

## Original Article

# Changes of plasma cardiac troponin T levels and calcitonin gene-related peptide levels after cardiac catheterization for congenital heart disease in children, and their relation to cardiac function

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**Abstract:** Cardiac troponin T (cTnT) indicates myocardial injury. Calcitonin gene-related peptide (CGRP) is an important regulator of the cardiovascular system. This study investigated plasma level of cTnT and CGRP before and after cardiac catheterization in congenital heart disease (CHD) patients, in order to study the correlation of plasma cTnT and CGRP levels with cardiac functions. Cardiac catheterization was performed on 125 CHD patients, of which 41 had Atrial septal defects (ASD), 42 had ventricular septal defects (VSD), and 42 had patent ductus arteriosus (PDA). Plasma cTnT and CGRP levels were detected and echocardiographic measurements were performed before and after surgery. After surgery, heart functions were significantly increased in all patients. Plasma cTnT levels were normal before surgery, and reached a peak 24 hours after surgery. In VSD patients, plasma cTnT levels were higher compared with the other two groups ( $P<0.05$ ). Plasma cTnT levels diminished to preoperative levels 48 hours after surgery in PDA patients, but were still higher for VSD and ASD patients. Before surgery, plasma CGRP levels were the highest in ASD patients, the lowest in PDA patients. Plasma CGRP levels elevated right after surgery, and peaked at 24 hours post-surgery ( $P<0.05$ ). It began to decrease, and returned to preoperative levels 3 months later. Plasma cTnT and CGRP levels showed a certain rhythm after cardiac catheterization in CHD patients. cTnT is associated with myocardial injury. CGRP reflects cardiac volume load and pressure load, and is correlated with heart functions.

**Keywords:** Pediatric congenital heart disease, cardiac troponin T, calcitonin gene-related peptide

## Introduction

Pediatric congenital heart disease (CHD) is a common clinical cardiovascular defect, and the incidence is relatively high in China. Atrial septal defects (ASD), ventricular septal defects (VSD), and patent ductus arteriosus (PDA) are the most common left-to-right shunt congenital heart defects [1, 2]. Compared with other therapies, cardiac catheterization excels because it causes less traumas, lead to less complications, and guarantees faster postoperative recovery. But there are some limitations in cardiac catheterization: catheters used in surgery will cause stretch; dilated balloon will tear the heart valves; sealing materials will be extruded and worn. Hence, cardiac catheterization can cause damages to myocardial tissues [3, 4]. The most common complication after cardi-

ac catheterization is myocardial injury, while most studies on postoperative myocardium injury have been performed shortly after surgery [5, 6]. The known myocardial damage indicators are creatine kinase (CK-MB) and cardiac troponins (cTnT and cTnI). CK-MB is an important indicator of myocardial necrosis, while cTnT and cTnI are specific myocardial antigens. Cardiac troponins are specific indicators for myocardial injury, because they are released at early time of minor myocardial damages and can stay long in the plasma. Hence, plasma cTn levels are extremely helpful in diagnosis of perioperative myocardial damages and evaluating the operation effect [7, 8]. Calcitonin gene-related peptide (CGRP) is a vasodilator, and CGRP containing nerve fibers are widely distributed in cardiovascular system. CGRP protects damaged endothelial cells and promotes func-

**Table 1.** Clinical characteristics of patients

	ASD patients	VSD patients	PDA patients	F/ $\chi^2$ value	P value
Number	41	42	42	-	-
Gender, mal:female	20:21	19:23	18:24	0.297	0.86
Age, year, mean $\pm$ SD	5.41 $\pm$ 1.43	5.53 $\pm$ 1.61	4.98 $\pm$ 1.52	1.543	0.11
Weight, kg, mean $\pm$ SD	20.54 $\pm$ 6.22	20.93 $\pm$ 6.64	20.73 $\pm$ 7.11	0.131	0.89
NYHA functional classification I~II	31	32	30	0.296	0.81
III~IV	10	10	12		

tional recovery of injured myocardium by multiple actions, including pulmonary vascular dilation, ischemic tissue protection, anti-arrhythmic, and inotropic effects [9, 10]. Abnormal shunts in CHD patients cause increased pulmonary blood flow, as well as elevated cardiac volume and pressure overload, resulting in changes in cardiac functions and neuroendocrine activation. This activated neuroendocrine will cause ischemia and hypoxia in CGRP containing nerve fibers, hence reduce CGRP Synthesis. At the same time, congestion in pulmonary circulation will increase pulmonary shear stress, and cause damages to vascular endothelial cells, reduce CGRP production, rise the concentration of vasoconstrictor substance, and deteriorate cardiac functions [11, 12]. There have been little studies on plasma level changes of CGRP before and after cardiac catheterization on children with CHD so far. In this study, we investigated plasma cTnT and CGRP levels before and after surgery in CHD patients, explored the changes in heart function before and after surgery, and discussed the correlations between plasma cTnT and CGRP levels and heart function.

## Materials and methods

### Patients

Between April 2012 and June 2015, interventional cardiac catheterization was successfully carried out on 125 children with congenital heart disease in the First Affiliated Hospital of Jiamusi University, of which 41 were ASD patients, 42 were VSD patients, and 42 were PDA patients. None of the patients had pulmonary hypertension. This study has been approved by the First Affiliated Hospital of Jiamusi University ethics committee. Potential risks were discussed with family members, and informed consents were signed before the surgery. Success of the therapy was judged by the following criteria after the surgery: occlude in good position,

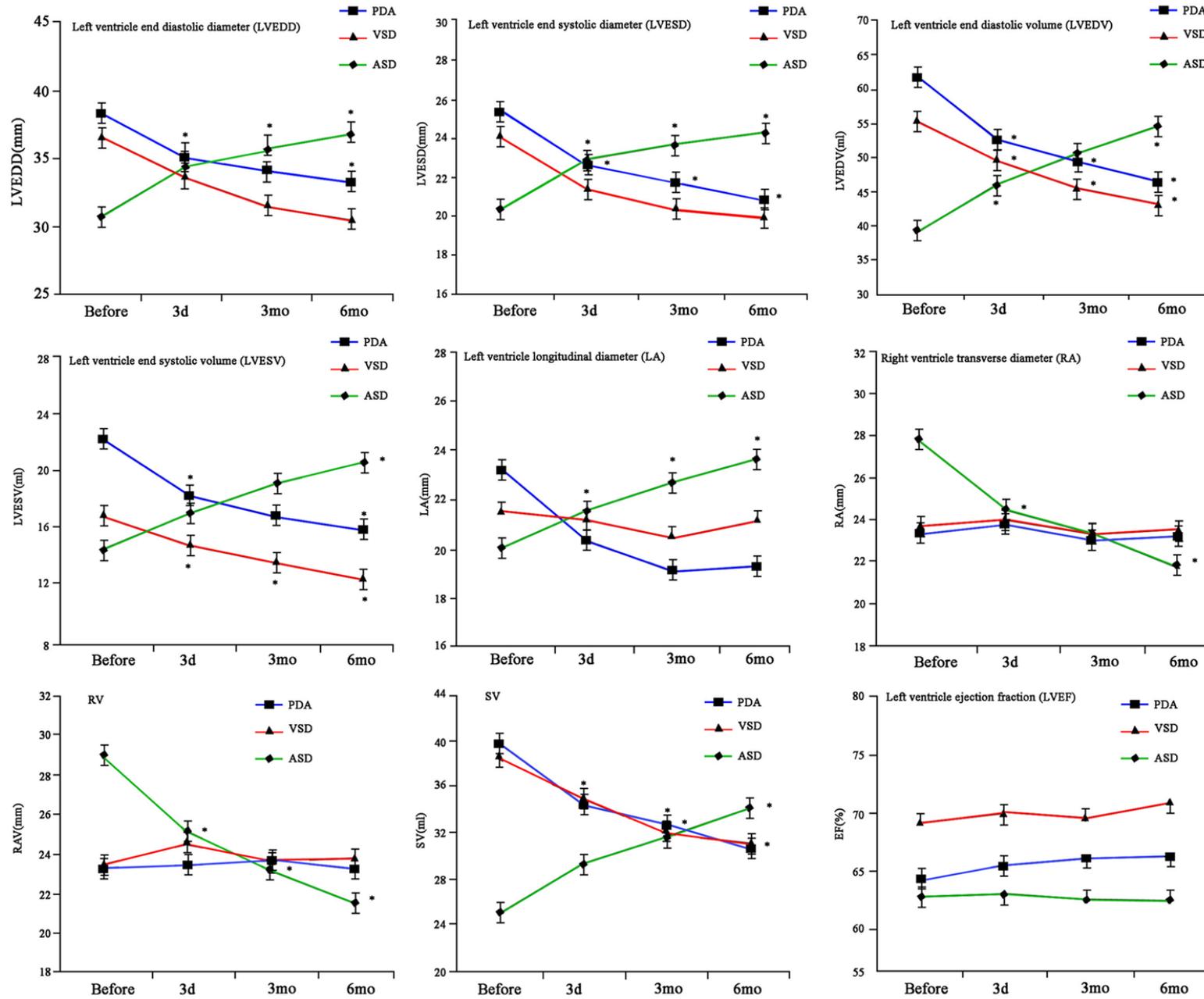
no new valvular regurgitations, no obvious blood flowing through ventricular septum, and no serious complications during hospitalization (death, dangerous arrhythmias, emergency surgery, etc.). Clinical characteristics for all three groups of patients are listed in **Table 1**.

*Inclusion and exclusion criteria for patients:* Patients in this study met these criteria: congenital heart disease patients with left-right shunts, who were suitable to accept the cardiac catheterization treatment which based on our guidelines, aged 2-10 years, no preoperative infections, no muscular diseases, with well-functioning liver and kidneys. Patients with any of the following criteria were excluded from this study: patients with two or more types of congenital heart disease; patients with infection or sepsis before, during or after surgery; patients who needed surgery for other diseases; patients with moderate-to-severe pulmonary hypertension; patients with serious complications during treatment.

### Measurements and data collection

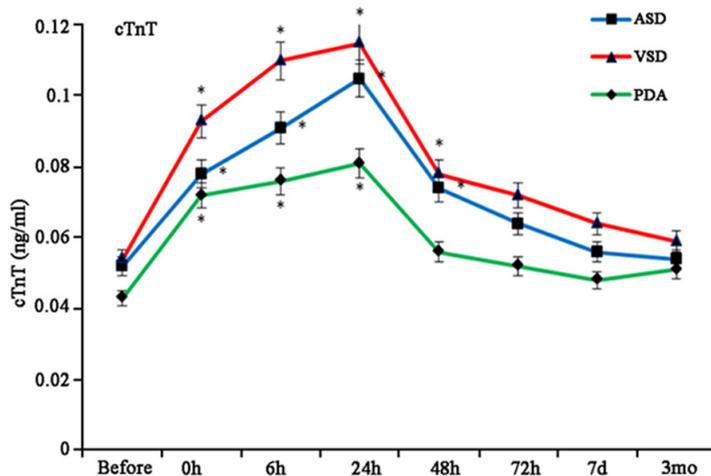
Echocardiographic measurements were given to each patient before and after surgery, and the following data were collected: left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic volume (LVESV), left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic volume (LVEDV), anteroposterior diameter of the left atrium (LA), right ventricular diameter (RV), right atrial diameter (RA), fractional shortening (FS), and stroke volume (SV). Plasma cTnT and CGRP levels were measured preoperatively and 0 h, 6 h, 24 h, 48 h, 72 h, 7 days, 3 months after the surgery, using ELISA assay according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Blood pressure, heart rate, and electrocardiogram were recorded before and after treatment.

## cTnT level and CHD



## cTnT level and CHD

**Figure 1.** Changes of cardiac structure and function in patients before and after cardiac catheterization. (ASD: autism spectrum disorder patients; VSD: ventricular septal defect patients; PDA: patent ductus arteriosus patients \* $P < 0.05$  before and after surgery).



**Figure 2.** Plasma cTnI changes before and after surgery. (ASD: autism spectrum disorder patients; VSD: ventricular septal defect patients; PDA: patent ductus arteriosus patients \* $P < 0.05$  before and after surgery).

### Statistical analyses

Data analyses were performed using SPSS 19.0 software. Data were presented as percentage. Statistical significance was analyzed using chi-square ( $\chi^2$ ) test or Chi-square goodness of fit test. Data were tested for normality first. If data was normally distributed, they would be presented as mean  $\pm$  standard deviation ( $\bar{x} \pm S$ ), one way analysis of variance would be used when comparing multiple groups, and Fisher's least significant difference (LSD) test would be used to compare between two groups. For data not normally distributed, they were presented as median and percentiles, and rank sum test was used to compare between groups. Repeated measures ANOVA was used to compare data from the same group of patients but collected at different time points, and correlations were assessed using Spearman correlation analysis. A  $P$ -value of  $< 0.05$  was considered statistically significant.

## Results

### Clinical observations

Cardiac catheterization was successfully given to all these groups of patients. There were no shortness of breath, fever, heart failure, or cyanosis after surgery. Routine blood test was

performed before and 48 h after surgery, showing no significant difference of white blood cell levels before and after surgery. X-ray chest film and ultrasound cardiogram revealed successful closure after surgery. There were no serious complications.

### Cardiac function changes

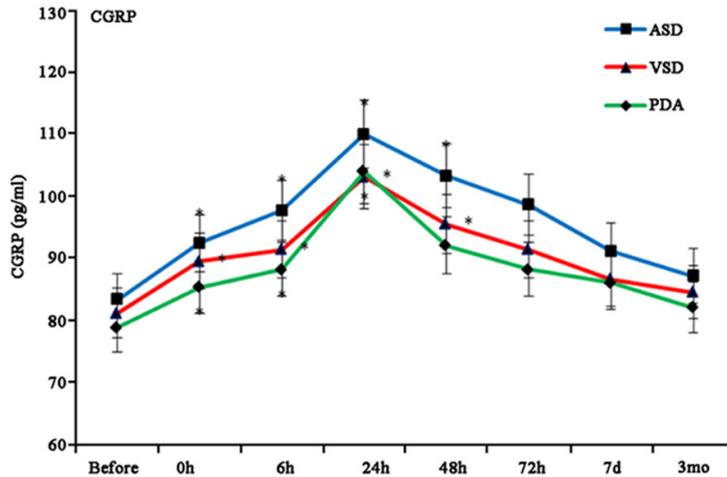
Before surgery, echocardiography results showed that VSD patients displayed a significant increase in left ventricles; left ventricles and left atria were notably larger in PDA children; right atrium and right ventricles were obviously enlarged in ASD patients. After surgery, there were great increases in heart functions in all three

groups of patients: right ventricular function improved in ASD patients; left ventricular function ameliorated in PDA patients; LVEDD, LVESD, LVEDV, LVESV, and SV values significantly decreased in PDA and VSD patient; there were no obvious changes in RA, RV, LVEF values. These data indicate that cardiac catheterization closure blocks abnormal blood flows in VSD and PDA patients, then relieves volume load and pressure load of left ventricle. In ASD patients, there were significant decreases in RA and RV dimensions 3 days after surgery ( $P < 0.05$ ), and these 2 numbers continued to decrease till 6 months postoperation; LA, SV, LVEDD, LVESD, LVEDV, and LVESV values increased significantly after surgery ( $P < 0.05$ ); no obvious changes in LVEF was observed. All data are displayed in **Figure 1**.

### Changes of plasma cTnI levels before and after therapy

Plasma cTnT levels were normal in all three groups of patients before surgery, and rose immediately after surgery ( $P < 0.05$ ). Plasma cTnT concentrations peaked 24 hours after surgery. Among all patients, the VSD group had higher plasma cTnT levels than normal, while the cTnT levels of the other two groups were within the normal range 24 hours after surgery. Plasma cTnT levels were always higher in VSD

## cTnT level and CHD



**Figure 3.** Plasma CGRP changes before and after surgery. (ASD: autism spectrum disorder patients; VSD: ventricular septal defect patients; PDA: patent ductus arteriosus patients \* $P < 0.05$  before and after surgery).

**Table 2.** Correlation between changes in plasma CGRP levels and cardiac functions

	PDA (r-value)	VSD (r-value)	ASD (r-value)
LVESD	0.613*	0.596*	0.578*
LVEDD	0.573*	0.561*	0.573*
LVESV	0.568*	0.583*	0.581*
LVEDV	0.517*	0.642*	0.623*
LA	0.518*	0.118	0.512*
RA	0.214	0.132	0.525*
SV	0.519*	0.536*	0.519*
RV	0.219	0.186	0.533*
LVEF	-0.224	-0.213	-0.247

\* $P < 0.05$ .

patients compared with the other two groups, either right after surgery, or 6 hours or 24 hours post surgery ( $P < 0.05$ ). Plasma cTnT levels dropped to preoperative levels 48 hours after surgery in PDA patients; while for VSD and ASD patients, the plasma cTnT levels decreased 48 hours after surgery, but were still higher than the preoperative levels. 72 h hours post surgery, cTnT levels returned to preoperative levels in all patients (**Figure 2**).

### Changes of plasma CGRP levels before and after surgery

Before surgery, compared among all three groups, plasma CGRP levels were the highest in ASD patients, the lowest in PDA patients, and in the middle in VSD patients. Plasma CGRP levels increased right after surgery, continued rising 6 hours after, and peaked at 24 hours post

surgery ( $P < 0.05$ ). Plasma CGRP dropped 48 hour after surgery, and returned to preoperative levels 3 months after surgery (**Figure 3**).

### Correlation of plasma CGRP level changes with cardiac functions

**Table 2** shows correlations between plasma CGRP level changes and cardiac functions by Spearman analysis. In PDA patients, CGRP levels were significantly associated with LA, LVESD, LVEDD, LVESV, LVEDV, and SV ( $P < 0.05$ ); while the correlations between CGRP and RV, RA, and LVEF did not reach statistical significance ( $P > 0.05$ ). In VSD patients, CGRP correlated with LVESD, LVEDD, LVESV, LVEDV, and SV ( $P < 0.05$ ); while there were no statistical significant correlations between CGRP and LA, RV, RA, or LVEF ( $P > 0.05$ ). In ASD patients, CGRP levels were correlated with LA, RV, LVEDD, RA, LVESD, LVESV, LVEDV, and SV ( $P < 0.05$ ); while the correlation between CGRP and LVEF was not significant ( $P > 0.05$ ).

### Discussion

Traditionally, surgery has been used to treat CHD patients, which is accompanied by big loss, long postoperative recovery, multiple complications, and high risk. With the development of interventional therapies, interventional cardiac catheterization has greatly improved the treatment of CHD. Cardiac catheterization offers many advantages over traditional surgery, but it still causes potential complications. The contrast agent, catheter, and guide wire used during cardiac cauterization procedure will irritate cardiac tissues, cause balloon dilation, and generate ruptured heart valves. Occluder implantation will cause frictions and extrusions to surrounding cardiac tissues; at the same time the immune responses caused by occluder can affect myocardial tissues at the cellular levels [13, 14]. Plasma cTnT levels increased in majority of CHD patients after cardiac catheterization. After radiofrequency ablation, the increases in plasma cTnT levels were negatively correlated with the weight and age of the patient. Plasma CK-MB and cTnT levels elevated after cardiac catheterization [15, 16].

As an endogenous vasodilator, CGRP is widely distributed in the lung and the cardiovascular system. It is involved in regulating varieties of physiological functions, including cardiac contraction, blood vessel dilation, endothelial cell apoptosis inhibition, and angiogenesis [17, 18]. CGRP protects vascular injury by down regulating RAAS and sympathetic activity, antagonizing vasoconstriction, and inhibiting smooth muscle cell proliferation. Plasma CGRP levels dropped as the progression of the diseases in patients with congenital heart disease and pulmonary hypertension, which was negatively associated with pulmonary artery pressure, but was strongly correlated the severity of the patient and poor prognosis [19, 20]. In the study, we investigated the changes of plasma cTnT and CGRP levels before surgery and 0 h, 6 h, 24 h, 48 h, 72 h, 7 d, and 3 months after surgery.

In this study, we found that plasma cTnT levels were within the normal range in all three groups of patients before surgery, and rose immediately after surgery with various degrees, suggesting that different procedures and the uses of different instruments to treat different types of CHD led to the variations in elevated plasma cTnT levels post surgery. During a surgical procedure, mechanical stimulations could increase plasma cTnT levels. In VSD patients, elevated cTnT levels were correlated with myocardial tissue injuries, which might be caused by the implanted VSD occluder and the mechanical stimulations during cardiac catheterization. The occluder might rub surrounding myocardial tissues during the motion of heart tissue (such as heart beats), resulting in regional myocardial edema. During cardiac catheterization operations, the mechanical stimulations could damage vascular endothelia, cause inflammation response, destroy myocardial cell integrity and increase its membrane permeability, which lead to the release of cTnT, because of its small molecular weight, into the blood, resulting a rise of plasma cTnT levels. For VSD patients, cardiac catheterization needed intracardiac imaging, and the occluder was implanted within the large vessels. Operations within large vessels were similar between VSD and PDA patients, while there were less intracardiac procedures in PDA patients compared to VSD patients. In PDA patients, occluder implantation and cardiac imaging were performed outside of the heart chamber. For ASD patients, the cardiac catheterization was ca-

ried out in atria and inferior vena cava, there was no need for contrast agent, and the occluder was located at the atrial septum. The severity of myocardial injury is associated with the size of occluder. The treatments for VSD patients were the most complicated among all three groups, so there were more damages in cardiac and endocardial tissues, and higher cTnT elevations in VSD patients after surgery compared with the other two groups. Our results demonstrate that there were no or minor damages to myocardial tissue after surgery, and the changes in cardiac markers before and after treatment were specified by the types of CHD.

Before surgery, plasma CGRP levels were the highest in ASD patients, the lowest in PDA patients, and in the middle in VSD patients. Plasma CGRP levels rose immediately after surgery, and continued increasing 6 hours after, and reached a peak at 24 hours post surgery. Plasma CGRP decreased 48 hour after surgery, and dropped to preoperative levels 3 months after surgery. Spearman analysis revealed that plasma CGRP levels were associated with cardiac functions, indicating that patients' plasma levels of CGRP may reflect cardiac volume load and pressure load, and that CGRP levels and cardiac functions are correlated. After PDA closure, inhibition factors for sympathetic activity were reduced under the pressures from circulation, pulmonary and cardiac chambers. Ischemia and hypoxia in CGRP nerve fibers were relieved, and plasma levels of CGRP were elevated. The closure of patent ductus arteriosus reduced the blood flow in pulmonary circulation, and alleviated cardiac stress and volume overload, which restored the endocrine changes caused by cardiac hemodynamic abnormalities. After surgery in VSD and ASD patients, we observed elevated plasma levels of CGRP, enhanced myocardial contractility, increased cardiac output, and improved heart functions. The elevated CGRP levels returned to normal after heart function improvement. The abnormal shunt in congenital heart disease patients caused pulmonary hypertension, hemodynamic changes, and reduced heart function, all of which affected plasma levels of CGRP. After treatment, the left-to-right blood flow was blocked, cardiac stress and capacity load were relieved, and heart functions were improved. The restored CGRP levels will cause a feedback effect on cardiovascular systems. CGRP levels may be an indirect indicator of the volume

load and pressure load in left ventricular in VSD and PDA patients, and an indicator of the changes of right heart in ASD patients, hence has great values for evaluation and prognosis after treatment.

In summary, plasma cTnT levels can be used to diagnose myocardial injuries after cardiac catheterization; plasma levels of CGRP are correlated with cardiac volume load and pressure load to some extent; changes in CGRP levels reflect changes in cardiac functions.

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#### Disclosure of conflict of interest

None.

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