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Effect of iloprost on biomarkers in patients with congenital heart disease-pulmonary arterial hypertension

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Summary

Some biomarkers play important roles in the endothelial dysfunction of patients with pulmonary arterial hypertension (PAH), including nitric oxide (NO), endothelin-1 (ET-1), asymmetric dimethylarginine (ADMA), galectin-3 (Gal-3), B-type natriuretic peptide (BNP), and uric acid (UA). However, studies on these biomarkers in pulmonary artery blood in congenital heart disease-PAH (CHD-PAH) and the effect of iloprost on the regulation of biomarkers are lacking. This study investigated potential CHD-PAH biomarkers and their association with the severity of disease. The effect of iloprost on the regulation of these biomarkers was also studied. A total of 31 patients with CHD-PAH were enrolled. Seven with positive effects of iloprost (the average reduction in mPAP 11.13±1.73 mm Hg) and 19 with negative effects of iloprost (the average reduction in mPAP 4.21±4.87 mm Hg; iloprost positive group [IPG] vs iloprost negative group [ING], $P<.01$) and five age-matched controls were studied. The pulmonary artery blood sample was collected before and after inhaling iloprost, and the plasma concentrations of Gal-3, ADMA, ET-1, and NO were measured. A significant positive linear relationship was observed between mPAP and plasma ET-1, BNP, ADMA, and UA levels in all patients with CHD-PAH. ET-1, ADMA, BNP, and UA levels had a significant linear relationship with mean pulmonary arterial pressure, which could be used to predict the severity of CHD-PAH. ET-1 might be a potential biomarker to pre-evaluate the effect of iloprost on CHD-PAH. Iloprost could affect the expression of Gal-3 and, therefore, the process of fibrosis could be influenced by iloprost.

KEYWORDS
congenital heart disease, iloprost, pulmonary arterial hypertension

1 | INTRODUCTION

Pulmonary arterial hypertension (PAH) is common in patients with congenital heart disease (CHD), which is still associated with considerable morbidity and mortality. The precise mechanism of CHD-PAH is still unclear. The left-to-right shunt due to CHD can increase the pulmonary blood flow, and the shear stress may be triggered, leading to the development of PAH. It is known that PAH can lead to progressive proliferation and migration of pulmonary vascular smooth cells.[1]

Many clinical and haemodynamic parameters were used to assess the severity of PAH and evaluate the effect of treatment. However,

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these parameters were associated with significant limitations, either because invasive procedures were required (eg, right heart catheterization), or because these parameters were subjective and difficult to objectify (eg, cardiac functional class, 6-minutes walk distance).

B-type natriuretic peptide (BNP) is a biomarker of heart function in the clinic, but no other biomarkers of CHD-PAH are available. This led to the investigation of the biomarkers of CHD-PAH. We chose biomarkers like nitric oxide (NO), endothelin-1 (ET-1), asymmetric dimethylarginine (ADMA), galectin-3 (Gal-3), BNP, and uric acid (UA) as our study objects which were reported on PAH patients.[2-6]

Iloprost, a vasodilator, is commonly used in treating patients with PAH. It is also a synthetic analogue of prostacyclin with few systemic adverse effects but with the additional advantages such as simple delivery, minimal toxicity, and dilation of the pulmonary artery through different cellular mechanisms.[7,8]

This study aimed at selecting effective biomarkers that could be used to assess the severity of CHD-PAH in patients and investigating the effect of iloprost at the level of biomarkers.

2 | RESULTS

2.1 | Clinical test characteristics of patients

Thirty-one patients with CHD-PAH were enrolled in this study. The clinical characteristics are shown in Table 1. No differences were found in demographic and clinical characteristics of the enrolled patients between the three groups, except the administration of endothelin-1 receptor antagonists (P=.04) and phosphodiesterase type-5 inhibitors (P=.01) and other hemodynamic parameters (mRAP [P=.03], PASP [P<.01], and pulmonary vascular resistance [PVR] [P=.02]). The average reduction in mPAP in IPG and ING was 11.13±1.73 and 4.21±4.87 mm Hg, respectively (P<.01).

2.2 | Linear analysis of biomarkers with mPAP

The linear regression with biomarkers and mPAP was calculated to evaluate the relationship between them (Figure 1). The level of biomarkers increased with the increase in mPAP (except galectin-3). A significant positive linear relationship was observed between mPAP and plasma ET-1 (r=.5, P=.004), BNP (r=.41, P=.02), ADMA (r=.35, P=.05), and UA (r=.49, P=.005) levels in patients with CHD-PAH. Meanwhile, no positive linear relationship was found between mPAP and plasma NO (r=.15, P=.40) and galectin-3 (r=.07, P=.15) levels.

2.3 | Effects of iloprost on biomarkers in pulmonary artery blood

Briefly, no difference in mean biomarker levels between CG-1 (mean level of biomarkers before occlusion) and CG-2 (mean level of biomarkers after occlusion) was observed (P>.05), implying that the effect of occlusion on the mean biomarker level could be ignored (Figure 2 and Table 2).

Moreover, the mean galectin-3 level decreased significantly after inhaling iloprost on IPG (P<.01), and a decrease in the mean galectin-3 level was observed in ING (P<.05) (Figure 2A). ADMA concentrations were not different in both IPG (IPG-1 [mean level of biomarkers before inhaling iloprost after occlusion] vs IPG-2 [mean level of biomarkers after inhaling iloprost and occlusion], P>.05) and ING (ING-1 [mean level of biomarkers before inhaling iloprost after occlusion] vs ING-2 [mean level of biomarkers after inhaling iloprost after occlusion], P>.05) (Figure 2B). The mean ET-1 level of IPG was significantly lower after inhaling iloprost than before inhaling iloprost (P<.05). However, interestingly, no significant difference was observed in the changes in the mean ET-1 level in ING (P>.05) (Figure 2C) after inhaling iloprost.

TABLE 1 Comparison of basic clinical characteristics of three groups

	CG	IPG	ING	*P* value
Number (male)	5 (1)	7 (0)	19 (5)	.32
Age (y)	51.42±6.14	48.57±12.56	49.52±14.43	.23
BMI (kg/m^2)	23.42±4.73	26.04±6.76	22.74±2.86	.39
ASD	3 (60%)	4 (57.1%)	9 (57.9%)	.83
VSD	0 (0%)	0 (0%)	2 (10.5%)	.51
PDA	2 (40%)	3 (42.9%)	8 (31.6%)	.91
HP (%)	3 (60%)	2 (28.6%)	4 (21.1%)	.25
ALT (U/L)	21.40±9.31	13.06±3.15	19.44±10.35	.21
Cr (μmol/L)	69.40±17.14	63.28±17.36	72.67±19.08	.52
BUN (mmol/L)	5.92±1.98	5.06±2.35	5.71±2.25	.76
eGFR (mL/min·1.73 m^2)	84.40±13.34	103.43±49.47	118.34±64.56	.72
GLU (mmol/L)	5.66±0.79	5.24±1.09	5.22±1.12	.71
HbA1c (%)	5.48±0.48	5.34±0.53	5.79±0.70	.27
TC (mmol/L)	4.85±0.20	3.81±0.45	3.82±0.19	.51
cTNT (ng/mL)	0.02±0.02	0.01±0.01	0.01±0.01	.27
CK-MB (U/L)	14.00±2.12	12.14±1.68	13.25±3.89	.60
CK-MM (U/L)	47.40±18.49	42. 50±20.96	47.75±35.78	.94
D-Dimmer (mg/L)	0.42±0.25	0.88±1.21	1.00±1.68	.74
CRP (mg/L)	5.10±2.25	1.30±2.31	5.98±11.57	.61
6MWD	311±28	305±43	276±111	.65
mRAP	2.67±0.66	2.14±0.38	6.88±5.23	.03*
PASP	55.8±13.6	55.0±9.4	93.6±19.9	<.01**
PVR	5.02±0.80	5.97±1.34	13.77±7.01	.02*
mPAP (mm Hg)	32.80±9.00	31.70±4.50	56.60±15.40	<.01**
ERAs (%)	0 (0%)	1 (14.3%)	10 (52.6%)	.04*
PDE-5 inhibitors (%)	0 (0%)	4 (57.1%)	14 (63.1%)	<.01**
Prostacyclin analogues (%)	0 (0%)	0 (0%)	3 (15.8%)	.35

CG, control group; IPG, Iloprost positive group; ING, Iloprost negative group; BMI, body mass index; ASD, atrial septal defect; VSD, ventriculap septal defect; PDA, patent ductus arteriosus; HP, hypertension; ALT, alanine aminotransaminase; Cr, creatine; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; GLU, glucose; HbA1c, haemoglobin A1c; TC, total cholesterol; cTNT, troponin T; CK, creatine kinase; CRP, C-reaction protein; 6MWD, 6 minutes walking distance; mRAP, mean right atrial pressure; PASP, pulmonary artery systolic pressure; PVR, pulmonary vascular resistance; ERA, endothelin receptor antagonism; PDE, phosphodiesterase.

Similar to ADMA, after inhaling iloprost, changes in the NO concentration were not significant in both IPG (P>.01) and ING (P>.01) (Figure 2D).

2.4 | Characteristics of biomarkers

The CG, ING and IPG data in Figure 3 are respectively derived from the CG1, ING1 and IPG1 in Figure 2. This part aimed to judge whether patients can be treated for occlusion and predict the treatment regimen by the concentrations of biomarkeroactive substances. A statistically significant decrease in the mean plasma BNP, ADMA, NO, and UA levels was observed in IPG compared with CG (P<.05). On the contrary, the mean plasma ET-1 and galectin-3 levels in CG were found to be progressively lower than those in IPG (P<.01) (Figure 3).

A significant difference was observed in the mean plasma BNP, ET-1, NO, ADMA, and UA levels between ING and CG (P<.01). Also, a difference in galectin-3 was observed between ING and CG (P<.05).

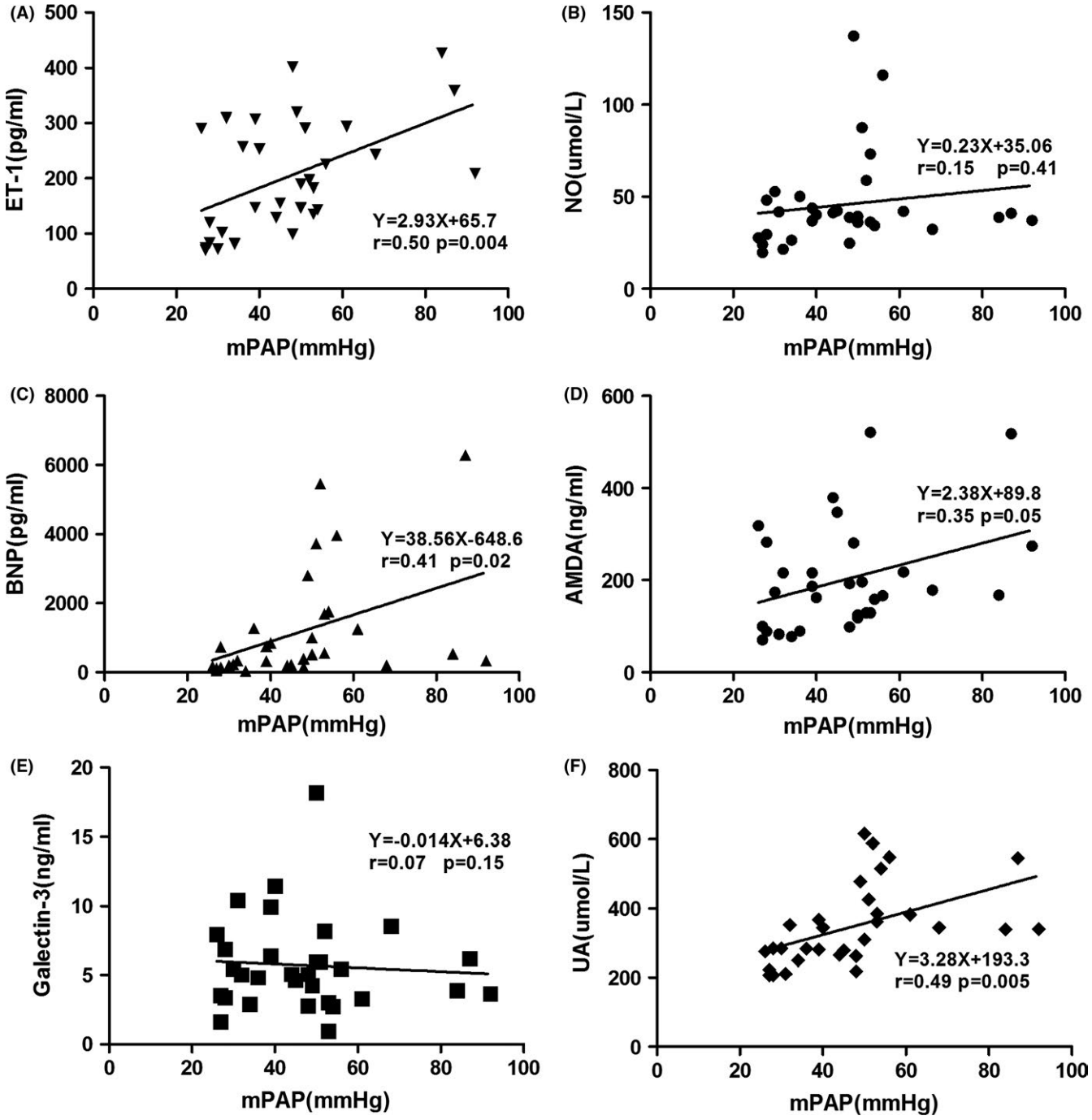

FIGURE 1 (A) Linear regression of mPAP and the ET-1 level (r=.50, P=.004). (B) Linear regression of mPAP and the NO level (r=.15, P=.4); (C) Linear regression of mPAP and the BNP level (r=.41, P=.02); (D) Linear regression of mPAP and the ADMA level (r=.35, P=.05); (E) Linear regression of mPAP and the Gal-3 level (r=.07, P=.15); (F) Linear regression of mPAP and the UA level (r=.49, P=.005)

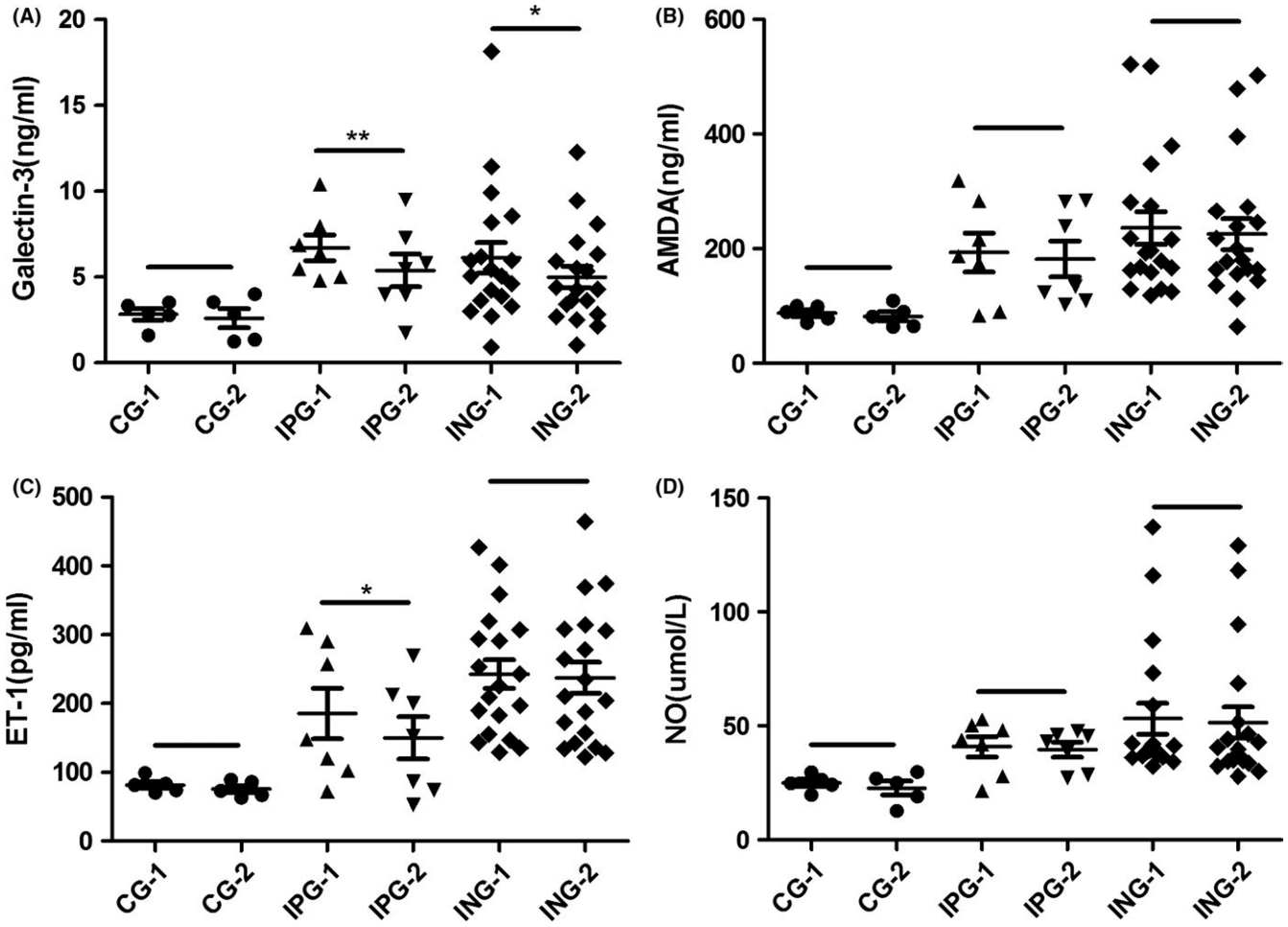

FIGURE 2 Comparisons of (A) Gal-3, (B) NO, (C) ET-1, and (D) ADMA level. (A) No difference of Gal-3 in CG (CG-1 vs CG-2; *P*>.05); Gal-3 were decreased in IPG and ING (IPG-1 vs IPG-2; *P*<.01) (ING-1 vs ING-2; *P*<.05). (B) No difference of NO in CG, IPG, ING (CG-1 vs CG-2; *P*>.05) (IPG-1 vs IPG-2; *P*>.05); (ING-1 vs ING-2; *P*>.05). (C) No difference of ET-1 in CG and ING (CG-1 and CG-2; *P*>.05) (ING-1 vs ING-2; *P*>.05), the level of ET-1 were decreased in IPG (IPG-1 vs IPG-2; *P*<.05). (D) No difference of ADMA in CG, IPG, ING (CG-1 vs CG-2; *P*>.05) (IPG-1 vs IPG-2; *P*>.05) (ING-1 vs ING-2; *P*>.05). CG-1, mean level of biomarkers before occlusion; CG-2, mean level of biomarkers after occlusion; IPG-1, mean level of biomarkers before inhaling iloprost after occlusion; IPG-2 mean level of biomarkers after inhaling iloprost and occlusion; ING-1, mean level of biomarkers before inhaling iloprost after occlusion; ING-2, mean level of biomarkers after inhaling iloprost after occlusion. **P*<.05, ***P*<.01

Interestingly, no difference in biomarkers between ING and IPG was found (*P*>.05), except BNP and UA, which were higher in ING than in IPG (*P*<.05) (Figure 3 and Table 3).

3 | DISCUSSION

PAH is defined as resting mPAP >25 mm Hg with a pulmonary capillary wedge pressure ≤15 mm Hg without an increase in cardiac output (CO).[9] The left-to-right shunt of CHD, which can increase the pulmonary blood flow, may be triggered leading to the development of PAH.

Endothelial dysfunction of pulmonary arteries, inflammation, and imbalance of biomarkeroactive substances in oxidative stress are the characteristics of PAH. The pathophysiological features of all forms of PAH are increasing proliferation and migration of pulmonary biomarkercular smooth cells.[10] Moreover, neurohormonal activation is also a significant pathogenic feature in CHD-PAH.[11]

Some biomarkers were found to be of prognostic value in PAH. The present study attempted to find out the biomarkers that could predict the severity of CHD-PAH. Another purpose was to find whether the iloprost could affect the levels of biomarkers.

Iloprost is vasodilator with less systemic side effects. It is less toxic and expands blood vessels through different mechanisms. It can change the concentration of biomarkeroactive substances in patients with biomarkercular endothelial dysfunction.[7,12]

The present study tested the levels of four biomarkers of pulmonary artery blood, which were more precise to predict the severity of CHD-PAH compared with those of venous blood. No previous studies measured the level of biomarkers in the pulmonary artery blood. CG was used to remove the influence of occlusion when evaluating the effect of iloprost on biomarkers. The levels of biomarkers in CG were compared with those in IPG and ING.

Table 1 shows that no differences were observed in the demographic and clinical characteristics between the three groups, except

TABLE 2 Camparison of biomarkers levels before and after inhaling iloprost

	Galectin-3 (μmol/L)	ADMA (ng/mL)	ET-1 (ng/mL)	NO (μmol/L)
CG-1	2.83±0.75	87.16±12.75	81.58±11.07	24.94±3.59
CG-2	2.60±1.25	81.81±18.96	75.64±11.38	22.71±6.84
IPG-1	6.70±1.98	193.08±89.02	185.54±97.40	40.89±11.80
IPG-2	5.39±2.52**	181.86±82.51	149.73±81.61*	35.13±12.59
ING-1	6.12±3.90	235.72±123.53	242.59±91.01	53.18±29.66
ING-2	5.00±2.76*	237.33±98.73	237.33±98.73	49.88±30.51

CG-1 vs CG-2; IPG-1 vs IPG-2; ING-1 vs ING-2;*P<.05, **P<.01.

the administration of endothelin receptor antagonists (P=.04) and phosphodiesterase type 5 inhibitors (P=.01) and other haemodynamic parameters (mRAP [P=.03], PASP [P<.01], PVR [P=.02]). The differences in mRAP, PASP, and PVR showed that the cardiac function and severity of the three groups were different, influencing the treatment regimens. All other factors that could influence the results were ignored. One study on 43 patients with CHD-PAH included 31 (72.1%) female patients compared with the present study comprising 25 (80.6%) female patients, with no significant difference (P>.05).[3] The average age of the patients in the present study was 49.6 years compared with 40.0 years in the aforementioned study.[3] The reason was that the present study included patients who were >18 years old, but the other study included patients >1 year old. Actually, the average age reported by several studies ranged from 39.5 to 48 years.[12]

Nitric oxide, a typical biomarkerodilator, is synthetized from L-Arg by NO synthase (NOS). Cyclic guanosine monophosphate (cGMP), which is biomarkerodilatory and antiproliferative, is produced while NO stimulates soluble guanylate cyclase. The endothelium could release the regular quantity of NO.[13] The progression of PAH is due to an imbalance between biomarkerodilation and biomarkeroconstriction in the pulmonary circulation, and NO is a biomarkerodilator.

Some studies showed that NO played a role in PAH. One study found no linear relationship between mPAP and NO concentration (r=.29, P>.05).[2] The present study also reported no linear relationship between mPAP and NO levels (r=.15, P=.4). Therefore, NO might be inappropriate as a predictor of mPAP. Iloprost could not affect the NO levels in patients with peripheral ischaemia.[8] The present results were consistent with previous reports, indicating that iloprost could not upregulate or downregulate the expression of NO in IPG and ING.

Figure 3 shows that the level of NO was higher in IPG and ING compared with CG. However, reduced NO bioavailability was believed to be one feature of all forms of PH, which led to reduced NO synthase (NOS),[14] oxidative stress,[15] and inhibition of NO synthesis.[16] Also, oxidative stress increased the production of free radicals, such as superoxide, which readily reacted with NO to form peroxynitrite, reducing NO levels.[15] One study showed that NO concentration was lower in patients with primary PH than in healthy, nonsmoking control subjects (P<.05).[14] However, researchers who established a model of pulmonary hypertension by increasing pulmonary blood flow found that increases in shear stress stimulated endothelial cells to produce several modulators of biomarkercular tone, including NO.[17] Another study reported that the NO levels in patients with CHD-PAH were higher than the levels in patients with CHD (P<.01).[18] It was hypothesized that patients with CHD-PAH, whose pulmonary blood flow increased, might have a higher concentration of NO because of compensation. However, more clinical trials were still required to prove this hypothesis.

One physiological characteristic of PAH is that the endothelium may be impaired. Therefore, after removing the influence of left-to-right shunt, if the mPAP is still >25 mm Hg, more NO is produced in the pulmonary artery to compensate. However, no difference was observed between IPG and ING, indicating that the increase in mPAP could not elevate the NO concentration. This finding needs more clinical trials and animal experiments for validation.

Asymmetric dimethylarginine, which is produced by methylation of arginine residues, competes with L-arginine, which regulates NOS. Theoretically speaking, ADMA can decrease the production of NO by the endothelium.

Three studies showed that ADMA levels were significantly elevated in patients with CHD-PAH compared with healthy controls.[2,19,20] The levels of ADMA in IPG and ING were higher than those in CG, but still no difference was found between IPG and ING. The studies using animal PAH models tested the relationship between the elevated plasma ADMA level and the development of PAH. Different mechanisms for explaining this increase were proposed. Some groups found an association of decreased dimethylarginine dimethylaminohydrolase-1 (DDAH1) levels with increased ADMA levels,[21,22] while others detected decreased DDAH2 levels.[23,24] Furthermore, protein arginine *N*-methyltransferase (PRMT) could be upregulated in mice with chronic hypoxia, and ADMA levels increased in tissues.[25]

Moreover, a significant positive relationship was observed between mPAP and ADMA levels in patients with CHD-PAH in our study (r=.35, P=.05). The mPAP and PVR index were positively correlated with the ADMA level (r=.762, P=.006; r=.603, P=.038, respectively).[26]

Another study showed that ADMA plasma concentrations in all patients with chronic thromboembolic pulmonary hypertension had a linear relationship with mPAP (r=.24, P=.005),[27] suggesting that ADMA could be a potential biomarker to evaluate the level of mPAP in patients with CHD-PAH. The possible explanation for this association might be that the NO/cGMP pathway regulated the pulmonary biomarkercular tone, which could be represented directly by mPAP. Interestingly, iloprost did not have an impact on the expression of ADMA, indicating that iloprost would not affect the L-arginine metabolism pathway immediately.

ET-1 is an amino acid peptide synthesized and released by endothelial cells, which binds to the endothelin receptor on the biomarkercular smooth muscle, causing an increase in cyclic adenosine monophosphate in smooth muscle and a long-lasting biomarkeroconstriction.

Several studies showed that plasma ET-1 levels were related to hemodynamic signs of heart failure and clinical outcomes.[28,29] However, few studies showed a relationship between ET-1 levels and clinical parameters in patients with CHD-PAH.

The present study found that ET-1 production displayed a significant positive relationship with pulmonary artery systolic pressure

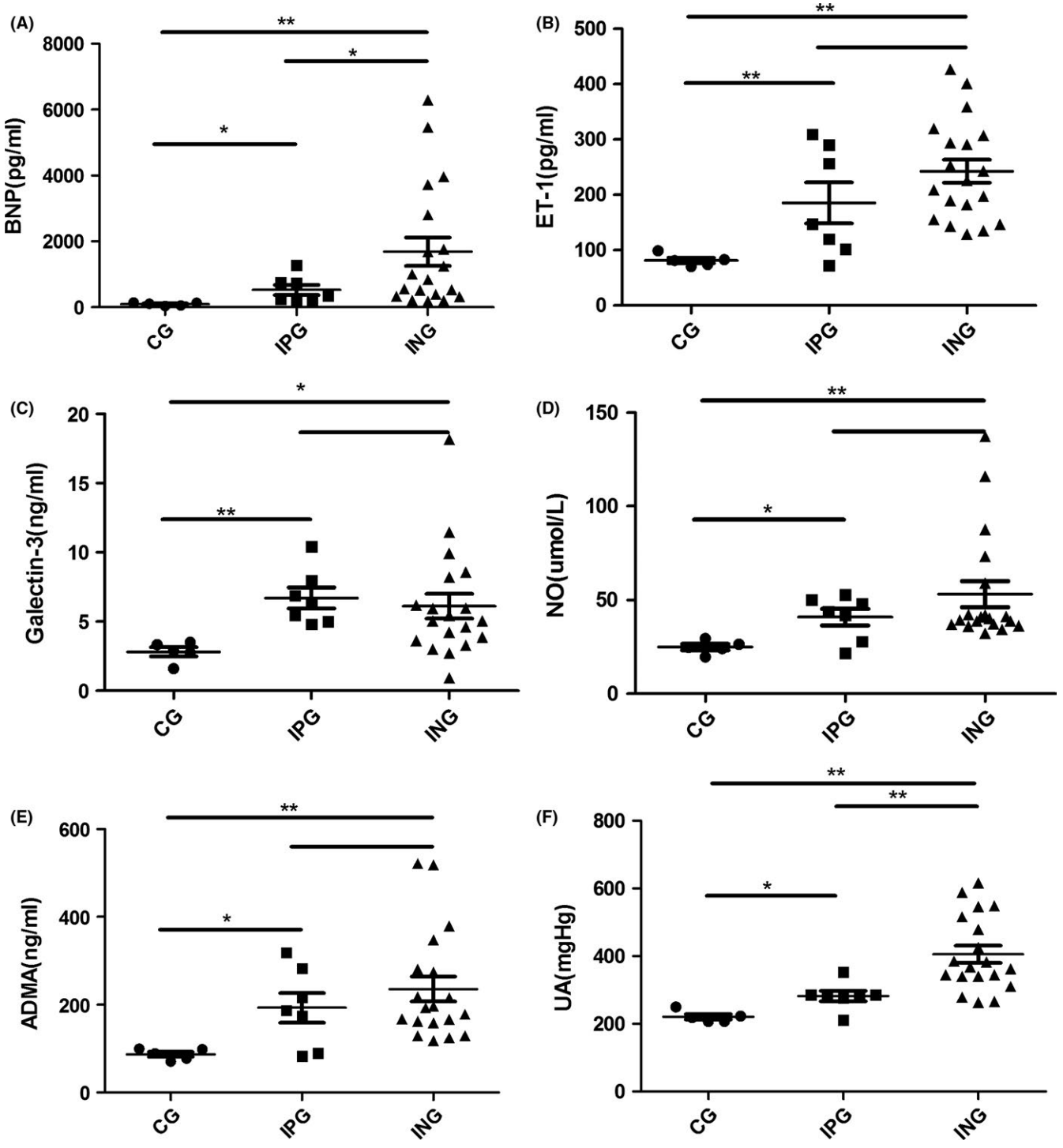

FIGURE 3 Comparisons of (A) BNP, (B) ET-1, (C) Gal-3, (D) NO, (E) ADMA, (F) UA level between any two groups. (A) BNP in CG was lower than IPG ($P<.05$) and ING ($P<.01$); BNP in IPG was lower than ING ($P<.05$). (B) ET-1 in CG was lower than IPG ($P<.01$) and ING ($P<.01$); no difference between IPG and ING ($P>.05$). (C) Gal-3 in CG was lower than IPG ($P<.01$) and ING ($P<.05$); no difference between IPG and ING ($P>.05$). (D) NO in CG was lower than IPG ($P<.01$) and ING ($P<.01$); no difference between IPG and ING ($P>.05$). (E) ADMA in CG was lower than IPG ($P<.05$) and ING ($P<.01$); no difference between IPG and ING ($P>.05$). (F) UA in CG was lower than IPG ($P<.05$) and ING ($P<.01$); no difference between IPG and ING ($P<.01$). *$P<.05$; **$P<.01$

($r=.8563$, $P<.0001$) as well as mean pulmonary artery pressure ($r=.8619$, $P<.0001$) in patients with CHD-PAH.[3] A significant positive linear relationship was also observed between mPAP and ET-1 levels in patients with CHD-PAH in this study ($r=.50$, $P=.004$).

Iloprost induced more potent biomarkerodilation compared with other prostacyclin mimetics.[30,31] However, the effect of iloprost on the change in the ET-1 level in patients with CHD-PAH was not reported. Figure 2 shows that iloprost had an impact only on IPG. Therefore,

TABLE 3 Mean level of biomarkers and mPAP of three groups

	CG	IPG	ING	*P* value
BNP (pg/mL)	97.86±46.61*	529.36±411.69★	1687.67±1876.10##	.05▲
ET-1 (ng/mL)	81.58±11.07**	185.54±97.40	242.59±91.01##	.19
NO (μmol/L)	24.94±3.59**	40.89±11.80	53.18±29.66##	.16
ADMA (ng/mL)	87.16±12.75*	193.08±89.02	235.72±123.53##	.25
Galectin-3 (μmol/L)	2.82±0.75**	6.7±1.98	6.12±3.90#	.40
UA (μmol/L)	221.00±7.89*	282.30±15.54★★	405.6±25.42##	.004▲▲
mPAP (mm Hg)	32.80±9.00	31.70±4.50★★	56.60±15.40##	.003▲▲

IPG vs CG *P<.05, **P<.01; ING vs IPG ★P<.05, ★★P<.01; ING vs CG #P<.05, ##P<.01; ANOVA ▲P<.05, ▲▲P<.01.

iloprost could lower mPAP by decreasing the level of ET-1 in a short time, providing a method to estimate the effect of iloprost on mPAP by measuring the ET-1 level.

One study reported no difference in the ET-1 level between healthy controls and patients with CHD but no PAH (P>.05). The ET-1 level was higher in patients with CHD-mild PAH than in patients with CHD but without PAH (P<.05) but lower in patients with CHD-severe PAH (P<.05).[3] The present study yielded a similar result but did not show any difference between IPG and ING.

Gal-3, produced by cardiac macrophages, is a β-galactosidase-binding lectin. Gal-3 mediates the influence of platelet-derived growth factor, which is associated with hypertrophy, hyperplasia, migration, biomarkercular remodelling, and apoptosis, thus contributing to the pathogenesis of PAH. Serum Gal-3 was significantly elevated in patients with PAH compared with healthy controls.[4] Gal-3 concentration was also higher in patients with idiopathic pulmonary arterial hypertension and connective tissue disease compared with controls.[32] The present study showed that the level of Gal-3 was higher in IPG and ING than in CG. However, no difference was found in the level of Gal-3 between IPG and ING. Nevertheless, iloprost had an impact on the level of Gal-3 in both IPG and ING groups. Serum galectin-3 levels were higher in patients with PAH having elevated right ventricular systolic pressure compared with healthy controls.[4] However, a linear relationship between Gal-3 and mPAP was not shown in the present study (r=.07, P=.15). A Gal-3 is a pro-fibrotic mediator. Its level increased in patients with PAH. The present results showed that Gal-3 levels decreased after inhaling iloprost in both IPG and ING, probably because iloprost not only played the role of a biomarkerodilator but also influenced the process of fibrosis. More basic animal and cell experiments are needed to verify this conclusion.

The plasma BNP levels were measured from the venous blood. BNP is the most important biomarker in terms of assessing clinical severity and prognosis of PAH. It was secreted by cardiac ventricles through a constitutive pathway and elevated according to the degree of myocardial stretch, damage and ischaemia.

BNP was proved to be a good predictor of prognosis for patients with PAH who also suffered from lung disease.[5,33] It had an established relationship with haemodynamics and the damage of cardiac function.[34,35] Moreover, BNP concentration could also be used to reflect the effect of PAH treatment[36] and served as a predictor of mortality in PH induced by lung diseases.[37]

Several studies showed that BNP concentrations were significantly lower in sex- and age-matched controls compared with patients with CHD-PAH.[38-40] Similarly, another study on children and adolescents with CHD-PAH yielded the same results: BNP levels were lower in normal controls than in patients with CHD-PAH (P<.001).[41] Moreover, BNP levels were higher in patients with CHD-PAH in IPG and ING than in CG. Plasma BNP from the pulmonary artery was correlated with mPAP (r=.73, P<.001), while venous plasma BNP was correlated with mPAP (r=.63, P<.001) in adults with CHD-PAH.[39] The linear relationship was positive for BNP (r=.41, P=.02), considering that most hospital could measure BNP. Therefore, BNP could be used as a potential biomarker to predict CHD-PAH progression and prognosis.

Elevated serum UA has recently been analyzed in patients with chronic heart or CHD. Several studies showed a close relationship between hyperuricemia and heart failure or other cardiobiomarkercular diseases.

One study showed the potential benefits of ET receptor antagonist in reducing serum UA. In a small uncontrolled open label study that enrolled inpatients with PAH, treatment with Bosentan for 6 months lowered serum UA from 5.94 to 5.12 mg/dL.[6]

Two studies reported that UA in patients with PAH[42,43] than in healthy subjects, which was coherent with the present results.

A linear relationship was reported between UA and mPAP in paediatric PAH patients (r=.63, P<.01).[43] This results also showed a linear relationship (r=.49, P=.006) between mPAP and UA.

The present study found that ET-1, ADMA, BNP, and UA levels had a significant linear relationship with mPAP, which could be used to predict disease progression and prognosis. Also, iloprost could affect plasma Gal-3, ET-1, and NO levels in ING and IPG. It was hypothesized that iloprost could lower mPAP by reducing the ET-1 level. Mo difference of mPAP was found between CG and IPG, but the levels of all biomarkers were higher in IPG than in CG, suggesting that these biomarkers might be used to predict disease progression and prognosis.

In conclusion:

1. ET-1, ADMA, BNP, and UA levels had a significant linear relationship with mean pulmonary arterial pressure, which could be used to predict the severity of CHD-PAH.
2. ET-1 can be a potential biomarker to pre-evaluate the effect of iloprost on CHD-PAH.
3. Iloprost can affect the expression of Gal-3 and, therefore, the process of fibrosis may be influenced by iloprost.
4. UA, BNP can be used as the differentiated biomarkers to distinguish three groups, to judge whether patients can be treated with occlusion and choose the treatment regimen for patients.

4 | METHODS

This prospective and observational study enrolled patients with CHD-PAH. The protocol was approved by the ethics committee of the

Zhongshan Hospital, Fudan University (Shanghai, China). Informed consent was obtained from all patients before undergoing the study procedure.

4.1 | Study design

A total of 31 Chinese patients with CHD-PAH undergoing transcatheter occlusion from March 2016 to August 2016 were enrolled in this study. All the patients were allocated into three groups according to the mean pulmonary artery pressure (mPAP), which was measured by right heart catheterization (RHC). Patients with mPAP <25 mm Hg after occlusion were allocated to the control group (CG). If the mPAP was >25 mm Hg after occlusion, the patients inhaled iloprost. The patients were allocated to the IPG if the mPAP was <25 mm Hg. Others were allocated to the ING.

The study was carried out at the Cardiovascular Division of the Zhongshan Hospital, Fudan University, Shanghai, China. The PAH was diagnosed as mPAP was >25 mm Hg according to the European Society of Cardiology (ESC) criteria. Patients eligible for inclusion were diagnosed with CHD-PAH (aged 18-75 years). All of the enrolled patients were treated by transcatheter occlusion.

The exclusion criteria were blood pressure less than 90/50 mm Hg or more than 170/110 mm Hg; comorbidities with congestive heart failure, cardiomyopathy, kidney disease, tumour, or autoimmune disease; other kinds of PAH; a major surgery or a history of trauma in a month; and liver transferase concentrations more than three times the normal level.

4.2 | Transcatheter closure

Congenital heart disease can be treated by transcatheter occlusion. The occluders were provided by Shanghai Shape Memory Alloy Co. Ltd. (SHSMA). RHC was applied in patients after percutaneous puncture from the femoral vein and artery. Haemodynamic parameters were recorded, including pulmonary artery systolic pressure (PASP), mPAP, right atrial pressure, systemic arterial pressure, and heart rate. Then, attempted transcatheter closure was applied to patients, and hemodynamic parameters were measured again. If the mPAP was <25 mm Hg, the device was released (CG, five patients which were successful in occlusion and did not need to take any medicine). Otherwise, acute pulmonary biomarkerodilator testing was performed. Iloprost was used as a biomarkerodilator drug. After inhaling aerosolized iloprost for 25 minutes, haemodynamic parameters were measured again. If the mPAP was <25 mm Hg, the occluder was released (IPG, 7 patients succeeded in occlusion and could be treated by CCBs); otherwise, the device was pulled back (ING, 19 patients which could not be treated not only by occlusion but also CCBs). Blood samples were collected from the pulmonary artery before and after inhaling iloprost and centrifuged at 1107 *g* for 10 minutes to obtain supernatants, which were stored at −80°C for further use.

Three criteria for positive acute pulmonary biomarkerodilator testing were as follows: (i) patients with mPAP <40 mm Hg after inhaling iloprost; (ii) average reduction of mPAP more than l0 mm Hg; and (iii) cardiac output after inhaling iloprost same or increased.

4.3 | Laboratory parameters

Biochemical and routine blood tests were conducted by the Department of Laboratory Medicine of Zhongshan Hospital. ET-1 (Jiancheng Bio, Nanjing, China), ADMA (CUSABIO, Wuhan, China), and galectin-3 (ALPCO, Shanghai, China) levels were measured in supernatants collected from the pulmonary artery using an enzyme-linked immunosorbent assay (ELISA) kit as recommended by the manufacturer. The detection range was 20-320 pg/mL, 7.8-500 ng/mL, and 0.47-30 ng/mL, respectively. NO (Beyotime, Shanghai, China) was measured by the cadmium reduction method with detection range 4-160 μmol/L. BNP and UA were excerpted from clinical data.

4.4 | Statistical analysis

Data were analyzed using SPSS version 16.0 (WPSS Ltd., Surrey, UK), and graphs were generated using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, CA, USA). All measurement data that fitted normal distribution were presented as mean±standard deviation. Comparisons among the three groups in Table 1 were assessed using the analysis of variance. Linear regression in Figure 1 between two variables was analyzed. Comparisons between groups in Figure 2 were assessed using the paired *t* test analysis. Comparisons between groups in Figure 3 were assessed using ANOVA post hoc test (Bonferroni). Statistical significance was assumed at $P<.05$.

4.5 | Limitations of the study

1. Since CHD-PAH is a rare disease, only 31 patients were included in this study. This was an extremely small sample size leading to errors in the results.
2. The blood sample collection time was not controlled precisely, changing the time interval of inhaling iloprost in variable patients.
3. This research was not a randomized trial and, therefore, it might be affected by researchers when grouping.

DISCLOSURE

The authors declared that they have no conflicts of interest to this work.

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