Abnormalities in blood coagulation, fibrinolysis, and platelet activation in adult patients after the Fontan procedure

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Background: Thrombosis occurs in up to 30% of patients with various forms of congenital single ventricle after the Fontan procedure. We investigated hemostatic abnormalities in adult Fontan patients.

Methods: Forty-eight Fontan patients between ages 18 and 40 years, including 10 (21%) patients with previous thromboembolism 5 to 15 years after surgery, and 35 control subjects matched for age and sex were studied. Coagulation factors and inhibitors, together with markers of fibrinolysis, platelets, and endothelial activation, were determined in peripheral venous blood plasma.

Results: Compared with control subjects, Fontan patients showed lower, although mostly within normal ranges, values of all coagulation factors, as well as reduced free protein S, in association with higher antithrombin and free tissue factor pathway inhibitor levels. Thrombin generation, reflected by prothrombin fragment 1.2, and platelet activation markers were increased in Fontan patients. The plasma clot lysis time was prolonged in Fontan patients, which was associated with an increased activity of thrombin-activatable fibrinolysis inhibitor. Fontan patients with previous thromboembolism had lower oxygen saturation, coagulation factors V and VIII, and free protein S, and increased von Willebrand factor, soluble CD40 ligand, and P-selectin. Other laboratory or clinical parameters were not associated with prior thrombotic episodes.

Conclusions: Adult Fontan patients are characterized by enhanced platelet activation and endothelial injury, heightened thrombin formation, and impaired fibrinolysis. Patients showed reduced free protein S levels, increased platelet activation, and endothelial damage after thromboembolic events observed late after Fontan surgery. Our findings indicate novel prothrombotic mechanisms in adult Fontan patients, which might help to optimize thromboprophylaxis. (J Thorac Cardiovasc Surg 2014;147:1284-90)

Fontan surgery has become the treatment of choice in patients with single-ventricle physiology. The purpose of this procedure is to normalize volume load of the ventricle and separate the pulmonary and systemic circulation to achieve normal or near-normal levels of blood oxygen. Several modifications of the Fontan surgery can provide excellent palliation for patients with various forms of single ventricles.¹ Long-term follow-up evaluation of Fontan patients showed an 85% survival rate.^{2,3} The longer the time since the procedure, the more frequently remote complications occur. In addition, thrombosis occurs in 8% to 33% of Fontan patients.²⁻⁵ Thrombi can be detected by

echocardiography in the systemic venous pathway, systemic ventricle, or ligated pulmonary artery. Asymptomatic pulmonary emboli have been reported in 17% of Fontan patients.⁶ Thrombosis risk factors reported in Fontan patients involve arrhythmias, slow venous flow, increased central venous pressure, cyanosis, ventricular dysfunction, liver injury, and effusion protein-losing enteropathy.^{5,7} A high prevalence of chronic venous insufficiency in adult Fontan patients also has been reported.⁸

Studies on alterations of blood coagulation after Fontan surgery conducted in children showed lower plasma levels of coagulation factor (F) V, VII, and VIII, along with low protein C, free protein S, and plasminogen levels.^{5,9-12} Mechanisms underlying thromboembolism in Fontan patients are unclear. Odegard et al¹³ showed that an increase in FVIII after Fontan surgery and a decrease in protein C levels were associated with a high risk of thrombosis in 37 patients (age, ≤ 49 mo) with hypoplastic left heart syndrome from stage I through Fontan completion. In Fontan patients ages 7 to 26 years, increased von Willebrand factor (vWF) levels have been observed, suggesting endothelial damage.¹⁴ It has been reported that efficiency of fibrinolysis is preserved in those patients based on normal plasma tissue-type plasminogen activator and plasminogen activator inhibitor-1 (PAI-1). We performed a comprehensive analysis

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Abbreviations and Acronyms

CLT	= clot lysis time
ELISA	= enzyme-linked immunosorbent assay
F	= factor
PAI-1	= plasminogen activator inhibitor-1
Sao ₂	= oxygen saturation
TAFI	= thrombin activatable fibrinolysis inhibitor
TFPI	= tissue factor pathway inhibitor
vWF	= von Willebrand factor

of blood coagulation, fibrinolysis, and platelet activation in adult Fontan patients. We investigated hemostatic abnormalities observed in adult Fontan patients to identify those associated with thromboembolism after the procedure.

PATIENTS AND METHODS

Study Participants

Forty-eight white Fontan patients (26 men, 22 women) ages 18 to 40 years and 35 apparently healthy control subjects matched for age and sex were included in the study. They were recruited between January 2011 and December 2011. The exclusion criteria were as follows: any acute illness, cancer, renal insufficiency (creatinine level >120 μ mol/L), diabetes mellitus, an acute vascular event, alcohol abuse, or pregnancy.

Echocardiography was performed in all the patients and single-ventricle systolic function was assessed.¹⁰ Oxygen saturation (SaO₂) was measured by pulse oximetry.

The diagnosis of deep vein thrombosis was established by a positive finding of color duplex sonography. The diagnosis of pulmonary embolism was based on the presence of typical symptoms and positive results of high-resolution spiral computed tomography. Stroke was diagnosed based on the World Health Organization criteria. In Fontan patients with previous thromboembolic events, thrombophilia screening including FV Leiden, 20210A prothrombin mutation, deficiencies in natural anticoagulants and antiphospholipid antibodies was performed.

The University Ethical Committee approved the study and patients provided written informed consent.

Laboratory Investigations

Fasting blood samples were collected into 0.1 volume of 3.2% trisodium citrate from the antecubital vein with minimal stasis on the same day that clinical data were recorded. In anticoagulated patients, blood was drawn at least 5 days after anticoagulation withdrawal. Citrated blood samples were centrifuged at 3000 g for 20 minutes and stored in aliquots at -80°C until further use. Red blood cell count, white blood cell count, platelet count, hematocrit level, total protein level, alanine aminotransferase level, creatinine level, C-reactive protein level, and international normalized ratio were assayed by routine laboratory techniques.

Coagulation Proteins

Fibrinogen was determined using the thrombin clotting time method. Prothrombin and coagulation factors (F)V, FVII, FVIII, FIX, and FX were measured by 1-stage clotting assays using factor-deficient plasmas (Siemens, Marburg, Germany). Antithrombin activity was measured using Berichrom (Siemens). Free (ie, active) tissue factor pathway inhibitor (free TFPI) was determined by an enzyme-linked immunosorbent assay (ELISA) (Diagnostica Stago, Asnieres, France). Protein C activity was measured using a chromogenic substrate assay (Siemens). Free protein S was determined using a latex ligand immunoassay (Instrumentation

Laboratory, Milan, Italy). The immunoenzymatic assay was used to determine plasma prothrombin fragments 1.2 (F1.2; Siemens).

Fibrinolytic Proteins

Plasma antiplasmin and plasminogen were measured by chromogenic assays (STA Stachrom 2 antiplasmin and STA Stachrom plasminogen; Diagnostica Stago). Plasma PAI-1 antigen levels were measured by an ELISA (American Diagnostica, Greenwich, Conn). Thrombin activatable fibrinolysis inhibitor (TAFI) antigen was determined with an ELISA (Chromogenix, Lexington, Mass). Plasma TAFI activity was measured by a chromogenic assay using the Actichrome Plasma TAFI Activity Kit (American Diagnostica). Soluble thrombomodulin was measured by an ELISA (Diagnostica Stago).

Fibrin clot lysis time (CLT) was measured as described.¹⁵ Briefly, citrated plasma was mixed with 15 mmol/L calcium chloride, 10,000-diluted human tissue factor (Innovin; Dade Behring), 12 μ mol/L phospholipid vesicles, and 60 ng/mL recombinant tissue-type plasminogen activator (Boehringer Ingelheim, Ingelheim, Germany). Turbidity of this mixture was measured at 405 nm at 37°C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents clot formation, to the midpoint of the maximum-turbid-to-clear transition, representing the lysis of a clot.

Platelet and Endothelial Cell Activation

vWF antigen (Diagnostica Stago), and 2 platelet activation markers, that is, soluble CD40 ligand (sCD40L; R&D Systems, Indianapolis, Ind), and P-selectin (R&D Systems) also were measured in plasma.

All the hemostatic measurements were performed by technicians blinded to the sample status. The coefficients of intra-assay and interassay variations were less than 9%.

Statistical Analysis

Data are expressed as the mean $\pm$ SD for normally distributed variables or as the median (interquartile range) for non-normally distributed variables. The Kolmogorov-Smirnov test was used to assess conformity with a normal distribution. Categorical values were analyzed using the χ^2 test. Continuous variables were compared by the Student *t* test when distributed normally or by the Mann-Whitney *U* test for non-normally distributed variables. Correlations between the individual parameters were calculated using the Pearson or Spearman rank correlation as appropriate. All clinical and laboratory variables that showed an association with thrombosis in the univariate model ($P < .05$) and did not show substantial correlations ($r \geq 0.4$) with another independent variable were included in the stepwise multiple logistic regression analysis. The statistical analyses were performed with the Statistica 9.0 software (StatSoft, Tulsa, Okla).

The study was powered to have a 90% chance of detecting a 10% difference in vWF using a *P* value of .001, based on the values of vWF in the previous article.¹⁴ To show a difference of this magnitude or greater, 28 patients were required in each group.

RESULTS

The majority of patients had the left ventricular type of single ventricle and they were in New York Heart Association class I or II (Table 1). The Fontan patients had a lower white blood cell and platelet counts, and a higher red blood cell count, hemoglobin level, alanine aminotransferase level, creatinine level, and international normalized ratio compared with the controls (Table 2).

Coagulation Factors and Inhibitors

Compared with the controls, Fontan patients had lower values (from 19% to 35%) of all coagulation factors and

TABLE 1. Patient characteristics

Variable	Fontan patients (n = 48)
Age at Fontan surgery (y)	5 (1-17)
Postoperative time (y)	18 (3-30)
Anatomic diagnosis, n (%)	
Tricuspid atresia	20 (42)
VSD, pulmonary atresia	9 (18.5)
Hypoplasia of right ventricle	10 (21)
Double-outlet right ventricle with hypoplastic left ventricle	9 (18.5)
Ventricle type, n (%)	
Right ventricle	9 (19)
Left ventricle	39 (81)
Type of Fontan, n (%)	
Atriopulmonary connection	16 (33.3)
Total cavopulmonary connection	29 (60.4)
Modified Fontan	3 (6.3)
Fenestration, n (%)	11 (23)
Systemic ventricle function, n (%)	
Good	25 (52)
Fair	16 (33)
Decreased	5 (11)
Poor	2 (4)
NYHA functional class, n (%)	
I	14 (29)
II	32 (67)
III	2 (4)
IV	0
Cardiac rhythm, n (%)	
Sinus rhythm	42 (88)
Atrial fibrillation	4 (8)
Pacemaker	1 (2)
Medication use, n (%)	
Warfarin	10 (21)
Aspirin	16 (33)
Prednisone	3 (6)
Mean oxygen SaO ₂ on room air (%)	92 (90-97)

Data are presented as median (interquartile range) or number (%). VSD, Ventricular septal defect; NYHA, New York Heart Association; SaO₂, oxygen saturation.

free protein S, however, most of the values were within normal ranges (Table 3). Interestingly, the patients had higher antithrombin and free TFPI levels, whereas the protein C level was similar in both groups. SaO₂ correlated positively with prothrombin ($r = 0.38$; $P = .009$), FV ($r = 0.49$; $P = .001$), FVIII ($r = 0.32$; $P = .03$), and FX ($r = 0.31$; $P = .03$), and free protein S ($r = -0.4$; $P = .005$). Compared with the controls, Fontan patients showed higher thrombin generation, reflected by increased prothrombin fragments F1.2. There was a positive correlation between F1.2 and age ($r = 0.32$; $P = .03$), but not with SaO₂ or clinical variables (data not shown).

Fibrinolysis

The Fontan patients had higher PAI-1 antigen levels than the controls, however PAI activity was similar in both

TABLE 2. Comparison of Fontan patients and controls

Variable	Controls (n = 36)	Fontan patients (n = 48)	P value
Age at enrollment (y)	27 (19-40)	25 (18-40)	.06
Male, n (%)	19 (57)	26 (54)	.23
BMI (kg/m ²)	22 ± 3	22 ± 3	.43
WBC (10 ³ /μL)	6.1 ± 1.4	5.5 ± 1.6	.023
RBC (10 ⁹ /μL)	4.9 [0.3]	5.2 [0.9]	.001
Hematocrit (%)	46 ± 3	46 ± 3	.84
Platelet count (10 ³ /μL)	226 ± 36	148 ± 48	<.001
ALT (IU/L)	24 ± 9	29 ± 12	.016
Total protein (g/dL)	73 [5]	74 [11]	.36
Creatinine (μmol/L)	69 ± 11	74 ± 15	<.001
CRP (mg/L)	1.6 [1.6]	1.6 [3.7]	.99
INR	0.97 [0.2]	1.1 [0.2]	.003

Data are presented as mean ± SD, median [interquartile range], or number (%). BMI, Body mass index; WBC, white blood cell; RBC, red blood cell; ALT, alanine aminotransferase; CRP, C-reactive protein; INR, international normalized ratio.

groups (Table 3). Interestingly, TAFI activity was higher by 24% ($P < .001$) in the patients despite comparable levels of TAFI antigen (Table 3). A global fibrinolysis marker, CLT, was 16% ($P < .001$) longer in the Fontan patients than in the controls.

CLT in the Fontan patients showed positive associations with PAI-1 antigen ($r = 0.71$; $P < .001$), TAFI activity ($r = 0.69$; $P < .001$), and prothrombin fragments F1.2 ($r = 0.74$; $P < .001$). TAFI activity correlated with PAI-1 activity ($r = -0.6$; $P < .001$), PAI-1 antigen ($r = 0.58$; $P < .001$), and F1.2 ($r = 0.41$; $P = .004$).

Platelet and Endothelial Activation Markers

sP-selectin and sCD40L were higher by 92% and 25%, respectively, in Fontan patients. We also found vWF to be 23% higher in Fontan patients (Table 3). There were inverse correlations between sP-selectin and SaO₂ ($r = -0.52$; $P < .001$), prothrombin ($r = -0.35$; $P = .01$), FV ($r = -0.4$; $P = .005$), FVIII ($r = -0.33$; $P = .02$), FIX ($r = -0.37$; $P = .01$), FX ($r = -0.38$; $P = .009$), and free protein S ($r = -0.48$; $P = .001$).

None of the clinical characteristics in the Fontan patients showed associations with fibrinolytic, platelet, or endothelial markers (data not shown).

Fontan Patients With Thrombosis

Previous arterial or venous thromboembolism occurred in 10 (21%) patients 5 to 15 years (median, 12 y) after the Fontan surgery. Three patients developed thrombi in the Fontan circulation, 4 patients had previous deep vein thrombosis, 1 patient had an isolated pulmonary embolism, and, finally, 2 patients had both previous ischemic stroke and deep vein thrombosis. Only 1 patient with a prior thromboembolism had thrombophilia (ie, heterozygous FV Leiden mutation).

TABLE 3. Coagulation factors/inhibitors, fibrinolysis variables, and platelet and endothelial activation markers in Fontan patients and controls

Variable	Controls (n = 36)	Fontan patients (n = 48)	P value
Coagulation factors and inhibitors			
FII (%)	109 ± 16	87 ± 17	<.001
FV (%)	104 ± 15	73 ± 24	<.001
FVII (%)	102 [14]	82 [18]	<.001
FVIII (%)	132 ± 28	108 ± 29	.001
FIX (%)	114 ± 20	75 ± 20	<.001
FX (%)	115 ± 21	87 ± 24	<.001
Fibrinogen (g/L)	3.2 ± 1.3	2.4 ± 0.5	.005
Prothrombin fragments F1.2 (pmol/L)	185 [31]	263 [74]	<.001
Antithrombin (%)	99 ± 14	109 ± 13	.001
Free TFPI (ng/mL)	17 [4]	25 [9]	<.001
Protein C (%)	105 [18]	103 [23]	.42
Free protein S (%)	95 [13]	85 [13]	<.001
Fibrinolysis variables			
α ₂ AP (%)	102 ± 9	100 ± 12	.29
Plasminogen (%)	102 ± 10	104 ± 12	.67
PAI-1 activity (IU/mL)	9 ± 1.1	9 ± 3	.28
PAI-1 antigen (ng/mL)	14 ± 4	26 ± 6	<.001
TAFI activity (μg/mL)	25 ± 4	31 ± 8	<.001
TAFI antigen (%)	106 ± 14	104 ± 11	.72
CLT (min)	80 ± 12	93 ± 14	<.001
Platelet and endothelial activation markers			
vWF (%)	122 ± 20	150 ± 28	<.001
sP-selectin (ng/mL)	142 ± 29	273 ± 69	<.001
sCD40L (pg/mL)	361 ± 76	450 ± 156	.006
TM (ng/mL)	3.9 [2.2]	3.0 [2.9]	.1

Data are presented as mean ± SD or median [interquartile range]. α₂AP, Antiplasmin; CLT, clot lysis time; sCD40L, soluble CD40 ligand; TM, thrombomodulin; F, factor; TFPI, free tissue factor pathway inhibitor; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin activatable fibrinolysis inhibitor; vWF, von Willebrand factor; sP-selectin, soluble P-selectin.

The Fontan patients with a history of thrombosis had a lower SaO₂. Moreover, lower values of FV, FVIII, and free protein S were observed. Interestingly, increased vWF, sCD40L, and P-selectin were found in these patients (Table 4). Other clinical and hemostatic parameters were not affected by a history of thrombosis. The multivariate model showed that free protein S and sCD40L were the only independent predictors of thromboembolic events late after Fontan surgery ($\chi^2 = 30.7$, $P < .001$; Table 5).

DISCUSSION

We present a comprehensive evaluation of blood coagulation, fibrinolysis, platelet, and endothelial cell activation in adult patients who underwent the Fontan procedure in their childhood or adolescence. The major findings of our study were uniformly decreased coagulation factors in adult Fontan patients with a paradoxically increased thrombin generation in the circulating blood, probably owing to enhanced platelet activation and endothelial injury coupled with a slightly lower inhibition of thrombin formation by reduced free protein S.

Our study clearly indicates that there are complex hemostatic disturbances in adult Fontan patients. On the one hand, they seem to favor bleeding by reduction of coagulation factor production, and on the other hand they

promote thrombosis through reduced protein S, hypofibrinolysis, impaired endothelial function, and increased platelet activation. However, the net effect of all the hemostatic disturbances in Fontan patients tilts the balance toward prothrombotic tendency.

Of note, we observed different patterns of hemostatic alterations in adult Fontan patients as compared with those reported previously in children mostly in the early postoperative period. In adult patients late after the Fontan surgery, we found significant reductions in coagulation factors including FVIII (all the individual alterations were mild and did not exceed the normal ranges). However, bleeding is fairly uncommon in Fontan patients, which suggests that despite a lower platelet count and coagulation factors, other potent mechanisms can overcome these alterations, leading to a prothrombotic state as evidenced by heightened thrombin generation and reflected by increased F1.2 levels. Interestingly, there were significantly increased levels of 2 coagulation inhibitors, liver-derived antithrombin and endothelium-derived free TFPI, in the Fontan patients as compared with controls. It might be hypothesized that this phenomenon is a compensatory mechanism aiming at restoring the hemostatic balance when larger amounts of thrombin are generated.

Of note, our study shows an impaired fibrinolytic potential in adult Fontan patients and hypofibrinolysis is

TABLE 4. Comparison between Fontan patients without and with thrombosis

Variable	Patients without thrombosis (n = 38)	Patients with thrombosis (n = 10)	P value
Age at enrollment, (y)	24 (18-40)	27 (18-36)	.18
Age at surgery, (y)	6.9 (1-16)	5.6 (2-17)	.19
Postoperative time, (y)	17 (3-30)	21 (14-27)	.09
Male, n (%)	20 (53)	6 (60)	.68
Anatomic diagnosis, n (%)			.2
Tricuspid atesia	16 (42)	4 (40)	
VSD, pulmonary atesia	6 (16)	4 (40)	
Hypoplasia of right ventricle	7 (18)	2 (20)	
Double-outlet right ventricle with hypoplasia of left ventricle	9 (24)	0 (0)	
Ventricle type, n (%)			.07
Right ventricle	9 (24)	0 (0)	
Left ventricle	29 (76)	10 (100)	
Type of Fontan, n (%)			.2
Atriopulmonary connection	16 (42)	0 (0)	
Total cavopulmonary connection	19 (50)	10 (100)	
Modified Fontan	3 (8)	0 (0)	
Systemic ventricle function, n (%)			.13
Good	21 (55)	4 (40)	
Fair	13 (34)	3 (30)	
Decreased	2 (6)	3 (30)	
Poor	2 (5)	0 (0)	
NYHA functional class, n (%)			.49
I	12 (31)	2 (20)	
II	25 (66)	7 (70)	
III	1 (3)	1 (10)	
IV	0	0	
SaO ₂ (%)	92 [6]	84 [8]	.003
Platelet count (10 ³ /μL)	142 [49]	152 [44]	.79
Hematocrit (%)	44 ± 7	49 ± 3	.07
Prothrombin (%)	89 ± 15	79 ± 22	.1
FV (%)	82 [25]	61 [20]	.058
FVII (%)	82 [19]	80 [15]	.64
FVIII (%)	113 [28]	82 [26]	.03
FIX (%)	75 [20]	67 [22]	.23
FX (%)	89 ± 25	80 ± 18	.28
Antithrombin (%)	110 ± 12	105 ± 15	.22
Protein C (%)	104 ± 26	102 ± 28	.85
Free protein S (%)	89 ± 10	70 ± 9	<.001
PAI-1 antigen (ng/mL)	26 ± 6	25 ± 6	.67
PAI-1 activity (IU/mL)	9 ± 3	9 ± 3	.65
TAFI antigen (%)	104 ± 11	104 ± 12	.93
TAFI activity (ug/mL)	31 ± 8	32 ± 9	.69
CLT (min)	91 [15]	89 [12]	.34
Prothrombin fragments F1.2 (pmol/L)	261 [76]	275 [73]	.88
Plasminogen (%)	104 ± 11	100 ± 15	.3
α ₂ AP (%)	99 ± 12	103 ± 11	.45
vWF (%)	144 ± 23	174 ± 33	.002
sP-selectin (ng/mL)	251 ± 55	353 ± 18	<.001
sCD40L (pg/mL)	417 ± 134	579 ± 173	.003

Data are presented as mean ± SD, median [interquartile range], or number (%). VSD, Ventricular septal defect; NYHA, New York Heart Association; TAFI, thrombin activatable fibrinolysis inhibitor; α₂AP, antiplasmin; sCD40L, soluble CD40 ligand; SaO₂, oxygen saturation; F, factor; PAI-1, plasminogen activator inhibitor-1; CLT, clot lysis time; vWF, von Willebrand factor; sP-selectin, soluble P-selectin.

driven mostly by increased TAFI activity. Increasing evidence indicates that increased TAFI levels occur in venous thromboembolism and ischemic stroke.^{16,17}

High TAFI activity in Fontan patients is a novel finding and it might contribute to late thromboembolism in this population. To assess fibrinolysis efficiency, we used the

TABLE 5. Univariate and multivariate predictors for thrombosis in Fontan patients

Variable	Univariate		Multivariate	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
SaO ₂ (%)	0.87 (0.78-0.97)	.015		
Free protein S (%)	0.81 (0.71-0.93)	.003	0.77 (0.63-0.94)	.012
P-selectin (ng/mL)	15.0 (2.6-85.2)	.003		
vWF (%)	1.04 (1.01-1.07)	.014		
sCD40L (pg/mL)	1.89 (1.16-3.09)	.012	2.71 (1.08-6.82)	.034
Factor VIII (%)	0.97 (0.94-0.99)	.049		

CI, Confidence interval; sCD40L, soluble CD40 ligand; vWF, von Willebrand factor; SaO₂, oxygen saturation.

plasma-based clot lysis assay, in which coagulation is initiated by a tissue factor without addition of human thrombin, and tissue-type plasminogen activator is used.¹⁸ In our study, CLT was prolonged in adult Fontan patients and correlated positively with PAI-1 antigen, TAFI activity, and F1.2. It remains to be established whether CLT might help identify subjects at increased risk of thromboembolic events among those patients.

In adult Fontan patients, nonpulsative flow in the pulmonary circulation, slow venous flow, and hypoxemia may damage endothelial cells. We observed evidence for endothelial injury in Fontan patients as reflected by increased plasma vWF levels. It has been postulated that vWF is a marker of endothelial dysfunction in contrast to thrombomodulin, which increases subsequent cell membrane injury.^{19,20}

Unexpectedly, we observed that Fontan patients with prior thromboembolism had lower FV and FVIII, both of which are associated with SaO₂, and free protein S. Cheung et al²¹ reported that systemic oxygen blood saturation correlated positively with plasma levels of anticoagulant and procoagulant factors, and those abnormalities preceded the Fontan procedure with a tendency to normalize after the surgery in association with improved systemic oxygenation.

A decrease of SaO₂ in adults after Fontan surgery may result from a right-to-left shunt through an atrial fenestration or venovenous collaterals. Atz et al²² observed lower rest SaO₂ without an increased risk of thrombosis during an 8-year follow-up period in Fontan patients with fenestration. In our study there were no differences in the prevalence of fenestration between the patients with and without a history of thrombosis. Desaturation also could reflect a progressive decrease in single-ventricle competence and an increase in pulmonary pressure.^{1,2,7} This mechanism is likely to cause lower SaO₂ in our patients.

A novel finding of the present study was increased platelet activation, as evidenced by higher sP-selectin and sCD40L in Fontan patients who experienced thromboembolic events. P-selectin, largely derived from activated platelets, is considered a marker of inflammation or platelet activation, but it also can induce procoagulant activity through increased tissue factor expression.²³ Despite significantly reduced coagulation factor levels, it is likely

that by providing a catalytic surface for prothrombinase complex formation, in combination with reduced levels of free protein S, activated platelets largely contribute to a prothrombotic tendency in Fontan patients. Of note, none of the patients with previous thromboembolism was taking aspirin at the time of blood collection, which might have resulted in increased platelet activation. There is evidence that low-dose aspirin is not potent enough to reduce circulating levels of platelet-derived proteins significantly (eg, sCD40L²⁴).

Several limitations of the study should be acknowledged. First, the number of patients in the study was small and the patients were heterogeneous with respect to cardiac diagnosis and the Fontan surgery type. However, our group is representative of the current growing adult Fontan patient population in the real-life clinical practice. Second, the true incidence of thrombosis may have been underestimated because we analyzed only symptomatic events. Because patients were studied post hoc after thromboembolic events, we cannot exclude that the abnormalities observed in this subset are to some extent secondary to thrombosis. However, given a long period of time that elapsed since the event, it is unlikely that the abnormalities are solely the result of past thromboembolism. Coagulation factors and inhibitors were not determined at the time of the index event, and it is known that transient increases in coagulation factors, particularly FVIII, and decreases in natural anticoagulants, especially antithrombin and protein C, are observed commonly during acute thrombosis. Therefore, changes during acute thrombosis are perceived as a poor predictor of overall function of the hemostatic system in human beings.

In conclusion, we found that adult Fontan patients are characterized by specific abnormalities in blood coagulation, enhanced platelet activation, and endothelial injury, associated with heightened thrombin formation and impaired fibrinolysis. Patients show reduced free protein S, increased platelet activation, and endothelial damage after thromboembolic events observed late after Fontan surgery. Further studies are needed to evaluate the potential benefits from combined antiplatelet and anticoagulant thromboprophylaxis in Fontan patients.

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