Ventricular fibrogenesis activity assessed by serum levels of procollagen type III N-terminal amino peptide during the staged Fontan procedure

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ABSTRACT

Objective: We tested the hypotheses that volume overload and cyanosis observed in the pre-Fontan single ventricular circulation are associated with increased ventricular fibrogenesis, that the Fontan procedure helps to reduce fibrogenesis, and that persistently increased fibrogenesis in the Fontan ventricle is associated with ventricular diastolic dysfunction.

Methods: Levels of serum amino-terminal procollagen type III, a marker of tissue fibrogenesis, were measured in 172 patients with single ventricle circulation and 149 controls. Patients were divided into 3 groups according to surgical stage: 59 patients after Blalock–Taussig shunt or pulmonary banding, 60 patients after Glenn surgery (Glenn group), and 53 patients after Fontan surgery (Fontan group).

Results: Serum amino-terminal procollagen type III levels were significantly higher among the 3 single ventricle groups than among control patients, but decreased with each surgical stage (0.604, 0.176, 0.143, and 0.073 U/mL, for Blalock–Taussig shunt or pulmonary banding, Glenn, Fontan, and controls, respectively). Severity of volume load and cyanosis were independent determinants of increased amino-terminal procollagen type III levels in patients before Fontan surgery, and persistently increased amino-terminal procollagen type III after Fontan surgery was associated with ventricular diastolic stiffening ($r = 0.494$, $P = .009$). Data also indicated close associations between amino-terminal procollagen type III levels and activation of the renin–angiotensin–aldosterone system, suggesting potential involvement of this hormonal system in the increased fibrogenesis after Fontan surgery.

Conclusions: These results suggest that serum amino-terminal procollagen type III may provide important diagnostic information on myocardial fibrosis in patients with single ventricle circulation and raise the possibility that ventricular fibrogenesis may be a potential therapeutic target in this population. (J Thorac Cardiovasc Surg 2016;151:1518–26)

PIIIP levels are associated with ventricular diastolic dysfunction.

Central Message

Serum procollagen type III may provide useful diagnostic and therapeutic information to improve prognosis in patients undergoing the Fontan procedure.

Perspective

Increased ventricular volume load and cyanosis before the Fontan procedure are associated with increased ventricular fibrotic activity. The Fontan procedure ameliorates this process, but ongoing fibrogenesis activation in some patients undergoing the Fontan is related to ventricular diastolic dysfunction. The effects of therapies that potentially suppress ventricular fibrogenesis warrant future study.

See Editorial Commentary page 1527.

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Although the Fontan procedure has greatly contributed to the improvement of mortality rates in patients with a single ventricle (SV) circulation, adverse outcomes become increasingly prevalent with time after this procedure.^{1–3}

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Abbreviations and Acronyms

ANOVA	= analysis of variance
BNP	= brain natriuretic peptide
BTS	= Blalock–Taussig shunt
EDP	= end-diastolic pressure
PAB	= pulmonary artery banding
PIIIP	= amino-terminal procollagen type III
Qp/Qs	= pulmonary-to-systemic blood flow
RAS	= renin–angiotensin–aldosterone system
SaO ₂	= arterial oxygen saturation
SV	= single ventricle

Ventricular dysfunction is one such adverse outcome, but the precise mechanisms for progression of ventricular dysfunction after Fontan surgery remain poorly understood. Collagen deposition in the myocardium (ventricular fibrosis) is a key feature of ventricular remodeling associated with ventricular dysfunction and failure,^{4,5} and abnormal hemodynamic load and hypoxia (cyanosis) are known to be important stimuli that promote cardiac fibrosis.^{6,7} During the staged interventions toward the Fontan procedure, various degrees of ventricular volume load and cyanosis coexist and markedly change with each palliative surgical stage. Therefore, it is possible that ventricular fibrogenic activity is enhanced in patients with SV circulation and is altered with advancing the palliative stage. In addition, persistently increased ventricular fibrogenesis even after amelioration of volume load and cyanosis with the Fontan procedure may be related to ventricular dysfunction.

To test these hypotheses, we assessed ventricular fibrogenic activity by measuring serum levels of amino-terminal procollagen type III (PIIIP), a useful marker of tissue fibrogenesis that correlates with myocardial collagen type III contents.^{8–10} Specifically, we tested whether volume overload or cyanosis observed in the pre-Fontan SV circulation is associated with increased ventricular fibrogenesis in patients before the Fontan procedure, whether enhanced fibrogenesis is effectively reduced by the Fontan procedure, which ameliorates fibrogenic stimuli (volume overload and cyanosis), and whether a persistent increase in fibrogenesis in the Fontan ventricle is associated with ventricular diastolic dysfunction.

MATERIALS AND METHODS

Patients

We enrolled 172 consecutive patients aged 10 years or less with an SV circulation who underwent cardiac catheterization and 149 volunteer children with noncardiac conditions (controls) aged 10 years or less who had been admitted to affiliated local hospitals. We selected this age group to avoid the effects of the age-associated nonlinear fluctuation of PIIIP levels on the analysis, because a previous report¹¹ and our own data indicated that PIIIP levels decrease linearly with age after birth but increase

significantly later at puberty (aged ~10 years), and then decrease thereafter, reaching levels similar to those in adults by 16 years of age. Patients with SV circulation were divided into 3 groups according to their clinical stages as follows: 59 had received a first-stage repair (Blalock–Taussig shunt [BTS] or pulmonary artery banding [PAB]; BTS/PAB group), 60 had undergone a bidirectional Glenn anastomosis or hemi-Fontan surgery as a second-stage palliation (Glenn group), and 53 had undergone the Fontan procedure (Fontan group). Before the Fontan surgery, all patients underwent cardiac catheterization for preoperative evaluation of the next surgical stage (ie, patients with BTS/PAB for the Glenn and patients with the Glenn for the Fontan surgery). Catheterization was also performed in patients undergoing the Fontan for the postoperative evaluation of Fontan hemodynamics, as is standard clinical practice in Japan and in our institutions. All study subjects had undergone appropriate clinical and laboratory evaluations to exclude other conditions associated with elevated serum PIIIP levels (eg, chronic liver disease, renal failure, pulmonary fibrosis, and extensive wounds). Control group children had been admitted to local hospitals affiliated to Asahikawa Medical University for the treatment of respiratory tract infections, asthma, or epilepsy, but had fully recovered from their illness at the time of study enrolment. Written informed consent was obtained from the parents of all patients, and the procedures were approved by the Committee on Clinical Investigation of the respective institutions (Saitama Medical University No. 13-034 and No. 972, and Asahikawa Medical University No. 1706).

Hemodynamic Measurements

Pressure measurement and blood sampling for the measurement of oxygen saturation were performed during catheterization, followed by angiography/ventriculography as necessary. The ratio of the pulmonary-to-systemic blood flow (Qp/Qs) was derived from oximetric measurements by applying Fick's principle. Ventricular pressure-area loops and relationships during caval occlusion were constructed in 22 randomly selected patients in the Fontan group to examine ventricular diastolic passive stiffness. The details of this methodology have been described.²

Measurement of the Serum Amino-Terminal Procollagen Type III Levels and the Renin–Angiotensin–Aldosterone System

The serum PIIIP levels were measured from blood samples obtained from the inferior vena cava during cardiac catheterization. Blood samples were immediately centrifuged after sampling, and the serum was stored at –80°C for subsequent analysis. The serum PIIIP levels were measured by immunoradiometry using a mouse monoclonal antibody and an IRMA kit for PIIIP measurement (Riagnost P-III-P, CIS Bio International, Ceze, France).¹² The inter- and intra-assay variations were 7% and 3%, respectively. To evaluate the status of the renin–angiotensin–aldosterone system (RAS), a potentially important hormonal system for inducing cardiac fibrosis,¹³ plasma levels of renin, angiotensin II, and serum aldosterone levels were measured using commercially available radioimmunoassay kits (Renin; FUJIREBIO Inc, Tokyo, Japan; Angiotensin2 set; FUJIREBIO Inc, SPAC-S Aldosterone Kit; FUJIREBIO Inc, respectively).

Statistical Analysis

All data were expressed as mean ± standard deviation. Spearman's rank correlation coefficient was used to assess the intervariable relationships. Serum PIIIP levels, expressed as log-transformed values, were compared between patients in the SV groups and control patients by analysis of variance (ANOVA) followed by the Dunnett's post hoc multiple comparison test. Stepwise multivariate linear regression analysis was performed to test the effects of age, hemodynamic load, and cyanosis on the serum PIIIP

levels. Correlation between continuously distributed variables was examined by linear regression analysis. A nonlinear effect of Qp/Qs on PIIP levels in the BTS/PAB group was tested by the random forests method.¹⁴ All analyses were performed using a commercially available statistical software package (SPSS for Windows, Version 22.0, IBM, New York, NY), except for the random forests analysis, which was carried out with R Version 3.0.1 and the package “random Forest SRC.”

RESULTS

Table 1 summarizes the characteristics of patients in each group, including demographic data, underlying cardiac lesions, and hemodynamics. The mean age of patients in the Fontan group was greater than that of those in the BTS/PAB and Glenn groups because of the nature of staged palliations, but was comparable to that of the controls. Distribution of the underlying cardiac lesion was similar among the 3 groups with SV circulation. The BTS/PAB group showed increased pulmonary flow, as evidenced by a mean Qp/Qs ratio of 1.6, demonstrating a ventricular volume overload. The arterial oxygen saturation (Sao₂) levels in the BTS/PAB and Glenn groups were significantly lower than those in the Fontan group ($P < .05$). The mean brain natriuretic peptide (BNP) levels in the BTS/PAB, Glenn, and Fontan groups were significantly higher than those in the controls ($P < .05$), reflecting the ventricular loading condition of each stage of SV circulation.

Serum Amino-Terminal Procollagen Type III Levels

Figure 1 shows age-associated changes in serum PIIP levels in our control patients, demonstrating that PIIP levels significantly decreased with advancing age (log

PIIP = $-0.024 \times \text{age} + 0.18$, $r = 0.51$, $P < .01$). **Figure 2, A** shows PIIP levels in all 4 groups. Serum PIIP levels were significantly higher in patient groups than in control patients (0.604 ± 0.322 U/mL for BTS/PAB, 0.176 ± 0.154 U/mL for Glenn, 0.143 ± 0.104 U/mL for Fontan, and 0.073 ± 0.129 U/mL for controls, $P < .01$ for each comparison between the SV group and control group by ANOVA). The difference in the serum PIIP levels also was observed when the effects of age were taken into account by analysis of covariance ($P < .01$ for group difference). Differences in the serum PIIP levels between the BTS/PAB and Glenn groups and between the BTS/PAB and Fontan groups were significant ($P < .05$, for both). The difference in serum PIIP levels between the Glenn and Fontan groups was borderline significant ($P = .08$). In 45 patients who had serial PIIP measurements throughout the palliative stages, levels of PIIP significantly decreased after the surgical stage advanced from BTS/PAB to Fontan (**Figure 2, B**) (BTS/PAB, 0.671 ± 0.306 U/mL; Glenn, 0.261 ± 0.145 U/mL; Fontan, 0.173 ± 0.108 U/mL; $P < .01$ between each stage by repeated-measures ANOVA). This demonstrates that tissue fibrogenesis significantly decreased with the surgical stages when ventricular volume load and cyanosis improved. One might suspect that the fibrogenesis response may be different between the single right and single left ventricles, but there were no significant differences between PIIP values at any surgical stage when compared between the single right and single left ventricles.

TABLE 1. Characteristics of healthy controls and patients with single ventricle circulation

	Control	BTS/PAB	Glenn	Fontan
n	149	59	60	53
Male/female	72/77	38/21	38/22	30/23
Age (y)	4.3 (0.5-10.0)	0.6 (0.2-1.9)†	1.9 (0.9-3.7)†	4.5 (1.7-11.3)
BNP (pg/mL)	10.1 ± 13.0	114.1 ± 148.8‡	63.4 ± 98.6‡	27.1 ± 36.4*
Underlying heart diseases (n)				
Tricuspid atresia		13	11	9
PA/IVS		11	10	12
SV				
Dominant left		6	7	5
Dominant right		13	17	14
HLHS		5	5	3
Functional SV		11	10	10
Hemodynamics				
Qp/Qs		1.6 ± 0.7	—	0.9 ± 0.1
VP (mm Hg)		84.5 ± 13.0	84.7 ± 14.2	100.2 ± 15.6
mean PAP (mm Hg)		17.3 ± 7.5	12.3 ± 3.7	12.9 ± 2.5
Sao ₂ (%)		83.5 ± 5.1‡	86.6 ± 6.1‡	93.4 ± 5.3

Data are mean ± standard deviation. Functional SV included unbalanced atrioventricular septal defects and double outlet of right ventricle with remote type of ventricular defect. BTS/PAB, Blalock-Taussig shunt/pulmonary artery banding; BNP, brain natriuretic peptide; PA/IVS, pulmonary atresia with intact ventricular septum; SV, single ventricle; HLHS, hypoplastic left heart syndrome; Qp/Qs, pulmonary to systemic blood flow ratio; VP, ventricular pressure; PAP, pulmonary artery pressure; Sao₂, arterial oxygen saturation. * $P < .05$ versus control. † $P < .01$ versus control. ‡ $P < .05$ versus Fontan group.

FIGURE 1. Relationship between serum PIIP levels and age in control patients (n = 149). PIIP, Amino-terminal procollagen type III.

Determinants of the Serum Amino-Terminal Procollagen Type III Levels

To further explore whether reduced fibrotic activity is directly associated with improvement of volume load and cyanosis, univariate and multivariate analyses were performed. As shown in Table 2, univariate analysis revealed significant correlations between the serum PIIP levels with age and Qp/Qs in the BTS/PAB group and with age and Sao₂ in the Glenn group. Because the lower levels of Qp/Qs inevitably would be associated with lower Sao₂ levels in the BTS/PAB group, the effects of cyanosis may have been masked by the counteracting effects of less severe volume load with lower Sao₂. In fact, scatter plots for PIIP versus Qp/Qs (Figure 3, A) appear to show a biphasic change, with increased PIIP levels at lower levels of Qp/Qs (<1). To test this possibility, we performed a nonparametric regression analysis using the random forests method, which is capable of accounting for a nonlinear effect of variables. As shown in Figure 3, B, Qp/Qs had a

significant effect on PIIP levels, after accounting for age. In addition, PIIP levels showed a U-shaped change according to the Qp/Qs values, with the lowest PIIP levels observed when the Qp/Qs was approximately 1.0 (0.87), suggesting the potential importance of cyanosis in the elevation of PIIP in patients with BTS/PAB and low pulmonary flow. In the patients undergoing the Fontan, Sao₂ levels did not have a statistically significant correlation with PIIP levels.

To further test whether the marked reduction in serum PIIP levels between the BTS/PAB and Fontan groups was biologically relevant, we examined determinants of the altered PIIP levels (Δ PIIP: PIIP value after Fontan surgery – PIIP value after BTS/PAB) by multivariate analysis. Changes in Qp/Qs, Sao₂, and age (Δ Qp/Qs, Δ Sao₂, and Δ age: value of each variable after Fontan surgery – that after BTS/PAB) and baseline PIIP values were included as independent variables. We found that Δ Qp/Qs and baseline PIIP were significant determinants of Δ PIIP between BTS and Fontan, with adjustment for Δ age ($R = 0.89$, $P < .01$). Furthermore, Δ PIIP was significantly correlated with Δ BNP (BNP value after Fontan – BNP value after BTS/PAB), a biomarker for cardiac load ($R = 0.61$, $P < .01$).

Serum Amino-Terminal Procollagen Type III Levels in the Fontan Circulation

To test whether increased fibrogenesis in the Fontan circulation is associated with ventricular diastolic dysfunction, the correlation between PIIP and ventricular diastolic function was examined. As shown in Figure 4, A, there was a significant correlation between serum PIIP levels and end-diastolic pressure (EDP) in the Fontan group ($\log \text{PIIP} = 0.017 \times \text{EDP} - 0.024$;

FIGURE 2. A, Serum PIIP levels for each group. Bars represent the mean values. B, Serial changes in PIIP levels in the BTS/PAB, Glenn, and Fontan groups. † $P < .05$ versus controls (right shoulder) and between the 2 groups stratified according to age (above the bars). PIIP, Amino-terminal procollagen type III; BTS/PAB, blalock–Taussig shunt/pulmonary artery banding.

TABLE 2. Univariate and multivariate regression analyses for determinants of serum amino-terminal procollagen type III levels

	BTS/PAB				Glenn				Fontan			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	<i>r</i>	<i>P</i> value	SE	<i>P</i> value	<i>r</i>	<i>P</i> value	SE	<i>P</i> value	<i>r</i>	<i>P</i> value	SE	<i>P</i> value
Age	−0.51	<.001*	−0.401	.002*	−0.529	<.001*	−0.371	.009*	−0.204	.074	−0.24	.544
Qp/Qs	0.508	<.001*	0.399	.002*	—	—	—	—	−0.121	.196	−0.548	.276
VP (mm Hg)	0.158	.141	0.158	.086	−0.196	.104	−0.037	.774	−0.127	.184	−0.477	.296
mean PAP (mm Hg)	0.173	.120	0.173	.867	0.081	.302	0.185	.153	−0.096	.249	0.202	.534
Sao ₂ (%)	0.105	.240	0.105	.921	−0.485	<.001*	−0.385	.007*	−0.136	.169	0.766	.129

BTS/PAB, Blalock–Taussig shunt/pulmonary artery banding; SE, standard error; Qp/Qs, pulmonary to systemic blood flow ratio; VP, ventricular pressure; PAP, pulmonary artery pressure; Sao₂, arterial oxygen saturation. **P* < .01.

R = 0.482, *P* = .004). EDP is an indicator of ventricular dysfunction. A significant relationship between serum PIIP levels and ventricular stiffness also was observed ($\log \text{PIIP} = 0.253 \times \beta + 0.030$; *R* = 0.494, *P* = .009) (Figure 4, B). These relationships also held true after controlling for the age of the patients (*P* < .05). Patients undergoing the Fontan randomly selected for this particular analysis had demographic and hemodynamic characteristics that were similar to those of the remaining Fontan cases (Table 3); thus, this randomly selected group of patients was representative of the whole Fontan group.

To further elucidate the pathologic significance and prognostic value of PIIP, we examined whether pre-Fontan PIIP levels prognosticated post-Fontan diastolic dysfunction. As shown in Figure 5, the pre-Fontan PIIP values were significantly correlated with levels of post-Fontan EDP ($\log \text{PIIP} = 0.013 \times \text{EDP} + 0.155$; *R* = 0.430, *P* = .040). This correlation persisted even when cardiopulmonary bypass time for the Fontan surgery (133 ± 34 minutes), a potentially important factor affecting

postoperative ventricular function, was taken into account by multivariate analysis (*P* = .036 for PIIP and *P* = .693 for bypass time).

Renin–Angiotensin–Aldosterone System Activation and Amino-Terminal Procollagen Type III in the Fontan Circulation

The relationship between serum PIIP levels and RAS activation in the Fontan group is shown in Figure 4, C–E. All RAS hormones positively correlated with levels of serum PIIP (renin: *R* = 0.429, *P* = .008; angiotensin II: *R* = 0.489, *P* = .002; aldosterone: *R* = 0.473, *P* = .003).

DISCUSSION

The present study provides the first evidence of increased serum levels of PIIP, a marker of tissue fibrosis, in children with SV circulation before and even after Fontan surgery. The PIIP levels decreased with advancing surgical stages as ventricular volume load and cyanosis were alleviated. Indeed, both severity of volume load and cyanosis were important determinants of the increased PIIP levels in

FIGURE 3. A, The relationship between serum PIIP levels and Qp/Qs in patients in the BTS or PAB group. B, Expected mean PIIP levels (black dotted line) and 95% confidence interval of mean PIIP levels (red dotted line) as a function of Qp/Qs based on random forests regression analysis. PIIP, Amino-terminal procollagen type III; Qp/Qs, pulmonary-to-systemic blood flow.

FIGURE 4. Relationship between PIIIP levels and EDP ($n = 32$) (A) and ventricular stiffness ($n = 22$) (B) in the Fontan group. Relationship between PIIIP levels and RAS in the Fontan group (C-E). PIIIP, Amino-terminal procollagen type III; EDP, end-diastolic pressure.

patients after BTS or PAB, and the severity of cyanosis was the determining factor of the increased PIIIP in patients with a Glenn circulation when ventricular volume load was largely eliminated. In addition, persistently increased PIIIP even after Fontan surgery was associated with ventricular diastolic stiffening, suggesting the potential importance of ventricular fibrosis in the development of ventricular diastolic dysfunction in patients after Fontan surgery. Data also suggested the potential involvement of RAS activation in the increased fibrogenesis after Fontan surgery. These results not only suggest that serum PIIIP may provide important diagnostic information on myocardial fibrosis but also raise the possibility that ventricular fibrogenesis may be a potential therapeutic target in patients with SV circulation.

Amino-Terminal Procollagen Type III in Single Ventricle Circulation

It has been demonstrated that the serum levels of PIIIP correlate significantly with myocardial collagen type III contents.^{15,16} Indeed, serum PIIIP levels are significantly elevated in pathologic conditions associated with enhanced myocardial collagen deposition (eg, cardiomyopathy,^{15,17} myocardial infarction,¹⁸ and hypertension¹⁹) and are decreased in response to therapies that

potentially inhibit cardiac fibrosis.^{17,20,21} In addition, several experimental studies have shown that abnormal hemodynamic loading,²² hypoxemia, and neurohumoral activation²³⁻²⁵ are known to stimulate collagen synthesis, thereby promoting myocardial fibrosis.^{6,26-30} Taken together with the present results, the increased serum PIIIP levels and their close association with hemodynamic load and hypoxia observed in this study strongly suggest increased ventricular fibrosis in patients with SV circulation, and thus serum PIIIP may offer a useful tool for the diagnosis of ventricular fibrotic activity in this population. The present results in patients with SV circulation were consistent with our previous study in simple forms of congenital heart disease, which demonstrated increased serum PIIIP levels in proportion to the magnitude of hemodynamic load or hypoxemia.⁹ Therefore, an increased hemodynamic burden and cyanosis appear to be a common pathway to promote ventricular fibrosis regardless of the type or anatomy of congenital heart disease.

In the BTS/PAB group, both ventricular volume load and cyanosis appeared to be important determinants of fibrotic tissue activity. Of note, PIIIP levels showed a U-shaped change according to the Qp/Qs values, with minimum PIIIP levels observed when Qp/Qs was 0.87 (Figure 3, A and B).

TABLE 3. Characteristics of Fontan cases with and without stiffness assessment

	P/V loop examination	Without P/V loop examination	P value
n	22	31	
M/F	12/10	18/13	
Age (y)	3.3 (1.7-10.0)	3.8 (2.1-11.3)	
BNP (pg/mL)	20.5 ± 4.0	27.7 ± 4.0	.301
Renin (pg/mL)	14.6 ± 1.4	25.6 ± 2.2	.063
Log angiotensin II (pg/mL)	1.2 ± 0.8	1.3 ± 0.5	.448
Log aldosterone (pg/mL)	2.4 ± 1.4	2.6 ± 0.6	.131
Underlying heart diseases (n)			
Tricuspid atresia	5	4	
PA/IVS	2	10	
SV			
Dominant left	2	3	
Dominant right	8	6	
HLHS	0	3	
Functional SV	5	5	
Hemodynamics			
Qp/Qs	0.76 ± 0.11	0.82 ± 0.17	.623
VP (mm Hg)	85.7 ± 10.3	92.5 ± 10.6	.973
mean PAP (mm Hg)	12.3 ± 8.0	12.4 ± 3.3	.697
Sao ₂ (%)	85.0 ± 6.3	87.8 ± 5.6	.779

Data are mean ± standard deviation. P/V, Pressure/volume; BNP, brain natriuretic peptide; PA/IVS, pulmonary artery/intact ventricular septum; SV, single ventricle; HLHS, hypoplastic left heart syndrome; Qp/Qs, pulmonary-to-systemic blood flow; VP, ventricular pressure; PAP, pulmonary artery pressure; Sao₂, arterial oxygen saturation.

Thus, Qp/Qs around this value appears to be an optimal shunt ratio when creating BTS or PAB in view of minimizing ventricular fibrosis due to coexisting ventricular volume load and cyanosis.

The optimal timing for Fontan completion has become earlier than originally suggested. This is based partly on the assumption that earlier volume unloading is beneficial to preserve ventricular function and thereby reduce late

complications.^{31,32} The present results indicating a progressive decline in PIIP levels with advancing palliative stages provide a supportive rationale for the prevailing strategy of earlier Fontan completion from the viewpoint of ventricular fibrosis. Conversely, earlier Fontan completion inevitably means that patients must experience a longer duration of elevated venous pressure. Because high venous pressure also is a main cause of late complications after the Fontan operation,³³ the optimal timing for Fontan completion should be determined with careful consideration of these conflicting effects. Serum PIIP levels at least may provide useful information about the ventricular structure to help determine the timing of the Fontan surgery.

The results of the present study indicating increased tissue fibrogenesis are consistent with several previous studies demonstrating myocardial fibrosis in patients with SV circulation. Histopathologic studies of tricuspid atresia after various types of palliative surgery,³⁴ including BT shunt, Glenn procedure, and Fontan surgery, reported more fibrotic left ventricle than age-matched normal hearts. Likewise, Padalino and colleagues⁷ reported that specimens obtained from patients after the Norwood procedure for hypoplastic left heart syndrome⁷ exhibited increased ventricular fibrosis compared with age- and weight-matched normal hearts. A study using magnetic resonance imaging with late gadolinium enhancement also demonstrated myocardial fibrosis in approximately one third of patients undergoing the Fontan.³⁵ No study has been performed in the population with congenital heart disease to directly compare serum PIIP levels with cardiac magnetic resonance imaging or endomyocardial biopsy. Such studies need to be conducted in the future to further clarify the pathophysiologic meaning of serum PIIP levels.

Amino-Terminal Procollagen Type III and Fontan Circulation

Our data also demonstrated that PIIP levels persistently increased even after the Fontan surgery when volume overload and cyanosis largely had been eliminated. It is well known that ventricular fibrosis is an important pathophysiology underlying ventricular diastolic dysfunction.^{25,36,37} Indeed, several clinical studies have described a close correlation between serum levels of PIIP and diastolic dysfunction in adults.³⁸ Consistent with these experimental and clinical data, we found a significant correlation between increased PIIP levels and ventricular diastolic stiffening with EDP elevation in patients with Fontan circulation (Figure 4, A and B). Because ventricular diastolic function may deteriorate with time after Fontan completion,³⁹ preventing ventricular fibrosis may offer a way to improve the prognosis of patients after Fontan surgery. In this sense, the relationship between PIIP and RAS activation (Figure 4, C-E) may suggest the potential efficacy of

FIGURE 5. Relationship between pre-Fontan PIIP levels and post-Fontan ventricular EDP. PIIP, Amino-terminal procollagen type III; EDP, end-diastolic pressure.

RAS inhibition to prevent ventricular fibrosis after the Fontan surgery. This notion is supported by numerous basic and clinical studies demonstrating the important role of the RAS in the development of myocardial fibrosis and the efficacy of RAS inhibition/blockade for the suppression of myocardial collagen synthesis.¹⁹⁻²¹ Effects of pharmacologic blockade of RAS on serum PIIIP levels, ventricular fibrosis, diastolic function, and prognosis in patients after Fontan surgery warrant further investigation.

Study Limitations

Ventricular pressure-area analysis to assess ventricular stiffness was performed in only a limited number of patients, although such patients appeared to represent the characteristics of the entire Fontan group (Table 2). Another limitation is the heterogeneous nature of each of the staged subcohorts. Although a significant association between PIIIP values and changes in cardiac load (Qp/Qs), as well as BNP, would support the biological relevance of the observed PIIIP levels, lack of cardiac tissue pathology data directly linking serum PIIIP levels is an important limitation of this study. In this sense, examining PIIIP levels in patients before BTS/PAB would be helpful to further test the biological relevance of serum PIIIP levels. Although we have only limited data for such patients, the average PIIIP value in the 3 patients who had PIIIP measurements before BTS/PAB was 0.627 ± 0.358 U/mL, which was slightly higher than in the BTS/PAB group (0.604 ± 0.322 U/mL). Two of these patients had serial data; PIIIP decreased from 0.74 to 0.49 U/mL in 1 patient who underwent PAB and from 0.20 to 0.17 U/mL in the other patient who underwent BTS. Further accumulation of data is needed. Finally, despite statistically significant differences, there was considerable overlap between PIIIP values among the groups, and the range of PIIIP levels that correlated with diastolic dysfunction was relatively small. Therefore, it is clear that further studies are needed to establish the clinical usefulness of PIIIP as a diagnostic and prognostic marker for quantitatively determining ventricular fibrotic activity. Development of highly sensitive measurement of PIIIP may be helpful.

CONCLUSIONS

Increased ventricular volume load and cyanosis before the Fontan procedure are likely to be associated with increased ventricular fibrotic activity. The Fontan procedure ameliorates this process, but there is still ongoing fibrogenesis activation that is related to increased ventricular diastolic stiffening and elevated EDP in some patients. The long-term outcomes of increased fibrogenesis and the effects of therapies that potentially suppress this process warrant future study.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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