Journal of Cardiology 75 (2020) 415–423

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Cyclophilin A as a biomarker for the therapeutic effect of balloon angioplasty in chronic thromboembolic pulmonary hypertension

Katsuya Kozu (MD PhD), Kimio Satoh (MD PhD FJCC)*, Tatsuo Aoki (MD PhD), Shunsuke Tatebe (MD PhD FJCC), Masanobu Miura (MD PhD), Saori Yamamoto (MD PhD), Nobuhiro Yaoita (MD PhD), Hideaki Suzuki (MD PhD), Toru Shimizu (MD PhD), Haruka Sato (MD PhD), Ryo Konno (MD), Yosuke Terui (MD), Kotaro Nochioka (MD PhD FJCC), Nobuhiro Kikuchi (MD PhD), Taijyu Satoh (MD PhD), Koichiro Sugimura (MD PhD), Satoshi Miyata (PhD), Hiroaki Shimokawa (MD PhD FJCC)

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

ARTICLE INFO

Article history:
Received 5 June 2019
Received in revised form 17 August 2019
Accepted 18 September 2019
Available online 12 October 2019

Keywords:
Brain natriuretic peptide
Hemodynamics
Pulmonary circulation
Pulmonary thromboembolism

ABSTRACT

Background: Although cardiac troponin and natriuretic peptide have been shown to decrease after balloon pulmonary angioplasty (BPA) with improved right ventricular afterload in chronic thromboembolic pulmonary hypertension (CTEPH), biomarkers to evaluate the effects of BPA independently of heart failure status remain to be developed.
Methods: In 39 consecutive CTEPH patients including 31 who underwent BPA, we measured plasma levels of cyclophilin A (CyPA), which we demonstrated is secreted from pulmonary vascular smooth muscle cells in response to mechanical stretch and hypoxia.
Results: CyPA levels were elevated in CTEPH patients (12.7, IQR: 7.6-16.0) compared with 8 thromboembolic controls with a history of venous thromboembolism (4.9, IQR: 2.4-11.2) or 18 healthy controls (4.1, IQR: 2.4-6.8) (both $p < 0.05$) and were linearly correlated with mean pulmonary arterial pressure ($r = 0.50$, $p = 0.0003$) and pulmonary vascular resistance ($r = 0.32$, $p = 0.026$). BPA reduced CyPA levels and tended to lower brain-type natriuretic peptide (BNP) levels ($p < 0.01$ and $p = 0.07$). When comparing the changes in CyPA before and after BPA in the two subgroups with higher (≥35 pg/mL) and normal (<35 pg/mL) BNP at baseline, CyPA decreased both in patients with higher BNP and those with normal BNP (both $p < 0.05$). In contrast, BNP decreased only in patients with higher BNP ($p < 0.05$). Also, CyPA decreased both in patients with lower (<25 kg/m^2) and higher (≥25 kg/m^2) body mass index (BMI) at baseline (both $p < 0.05$), whereas BPA tended to reduce BNP in patients with lower BMI ($p = 0.12$) but not in those with higher BMI ($p = 0.55$).
Conclusions: CyPA could be a useful biomarker to evaluate the effects of BPA even in patients with normal BNP or high BMI.

Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is characterized by the persistence of stenosis and obstruction of the pulmonary arteries as organized thrombi and fibrous tissue with resultant pulmonary hypertension [1]. Pulmonary endarterectomy remains the gold-standard treatment for CTEPH with organized thrombi in the main, lobar, or segmental pulmonary arteries [2]. Recent studies reported that non-operated CTEPH patients had a poorer prognosis compared with operated CTEPH patients [3]. We and others have recently demonstrated the beneficial effects of balloon pulmonary angioplasty (BPA) for CTEPH patients, including the beneficial hemodynamic and prognostic effects for inoperable CTEPH patients more than those which were previously reported [4–6]. Indeed, the use of BPA for CTEPH has been expanding

* Corresponding author: Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.
E-mail address: satoh-k@cardio.med.tohoku.ac.jp (K. Satoh).

https://doi.org/10.1016/j.jjcc.2019.09.010
0914-5087/

worldwide [4–7]. Although non-invasive diagnostic methods have recently attracted much attention [8], we still need an invasive examination by right heart catheterization to evaluate the effectiveness of BPA. Kriechbaum et al. reported that cardiac troponin and N-terminal pro-B-type natriuretic peptide decreased after BPA indicating that reducing right ventricular afterload has led to lessening right ventricular wall stress and myocardial damage [9,10]. However, non-invasive biomarkers for evaluation of BPA therapy independent of heart failure status have not been established.

Cyclophilin A (CyPA) was initially discovered as a binding partner of the immunosuppressive drug cyclosporine in 1984 [11]. Intracellular CyPA plays essential roles in protein folding and trafficking of extracellular signal-regulated kinase 1/2 and apoptosis-inducing factor [12,13]. CyPA is secreted from endothelial cells [14], cardiac fibroblasts [15], adipocytes [16], macrophages [15], activated platelets [17,18], and is secreted from vascular smooth muscle cells (VSMCs) in response to several stimuli including mechanical stretch, hypoxia, and oxidative stress [19]. We found that intracellular and extracellular CyPA promotes intimal thickening, abdominal aortic aneurysms, atherosclerosis, and cardiac hypertrophy in mice [20,21]. The secretion of CyPA is regulated by the activation of Rho-kinase [22], which plays a crucial role in inflammation, vascular contraction, and the development of cardiovascular diseases [23,24]. Additionally, we have demonstrated that plasma levels of CyPA are significantly higher in patients with coronary artery disease in proportion to the severity of the disorder [20,21]. Notably, Zuern et al. reported that CyPA expression in myocardial biopsy samples is an independent predictor of clinical outcome in patients with heart failure [25]. A previous study showed that CyPA expression increased in a time-dependent manner in the pulmonary microvascular walls in hypoxia-induced pulmonary hypertension (PH) in wild-type mice [26]. Also, CyPA was also demonstrated to be strongly expressed in the remodeled pulmonary microvasculature in patients with pulmonary arterial hypertension (PAH) undergoing lung transplantation [26]. Importantly, plasma CyPA levels in patients with PAH were increased parallel to the severity of pulmonary vascular resistance (PVR), and high plasma CyPA levels in those patients were associated with poor outcomes including death and lung transplantation [26].

In the present study, we thus aimed to evaluate plasma CyPA levels also in CTEPH patients and to examine the change of CyPA before and after BPA for determining whether CyPA could be useful to assess the effect of BPA.

Methods

Study population

The ethical review board of Tohoku University approved the study protocol, and written informed consent was obtained from all patients (No. 2008-470, 2015-1-191). We enrolled a total of 39 CTEPH patients including 31 patients who underwent BPA in our hospital from January 2010 to April 2017. They were diagnosed as having inoperable CTEPH based on the standard criteria [27]. CTEPH patients were compared with 8 thromboembolic controls and 18 healthy controls. Thromboembolism controls were patients who had a history of venous thromboembolism (VTE) with findings of PH on echocardiography or with residual thrombi on scintigraphy or contrast-enhanced computed tomography but had mean pulmonary arterial pressure (mPAP) <25 mmHg on right heart catheterization. Healthy controls were patients who underwent coronary arteriography for examination of chest pain. In all the CTEPH patients and thromboembolic controls, we examined hemodynamic parameters by right heart catheterization with measurements of plasma levels of CyPA and brain natriuretic peptide (BNP). In 31 CTEPH patients treated with BPA, we also compared the data before and after the BPA procedure.

BPA

We performed BPA via the right femoral vein to treat pulmonary arterial branches [4,28]. We selected targeted vessels based on comprehensive findings, including webs, bands, abrupt narrowing, and complete obstructions, obtained by pulmonary angiography, 3D-reconstructed computed tomography, and intravascular imaging modalities including optical computed tomography [4,28]. The procedures were repeated more than 4 weeks apart in all patients, and additional BPA was repeated until mPAP decreased below 30 mmHg or no more treatable lesions were found [4,28]. Treating physicians were not aware of the CyPA level while making management decisions.

Measurement of plasma CyPA levels

In CTEPH patients, fasting blood samples for the measurement of CyPA were drawn from the sheath immediately after being inserted into a vein in both timings of diagnostic catheterization and BPA session. The blood samples were also drawn at the beginning of the catheterization from the sheath in VTE controls and a peripheral vein in healthy controls. Plasma samples were collected using EDTA and centrifuged for 10 min at 2,500 g within 30 min of collection, and aliquots were stored at −80 °C. CyPA was measured with the use of an immunoassay based on the sandwich technique according to the protocol (Human Cyclophilin A ELISA Kit, CSB-E09920 h, Cusabio, Wuhan, China) [21]. The detection limit was 0.78 ng/ml.

Statistical analysis

Continuous variables are expressed as the mean ± SD or median (interquartile range), and categorical variables as the number (%). Means, medians, and percentages were compared using paired t-test, Wilcoxon signed-rank test, and χ^2 or Fisher exact test, as appropriate. Values of $p < 0.05$ were considered to be statistically significant for all analyses. All analyses were performed with JMP Pro 12.2.0 (Japanese version, SAS Institute Inc., Tokyo, Japan).

Results

Patient characteristics

Baseline characteristics and hemodynamic profile of the CTEPH patients, VTE controls, and healthy controls are shown in Table 1. The mean age of CTEPH patients was 63 ± 12 years, and 87% were female. The proportion of females was higher in the CTEPH cohort than in the other two groups. Of the patients with CTEPH, 46% had hypertension, 23% diabetes mellitus, and 38% dyslipidemia, frequencies that were comparable to the other two controls. Mean PAP, cardiac index, and the median value of PVR in CTEPH patients were 34 ± 8 mmHg, 2.5 ± 0.7 L/min/m^2, and 528 [inter-quartile range (IQR), 398-688] dynes/sec/cm^5, respectively, which were worse than VTE controls. CTEPH patients underwent a median of 2 sessions of BPA per patient (IQR, 1–3), and the median follow-up period before and after BPA was 1.8 years. Their characteristics and medications before and after BPA are shown in Table 2. The use of PH-specific medications was comparable before and after BPA.

Hemodynamic parameters before and after BPA

BPA significantly improved the hemodynamic status, including mPAP (34 ± 7 to 26 ± 5 mmHg) and PVR (562 ± 252 to 354 ± 124

Table 1
Patient characteristics.

	CTEPH	VTE controls	Healthy controls	*p*-Value CTEPH vs VTE controls	*p*-Value CTEPH vs Healthy controls
N	39	8	18		
Age (years)	63 ± 12	62 ± 10	56 ± 13	0.83	0.06
Female (%)	34 (87)	2 (25)	10 (56)	0.001	0.02
Body mass index	23 ± 4	24 ± 2	24 ± 3	0.64	0.63
eGFR (mL/min/1.73 m^2)	63 ± 16	67 ± 30	71 ± 15	0.69	0.07
D-dimer (μg/mL)	0.5 (0.5–0.7)	1.1 (0.5–1.8)	0.6 (0.5–0.9)	0.02	0.12
BNP (pg/mL)	36 (14–50)	25 (8–55)	19 (6–40)	0.44	0.11
WHO FC III or IV (%)	5 (13)	1 (13)	0	1.00	0.17
Medical history					
Hypertension	18 (46)	5 (63)	11 (61)	0.46	0.40
Diabetes	9 (23)	3 (38)	7 (39)	0.40	0.34
Dyslipidemia	15 (38)	2 (25)	11 (61)	0.69	0.15
Hemodynamics					
mPAP (mmHg)	34 ± 8	15 ± 3	NA	<0.0001	NA
PAWP (mmHg)	9 ± 3	8 ± 4	NA	0.47	NA
RAP (mmHg)	5 ± 3	5 ± 2	NA	0.81	NA
CI (L/min/m^2)	2.5 ± 0.7	3.0 ± 0.6	NA	0.03	NA
PVR (dynes/sec/cm^5)	528 (398–688)	114 (111–140)	NA	<0.0001	NA
Heart rate (bpm)	70 ± 11	72 ± 9	NA	0.62	NA
SaO_2 (%)	92 ± 5	96 ± 4	NA	0.10	NA
SvO_2 (%)	67 ± 6	70 ± 10	NA	0.11	NA

Data are presented as n (%), mean ± SD or median (interquartile range), unless otherwise stated. BNP, brain natriuretic peptide; CI, cardiac index; CTEPH, chronic thromboembolic pulmonary hypertension; eGFR, estimated glomerular filtration rate; mPAP, mean pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SaO_2, arterial oxygen saturation; SvO_2, mixed venous oxygen saturation; VTE, venous thromboembolism; WHO FC, World Health Organization functional class.

dynes/sec/cm^5), and heart rate (72 ± 10 to 67 ± 9 bpm) (Fig. 1). Before and after BPA, no significant change was noted in right atrial pressure, cardiac index, or mixed venous oxygen saturation.

Plasma CyPA levels

Plasma CyPA levels were elevated in CTEPH patients (12.7, IQR: 7.6–16.0) compared with thromboembolic controls (4.9, IQR: 2.4-11.2, $p = 0.04$) and with healthy controls (4.1, IQR: 2.4-6.8, $p < 0.001$) (Fig. 2A). There was no significant difference between thromboembolic controls and healthy controls. In 31 CTEPH patients who underwent BPA, the procedure reduced plasma CyPA levels (12.0, IQR: 8.4-15.8 to 4.7, IQR: 0.8-8.4, $p < 0.0001$) (Fig. 2B), and tended to reduce plasma BNP levels (26, IQR: 13-47 to 19, IQR: 9-36, $p = 0.07$) (Fig. 2C). In CTEPH patients before BPA and VTE controls at baseline, plasma CyPA levels were linearly correlated with mPAP ($r = 0.50$, $p = 0.0003$) and PVR ($r = 0.32$, $p = 0.026$) (Fig. 3A, B). Similarly, plasma BNP levels were correlated with mPAP ($r = 0.40$, $p = 0.006$) and PVR ($r = 0.57$, $p < 0.0001$) (Fig. 3C, D).

Table 2
Changes in patient characteristics and medications before and after BPA.

	Pre BPA	Post BPA	p-Value
Hemoglobin (g/dL)	13.2 ± 1.4	12.6 ± 1.4	0.03
Creatinine (mg/dL)	0.78 ± 0.20	0.74 ± 0.19	0.14
Estimated GFR (mL/min/1.73 m^2)	64 ± 16	67 ± 16	0.39
Uric acid (mg/dL)	5.8 ± 2.1	5.3 ± 1.5	0.08
Aspartate aminotransferase (IU/L)	24 ± 13	21 ± 8	0.47
Alanine aminotransferase (IU/L)	21 ± 17	16 ± 7	0.09
Gamma-glutamyl transpeptidase (IU/L)	21 (15–41)	18 (14–27)	0.10
Triglyceride (mg/dL)	109 ± 43	118 ± 64	0.61
LDL-cholesterol (mg/dL)	115 ± 33	111 ± 40	0.30
HDL-cholesterol (mg/dL)	59 ± 16	62 ± 16	0.16
Hemoglobin A1c (%)	6.0 ± 0.6	6.0 ± 0.6	0.98
High-sensitivity CRP (mg/dL)	0.08 (0.05–0.14)	0.05 (0.03–0.09)	0.21
D-dimer (μg/mL)	0.5 (0.5–0.9)	0.5 (0.5–0.6)	0.15
Pulmonary vasodilators			
PDE-5i	14 (45)	14 (45)	1.00
ERA	6 (19)	8 (26)	0.16
Oral prostacyclin analogues	14 (45)	13 (42)	0.56
Epoprostenol	3 (10)	1 (3)	0.16
sGC stimulator	6 (19)	8 (26)	0.16
Calcium channel blockers	8 (26)	7 (23)	0.56
Statins	7 (23)	10 (32)	0.08
Diuretics	14 (45)	10 (32)	0.10
Anticoagulants	31 (100)	31 (100)	1.00

Data are presented as n (%), mean ± SD or median (interquartile range), unless otherwise stated. BPA, balloon pulmonary angioplasty; CRP, C-reactive protein; ERA, endothelin receptor antagonists; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PDE-5i, phosphodiesterase-5 inhibitors; sGC, soluble guanylate cyclase.

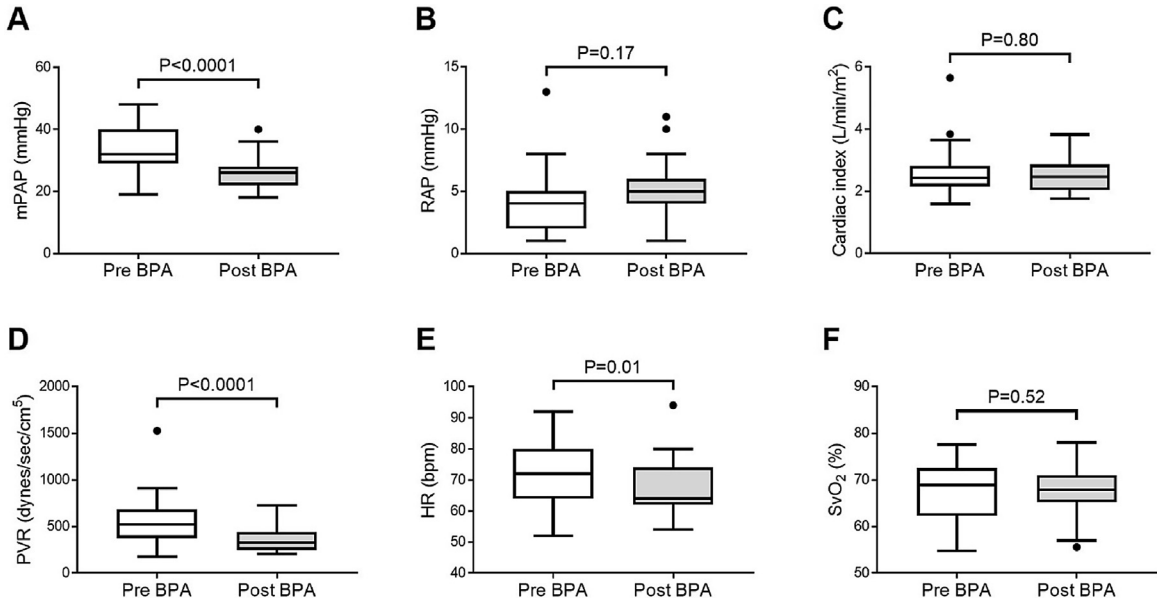

Fig. 1. Hemodynamic parameters before and after balloon pulmonary angioplasty (BPA) in chronic thromboembolic pulmonary hypertension patients (n = 31). (A) Mean pulmonary arterial pressure (mPAP). (B) Right atrial pressure (RAP). (C) Cardiac index. (D) Pulmonary vascular resistance (PVR). (E) Heart rate (HR). (F) Mixed venous oxygen saturation (SvO_2).

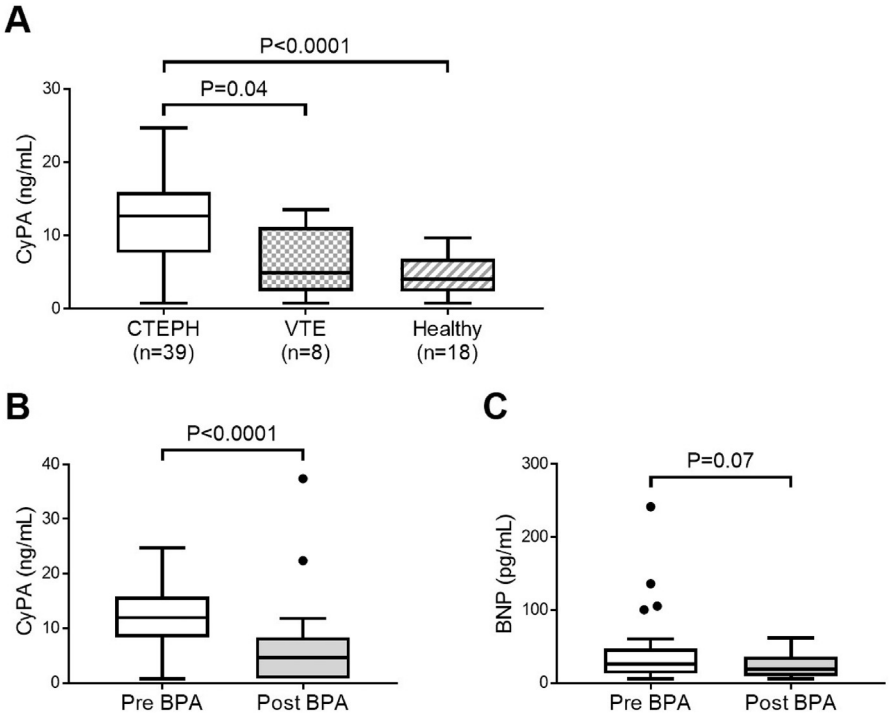

Fig. 2. Plasma cyclophilin A (CyPA) Levels. (A) Plasma CyPA levels were significantly elevated in patients with chronic thromboembolic pulmonary hypertension (CTEPH) compared with patients with a history of venous thromboembolism (VTE) without pulmonary hypertension or healthy controls. (B and C) Balloon pulmonary angioplasty (BPA) markedly reduced CyPA (B), but only tended to reduce brain natriuretic peptide (BNP) (C) (n = 31).

Differences as a biomarker between BNP and CyPA

We further compared the changes in plasma CyPA levels before and after BPA in the two subgroups with higher (≥35 pg/ml, $n = 12$) and normal (<35 pg/ml, $n = 19$) levels of BNP at baseline according to the upper limit of normal in heart failure [29]. Patients with higher BNP and those with normal BNP at baseline both showed decreased plasma levels of CyPA after BPA (10.7, IQR: 8.2-15.7 to 3.3, IQR: 0.8-8.2, $p = 0.0005$; 12.8, IQR: 8.4-15.8 to 4.7, IQR: 0.8-10.8, $p = 0.013$, respectively) (Fig. 4A). In contrast, patients with higher BNP at baseline showed decreased plasma levels of BNP (54, IQR: 39-104 to 36, IQR: 9-46, $p = 0.016$) but not in those with normal BNP at baseline (14, IQR: 9-25 to 15, IQR: 9-21, $p = 0.87$) (Fig. 4B). Additionally, we compared the changes in plasma CyPA levels before and after BPA in the two subgroups with lower (<25 kg/m^2, $n = 22$) and higher (≥25 kg/m^2, $n = 9$) levels of body mass index (BMI) at baseline. BPA reduced CyPA both in patients with lower BMI and those with higher BMI (12.2, IQR: 8.2-15.6 to 4.6, IQR:

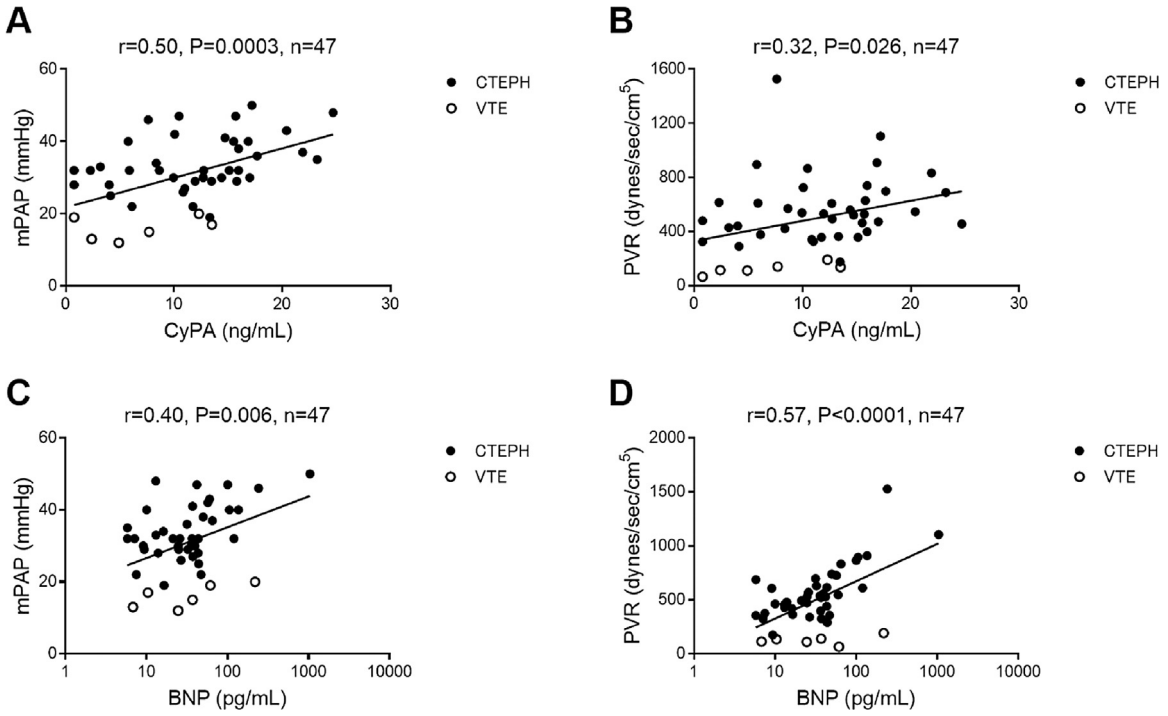

Fig. 3. Relationship between cyclophilin A (CyPA) or brain-type natriuretic peptide (BNP) levels and hemodynamic parameters at baseline. In chronic thromboembolic pulmonary hypertension (CTEPH) patients and those with a history of venous thromboembolism (VTE) without pulmonary hypertension, plasma CyPA levels linearly correlated with (A) baseline mean pulmonary arterial pressure (mPAP) and (B) pulmonary vascular resistance (PVR). BNP also correlated with (C) baseline mPAP and (D) PVR.

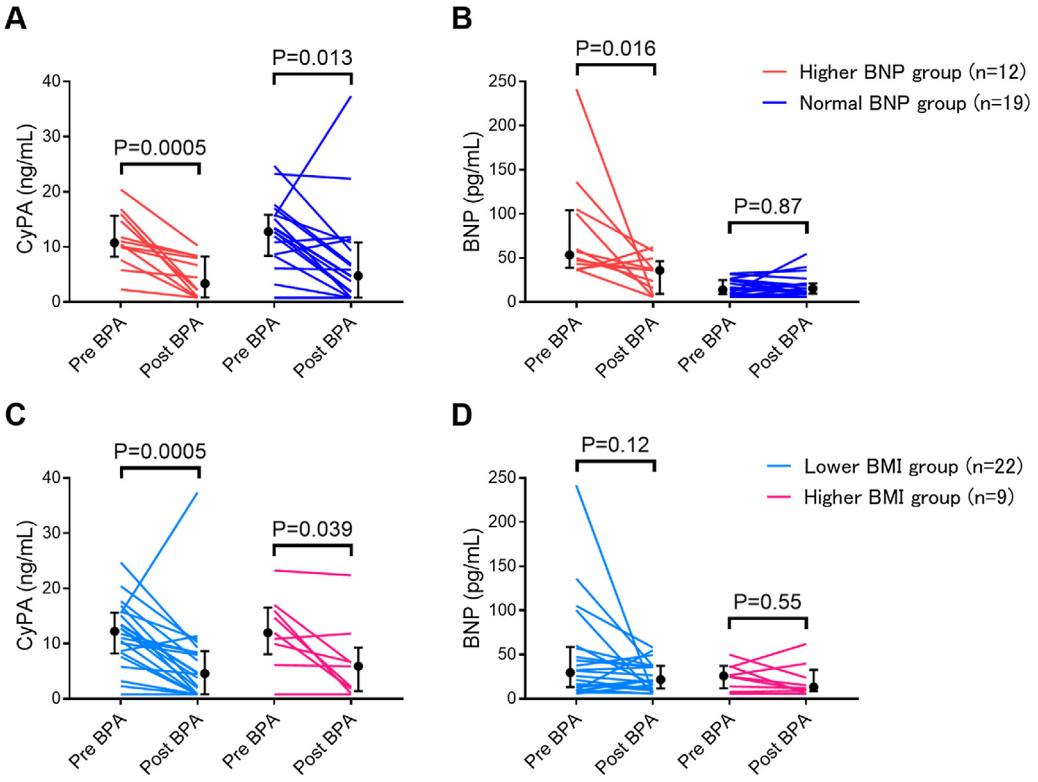

Fig. 4. Differences as a biomarker between brain-type natriuretic peptide (BNP) and cyclophilin A (CyPA) in chronic thromboembolic pulmonary hypertension by BNP or by body mass index (BMI) at baseline. (A) Patients with both higher (≥35 pg/mL) and normal (<35 pg/mL) levels of BNP at baseline showed a significant decrease in plasma levels of CyPA after balloon pulmonary angioplasty (BPA). (B) Patients with higher BNP at baseline showed a significant reduction in plasma levels of BNP but not in those with normal BNP after BPA. (C) In patients with both lower (<25 kg/m^2) and higher (≥25 kg/m^2) BMI, BPA significantly reduced CyPA. (D) In patients with lower BMI at baseline, BPA tended to decrease plasma BNP levels but not in those with higher BMI.

Table 3
Changes in hemodynamics before and after BPA according to BNP or BMI at baseline

	Higher BNP group (≥35 pg/mL) (n = 12)			Normal BNP group (<35 pg/mL) (n = 19)		
	Pre BPA	Post BPA	*p*-Value	Pre BPA	Post BPA	*p*-Value
mPAP (mmHg)	37 ± 8	27 ± 6	0.0005	31 ± 6	25 ± 5	0.0005
PVR (dynes/sec/cm^5)	670 (526–888)	387 (258–474)	0.002	463 (364–570)	309 (244–395)	0.0002
	Lower BMI group (<25 kg/m^2) (n = 22)			Higher BMI group (≥25 kg/m^2) (n = 9)		
	Pre BPA	Post BPA	*p*-Value	Pre BPA	Post BPA	*p*-Value
mPAP (mmHg)	35 ± 8	26 ± 5	<0.0001	31 ± 6	27 ± 6	0.004
PVR (dynes/sec/cm^5)	520 (362–705)	321 (253–446)	0.0001	521 (424–613)	328 (215–455)	0.004

Data are presented as mean ± SD or median (interquartile range), unless otherwise stated.
BMI, body mass index; BNP, brain natriuretic peptide; BPA, balloon pulmonary angioplasty; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance.

0.8-8.6, $p = 0.0005$; 12.0, IQR: 8.0-16.5 to 5.9, IQR: 1.4-9.3, $p = 0.039$, respectively) (Fig. 4C). On the other hand, BPA tended to reduce BNP in patients with lower BMI (29, IQR: 12-58 to 21, IQR: 10-36, $p = 0.12$) but not in those with higher BMI (25, IQR: 11-36 to 12, IQR: 8-32, $p = 0.55$) (Fig. 4D). The changes in hemodynamics between 2 groups according to BNP or BMI at baseline were comparable before and after BPA (Table 3).

Discussion

The novel findings of this study are as follows: 1) plasma CyPA levels were significantly elevated in CTEPH patients compared with VTE controls and healthy controls, 2) CyPA levels were correlated with mPAP and PVR, 3) BPA reduced CyPA regardless of the levels of BNP at baseline, whereas there was no significant change in BNP levels in patients with normal BNP levels at baseline, and 4) BPA reduced CyPA regardless of BMI at baseline, whereas there was no apparent change in BNP in patients with high BMI at baseline. To the best of our knowledge, this is the first study that demonstrates that plasma levels of CyPA in CTEPH patients could be a useful biomarker to evaluate the effects of BPA even in patients with normal BNP levels or high BMI.

The role of CyPA in the pathophysiology of CTEPH

The organized thrombi in CTEPH patients were considered to be originated from acute pulmonary thromboembolism [1]. However, its pathophysiological mechanism has not been fully elucidated [30]. Since the frequency of transition from acute pulmonary thromboembolism to CTEPH is reported to be between 0.1% and 3.8% [31,32], the pathophysiology other than coagulation and fibrinolysis abnormalities may be involved in the onset mechanism of CTEPH.

Inflammation is now increasingly recognized to play a key role in the development of PAH [33]. Here, similar findings are recently accumulating in CTEPH patients [34]. Several reports of a series of CTEPH patients who underwent pulmonary endarterectomy showed the presence of inflammation in resected pulmonary artery tissue, such as neointima, angiogenesis, increased cellularity within thrombi around newly formed recanalizing vessels, and atherosclerotic lesions containing macrophage-derived foam cells with lipid droplets [35,36]. Moreover, chronic inflammatory disease including inflammatory bowel disease and osteomyelitis has been associated with an increased risk of CTEPH [37]. Indeed, inflammatory markers including C-reactive protein, interleukin-10, monocyte chemotactic protein-1, macrophage inflammatory protein-1α, and matrix metalloproteinase-9 have been reported to be increased in CTEPH patients compared with healthy controls [36].

Additionally, we have recently demonstrated that the thrombin-activatable fibrinolysis inhibitor plays a crucial role in the development of CTEPH and promotes the secretion of inflammatory cytokines [34,38]. Moreover, CyPA is secreted from endothelial cells, VSMCs, adipocytes, macrophages, and activated platelets [14–18], and stimulates VSMCs through basigin, which is an extracellular receptor for CyPA. Extracellular CyPA promotes cell proliferation and inflammation and induces inflammatory cell migration [26].

In CTEPH patients, pulmonary vasculature is exposed to pressure overload, shear stress, and hypoxia [34]. In the present study, plasma CyPA levels were significantly correlated with mPAP and PVR. Here, vascular reactive oxygen species (ROS) formation can be stimulated by mechanical stretch, pressure, shear stress, hypoxia, and growth factors, all of which activate the Rho-kinase system [39]. Importantly, excessive and continuous activation of Rho-kinase promotes the secretion of CyPA [22], which generates a vicious cycle of ROS augmentation, affecting endothelial cells, VSMCs, and inflammatory cells [23]. The enhanced expression of CyPA has been shown in pulmonary vascular lesions in both experimental PH models and PAH patients [26]. Moreover, acetylated CyPA accelerates PH by augmenting oxidative stress and inflammation [40]. Interestingly, the antibody-based blockade of extracellular CyPA inhibited thrombosis and thrombi-related inflammation without affecting blood homeostasis in vitro and in vivo [41]. These findings highlight the potential role of CyPA through inflammation and ROS in the development of CTEPH.

The effects of BPA on the secretion of CyPA

The present results provide the mechanistic insights into the CyPA-mediated hemodynamic assessment and effectiveness after BPA. According to a report of measuring pulmonary artery flow by phase contrast magnetic resonance imaging after BPA in one lung, PVR and mPAP were decreased in both lungs after BPA [42]. In contrast, pulmonary artery flow was increased only in the treatment side and was reduced in the non-treatment side, suggesting that the decrease in PVR on the non-treatment side was the indirect effect exerted by hemodynamic unloading and reducing inflammation which could regress the progression of the small-vessel arteriopathy in non-treatment side vasculature [42]. Moreover, lumen diameter and blood flow were improved even more after 3 months than immediately after BPA, although no additional procedure was performed at the lesion [43]. An autopsy of a PAH patient at 9 years after single-lung transplantation gives

support to those findings of BPA-mediated reverse remodeling of the pulmonary vasculature in CTEPH [44]. The case with severe PAH has shown that single-lung transplantation shifted the pulmonary arterial flow from the native diseased lung into the lung allograft soon after transplantation, which gradually normalized over the years [44]. Furthermore, an autopsy revealed the amelioration of the small-vessel arteriopathy including vessel occlusion and plexiform lesion in the native diseased lung compared to the lung resected 9 years previously [44]. These findings may indicate that pulmonary unloading by BPA reduces shear stress and improves oxygenation, and soothing inflammation in the process of pulmonary vascular reverse remodeling, resulting in less ROS production and CyPA secretion.

Next, it must be noticed that right heart function is closely linked to the prognosis of CTEPH patients [45]. Patient outcome is mostly determined by the adaptation of the right ventricle to pressure load [45]. The increase in pressure overload leads to an increase in wall stress, causing right ventricular hypertrophy, and eventually cardiac contractile force decreases, resulting in right ventricular dilation, for which maladaptive neurohumoral signaling, oxidative stress, and inflammatory responses may be involved [46]. Although no significant change was noted in right atrial pressure or cardiac index after BPA at rest, recent studies have shown the amelioration of exercise tolerance [4] and indices of right heart function as evaluated by echocardiography [47] and magnetic resonance imaging [48] after BPA. Here, CyPA has a direct hypertrophic effect on cardiac myocytes and stimulates the proliferation of cardiac fibroblasts, which also secrete CyPA, in response to angiotensin II [15]. Remarkably, basigin, a receptor for CyPA, is upregulated in pressure-overloaded heart and augments inflammation and oxidative stress and mediates mechanical stretch- and angiotensin II-induced activation of cardiac fibroblasts and inflammatory cytokine secretion [49]. We further demonstrated that CyPA and basigin induce cardiac hypertrophy, failure, and postcapillary PH [50]. Importantly, treatment with celastrol which downregulates the expressions of CyPA and basigin in the heart and the lung ameliorated cardiac dysfunction and postcapillary PH [50]. Thus, BPA-mediated reduction in plasma CyPA levels could have been caused by lowering CyPA production from cardiac fibroblasts in the right ventricle due to reduced pressure-overload.

Comparisons between CyPA and BNP

BNP is useful as a biomarker for CTEPH [51], and the reduction of N-terminal pro-B-type natriuretic peptide after BPA is proof of the procedural success of BPA [9]. These findings indicate that BNP is primarily secreted from cardiomyocytes in response to mechanical stretch [52]. On the other hand, we consider that CyPA is mainly secreted from VSMCs [19,53] and cardiac fibroblasts in CTEPH patients [15,49]. BNP is frequently shown as logarithms when correlating with hemodynamic parameters [54,55], in contrast, CyPA had a linear correlation with mPAP and PVR, suggesting the potential as a more sensitive biomarker to detect the slight loading on pulmonary VSMCs and right ventricles. Since actively lowering mPAP has been demonstrated to be quite successful for improving prognosis in PAH patients [56], the characteristics of CyPA may be useful for treating CTEPH.

In this study, BPA reduced plasma CyPA levels regardless of BMI before BPA, whereas there was no significant change in BNP levels in patients with high BMI. Large-scale epidemiological studies demonstrated that natriuretic peptide levels are influenced by obesity, which is called “natriuretic handicap” [57]. This paradoxical phenomenon is explained by increased clearance of natriuretic peptides in adipose tissue in obese subjects, the secretion of bioactive substances by adipose tissue that affects natriuretic peptide production in the heart, alterations in sex hormone production and activity, and insulin resistance [57]. Conversely, plasma CyPA levels were higher in patients with type 2 diabetes irrespective of the presence of coronary artery disease [58]. An experimental study demonstrated that glucocorticoids increased, and free fatty acids, insulin-like growth factor-1, and insulin decreased the expression of the growth hormone stimulatory receptors, growth hormone-releasing hormone receptor, and ghrelin-receptor, without significantly altering CyPA mRNA levels [59]. It is tempting to speculate that plasma CyPA may not be suppressed by obese-related hormones, however, this hypothesis needs scrutiny.

Study limitations

Several limitations should be mentioned in the present study. First, the period of follow-up and the number of BPA procedures differed among patients. Second, evaluation of right ventricular function, pulmonary vascular function, and inflammatory state with modality other than right heart catheterization may result in a more detailed estimation of the source of plasma CyPA. Finally, since CyPA has been shown to have a close relationship with several cardiovascular diseases [60,61] and liver disease [62,63], it can be influenced by other comorbidities before and after BPA. In this study, there were 5 patients with hepatic disorders, including cholelithiasis in 3, alcoholic liver disease in 1, and unknown bile duct dilatation in 1. However, no significant change was noted in the hepatobiliary enzymes before and after BPA. The usefulness of CyPA as a biomarker for liver disease needs to be validated in another study.

Conclusions

In the present study, we were able to demonstrate that plasma levels of CyPA could be a useful biomarker to evaluate the effects of BPA even in patients with normal BNP levels or high BMI.

Sources of Funding

This work was supported in part by the grants-in-aid for Scientific Research (15H02535, 15H04816, and 15K15046) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan, the grants-in-aid for Scientific Research from the Ministry of Health, Labour, and Welfare, Tokyo, Japan (10102895), and the grants-in-aid for Scientific Research from the Japan Agency for Medical Research and Development, Tokyo, Japan (15ak0101035h0001, 16ek0109176h0001, 17ek0109227h0001).

Disclosure

The authors declare that there is no conflict of interest.

Acknowledgments

We are grateful to the laboratory members in the Department of Cardiovascular Medicine of the Tohoku University for valuable technical assistance, especially to Yumi Watanabe, Ai Nishihara, and Hiromi Yamashita.

References

[1] Delcroix M, Vonk Noordegraaf A, Fadel E, Lang I, Simonneau G, Naeije R. Vascular and right ventricular remodelling in chronic thromboembolic pulmonary hypertension. Eur Respir J 2013;41:224–32.
[2] Corsico AG, D'Armini AM, Cerveri I, Klersy C, Ansaldo E, Niniano R, et al. Long-term outcome after pulmonary endarterectomy. Am J Respir Crit Care Med 2008;178:419–24.
[3] Delcroix M, Lang I, Pepke-Zaba J, Jansa P, D'Armini AM, Snijder R, et al. Long-term outcome of patients with chronic thromboembolic pulmonary hypertension: results from an international prospective registry. Circulation 2016;133:859–71.

[4] Aoki T, Sugimura K, Tatebe S, Miura M, Yamamoto S, Yaoita N, et al. Comprehensive evaluation of the effectiveness and safety of balloon pulmonary angioplasty for inoperable chronic thrombo-embolic pulmonary hypertension: long-term effects and procedure-related complications. Eur Heart J 2017;38:3152–9.
[5] Inami T, Kataoka M, Shimura N, Ishiguro H, Yanagisawa R, Taguchi H, et al. Pulmonary edema predictive scoring index (PEPSI), a new index to predict risk of reperfusion pulmonary edema and improvement of hemodynamics in percutaneous transluminal pulmonary angioplasty. JACC Cardiovasc Interv 2013;6:725–36.
[6] Mizoguchi H, Ogawa A, Munemasa M, Mikouchi H, Ito H, Matsubara H. Refined balloon pulmonary angioplasty for inoperable patients with chronic thromboembolic pulmonary hypertension. Circ Cardiovasc Interv 2012;5:748–55.
[7] Madani M, Ogo T, Simonneau G. The changing landscape of chronic thromboembolic pulmonary hypertension management. Eur Respir Rev 2017;26. pii:170105.
[8] Ogawa A, Takahashi Y, Matsubara H. Clinical prediction score for identifying patients with pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis. J Cardiol 2018;72:255–60.
[9] Kriechbaum SD, Wiedenroth CB, Wolter JS, Hutz R, Haas M, Breithecker A, et al. N-terminal pro-B-type natriuretic peptide for monitoring after balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension. J Heart Lung Transplant 2018;37:639–46.
[10] Kriechbaum SD, Wiedenroth CB, Keller T, Wolter JS, Ajnwojner R, Peters K, et al. Dynamics of high-sensitivity cardiac troponin T during therapy with balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension. PLoS One 2018;13e0204683.
[11] Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science 1984;226:544–7.
[12] Pan H, Luo C, Li R, Qiao A, Zhang L, Mines M, et al. Cyclophilin A is required for CXCR4-mediated nuclear export of heterogeneous nuclear ribonucleoprotein A2, activation and nuclear translocation of ERK1/2, and chemotactic cell migration. J Biol Chem 2008;283:623–37.
[13] Zhu C, Wang X, Deinum J, Huang Z, Gao J, Modjtahedi N, et al. Cyclophilin A participates in the nuclear translocation of apoptosis-inducing factor in neurons after cerebral hypoxia-ischemia. J Exp Med 2007;204:1741–8.
[14] Fan LM, Douglas G, Bendall JK, McNeill E, Crabtree MJ, Hale AB, et al. Endothelial cell-specific reactive oxygen species production increases susceptibility to aortic dissection. Circulation 2014;129:2661–72.
[15] Satoh K, Nigro P, Zeidan A, Soe NN, Jaffre F, Oikawa M, et al. Cyclophilin A promotes cardiac hypertrophy in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2011;31:1116–23.
[16] Wang P, Mariman E, Keijer J, Bouwman F, Noben JP, Robben J, et al. Profiling of the secreted proteins during 3T3-L1 adipocyte differentiation leads to the identification of novel adipokines. Cell Mol Life Sci 2004;61:2405–17.
[17] Seizer P, Gawaz M, May AE. Cyclophilin A and EMMPRIN (CD147) in cardiovascular diseases. Cardiovasc Res 2014;102:17–23.
[18] Seizer P, Fuchs C, Ungern-Sternberg SN, Heinzmann D, Langer H, Gawaz M, et al. Platelet- bound cyclophilin A in patients with stable coronary artery disease and acute myocardial infarction. Platelets 2016;27:155–8.
[19] Jin ZG, Melaragno MG, Liao DF, Yan C, Haendeler J, Suh YA, et al. Cyclophilin A is a secreted growth factor induced by oxidative stress. Circ Res 2000;87:789–96.
[20] Satoh K. Cyclophilin A in cardiovascular homeostasis and diseases. Tohoku J Exp Med 2015;235:1–15.
[21] Satoh K, Fukumoto Y, Sugimura K, Miura Y, Aoki T, Nochioka K, et al. Plasma cyclophilin A is a novel biomarker for coronary artery disease. Circ J 2013;77:447–55.
[22] Suzuki J, Jin ZG, Meoli DF, Matoba T, Berk BC. Cyclophilin A is secreted by a vesicular pathway in vascular smooth muscle cells. Circ Res 2006;98:811–7.
[23] Shimokawa H, Satoh K. 2015 ATVB Plenary Lecture: translational research on rho-kinase in cardiovascular medicine. Arterioscler Thromb Vasc Biol 2015;35:1756–69.
[24] Shimokawa H, Sunamura S, Satoh K. RhoA/Rho-kinase in the cardiovascular system. Circ Res 2016;118:352–66.
[25] Zuern CS, Muller KA, Seizer P, Geisler T, Banya W, Klingel K, et al. Cyclophilin A predicts clinical outcome in patients with congestive heart failure undergoing endomyocardial biopsy. Eur J Heart Fail 2013;15:176–84.
[26] Satoh K, Satoh T, Kikuchi N, Omura J, Kurosawa R, Suzuki K, et al. Basigin mediates pulmonary hypertension by promoting inflammation and vascular smooth muscle cell proliferation. Circ Res 2014;115:738–50.
[27] Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J 2016;37:67–119.
[28] Sugimura K, Fukumoto Y, Satoh K, Nochioka K, Miura Y, Aoki T, et al. Percutaneous transluminal pulmonary angioplasty markedly improves pulmonary hemodynamics and long-term prognosis in patients with chronic thromboembolic pulmonary hypertension. Circ J 2012;76:485–8.
[29] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;37:2129–200.
[30] Lang IM, Pesavento R, Bonderman D, Yuan JX. Risk factors and basic mechanisms of chronic thromboembolic pulmonary hypertension: a current understanding. Eur Respir J 2013;41:462–8.
[31] Pengo V, Lensing AW, Prins MH, Marchiori A, Davidson BL, Tiozzo F, et al. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. N Engl J Med 2004;350:2257–64.
[32] Ende-Verhaar YM, Cannegieter SC, Vonk Noordegraaf A, Delcroix M, Pruszczyk P, Mairuhu AT, et al. Incidence of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: a contemporary view of the published literature. Eur Respir J 2017;49. pii:1601792.
[33] Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. Circ Res 2014;115:165–75.
[34] Satoh T, Satoh K, Yaoita N, Kikuchi N, Omura J, Kurosawa R, et al. Activated TAFI promotes the development of chronic thromboembolic pulmonary hypertension: a possible novel therapeutic target. Circ Res 2017;120:1246–62.
[35] Bernard J, Yi ES. Pulmonary thromboendarterectomy: a clinicopathologic study of 200 consecutive pulmonary thromboendarterectomy cases in one institution. Hum Pathol 2007;38:871–7.
[36] Quarck R, Wynants M, Verbeken E, Meyns B, Delcroix M. Contribution of inflammation and impaired angiogenesis to the pathobiology of chronic thromboembolic pulmonary hypertension. Eur Respir J 2015;46:431–43.
[37] Bonderman D, Jakowitsch J, Adlbrecht C, Schemper M, Kyrle PA, Schonauer V, et al. Medical conditions increasing the risk of chronic thromboembolic pulmonary hypertension. Thromb Haemost 2005;93:512–6.
[38] Yaoita N, Satoh K, Satoh T, Sugimura K, Tatebe S, Yamamoto S, et al. Thrombin-activatable fibrinolysis inhibitor in chronic thromboembolic pulmonary hypertension. Arterioscler Thromb Vasc Biol 2016;36:1293–301.
[39] Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, et al. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nature Med 2009;15:649–56.
[40] Xue C, Sowden M, Berk BC. Extracellular cyclophilin A, especially acetylated, causes pulmonary hypertension by stimulating endothelial apoptosis, redox stress, and inflammation. Arterioscler Thromb Vasc Biol 2017;37:1138–46.
[41] von Ungern-Sternberg SNI, Vogel S, Walker-Allgaier B, Geue S, Maurer A, Wild AM, et al. Extracellular cyclophilin A augments platelet-dependent thrombosis and thromboinflammation. Thromb Haemost 2017;117:2063–78.
[42] Hosokawa K, Abe K, Horimoto K, Yamasaki Y, Nagao M, Tsutsui H. Balloon pulmonary angioplasty relieves haemodynamic stress towards untreated-side pulmonary vasculature and improves its resistance in patients with chronic thromboembolic pulmonary hypertension. EuroIntervention 2018;13:2069–76.
[43] Nagayoshi S, Ogawa A, Matsubara H. Spontaneous enlargement of pulmonary artery after successful balloon pulmonary angioplasty in a patient with chronic thromboembolic pulmonary hypertension. EuroIntervention 2016;12e1435.
[44] Levy NT, Liapis H, Eisenberg PR, Botney MD, Trulock EP. Pathologic regression of primary pulmonary hypertension in left native lung following right single-lung transplantation. J Heart Lung Transplant 2001;20:381–4.
[45] Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. Chest 2009;135:794–804.
[46] Voelkel NF, Quaife RA, Leinwand LA, Barst RJ, McGoon MD, Meldrum DR, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation 2006;114:1883–91.
[47] Broch K, Murbraech K, Ragnarsson A, Gude E, Andersen R, Fiane AE, et al. Echocardiographic evidence of right ventricular functional improvement after balloon pulmonary angioplasty in chronic thromboembolic pulmonary hypertension. J Heart Lung Transplant 2016;35:80–6.
[48] Sato H, Ota H, Sugimura K, Aoki T, Tatebe S, Miura M, et al. Balloon pulmonary angioplasty improves biventricular functions and pulmonary flow in chronic thromboembolic pulmonary hypertension. Circ J 2016;80:1470–7.
[49] Suzuki K, Satoh K, Ikeda S, Sunamura S, Otsuki T, Satoh T, et al. Basigin promotes cardiac fibrosis and failure in response to chronic pressure overload in mice. Arterioscler Thromb Vasc Biol 2016;36:636–46.
[50] Sunamura S, Satoh K, Kurosawa R, Ohtsuki T, Kikuchi N, Elias-Al-Mamun M, et al. Different roles of myocardial ROCK1 and ROCK2 in cardiac dysfunction and postcapillary pulmonary hypertension in mice. Proc Natl Acad Sci USA 2018;115:E7129–38.
[51] Simonneau G, D'Armini AM, Ghofrani HA, Grimminger F, Jansa P, Kim NH, et al. Predictors of long-term outcomes in patients treated with riociguat for chronic thromboembolic pulmonary hypertension: data from the CHEST-2 open-label, randomised, long-term extension trial. Lancet Respir Med 2016;4:372–80.
[52] Kinnunen P, Vuolteenaho O, Uusimaa P, Ruskoaho H. Passive mechanical stretch releases atrial natriuretic peptide from rat ventricular myocardium. Circ Res 1992;70:1244–53.
[53] Liao DF, Jin ZG, Baas AS, Daum G, Gygi SP, Aebersold R, et al. Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells. J Biol Chem 2000;275:189–96.
[54] Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S, et al. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. J Am Coll Cardiol 1998;31:202–8.

[55] Helgeson SA, Imam JS, Moss JE, Hodge DO, Burger CD. Comparison of brain natriuretic peptide levels to simultaneously obtained right heart hemodynamics in stable outpatients with pulmonary arterial hypertension. Diseases 2018;6. pii:E33.
[56] Akagi S, Matsubara H, Nakamura K, Ito H. Modern treatment to reduce pulmonary arterial pressure in pulmonary arterial hypertension. J Cardiol 2018;72:466–72.
[57] Clerico A, Giannoni A, Vittorini S, Emdin M. The paradox of low BNP levels in obesity. Heart Fail Rev 2012;17:81–96.
[58] Ramachandran S, Venugopal A, Kutty VR, Vinitha A, Divya G, Chitrasree V, et al. Plasma level of cyclophilin A is increased in patients with type 2 diabetes mellitus and suggests presence of vascular disease. Cardiovasc Diabetol 2014;13:38.
[59] Luque RM, Gahete MD, Valentine RJ, Kineman RD. Examination of the direct effects of metabolic factors on somatotrope function in a non-human primate model, Papio anubis. J Mol Endocrinol 2006;37:25–38.
[60] Nigro P, Pompilio G, Capogrossi MC. Cyclophilin A: a key player for human disease. Cell Death Dis 2013;4:e888.
[61] Ohtsuki T, Satoh K, Omura J, Kikuchi N, Satoh T, Kurosawa R, et al. Prognostic impacts of plasma levels of cyclophilin A in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2017;37:685–93.
[62] Naoumov NV. Cyclophilin inhibition as potential therapy for liver diseases. J Hepatol 2014;61:1166–74.
[63] Mutlu HH, Caklili OT, Coskunpinar E. Serum concentrations of cyclophilin A in patients with nonalcoholic fatty liver disease. Acta Gastroenterol Belg 2017;80:3–7.