Prospective longitudinal study of coagulation profiles in children with hypoplastic left heart syndrome from stage I through Fontan completion

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Objective: The risk for thrombosis is increased after the Fontan operation. It is unknown whether children with univentricular heart disease have an intrinsic coagulation anomaly or acquire a defect in coagulation during the course of the staged repair. This prospective, longitudinal study evaluated changes in coagulation profiles in a cohort of patients with hypoplastic left heart syndrome from stage I palliation through completion of the Fontan operation.

Methods: Thirty-seven patients with hypoplastic left heart syndrome were enrolled prospectively, and the concentration of factors II, V, VII, VIII, IX, X, proteins C and S, fibrinogen, antithrombin, serum albumin, and liver enzymes were measured before stage I palliation (mean age 4 ± 2 days), before bidirectional Glenn (mean age 5.9 ± 1.8 months), before the Fontan procedure (mean age 27.1 ± 6.6 months), and after the Fontan procedure (mean age 49 ± 17.6 months). Healthy children were used as age-matched controls for coagulation factors. Demographic, hemodynamic variables, and elapsed time after the Fontan procedure were evaluated as possible predictors of coagulation abnormalities.

Results: Significantly lower levels of both procoagulation and anticoagulation factors were demonstrated through to completion of the Fontan procedure. After the Fontan procedure, there was a significantly higher factor VIII level ($P < .005$) but no correlation with hemodynamic variables or liver function.

Conclusion: This longitudinal study in patients with identical cardiac disease and staged surgical procedures confirms the increase in factor VIII level after the Fontan procedure. This is an acquired defect, and although the cause remains to be determined, monitoring factor VIII levels after the Fontan operation could indicate a subset of patients at risk for thrombosis.

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As early outcome after the Fontan operation continues to improve, the focus has changed from survival to long-term morbidity, prognosis, and quality of life.[1-3] A major factor contributing to both early and late morbidity and mortality after the Fontan operation is the potential for thromboembolic complications.[1,4,5] The incidence of thromboembolic events in patients with Fontan physiology is uncertain but has been reported to be as high as 20% to 33%.[4, 6-9] The etiology is not completely understood and is likely to be multifactorial, including the nature of the Fontan circulation with elevated central venous pressure, low flow with possible stasis through the atrial baffle and pulmonary circulation, atrial dysrhythmias, ventricular dysfunction, hepatic dysfunction, and altered resting venous tone. It has also been demonstrated that lower levels of both procoagulant and an-

ticoagulant factors precede the Fontan operation, and it has been speculated that a ''functional balance'' may exist that reduces the risk for thrombosis during the earlier staged palliation for single ventricle cardiac defects.[10,11]

A possible hypercoagulable state after the Fontan procedure predisposing to thrombus formation has been postulated, secondary to low levels of the naturally occurring anticoagulants protein C, protein S, and antithrombin,[12,13] and elevation of factor VIII.[14] Interpretation of these studies is limited because they included heterogeneous patient populations, often with various modifications of the Fontan operation, and at a variable point in time after the Fontan procedure.

The purpose of this single-center, prospective, longitudinal study was to follow up a homogeneous cohort of children with hypoplastic left heart syndrome (HLHS) undergoing identical staged procedures from the Norwood operation to after completion of the Fontan operation to determine whether there may be an intrinsic or acquired anomaly in the coagulation profile causing hypercoagulability in these patients.

METHODS

After institutional review board approval and informed parental consent had been obtained, 37 neonates with the diagnosis HLHS were enrolled in this single-center prospective study. Patients were followed up from 1998 to

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Abbreviations and Acronyms
BDG = bidirectional Glenn
HLHS = hypoplastic left heart syndrome

2006; patients were excluded if they had other known congenital abnormalities or syndromes and had pre-existing or known family history of hematologic disorder or coagulopathy.

All patients underwent identical staged surgical procedures. Neonates underwent initial stage I palliation with a Norwood procedure using a 3.5-mm polytetrafluoroethylene (Gore-Tex; W. L Gore & Associates, Inc, Flagstaff, Ariz) right modified Blalock–Taussig shunt. The second stage operation was a bidirectional Glenn cavopulmonary connection (BDG) performed on cardiopulmonary bypass. The Fontan procedure consisted of a fenestrated lateral tunnel, cavo–cavo–pulmonary connection, performed with a 0.4-mm thickness polytetrafluoroethylene cardiovascular patch and fenestrated with a 4-mm punch hole.

Postoperative and interstage anticoagulation management was at the discretion of each patient's physician. Blood samples from all patients (7 mL) were obtained the day of the operation before each stage (stage I, BDG, and Fontan) after induction of general anesthesia and in the catheterization laboratory after catheter placement for the post-Fontan evaluation. Those patients receiving aspirin and/or warfarin sodium (Coumadin) as prophylaxis for thrombosis had these drugs withheld for an appropriate time period before procedures. Hemoglobin, hematocrit, platelet count, prothrombin time, and activated partial prothrombin time were measured immediately, and this information was available to clinicians caring for the patients. The remaining plasma was stored at −70°C in 200-μL aliquots for batch analysis of the other coagulation assays; this information was not available to clinicians during the admission of each patient. The circulating anticoagulant factors measured were protein C, protein S, and antithrombin; the procoagulant factors measured were II, V, VII, VIII, IX, X, and fibrinogen. All samples were analyzed by identical techniques, in the same laboratory, and by the same technician (R.A.C.); the techniques for analysis along with coefficients of variation have been reported elsewhere.[10,11,14]

To determine whether the increase in factor VIII levels observed after the Fontan operation could have been due to an acute inflammatory response, we measured the level of von Willebrand factor post hoc in the available remaining frozen serum in 11 of the Fontan patients. An increase in both the factor VIII and von Willebrand factor levels would be expected in an acute phase response. von Willebrand factor level was measured by a immunoturbidimetric assay of von Willebrand factor (STA-Liatest VWF:Ag kit; Diagnostica Stago, Asnieres France; coefficients of variation, 1.9% and 2.7%).

Because altered hepatic dysfunction can contribute to coagulation factor abnormalities, serum alkaline phosphatase, gamma-glutamyl transferase, alanine transaminase, aspartate transaminase, total bilirubin, albumin, and total protein were measured in all patients and compared with normal values for our laboratory.

Hemodynamic Variables

Ventricular, atrioventricular valve, and semilunar valve function was assessed by 2-dimensional and Doppler echocardiographic examination for all patients before data sampling. Cardiac catheterization was routinely performed before the BDG and the fenestrated Fontan procedures, but not before the Norwood procedure in neonates. Catheterization data were also available for all 20 of the post-Fontan patients. Data obtained at the time of cardiac catheterization included superior vena cava oxygen saturation, the ratio of pulmonary to systemic blood flow, superior vena cava pressure, pulmonary artery pressure, pulmonary vascular resistance, and systemic ventricular end-diastolic pressure. The time after the Fontan operation

was also examined as a potential variable contributing to coagulation abnormalities.

Age-Matched Control Coagulation Parameters

Developmental hemostasis, or maturation of the coagulation system in infants and children, is widely recognized.[15,16] To establish age-matched reference ranges, we obtained informed written parental consent to draw blood samples for coagulation factor assays in healthy infants and children undergoing minor day surgery. We used published control values for neonates because of a state of Massachusetts requirement limiting research in newborn subjects unless potential direct benefit can be demonstrated by participating in a clinical study. The available normative ranges for neonates and infants vary according to the techniques and reagents used to measure coagulation factor levels,[15,16] and we chose to use the reference range described by Andrew and associates[16] because it provided values (mean age 2 days) with similar age distributions to our patients (mean age 2 days). Beyond neonates, there were a total of 90 control subjects, 30 for each age-matched control group. The mean ages for the groups were 7.8 ± 2.2 months, 26 ± 12 months, and 8.3 ± 2.9 years.

A total of 1.8 mL of blood was taken from each control patient, and after centrifuge, the plasma was stored at −70°C for subsequent batch analyses in the same laboratory using the techniques as described above. Normal ranges in these age-matched control groups were based on the empirical 95% confidence intervals and have been described in a previous manuscript.[17]

Because of technical problems, we were unable to obtain complete samples to measure prothrombin time and activated partial thromboplastin time in all of the control patients, and to use a consistent reference range we chose the range of normal for age as described by Andrew and colleagues.[16]

Statistical Analysis

Factor levels at each stage were compared with values from control patients by the 2-sample Student t test after verifying normality. Univariate and multivariable logistic regression using maximum likelihood estimation was performed to identify potential predictors of a coagulation abnormality. Variables evaluated included age, weight, gender, systemic ventricular end-diastolic pressure, superior vena cava oxygen saturation, superior vena cava pressure, ratio of pulmonary to systemic blood flow, pulmonary artery pressure, pulmonary vascular resistance, ventricular function, atrioventricular valve regurgitation, and time after the Fontan operation. In comparing coagulation factors between patients and age-matched controls, we used a 2-tailed Bonferroni-adjusted P value of .005 as the criterion for statistical significance inasmuch as there were 10 variables ($P = .05/10 = .005$) provided this conservative α-level was chosen to minimize the risk of type I (false positive) errors.[18,19] For those patients in whom we were able to obtain complete coagulation factor levels at each study point, a repeated-measures linear mixed model, with compound symmetry to model the covariance, was applied to assess changes over time in coagulation variables from stage I through post-Fontan completion. Statistical analysis was conducted with the SPSS software package (version 6.0; SPSS Inc, Chicago, Ill).

RESULTS

The study population with the mean age and weight at the time of each procedure is shown in Figure 1. Coagulation factor analysis could not be performed in 1 of the patients at the time of stage I, in 1 BDG patient, and in 1 post-Fontan patient because of sampling error. In addition to death (n = 5) or heart transplantation (n = 1), 11 patients were lost to follow-up because they were out-of-state referrals who had undergone the stage I palliation and/or BDG at our institution and subsequent procedures elsewhere. Post-Fontan patients were followed up at a mean of 27 ± 17.8 months after
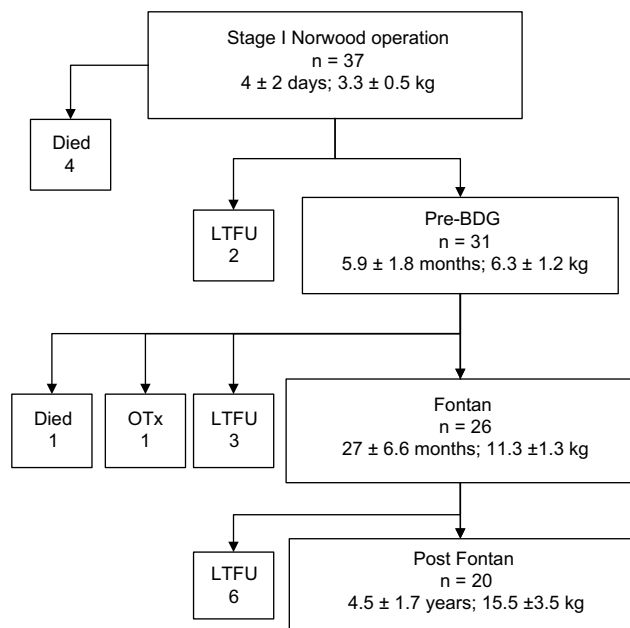
FIGURE 1. Number of study patients, mean age and mean weight at each stage *LTFU,* Lost to follow-up; *BDG,* bidirectional Glenn procedure; *OTx,* orthotopic heart transplant.

the Fontan procedure, at the time of fenestration closure in the catheterization laboratory (n = 16), a clinic visit (n = 2), in the operating room during a permanent pacemaker placement (n = 1), or during a sternal wire removal (n = 1).

Four neonates died while in the hospital after the Norwood procedure, none as a result of acute thrombosis. The coagulation factor levels in these 4 patients were similar to the other patients before the Norwood procedure who survived to discharge. All survivors (89%) were discharged home receiving aspirin as thrombosis prophylaxis.

There were no interstage deaths between the Norwood operation and the BDG procedure. One patient was admitted to the hospital with severe cyanosis at 2 months of age, a thrombosed modified Blalock–Taussig shunt was diagnosed and reopened by balloon dilation, and the patient was discharged home receiving aspirin and warfarin; no coagulation factors were measured at this admission.

All 31 patients who underwent the BDG procedure recovered well and were discharged receiving aspirin for thrombosis prophylaxis. There was 1 interstage death between the BDG and Fontan procedures. This patient died suddenly at home at the age of 18 months. An autopsy failed to find any particular cause of sudden death. One patient had severe right ventricular dysfunction after the BDG procedure and underwent successful heart transplantation.

All patients survived to discharge after the Fontan procedure, and the fenestrations were open by echocardiography at the time of discharge in all but 1 patient. The majority of patients (22/26) were discharged receiving aspirin alone; the other 4 patients were discharged receiving aspirin and warfarin or aspirin and clopidogrel (Plavix). In 1 patient a thrombus developed within the baffle, detected by echocardiography 2 months after the Fontan operation. The patient was treated with warfarin and aspirin and the clot resolved. Two patients needed cardiac transplantation after the Fontan operation: both had failing Fontan physiology, and both successfully underwent transplantation. The coagulation factor levels in these patients were similar to those of the other patients in the post-Fontan group.

Coagulation Factors

The comparison of coagulation factor levels between control subjects and all patients at each stage of surgical repair and post-Fontan period are summarized in Table 1. Lower concentrations of procoagulant and anticoagulant factors are seen before each staged surgical procedure. Although the levels of protein C and factors II, V, VII, and X remained significantly lower than in the age-matched controls after the Fontan operation ($P < .005$), the factor VIII level was significantly higher ($P < .005$), and 8 (42%) patients had factor VIII levels greater than 160% activity (Figure 2); von Willebrand factor levels were not elevated in any of these 8

TABLE 1. Comparison of coagulation factors for all patients with controls at each staged surgical procedure through to after Fontan completion

Variable	Stage I (n = 36)	Controls*	Pre-BDG (n = 30)	Controls (n = 30)	Pre-Fontan (n = 26)	Controls (n = 30)	Post-Fontan (n = 19)	Controls (n = 30)
Fibrinogen (mg/L)	191 ± 77†	270 ± 54	210 ± 71	251 ± 68	302 ± 84	276 ± 53	336 ± 93	279 ± 61
Antithrombin (%)	34 ± 12†	78 ± 15	76 ± 15†	106 ± 13	93 ± 13†	105 ± 16	97 ± 16	108 ± 11
Protein C (%)	19 ± 8†	43 ± 11	43 ± 14†	81 ± 17	61 ± 14†	98 ± 20	58 ± 15†	95 ± 22
Protein S (%)	34 ± 15†	63 ± 15	77 ± 19	89 ± 20	76 ± 23	86 ± 15	80 ± 22	87 ± 16
Factor II (%)	39 ± 14†	68 ± 17	66 ± 13†	90 ± 12	79 ± 13†	93 ± 10	82 ± 10	95 ± 15
Factor V (%)	67 ± 19†	98 ± 18	80 ± 16†	117 ± 18	87 ± 19†	109 ± 16	71 ± 17†	103 ± 24
Factor VII (%)	33 ± 13†	90 ± 24	47 ± 14†	88 ± 20	57 ± 15†	88 ± 18	48 ± 14†	92 ± 27
Factor VIII (%)	57 ± 22†	91 ± 33	68 ± 25	77 ± 20	95 ± 40	85 ± 22	167 ± 88†	96 ± 32
Factor IX (%)	33 ± 12†	51 ± 15	54 ± 22	66 ± 15	63 ± 14	69 ± 11	71 ± 18	80 ± 21
Factor X (%)	34 ± 10†	59 ± 14	66 ± 14†	95 ± 14	74 ± 14†	93 ± 11	72 ± 15†	90 ± 17

*Published control values for neonates.[16] †Statistically significant, $P < .005$ (Bonferroni criterion); *n*, number of patients who had blood samples drawn at each stage. Mean age for pre-BDG controls 7.8 ± 2.2 months, Pre-Fontan controls 2.6 ±12 months and post-Fontan controls 8.3 ±2.9 years. *BDG,* Bidirectional Glenn.
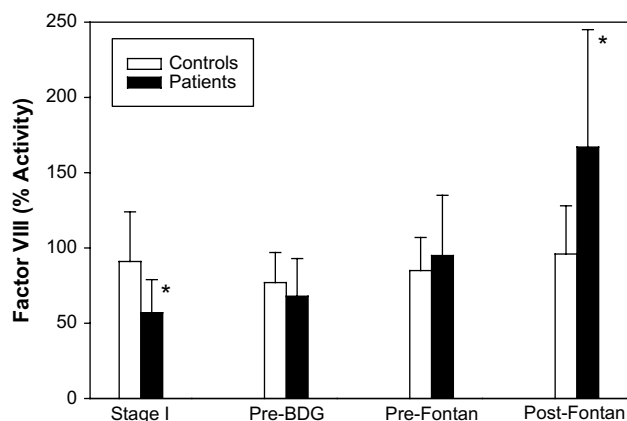
FIGURE 2. Factor VIII level in all study patients compared with age-matched controls at each of the four stages. Before the Norwood operation, patients had a factor VIII level significantly lower than controls. After the Fontan operation, there was a significant increase in *factor VIII level ($P <$ .005).
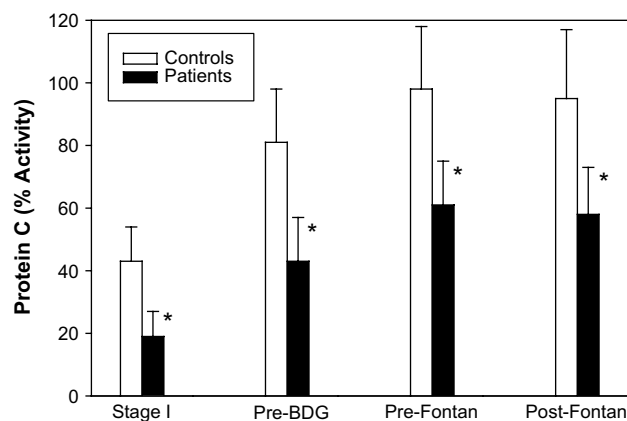

FIGURE 3. Protein C level in all study patients compared with age-matched controls at each of the four stages. *Protein C was significantly lower than in controls ($P <$ .005).

patient. Protein C levels across all four stages of the study compared with controls are shown in Figure 3; 4 patients had a protein C activity level less than 50%. No patient demonstrated clinical evidence for a thromboembolic event, and intracardiac thrombus was not detected by transthoracic or transesophageal echocardiography on routine follow-up.

It was possible to collect a full coagulation profile at each of the four stages of the study period in only 17 of the 37 enrolled subjects because of the limitations outlined above. The changes in coagulation factor concentrations in these 17 patients over time are shown in Table 2. The change in factor VIII concentration for these 17 patients is shown in Figure 4; the increase in factor VIII levels after the Fontan procedure was not related to the level before the Fontan operation in these patients.

Hemodynamic variables and laboratory values at each stage are shown in Tables 3 and 4. With the use of univariate and multivariate logistic regression, no relationship was demonstrated between significantly lower or higher factor levels and gender, weight, hemodynamic variables, liver function abnormalities, serum albumin, and the time interval after the Fontan procedure (all $P >$ .20).

DISCUSSION

This prospective, longitudinal study of coagulation profiles in a homogeneous cohort of patients with HLHS undergoing identical surgical procedures confirms the risk for a significant increase in factor VIII level after the Fontan operation and, in association with a significantly lower protein C level, indicates an acquired prothrombotic risk in patients with Fontan physiology.

The implications for thrombosis after the Fontan operation have been reported.[1,3,5,20,21] du Plessis and coworkers[5]

reported an incidence of cerebral vascular accidents of 2.6% in a cohort of 605 patients undergoing the Fontan procedure between 1978 and 1993, although the cause of these events could not be determined. The long-term survival, modes of death, and predictors of mortality in 261 patients over a median follow-up of 12.1 years after the Fontan operation was recently reported by Khairy and coworkers.[3] A thromboembolic event was deemed the cause of late death in 3% of those patients who had survived the immediate perioperative period after the Fontan operation, and it was the mode of death in 25% of the patients who died. In this study, death was considered thromboembolic in nature if thrombus was identified either clinically or on autopsy within the systemic venous or pulmonary artery circulation, but the frequency of subclinical thrombus formation or systemic thromboembolic events was not reported. In the studies by both du Plessis and Khairy's groups, anticoagulation practices were not standardized.

Other retrospective cohort studies have also reported on the frequency of thromboembolic events after the Fontan operation. Coon and colleagues[20] reported an incidence of thromboembolic events in 8.8% of 592 patients who had undergone a Fontan procedure. Anticoagulation practices were not standardized, and they demonstrated that thrombus formation occurred with equal frequency in all types of surgical modifications to the Fontan procedure and occurred in the Fontan pathway as well as the systemic venous atria. A recent study by d'Udekem and associates[1] of 305 patients who had undergone the Fontan procedure in the current surgical era with either an intracardiac or extracardiac lateral tunnel technique reported a freedom from thromboembolic events at 15 years after the Fontan procedure of 94.3%; patients in this study who had a thromboembolic event did so despite receiving warfarin for longer-term anticoagulation. Kaulitz and coworkers[21] reported an incidence of 7% of thrombotic events in 142 patients after the Fontan

TABLE 2. Coagulation factor levels (mean ± SD) over time for the subgroup of 17 patients who had complete samples drawn at each stage

Variable	Stage I	Pre-BDG	Pre-Fontan	Post-Fontan
Fibrinogen (mg/L)	200.5 ± 70	203.5 ± 86	292.8 ± 76	350.2 ± 93
Antithrombin (%)	36.8 ± 12	75.4 ± 18*	93.1 ± 16†	97.6 ± 17
Protein C (%)	17.7 ± 9	43.2 ± 12*	60.2 ± 16†	56.9 ± 15
Protein S (%)	34.1 ± 17	81.3 ± 16*	71.1 ± 18	76.3 ± 19
Factor II (%)	37.5 ± 14	64.7 ± 15*	77.8 ± 13	81.9 ± 11
Factor V (%)	67.9 ± 19	79.1 ± 15	86.9 ± 21	71.8 ± 19
Factor VII (%)	32.7 ± 14	49.3 ± 13*	57.9 ± 15	49.2 ± 14
Factor VIII (%)	50.3 ± 21	63.5 ± 26	90.9 ± 36	175.5 ± 89‡
Factor IX (%)	32.5 ± 14	47.2 ± 17	62.9 ± 13	72.5 ± 18
Factor X (%)	33.2 ± 9	63.6 ± 15*	71.5 ± 13	72.4 ± 16

Data are mean ± SD. *BDG,* Bidirectional Glenn. The following symbols denote significant changes (*P* < .005) between selected stages: *Stage I to pre-BDG; †Pre-BDG to pre-Fontan; ‡Pre-Fontan to post-Fontan.
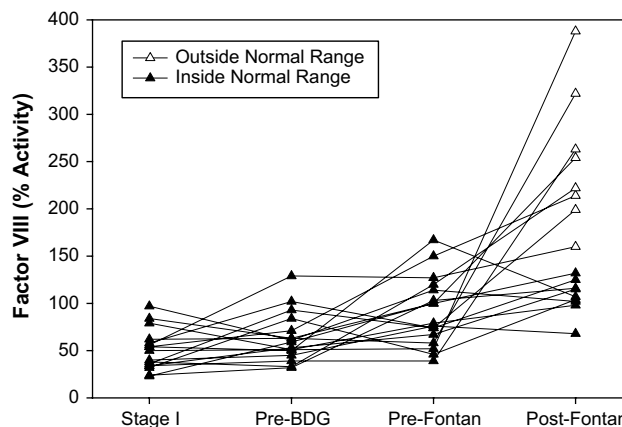

FIGURE 4. Change in factor VIII concentration in the subgroup of 17 patients who were sampled at each of the four stages. The increase in factor VIII level after the Fontan procedure was not related to the level before the Fontan operation.

procedure, despite their receiving prophylactic anticoagulation treatment.

Although evidence-based guidelines for anticoagulation in children are available,[22] there remains variability in practice regarding the optimal long-term anticoagulation prophylaxis after the Fontan operation.[23] Jacobs and colleagues[24] evaluated the efficacy of a strategy to reduce thromboembolic events with aspirin alone, concluding that more aggressive anticoagulation regimens seemed unwarranted, at least on the basis of intermediate follow-up. Recently, comprehensive guidelines for anticoagulation in children recommended either aspirin or a vitamin K antagonist for longer-term management in children with Fontan physiology.[25] In our study, all patients after the Norwood and BDG procedures, and 22 of the 26 Fontan patients, were discharged home receiving aspirin prophylaxis as the primary anticoagulation strategy. However, the decision for longer-term anticoagulation therapy is influenced by the anticipated risk for thrombosis, often based on the surgical technique, presence of arrhythmias, and a patient's functional status. However, it is also apparent that prophylactic long-term anticoagulation does not necessarily mitigate the risk for a later thromboembolic event in Fontan patients.[1,3,26] Owing to the multiple factors contributing to this risk, it is unlikely that a single therapy or drug will provide complete prophylaxis. Although needing further study, it is possible that monitoring for an elevated factor VIII level may provide a method to identify post-Fontan patients at risk for thrombosis.

Abnormalities or an imbalance of both procoagulant and anticoagulant proteins as a cause of a hypercoagulable state in children who have previously undergone the Fontan operation have been described, including low levels of the naturally occurring anticoagulants protein C, protein S, and antithrombin,[6-9] and more recently an increase in factor VIII level.[14,27] Interpretation from some of these studies is limited, however, because subjects were studied at a single

point in time, assay techniques were not controlled, and age-matched controls were not used, which is particularly important given the maturation of the hemostatic system over the first several years of life.[15,16,28] We[10,11,14] addressed such limitations in our previous work in which we reported changes in coagulation factors after staged surgical palliation, but the subjects in these studies included heterogeneous diagnoses and had undergone different surgical procedures.

TABLE 3. Hemodynamic variables at each stage

	Pre-BDG	Pre-Fontan	Post-Fontan
Echocardiography data (n, %)	*n = 31*	*n = 26*	*n = 20*
Ventricular function			
Normal	22 (71%)	22 (85%)	17 (85%)
Mildly depressed	8 (26%)	4 (15%)	2 (10%)
Moderately–severely depressed	1 (3%)	0	1 (5%)
AVVR			
No regurgitation	15 (48%)	15 (58%)	8 (40%)
Trivial	13 (42%)	9 (35%)	10 (50%)
Mild–moderate	3 (10%)	2 (7%)	2 (10%)
Catheterization data (mean ± SD)	*n = 29*	*n = 23*	*n = 20*
EDp (mm Hg)	9.1 ± 3.1	9.9 ± 2.9	8.2 ± 1.9
SVCp (mm Hg)	7.5 ± 2.8	13.2 ± 3.3	13.0 ± 3.8
TPG mmHg		4.9 ± 2.4	4.9 ± 2.7
PVR (Wood units)	1.8 ± 0.8	1.9 ± 0.8	1.6 ± 0.7
Qp/Qs	1.2 ± 0.4	0.7 ± 0.1	0.8 ± 0.1
Pao₂ (mm Hg)	43.4 ± 4.3	56.4 ± 10.9	61.3 ± 6.7
Svo₂ (%)	50.5 ± 7.7	61.0 ± 11.3	65.5 ± 5.7
Spo₂ (%)	76.4 ± 6.0	86.6 ± 3.8	89.4 ± 3.8

AVVR, Atrioventricular valve regurgitation; *BDG,* bidirectional Glenn; *EDp,* end-diastolic pressure; *Pao₂,* arterial oxygen tension; *PVR,* pulmonary vascular resistance; *Qp/Qs,* pulmonary to systemic blood flow; *Spo₂,* oxygen saturation; *Svo₂,* superior vena cava oxygen saturation; *TPG,* transpulmonary gradient.

TABLE 4. Laboratory values at each stage

	Stage I	Pre-BDG	Pre-Fontan	Post-Fontan	Normal range
Hematocrit (%)	39.7 ± 5.8	41.0 ± 3.9	39.9 ± 3.4	39.8 ± 4.0	33–55
Platelet (×10³)	197.4 ± 67.5	282 ± 77.9	256 ± 75.1	260 ± 59.9	130–400
PT (s)	14.8 ± 1.8	13.0 ± 1.0	12.4 ± 0.8	13.2 ± 1.1	11.0–13.0
aPTT (s)	67.2 ± 15.8	49.5 ± 14.2	39.1 ± 10.6	33.0 ± 10.0	27–37
Albumin (g/dL)	2.8 ± 0.5	3.3 ± 0.4	3.6 ± 0.4	3.9 ± 0.5	3.0–4.6
AST (U/L)	45.4 ± 40	31.9 ± 13.4	37.6 ± 20.1	38.0 ± 11.0	2–40
ALT (U/L)	46.2 ± 78.4	18.2 ± 6.9	25.8 ± 38.3	22.2 ± 10.2	0–35
GGTP (U/L)	45.3 ± 21.2	12.6 ± 7.4	21.8 ± 62.9	39.5 ± 30.2	5–40
Bilirubin (mmol/L)	6.8 ± 3.7	0.5 ± 0.2	0.4 ± 0.2	0.6 ± 0.5	0.3–1.2

ALT, Alanine aminotransferase; *AST*, aspartate aminotransferase; *BDG*, bidirectional Glenn; *GGTP*, gamma-glutamyl transpepsidase; *aPTT*, activated partial thromboplastin time; *PT*, prothrombin time.

The advantage of this study, therefore, is the longitudinal and prospective design that allowed observation of the changes in coagulation factor abnormalities over time in a consistent cohort of patients with the same diagnosis undergoing identical procedures. Although significantly lower levels of both procoagulant and anticoagulant factors were observed at all stages compared with those of healthy controls, it appeared they matured over time in a similar pattern to control subjects, albeit at a slower rate. The maturation in coagulation factor levels is also similar to the hemostatic maturation reported in patients who have undergone a two-ventricle repair,[17] which suggests that the maturation of coagulation factors over time is not specifically related to diagnosis. The conversion from a state of relatively low factor VIII levels early in the course of single ventricle palliation to an increase in factor VIII level after the Fontan operation indicates an acquired disturbance specifically related to the Fontan physiology; the low von Willebrand factor levels measured in the post-Fontan patients with elevated factor VIII levels suggests that the increase in factor VIII was not an acute phase response in these patients. There were no relationships between factor levels and hemodynamic variables at any of the stages in our study. In an earlier study, we[14] reported a correlation between increased central venous pressure and an elevated factor VIII level in post-Fontan patients, but we could not find any such relationship in this longitudinal study. Hepatic synthetic dysfunction (eg, reduced albumin) or evidence of hepatocellular dysfunction (eg, bilirubin, transaminase) did not appear to be associated with coagulation abnormalities either, an important observation given the prominent role of the liver in the synthesis of procoagulants and anticoagulants and the potential for altered liver blood flow and oxygen delivery in these patients.

The importance of an elevated factor VIII level as an independent risk factor for both primary and recurrent venous thrombosis in adult patients without cardiac defects has been reported.[29-31] Factor VIII levels greater than 150% are associated with a 5- to 6-fold increased risk for venous thrombosis when compared with levels below 100%.[32] Goldenberg and coworkers[33] reported that children with increased factor VIII levels were also at increased risk for thrombosis. Elevated factor VIII, D-dimer, or both at diagnosis of thrombosis, and a persistent elevation in children with thrombosis after standard-duration anticoagulant therapy, predicted a poor outcome (ie, lack of thrombus resolution, recurrent thrombosis, or development of a postthrombotic syndrome).

The cause of increased factor VIII levels in patients with the Fontan circulation is unknown. Liver disease can be associated with markedly elevated plasma factor VIII levels,[34,35] whereas the synthesis of many other coagulation factors is reduced; it has been suggested that the increase of plasma factor VIII concentrations may be due to up-regulation of factor VIII synthesis by inflamed liver sinusoidal endothelium. The expression of factor VIII mRNA is particularly prominent in the endothelial lining of the liver sinusoids,[36] and although speculative, it is possible that patients with Fontan physiology may have chronically elevated central and hepatic venous pressure, which could contribute to an increase in factor VIII production from liver sinusoidal endothelium. It has also been recently reported that Fontan patients are at increased risk for liver fibrosis and cirrhosis,[37] although no relationship with factor VIII levels was studied.

It is also important to note that the protein C levels were significantly lower in our patients throughout the different stages. Inasmuch as a high factor VIII level is an independent risk factor for thrombosis in children and adults and a low protein C level also predisposes for thrombosis, this combination along with the Fontan physiology may place these patients at a significantly higher risk for a thromboembolic event.

There are limitations to our study because of sample size and a relatively short follow-up time after the Fontan procedure. Eleven patients were lost to follow-up. When designing the study, we did not confine enrollment to patients from our geographic area; however, if we had done so, the

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number of patients with complete data results would have been higher. An elevated factor VIII level could be a marker of risk for thrombosis, but we are unable to provide recommendations for longer-term anticoagulation. A prospective, randomized study treating post-Fontan patients who have an elevated factor VIII level with or without longer-term anticoagulation therapy is needed to answer this question. Although we noted no clinical evidence for thrombosis in our patients, we did not rule out the presence of intracardiac thrombi with detailed examination such as that provided by transesophageal echocardiography. Furthermore, measuring changes in coagulation factors over time does not provide a complete evaluation of risk for thrombus formation. We did not perform specific platelet function studies nor assess clot formation by thromboelastography. Finally, we did not examine for specific coagulation protein polymorphisms such as factor V Leiden; and it is unknown whether the frequency of specific polymorphisms is increased in children with congenital heart disease.

In summary, on the basis of the known risk for thromboembolic events after the Fontan procedure, along with the increase in factor VIII level demonstrated in this study and the evidence that high levels of factor VIII may be an independent risk factor for thromboembolic events, monitoring factor VIII levels may define a group of at-risk Fontan patients who may benefit from long-term anticoagulation prophylaxis. Further work is necessary to determine whether there may be a critical factor VIII level associated with thromboembolic risk in these patients, and the interaction between elevated factor VIII levels and low levels of native circulation inhibitors of coagulation, such as protein C or prothrombotic genetic polymorphisms, contributing to thromboembolic risk, needs to be determined.

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