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Clinical usefulness of endothelial progenitor cells in predicting the efficacy of riociguat in chronic thromboembolic pulmonary hypertension

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ABSTRACT

Endothelial dysfunction is important in the pathology of pulmonary hypertension, and circulating endothelial progenitor cells (EPCs) have been studied to evaluate endothelial dysfunction. In patients with chronic thromboembolic pulmonary hypertension (CTEPH), riociguat reportedly increases the number of circulating EPCs. However, the relationship between EPC numbers at baseline and changes in clinical parameters after riociguat administration has not been fully elucidated. Here, we evaluated 27 treatment-naïve patients with CTEPH and analyzed the relationships between EPC number at diagnosis and clinical variables (age, hemodynamics, atrial blood gas parameters, brain natriuretic peptide, and exercise tolerance) before and after riociguat initiation. EPCs were defined as $CD45^{dim}$ $CD34^{+}$ $CD133^{+}$ cells and measured by flow cytometry. A low number of circulating EPCs at diagnosis was significantly correlated with increased reductions in mean pulmonary arterial pressure (mPAP) (correlation coefficient = 0.535, P = 0.004) and right atrial pressure (correlation coefficient = 0.618, P = 0.001) upon riociguat treatment. We then divided the study population into two groups according to the mPAP change: a weak-response group (a decrease in mPAP of 4 mmHg or less) and a strong-response group (a decrease in mPAP of more than 4 mmHg). The number of EPCs at diagnosis was significantly lower in the strong-response group than in the weak-response group (P = 0.022), but there were no significant differences in other clinical variables or in medication profiles. In conclusion, circulating EPC numbers could be a potential predictor of the therapeutic effect of riociguat in CTEPH patients.

Keywords: pulmonary arterial pressure, right atrial pressure, endothelial dysfunction

Abbreviations:
BPA: balloon pulmonary angioplasty
EPC: endothelial progenitor cell
CTEPH: chronic thromboembolic pulmonary hypertension
mPAP: mean pulmonary arterial pressure
PAH: pulmonary arterial hypertension
PEA: pulmonary endarterectomy

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INTRODUCTION

Pulmonary hypertension is an intractable disease caused by increased pulmonary arterial pressure and leading to right heart failure. Pulmonary hypertension is classified according to pathogenesis into five groups, two of which are pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH).[1] Endothelial dysfunction plays an important role in the initiation and progression of pulmonary hypertension and has been investigated to develop novel treatments for this disease.[2,3] Endothelial progenitor cells (EPCs) have been well studied as part of the evaluation of endothelial dysfunction. These cells are derived from bone marrow and migrate to injured vessels to repair the endothelium.[4,5]

Several reports have shown that the number of circulating EPCs in PAH significantly increases after treatment with pulmonary vasodilators.[6,7] These findings may represent an effect of pulmonary vasodilators on endothelial dysfunction. Interestingly, a similar finding has been reported in CTEPH.[8] Riociguat is a pulmonary vasodilator, the use of which in a randomized controlled trial led to marked improvements in exercise capacity and hemodynamic status in patients with CTEPH.[9] In one study, the number of circulating EPCs was higher in CTEPH patients who received riociguat than in treatment-naïve patients; riociguat enhanced the protective role of EPCs, leading to the prevention of remodeling in CTEPH.[8] Thus, riociguat might mitigate endothelial dysfunction in CTEPH, as reflected by the number of EPCs after treatment initiation. However, the relationship between the number of EPCs at diagnosis and changes in clinical parameters after administration of riociguat has not been elucidated fully. Here, we investigated the association between the number of circulating EPCs at diagnosis and the therapeutic effect of riociguat in patients with CTEPH.

METHODS

Study population

We assessed treatment-naïve patients who were diagnosed with CTEPH at Nagoya University Hospital (Nagoya, Japan) between April 2015 and February 2020. CTEPH was diagnosed according to the 2015 guidelines of the European Society of Cardiology – European Respiratory Society.[10] Pulmonary hypertension was confirmed via right heart catheterization and was defined as a mean pulmonary arterial pressure (mPAP) ≥25 mmHg and pulmonary arterial wedge pressure ≤15 mmHg. The diagnosis of CTEPH was obtained after at least 3 months of effective anticoagulant therapy.

Among these patients, we evaluated those who were treated with riociguat after diagnosis and underwent reevaluation through right heart catheterization before balloon pulmonary angioplasty (BPA) or pulmonary endarterectomy (PEA). To eliminate factors other than riociguat that could affect the numbers of circulating EPCs, we excluded patients who took pulmonary vasodilators or statins, or both, before diagnosis; who were treated with other pulmonary vasodilators other than riociguat after diagnosis; or who had diabetes, chronic kidney disease stage 4 or 5, chronic obstructive pulmonary disease, or interstitial pneumonia.[6,7,11-15] In addition, patients with D-dimer levels of ≥1.0 μg/mL (Sysmex, Kobe, Japan) at either diagnosis or reevaluation were excluded to exclude any influence of acute thrombosis.

This study was approved by the ethics committee of Nagoya University Hospital (approval no. 2016-0372). Informed consent was implied by allowing subjects to opt out of the study; no patient opted out.

Measurement of circulating EPCs

At the time of right heart catheterization for diagnosis, venous blood samples were collected from each patient for routine hematology, with the residual sample drawn into heparinized tubes. EPCs were then quantified by flow cytometry; EPCs were defined as $CD45^{dim}$ $CD34^{+}$ $CD133^{+}$ cells. In brief, venous blood samples were washed with phosphate-buffered saline supplemented with 0.2% of bovine serum albumin and centrifuged at 530 *g* for 5 min, after which the plasma was removed. EPCs were labeled with allophycocyanin–anti-CD45 (Life Technologies, Carlsbad, CA, USA), phycoerythrin cyanine 5.1-anti-CD34 (Beckman Coulter, Brea, CA, USA), and phycoerythrin–anti-CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany). After incubation of the samples for 40 min at 4 °C, the red cells were lysed and the samples were washed again with phosphate-buffered saline supplemented with 0.2% bovine serum albumin. At most, 100,000 events were collected with the flow cytometer. The number of EPCs was calculated, along with the ratio of EPCs to $CD45^{+}$ cells and the number of white blood cells measured through automated hemocytology.

Statistical analyses

All statistical analyses were performed by using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation or median with the interquartile range, as appropriate. Categorical variables are presented as counts or percentages, or both. Correlations between number of circulating EPCs and clinical variables were analyzed by using Spearman's correlation test. To evaluate the differences between patients whose mPAP values decreased considerably upon treatment with riociguat and those whose values decreased only slightly, the paired *t*-test, Mann–Whitney U-test, and chi-squared test were used. Receiver-operator-characteristic curve analyses were performed to identify the potential cut-off value of the number of circulating EPCs at diagnosis for predicting a strong response to riociguat in terms of improvement in mPAP. Hazard ratios with 95% confidence intervals were determined. In all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

A total of 27 patients with CTEPH were enrolled in the study. Table 1 shows their characteristics at diagnosis and at reevaluation after initiation of riociguat. The median (interquartile range) interval between diagnosis and reevaluation was 189 (133–224) days. At reevaluation, the median (interquartile range) dose of riociguat was 7.5 (6.0–7.5) mg/day.

Correlation analysis between number of circulating EPCs and clinical variables

We investigated the relationship between the number of circulating EPCs at diagnosis and clinical variables. At diagnosis, there was no significant correlation between the number of EPCs and age, hemodynamic parameters, atrial blood gas analysis parameters, brain natriuretic peptide level, or exercise tolerance (Table 2). The number of circulating EPCs at diagnosis was significantly correlated with the changes in mPAP between before and after initiation of riociguat (correlation coefficient = 0.535, $P = 0.004$) and in right atrial pressure (RAP) between before and after initiation of riociguat (correlation coefficient = 0.618, $P = 0.001$; Table 3). Patients with lower EPC numbers at diagnosis tended to have greater reductions in mPAP and RAP after riociguat treatment.

Table 1 Patient characteristics at baseline and after initiation of riociguat

	Baseline	**After initiation of riociguat**
Age (years)	61.6 ± 15.7	
Female	17 (63%)	
Hemodynamics		
mPAP (mmHg)	43.4 ± 9.3	36.4 ± 9.0
RAP (mmHg)	7.4 ± 3.0	6.1 ± 3.1
CI (L/min/m^2)	1.9 ± 0.5	2.4 ± 0.6
SvO_2 (%)	60.7 ± 7.4	65.3 ± 6.9
PVR (WU)	12.7 ± 6.0	7.5 ± 3.8
PAWP (mmHg)	8.2 ± 3.6	10.2 ± 3.2
mSAP (mmHg)	95.0 ± 14.0	83.2 ± 12.6
Arterial blood gas analysis		
PaO_2 (Torr)	61.4 ± 7.0	61.3 ± 9.3
A-a DO_2 (Torr)	48.9 ± 9.7	47.4 ± 12.5
BNP (ng/L)	104.8 (20.8–381.4)	22.6 (7.5–144.2)
Cardiopulmonary exercise testing		
Peak VO_2 (mL/min/kg)	12.8 ± 3.3 (n = 20)	17.0 ± 3.1 (n = 11)
VE vs VCO_2 slope	50.3 ± 13.7 (n = 20)	39.1 ± 7.7 (n = 11)
Endothelial progenitor cells (no./mL)	633 (328–1089)	

A-a DO_2: alveolar-arterial oxygen difference
BNP: brain natriuretic peptide
CI: cardiac index
mPAP: mean pulmonary arterial pressure
mSAP: mean systemic arterial pressure
PaO_2: partial pressure of arterial oxygen
PAWP: pulmonary artery wedge pressure
Peak VO_2: peak oxygen uptake
PVR: pulmonary vascular resistance
RAP: right atrial pressure
SvO_2: mixed venous oxygen saturation
VE vs VCO_2 slope: ventilatory equivalent versus carbon dioxide output slope
WU: wood units
Data are presented as n (%), mean ± standard deviation, or median (interquartile range).

Table 2 Spearman correlation analysis between number of circulating endothelial progenitor cells and clinical variables at baseline

	Correlation coefficient	***P***
Age (years)	–0.286	0.148
Hemodynamics		
mPAP (mmHg)	0.235	0.238
RAP (mmHg)	–0.038	0.849
CI (L/min/m^2)	–0.047	0.815
SvO_2 (%)	–0.223	0.263
PVR (WU)	0.033	0.872
PAWP (mmHg)	–0.227	0.264
mSAP (mmHg)	0.018	0.930
Arterial blood gas analysis		
PaO_2 (Torr)	–0.135	0.501
A-a DO_2 (Torr)	0.066	0.743
BNP (ng/L)	0.164	0.413
Cardiopulmonary exercise testing[a]		
Peak VO_2 (mL/min/kg)	–0.068	0.774
VE vs VCO_2 slope	0.116	0.627

A-a DO_2: alveolar-arterial oxygen difference
BNP: brain natriuretic peptide
CI: cardiac index
mPAP: mean pulmonary arterial pressure
mSAP: mean systemic arterial pressure
PaO_2: partial pressure of arterial oxygen
PAWP: pulmonary artery wedge pressure
Peak VO_2: peak oxygen uptake
PVR: pulmonary vascular resistance
RAP: right atrial pressure
SvO_2: mixed venous oxygen saturation
VE vs VCO_2 slope: ventilatory equivalent versus carbon dioxide output slope
WU: wood units
[a] Cardiopulmonary exercise testing was performed in 20 patients at baseline.

Table 3 Spearman correlation analysis between number of circulating endothelial progenitor cells at diagnosis and changes in clinical variables between before and after initiation of riociguat

	Correlation coefficient	*P*
Hemodynamics		
ΔmPAP (mmHg)	0.535	0.004
ΔRAP (mmHg)	0.618	0.001
ΔCI (L/min/m^2)	–0.006	0.977
ΔSvO_2 (%)	0.140	0.496
ΔPVR (WU)	0.179	0.380
ΔPAWP (mmHg)	0.251	0.215
ΔmSAP (mmHg)	0.321	0.126
Arterial blood gas analysis		
ΔPaO_2 (Torr)	–0.022	0.912
ΔA-a DO_2 (Torr)	0.162	0.418
Δ**BNP** (ng/L)	0.074	0.713
Cardiopulmonary exercise testing[a]		
ΔPeak VO_2 (mL/min/kg)	–0.179	0.702
ΔVE vs VCO_2 slope	–0.321	0.482

A-a DO_2: alveolar-arterial oxygen difference
BNP: brain natriuretic peptide
CI: cardiac index
mPAP: mean pulmonary arterial pressure
mSAP: mean systemic arterial pressure
PaO_2: partial pressure of arterial oxygen
PAWP: pulmonary artery wedge pressure
Peak VO_2: peak oxygen uptake
PVR: pulmonary vascular resistance
RAP: right atrial pressure
SvO_2: mixed venous oxygen saturation
VE vs VCO_2 slope: ventilatory equivalent versus carbon dioxide output slope
WU: wood units
[a] Cardiopulmonary exercise testing was performed in seven patients before and after administration of riociguat.

Comparison between patients with large and small reductions in mPAP

To validate the factors associated with the reduction of mPAP upon riociguat treatment, we divided the study population into two groups according to the change in mPAP, namely a strong-response group (a decrease in mPAP of more than 4 mmHg; n = 15) and a weak-response group (a decrease in mPAP of 4 mmHg or less; n = 12). We determined the mPAP cut-off value of –4 mmHg by considering the results of Chronic Thromboembolic Pulmonary Hypertension Soluble Guanylate Cyclase–Stimulator Trial 1 (CHEST-1) and a meta-analysis of the efficacy of riociguat[9,16]; the mean decrease in mPAP with riociguat treatment was 4 mmHg in both studies. There were no significant between-group differences in age, sex, hemodynamic parameters, atrial blood gas parameters, blood parameters such as brain natriuretic peptide and creatinine level, and exercise tolerance at diagnosis. Duration of anticoagulant therapy before diagnosis did not

differ significantly between groups. In contrast, the number of circulating EPCs at diagnosis was significantly lower in the strong-response group than in the weak-response group (P = 0.022; Table 4). In addition, we explored differences in medication profiles at reevaluation. Rates of use of diuretics such as loop diuretics, spironolactone, and tolvaptan were comparable between the two groups. In our study population, only one patient received a thiazide diuretic. The doses of riociguat and diuretics did not differ between groups.

Finally, we performed a receiver-operator-characteristic curve analysis to determine the cut-off point for the number of circulating EPCs to maximize the predictive value of a decrease in mPAP of greater than 4 mmHg with riociguat (Fig. 1). The optimal threshold of circulating EPCs was 576/mL, with a maximum area under the curve of 0.761 (sensitivity, 91.7%; specificity, 60.0%).

Table 4 Comparison between patients with a decrease in mean pulmonary arterial pressure of more than 4 mmHg (strong response group) and those with a decrease of 4 mmHg or less (weak-response group) upon riociguat treatment

	Weak-response group (n = 12)	**Strong-response group** (n = 15)	***P***
Clinical data at baseline			
Age (years)	59.5 ± 16.8	63.2 ± 15.1	0.448
Female	6 (50%)	11 (73%)	0.199
Hemodynamics			
mPAP (mmHg)	40.7 ± 6.4	45.5 ± 10.9	0.270
RAP (mmHg)	7.5 ± 2.4	7.3 ± 3.5	0.846
CI (L/min/m^2)	1.8 ± 0.5	1.9 ± 0.5	0.557
SvO_2 (%)	60.5 ± 7.7	60.9 ± 7.5	0.878
PVR (WU)	11.8 ± 6.2	13.3 ± 6.0	0.552
PAWP (mmHg)	8.5 ± 3.8	8.0 ± 3.6	0.757
mSAP (mmHg)	89.4 ± 10.9	99.1 ± 14.8	0.079
Arterial blood gas analysis			
PaO_2 (Torr)	61.7 ± 5.9	61.1 ± 8.0	0.837
A-a DO_2 (Torr)	46.4 ± 10.1	50.9 ± 9.3	0.244
Blood tests			
BNP (ng/L)	147.8 (20.9–561.1)	104.8 (20.8–283.0)	0.770
Creatinine (mg/dL)	0.9 ± 0.2	0.8 ± 0.3	0.393
Hemoglobin (g/dL)	14.9 ± 2.3	13.8 ± 1.8	0.196
Cardiopulmonary exercise testing			
Peak VO_2 (mL/min/kg)	12.9 ± 4.1 (n = 9)	12.8 ± 2.6 (n = 11)	0.924
VE vs VCO_2 slope	47.4 ± 13.9 (n = 9)	52.7 ± 13.7 (n = 11)	0.406
Duration of anticoagulant therapy (days)	232 (137–408)	158 (111–270)	0.294
Endothelial progenitor cells (no./mL)	938 (623–1138)	450 (302–886)	0.022

Medication profile at reevaluation			
Use of diuretics			
Loop diuretic	8 (67%)	12 (80%)	0.364
Spironolactone	3 (25%)	7 (47%)	0.226
Tolvaptan	2 (17%)	1 (7%)	0.414
Drug dose			
Riociguat (mg/day)	7.5 (6.4–7.5)	6.0 (6.0–7.5)	0.126
Loop diuretic (mg/day)[a]	20 (0–40)	20 (10–20)	0.698

A-a DO_2: alveolar–arterial oxygen difference
BNP: brain natriuretic peptide
CI: cardiac index
mPAP: mean pulmonary arterial pressure
mSAP: mean systemic arterial pressure
PaO_2: partial pressure of arterial oxygen
PAWP: pulmonary artery wedge pressure
Peak VO_2: peak oxygen uptake
PVR: pulmonary vascular resistance
RAP: right atrial pressure
SvO_2: mixed venous oxygen saturation
VE vs VCO_2 slope: ventilatory equivalent versus carbon dioxide output slope
WU: wood units
Data are presented as n (%), mean ± standard deviation, or median (interquartile range).
[a] Loop diuretic doses were converted to furosemide-equivalent doses; 30 mg of azosemide was converted to 20 mg of furosemide.

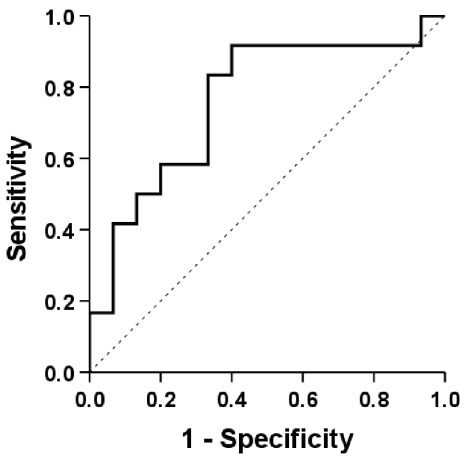

Fig. 1 ROC curve of the number of circulating EPCs for predicting a decrease in mPAP of more than 4 mmHg upon riociguat treatment

EPC: endothelial progenitor cell
mPAP: mean pulmonary arterial pressure
ROC: receiver operating characteristic

DISCUSSION

Here, we investigated the association between number of circulating EPCs at diagnosis and the therapeutic effect of riociguat in patients with CTEPH. A lower number of circulating EPCs, defined as $CD45^{dim}$ $CD34^{+}$ $CD133^{+}$ cells, at diagnosis was significantly correlated with a greater reduction in mPAP and RAP upon riociguat treatment. Furthermore, the number of circulating EPCs at diagnosis was significantly lower in patients with a greater reduction of mPAP with riociguat than in those with a smaller reduction, although no other clinical parameters or medication profiles differed significantly between the groups. These results show that the number of circulating EPCs could be a useful marker for predicting the therapeutic effect of riociguat.

mPAP is a prognostic factor in patients with CTEPH: a reduction in mPAP leads to prognostic improvement.[17,18] Although PEA and BPA are established treatments that can reduce mPAP, these treatments can be performed at only a few institutions. Therefore, medical therapy, including the use of pulmonary vasodilators, is an important initial choice. One report suggested that pulmonary vasodilators contributed to increased survival among patients with inoperable CTEPH, and survival has not differed among patients treated with medical therapy, PEA, and BPA in recent years.[19] In addition, a higher mPAP is a significant predictor of lung injury after BPA.[20] Therefore, medical therapy before BPA is thought to be important to reduce the risk of complications. Presently, riociguat is a pulmonary vasodilator that is covered by health insurance for patients with CTEPH in Japan. Its hemodynamic improving effect, including mPAP reduction, varies among patients, and predictors of this effect have been unavailable until recently.

Two factors contribute to increasing pulmonary arterial pressure in patients with CTEPH: chronic pulmonary thromboembolism and secondary small-vessel arteriopathy.[21] CTEPH is characterized by chronic thromboembolic obstructions and stenoses. These reduce the capacity of the pulmonary vascular bed and increase pulmonary arterial pressure, and they cause abnormal vascular remodeling of the small pulmonary arteries in the patent pulmonary vascular bed.[22] The morphologic characteristics of the small-vessel arteriopathy in CTEPH are similar to those in idiopathic PAH: both of these conditions are characterized by medial hypertrophy, intimal proliferation, and plexiform lesions.[21] PEA and BPA are treatments for chronic pulmonary thromboembolism, whereas pulmonary vasodilators combat arteriopathies similar to those in PAH. Therefore, the effect of riociguat in patients with CTEPH likely is related to the degree of small-vessel arteriopathy; however, this would be difficult to examine.

Our study showed that the beneficial effect of riociguat on mPAP in patients with CTEPH was related to the number of circulating EPCs before treatment. Therefore, the number of circulating EPCs may reflect the degree of small-vessel arteriopathy. In PAH, the number of circulating EPCs decreases as a consequence of homing and recruitment to repair the injured endothelium of pulmonary vessels.[6,7,23-26] Therefore, the number of circulating EPCs in CTEPH may also decrease because of homing and recruitment in accordance with the degree of small-vessel arteriopathy. Indeed, Yao et al[27] investigated endarterectomized tissues after PEA in patients with CTEPH and showed that cells in the vascular tissues distal to the thromboemboli expressed higher levels of CD34 and CD133 than those in the proximal vascular tissues and in normal pulmonary artery smooth muscle cells. On the basis of these findings, the number of circulating EPCs in CTEPH may reflect the degree of arteriopathy and be indirectly associated with the effect of riociguat, as our findings suggest.

Our study had several limitations. First, it was a single-center study with a small sample size. Secondly, we did not evaluate thrombus changes in the pulmonary arteries between before and after administration of riociguat, and thrombolysis might have led to some improvement in hemodynamics. (To avoid this potential confounder, we excluded patients with high levels of D-

dimer at either diagnosis or reevaluation.) Thirdly, we did not measure the number of circulating EPCs at reevaluation, such that any riociguat-induced change in EPC number was not assessed. In addition, we did not assess markers other than CD34 and CD133 that could be associated with EPCs. Finally, the number of circulating EPCs is reportedly altered by such conditions as diabetes, chronic kidney disease, chronic obstructive pulmonary disease, and interstitial pneumonia,[12-15] as well as by several medications, including pulmonary vasodilators and statins.[6,7,11] We therefore excluded subjects with these diseases or receiving these medications, but other factors may still have influenced the number of EPCs. In addition, standardized methods for defining and measuring EPCs are unavailable. Further consideration of these issues is needed before the number of circulating EPCs can be used as a marker.

CONCLUSION

A low number of circulating EPCs, defined as $CD45^{dim}$ $CD34^{+}$ $CD133^{+}$ cells, at diagnosis was significantly associated with increased reductions in mPAP and RAP after the initiation of riociguat in patients with CTEPH. Moreover, the number of EPCs at baseline appeared to be the only predictor of the degree of reduction in mPAP upon riociguat treatment. These findings suggest that EPC numbers could be used to predict the therapeutic effect of riociguat in patients with CTEPH.

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CONFLICT OF INTEREST

All authors report that they have no conflict of interest to disclose.

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