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The effects of corrective surgery on endothelial biomarkers and anthropometric data in children with congenital heart disease

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Abstract

Objective: To investigate the influence of surgical correction on biomarkers of endothelial dysfunction in children with congenital heart disease and to evaluate anthropometric data.

Methods: Children with pulmonary hypertension (PH) or Tetralogy of Fallot (TOF) who were scheduled for corrective surgery were enrolled in this prospective study. Age-matched healthy children were included as controls. Demographic, haemodynamic and cardiac ultrasonography data were collected. Blood samples were taken pre-surgery, 24–48 hours post-surgery and again 3–6 months later. Several biomarkers (protein C, soluble platelet selectin [CD62P], soluble endothelium selectin [CD62E], soluble leukocyte selectin [CD62L], plasma von Willebrand Factor [vWF] atrial natriuretic peptide [ANP], brain natriuretic peptide[(BNP] and insulin-like growth factor-1 [IGF-1]) were measured.

Results: Sixty-three children (32 with PH, 15 with TOF, and 16 controls) were enrolled. No significant differences between the PH and TOF groups were observed in the expression of biomarkers pre- and post-surgery. IGF-1 levels were closely related to anthropometric data, particularly those children with PH. Expression of IGF-1 and weight/height normalized after corrective surgery.

Conclusions: No significant endothelial dysfunction was observed in children with PH or TOF before or after corrective surgery. Significant retardation of growth, particularly weight, was found before surgery and may be related to IGF-1 suppression.

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Introduction

Worldwide, congenital heart defects (CHDs) occur in approximately 0.8% of live births and in 30%–50% of cases the malformations are severe enough to warrant more than one surgical procedure during early childhood.[1] Depending on whether patients clinically exhibit cyanosis, these defects can be categorized into cyanotic and acyanotic.[1]

In acyanotic CHD, the back-leak of blood from the systemic to pulmonary circulation via the cardiovascular defect, namely left-to-right shunt, is the most common aetiological cause.[2] Ventricular septal defect (VSD), atrial septal defect (ASD) patent ductus arteriosus (PDA) and endocardial cushion defect (ECD) are common CHDs in young children. If the shunt is persistent and substantial, the overload of blood increases the pulmonary pressure and may damage the pulmonary vasculature.[2] Surgical intervention to correct the shunt defect can amend the overloaded pulmonary circulation and pulmonary hypertension (PH) but if the overloaded pulmonary circulation remains untreated, irreversible PH (i.e., Eisenmenger syndrome) inevitably develops.[2]

In cyanotic CHD, venous deoxygenated blood bypasses the pulmonary circulation and is shunted to the left side of the heart where it mixes with oxygenated blood. [1] In children aged >12 months, Tetralogy of Fallot (TOF) is the most common cause of cyanotic CHD and accounts for 10% of all CHD cases.[2] This condition is a constellation of four abnormalities: VSD; pulmonary stenosis; right ventricular hypertrophy; overriding of aorta. The optimal protocol for surgical correction has not been established.[2] In children older than 6 months, establishing adequate pulmonary flow is crucial for total correction.[3] If adequate pulmonary flow is not established, palliative surgery, such as Blalock–Taussig shunt, is the initial therapeutic strategy to increase pulmonary flow temporarily.[1] The feasibility of total operative repair is largely dependent on the underlying distribution and size of the pulmonary arteries.[4,5]

The endothelium is composed of a single layer of endothelial cells lining the whole vasculature and endothelial cells have been shown to have both metabolic and synthetic functions.[6] By secreting numerous cell products, endothelial cells can influence cellular functions throughout the body.[6] Studies have reported that endothelial dysfunction may occur as a result of persistent hypoxemia and/or PH.[7,8] Coagulopathy, aggravated PH and further vascular remodelling may develop as a consequence of the dysfunction.[9,10] To our knowledge, no studies have previously compared endothelial dysfunction before and after surgical correction in children with CHD and either hypoxemia or PH. The primary aim of this study was to investigate the influence of surgical correction on biomarkers of endothelial dysfunction in children with long-term PH or hypoxemia. The secondary aim was to evaluate anthropometric data pre- and post-surgery.

Methods

This prospective, case-controlled study was conducted at at Chang Gung Memorial Hospital from July 2010 to January 2014. Children less than 2 years of age with CHD who were scheduled for corrective surgery for PH or TOF were considered eligible for this study. CHD was pathologically

confirmed by cardiac ultrasonography or catheterization. Children with comorbid haematological disorders were excluded from the study. The PH group included children with left-to-right shunt. The hypoxemia group included children with TOF and hypoxemia with a pulmonary index less than 200 mm^2/m^2.[2] Age-matched healthy children without pre-existing haematological or cardiovascular diseases who were admitted for elective procedures, such as panendoscopy and bronchoscopy, were included in the control group.

Demographic data were collected for all subjects. Peripheral saturation of oxygen was determined using pulse oximetry and prothrombin time (PT) and activated prothrombin time (aPTT) were determined from hemograms and basic coagulation studies. In most cases, morphologic characteristics of the congenital cardiac malformations were defined using Doppler echocardiography, although angiography was necessary in some cases. Cardiac catheterization was used to determine haemodynamic data.

For the PH and TOF groups, blood samples were obtained immediately after anaesthesia induction for cardiac catheterization, 24–48 hours post-operation in the intensive care unit (after discontinuation of anticoagulants) and during the convalescent period (approximately 3–6 months after corrective surgery). For the controls, blood samples were collected during the pre-procedure assessment. Anthropometric measurements were obtained for the PH and TOF groups at cardiac catheterization and during convalescence. For each participant, body weight and length were interpreted using the Z-score classification system.[11]

Protein C activity was measured using functional clotting assays and on the basis of the prolongation of the aPTT by using the Staclot Protein C Kit (Diagnostica Stago, Asnieres-Sur-Seine, France) according to the manufacturer's instructions. The concentrations (ng/mL) of the selectin class of soluble adhesion molecules (i.e., soluble platelet selectin [CD62P], soluble endothelium selectin [CD62E] and soluble leukocyte selectin [CD62L]) were determined using a commercial ELISA assay (R&D Systems, Minneapolis, Minnesota, USA). Concentrations of plasma von Willebrand Factor (vWF) were measured using the IMUBIND® von Willebrand Factor ELISA Kit (American Diagnostica, Stamford, Connecticut, USA). Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were analysed using ELISA kits (Bachem, Torrance, California, USA). Insulin-like growth factor-1 (IGF-1) levels were also measured using an ELISA kit (Alpco Diagnostics, Salem, New Hampshire, USA).

The study was approved by the Ethics Committee of Chang Gung Memorial Hospital (98-3284B) and informed consent was obtained from the parents or legal guardians of the children.

Statistical analyses

Between-group comparisons were performed using Student's *t* test for continuous variables and Fisher's exact test for categorical variables. A P value of < 0.05 was considered statistically significant. All analyses were performed using IBM SPSS software (version 20 for Windows®; IBM Corp, Armonk, NY, USA).

Results

Sixty-three children (32 with PH, 15 with TOF and 16 controls) were enrolled into the study. In the control group, 3 children required panendoscopies (chronic abdominal pain [$n = 1$], oesophagitis [$n = 1$] and peptic ulcer [$n = 1$]) and 13 required bronchoscopies (noisy breath [$n = 6$)]and recurrent wheeze [$n = 7$]).

There were no differences between groups in terms of age, sex, or platelet levels (Table 1). However, a high haemoglobin

Table 1. Demographic and diagnostic data for children with congenital heart disease and healthy age-matched controls.

	Congenital Heart disease $n = 47$		
	PH $n = 32$	TOF $n = 15$	Controls $n = 16$
Demographic data			
Age, months	9.9 ± 12.3	11.7 ± 3.2	12.4 ± 7.7
Boys	20 (63)	9 (60)	12 (75)
Haemoglobin, g/dL	11.9 ± 1.5	16.7 ± 1.9***	11.9 ± 1.1
Platelet, $x10^3/\mu L$	335 ± 103	324 ± 93	376 ± 116
INR	1.12 ± 0.08*	1.06 ± 0.06	1.02 ± 0.08
Diagnostic data			
O_2 pre-operation, %	94.5 ± 2.4	81.8 ± 4.4†††	
PA pre-operation, mmHg	40.2 ± 14.4	12.0 ± 3.3†††	
Qp/Qs pre-operation	2.6 ± 1.0		
Cardiovascular defect			
VSD	16	–	
VSD + ASD	9	–	
ASD	2	–	
VSD + ASD + PDA	2	–	
PDA	1	–	
VSD + CoA	1	–	
ECD	1	–	

Values are shown as mean ± standard error, *n* or *n* (%).
*$P < 0.05$ and ***$P < 0.001$ compared with the control group; †††$P < 0.001$ compared with the PH group.
Abbreviations: PH, pulmonary hypertension; TOF, Tetralogy of Fallot; INR, international normalized ratio of prothrombin time; O_2, oxygen saturation; PA, pulmonary artery; Qp/Qs, perfusion of pulmonary system/perfusion of systemic system; VSD, ventricular septal defect; ASD, atrial septal defect; PDA, patent ductus arteriosus; CoA, coarctation of aorta; ECD, endocardial cushion defect

level in the TOF group and mild prolonged PT in the PH group were detected. As expected, the pulmonary artery pressure was high in the PH group and oxygen saturation was low in the TOF group. Most children in the PT group had VSD or VSD +ASD (Table 1).

Among the various blood biomarkers, only CD62P and IGF-1 levels showed statistically significant differences from the control group before corrective surgery (Table 2). Following corrective surgery, CD62E and CD62L decreased rapidly in the PH and TOF groups by comparison with control values, only the reduction of CD62E in the TOF group was nonsignificant (Table 3). However, the initially reduced expression of CD62P normalized to control levels post-surgery. Interestingly, the reduced expression of IGF-1 persisted after surgery in both the PH and TOF groups. Nevertheless, the IGF-1 levels in both groups had normalized approximately 3–6 months post-surgery (Table 4).

Immediately post-surgery, BNP levels were elevated in both CHD groups by comparison with controls; the levels in the TOF group were statistically significantly higher than controls ($P < 0.001$]) (Table 3). During the 3–6 months convalescence period, serum concentrations of biomarkers in both CHD groups were comparable with

Table 2. Serum levels of several biomarkers in children with congenital heart disease and healthy age-matched controls before corrective surgery.

	Congenital Heart disease		
	PH $n = 32$	TOF $n = 15$	Controls $n = 16$
Biomarkers			
ANP, pg/ml	175 (32–372)	72 (33–112)	34 (18–50)
BNP, pg/ml	47 (23–71)	59 (23–95)	34 (17–52)
vWF, mU/ml	1107 (982–1233)	962.1 (786–1138)	1203 (699–1707)
Protein C, ng/ml	2383 (641–4124)	401 (173–630)	2835 (330–5339)
CD62E, ng/ml	50.9 (43.7–58.1)	61.1 (47.2–75.0)	61.1 (47.6–74.6)
CD62P, ng/ml	39.9 (36.8–43.0)***	40.1 (35.4–44.7)**	54.0 (45.4–62.5)
CD62L, ng/ml	1907 (1791–2023)	1887 (1653–2121)	2197 (1920–2473)
IGF-1, ng/ml	47.7 (35.0–60.5)**	52.8 (37.1–68.5)*	92.7 (61.6–123.7)

The results are expressed as mean (95% Confidence Intervals)
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the control group.
Abbreviations: PH, pulmonary hypertension; TOF, Tetralogy of Fallot; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; vWF, von Willebrand Factor; CD62E, soluble endothelium selectin; CD62P, soluble platelet selectin; CD62L, soluble leukocyte selectin; IGF-1, insulin-like growth factor-1

Table 3. Serum levels of several biomarkers in children with congenital heart disease and healthy age-matched controls 24–48 hours after corrective surgery.

	Congenital Heart disease		
	PH $n = 28$	TOF $n = 12$	Controls $n = 16$
Biomarkers			
ANP, pg/ml	43 (23–63)	52 (29–75)	34 (18–50)
BNP, pg/ml	56 (36–77)####	199 (72–327)**	34 (17–52)
vWF, mU/ml	1589 (1386–1792)	2249 (1398–3100)	1203 (699–1707)
Protein C, ng/ml	1581 (32.0–3355)	190 (33–346)	2835 (330–5339)
CD62E, ng/ml	39.0 (32.5–45.6)**	45.0 (30.4–59.6)	61.1 (47.6–74.6)
CD62P, ng/ml	60.4 (52.5–68.4)	74.5 (51.3–97.7)	54.0 (45.4–62.5)
CD62L, ng/ml	1333 (1225–1441)***	1311 (1128–1493)***	2197 (1920–2473)
IGF-1 (ng/ml)	32.5 (19.5–45.6)***	42.6 (21.5–63.6)*	92.7 (61.6–123.7)

The results are expressed as mean (95% Confidence Intervals)
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the control group; ####$P < 0.001$ compared with the TOF group.
Abbreviations: PH, pulmonary hypertension; TOF, Tetralogy of Fallot; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; vWF, von Willebrand Factor; CD62E, soluble endothelium selectin; CD62P, soluble platelet selectin; CD62L, soluble leukocyte selectin; IGF-1, insulin-like growth factor-1

Table 4. Serum levels of several biomarkers in children with congenital heart disease and healthy age-matched controls 3–6 months after corrective surgery.

	Congenital Heart disease		
	PH $n = 17$	TOF $n = 7$	Controls $n = 15$
Biomarkers			
ANP, pg/ml	29 (5–52)	91 (8–191)	34 (18–50)
BNP, pg/ml	39 (9–68)	60 (7–114)	34 (17–52)
vWF, mU/ml	1021 (861–1181)	1123 (772–1475)	1203 (699–1707)
Protein C, ng/ml	558 (294–823)	942 (145–1741)	2835 (330–5339)
CD62E, ng/ml	51.0 (42.0–60.0)	52.3 (37.2–67.4)	61.1 (47.6–74.6)
CD62P, ng/ml	39.4 (34.4–44.3)	41.9 (30.8–53.0)	54.0 (45.4–62.5)
CD62L, ng/ml	2080 (1822–2337)	1722 (1512–1932)	2197 (1920–2473)
IGF-1, ng/ml	87.4 (64.3–110.4)	70.1 (24.0–116.2)	92.7 (61.6–123.7)

The results are expressed as mean (95% Confidence Intervals)

Abbreviations: PH, pulmonary hypertension; TOF, Tetralogy of Fallot; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; vWF, von Willebrand Factor; CD62E, soluble endothelium selectin; CD62P, soluble platelet selectin; CD62L, soluble leukocyte selectin; IGF-1, insulin-like growth factor-1

those in the control group (Table 4). Differences in endothelial biomarkers pre- and 3–6 months post-surgery are shown graphically in Figure 1.

To evaluate changes in IGF-1 expression with growth, Z scores from height and weight data were assessed pre- and 3–6 months post-surgery. Before corrective surgery, significant growth failure was observed in children with CHD, particularly in the PH group (Table 5). However, values taken after the 3–6 months convalescence period, showed that growth had normalized after corrective surgery (Table 5). In the PH group, weight gain had markedly improved during the convalescent period ($P < 0.0001$).

Discussion

Several biomarkers of endothelial dysfunction were analysed pre- and post-surgery in children with CHD and either hypoxemia or PH in this single-centre cohort study. Different expression patterns were found for various adhesion molecules, namely, CD62E, CD62P, and CD62L, before and after corrective surgery. Although all children with CHD had significantly lower levels of CD62P compared with controls pre-surgery, irrespective of the pathogenesis of the initial heart defect (i.e., PH or hypoxemia) no significant differences were observed between the patient groups in most biomarkers of endothelial dysfunction. Notably, the significant reduction of IGF-1 levels in both CHD groups before surgery, normalized 3–6 months after surgical correction of the heart defects.

Natriuretic peptides, particularly ANP and BNP, play crucial roles in sodium and water homeostasis and are correlated with several diseases with cardiovascular dysfunction.[12] Before corrective surgery, although no statistically significant differences were observed in natriuretic peptides between controls and patient groups, ANP and BNP levels tended to be higher in the PH and TOF groups compared with controls. Our results are similar to other reports that have shown elevated BNP levels in children with TOF.[13] In this present study, significantly elevated BNP expression was

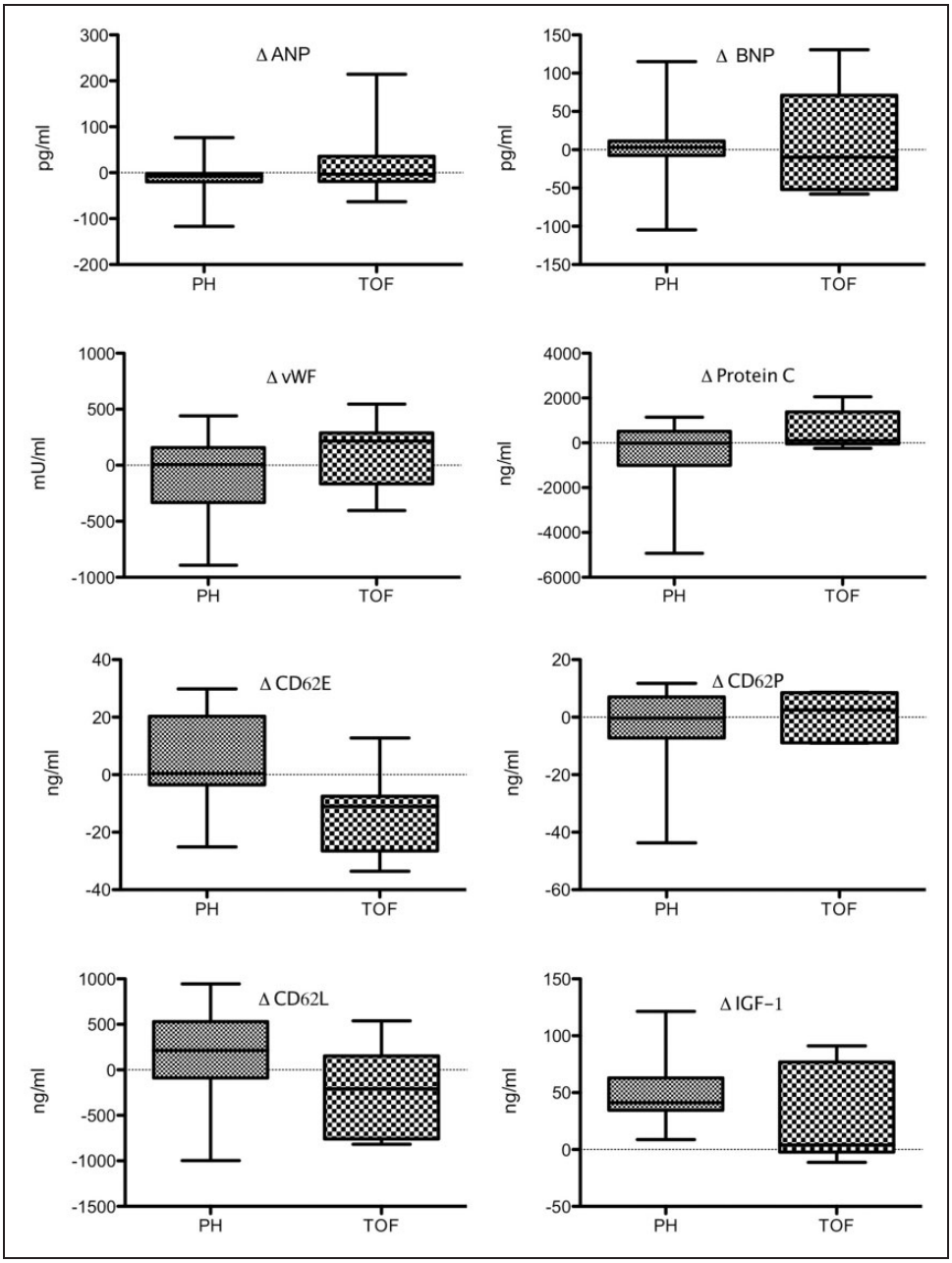

Figure 1. Changes in biomarkers before and 3–6 months after surgical intervention in children with congenital heart disease separated into those with pulmonary hypertension (PH) and those with Tetralogy of Fallot (TOF).

Pre-surgery, there were 32 patients in the PH group and 15 in the TOF group; 3–6 months post-surgery, there were 17 patients in the PH group and 7 in the TOF group.

Abbreviations: PH, pulmonary hypertension; TOF, Tetralogy of Fallot; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; vWF, von Willebrand Factor; CD62E, soluble endothelium selectin; CD62P, soluble platelet selectin; CD62L, soluble leukocyte selectin; IGF-1, insulin-like growth factor-1

Table 5. Z-scores for weight and length before and after corrective surgery.

		Pre-operation Z scores	Convalescence (3–6 months after corrective surgery Z scores	Statistical significance pre- vs post surgery
PH $n = 32$	Weight	-1.83 ± 0.85*** (−3.50– 0.88)	-0.83 ± 1.00 (−2.20–2.20)	$P < 0.0001$
	Length	-1.03 ± 1.31* (−2.60–2.17)	-0.69 ± 1.25 (−2.33–2.4)	*ns*
TOF $n = 15$	Weight	-1.66 ± 1.26** (−3.5–0.90)	-0.72 ± 1.32 (−2.40–.98)	*ns*
	Length	-0.89 ± 1.37 (−2.45–.36)	-0.30 ± 1.30 (−2.20–1.50)	*ns*
Controls $n = 16$	Weight	0.02 ± 1.30 (−2.1–2.2)	–	–
	Length	-0.01 ± 1.58 (−2.1–2.1)	–	–

The results are expressed as mean ± SE (95% Confidence Intervals)
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the control group.
Abbreviations: PH, pulmonary hypertension; TOF, Tetralogy of Fallot

observed in the TOF group soon after corrective surgery. This finding may have been related to ventricular injury secondary to transventricular repair which is generally adopted by our surgical team to reduce the relatively high incidence of recurrent obstruction of right ventricular outflow in transatrial repair.[14]

Coagulation abnormalities, such as polycythaemia, thrombocytopenia, platelet dysfunction, disseminated intravascular coagulation, decreased coagulation factor production and primary fibrinolysis, have been observed in patients with cyanotic congenital heart diseases.[15] In the current study, although significant mild prolonged PT was observed in patients with PH, no differences were observed in protein C, vWF levels and the platelet count between the PH and TOF groups. Elevated vWF levels have been shown to be related to the severity of hypoxemia in adults with Eisenmenger syndrome. [16] Therefore, the lack of effect on vWF levels observed in this present study may be related to none or mild endothelial dysfunction because the patients did not have Eisenmenger syndrome or prolonged hypoxemia.

Endothelial cells regulate the trafficking of leukocytes via adhesion molecules, such as CD62 (selectin), as well as intercellular and vascular adhesion molecules. The adhesion molecules CD62E, CD62P, and CD62L are mainly expressed by the endothelium, platelets and leukocytes, respectively. Low levels of soluble CD62L (sCD62L) and high levels of sCD62E have been reported in adults with primary PH.[17] In this current study, although a mildly lower level of sCD62L was observed in patients with TOF and PH by comparison with controls, no difference was observed between groups in sCD62E levels. In addition, the pulmonary pressure of the PH group was much lower than that observed in a previous study in adults [17] (i.e., 40.2 ± 14.4 *vs.* 97.5 ± 6.6 mmHg). Therefore, we suggest that perhaps low pulmonary pressure may affect levels of sCD62s.

Soon after surgery, sCD62L levels were significantly decreased in both the PH and

TOF groups compared with controls. In another study, patients with major trauma presented with a significant reduction in plasma concentrations of sCD62L within the first 12 hours after trauma.[18] The authors of that study suggested that the reduction may be attributed to the activation of endothelial ligands with subsequent cell–endothelial or sCD62L–endothelial interaction.[18] Likewise, the decrease in sCD62L noted in our study may have been caused by injury as a result of major surgery.

Previous studies have shown that the expression of sCD62P, a marker of platelet activation and vessel wall injury, increased in patients with primary PH and in those with cyanotic CHD and secondary erythrocytosis.[19] However, in this present study, no differences were observed in sCD62P levels between the PH and TOF groups. As mentioned previously, the relatively lower pulmonary pressure in the PH group may have accounted for this observation. Interestingly, in the TOF group, secondary erythrocytosis was not as prominent as in a previous study. [20]

Children with acyanotic CHD, particularly those with increased pulmonary flow, have previously been shown to have significant retardation in growth.[21] It has been suggested that the ensuing congestive heart failure is closely related to growth failure. [21] In the current study, significant growth failure was observed in patients with CHD, particularly in those with PH. Our study also showed that before and soon after corrective surgery, IGF-1 expression was significantly decreased in both the PH and TOF groups by comparison with controls and the suppression tended to be greater in the PH group. Interestingly, the reduction of IGF-1 level was normalized in both groups over the convalescent period and appeared to correlate with changes in growth. We suggest that in these patients, particularly the PH group, IGF-1 levels may be used as a growth indicator. Similar results have been reported previously in children with acyanotic CHD.[22]

The study had some potential limitations. Firstly, although patients in the control group did not have CHD, their health status was unclear. However, an extensive literature search confirmed that there was no association between endothelium dysfunction and healthy control subjects. Secondly, before surgery some diuretics and inotropics were used in children in the PH group but children in the TOF group did not receive any medication. Although endothelial vasodilatory function has been shown to be unaffected by diuretics and inotropics, [23] their effects on endothelial biomarkers are unknown. Moreover, the different surgical interventions used in the present study may have resulted in diverse effects on the endothelium. For example, a recent study using a mouse model has shown cardiac surgery can increase microparticles and inhibit endothelium-dependent vasodilation.[24] Therefore, further studied are required to substantiate our findings.

In conclusion, although inconsistent pattern of CD62 expression were observed before and after corrective surgery, no significant differences in several endothelial biomarkers were observed between children with PH or TOF. Prior to corrective surgery, a significant retardation in growth, particularly weight gain, was observed in both groups of children with CHD. The growth delay and subsequent normalization after surgery was closely related to IGF-1 levels. The precise role of IGF-1 in the growth of children with CHD requires further investigation.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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