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

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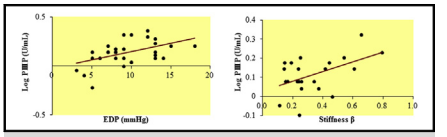
ABSTRACT

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.

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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 report[11] 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