

# Decreased haptoglobin levels inversely correlated with pulmonary artery pressure in patients with pulmonary arterial hypertension

## A cross-sectional study

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### Abstract

We investigated the serum haptoglobin levels in patients with pulmonary arterial hypertension (PAH) based on the hypothesis that haptoglobin levels would reflect subclinical hemolysis due to microangiopathy in pulmonary arterioles.

This cross-sectional study included 3 groups of patients attending Hokkaido University Hospital: PAH, chronic thromboembolic pulmonary hypertension (CTEPH), and connective tissue diseases (CTD) without PAH (CTD-non-PAH) group. Serum haptoglobin levels were measured by standardized turbidimetric immunoassay in all patients. Demographic data, laboratory results, right heart catheter, and echocardiographic findings were extracted from the medical records. Decreased haptoglobin levels were defined as below 19 mg/dL based on the 95th percentile of healthy controls.

Thirty-five patients in PAH group including 11 with idiopathic PAH (IPAH) and 24 with CTD-associated PAH (CTD-PAH), 27 in CTEPH group, and 32 in CTD-non-PAH group were analyzed. Serum haptoglobin levels in PAH group (median 66 mg/dL) were significantly lower than those in CTEPH group (median 94 mg/dL,  $P = .03$ ) and CTD-non-PAH group (median 79 mg/dL,  $P = .03$ ). The prevalence of decreased haptoglobin levels was 26% in PAH group, 15% in CTEPH group, and 6% in CTD-non-PAH group. Serum haptoglobin levels had a significant negative correlation ( $r = -0.66$ ,  $P < .001$ ) with mean pulmonary artery pressure in PAH group, particularly in CTD-PAH subgroup ( $r = -0.74$ ,  $P < .001$ ), but no correlation in IPAH subgroup ( $r = -0.52$ ,  $P = .13$ ) and in CTEPH group ( $r = -0.17$ ,  $P = .41$ ). Follow-up cases of CTD-PAH showed lowering pulmonary artery pressure led to normalizing serum haptoglobin levels.

Serum haptoglobin levels decreased in PAH patients and inversely correlated with pulmonary artery pressure in CTD-PAH patients, suggesting their potential as a surrogate marker for CTD-PAH.

**Abbreviations:** CTD = connective tissue diseases, CTD-non-PAH = connective tissue diseases without pulmonary arterial hypertension, CTD-PAH = connective tissue diseases associated pulmonary arterial hypertension, CTEPH = chronic thromboembolic pulmonary hypertension, ePASP = pulmonary artery systolic pressure estimated by echocardiography, IPAH = idiopathic pulmonary arterial hypertension, LDH = lactate dehydrogenase, mPAP = mean pulmonary artery pressure, PAH = pulmonary arterial hypertension, PH = pulmonary hypertension, RHC = right heart catheter.

**Keywords:** connective tissues diseases, haptoglobin, microangiopathy, pulmonary arterial hypertension

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## 1. Introduction

Pulmonary hypertension (PH) is an increased blood pressure in pulmonary arteries and affects the right side of the heart, being diagnosed when mean pulmonary artery pressure (mPAP) measured by right heart catheter (RHC) is 25 mm Hg or greater. PH occurs as an idiopathic disease of the pulmonary arterioles or as a complication of various diseases, such as connective tissue diseases (CTD) and chronic pulmonary thromboembolism. The current Nice classification of PH distinguishes 5 subgroups: pulmonary arterial hypertension (PAH), PH due to left heart disease, PH due to lung diseases, chronic thromboembolic PH (CTEPH), and PH with unclear multifactorial mechanisms.<sup>[1]</sup>

Among these subgroups, PAH is characterized by abnormalities in the small arteries of pulmonary vasculature.<sup>[2,3]</sup> The histological features of PAH include remodeling of the small pulmonary arterial walls and microthrombosis in the pulmonary arterioles. Microangiopathy in the pulmonary arterioles is one of key pathophysiology of PAH.<sup>[4–6]</sup>

PAH is of great clinical significance because of its high mortality<sup>[7,8]</sup> and poor quality of life.<sup>[9]</sup> Although recent development of targeted therapy for patients with PAH has improved their prognosis, strategy for early diagnosis and accurate evaluation of disease progression, particularly with noninvasive tools, are still on unmet needs. The DETECT study has recently showed an evidence-based algorithm to detect PAH in CTD patients.<sup>[10]</sup> In this algorithm, echocardiography and RHC are recommended according to the total risk points calculated using the results of several noninvasive tests, such as laboratory examination, electrocardiogram, and pulmonary function tests. This detection algorithm is highly sensitive (96%) to detect high-risk population of PAH, but may lead to unnecessary RHC, reflected by its low specificity (48%). An additional noninvasive marker is required to improve the screening yield of PAH.

Moreover, the degree of microangiopathy in the pulmonary arterioles is associated with vasodilator response, severity, and prognosis of PAH. Considering the pathophysiological profile, anticoagulation might lead to favorable outcome in PAH patients with features of thrombotic microangiopathy.<sup>[6]</sup> However, in clinical practice, it is difficult to assess the degree of microangiopathy in the pulmonary arterioles in PAH patients because it needs to be defined and evaluated histologically. Noninvasive surrogate tools are desired.

Haptoglobin is a plasma protein mainly produced by hepatocytes, which binds free hemoglobin released from erythrocytes and protects the kidneys from damage induced by hemoglobin.<sup>[11]</sup> The haptoglobin levels are extremely sensitive to detect intravascular hemolysis including thrombotic microangiopathy.<sup>[12]</sup>

We hypothesized that serum haptoglobin levels decreased in patients with PAH due to subclinical hemolysis related with microangiopathy in the pulmonary arterioles. The aim of this study was to investigate the serum haptoglobin levels in patients with PAH as a potential marker.

## 2. Methods

### 2.1. Patients and methods

This cross-sectional study was composed of 3 groups of patients: PAH, CTEPH, or CTD without PAH (CTD-non-PAH) group. PAH and CTEPH groups included consecutive patients with idiopathic PAH (IPAH), CTD-associated PAH (CTD-PAH), or CTEPH who were attending Cardiopulmonary Department of

Hokkaido University Hospital in 2015. PAH was diagnosed based on RHC findings.<sup>[1]</sup> CTEPH was diagnosed based on RHC, enhanced-computed tomography scanning, and ventilation-perfusion scintigraphy findings.<sup>[1]</sup> CTD-non-PAH group included CTD patients who were attending Rheumatology Department of Hokkaido University Hospital during the same period and underwent echocardiography according to the DETECT algorithm<sup>[10]</sup>; in this group, pulmonary artery systolic pressure estimated by echocardiography (ePASP) was <36 mm Hg.<sup>[13]</sup> This study was performed in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. Approval was obtained from the Local Ethics Committee (approval number: 015-0535). All study participants provided informed consent. Serum haptoglobin levels were measured by standardized turbidimetric immunoassay in all patients between August 2015 and August 2016. Demographic data, laboratory results of the same time as haptoglobin test, and echocardiography and RHC findings nearest haptoglobin test were extracted from the medical records. Decreased haptoglobin levels were defined as <19 mg/dL of serum haptoglobin concentration based on the 95th percentile of healthy volunteers according to the manufacture's instruction.

### 2.2. Statistical analysis

Continuous variables were compared using the Wilcoxon test, and correlation between 2 continuous variables was assessed using Spearman rank correlation coefficients. Subgroup analysis was performed in PAH group. We compared CTD-PAH and IPAH subgroups. An influence of anticoagulation and immunosuppressant was assessed as sensitivity analyses. Statistical significance was defined as the probability value <0.05. All analyses were performed using the JMP Pro software (ver. 12.0; SAS Institute Inc., Cary, NC).

## 3. Results

### 3.1. Patients' characteristics

Among 96 patients enrolled, 2 were excluded due to the presence of mechanical valve or the complication of autoimmune hemolytic anemia with positive Coombs test. Thirty-five patients in PAH group including 11 patients with IPAH and 24 with CTD-PAH, 27 in CTEPH group, and 32 in CTD-non-PAH group were analyzed. Patient's characteristics were summarized in Table 1.

Age in CTEPH group was higher compared with other groups. All groups had female predominance. RHC was performed median 7 months (quartile: 3–20 months) apart from haptoglobin tests, and ePASP was measured median 4 months (quartile: 2–7 months) apart from haptoglobin tests. The patients included in the study had a long duration of PH (median: 7 years, quartile: 4–11 years). Cardiac index in CTEPH group was lower than that in PAH group. Vasodilators were used in 87% of PAH patients and in 56% of CTEPH patients. Immunosuppressants were used concomitantly in 49% of PAH patients. All CTEPH patients and 29% of PAH patients were given anticoagulant. Pulmonary endarterectomy had been performed in 30% of CTEPH patients. Serum haptoglobin levels were tested in 3 patients with CTD-PAH before starting treatments of PAH.

None had positive direct Coombs test. Albumin and cholinesterase levels to assess liver function were within normal range in all patients. High C-reactive protein levels (>0.3 mg/dL) suggesting inflammatory condition were not observed in any patients.

**Table 1**  
**Patients' characteristics.**

	PAH	CTEPH	CTD-non-PAH
Number	35	27	32
Age	47 [41–57]	64 [53–75]	47.5 [35–62]
Sex (F:M)	34: 1	18: 9	27: 5
Duration of PH (y)	7.3 [3.9–10.7]	5.7 [3.3–11.5]	
Underlying disease			
IPAH	11 (31%)		
SSc	8 (23%)	1 (4%)	6 (19%)
SLE	7 (20%)		14 (44%)
MCTD	8 (23%)		8 (25%)
DM	1 (3%)		1 (3%)
SS		1 (4%)	3 (9%)
APS		4 (14%)	
RA		2 (7%)	
RHC findings			
mPAP (mm Hg)	31 [22–42]	28 [24–36]	
CI (L/min/m <sup>2</sup> )	3.2 [2.6–3.5]	2.5 [2.2–3.0]	
PVR (dyne-s/cm <sup>5</sup> )	409 [249–555]	383 [283–695]	
RAP (mm Hg)	5 [3–6]	4 [3–6]	
PCWP (mm Hg)	8 [6–10]	8 [6–10]	
Interval of tests* (mo)	7.1 [3–23]	6.5 [4–11]	
ePASP (mm Hg)	41 [31–55]	44 [30–59]	24 [19–28]
Interval of tests† (mo)	3.1 [1.6–6.3]	3.5 [0.8–3.3]	4.5 [2.6–7.4]
Functional class (I/II/III/IV)	11/17/6/0	9/9/9/0	
6MWD (m)	410 [352–507]	411 [358–510]	
BNP (pg/mL)	20.8 [8.1–67.3]	14.5 [7.6–37.6]	
Vasodilators			
Triple therapy	3 (9%)	0 (0%)	
Double therapy	24 (69%)	5 (19%)	
PDE5-I/Riociguat	3 (9%)	10 (37%)	
ERA	0 (0%)	0 (0%)	
Immunosuppressant	17 (49%)	2 (7%)	26 (81%)
Anticoagulation	10 (29%)	27 (100%)	5 (16%)
Endarterectomy		8 (30%)	
Positive Coombs test	0 (0%)	0 (0%)	0 (0%)
Albumin (mg/dL)	4.0 [3.7–4.4]	4.3 [4.1–4.6]	3.9 [3.6–4.3]
Cholinesterase (U/L)	282 [221–310]	334 [281–365]	298 [251–327]
C-reactive protein (mg/dL)	0.04 [0.02–0.08]	0.07 [0.02–0.19]	0.06 [0.02–0.13]

All data are shown as median [quartile] or number (%).

PAH = pulmonary arterial hypertension, CTEPH = chronic thromboembolic pulmonary hypertension, CTD-non-PAH = connective tissue diseases without pulmonary arterial hypertension, IPAH = idiopathic pulmonary arterial hypertension, SSc = systemic sclerosis, SLE = systemic lupus erythematosus, MCTD = mixed connective tissue disease, DM = dermatomyositis, SS = Sjögren's syndrome, APS = antiphospholipid syndrome, RA = rheumatoid arthritis, mPAP = mean pulmonary artery pressure, CI = cardiac index, PVR = pulmonary vascular resistance, RAP = right atrial pressure, PCWP = pulmonary capillary wedge pressure, ePASP = pulmonary artery systolic pressure estimated by echocardiography, 6MWD = 6-minute walk distance, BNP = brain natriuretic peptide, PDE5-I = phosphodiesterase-5 inhibitors, ERA = endothelin receptor antagonists.

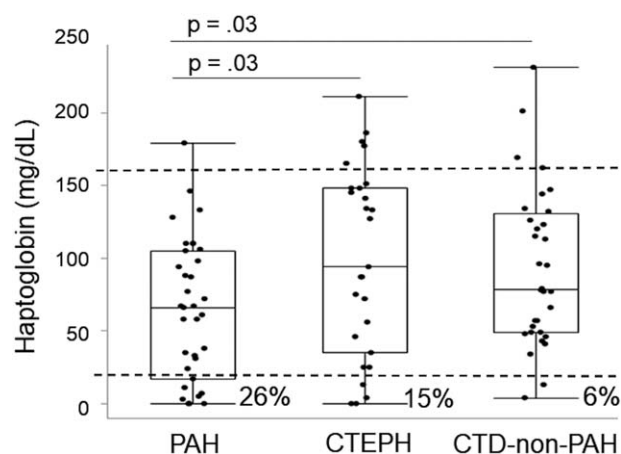
\* Interval between haptoglobin test and right heart catheter.

† Interval between haptoglobin test and echocardiography.

### 3.2. Serum haptoglobin levels in PAH or CTEPH patients

Serum haptoglobin levels in PAH group (median 66 mg/dL, quartile [17–105]) were significantly lower than those in CTEPH group (median 94 mg/dL, quartile [35–148],  $P = .03$ ) and CTD-non-PAH group (median 79 mg/dL, quartile [49–131],  $P = .03$ ). The prevalence of decreased haptoglobin levels was 26% in PAH group, 15% in CTEPH group, and 6% in CTD-non-PAH group (Fig. 1).

Serum haptoglobin levels in PAH group had a significant negative correlation with mPAP ( $r = -0.66$ ,  $P < .001$ ) and ePASP ( $r = -0.57$ ,  $P < .001$ ). In contrast, serum haptoglobin levels in CTEPH groups had no significant correlation with mPAP ( $r = -0.17$ ,  $P = .41$ ) and ePASP ( $r = -0.08$ ,  $P = .71$ ) (Fig. 2).



**Figure 1.** The box-plot diagrams show the serum haptoglobin levels in each group of patients: PAH, CTEPH, and CTD-non-PAH group. The bottom and top of the box represent the first and third quartiles, and the band inside the box shows the median. Whiskers represent the minimum and maximum of all of the data. The percentages show the prevalence of serum haptoglobin levels lower than 95th percentile of healthy controls in each group. CTD-non-PAH = connective tissue diseases without pulmonary arterial hypertension, CTEPH = chronic thromboembolic pulmonary hypertension, PAH = pulmonary arterial hypertension.

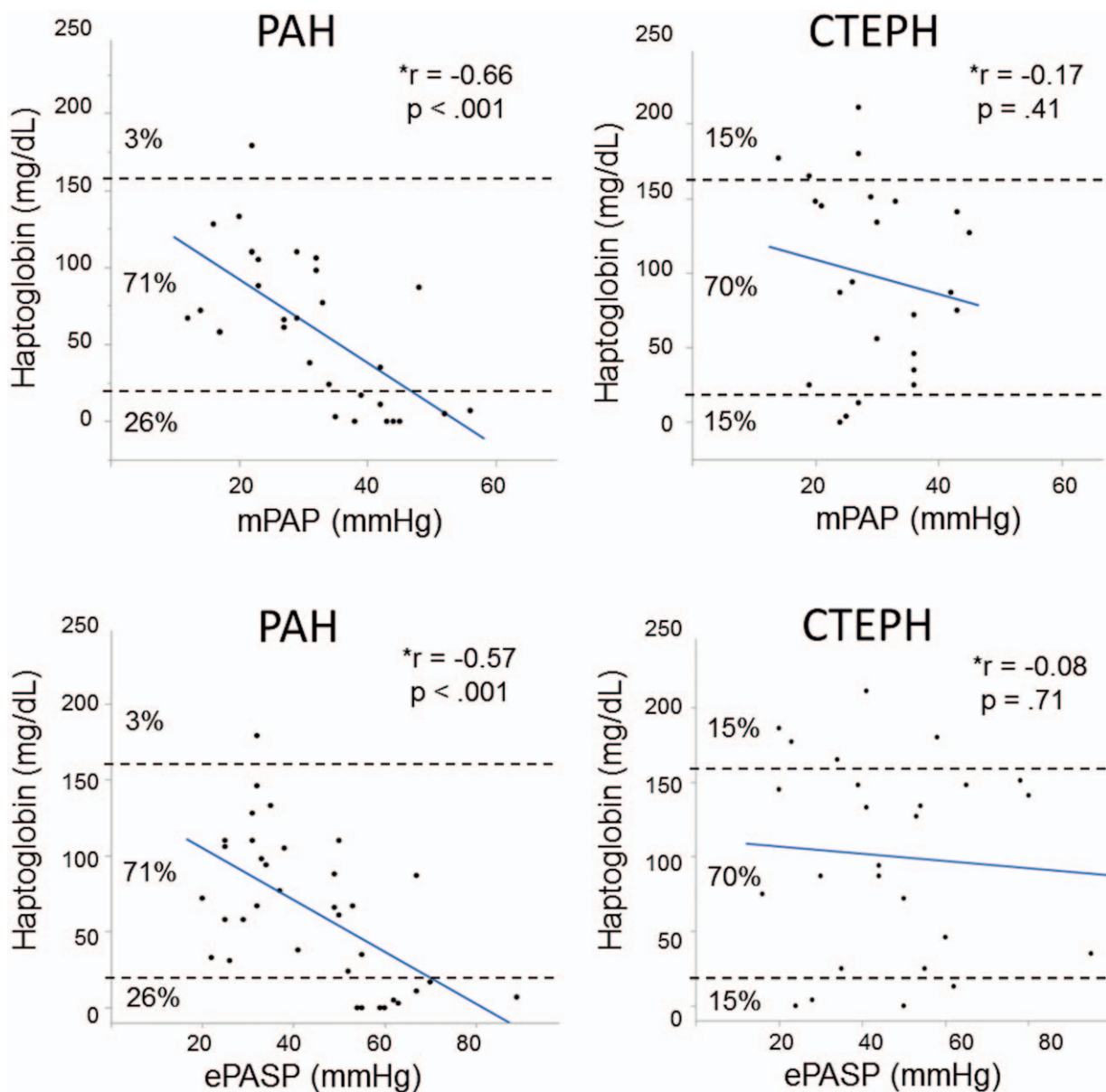
Serum haptoglobin levels had a strong negative correlation with mPAP in CTD-PAH subgroup ( $r = -0.74$ ,  $P < .001$ ), but no significant correlation in IPAH subgroup ( $r = -0.52$ ,  $P = .13$ ) (Fig. 3A). Serum haptoglobin levels had a significant correlation with mPAP regardless of anticoagulation in PAH group (Fig. 3B). Serum haptoglobin levels also had a significant correlation with mPAP regardless of immunosuppressant in CTD-PAH group (Fig. 3C).

Serum lactate dehydrogenase (LDH) levels (normal: 107–230), another marker of hemolysis, were significantly elevated in PAH patients with decreased haptoglobin levels (median 230 U/L, quartile [194–256]) compared with those with normal haptoglobin levels (median 180 U/L, quartile [146–203],  $P = .009$ ) (Fig. 4). Total bilirubin levels, uric acid levels, hemoglobin, and platelets in PAH group were within almost normal range and had no significant difference between patients with decreased and normal haptoglobin levels.

### 3.3. Follow-up study

We could follow up serum haptoglobin levels of 3 patients with CTD-PAH in whom first haptoglobin tests were performed before starting treatments of PAH. These follow-up cases showed lowering ePASP led to normalizing serum haptoglobin levels, as shown in Figure 5A.

A representative clinical course is presented in Figure 5B. A 45-year-old Japanese woman was admitted to our department for the evaluation of cardiopulmonary diseases as she had 3 months' history of shortness of breath. She was given the diagnosis of systemic sclerosis based on hardening fingers, Raynaud's phenomenon, and positive anti-U1 ribonucleic protein antibodies. The diagnosis of PAH was confirmed by RHC (mPAP: 45 mm Hg, pulmonary artery wedge pressure: 5 mm Hg, pulmonary vascular resistance: 11.8 Wood unit). Serum haptoglobin level was below the detection limit ( $< 2$  mg/dL). After the administration of tadalafil (40 mg/d) and macitentan (10 mg/d), mPAP was decreased from 45 to 26 mm Hg. Serum haptoglobin level



**Figure 2.** The correlation between serum haptoglobin levels and mPAP or ePASP in PAH or CTEPH group. The continuous lines show regression line. The broken horizontal lines show upper or lower limit of serum haptoglobin levels established using 95th percentile of healthy controls. The percentages show the prevalence of increased, normal, or decreased haptoglobin levels in each group. CTEPH = chronic thromboembolic pulmonary hypertension, ePASP = pulmonary artery systolic pressure estimated by echocardiography, mPAP = mean pulmonary artery pressure, PAH = pulmonary arterial hypertension. \*Spearman rank correlation coefficient.

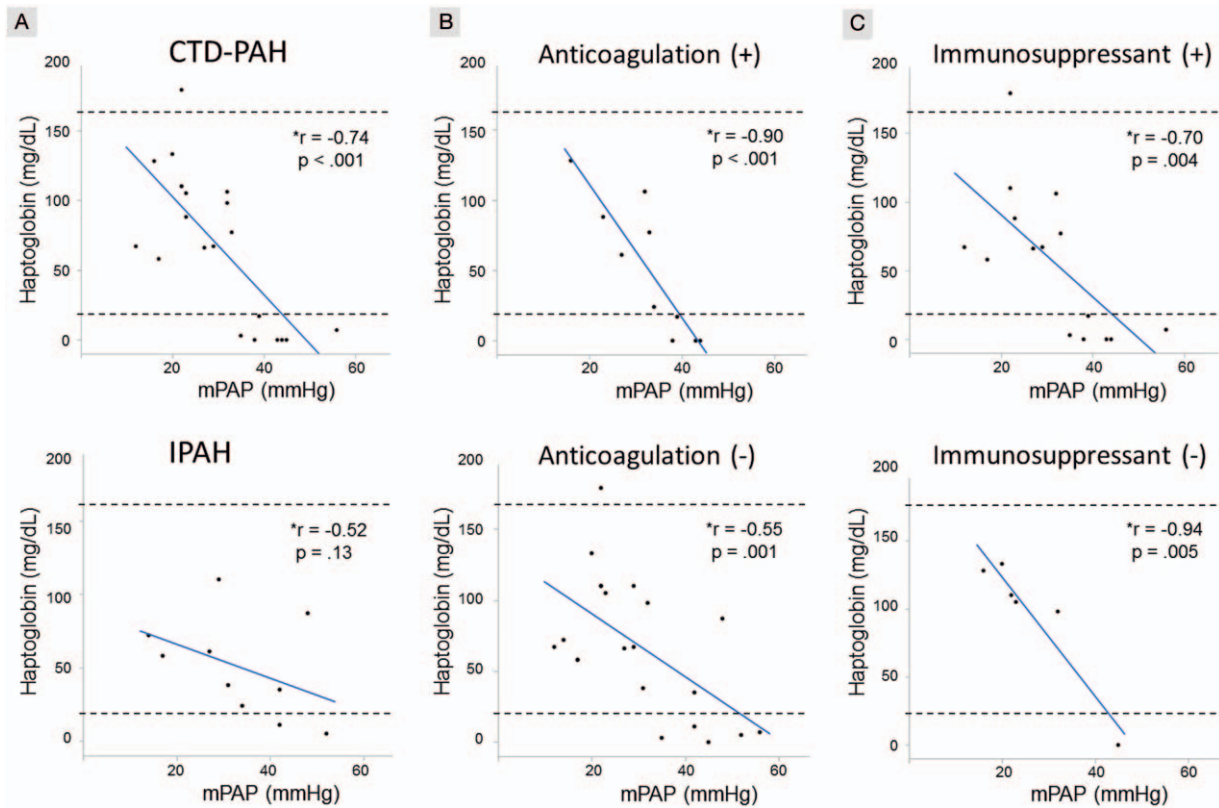
gradually increased and finally normalized along the improvement of PAH.

#### 4. Discussion

In this study, we newly showed decreased haptoglobin levels in patients with PAH. Furthermore, haptoglobin levels inversely correlated with pulmonary artery pressure, and lowering pulmonary artery pressure led to normalizing haptoglobin levels in CTD-PAH patients.

The decreased haptoglobin levels in PAH patients may reflect microangiopathy and subsequent subclinical hemolysis in the pulmonary arterioles.<sup>[6]</sup> Serum LDH levels, another marker of hemolysis,<sup>[12]</sup> were elevated in our PAH patients in parallel with

decreased haptoglobin levels. Consistent with these findings, serum uric acid and bilirubin levels, other hemolytic markers, have been reported to be elevated in PAH patients and associated with its severity and prognosis,<sup>[14,15]</sup> although our study could not confirm. Moreover, it was reported that free hemoglobin levels were increased in PAH patients, supporting the presence of subclinical hemolysis in the pulmonary arterioles.<sup>[16]</sup> Free hemoglobin reduces the bioavailability of nitric oxide, acting as a scavenger, and subsequently induces vasoconstriction. Haptoglobin can influence the bioavailability of nitric oxide to trap free hemoglobin.<sup>[17]</sup> Thus, serum haptoglobin levels may be a potential marker for the severity of PAH reflecting the degree of microangiopathic hemolysis, released free hemoglobin levels, and the bioavailability of nitric oxide.

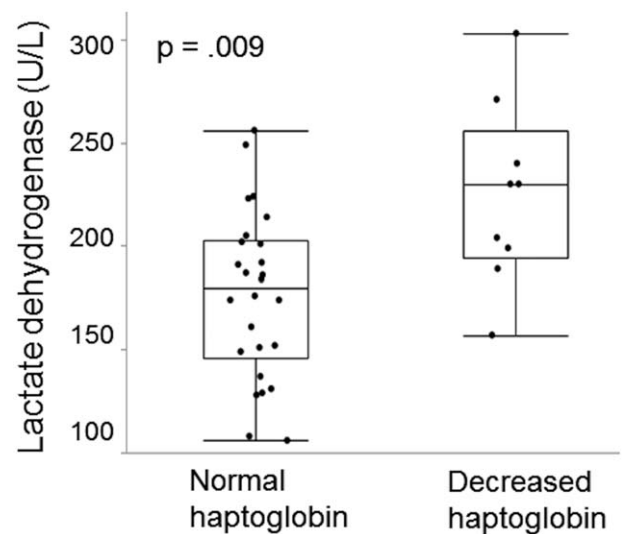


**Figure 3.** Subanalysis on the correlation between serum haptoglobin levels and mPAP in CTD-PAH or IPAH (A), PAH with anticoagulation or PAH without anticoagulation (B), and CTD-PAH with immunosuppressant or CTD-PAH without immunosuppressant (C). CTD-PAH = connective tissue diseases associated pulmonary arterial hypertension, IPAH = idiopathic pulmonary arterial hypertension, mPAP = mean pulmonary artery pressure. \*Spearman rank correlation coefficient.

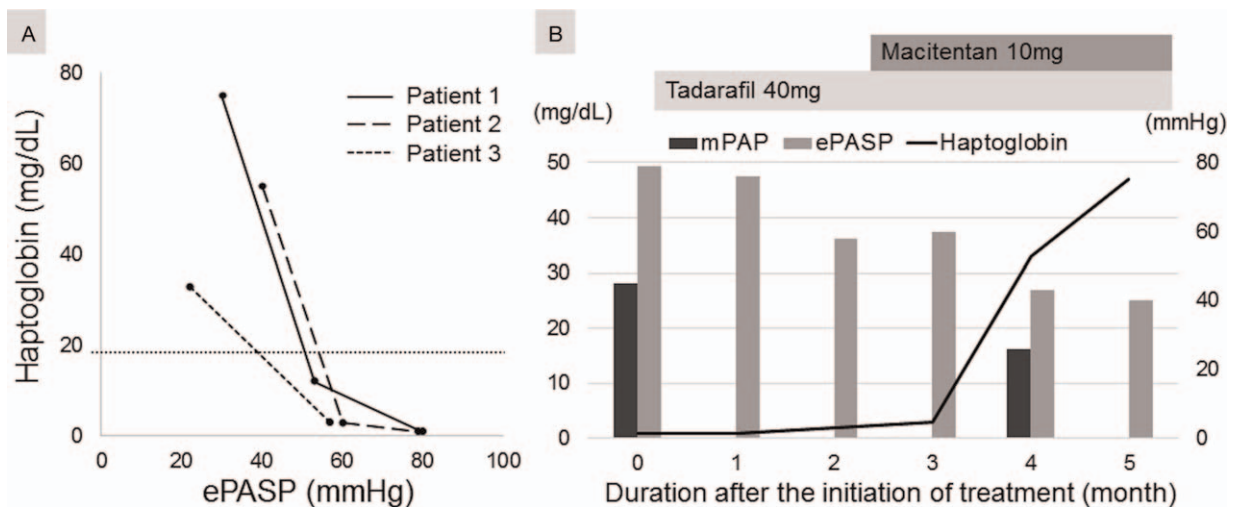
Another possible explanation for decreased haptoglobin levels in PAH patients is mechanical hemolysis caused by PH-induced tricuspid valve regurgitation jet.<sup>[18,19]</sup> Hemolysis in prosthetic valve regurgitation has been well recognized; however, hemolysis following native valve regurgitation has been seldom reported. A collision with hard stuff, such as the prosthetic valve and thrombosis, as well as free jet is required to cause hemolysis.<sup>[20]</sup> Therefore, mechanical hemolysis through tricuspid valve regurgitation is unlikely to occur in most of PAH patients.

Serum haptoglobin levels could be affected by various conditions other than hemolysis. Decreased haptoglobin levels are also observed in liver dysfunction, malnutrition, or congenital hypohaptoglobinemia, whereas haptoglobin increases in inflammatory condition. Especially, CTD patients could have various complications influencing serum haptoglobin level independently of PAH. This study included CTD-PAH patients dominantly in PAH group. To decrease the bias about CTD, we included CTD-non-PAH patients as a control group. As shown in Figure 1, decreased haptoglobin levels were rarely observed in CTD-non-PAH patients (6%), and were less frequent compared with PAH patients (26%). In addition, we ruled out the major complications of CTD influencing serum haptoglobin levels, such as autoimmune hemolytic anemia, autoimmune hepatitis, and systemic thrombotic microangiopathy, by Coombs test, serum albumin level, serum cholinesterase level, and blood cell count. Moreover, serum haptoglobin levels were correlated with

pulmonary artery pressure in CTD-PAH patients. Based on these findings, it is likely that decreased haptoglobin levels are associated with the presence and degree of PAH rather than other complications of CTD.



**Figure 4.** The box-plot diagrams show the serum lactate dehydrogenase levels in pulmonary arterial hypertension patients with normal haptoglobin levels or those with decreased ones.



**Figure 5.** (A) Transition of serum haptoglobin levels in 3 patients. (B) A representative clinical course of a patient with pulmonary arterial hypertension associated with systemic sclerosis. ePASP = pulmonary artery systolic pressure estimated by echocardiography, mPAP = mean pulmonary arterial pressure.

Anemia and thrombocytopenia were not overt in our PAH patients. We speculate that blood cell count in a total body may not be affected in PAH due to microangiopathy in minor extent, limited in the pulmonary arterioles. Localized microangiopathy to be defined histologically is, in this sense, similar to kidney-limited thrombotic microangiopathy.<sup>[21,22]</sup> It should be discriminated from systemic thrombotic microangiopathy characterized by microangiopathic hemolytic anemia and thrombotic thrombocytopenia<sup>[23]</sup>; however, microangiopathic hemolytic anemia and thrombotic thrombocytopenia in the patients with extremely severe PAH were previously described in the literatures.<sup>[4,5]</sup>

CTEPH is defined as persistent PH caused by chronic thrombotic occlusion of major to small pulmonary arteries. Histological findings of CTEPH show organized thrombotic lesions, intimal thickening, and fibrosis of the small pulmonary arterioles. Thrombotic microangiopathy could be another important pathophysiology of CTEPH in addition to thrombotic occlusion of major pulmonary arteries.<sup>[24]</sup> In addition, it was reported that serum haptoglobin levels were decreased in patients at high risk for pulmonary embolism.<sup>[25]</sup> However, in our CTEPH patients, the correlation between haptoglobin levels and mPAP was not observed. Serum haptoglobin levels were even high in 15% of the CTEPH patients. A previous study showed the increased haptoglobin levels in patients with acute pulmonary embolism, but the increase was counterbalanced by increased pulmonary arterial pressure.<sup>[19]</sup> Pulmonary embolism causes an induction of haptoglobin and may mask an underlying hemolysis.

This study includes some potential limitations. First, it was conducted at a single center, had a small sample size, and used a cross-sectional design. Moreover, patients included in the study had a long duration of PH, and already had been on treatment of PH. The findings of this study require to be confirmed by multicenter, large sample, and prospective longitudinal studies. Secondly, this study lacks histological confirmations even though it is difficult to perform the lung biopsy in patients with PH. We need to evaluate the relation between serum haptoglobin levels and histological findings. Thirdly, we cannot discuss the relation of cause and effect in the cross-sectional study. It is known that

hemolytic disorders cause pulmonary hypertension reversely.<sup>[26]</sup> Besides, we cannot deny the presence of Coombs-negative autoimmune hemolytic anemia<sup>[27]</sup> in our patients.

## 5. Conclusions

We first report the decreased haptoglobin levels correlated with pulmonary artery pressure in patients with CTD-PAH. This finding may reflect microangiopathy in the pulmonary arterioles as one of the important pathophysiology of PAH. In addition, this is the pilot study suggesting the usefulness of serum haptoglobin levels as a potential surrogate marker for CTD-PAH.

## References

- [1] Galie N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Rev Esp Cardiol (Engl Ed)* 2016;69:177.
- [2] Tuder RM, Abman SH, Braun T, et al. Development and pathology of pulmonary hypertension. *J Am Coll Cardiol* 2009;54:53–9.
- [3] Irzyk K, Bienias P, Rymarczyk Z, et al. Assessment of systemic and pulmonary arterial remodelling in women with systemic sclerosis. *Scand J Rheumatol* 2015;44:385–8.
- [4] Stuard ID, Heusinkveld RS, Moss AJ. Microangiopathic hemolytic anemia and thrombocytopenia in primary pulmonary hypertension. *N Engl J Med* 1972;287:869–70.
- [5] Jubelirer SJ. Primary pulmonary hypertension. Its association with microangiopathic hemolytic anemia and thrombocytopenia. *Arch Intern Med* 1991;151:1221–3.
- [6] Johnson SR, Granton JT, Mehta S. Thrombotic arteriopathy and anticoagulation in pulmonary hypertension. *Chest* 2006;130:545–52.
- [7] Ngian GS, Stevens W, Prior D, et al. Predictors of mortality in connective tissue disease-associated pulmonary arterial hypertension: a cohort study. *Arthritis Res Ther* 2012;14:R213.
- [8] Hesselstrand R, Wildt M, Ekmechag B, et al. Survival in patients with pulmonary arterial hypertension associated with systemic sclerosis from a Swedish single centre: prognosis still poor and prediction difficult. *Scand J Rheumatol* 2011;40:127–32.
- [9] Morrisroe K, Huq M, Stevens W, et al. Determinants of unemployment amongst Australian systemic sclerosis patients: results from a multicentre cohort study. *Clin Exp Rheumatol* 2016;34(Suppl. 100):79–84.
- [10] Coghlan JG, Denton CP, Grunig E, et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis* 2014;73:1340–9.

- [11] Dobryszcka W. Biological functions of haptoglobin—new pieces to an old puzzle. *Eur J Clin Chem Clin Biochem* 1997;35:647–54.
- [12] Barcellini W, Fattizzo B. Clinical Applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. *Dis Markers* 2015;2015:635670.
- [13] McLaughlin VV, Archer SL, Badesch DB, et al. ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. *J Am Coll Cardiol* 2009;53:1573–619.
- [14] Voelkel MA, Wynne KM, Badesch DB, et al. Hyperuricemia in severe pulmonary hypertension. *Chest* 2000;117:19–24.
- [15] Gong JN, Zhai ZG, Yang YH, et al. Serum bilirubin and 6-min walk distance as prognostic predictors for inoperable chronic thromboembolic pulmonary hypertension: a prospective cohort study. *Chin Med J (Engl)* 2015;128:3125–31.
- [16] Brittain EL, Janz DR, Austin ED, et al. Elevation of plasma cell-free hemoglobin in pulmonary arterial hypertension. *Chest* 2014;146:1478–85.
- [17] Dahan I, Farber E, Thauho N, et al. Interaction between the haptoglobin 2 phenotype and diabetes mellitus on systolic pulmonary arterial pressure and nitric oxide bioavailability in hemodialysis patients. *J Diabetes Res* 2015;2015:613860.
- [18] Zagorski J, Marchick MR, Kline JA. Rapid clearance of circulating haptoglobin from plasma during acute pulmonary embolism in rats results in HMOX1 up-regulation in peripheral blood leukocytes. *J Thromb Haemost* 2010;8:389–96.
- [19] Kline JA, Marchick MR, Hogg MM. Reduction in plasma haptoglobin in humans with acute pulmonary embolism causing tricuspid regurgitation. *J Thromb Haemost* 2009;7:1597–9.
- [20] Garcia MJ, Vandervoort P, Stewart WJ, et al. Mechanisms of hemolysis with mitral prosthetic regurgitation. Study using transesophageal echocardiography and fluid dynamic simulation. *J Am Coll Cardiol* 1996;27:399–406.
- [21] Tomov S, Lazarchick J, Self SE, et al. Kidney-limited thrombotic microangiopathy in patients with SLE treated with romiplostim. *Lupus* 2013;22:504–9.
- [22] Barrera-Vargas A, Rosado-Canto R, Merayo-Chalico J, et al. Renal thrombotic microangiopathy in proliferative lupus nephritis: risk factors and clinical outcomes: a case-control study. *J Clin Rheumatol* 2016;22:235–40.
- [23] Arnold DM, Patriquin CJ, Nazy I. Thrombotic microangiopathies: a general approach to diagnosis and management. *CMAJ* 2017;189:E153–9.
- [24] Dorfmueller P, Gunther S, Ghigna MR, et al. Microvascular disease in chronic thromboembolic pulmonary hypertension: a role for pulmonary veins and systemic vasculature. *Eur Respir J* 2014;44:1275–88.
- [25] Insenser M, Montes-Nieto R, Martinez-Garcia MA, et al. Identification of reduced circulating haptoglobin concentration as a biomarker of the severity of pulmonary embolism: a nontargeted proteomic study. *PLoS One* 2014;9:e100902.
- [26] Machado RF, Gladwin MT. Pulmonary hypertension in hemolytic disorders: pulmonary vascular disease: the global perspective. *Chest* 2010;137:30s–8s.
- [27] Thedsawad A, Taka O, Wanachiwanawin W. Significances of red cell bound immunoglobulin G as detected by flow cytometry in patients with Coombs-negative immune hemolysis. *Transfus Med* 2016;26:130–7.