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ORIGINAL ARTICLE

WILEY Congenital Heart Disease

Hemodynamic, biological, and right ventricular functional changes following intraatrial shunt repair in patients with flow-induced pulmonary hypertension

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Abstract

Objectives: Atrial septal defects may result in pulmonary hypertension and right heart remodeling. We analyzed improvements in patients with flow-induced pulmonary hypertension and the activation of endothelial progenitor cells after flow reduction.

Design: This prospective cohort study included 37 patients who were admitted for an occluder implantation. Blood samples were collected before and after the procedure. We determined the number of endothelial progenitor cells in outgrowth colonies and serum Hsp27 concentrations. Daily performance and cardiothoracic ratio were reevaluated later.

Results: Closure of the defect significantly reduced the pulmonary pressure and B-type natriuretic peptide levels. The cardiothoracic ratio and daily performance status also improved. The number of endothelial progenitor cell outgrowth colony-forming units significantly increased and was positively correlated with daily performance. In patients with enhanced colony formation, Hsp27 levels were significantly increased.

Conclusions: The implantation of an occluder successfully improved hemodynamic, right ventricular, and daily performance. Qualitative enhancement of colony formation for endothelial progenitor cells was also noted and positively correlated with daily performance. Closure of defects may serve as a valid, reliable model to obtain a deeper understanding of the modulation of endothelial progenitor cell activity and its relationship with pulmonary hypertension prognosis.

KEYWORDS

atrial septal defect, endothelial progenitor cells, pulmonary hypertension

1 | INTRODUCTION

Pulmonary hypertension (PH) is often a progressive disease with limited treatment options. In some secondary PH disorders, the combination of increased pulmonary blood flow due to left-to-right shunt congenital heart defects or the presence of a systemic arteriovenous shunt forms a unique pulmonary arteriopathy that leads to PH.[1,2] The characteristic vascular changes associated with pulmonary arteriopathy in patients with flow-induced PH include intimal hyperplasia/fibrosis, medial hypertrophy, extensive extracellular matrix modulation, and, in more severe cases, the formation of plexiform lesions.[2,3] These pathophysiological changes decrease the compliance of the pulmonary vasculature and alter pulmonary arterial vasoreactivity.[4] Atrial septal defects (ASDs) are one of the main causes of flow-induced PH. Right heart failure usually develops after periods of long-term increased blood flow into the right heart chambers. In clinical practice, ASD closure is recommended when the blood flow in the pulmonary artery exceeds 1.5 times the left ventricular cardiac output. Most secundum ASDs are amenable to closure by an endovascular approach. Although ASD occlusion has been shown to improve the patients' physical status and right ventricular function,[5] there have been few integrated investigations on the physiological changes and development of prognostic biological markers in patients with ASD-associated PH. Recent research has shown that endothelial progenitor cells (EPCs) play an important role in endothelial injury repair and directly participate in postnatal vasculogenesis and angiogenesis in systemic vascular beds.[6,7] Animal studies have also shown that EPC administration can prevent experimental PAH.[7] We hypothesized that dynamic changes in EPCs occur after flow reduction in flow-induced PH and that this correlates with prognosis. The aim of this prospective observational clinical study was to characterize any functional improvements and to correlate physiological changes with biological markers in patients treated with transcatheter ASD occlusion.

2 | MATERIALS AND METHODS

2.1 | Study design

This prospective, case–control and interventional study was conducted at the National Cheng Kung University Hospital and had been approved by the ethics committee and the Institutional Review Board (IRB) of the National Cheng Kung University Hospital, Taiwan. We obtained written informed consent from all participants after the interview procedures were fully explained to them and their legal representatives. We included successive patients (age range: 18–60 years) who had been admitted because of secundum ASD with elevated pulmonary pressure. All patients underwent transcatheter ASD closure with the implantation of an Amplatzer occluder under general anesthesia. The baseline demographic data of the patients are summarized in Table 1. In addition, one patient with a patent foramen ovale (PFO) and another patient with an enlarged ASD were included in the present study to serve as positive and negative controls (Table 2) for the occluder applications, respectively. Echocardiography and blood sample collections were performed before and after the Amplatzer occluder (AGA Medical Corp., Golden Valley, MN) application.

TABLE 1 Patient demographic data

Characteristics	n or Mean ± SD
Age (years)	49.2 ± 2.5
Gender (M:F)	4:33
ASD size (cm)	2.4 ± 0.1
Q_P/Q_S	2.9 ± 0.2
PAP (mm Hg)	53.1 ± 4.0
TAPSE	2.1 ± 0.3
NYHA Fc I:II:III:IV (n)	2:32:3:0
6-MWD (m)	443.9 ± 16.4
Cardiothoracic ratio (%)	57.4 ± 1.4
BNP (pg/mL)	191.4 ± 53.3

Abbreviations: 6-MWD, 6-min walk distance; ASD, atrial septal defect; BNP, B-type natriuretic peptide; NYHA Fc, New York Heart Association functional class; PAP, pulmonary artery pressure (estimated by transthoracic echocardiography); Q_P/Q_S, pulmonary-to-systemic flow ratio; TAPSE, tricuspid annular plane systolic excursion.

2.2 | Exclusion criteria

We excluded any patients who were unwilling to participate or who had PH and right heart failure because of causes other than an ASD-related left-to-right shunt, advanced liver disease, chronic renal disease (serum creatinine $\geq$ 3 mg/dL), global heart failure, malignancy, pregnancy, hematological disorders, or poor echocardiographic views.

TABLE 2 Characteristics of two control patients[a]

	Patient 1	Patient 2
Patient characteristics and baseline measurements		
Age (years)	57	58
Gender	Male	Female
Diagnosis	PFO	Large ASD
sPAP (mm Hg)	28	80
Q_P/Q_S	1.1	2.87
BNP(pg/mL)	13.6	286
EPC CFU	1	0
After application of ASD occluder		
Outcomes of ASD occlusion	Successful	Failure
sPAP (mm Hg)	24	80
BNP (pg/mL)	6.6	387
EPC CFU	5	2

Abbreviations: BNP, B-type natriuretic peptide; CFU, colony formation unit; EPC, endothelial progenitor cell; NYHA Fc, New York Heart Association functional class; PFO, patent foramen ovale; sPAP, systolic pulmonary artery pressure (estimated by transthoracic echocardiography); Q_P/Q_S, pulmonary-to-systemic flow ratio.

[a]These two patients received application of an ASD occluder without causing significant changes in the pulmonary artery blood flow after the procedure. Readers are referred to the manuscript text for a more detailed description of the clinical outcomes.

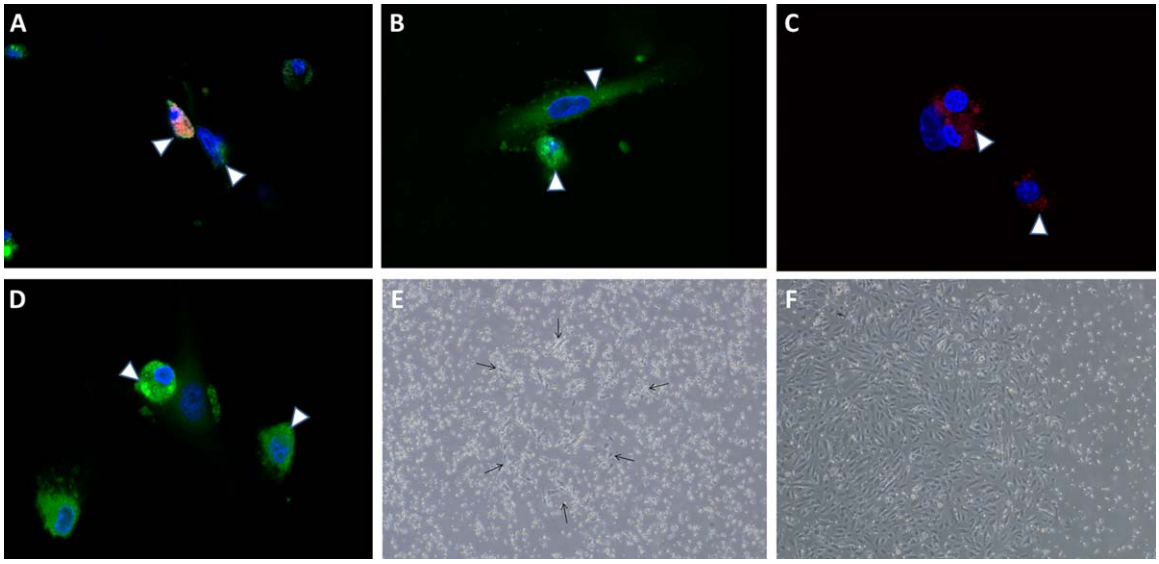

FIGURE 1 In vitro characterization of circulating endothelial progenitor cells (EPCs). A, These endothelial progenitors incorporated acetyl-LDL (red fluorescence) and isolectin (green fluorescence). The expressions of CD34—B, vascular endothelial growth factor receptor (VEGFR)-2—C, and Von Willebrand factor—D were detected on cultured EPCs. The cellular markers were stained in red or green fluorescence (arrowheads), and the nuclei were counterstained in blue fluorescence using a fluorescence confocal microscopy. E, The formation of an early EPC outgrowth colony (delineated by arrows) within 14 days after plating—F. After culturing for another week, EPCs outgrew into the typical monolayer "cobblestone" appearance of endothelial cells

2.3 | Implantation of the Amplatzer ASD occluder

The operator inserted the delivery catheter into the femoral vein and advanced it into the right atrium under general anesthesia and fluoroscopic guidance. The left atrial disc was delivered with the self-centering connecting stalk. Finally, the surgeon left the connecting stalk across ASD. Using transesophageal echocardiography, the surgeon confirmed successful occlusion of ASD with the cessation of blood flow across the septal defect.

2.4 | Echocardiography, chest radiography, and blood sampling

The transthoracic and transesophageal echocardiography were performed by experienced cardiologists. The pulmonary artery pressure was estimated by measuring the peak velocity of the jet of tricuspid regurgitation. The transthoracic echocardiography was reassessed within 24 h after ASD closure. Blood samples (25 mL) were collected under general anesthesia immediately before and within 60 min after the implantation of the ASD occluder. The radiographical cardiothoracic ratio was reassessed after 6 months.

2.5 | Isolation and characterization of EPCs

Peripheral blood mononuclear cells were isolated using density gradient centrifugation with Ficoll-Paque PLUS medium (Amersham Biosciences, Piscataway, NJ). Washed mononuclear cells were plated on a 100-mm plate coated with human fibronectin (Sigma-Aldrich Co., St. Louis, MO) in endothelial growth medium-2 (EGM-2, Cambrex, East Rutherford, NJ); the nonadherent cells were removed 3 days after plating. The numbers of EPC outgrowth colonies that developed within 2 weeks after culture were recorded. An outgrowth EPC colony was defined as a grouping of well-circumscribed monolayer cells with a cobblestone appearance,[8] as visualized under a light microscope (Figure 1). Early outgrowth cells were incubated with acetyl-LDL (Molecular Probes, Waltham, MA) and isolectin (Molecular Probes) and observed under a fluorescence microscope. The uptake of acLDL and isolectin is a functional marker for endothelial cells. In a separate experiment, isolated mononuclear cells were cultured on four-well glass slides coated with human fibronectin. The early attached EPCS were stained with specific endothelial cell markers, including CD34 (Abbiotec, San Diego, CA), vascular endothelial growth factor receptor-2 (BioLegend, San Diego, CA), and *Von Willebrand factor* (Bioss Inc., Woburn, MA); the immunofluorescent densities were analyzed by confocal microscopy.

2.6 | Quantification of circulating EPCs

Freshly isolated mononuclear cells were stained with fluorescent conjugated antibodies CD34 (eBioscience, Waltham, MA) and VEGF receptor-2/KDR (Epitomics, Burlingame, CA). Cell fluorescence was immediately measured under flow cytometry.[9]

2.7 | Serum concentrations of Hsp27

The concentrations of serum Hsp27 before and after the application of the ASD occluder were determined with a commercially available sandwich ELISA kit using the colorimetric method (Abcam, Cambridge, United Kingdom).[10]

2.8 | Biochemical analysis

Using the Triage BNP test, the serum concentrations of B-type natriuretic peptide (BNP) were measured immediately before and within 60 min after the application of the ASD occluder, in accordance with the manufacturer's instructions (Biosite, San Diego, CA).

2.9 | Daily performance evaluations

We evaluated the patients' daily performance at admission and a month after the procedure using the New York Heart Association (NYHA) functional classifications and the 6-min walk test.

2.10 | Statistical analysis

All values were expressed as mean ± SD or mean ± SEM, as appropriate. Wilcoxon signed-rank tests were performed for the comparison of measurement before and after ASD occlusion. The relationships between two continuous variables were determined by Pearson analysis. A P value of $<.05$ was considered statistically significant.

3 | RESULTS

In total, 37 patients who had been admitted for transcatheter ASD closure were enrolled in this study (Table 1). The average systolic pulmonary artery pressure was 53.1 mm Hg, and most of the patients were in NYHA functional class II before undergoing ASD occlusion. The right ventricular function in these patients was mostly normal, as assessed by the tricuspid annular plane systolic excursion (TAPSE) under echocardiography (>1.8 cm) (Table 1). One patient with PFO underwent implantation of an occluder for the prevention of a thromboembolic stroke. There was no increase in the systolic pulmonary artery pressure, and there was no evidence of pulmonary artery remodeling in this patient (Table 2). Another patient with an enlarged ASD received general anesthesia before occluder implantation (Table 2). However, the device was removed because of incomplete ASD occlusion under transesophageal echocardiography. The pulmonary artery pressure and pulmonary blood flow remained elevated in this patient after the procedure (Table 2), and she was rescheduled for patch repair of ASD using a cardiopulmonary bypass.

TABLE 3 Changes following intra-atrial shunt repair

Measurement	Baseline	Post-occluder	P value
sPAP (mm Hg)	53.13 ± 4.04	39.13 ± 3.88	.001
EPC numbers (% MNC)	0.10 ± 0.02	0.15 ± 0.03	.083
EPC CFU (colonies/100-mm disc)	0.79 ± 0.25	21.21 ± 4.22	<.001
BNP (pg/mL)	191.38 ± 53.31	160.94 ± 45.79	.006
Cardiothoracic ratio (%)[a]	57.44 ± 1.41	54.68 ± 1.69	.001
6-minute walk test (m)	443.9 ± 16.47	468.55 ± 15.20	.074
NYHA Fc I:II:III:IV (n)	2:32:3:0	30:7:0:0	

Abbreviations: BNP, B-type natriuretic peptide; CFU, colony formation unit; EPC, endothelial progenitor cell; MNC, mononuclear cells; PAP, systolic pulmonary artery pressure (estimated by transthoracic echocardiography).
[a]Cardiothoracic ratios were measured before (baseline) and 1 month after application of an ASD occluder (post-occluder). Data were analyzed by the Wilcoxon signed-rank test and is presented as mean ± SEM.

Within 24 h after the ASD closure, the pulmonary arterial pressure was significantly reduced (53.13 ± 4.04 vs 39.13 ± 3.88 mm Hg, $P = .001$; Table 3 and Figure 2A). We isolated EPCs and obtained the outgrowth colonies. The cultured EPCs exhibited the phenotypic and genotypic characteristics of their endothelial progenitors (Figure 1). The closure of ASD did not significantly affect the numbers of circulating EPCs in these patients (0.10 ± 0.02 vs 0.15 ± 0.03% of MNCs, before vs after ASD occlusion. $P = .083$; Table 3). Compared with the blood samples collected before the procedure, the numbers of EPC outgrowth colonies were significantly increased after successful occluder application (0.79 ± 0.25 vs 21.21 ± 4.22 colonies/100-mm culture disc, before vs 60 min after ASD occlusion: $P < .001$; Table 3

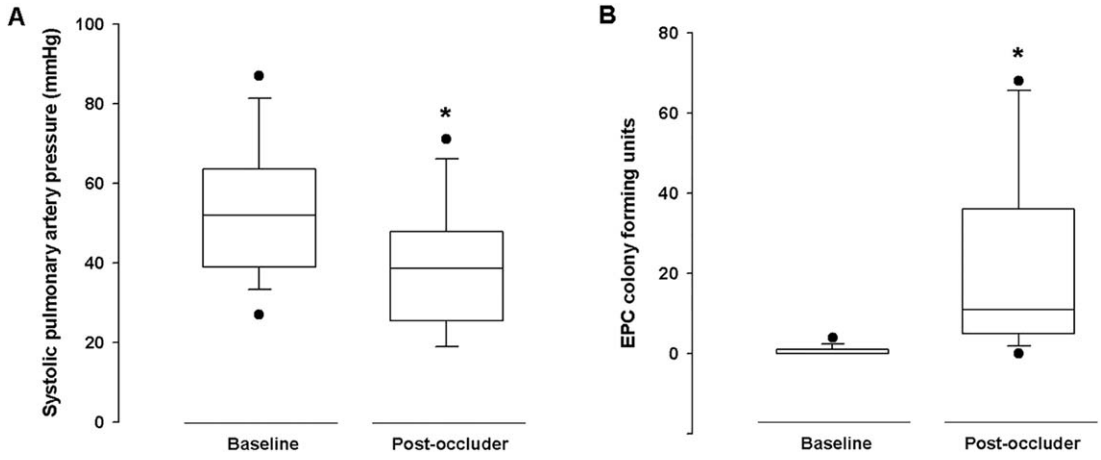

FIGURE 2 A. Reduction of systolic pulmonary artery pressure (sPAP) after application of an Amplatzer occluder. B. Increase in endothelial progenitor cell colony-forming units (EPC CFUs) after the application of an Amplatzer occluder. *$P < .05$ compared with baseline. Data were analyzed by the Wilcoxon signed-rank tests

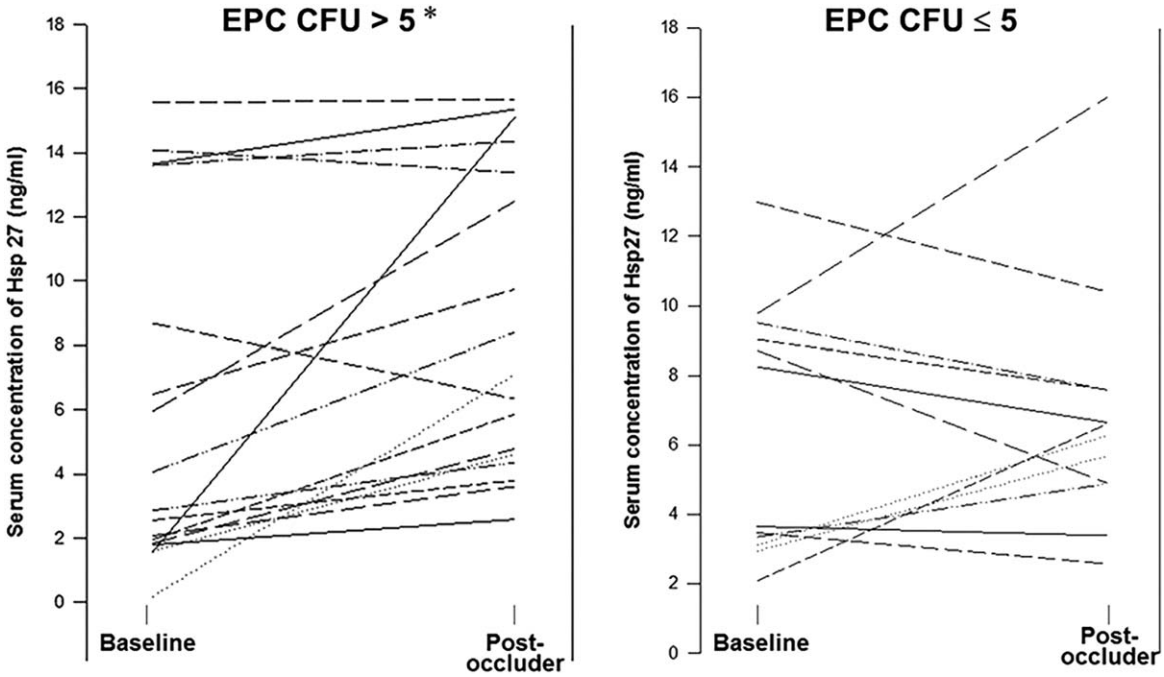

FIGURE 3 In patients with enhanced EPC colony formation (CFU > 5), serum Hsp27 levels were significantly increased. *$P < .05$ compared with baseline. Data were analyzed by the Wilcoxon signed-rank tests

and Figure 2B). On the other hand, the numbers of circulating EPCs and outgrowth colonies were similar between the two study time-points in the patient with PFO and the patient with failed ASD occlusion (Table 2). In patients with enhanced EPC colony formation (CFU > 5), Hsp27 serum levels were also significantly increased (Figure 3). However, the Hsp27 concentrations poorly correlated with the number of EPC outgrowth colonies ($r^2 = .289$, $P = .26$).

We assessed functional changes in patients who underwent ASD occlusion using different approaches. The patients' daily performance was assessed using the NYHA functional classification, and 83.8% of the patients had a significant improvement (Table 3). The 6-min walk distance measured at a month after the procedure was well-correlated with the number of EPC outgrowth colonies ($r^2 = .831$, $P = .031$). The pressure loading and remodeling of the right ventricle were assessed with the BNP and the radiographical cardiothoracic ratio. The serum concentrations of BNP decreased following the placement of the occluder (191.38 ± 53.31 vs 160.94 ± 45.79 pg/mL, before vs 60 min after ASD occlusion, $P = .006$; Table 3). The radiographic cardiothoracic ratio was also significantly decreased 6 months after the procedure (57.44 ± 1.41 vs 54.98 ± 1.69%, $P = .001$; Table 3).

4 | DISCUSSION

Our study demonstrated that the occlusion of ASD significantly reduced pulmonary artery pressure. Although TAPSEs measured were not different from the baseline values (2.12 ± 0.33 vs 1.9 ± 0.18, $P = .427$, respectively), the cardiothoracic ratio, functional performance, and plasma BNP levels were significantly improved after intervention, indicating the improvement of the global function of right ventricle. These improvements suggested that PH and cardiopulmonary remodeling secondary to a left-to-right shunt may be mostly reversible.

The other novel finding in the present study was that the cessation of the excessive pulmonary blood flow was associated with increased colony formation of isolated EPCs in patients with ASD-associated PH. Endothelial dysfunction is a hallmark of PH, and a recent study revealed that the number of circulating EPCs was reduced in Eisenmenger patients; these cells were increased after sildenafil treatment, indicating that circulating EPCs could be a valid biomarker for the decrease in pulmonary artery pressure.[11] On the other hand, Xu et al reported that transcatheter ASD closure for congenital heart disease did not significantly alter the levels of circulating EPCs before and after closure.[12] Xu et al also proposed that transcatheter ASD closure did not cause significant damage to the vascular wall or endothelial activation. However, these studies did not compare the biological function of EPCs before and after shunt repair or blood flow cessation.

The shear stress generated by the luminal blood flow is the most important mechanical force that regulates vascular tone and endothelial function. In addition, changes in the luminal blood flow rate may modulate the numbers and functioning of the endothelial stem/progenitor cells.[13,14] Shunt closure alters the left-to-right flow. The number of colony formation units is generally considered an in vitro functional assessment of circulating EPCs.[15] Shortly (within an hour) after the cessation of blood flow in ASD, EPC CFUs were significantly enhanced, whereas the numbers of circulating EPCs were similar before and after the implantation of the ASD occluder. The qualitative changes in EPCs can be regulated by a number of physiological factors, particularly in response to shear force.[13,16] ASD occlusion significantly reduced the pulmonary blood flow; therefore, the shear stress on the pulmonary

artery vascular wall had decreased. Although it has been indicated that fluid shear stress induces adhesion, proliferation, tube formation, and differentiation of cultured EPCs,[13] there are currently no in vivo data to clarify the true effect of shear stress on circulating EPC activation. In fact, the culturing conditions were incomparable with the in vivo physiology as the mobilized EPCs circulated in the peripheral blood instead of adhering to the cultured interfaces. One of the major challenges of this clinically important phenomenon was the lack of a valid and reliable animal or human study model that could be used to determine the effects of shear force on the EPC cellular biology. In the present report, we demonstrated that the implantation of an Amplatzer occluder in patients with left-to-right shunt ASDs consistently enhanced the colony formation of isolated EPCs; therefore, this could serve as an ideal in vivo study model for the investigation of flow-induced EPC activation.

In this study, we also tried to correlate the potential proangiogenic cytokines and biological activity of EPCs in the peripheral blood circulation. Hsp27 is commonly found in tumor microenvironments, and has been shown to promote angiogenesis through TLR3-dependent calcium entry and NF-κB activation in human endothelial cells; this leads to VEGF secretion, autocrine or paracrine VEGFR2 activation, cell migration, and angiogenesis.[17] In addition, augmentation of the systemic or local levels of Hsp27 significantly enhances re-endothelialization after vascular injury.[18] Therefore, we measured serum Hsp27 levels before and after the occlusion of ASD and correlated the changes in Hsp27 levels with the EPC colony-forming units. Our results showed that the serum (ie, extracellular) Hsp27 concentrations were significantly increased in patients with elevated biological EPC activity (ie, CFU $>$ 5 colonies), but the serum concentrations were similar in patients with lower EPC activity (ie, CFU $<$ 5 colonies). Although these findings did not prove a direct causal relationship, our study reinforced the view that extracellular Hsp27 could potentially induce the circulating EPC activity during proangiogenic conditions.

To identify the net effect of shear stress on the activation of circulating EPCs, several approaches were utilized to rule out potential confounding factors. The numbers of CD34+/KDR+ cells were quantified before and after the procedures, and the results showed that application of the ASD occluder did not significantly affect the quantification of EPCs in the circulation. Circulating EPCs could also be activated by an indwelling catheter and the implantation of occluder devices. We enrolled a patient with PFO, who did not have flow-induced PH, for closure of PFO using the occluder to prevent any cerebral thromboembolic events.[19] This study also included another patient with an enlarged ASD; however, the device was eventually removed because of placement failure. These patients received identical procedures as the patients who underwent ASD occlusion, including general anesthesia, femoral vein catheterization, administration of heparin, and occluder implantation. EPC CFUs of these two patients were only marginally increased after the procedure (1 to 5 and 0 to 2), which were incomparable with the significant increase of CFUs in the flow-induced pulmonary hypertension patients with eventually reduced pulmonary pressures. Although it is unpractical to carry out statistical analysis with data collected from these two positive control patients, the huge difference in EPC-CFUs between the study and positive control group might suggest that the implantation procedures and devices were unlikely to have affected the EPC activity in the present study conditions.

5 | LIMITATIONS

This study was conducted in a prospective, case–control manner. Therefore, these observational results could have been confounded by a wide range of physiological factors. Although we included two patients as controls in this study to eliminate the effects of instrumentation, perioperative drugs (such as heparin) and anesthesia, there could have been reactions to the foreign bodies or to the scraping of the vascular wall during the catheterization. In addition, the nature of outgrowth endothelial colonies was not completely identified. Sloughed-off endothelial cells may have formed the endothelial colonies in these culture conditions instead of the circulating EPCs in the blood. Nevertheless, we followed the general protocol for isolating and culturing EPCs in the peripheral blood, and the obtained outgrowth EPCs exhibited the typical phenotyping of their endothelial progenitors (Figure 1). Since endothelial colony formation was not increased in PFO patients receiving catheterization, the formation of colonies after application of ASD occluder in patients with flow-induced pulmonary hypertension was therefore unlikely to be derived from the mature endothelial cells in the circulation.

6 | CONCLUSIONS

This report demonstrated that the closure of a left-to-right shunt successfully stopped the excessive pulmonary blood flow and reduced the pulmonary artery pressure. Most patients with flow-induced PH secondary to an ASD experienced improvement in their daily performance and effort tolerance shortly after ASD occlusion. The suppression of plasma BNP levels and the cardiothoracic ratio following the cessation of left-to-right shunt suggested improvement in the right ventricular performance, although these biochemical or radiographic findings might not inevitably infer the clinically significant improvement in physical status. Another novel finding of our present study was that the reduction of excessive pulmonary blood flow after the occluder implantation was associated with the qualitative enhancement of colony formation of the circulating EPCs and increased serum Hsp27 concentrations. Increased EPC colony formation was positively correlated with the patients' daily performance. Since the numbers of colony-forming units were considered a surrogate parameter for the biological function of EPCs, our results indicated that acute reduction of pulmonary blood flow enhanced the biological function of circulating EPCs. We also proposed that extracellular Hsp27 could be a proangiogenic cytokine that regulated the biological activity of the circulating EPCs. Further studies will be required to obtain a deeper understanding of EPC activity modulation and the relationships between EPCs and PH prognosis. However, the results of this study indicated that the application of the Amplatzer

occluder in patients with left-to-right shunt ASD may serve as a valid, reliable clinical model for further investigation.

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CONFLICT OF INTERESTS

The authors confirm that there was no conflict of interest while conducting this research.

AUTHORS CONTRIBUTIONS

Conceptualized and designed the study: Hsu, Wang, Chen, Lam

Collected data: Hsu, Roan, Wang, Wu

Analyzed data: Hsu, Roan, Huang, Shih, Chen, Lam

Drafted the initial manuscript: Hsu

Revised the manuscript: Hsu, Roan, Wang, Huang, Wu, Chen, Lam

Approved the final manuscript as submitted: Hsu, Roan, Wang, Huang, Shih, Wu, Chen, Lam

REFERENCES

[1] Abassi Z, Nakhoul F, Khankin E, Reisner SA, Yigla M. Pulmonary hypertension in chronic dialysis patients with arteriovenous fistula: pathogenesis and therapeutic prospective. *Curr Opin Nephrol Hypertens*. 2006;15(4):353–360.

[2] Hoffman JI, Rudolph AM, Heymann MA. Pulmonary vascular disease with congenital heart lesions: pathologic features and causes. *Circulation*. 1981;64(5):873–877.

[3] Fishman AP. Changing concepts of the pulmonary plexiform lesion. *Physiol Res*. 2000;49(5):485–492.

[4] Mathew R, Gewitz MH. Pulmonary hypertension in infancy and childhood. *Heart Dis*. 2000;2(5):362–368.

[5] Khan AA, Tan JL, Li W, et al. The impact of transcatheter atrial septal defect closure in the older population: a prospective study. *JACC Cardiovasc Interv*. 2010;3(3):276–281.

[6] Asahara T, Kawamoto A. Endothelial progenitor cells for postnatal vasculogenesis. *Am J Physiol Cell Physiol*. 2004;287(3):C572–C579.

[7] Zhao YD, Courtman DW, Deng Y, Kugathasan L, Zhang Q, Stewart DJ. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res*. 2005;96(4):442–450.

[8] Heys JJ, Holyoak N, Calleja AM, Belohlavek M, Chaliki HP. Revisiting the simplified Bernoulli equation. *Open Biomed Eng J*. 2010;4:123–128.

[9] Roan JN, Fang SY, Chang SW, et al. Rosuvastatin improves vascular function of arteriovenous fistula in a diabetic rat model. *J Vasc Surg*. 2012;56(5):1381–1389 e1.

[10] Zimmermann M, Mueller T, Dieplinger B, et al. Circulating heat shock protein 27 as a biomarker for the differentiation of patients with lung cancer and healthy controls–a clinical comparison of different enzyme linked immunosorbent assays. *Clin Lab*. 2014;60(6):999–1006.

[11] Diller GP, van Eijl S, Okonko DO, et al. Circulating endothelial progenitor cells in patients with Eisenmenger syndrome and idiopathic pulmonary arterial hypertension. *Circulation*. 2008;117(23):3020–3030.

[12] Xu MG, Meng XC, Li BN, Liu C. The circulating level of endothelial progenitor cells after transcatheter closure of congenital heart disease in children. *Pediatr Cardiol*. 2013;34(6):1344–1349.

[13] Obi S, Yamamoto K, Shimizu N, et al. Fluid shear stress induces arterial differentiation of endothelial progenitor cells. *J Appl Physiol (1985)*. 2009;106(1):203–211.

[14] Ando J, Yamamoto K. Vascular mechanobiology: endothelial cell responses to fluid shear stress. *Circ J*. 2009;73(11):1983–1992.

[15] Alaiti MA, Ishikawa M, Costa MA. Bone marrow and circulating stem/progenitor cells for regenerative cardiovascular therapy. *Transl Res*. 2010;156(3):112–129.

[16] Yamamoto K, Takahashi T, Asahara T, et al. Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. *J Appl Physiol (1985)*. 2003;95(5):2081–2088.

[17] Thuringer D, Jego G, Wettstein G, et al. Extracellular HSP27 mediates angiogenesis through Toll-like receptor 3. *FASEB J*. 2013;27(10):4169–4183.

[18] Ma X, Hibbert B, McNulty M, et al. Heat shock protein 27 attenuates neointima formation and accelerates reendothelialization after arterial injury and stent implantation: importance of vascular endothelial growth factor up-regulation. *FASEB J*. 2014;28(2):594–602.

[19] Sievert H, Horvath K, Zadan E, et al. Patent foramen ovale closure in patients with transient ischemia attack/stroke. *J Interv Cardiol*. 2001;14(2):261–266.

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