in response to medical therapy during follow-up predicted poor prognosis. These results indicate that serum SeP levels are a useful diagnostic and prognostic biomarker in patients with PAH.

### Newly Developed SPIA System As an Easy and Exact Tool to Measure Serum SeP Levels

Recently, there is mounting evidence of new biomarkers for distinguishing patients with PAH from controls, assessing the severity, and predicting the prognosis of the disease.<sup>16,18–21</sup> In addition to higher sensitivity and specificity, better reproducibility, and smaller variation, we need to consider a good cost-effectiveness and a procedure easy-to-use as a new biomarker. In this respect, we demonstrated that measuring serum SeP levels in patients with PAH showed high sensitivity and specificity, well reproducibility, and minimal variation. To measure serum SeP levels, several different assays such as ELISA have been developed. However, most of them are time-consuming and inconvenient for clinical use.<sup>41</sup> Furthermore, since the antibodies used in these assay systems recognize only single terminal of SeP, the assays could capture not only intact SeP but also detect artificially generated N-terminal or C-terminal fragments of SeP, resulting in substantial variation and low reproducibility.<sup>32</sup> Thus, in the present study, we used a newly developed SPIA system and demonstrated that our assay system could distinguish and predict long-term prognosis of patients with PAH with good sensitivity, specificity, and reproducibility, which seem to be superior to the established biomarkers for PAH, such as BNP, hsCRP, and estimated glomerular filtration rate.<sup>16,42,43</sup> Indeed, BNP increases in



**Figure 4. Correlation between absolute change in SeP (Selenoprotein P) and various hemodynamic parameters of patients with pulmonary arterial hypertension (PAH) in response to PAH therapies.**

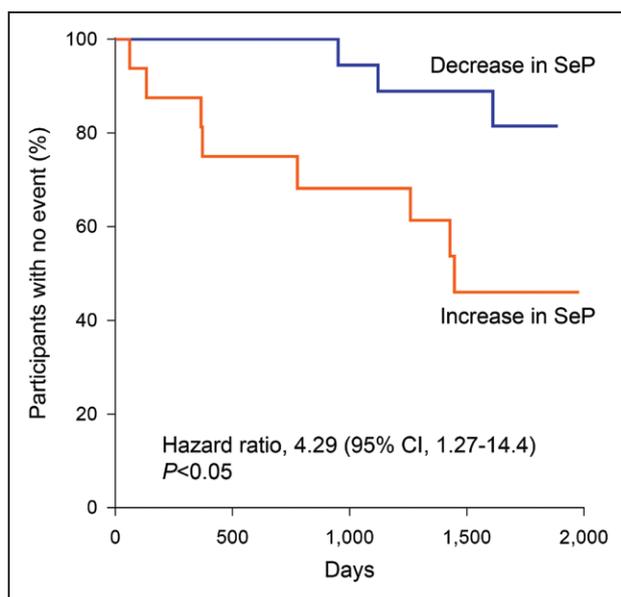
Treatment response, showing the correlation between absolute change in serum SeP levels ( $\Delta\text{SeP}$ ; mg/L) and absolute change in mean pulmonary arterial pressure ( $\Delta\text{mPAP}$ ; mmHg), pulmonary vascular resistance ( $\Delta\text{PVR}$ ; dyne/s per  $\text{cm}^{-5}$ ), and cardiac index ( $\Delta\text{CI}$ ; L/min per  $\text{m}^2$ ) in 34 patients who underwent cardiac catheterization with sample collection before and after initiation or uptitration of PAH therapies.

response to right heart failure but also is influenced by left heart failure and chronic kidney disease as well, while many types of inflammatory diseases can change the hsCRP level.<sup>15,16</sup> In contrast, SeP is produced by excessively proliferating PAH-PASMCs, which makes SeP as a novel biomarker with good sensitivity and specificity for discriminating patients with PAH from controls.<sup>28</sup> Moreover, the SPIA system is a fully automated assay that can

be used in conjunction with various commercial analyzers. This assay system allows clinicians to simultaneously measure multiple examination items and saves time and labor compared with other assays such as ELISA.

### Serum SeP Levels As a Novel Biomarker of PAH

Currently available therapies for PAH comprise 3 types of drugs (phosphodiesterase-5 inhibitors, endothelin receptor antagonists, and prostacyclin analogues), and the number of new drugs are gradually increasing.<sup>44–46</sup> Based on the AMBITION trial (the ambrisentan and tadalafil in patients with pulmonary arterial hypertension trial), which demonstrated clinical benefit of initial combination therapy of ambrisentan and tadalafil for PAH, recent guidelines recommend the initial combination therapy in treatment-naïve patients with PAH.<sup>39,47</sup> In addition, the frequent follow-up tests using several modalities, such as cardiac imaging (eg, cardiac magnetic resonance imaging) and right heart catheterization, can contribute substantially to the PAH cost burden.<sup>45</sup> The initial use of multiple PAH-specific drugs and frequent medical checkup increase the medical costs of PAH therapy. In the present study, we showed that absolute change in serum SeP levels during follow-up strongly correlated with the absolute change in several hemodynamic parameters such as mPAP, PVR, and CI. As we have previously shown that SeP induces proliferative and antiapoptotic phenotypes in PAH-PASMCs, the increase in serum SeP levels could be a reflection of PAH-PASMCs proliferation and worsening of PAH.<sup>28</sup> Altogether, serum SeP levels are a possible novel biomarker that could save healthcare expenditure for the treatment of PAH.



**Figure 5. Kaplan-Meier curves based on the absolute change in SeP (selenoprotein P) in response to pulmonary arterial hypertension (PAH) therapy.**

Kaplan-Meier curve of the 2 groups of PAH according to the change in serum SeP levels after initiation or uptitration of PAH therapy ( $n=16$ , increased in SeP;  $n=18$ , decreased in SeP). Baseline characteristics of samples can be found in Table 3. The outcome was composite of death from any cause and lung transplantation. Statistical significance was assessed using log-rank test.

## Significance of SeP Upregulation in the Serum of Patients With PAH

Multiple mechanisms seem to be involved in the pathogenesis of pulmonary vascular remodeling in PAH, including growth factors and cytokines, BMPR2 mutation, dysregulation of mitochondrial homeostasis, and cellular energy metabolism.<sup>2,48</sup> PAH-PASMCs have cancer-like phenotypes, including excess proliferation, resistance to apoptosis, and metabolic change to glycolysis (Warburg effect).<sup>49,50</sup> The mechanism for these pathological phenotypes in PAH-PASMCs may be caused, at least in part, by dysregulated mitochondrial homeostasis and resultant shift of energy metabolism.<sup>49</sup> SeP is a secreted protein mainly produced by hepatocytes but also from many other types of cells to maintain cellular redox state and metabolism.<sup>24–27</sup> We have recently demonstrated that SeP is upregulated and secreted from PAH-PASMCs and that SeP overexpression is linked to the development and progression of PAH by increasing cell proliferation and resistance to apoptosis like cancer cells.<sup>28</sup> Moreover, experimental studies with genetically modified mice showed that SeP induces PASM proliferation, pulmonary endothelial dysfunction, and inflammatory cytokine dysregulation, promoting the development of PH.<sup>28</sup> Recently, it has been reported that SeP is upregulated in the liver of patients with type 2 DM and that SeP downregulates AMPK activity, which is the key molecule of metabolic switch.<sup>29</sup> We also have shown that endothelial AMPK plays a crucial role in suppressing the development of hypoxia-induced PH, which can be achieved through AMPK activation by metformin.<sup>51</sup> Indeed, it has been reported that AMPK regulates SeP expression from hepatocytes and serum SeP levels are associated with the development of insulin resistance.<sup>52–54</sup> Additionally, it has also been reported that patients with PAH have insulin resistance compared with healthy controls.<sup>29,55,56</sup> These findings may link the upregulation of SeP to the dysregulation of cellular energy metabolism and proproliferative and antiapoptotic phenotype in PAH. Recently, there are several reports which have shown that higher serum SeP levels were associated with the favorable outcomes of left heart failure, which is quite different from the case of right heart failure shown in the present study.<sup>57,58</sup> These findings indicate that serum SeP is not just a selenium transporter or supplier and it could have different function between the right and left heart diseases, which suggests the usability of SeP levels for right heart disease as an important biomarker.

### Future Perspectives

Although the treatment for PAH has been recently improved, it is still difficult to early diagnose the disorder. PAH is a rare disease with an estimated prevalence of 52 cases per million.<sup>59</sup> However, the prevalence of PAH has

been rapidly increasing along with medical awareness and is higher in patients with lung disease, congenital heart disease, collagen disease, and infectious disorders.<sup>5</sup> Moreover, it is estimated that the prevalence of whole PH is about 1% of the general population, which increases to 10% of individuals aged over 65 years.<sup>5</sup> In the last 20 years, efforts have been made to develop effective therapies for patients with PAH.<sup>60</sup> However, we still have limited therapeutic strategies and no treatments for radical cure are available for patients with PAH.<sup>60</sup>

In the present study, we were able to demonstrate that serum SeP levels were significantly elevated in patients with PAH with good reproducibility and minimum variation. In addition, higher serum SeP levels predicted a poor outcome. Based on these results, serum SeP levels can be used as a novel biomarker for PAH and are useful to evaluate the therapeutic effect of medical treatments without any invasive examinations. Moreover, using a combination of SeP inhibitors and serum levels of SeP, we may find good candidates among patients with PAH that can be used to demonstrate the effectiveness of this strategy in future. Indeed, we have recently found a SeP inhibitor, sanguinarine, which inhibits the development of PH in animal models.<sup>28</sup> By targeting SeP, we will be able to develop early diagnostics and novel therapeutic agents for the disease.

### Study Limitations

Several limitations should be mentioned for the present study. First, the number of patients in the present study was relatively small. However, despite this small sample size, serum SeP levels had a significant prognostic impact in patients with PAH. Second, BNP and several hemodynamic parameters (eg, mPAP and CI) were not correlated with all-cause death or lung transplantation. This could be because most of the patients with PAH enrolled in the present study had already been controlled by medications. Finally, right ventricular function such as right ventricular ejection fraction was not investigated in the present study. Indeed, we found that the total cardiac function represented by CI and prognosis of PAH were significantly correlated with serum SeP levels; therefore, right ventricular function could also have some correlation with the SeP levels. Further prospective analysis in a large cohort is needed to elucidate the importance of serum SeP levels.

### Conclusions

In the present study, we were able to demonstrate for the first time that serum SeP levels are elevated in patients with PAH and that serum SeP levels can predict long-term prognosis of the disorders. Importantly, the present study also demonstrates that assessing absolute changes in SeP during the follow-up can provide useful information

on therapeutic effects of multiple combination therapies, prevent excess invasive tests and healthcare costs, and further enhance the prognostic impacts of patients with PAH. Thus, serum SeP levels could be a novel and useful biomarker in the management of patients with PAH.

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### Disclosures

None.

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