EVALUATION OF HEMOSTATIC AND COAGULATION FACTOR ABNORMALITIES IN PATIENTS UNDERGOING THE FONTAN OPERATION

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Objective: Low-velocity and nonlaminar flow patterns in the Fontan circulation, as well as abnormal liver function in some patients, may partly account for the coagulation abnormalities seen. We examined (1) coagulation factor abnormalities before and after the Fontan procedure and (2) regional coagulation factor abnormalities in the Fontan circulation.

Methods: Levels of factors V, VII, VIII, X, antithrombin III, prothrombin fragment F1+2, protein C, and protein S were measured in 2 groups of patients: In 14 patients undergoing the Fontan procedure, blood was analyzed before the operation and 5 days after the operation (group 1). The median age in this group was 3.2 years. In 10 patients who had undergone the Fontan procedure, cardiac catheterization was performed and samples were taken from the femoral vein, inferior vena cava, right atrium, and pulmonary artery (group 2). The median age in this group was 6.2 years and the median follow-up from the Fontan procedure was 4.1 years.

Results: In group 1 a significant increase was noted postoperatively in the concentration of factor VIII ($P < .001$), factor X ($P < .001$), and prothrombin fraction F1+2 ($P < .001$). A significant decrease in the levels of antithrombin III ($P < .001$), protein C ($P < .004$), and protein S ($P < .02$) was also found. The increase in factors VIII and X persisted at 4 years' follow-up in group 2 patients. In group 2, no significant regional differences were observed between the coagulation factors measured at different sites.

Conclusions: There is an increased tendency toward coagulation after the Fontan procedure. A prothrombotic state is supported by thrombin generation associated with reduced antithrombin III concentration. This increase in coagulation may contribute to the early and late risks of thromboembolism observed after the Fontan procedure. We did not find any regional differences in coagulation abnormalities in patients late after the Fontan procedure. Therefore, the mechanisms and causes of the coagulation abnormalities remain unclear. (J Thorac Cardiovasc Surg 2000;120:778-82)

The Fontan operation was initially reported for the surgical management of tricuspid atresia in 1971.¹ Since 1971, various modifications have been introduced and now cavopulmonary anastomosis is used as a defin-

itive procedure for many cardiac anomalies in which the size or function of only one ventricle is adequate. One of the recognized complications after the Fontan procedure is thromboembolism both early and late after the operation. Along with arterial embolization to the central nervous system resulting in persistent neurologic deficit, pulmonary thromboembolism has also been reported.^{2,3} According to Virchow's triad (characterized by loss of endothelial cell function, slow or turbulent blood flow, and hypercoagulability state), the risk of thromboembolism could be increased in this setting by hemodynamic factors, the presence of artificial material, or coagulation abnormalities. A number of authors have analyzed a variety of possible predisposing factors, including demographic and surgical factors

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Table I. Morphologic diagnosis of 14 patients who underwent the Fontan procedure

Diagnosis	No.
Unbalanced common atrioventricular canal	1
Tricuspid atresia	3
Double-outlet right ventricle, pulmonary atresia	6
Double-inlet left ventricle	3
Hypoplastic left heart (stage III)	1

(patient age at operation; the original morphologic features; type of Fontan procedure, including the presence or absence of fenestration; type of material used for the conduit; use of valved or nonvalved conduit) and hemodynamic factors.⁴ No single predisposing factor has emerged from these studies. This led us to believe that coagulation abnormalities may play a role. Recent evidence suggests that this may indeed be the case.^{5,6}

In the adult population, regional left atrial coagulation activity may be increased in mitral stenosis and may contribute to the pathophysiology of left atrial thrombus.⁷ Furthermore, the increase in left atrial coagulation has been shown to be associated with the echocardiographic phenomenon of left atrial spontaneous echo contrast.⁸ Similarly, abnormal flow patterns in the Fontan circulation as demonstrated by spontaneous echo contrast may partly account for the coagulation abnormalities seen.

Venous pressure in the liver is increased by the Fontan procedure. Since procoagulant and anticoagulant factors are produced in the liver, the increased venous pressure of Fontan physiology might change the usual balance of procoagulant and anticoagulant activity. Hemodynamic factors might also have an impact by affecting platelet aggregation with different intensities of turbulence. Increase in platelet hyperactivity due to increased wall shear stress and flow disturbance can contribute to a prothrombotic state.

We set out to assess the overall thrombotic status of patients who have undergone the Fontan procedure and its modifications and to assess any regional variability in the Fontan pathway.

Patients and methods

Two groups of patients were studied.

Group 1: Fourteen patients after the Fontan procedure.

The median age in this group was 3.2 years (range, 14 months–12 years). Their morphologic diagnoses are shown in Table I. Twelve patients underwent total cavopulmonary connection and 2 patients underwent an extracardiac Fontan procedure. In this group, blood was taken before the operation and 5 days after the operation. These time frames were cho-

sen to avoid extra venipunctures and to use the existing lines placed during anesthesia. None of the patients was receiving heparin or warfarin sodium (Coumadin). An activated clotting time of more than 700 seconds was achieved before cardiopulmonary bypass was begun. All patients who received aprotinin during or after the operation were excluded from the study. Five patients received fresh frozen plasma and 1 received platelets within 4 hours of the operation.

Group 2: Ten patients who were admitted for elective cardiac catheterization at different points of follow-up after their Fontan procedure. All patients had undergone total cavopulmonary connection. The median age in this group at the time of the study was 6.2 years (range, 3–18 years) and the median time from the Fontan repair was 4.1 years (range, 2–14 years). At the time of the elective cardiac catheterization, blood was taken from the site of entry via the catheter for baseline measurements, at the level of the renal veins, in the right atrium, and in the left pulmonary artery. All samples were taken before administration of heparin.

Blood samples were examined for coagulation factors V, VII, VIII, and X, antithrombin III, prothrombin fragment (F1+2), protein C, and protein S. Patients with history of thromboembolism and clotting abnormalities, patients receiving warfarin, aspirin, or heparin, and those with protein-losing enteropathy were excluded from the study.

Assay procedure. Samples for factors VIII, V, VII, and X and protein C were analyzed with an Electra 1000C coagulation instrument (Hemoliance, Pleasantville, NY). Factors V, VII, and X were measured with Dade deficient plasmas and thromboplastin C plus reagent (Dade Behring Inc, Newark, Del).

Protein C was assessed by a functional clotting assay based on prolongation of the activated partial thromboplastin time with a protein C activator. The reference range (66%–129%) was developed in-house from normal subjects in the age range of 2 years to adult. Protein S was measured by an enzyme immunoassay for free protein S with the use of the Asserachrome free protein S kit from Diagnostica Stago, Asnieres-Sur-Seine, France. The reference range (66%–129%) was developed in-house from normal subjects in the age range of 2 years to adult.

Antithrombin levels were measured on a Hitachi 911 chemistry analyzer (Roche/Boehringer Mannheim, Division of Roche Diagnostics, Mannheim, Germany) with the Coamatic Antithrombin kit (Chromogenix, Molndal, Sweden). This is a chromogenic method in which plasma is incubated with an excess of factor Xa in the presence of heparin. The residual quantity of factor Xa is determined by age hydrolysis of the chromogenic substrate S-2765 and is inversely proportional to the antithrombin level in the plasma. Reference range is based on in-house study of patients being treated for ear, nose, and throat problems (80%–130%).

The F1+2 was measured by an enzyme immunoassay technique by means of the Enzygnost F1+2 micro kit (Behring Diagnostics GmbH, Marburg, Germany). A reference range from 140 adults was used (0.4–1.1 nmol/L).

Statistical analysis. Data are presented in terms of the mean and standard deviation since all variables conformed to

Table II. Profile of coagulation factors and protein levels for group 1 patients ($n = 14$) measured both before and after the Fontan operation

	Before Fontan (mean $\pm$ SD)	After Fontan (mean $\pm$ SD)	P value
Factor V (%)	97.6 $\pm$ 26.0	86.6 $\pm$ 27.8	.22
Factor VII (%)	57.7 $\pm$ 20.4	40.8 $\pm$ 19.5	.002
Factor VIII (%)	134.1 $\pm$ 40.8	210.3 $\pm$ 55.2	<.001
Factor X (%)	58.6 $\pm$ 13.3	72.6 $\pm$ 14.2	<.001
Antithrombin III (%)	92.0 $\pm$ 14.6	74.5 $\pm$ 14.1	<.001
F1+2 (nmol/L)	0.60 $\pm$ 0.24	1.54 $\pm$ 0.63	<.001
Protein C (%)	80.1 $\pm$ 9.8	65.6 $\pm$ 11.7	.004
Protein S (%)	58.2 $\pm$ 13.9	48.1 $\pm$ 19.1	.02

F1+2, Prothrombin fragment.

Table III. Regional measurements for group 2 (post-Fontan) patients ($n = 10$)

	Femoral vein (mean $\pm$ SD)	Renal vein (mean $\pm$ SD)	Right atrium (mean $\pm$ SD)	LPA (mean $\pm$ SD)	P value
Factor V (%)	63.0 $\pm$ 18.9	61.6 $\pm$ 18.5	61.8 $\pm$ 18.9	59.2 $\pm$ 13.2	.6
Factor VII (%)	49.3 $\pm$ 16.3	47.6 $\pm$ 17.1	47.5 $\pm$ 16.4	43.5 $\pm$ 14.0	.36
Factor VIII (%)	187.4 $\pm$ 48.2	175.3 $\pm$ 43.2	171.2 $\pm$ 37.4	167.3 $\pm$ 45.7	.14
Factor X (%)	63.4 $\pm$ 12.0	62.4 $\pm$ 9.7	62.5 $\pm$ 9.5	63.2 $\pm$ 9.8	.73
Antithrombin III (%)	91.6 $\pm$ 12.7	92.3 $\pm$ 12.8	92.1 $\pm$ 12.3	89.3 $\pm$ 9.9	.56
F1+2 (nmol/L)	0.74 $\pm$ 0.50	0.34 $\pm$ 0.11	0.33 $\pm$ 0.10	0.37 $\pm$ 0.10	.09
Protein C (%)	87.8 $\pm$ 16.2	93.9 $\pm$ 20.1	92.8 $\pm$ 16.7	91.3 $\pm$ 18.9	.27
Protein S (%)	72.5 $\pm$ 15.7	73.0 $\pm$ 16.8	71.8 $\pm$ 13.7	71.0 $\pm$ 16.6	.52

LPA, Left pulmonary artery; F1+2, prothrombin fragment.

a normal distribution. Measurements of coagulation factors and protein levels before and after the Fontan operation were compared by paired t tests. Repeated-measures analysis of variance with a Greenhouse-Geisser F test for small samples⁹ was used to determine post-Fontan differences among 4 regional sites. Analysis was performed by means of the SAS version 6.12 software package (SAS Institute, Inc, Cary, NC). Power analysis indicated that the 14 patients studied would provide more than 90% power to detect an effect size of 1.0 between pre-Fontan and post-Fontan measurements. Our sample size of 10 patients in whom post-Fontan regional site data were obtained provided 68% power (nQuery Advisor, version 3.0, Statistical Solutions, Boston, Mass).

Results

Group 1 had a significant early increase, 5 days after the Fontan procedure, in coagulation factors VIII ($P < .001$), X ($P < .001$), and F1+2 relative to the preoperative levels, whereas the levels of factor VII ($P = .002$) and antithrombin III ($P < .001$) decreased. Concentrations of both protein C and protein S were significantly lower in the postoperative period ($P = .004$ and $P = .02$, respectively) (Table II).

In group 2, no regional coagulation differences were detected at the 4 sites examined late after the Fontan

procedure (Table III). The postoperative values in group 1 were compared with the baseline measurements in this group to assess whether coagulation abnormalities persisted beyond the early postoperative period (Table IV). The increase in factors VIII and X persisted at 4 years' follow-up. Factor VII remained reduced.

Discussion

Thromboembolism is a major cause of morbidity and mortality after the Fontan procedure and its modifications. The reported incidence of this complication has been as high as 20% in some series.¹⁰ In this study we have attempted to determine whether a prothrombotic condition is present after the Fontan procedure and whether any regional coagulation abnormalities are present in the Fontan pathway. It is unlikely that the results early after the Fontan procedure are affected in the 5 patients who received fresh frozen plasma, since its half-life is 6 hours.

We have shown a significant increase in coagulation factors and a reduction in natural anticoagulants after the Fontan procedure. Furthermore, we have shown a significant increase in F1+2 concentration associated with reduced antithrombin III level. An increase in

Table IV. Comparison of patients in whom measurements were made in the early postoperative period (group 1) and patients who had undergone the Fontan procedure several years earlier (group 2)

	Group 1 (n = 14) (mean ± SD)	Group 2 (n = 10) (mean ± SD)	P value
Factor V (%)	86.6 ± 7.4	63.4 ± 6.2	.05
Factor VII (%)	40.8 ± 5.2	48.1 ± 5.8	.38
Factor VIII (%)	210.3 ± 14.8	177.4 ± 14.5	.16
Factor X (%)	58.6 ± 3.6	62.7 ± 3.7	.46
Antithrombin III (%)	74.6 ± 3.8	91.8 ± 4.4	.009
F1+2 (nmol/L)	1.54 ± 0.17	0.47 ± 0.06	<.001
Protein C (%)	65.6 ± 3.2	91.6 ± 6.0	<.001
Protein S (%)	48.1 ± 5.1	71.9 ± 5.2	.007

In group 2, blood samples were obtained from an average of 4 sites. F1+2, Prothrombin fragment.

F1+2 has previously not been reported. Thrombin generation is one of the most important contributors to a prothrombotic state in both venous and arterial thrombosis. Thrombin is the most active platelet-aggregating agent, and by forming fibrin strands it stabilizes the fragile platelet-rich thrombus. A highly significant increase in thrombin generation coupled with reduced antithrombin III levels is the best and most reliable indicator of a prothrombotic state.

Some of these changes persisted in a second group of patients up to 14 years after the Fontan operation. We found no regional coagulation abnormalities in the Fontan pathway in this latter group.

Protein C, a vitamin K-dependent protein, is a natural anticoagulant that is synthesized in the liver.¹¹ After activation by thrombin, protein C is a potent inhibitor of the coagulation cascade stimulating fibrinolysis. Protein S acts as a cofactor in this pathway, increasing fibrinolysis.¹² Therefore, deficiency of these proteins can cause an increase in coagulation. Protein C with or without protein S deficiency, either inherited or acquired, has been associated with increased thrombotic activity.^{13,14}

Increase in coagulation status after the Fontan procedure has previously been reported.^{5,6,15} Some of these findings have been criticized for not having used an age-appropriate range for control values.¹⁶ However, even when age-appropriate ranges were used, these coagulation abnormalities persisted. Cromme-Dijkhuis and colleagues⁵ reported that these coagulation abnormalities tended to resolve as duration of follow-up lengthened. In the present study, we have shown that some of these coagulation abnormalities persisted at a median follow-up of 4 years after the Fontan repair.

An interesting observation of our study was that factor VII levels not only did not increase but decreased after the Fontan procedure and this decrease persisted at follow-up (group 2 patients). Experimental evidence suggests that very low concentrations of factor VII can

support tissue factor-induced coagulation in pathologic states.¹⁷ Furthermore, the risk of thromboembolism has been shown to increase in patients with hereditary factor VII deficiency.¹⁸

We have shown a generalized increase in coagulation in the early postoperative period after the Fontan procedure. Some of these changes have persisted at 4 years' follow-up. The practical implication of this increase may be that long-term administration of anticoagulants after the Fontan operation is indicated.

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