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Thromboembolic Complications After Fontan Procedures: Comparison of Different Therapeutic Approaches

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Background. Although patients after Fontan procedure have a high incidence of thromboembolic complications, anticoagulant therapy is not handled uniformly. We analyzed the frequency and clinical relevance of thromboembolism after Fontan procedure and compared different therapeutic approaches.

Methods. From 1986 to 1998, 101 patients (mean age, 7.3 ± 8.1 years) underwent Fontan type procedure (modified Fontan, n = 40; total cavopulmonary connection, n = 61). In 85 of 87 survivors, transthoracic echocardiography was performed; and in 31 transesophageal echocardiography and/or angiography was performed. Mean follow-up was 5.7 ± 3.5 years. Three groups with different anticoagulant regimen were compared: group I without medication (n = 45), group II with acetylsalicylic acid therapy (n = 14) and group III with Coumadin (n = 26).

Results. Thromboembolic events occurred in 13 of 85 patients (15.3%; 3.3 events/100 patient-years). Type of operation as well as other known risk factors had no influence on the rate of thromboembolism. Within the first postoperative year, seven of 13 events occurred. A second peak developed beyond 10 years of follow-up. Patients benefit significantly from Coumadin compared with those who did not receive any medication, with similar results in the entire population and the subgroup of patients with total cavopulmonary connection (log-rank, $p = 0.031$ and $p = 0.033$, respectively). With 4.2 events/100 patient-years, the cumulative event rate was substantially higher in group I than with 1.6 in group II and with 1.1 in group III. No relevant bleeding complications occurred.

Conclusions. Thromboembolism is frequent after Fontan procedure, with a peak during the first postoperative year and another peak beyond 10 years of follow-up. Coumadin is the most effective prophylactic therapy in preventing thromboembolism. Therefore, we suggest initial oral anticoagulation therapy in patients with Fontan type operation.

(Ann Thorac Surg 2002;74:556–62)

The Fontan procedure was first described by Francis Fontan in 1971 [1]. It is currently, in modified form, a well-established definitive surgical palliation for patients with complex cardiac malformations, in cases in which biventricular circulation cannot be achieved [2, 3]. Among various modifications, a significant alteration of the surgical technique was introduced by de Leval and associates [4] in 1988, when they described the total cavopulmonary connection. Over the last decade, hospital mortality has decreased substantially; therefore, late mortality and, especially, late morbidity are now of greater interest. Besides arrhythmias, dysfunction of the systemic ventricle and protein losing enteropathy, thromboembolic complications have a major impact on long-term prognosis [5–8]. Mortality after thromboembolic events in Fontan patients has been reported to be as high as 25% [7]. This high mortality is explained by the fact that any increase of pulmonary vascular resistance leads to deleterious hemodynamic impairment. Apart from venous thromboembolic complications, stroke or arterial embolization have been described in 3% to 19% of all patients [6, 8–10].

Despite the documented frequency and clinical impact of thromboembolic complications, no consensus is found in the literature regarding anticoagulation practices regarding prophylaxis, methods (ie, Coumadin vs antiplatelet agents), or duration of therapy [7, 11, 12]. The aim of the present study was to analyze retrospectively the frequency, time course, clinical relevance, and origin of thromboembolic complications after Fontan-type surgery. Different methods of anticoagulation for prophylaxis were compared for the entire population and for the subgroup of patients who underwent the currently favored total cavopulmonary connection (TCPC).

Patients and Methods

Between January 1986 and December 1998, 101 patients (55 male, mean age: 7.3 ± 8.1 years) underwent modified

Accepted for publication April 10, 2002.

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Published by Elsevier Science Inc

0003-4975/02/$22.00
PII S0003-4975(02)03677-9 21

Fontan procedure for various types of univentricular heart conditions. Preoperative morphologic diagnoses included tricuspid atresia (n = 53), double inlet ventricle (n = 25), and complex malformations (n = 23). The group of patients with complex malformations consisted mainly of patients with criss-cross heart (n = 5), complex forms of congenitally corrected transposition of the great arteries (n = 4), or atrial isomerism (n = 4).

Operative Technique

Two different operative techniques were used to direct the systemic venous return to the pulmonary arteries. An anastomosis between the roof of the right atrium and the pulmonary artery (modified Fontan procedure) was performed in 40 patients. In 26 of these patients, the anastomosis was created with autologous atrial tissue only. In the remaining 14 patients, atrial tissue was not sufficient, and a Gore-Tex patch (W. L. Gore & Associates, Flagstaff, AZ) had to be added. Fenestration was created surgically in 1 patient to allow decompression of the systemic venous atrium. Starting in 1991, the concept of modified Fontan has changed in favor of TCPC, usually carried out by creating an intraatrial tunnel to conduct inferior vena cava blood to the superior vena cava orifice. In 27 of 61 patients with TCPC, the intraatrial tunnel could be created with autologous tissue only. A fenestration was performed in 12 of 61 patients.

Anticoagulation Regimen

Until October 1995, patients with Fontan procedure received either no prophylactic medication or antiplatelet therapy in the postoperative period. Only in 1 patient with a history of deep venous thrombosis was Coumadin (Du Pont Pharmaceuticals, Wilmington, DE) therapy started. After October 1995, we started using intravenous heparin therapy immediately after operation. Then all except 2 patients received Coumadin early postoperatively before hospital discharge. Two patients were placed on acetylsalicylic acid (ASA) only, as their parents declined Coumadin therapy. Because of the initial anticoagulant regimen, patients were divided into three groups: patients without any medical prophylaxis (group I, n = 45); patients treated with ASA at a dose of 2 to 3 mg/kg/d (group II, n = 14); and patients with Coumadin therapy (target international normalized ratio [INR] between 2.2 and 2.7; group III, n = 26).

Follow-Up

Follow-up included clinical examination, electrocardiography, and transthoracic echocardiography (TTE) (Agilent Sonos 5500, Andover, MA) once per year. In addition, transesophageal echocardiography (TEE) was performed in 31 patients. Cardiac catheterization was occasionally performed to evaluate the hemodynamic situation of the patient. Follow-up information was available in 85 of the 87 hospital survivors. The mean follow-up for the entire population was 5.7 ± 3.5 years (range, 10 months to 15 years).

Statistical Analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (version 10.0; SPSS Institute, Chicago, IL). Data are expressed as mean ± standard deviation unless otherwise indicated. Differences between groups were determined using χ^2, Fisher's exact, and unpaired Student's t tests. Multiple logistic regression analysis was performed to identify predictors of thromboembolic events. The Kaplan-Meier method was used to estimate event-free probabilities. Values of p less than 0.05 were considered to be statistically significant. Thromboembolic events were analyzed for the study population and for the three prophylactic groups separately. When comparing the three prophylactic approaches, follow-up was limited to the duration of each patient's initial prophylactic approach, and periods after change of therapy were excluded. Comparison of the groups was performed after 3 and 5 years using the log-rank test. Beyond a follow-up period of 5 years, a comparison was not meaningful because of the small number of patients in groups II and III.

Results

Outcome

Overall hospital mortality was 13.9% (14 of 101 patients), but was substantially lower (4%, 2 patients) in the last 50 patients. None of the early deaths was caused by thromboembolic complications. Of four late deaths, in one an acute pulmonary artery embolism was the cause 41 months after TCPC. Overall survival was 85% at 1 year, 82% at 5 years, and 79% at 10 years, respectively. Clinical condition was excellent or good in 94% and reduced in 6% at last follow-up.

Thromboembolic Events

Thromboembolic events or thrombus formation were detected in 13 of 85 hospital survivors (15.3%), for an overall rate of 3.3 events per 100 patient-years.

Eight patients had a clinical episode of thromboembolism (symptomatic thrombus) (Table 1). Two patients suffered from stroke with left-sided hemiplegia. The postevent cardiac catheterization of the normal contracting systemic ventricle failed to detect thrombi, but revealed a residual pulmonary artery trunk or a rudimentary, previously subpulmonary ventricle in both patients. Both patients did not have a fenestration. Symptomatic events in the venous circulation included a 24-year-old women with erysipelas and severe protein losing enteropathy. She experienced extensive superior vena cava thrombosis originating at a central venous line inserted for albumin substitution and died after pulmonary artery embolism despite Coumadin therapy. Five months postoperatively and 2 months after withdrawal of Coumadin therapy, a 32-year-old woman experienced pulmonary embolism of the right middle and lower lobe. After resumption of anticoagulant therapy, she recovered without persistent perfusion defects or elevated pressure in the venous circulation. In another patient, a symptom-

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Table 1. Data of Patients With Symptomatic Thromboembolic Events

	Systemic		Venous					
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Anatomy	TA	DIV, L-TGA	DIV, L-TGA, PS, MS	Re-ISO, TA, PS, PAPVR	DIV, L-TGA, PAB	TA	TA	TA
Type of surgery	Fontan	TCPC	TCPC	TCPC	TCPC	Fontan	Fontan (RA-PA-Conduit)	Fontan
Thromboembolic event	Stroke	Stroke	Thrombus SVC-PA	Innominate vein thrombosis	Pulmonary embolism	RA Thrombus	RA/Conduit thrombosis	RA Thrombus
Interval to surgery (mo)	1	2	41	3	5	175	136	162
Age at event (y)	0.9	5	24	6	33	17	15	18
Anticoagulant prophylaxis	No	ASA	Coumadin	No	No	No	No	No
Outcome	Treated with Heparin	Treated with Heparin and Coumadin	Died	Treated with Heparin	Treated with Heparin and Coumadin	Treated with Heparin and Coumadin	Thrombectomie and Conversion in TCPC	Thrombectomie and Conversion in TCPC

ASA = acetylsalicylic acid; DIV = double inlet ventricle; ISO = isomerism; L-TGA = levotransposition of the great arteries; MS = mitral stenosis; PA = pulmonary artery; PAB = banding of the pulmonary artery trunk; PAPVR = partial anomalous pulmonary venous return; PS = pulmonary stenosis; RA = right atrium; SVC = superior vena cava; TA = tricuspid atresia; TCPC = total cavopulmonary connection.

atic venous thrombosis of the innominate vein resistant to thrombolytic therapy led to a long-standing chylothorax. Two patients with excessively enlarged right atrium developed atrial flutter and life-threatening right atrium–pulmonary artery conduit thrombosis in 1 case and a mobile right atrial thrombosis obstructing the pulmonary artery anastomosis in the other, necessitating urgent operation with conversion to total cavopulmonary connection more than 10 years after modified Fontan operation. Both patients were doing well at last follow-up 12 and 18 months after the event. One patient suffered from atrial flutter 14.5 years after modified Fontan operation. A thrombus at the lateral portion of the enlarged right atrium was revealed with TEE, and the patient received heparin and amiodarone. On TEE before discharge, the thrombus had resolved. The patient was discharged on a regimen of Coumadin and was doing well 8 months thereafter.

In 5 patients a thrombus was detected without clinical suspicion (asymptomatic thrombus) (Table 2). A large thrombus adherent to a central venous catheter in the superior vena cava was observed during angiography of the cavopulmonary anastomosis on postoperative day 6. Local thrombolytic therapy with recombinant tissue plasminogen activator was successful and showed a good long-term result as documented by angiography 1 year later. In 1 patient, TTE demonstrated an echogenic thrombus in the residual pulmonary artery trunk. Three asymptomatic thrombi either in the proximal pulmonary artery trunk (n = 2) or in the right atrium (n = 1) were diagnosed by TEE. All of them had been missed using TTE alone.

Time Course of Thromboembolic Events

Freedom from thromboembolism over the follow-up period is depicted in Figure 1. More than half (seven of 13 events) of all observed thromboembolic events occurred within the first year after modified Fontan procedure and 70% within the third year. After 3.5 years a plateau is reached, with no additional risk of thromboembolism over several years. A second peak of thromboembolic events appeared beyond 10 years of follow-up. Three of 11 patients with follow-up of more than 10 years experienced a thromboembolic event. All patients had an excessively enlarged right atrium after modified Fontan operation and new onset of atrial flutter. It was unclear whether the atrial arrhythmia occurred first or was followed by the thromboembolism.

Risk Factors

The thromboembolic events were not correlated with the following potential risk factors: age at operation, sex, presence of fenestration, use of patch material, closed main pulmonary artery pouch, protein losing enteropathy, dysfunction of the systemic ventricle, or atrial arrhythmias. Although not statistically significant, it is important to note that atrial arrhythmias were present in all 3 patients with a follow-up of more than 10 years and symptomatic thromboembolic events. Thrombus formation was equally detected after modified Fontan proce-

Table 2. Data of Patients With Asymptomatic Thrombus Formation

	Patient Number				
	1	2	3	4	5
Anatomy	TA	DIV, PS, MS	DIV, L-TGA, PS	Re-ISO, TA, TGA, PS	TA
Type of surgery	TCPC	TCPC	TCPC	TCPC	Fontan
Thrombus	Thrombus SVC	Thrombus residual PA trunk	Thrombus residual PA trunk	Thrombus residual PA trunk	RA Thrombus
Interval to surgery (mo)	0.2	0.2	23	32	7
Age at event (y)	1.6	5	16	4	11
Anticoagulant prophylaxis	No	no	No	no	No
Outcome	Treated with Heparin and Coumadin	Treated with Heparin	Treated with Heparin and Coumadin	Treated with Heparin and Coumadin	Treated with Heparin and Coumadin

DIV = double inlet ventricle; ISO = isomerism; L-TGA = levotransposition of the great arteries; MS = mitral stenosis; PA = pulmonary artery; PS = pulmonary stenosis; SVC = superior vena cava; TA = tricuspid atresia; TCPC = total cavopulmonary connection

dure or TCPC (15.1% vs 15.6%), although the duration of follow-up was significantly longer after modified Fontan (6.5 ± 4.6 years vs 3.5 ± 2.1 years, $p = 0.0001$). Moreover, no patient received Coumadin after modified Fontan. Freedom from thrombus formation at 3 and 5 years after modified Fontan operation was 94% and 94%, compared with 91% and 85% after TCPC. There was no significant difference between these groups ($p = 0.43$ at the third postoperative year, $p = 0.201$ at the fifth postoperative year). Minor protein C and S deficiency was observed in some patients with events, but coagulation abnormalities have not been studied systematically in the whole population.

Thromboembolic Events Under Different Anticoagulant Regimen

The majority of thromboembolic events (n = 10) occurred during follow-up without antithrombotic prophylaxis (group I). The ASA medication (group II) did not prevent systemic embolization in 1 child. One patient had a pulmonary artery embolism despite adequate Coumadin therapy (group III). Comparison of the three groups with regard to thromboembolic events and possible risk factors are shown in Table 3. The cumulative event rate in group I was 4.2 events/100 patient-years, which is 3.8 times higher than 1.1/100 patient-years in group III. Group II had an intermediate rate of thromboembolic events with 1.6 events/100 patient-years. There were no relevant bleeding complications or other severe side effects of antithrombotic treatment in both groups II and III. Thromboembolic event-free survival with regard to the initial anticoagulant regimen is depicted in Figure 2. Up to the third postoperative year, patients benefitted significantly from Coumadin compared with patients in group I ($p = 0.031$ at the third postoperative year), and showed a tendency toward better event-free survival compared with patients in ($p = 0.17$). Thereafter, although not statistically significant, possibly because of the small absolute number of patients and events, there was a tendency in favor of Coumadin prophylaxis than without medication ($p = 0.12$ at 5 years).

In addition, a comparison of the different therapeutic approaches for the subgroup of patients with TCPC was performed to eliminate the interaction of duration of follow-up and type of operation on our results. The mean follow-up was comparable in all three groups (group I, n = 21: 3.5 ± 2.8 years; group II, n = 6: 2.9 ± 2.5 years; group III, n = 26: 3.6 ± 1.3 years; $p = 0.82$). Thromboem-

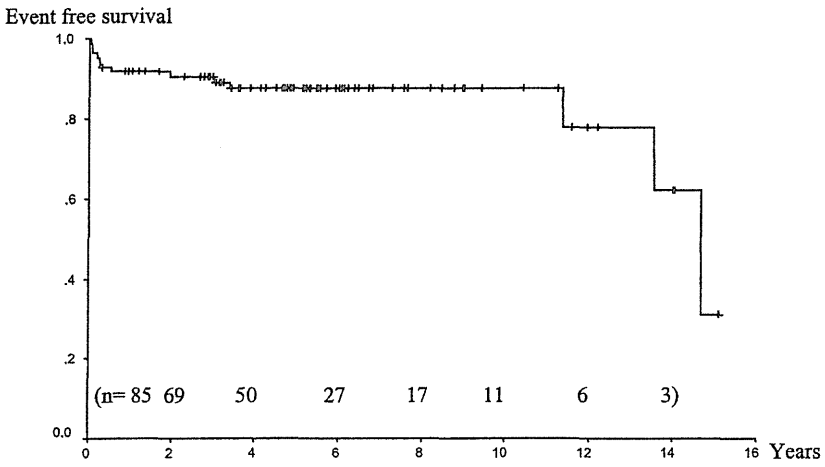

Fig 1. Freedom from thromboembolism in 85 patients with modified Fontan operation. Data in parentheses *indicate numbers of patients at risk.*

Table 3. Comparison of the Initial Anticoagulant Regimen With Regard to Thromboembolic Events and Possible Risk Factors

	Group I (n = 45)	Group II (n = 14)	Group III (n = 26)	p Value
Thromboembolic events[a]	10 (22%)	1 (7%)	1 (4%)	0.072
Mean follow-up (y)[b]	5.3 ± 4.5	4.4 ± 2.8	3.6 ± 1.3	0.134
Type of operation				0.001
TCPC	21	6	26	
Modified Fontan	24	8	0	
Atrial arrhythmias	13 (28%)	2 (14%)	3 (12%)	0.272
Impaired LV function	2 (4%)	1 (7%)	1 (4%)	0.916
Age at operation (y)	8.8 ± 9.4	5.8 ± 7.4	5.9 ± 7.1	0.287

[a] The number of thromboembolic events of group I to III do not match with the total number because of exclusion of one thromboembolic event occurring 2 months after withdrawal of Coumadin. p = 0.036 between group I and group III. [b] p = 0.018 between group I and group III.

bolic event-free survival at 3 and 5 years was 72% in group I, 83% in group II, and 100% and 93% in group III. Patients treated with Coumadin showed significant benefit compared with untreated patients (log-rank test, p = 0.033).

Comment

Thrombotic and thromboembolic complications after Fontan-type procedures are a common and serious problem. In the present study, the incidence during long-term follow-up was 13 of 85 consecutive patients (15.3%; 3.3 events/100 patient years). This is similar to most other recent studies, which have reported a 10% to 20% prevalence of thromboembolic complications in this patient population [6–8, 11, 12].

Of all patients with thrombus formation in our study, 60% experienced a symptomatic event, whereas asymptomatic thrombus was found by chance in the others (mostly detected only by means of TEE). In the past, TEE has been proved to be superior to TTE for the diagnosis of thrombus formation in Fontan patients [13, 14]. Thus, our study (as most previous studies) may underestimate the true frequency of asymptomatic thrombus formation because TEE was not used systematically. This is mainly due to the fact that the majority of patients were children who required in a high percentage general anesthesia to undergo TEE. Therefore, TEE was performed in selected patients only.

Risk Factors for Thromboembolism

In 1986, Matthews and coworkers [10], in a small study group, already recognized that Fontan patients are at risk for postoperative cerebrovascular events. Three of 16 patients (19%) who survived the early postoperative period experienced stroke within 3 months after surgery. The substantially lower prevalence of stroke of 2.3% in the present study was similar to a rate of 2.6% reported in a large study with 645 patients [9]. Systemic embolic complications require either a residual right-to-left shunt at the atrial level with paradoxical embolism of primarily venous thrombi or low-flow areas in connection to the systemic ventricle, with a potential for thrombus formation and its subsequent embolization. In recent studies, thrombus formation in the residual pulmonary artery trunk was detected in 4% to 6% of cases [11, 15]. Thrombotic material may reach the systemic circulation if pulmonary valve regurgitation is present. Similarly, angiography of the systemic ventricle revealed the potential source of postoperative embolic stroke in our 2 patients: long-standing opacification of the residual pulmonary artery trunk, indicating low-flow areas. In addition, asymptomatic thrombus formation in the residual pulmonary artery trunk was found in 3 other patients. As a consequence finding a persistent flow in the residual pulmonary artery trunk, oversewing or even excision of the pulmonary valve have been proposed to prevent from systemic embolization out of this area [11, 15, 16].

Other potential risk factors for venous and systemic thrombosis or embolic events after Fontan-type procedures have been described in literature. Morphologic and functional factors such as patch material used for the lateral tunnel, fenestration, excessive right atrial enlargement, severe dysfunction of the systemic ventricle, as well as supraventricular tachycardia or atrial flutter may

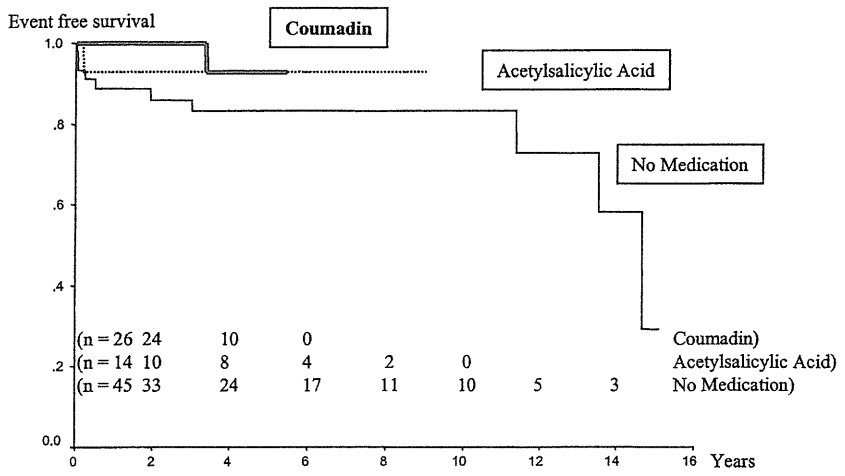

Fig 2. Kaplan-Meier plot for the three initial prophylactic approaches (n= 85): patients with Coumadin therapy (boldface solid line), *with acetylsalicylic acid* (broken line), *and without anticoagulant prophylaxis* (solid line). Data in parentheses *indicate numbers of patients at risk. Log-rank test between Coumadin and no medication:* p = *0.031 after 3 years and* p = *0.12 after 5 years. Log-rank test between Coumadin and acetylsalicylic acid:* p = *0.17 after 3 years and* p = *0.67 after 5 years.*

promote thrombus formation in the right or left atrium [6, 8, 9, 17]. None of these factors could be identified in our study. As in recent studies [8, 18], the type of operation (modified Fontan, TCPC) had no influence on the actuarial freedom from thrombus formation.

Coagulation abnormalities predisposing for thromboembolic events have been demonstrated in patients after Fontan-type procedures. It was recommended that these patients should be treated with anticoagulation [19, 20]. In-dwelling central venous catheters were a possible source for thrombus formation. Therefore, it was our policy throughout the study to remove the central venous catheter as soon as possible.

Time Course of Thromboembolic Events

Rosenthal and colleagues [8] observed a fairly constant risk for thromboembolism up to 14 years after surgery. In contrast, the majority of events in our study occurred within the first postoperative year, as also reported by others [6, 10, 11]. After 3.5 years a plateau was reached followed by a second peak beyond 10 years of follow-up.

The number of patients who survive more than 10 years after Fontan procedure will increase constantly in the near future. Therefore, therapeutic and prophylactic concepts to avoid thromboembolic complications and arrhythmias in patients at special risk are needed. Recently, the conversion from Fontan connection to intracardiac TCPC has been described as a therapeutic approach in patients with excessive enlargement of the venous atrium to improve blood flow characteristics and to prevent from thrombus formation and atrial arrhythmias [21, 22]. In 2 of our patients, this conversion has been performed successfully. Another therapeutic option, used in one of our patients, could be the treatment with antiarrhythmic medication and anticoagulant therapy. However, the best management for these patients is still unknown, and further studies investigating long-term outcome are urgently required to find a consensus on type and duration of prophylactic anticoagulant therapy.

Prophylactic Antithrombotic Therapy

Prophylactic anticoagulation therapy is frequently recommended [11, 12, 14, 20], but at this time there is no prospective study investigating different anticoagulation regimen in Fontan patients. There are several studies and case reports on the value of anticoagulant prophylaxis in Fontan patients. Nevertheless, systemic or venous thromboembolic events have been reported even during ASA or Coumadin therapy in selective cases [14, 18]. However, all of these studies did not compare different therapeutic approaches. Because the therapeutic regimen in our center changed in 1995, we were able to compare different prophylactic approaches over a follow-up period of 5 years postoperatively. Since October 1995, all except 2 of our patients received heparin immediately after operation, followed by Coumadin. We kept the INR target level between 2.2 and 2.7. When comparing the different therapeutic approaches in our entire population, patients with Coumadin were shown to have significantly fewer thromboembolic complications up to the third year when compared to patients without medication. Thereafter, we saw a tendency in favor of Coumadin prophylaxis as the most effective regimen. The cumulative thromboembolic event rate was approximately 4 times higher in patients without medication than with Coumadin. Patients with ASA medication had an intermediate state with a higher rate than with Coumadin, but a lower rate than without medication. Because the modified Fontan procedure has now been abandoned by most surgeons, we consequently compared the different therapeutic approaches in the subgroup of patients with TCPC. Hereby, we eliminated the possible interaction of duration of follow-up and type of operation on our results. Patients benefit significantly from Coumadin compared with no medication. However, the absolute number of thromboembolic events in general, and of patients with ASA medication, is very limited, and constitutes the major limitation of this study. The potential benefit of ASA compared with no medication, and its role in relation to Coumadin, must be reproduced in a larger patient sample as part of a multicenter clinical trial. So far, we believe that our data support the concept of early postoperative anticoagulation using heparin followed by oral Coumadin therapy after Fontan-type surgery. The benefit of Coumadin therapy must, of course, be weighed against its risk. In recent studies, the risk of bleeding complications in children with mechanical valves and long-term Coumadin therapy is known to be within a range of 0.8 to 4.0 events/100 patient-years, depending on and increasing with the target INR [23, 24]. Thus, it is even more important to quantify exactly the benefit of anticoagulant therapy in a higher number of children after Fontan procedure. Furthermore, no information on the optimal target INR is available.

In conclusion, thromboembolic events are commonly observed after Fontan-type procedures. In our patient population, thromboembolic events were detected in 13 of 85 hospital survivors, with a peak each during the first postoperative year and beyond 10 years of follow-up. When comparing different anticoagulant regimens, Coumadin was the most effective prophylactic therapy for preventing thromboembolism. Therefore, we suggest initial oral anticoagulation in patients undergoing Fontan-type operations.

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International Journal of Cardiology 168 (2013) 3940–3943

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Effect of Aspirin and warfarin therapy on thromboembolic events in patients with univentricular hearts and Fontan palliation

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ARTICLE INFO

Article history:
Received 25 May 2013
Accepted 29 June 2013
Available online 17 July 2013

Keywords:
Fontan
Thromboembolism
Thrombus
Aspirin
Warfarin
Anticoagulation

ABSTRACT

Background: Patients with univentricular hearts and Fontan palliation are at risk for thromboembolic complications. While aspirin and warfarin therapies are currently the mainstay of prophylaxis, controversy exists as to the optimal prevention strategy.
Methods: A cohort study was conducted on the New England registry of patients born in 1985 or earlier with Fontan surgery at Boston Children's Hospital, in order to assess and compare the effect of prophylactic aspirin and warfarin on incident thromboembolic events.
Results: A total of 210 qualifying patients (49% male) underwent Fontan surgery at a median age of 8.5 years: 48.6% had a right atrium to pulmonary artery anastomosis, 11% a right atrium to right ventricle conduit, 38.6% a lateral tunnel, and 1.9% an extracardiac conduit. No thromboembolic prophylaxis was prescribed to 50.0%, whereas 24.3% received aspirin, and 25.7% warfarin. In multivariate analyses, lack of aspirin or warfarin was associated with a significantly higher thromboembolic event rate when compared to therapy with either [hazard ratio 8.5, 95% confidence interval (3.6–19.9), $P < 0.001$], with no difference between the two treatment strategies ($P = 0.768$). Twenty-year freedom from thromboemboli was 86% versus 52% in patients with and without thromboprophylaxis, respectively. Other factors independently associated with thromboemboli were a low post-operative cardiac index [hazard ratio 2.6, 95% confidence interval (1.2, 5.9)] and atrial fibrillation or flutter [hazard ratio 3.1, 95% confidence interval (1.2, 8.0)].
Conclusions: Prophylaxis with either aspirin or warfarin was associated with a significantly lower rate of incident thromboembolic events following Fontan palliation, with no difference between the two therapies.

1. Introduction

The Fontan procedure encompasses a family of related operations [1–7] with the common goal of palliating the "univentricular heart" [6] by rerouting systemic venous return directly to the pulmonary arterial circulation. Outcomes have improved considerably over the last 25 years such that patients are now increasingly surviving into adulthood, exposing them to various longer-term complications, including heart failure, arrhythmias, and thrombosis [4–6,8–12]. Thromboembolic complications are a well-recognized source of morbidity [13–15] and mortality [9] in patients with Fontan physiology. Thromboembolic death may occur suddenly or be preceded by other thromboembolic events, which may or may not be symptomatic [16–18]. As many as 25% of clinical thromboembolic events in patients with Fontan surgery are fatal [16,18]. While the prevention of thromboembolic events is a desirable clinical goal, controversy exists as to the optimal prophylaxis strategy [16,18–25]. Given the paucity of evidence regarding prophylactic therapy with newer antithrombotic regimens in this patient population [24], aspirin (ASA) or warfarin remain the mainstay of antiplatelet or anticoagulant therapy. We, therefore, sought to evaluate and compare the effect of prophylactic ASA and warfarin on incident thromboembolic events in a cohort of patients with Fontan palliation.

2. Methods

2.1. Study cohort

The New England Fontan registry included all patients who lived in the New England area, were born before 1985, and had Fontan surgery between April 1973 and July 1991 at Boston Children's Hospital. Details of this cohort have been previously described [9,10]. Patients with surgery limited to cavopulmonary shunts were ineligible, including those with an interrupted inferior vena cava and azygos extension to a superior vena cava who underwent a bidirectional cavopulmonary shunt. For the current analysis, patients with early post-operative death (<30 days) or a thromboembolic event within the first 14 days of Fontan surgery were excluded [26,27]. The study

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http://dx.doi.org/10.1016/j.ijcard.2013.06.058

protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee.

2.2. Definitions

Systemic ventricular morphology (left, right, or both) and atrioventricular valve anatomy were categorized in accordance with previously defined nomenclature [28] on the basis of preoperative studies and surgical observations. The type of Fontan procedure was classified as right atrial (RA) to pulmonary artery (PA) anastomosis, RA to right ventricle (RV) connection, intracardiac lateral tunnel (LT), or extracardiac conduit (ECC). In all cases, surgery involved separating systemic from pulmonary venous return by excluding the systemic venous return from the systemic ventricle, with or without a residual atrial communication or baffle fenestration. Fontan fenestration, surgical arrhythmia ablation, and concomitant surgery for associated anomalies were recorded as separate variables.

The "era" of the Fontan procedure was classified as "early" if surgery was performed prior to 1985 and "late" if in 1985 or after. "Revision" was defined as a repeat operation that sought to correct a defect in the original Fontan palliation. A surgery that sought to transform an earlier Fontan into another type of Fontan was termed a "conversion". Atrial fibrillation was defined as the absence of consistent P waves on electrocardiography, with rapid oscillations or fibrillatory waves typically associated with an irregular ventricular response. Intra-atrial re-entry/atrial flutter was defined as a regular atrial rhythm with a constant atrial rate ≥200 bpm.

2.3. Outcomes

The primary outcome was the occurrence of a fatal or non-fatal thromboembolic event, whether within the systemic or pulmonary circulation. All clinical thromboembolic events required documentation by non-invasive imaging, angiography or post-mortem evaluation. Death was considered thromboembolic if deemed secondary to thrombus identified either clinically or posthumously. The site of thromboembolism was further classified in one or more of the following locations: right atrium/Fontan, superior vena cava, inferior vena cava, systemic venous atrium, pulmonary artery, or other.

2.4. Statistical Analyses

Continuous variables are summarized by median values and interquartile ranges (IQR; 25th, 75th percentile). Categorical variables are presented as frequencies and percentages. Baseline comparisons between patients with no thromboembolic prophylaxis, ASA, and warfarin therapy were performed by Kruskal-Wallis, chi-squared, or Fisher Exact tests when appropriate. Survival free from a first thromboembolic event according to the type of thromboembolic prophylaxis was plotted using the Kaplan–Meier method and compared by the log-rank statistic. Time 0 was defined as time of Fontan surgery, after which patient-years were accrued until occurrence of the primary outcome or upon censoring. Censoring occurred at the last follow-up visit, upon takedown of the Fontan circulation, or at the time of Fontan conversion, cardiac transplantation, or non-thromboembolic death.

Predictors of thromboembolic events were explored in univariate and multivariate Cox regression analyses, from which hazard ratios and 95% confidence intervals (CI) were generated. For multivariate analyses, an automated stepwise algorithm was used, with levels of significance for entry and staying in the model of 0.25. The type of prophylactic therapy and presence of atrial fibrillation or flutter were forced into the model as covariates. In order to test the robustness of the primary analysis, several sensitivity analyses were performed including 1) omitting reoperation (revision or conversion surgery) from the censoring definition, 2) varying the exclusion period for early thromboembolic events from 14 to 30 days, and 3) varying the early death exclusion criterion from 30 to 14 days.

Two-tailed values of P-values <0.05 were considered statistically significant. All statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, North Carolina).

3. Results

3.1. Baseline characteristics

A total of 210 patients, 49.0% of whom were male, underwent a first Fontan surgery at a median age of 8.5 (IQR 4.8, 15.2) years. Seventy-two (34.3%) patients had tricuspid atresia, 51 (24.3%) a double-inlet left ventricle, and 4 (1.9%) hypoplastic left heart syndrome. The single ventricle was morphologically left in 70.5%. The type of first Fontan procedure was a RA to PA connection in 102 (48.6%) patients, RA to RV conduit in 23 (11.0%), lateral tunnel in 81 (38.6%), and extracardiac tunnel in 4 (1.9%). No thromboembolic prophylaxis was prescribed in 50.0%, 24.3% received ASA, and 25.7% warfarin-based therapy. Patient characteristics and clinical events during follow-up according to the prophylaxis strategy are presented in Table 1.

3.2. Clinical Events

Over a median follow-up of 14.5 years (IQR 8.7–18.4 years), 25 (9.5%) patients had an eventual surgical conversion, 5 (2.4%) had at least one surgical revision, 23 (11%) were transplanted, and 34 (16%) died. Overall, a total of 40 (19.%) patients experienced a thromboembolic event, 5 (12.5%) of which were fatal. The thromboembolic

Table 1
Patient characteristics.

	All Patients N = 210	No Prophylaxis N = 105	ASA N = 51	Warfarin N = 54	P-value†
Age at Fontan, years*	8.5 (4.8, 15.2)	7.0 (4.3, 13.1)	6.8 (4.5, 15.9)	11.3 (8.0, 17.6)	0.0006
Male, N (%)	103 (49)	49 (47)	26 (51)	28 (52)	0.7889
Type of congenital heart disease, N (%)					0.4715
Tricuspid atresia	72 (34)	36 (34)	13 (25)	23 (43)	
Double-inlet left ventricle	51 (24)	26 (25)	14 (27)	11 (20)	
Hypoplastic left heart syndrome	4 (2)	1 (1)	1 (2)	2 (4)	
Other	83 (40)	42 (40)	23 (45)	18 (33)	
Systemic right ventricle, N (%)	55 (26)	26 (25)	14 (27)	15 (28)	0.8773
Type of Fontan, N (%)					0.0008
Lateral tunnel	81 (39)	35 (33)	28 (55)	18 (33)	
RA-PA anastomosis	102 (49)	58 (55)	22 (43)	22 (41)	
RA-RV connection	23 (11)	11 (10)	0 (0)	12 (22)	
Extracardiac conduit	4 (2)	1 (1)	1 (2)	2 (4)	
Early surgical era (pre-1985), N (%)	69 (33)	36 (34)	7 (14)	26 (48)	0.0006
Low cardiac index (<2.35 mL/min/m^2), N (%)	80 (38)	35 (33)	20 (39)	25 (46)	0.2771
Atrial fibrillation or flutter, N (%)	95 (45)	33 (31)	24 (47)	38 (70)	<0.0001
Ablation procedure, N (%)	49 (23)	17 (16)	10 (19)	22 (41)	0.0032
Follow-up, years*	14.5 (8.6, 18.1)	12.0 (4.8, 16.4)	15.8 (13.5, 18.7)	17.1 (10.9, 20.1)	<0.0001
Redo-surgery, N (%)					
Fontan revision	5 (2)	1 (1)	1 (2)	3 (6)	0.1902
Fontan conversion	20 (10)	8 (8)	5 (10)	7 (13)	0.5594
Transplantation	23 (11)	8 (8)	6 (12)	9 (17)	0.1962
Thromboembolic event, N (%)	40 (19)	28 (27)	5 (10)	7 (13)	0.0207

ASA denotes aspirin; RA, right atrium; PA, pulmonary artery; RV, right ventricle.
* Continuous variables are summarized by median and interquartile range (25th, 75th percentile).
† Comparison between the three prophylaxis groups.

events consisted of thrombus within the RA/Fontan in 25 patients (62.5%), limited to the PA in 3 (7.5%), the superior vena cava in 2 (5%), and the pulmonary venous atrium in 3 (7.5%). No clots were identified within the inferior vena cava and precise location information was missing for the remaining 7 (17.5%) cases. In one patient with a fatal thromboembolic event, thrombolysis with recombinant tissue plasminogen activator was unsuccessful, and a second patient died despite an attempted thrombectomy.

3.3. Prophylactic strategy and thromboembolic events

Overall, 28 (26.7%) thromboembolic events occurred among 105 patients without any prophylaxis (median follow-up 12.0 years), 5 occurred among the 51 (9.8%) patients on ASA therapy (median follow-up 15.8 years), and 7 occurred among 54 (13.0%) patients on warfarin-based therapy (median follow-up 17.1 years). In the 7 patients receiving warfarin, the international normalized ratio (INR) was therapeutic (≥2.0) in 1, subtherapeutic in 2, and unknown in 4 at the time of the thromboembolic event.

Univariate and multivariate predictors of incident thromboembolic events are summarized in Table 2. In univariate analyses, warfarin was associated with a significantly lower thromboembolic event rate [hazard ratio 0.39, 95% CI (0.16, 0.96), P = 0.040]. Aspirin was associated with a numerically similar albeit non-significant reduction in the thromboembolic event rate [hazard ratio 0.37, 95% CI (0.13, 1.06), P = 0.063]. In multivariate analyses, there was no significant advantage of warfarin compared to ASA therapy (P = 0.768). The lack of ASA or warfarin was associated with a highly significant 8.5-fold increase in the thromboembolic event rate when compared to therapy with either [adjusted hazard ratio 8.49, 95% CI (3.64–19.86), P < 0.0001]. The Kaplan-Meyer plot depicting freedom from a first thromboembolic event is shown in Fig. 1. In patients without aspirin or warfarin therapy, freedom from a thromboembolic event was 92% at 10 years and 52% at 20 years. For those receiving aspirin or warfarin prophylaxis, corresponding 10 and 20-year event-free survival rates were 94% and 86%, respectively.

Other factors independently associated with a higher thromboembolic event rate in multivariate analyses were a low post-operative cardiac index [adjusted hazard ratio 2.63, 95% CI (1.17, 5.93), P = 0.0199] and a diagnosis of atrial fibrillation or flutter [adjusted hazard ratio 3.10, 95% CI (1.20, 7.96), P = 0.0190]. Prespecified sensitivity analyses with alternate group assignment algorithms and exclusion criteria did not alter the results appreciably.

4. Discussion

The key findings of this analysis from the New England Fontan registry are that 1) prophylaxis with either aspirin or warfarin is associated with a significantly lower incident thromboembolic event rate; 2) no signal favored one therapy over the other; 3) the residual risk of thromboemboli remains substantial despite ASA or warfarin therapy;

Table 2
Factors associated with incident thromboembolic events.

Characteristic	Hazard ratio	95% CI	P-value
Univariate			
ASA therapy	0.37	0.13, 1.06	0.0633
Warfarin therapy	0.39	0.16, 0.96	0.0400
ASA or warfarin therapy	0.25	1.88, 8.46	0.0003
Low cardiac index (<2.35 mL/min/m^2)	2.33	1.16, 4.68	0.0172
Atrial fibrillation or flutter	2.44	1.10, 5.43	0.0290
Multivariate			
Lack of ASA or warfarin therapy	8.49	3.63, 19.86	<0.0001
Low cardiac index (<2.35 mL/min/m^2)	2.63	1.17, 5.93	0.0199
Atrial fibrillation or flutter	3.10	1.20, 7.96	0.0190

CI denotes confidence interval; ASA, aspirin.

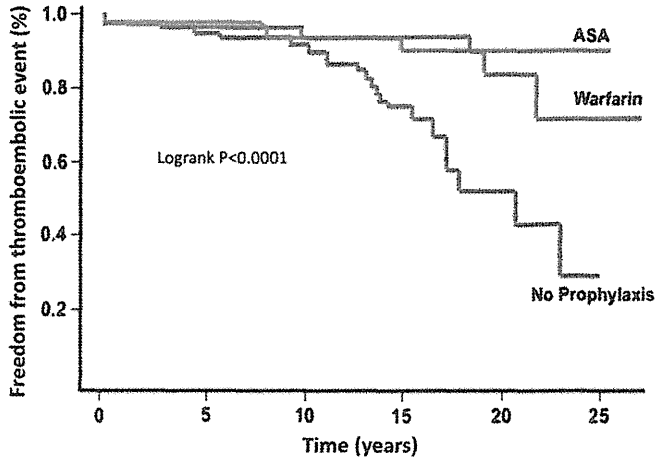

Fig. 1. Freedom from thromboemboli according to the type of prophylactic therapy. The Kaplan–Meier curves depict freedom from an incident thromboembolic event according to whether patients received thromboprophylaxis with aspirin (ASA), warfarin, or neither.

and 4) other factors associated with a higher risk of thromboembolic events include a low post-operative cardiac index and a history of atrial fibrillation or flutter.

The high incidence and the type of thromboembolic events observed in our patient population (i.e., 48% at 20 years in patients without prophylactic therapy) are consistent with previous reports [9,13,15,16,18,25–27]. During the study period, no formal departmental policy was implemented for anticoagulation therapy, although standard indications (e.g., atrial tachyarrhythmias) were generally respected. The major reduction in the thromboembolic event rate with ASA or warfarin therapy (i.e., 14% at 20 years in patients without prophylactic therapy, representing a 71% unadjusted relative risk reduction) would suggest that some form of prophylaxis should be considered as the mainstay treatment for patients with Fontan palliation, barring contraindication.

The optimal thromboprophylaxis strategy remains a source of controversy [16,19,22]. The observed lack of superiority of one strategy over the other is consistent with a recent clinical trial that compared ASA to heparin/warfarin therapy in children during the first two years after Fontan surgery [16]. Our study extends these findings to an older population with over 14 years of follow-up. Reasons as to why warfarin therapy does not outperform ASA in this patient population remain speculative and may reflect the multifactorial mechanisms of thrombus formation in Fontan patients, including decreased cardiac output, abnormal venous flow, prosthetic material, blind cavities, lack of atrioventricular synchrony, coagulation defects, and platelet abnormalities [16,18,29–31].

Alternatively, the lack of superiority of prophylactic anticoagulation versus antiplatelet therapy may be due, in part, to difficulties achieving and maintaining therapeutic INR levels [16,18,32,33]. In an exploratory analysis of the pediatric Fontan study, patients on warfarin who often failed to meet targeted INR levels experienced a significantly higher rate of thromboemboli than those who consistently achieved targeted INR levels or received ASA therapy [18]. Our results and others suggest that alternate thromboprophylaxis strategies should be explored in light of the high prevalence of thromboembolic events in this patient population, difficulties achieving consistent protection with warfarin therapy, and the considerable residual risk that remains in patients treated with warfarin or ASA.

4.1. Study limitations

The study is observational in nature and subject to the limitations inherent to retrospective designs, particularly with regards to unmeasured

potential confounders. INR values were not routinely collected during the >14-year follow-up, precluding exploratory analyses regarding the prevalence of subtherapeutic anticoagulation and its potential impact. In addition, study power was limited by the 40 thromboembolic events. The final Cox regression model contained 4 covariates in addition to the presence or absence of prophylactic therapy and history of atrial tachyarrhythmias, resulting in 6 degrees of freedom. Such a model may be at risk of "over-fitting", since fewer than 10 outcome events occurred per predictor variable. However, this approach was deemed justified given the potential for important confounding in this cohort and our impression that a model that did not adequately control for such would have limited utility. Moreover, simulation studies suggest that problems are seldom encountered with 5–9 events per predictor variable [34].

5. Conclusion

A high rate of incident thromboembolic events was observed in the New England cohort of Fontan patients followed for over 14 years. Prophylactic therapy with ASA or warfarin was associated with a significant reduction in the thromboembolic event rate, with the two treatment strategies yielding similar outcomes. Despite protective therapy, patients remained at substantial residual risk for thromboembolic events. Adequately powered prospective trials comparing these and other prophylactic antithrombotic regimens in this high-risk population are warranted. In the interim, in light of these results, it would appear reasonable to favor some form of routine thromboprophylaxis.

Statement of authorship

The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Conflict of interest disclosures

Dr. Khairy has received research support for an investigator-initiated grant from Boehringer Ingelheim.

Acknowledgement

Drs. Susan Fernandes, Michael Landzberg, and Paul Khairy received research support from the Dunlevie Foundation.

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Pediatr Cardiol (2013) 34:262–272
DOI 10.1007/s00246-012-0431-4

ORIGINAL ARTICLE

Thromboembolic Complications in Fontan Patients: Population-Based Prevalence and Exploration of the Etiology

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Received: 2 May 2012 / Accepted: 29 June 2012 / Published online: 28 July 2012

Abstract After the Fontan procedure, patients face an increased risk for thromboembolic events (TE). The etiology for this increased thrombogenecity is incompletely understood. This study aimed to determine the prevalence of TE in Danish Fontan patients and to bring new insights into the etiology of TE. Using a population-based design, we retrospectively identified all TEs in 210 Fontan patients. Whole blood assays (thromboelastography, thromboelastography functional fibrinogen and Multiplate) reflecting global hemostasis, clot strength and platelet aggregation were analyzed prospectively in 112 patients and plasma was analyzed in 76 patients for biomarkers reflecting endothelial-, glycocalyx-, platelet-, and fibrinolysis function (histone-complexed DNA fragments, Protein C, soluble CD40 ligand, soluble thrombomodulin, syndecan-1, tissue-type plasminogen activator). The results were compared in groups stratified according to age, antithrombotic therapy, TE, and glycocalyx degradation (syndecan-1 $<$ or $\geq$ median). Correlation between biomarkers and demographic-, anatomical-, clinical- and biochemical parameters was investigated. The prevalence of TE was 8.1 % after a mean follow-up of 8.4 years. None of the stratified groups demonstrated evidence of hypercoagulability in the whole blood assays and no unexpected significant differences were found between the groups. All biomarkers, except protein C, correlated with one another and after stratification of glycocalyx degradation only syndecan-1 levels $\geq$ median correlated with other biomarkers. The prevalence of TEs was 8.1 % after mean follow-up of 8.4 years. Overall, the hemostatic profile appeared normal, however, in a subset of patients, evidence of some endothelial activation/damage including glycocalyx degradation and fibrinolysis was found, identifying a potentially more thrombogenic group.

Keywords Fontan procedure · Thrombosis · Prevalence · Hematology · Coagulation

Introduction

After Fontan surgery, patients face an increased risk for thromboembolic events (TEs), the prevalence of which varies from 3 to 33 % depending on study design, imaging technique, and duration of follow-up [2, 6, 7, 16, 20, 30–32]. The etiology for this apparently increased thrombogenecity is incompletely understood, but is likely to be multifactorial, potentially involving all three factors of Virchow's triad: hypercoagulability, abnormal hemodynamics, and endothelial injury/dysfunction. Decreased levels of protein C, protein S, antithrombin III, ND coagulation factor S II, VII, and X, as well as increased platelet reactivity, have been described in Fontan patients [7, 12, 26, 33]. Studies evaluating the impact of these altered hemodynamics are sparse. Modifications of Fontan surgery (from the atriopulmonary

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anastomosis to the lateral tunnel and extracardiac conduit total cavopulmonary anastomosis) showed no change in the prevalence of TE [6, 29, 30]. The vascular endothelium is instrumental for balancing hemostasis [5, 22] as is the anticoagulant surface layer, the glycocalyx that protects the endothelium [24, 27]. Impaired endothelial-mediated vasodilation has been reported in Fontan patients [10, 18] as have signs of impaired endothelial function as assessed by plasma levels of endothelial derived biomarkers [3, 15]. However, the combined effect of the abnormalities of coagulation and endothelial function on thrombus formation is unclear, and correlation to TE has not been shown.

The aim of this study was to determine a population-based prevalence of TE in Danish Fontan patients. Furthermore, we sought to bring new insights into the etiology of TE and to describe the global hemostasis as well as the separate factors of the hemostasis, such as endothelial and glycocalyx function, platelet activation, fibrinogen function, and fibrinolysis. Finally, we aimed to compare patients stratified according to age, antithrombotic therapy, previous TE, and glycocalyx degradation and to investigate correlations between parameters of hemostasis and demographic, anatomical, and clinical parameters.

Materials and Methods

The study consisted of two parts: a population-based study to determine the prevalence of TE and a cross-sectional study to explore the etiology of TE. The population-based study covers the period from January 1, 1990, to December 31, 2010. The local Ethics Committee approved the study (approval no. H-C-2009-039), and all patients/guardians gave informed consent.

Identification of Fontan Patients

Fontan surgery as a routine was implemented in the early 1990s in the two tertiary pediatric cardiology centers in Denmark: Rigshospitalet (Copenhagen) and Aarhus University Hospital (Skejby, Aarhus). All surgeries were registered in local registries, and all patients were followed-up in the outpatient departments in the two centers or at Odense University Hospital (Odense). Because the study started in January 2011, information on each patient was collected from the medical records and through patient interviews at the next clinical outpatient visit. Previous TE had to be confirmed by imaging modalities, and previous supraventricular tachycardia (SVT) had to be confirmed by electrocardiography. SVT was defined as three or more QRS complexes with QRS duration $\leq$120 ms and heart rate >100 bpm and included intra-atrial re-entrant tachycardia, ectopic atrial tachycardia, atrioventricular re-entrant tachycardia, and atrial flutter/fibrillation. As a standard procedure, all of the patients received vitamin K antagonist (VKA) therapy 6 months after Fontan surgery. Subsequently patients either continued with VKA therapy, antiplatelet therapy, heparin, or no antithrombotic therapy all at the discretion of the cardiologist. At clinical visits in the outpatient department, transthoracic echocardiogram (TTE), but no other imaging, was performed routinely.

Blood Sampling

In the cross-sectional study, blood was sampled on arrival at the clinical outpatient visit at Rigshospitalet (Copenhagen). Patients visiting the Outpatient Department at Aarhus University Hospital (Skejby, Aarhus) did not participate in the cross-sectional study because not all blood analyses could be performed. Blood was sampled in ethylene diamine tetraacetic acid (EDTA), plasma, and serum tubes. Blood samples were obtained with minimal or no stasis (<30 s), ice-cooled, and centrifuged at 2,000×*g* for 10 min. EDTA plasma samples were frozen within 1 h and stored at −80 °C until analyzed. Antithrombotic medicine was not paused before blood sampling.

Biochemical Parameters

Biochemical parameters were processed in a standardized laboratory in 112 patients. The parameters included hemoglobin, hematocrit, platelet count, albumin, *N*-terminal pro b-type natriuretic peptide (NT-proBNP), alanine aminotransferase, lactate dehydrogenase, alkaline phosfatase, homocystein, antithrombin, D-dimer, protein S, protein C, international normalized ratio (INR), activated partial thromboplastin time (APTT), factor V Leiden gene (Arg506Gln) and factor II gene mutation (nucleotide20210), and plasma fibrinogen.

Thromboelastography

The global hemostasis of whole blood was assessed in 112 patients by thromboelastography (TEG) analysis using a computerized thromboelastograph (model 5000 and software version 4.1.73 [database version 1.0.16]; Haemoscope, Skokie, IL). All preparations were measured on TEG by adding 1 ml citrated whole blood into a kaolin vial, gently mixing and then transferring 340 µl of the kaolin-activated sample to a prewarmed (37 °C) plain TEG cup loaded with 20 µl 0.2 mol/l calcium chloride (final concentration 11.1 mmol/l) for recalcification. Analysis was started 90–120 min after collection. The TEG variables recorded were reaction time (R) (rate [min] of initial fibrin formation reflecting plasma clotting factors), the alpha-angle (α) (rate [°] of clot growth reflecting platelet

function, fibrinogen, and plasma components residing on the platelet surface), maximum amplitude (MA) (maximum clot strength reflecting the maximum dynamic properties of the platelet–fibrin interaction), and lysis 30 (LY30) (rate of amplitude decrease reflecting clot stability).

TEG Functional Fibrinogen

The contribution of fibrinogen function to clot strength of whole blood was analyzed in 103 patients by TEG functional fibrinogen (FF) (Haemoscope, Skokie, IL) analysis (sampled in 112 patients but analyses failed in nine). The FF reagent activates the extrinsic pathway using tissue factor and inhibits platelet aggregation using a platelet inhibitor (ReoPro). ReoPro binds to the GPIIb/IIIa receptors and fully inhibits the platelets ability to interact with fibrin and von Willebrand factor, thereby excluding their contribution to clot strength, measuring only the FF contribution (MAf) to clot strength. In TEG FF, whole blood was recalcified with 20 mmol/l $CaCl_2$, and FF reagent was carefully mixed by inverting the tube five times before loading the contents to the TEG cup containing $CaCl_2$. Analysis was started 90–120 min after collection.

Impedance Aggregometry (Multiplate)

Platelet aggregation of whole blood was analyzed in 110 patients by impedance aggregometry using a multiple platelet function analyzer (Multiplate, Dynabyte Medical, Munich, Germany) (Multiplate Analyzer, Software version 2.02.11; Dynabyte GmbH) (sampled in 112 patients but analyses failed in two). All samples of heparinized whole blood were analyzed according to the manufacturer's instructions 90–120 min after collection. Platelet aggregation was determined in response to commercially available test reagents (Dynabyte GmbH). Briefly, 300 µl of the adjusted sample was mixed with 300 µl NaCl (ASPItest) or NaCl–$CaCl_2$ (TRAPtest, ADPtest) and 20 µl platelet agonist (TRAPtest [thrombin-receptor activating peptide -6; final concentration, 32 µmol/l], ADPtest [adenosine 5′-diphosphate, 6.5 µmol/l], and ASPItest [arachidonic acid, 0.5 mmol/l]). The increase in impedance by the attachment of platelets onto the Multiplate sensors is transformed to arbitrary aggregation units and plotted against time. For each applied platelet agonist, the area under the aggregation curve (AU × min or U [1 U = 10 AU × min]) after 6 min of analysis was recorded by the Multiplate analyzer.

Soluble Biomarkers

Enzyme-linked immunosorbent assay (ELISA) was performed in a subset of 76 randomly selected patients. Soluble biomarkers were measured in uniplicate in thawed EDTA plasma by commercially available immunoassays according to the manufacturer's recommendations: syndecan-1 to reflect glycocalyx degradation, soluble thrombomodulin (sTM) to reflect endothelial damage, protein C to reflect natural anticoagulation, soluble CD40 ligand (sCD40L) to reflect platelet activation, tissue-type plasminogen activator (tPA) to reflect fibrinolysis, and histone-complexed DNA fragments (hcDNAf) to reflect cell damage. In each patient, all biomarkers were measured corresponding to a total of $6 \times 76 = 456$ measurements with 14 (3.1 %) missing measurement values. Syndecan-1 (sCD138; Diaclone SAS, Besancon, France [LLD 2.56 ng/ml]), sTM (Nordic Biosite, Copenhagen, Denmark [LLD 0.38 ng/ml]), hcDNAf (Cell Death Detection ELISA; Roche, Hvidovre, Denmark [LLD not stated, relative quantification]), protein C (Helena Laboratories, Beaumont, TX [LLD 5 % relative to reference plasma]), tPA (American Diagnostica, Stanford, CT [LLD 1 ng/ml]), and sCD40L (R&D Systems Europe [LLD 4.2 pg/ml]) were measured in plasma.

Due to changes in the hemostatic profile according to age and antithrombotic therapy, results of the TEG, TEG FF, Multiplate, and biomarkers were compared in patient groups, stratified according to age (≤16 years or >16 years) and antithrombotic therapy (antiplatelet therapy, VKA, heparin or no antithrombotic therapy). In search of Fontan groups at increased risk of TE, we compared patient groups stratified according to previous TE and degree of glycocalyx degradation (syndecan-1 level ≥ median or < median).

TEG parameters in patients ≤16-years-old were compared with reference values from 51 healthy children [25], whereas patients >16-years-old were compared with reference values obtained from 59 healthy adult blood donors [13]. TEG FF and Multiplate parameters in patients >16-years-old were compared with reference values [9]. To our knowledge, no reference data on TEG FF and Multiplate in children have been published. The only available reference values of syndecan-1 levels were measured in 110 male subjects with a median age of 64 years [1]. Indicators of hypercoagulability from TEG include decreased R time and increased α and MA; indicators of hypercoagulability from TEG FF include increased MAf; and indicators of hypercoagulability from Multiplate include increased ASPItest, TRAPtest, and ADPtest.

Statistical Analysis

Continuous variables are summarized by mean ± SD or median and interquartile range (IQR; 25–75th percentiles) depending on normality of distribution. Categorical variables are represented by frequencies and percentages. Group comparisons of continuous variables were performed using parametric methods whenever possible.

Therefore, nonnormally distributed variables were transformed (logarithm10) to approximate a normal distribution. A curve reflecting freedom from TE was generated using Kaplan–Meier analysis. In Fontan groups stratified according to antithrombotic therapy, blood parameters were compared using one-way analysis of variance (ANOVA), and in Fontan groups stratified according to age, TE, and syndecan-1 level, blood parameters were compared using unpaired Student t test. Correlations between each biomarker and demographic, anatomical, clinical, and biochemical parameters were investigated by Pearson correlation. Separate analyses of correlation were performed in the groups with high and low syndecan-1 levels. The correlation value of whole-blood assays, biomarkers, and biochemical parameters for TE was investigated by logistic regression analysis. Because there were many statistical tests, $p < 0.01$ were considered statistically significant. Analyses were performed using Stata 11.0 software (Stata LP, College Station, TX).

Results

Prevalence

During the study period, 210 Fontan surgeries were performed in Denmark: 124 at Rigshospitalet (Copenhagen) and 86 at Aarhus University Hospital (Skejby, Aarhus) (Fig. 1). Mean follow-up was 8.4 ± 5.7 years, and 16 patients (8 %) died: three from TE, five from other Fontan-related complications, and eight from unknown reasons (autopsy was not performed). TE was identified in 17 patients (12 clinical and five routine TTE); hence, the overall prevalence of TE was 8.1 % (5.7 % for clinical TE). Mortality from TE was 17.6 % (25.0 % for clinical TE). Location of the thrombus/embolus was cerebral in nine patients, inferior vena cava–to–pulmonary artery tunnel in four patients, intrapulmonary in two patients, and intracardiac in two patients. Actuarial freedom from TE was 98.6 % at 6 months, 97.6 % at 1 year, 95.5 % at 3 years, 94.9 % at 5 years, 92.8 % at 10 years, 84.2 % at 15 years, and 84.2 % at 20 years after surgery (Fig. 2). Mean time since Fontan surgery to TE was 5.0 ± 5.5 years. Of the 14 patients alive with TE, 4 (29 %) had SVT before TE. Of the remaining 167 patients alive, SVT was noted in 35 patients (21 %) (Chi-square $p = 0.60$). Demographic and clinical data of the Fontan groups, stratified according to vital status and analyzed blood samples, are listed in Table 1.

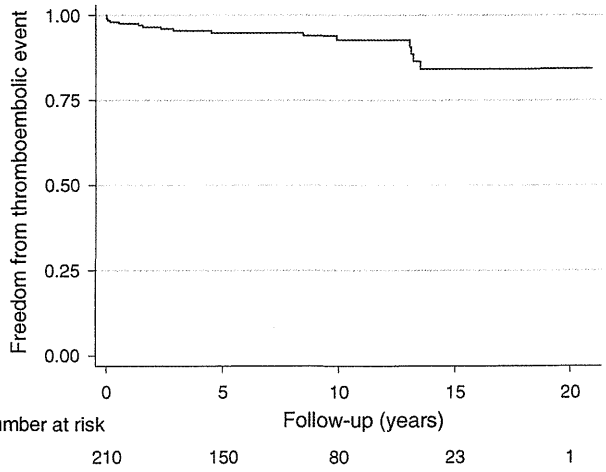

Fig. 2 Freedom from TE after Fontan surgery

Global Hemostasis

TEG results stratified by age, antithrombotic therapy, TE, and syndecan-1 level are listed in Table 2. None of the groups showed evidence of hypercoagulability compared with reference data, and no statistically significant differences were found between groups. Only four patients (3 %) were classified as hypercoagulable based on TEG, and none of them experienced a TE. Three patients with protein-losing enteropathy (all on heparin) were investigated, and none of them were classified as hypercoagulable.

Separate Factors of Hemostasis

Biochemical Parameters

Median value of all biochemical parameters mentioned in Materials and Methods were within normal reference intervals for age and sex (data not shown). In particular, normal parameters of liver function and protein S, protein C, INR, APTT, plasma fibrinogen, and D-dimer were found. One patient (0.9 %) was heterozygote for F2 gene

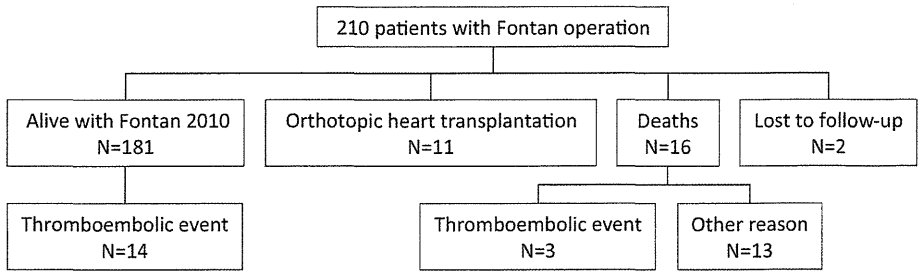

Fig. 1 Outcome of patients with Fontan surgery in Denmark between 1990 and 2010

Table 1 Demographic data of four Fontan groups

Demographic data	Group 1 ($n = 210$)	Group 2 ($n = 181$)	Group 3 ($n = 112$)	Group 4 ($n = 76$)
Age (years)		14.1 (7.2)	13.7 (7.3)	13.7 (7.3)
Male	124 (59)	110 (61)	64 (57)	42 (55)
Post-Fontan follow-up (years)	8.4 (5.7)	8.7 (4.8)	8.6 (4.7)	8.5 (4.7)
Age at Fontan surgery (years)	5.0 (4.8)	5.3 (5.4)	5.0 (5.7)	5.1 (5.8)
Type of Fontan				
RA–PA anastomosis	4 (2)	1 (1)	0	0
Lateral tunnel	95 (45)	77 (43)	54 (48)	37 (49)
Extracardiac conduit	111 (53)	103 (57)	58 (52)	39 (51)
Fenestration				
Patent		18 (10)	11 (10)	7 (9)
Closed/none		163 (90)	101 (90)	69 (91)
Anatomical diagnosis				
AVSD	23 (11)	17 (9)	10 (9)	6 (8)
DILV	40 (19)	38 (21)	19 (17)	14 (18)
HLHS	27 (13)	18 (10)	15 (13)	10 (13)
PA–IVS	21 (10)	17 (9)	13 (12)	8 (12)
TA	51 (24)	48 (27)	31 (28)	25 (33)
Other functional UVH	48 (23)	43 (24)	24 (21)	12 (16)
Post-Fontan TE				
Yes	17 (8)	14 (8)	6 (5)	5 (7)
No	193 (92)	167 (92)	106 (95)	71 (93)
Time since surgery to TE (years)	5.0 (5.5)	5.8 (5.7)	8.2 (6.1)	9.5 (5.7)
Antithrombotic therapy				
Antiplatelet therapy		104 (57)	75 (67)	51 (67)
Vitamin K antagonist		48 (27)	27 (24)	18 (24)
Unfractionated heparin		3 (2)	3 (3)	2 (3)
None		26 (14)	7 (6)	5 (7)

Data are presented as mean (SD) or *n* (%)

Group 1 patients operated in Denmark 1990–2010; *group 2* patients alive at the time of study; *group 3* patients from group 2 in whom TEG and biochemical parameters were analyzed; and *group 4* Fontan patients from group 3 in whom biomarkers were analyzed

RA–PA right atrium–to–pulmonary artery; *AVSD* atrioventricular septal defect; *DILV* double-inlet left ventricle; *HLHS* hypoplastic left heart syndrome; *PA–IVS* pulmonary valve atresia and intact ventricular septum; *TA* tricuspid atresia; *UVH* univentricular heart; *TE* thromboembolic event

mutation (ga), and seven patients (6.3 %) were heterozygote for F5 gene mutation (arg–gln). None of the heterozygote patients had a previous TE, and none of them were classified as hypercoagulable based on TEG.

TEG FF and Multiplate

TEG FF and Multiplate results stratified by age, antithrombotic therapy, TE, and syndecan-1 level are listed in Table 2. None of the groups showed evidence of hypercoagulability regarding clot strength (TEG FF) and platelet activation (Multiplate), and no significant differences were found between groups except for those expected from any type of antithrombotic therapy. Furthermore, we compared TEG, TEG FF, and Multiplate in patients receiving VKA therapy stratified according to TE and found no significant difference.

Biomarkers

Median value of syndecan-1 was higher in Fontan patients (46.4 ng/ml) compared with nonmatched reference value (17 ng/ml) [1]. In patients ≤16-years-old, the median was 53.7 ng/ml (IQR 38.8–64.4), and in patients >16-years-old the median was 37.7 ng/ml (IQR 24.6–57.8) ($p = 0.09$). Table 3 lists levels of biomarkers by age group, antithrombotic therapy, TE, and syndecan-1 level. In the group with high syndecan-1 levels (syndecan-1 ≥ median) compared with the group having low syndecan-1 levels, a tendency toward higher levels of all biomarkers, except protein C, was found. However, in the group with high syndecan-1 levels, only the differences between syndecan-1 and hcDNAf ($p < 0.001$) and sCD40L ($p = 0.009$) in patients ≤16-years-old and hcDNAf ($p = 0.01$) in patients >16-years-old were significant. Differences in biomarkers were not found between any of the other stratified groups (except, as expected, lower protein C in patients on VKA).

Correlation

Correlations between each biomarker and demographic parameters (age, sex, age at Fontan surgery), anatomical parameters (type of heart defect, type of Fontan surgery),

Table 2 Comparisons of TEG, TEG FF, and Multiplate in Fontan patients by age group, antithrombotic therapy, previous TE, and syndecan-1 level

Characteristics	TEG				TEG FF	Multiplate		
	R (s)	Angle (°)	MA (mm)	LY30 (%)	MAf (mm)	ASPI test	TRAP test	AD test
Antithrombotic therapy (≤16 years)								
Antiplatelet ($n = 53$)	7.8 (7.2–8.6)	60.7 (56.2–64.4)	61.9 (56.8–63.9)	0.9 (0.1–2.0)	16.0 (12.5–17.9)	34 (26–47)	110 (91–121)	88 (71–98)
VKA ($n = 14$)	9.0 (7.1–10.6)	60.8 (50.6–64.2)	64.7 (56.4–68.3)	0.5 (0.0–0.9)	15.5 (13.9–17.0)	79 (58–99)	98 (74–116)	67 (52–92)
Heparin ($n = 3$)	7.8 (7.8–8.9)	61.0 (49.7–61.5)	62.7 (59.4–66.5)	5.2 (0.0–6.2)	15.4 (14.8–16.5)	83 (23–114)	117 (110–117)	91 (87–94)
None ($n = 1$)	10.5	49.4	57.9	0.6	11.7	68	78	63
ANOVA (p)	0.10	0.20	0.67	0.92	0.49	**<0.001**	0.47	0.15
Antithrombotic therapy (>16 years)								
Antiplatelet ($n = 22$)	8.9 (8.2–10.2)	51.7 (47.3–61.5)	57.5 (53.8–61.6)	0.2 (0.0–1.1)	14.3 (11.7–18.7)	42 (31–60)	104 (95–111)	76 (64–87)
VKA ($n = 13$)	10.5 (9.0–12.0)	49.7 (44.3–54.4)	62.3 (51.0–65.6)	0.1 (0.0–0.7)	14.7 (12.9–18.8)	75 (54–94)	113 (88–117)	77 (59–90)
Heparin ($n = 0$)								
None ($n = 6$)	8.5 (8.1–11.2)	46.9 (34.0–59.0)	55.1 (51.5–59.9)	0.7 (0.1–1.0)	14.5 (8.1–15.2)	78 (69–100)	105 (93–119)	80 (73–85)
ANOVA (p)	0.02	0.35	0.77	0.44	0.48	**0.007**	0.99	0.95
Previous TE (≤16 years)								
Yes ($n = 3$)	8.9 (7.8–11.9)	49.7 (45.8–63.4)	64.5 (56.4–66.5)	2.4 (1.6–6.2)	15.4 (8.1–18.3)	77 (23–81)	109 (95–110)	62 (61–91)
No ($n = 68$)	7.8 (7.3–9.2)	60.7 (55.8–64.2)	61.9 (57.4–64.9)	0.7 (0.0–1.9)	15.8 (12.5–17.7)	39 (29–62)	109 (88–120)	87 (69–95)
Student t test (p)	0.15	0.08	0.62	0.10	0.45	0.56	0.95	0.39
Previous TE (>16 years)								
Yes ($n = 3$)	9.0 (8.8–10.3)	50.4 (49.0–54.4)	62.5 (54.1–65.6)	0.0 (0.0–0.7)	18.6 (13.6–23.6)	75 (15–84)	119 (69–135)	73 (37–84)
No $n = (38)$	9.2 (8.2–10.8)	51.5 (45.8–58.4)	57.3 (51.5–62.2)	0.4 (0.0–1.0)	14.6 (11.6–18.5)	60 (38–88)	104 (95–113)	78 (62–87)
Student t test (p)	0.76	0.93	0.37	0.89	0.30	0.78	0.74	0.26
Syndecan-1 level (≤16 years)								
≥Median ($n = 23$)	8.0 (7.5–9.8)	58.9 (49.9–63.4)	61.9 (57.4–65.6)	1.1 (0.4–2.7)	15.6 (12.6–17.6)	39 (26–68)	102 (88–112)	87 (67–91)
<Median ($n = 22$)	7.9 (7.2–9.1)	61.0 (58.8–64.2)	62.3 (58.0–65.1)	0.6 (0.0–1.4)	17.2 (12.3–19.5)	41 (29–62)	116 (84–125)	85 (73–95)
Student t test (p)	0.49	0.10	0.97	0.09	0.55	0.96	0.54	0.69
Syndecan-1 level (>16 years)								
≥Median ($n = 12$)	8.8 (8.4–9.5)	50.2 (48.0–61.7)	58.5 (53.3–64.2)	0.3 (0.0–1.5)	16.2 (11.6–20.4)	56 (36–76)	104 (97–111)	80 (61–85)
<Median ($n = 11$)	10.1 (9.0–11.2)	47.1 (44.9–54.3)	55.7 (54.0–62.2)	0.1 (0.0–0.5)	13.7 (11.7–18.4)	49 (31–78)	97 (88–104)	71 (63–76)
Student t test (p)	0.16	0.05	0.30	0.26	0.46	0.66	0.50	0.32
Reference, 95 % CI (≤16 years)	2.3–10.3	49.4–75.8	55.0–71.3	0–1.7				
Reference, 95 % CI (>16 years)	3–8	55–78	51–69	0–8	11–24.4	79–141	92–151	55–117

Angle = α angle

TEG FF was missing in nine patients and Multiplate in two patients. Data are presented as median (IQR). Comparisons between groups were performed after logarithm transformation. p is *bolded* if <0.01

TEG thrombelastography, *TEG FF* TEG functional fibrinogen, *R* reaction time, *MA* maximum amplitude, *LY30* Lysis 30, *MAf* contribution of functional fibrinogen to maximum amplitude, *ASPI test* arachidonic acid test, *TRAP test*: thrombin receptor activating peptide test, *ADP test* adenosine diphosphate test, *VKA* Vitamin K antagonist, *ANOVA* analysis of variance, *TE* thromboembolic event

Table 3 Comparisons of biomarkers in Fontan patients by age group, antithrombotic therapy, previous TE, and syndecan-1 level

Characteristics	Biomarker					
	Syndecan-1 (ng/mL)	Protein C (%)	sTM (ng/mL)	sCD40L (pg/mL)	tPA (ng/mL)	HcDNAf (%)
Antithrombotic therapy (≤16 years)						
Antiplatelet ($n = 36$)	50.9 (34.7–63.9)	98.6 (85.6–115.7)	5.4 (4.4–6.2)	91.6 (57.8–226.2)	4.9 (3.9–6.2)	2.6 (1.2–4.7)
VKA ($n = 9$)	59.9 (39.0–97.2)	58.5 (53.7–73.6)	3.5 (2.8–5.3)	106.8 (55.3–343.3)	4.3 (3.9–5.57)	2.5 (1.8–3.9)
Heparin ($n = 2$)	52.5 (44.7–60.3)	152.6 (123.4–182.0)	5.4 (5.1–5.6)	198.0 (144.9–251.2)	4.1 (3.2–5.0)	2.2 (1.3–3.1)
None ($n = 1$)	58.6	111.3	4.2	440.1	4.8	1.4
ANOVA (p)	0.95	**<0.001**	0.39	0.74	0.71	0.89
Antithrombotic therapy (>16 years)						
Antiplatelet ($n = 14$)	43.4 (24.6–132.0)	124.1 (115.6–137.0)	4.2 (3.7–5.3)	117.5 (67.8–243.8)	5.3 (4.3–6.6)	2.4 (1.1–8.9)
VKA ($n = 9$)	32.8 (14.5–47.1)	63.4 (49.9–80.2)	2.9 (2.7–4.2)	142.9 (109.5–165.8)	4.2 (4.0–4.9)	1.4 (0.9–2.2)
Heparin ($n = 0$)						
None ($n = 5$)	27.8 (25.5–84.1)	106.6 (80.2–137.8)	3.2 (3.2–4.1)	198.6 (80.6–369.5)	8.5 (5.8–9.5)	1.8 (1.5–2.0)
ANOVA (p)	0.19	**<0.001**	0.20	0.77	0.17	0.32
Previous TE (≤16 years)						
Yes ($n = 2$)	133.8 (60.3–207.3)	124.9 (67.8–182.0)	6.2 (5.1–7.2)	105.7 (66.5–144.9)	5.7 (3.2–8.3)	5.3 (1.3–9.2)
No ($n = 46$)	52.1 (35.5–64.4)	97.1 (84.2–113.2)	5.2 (3.9–6.1)	108.1 (55.3–245.2)	4.8 (3.9–6.1)	2.5 (1.4–4.1)
Student t test (p)	0.19	0.17	0.51	0.67	0.80	0.63
Previous TE (>16 years)						
Yes ($n = 3$)	35.8 (18.9–52.6)	96.7 (68.9–113.7)	3.5 (3.0–5.4)	156.0 (154.6–165.8)	4.2 (4.1–4.2)	1.8 (0.8–2.0)
No ($n = 25$)	37.7 (24.6–80.1)	116.7 (77.9–130.7)	3.8 (2.9–4.8)	120.2 (77.3–279.0)	5.4 (4.3–8.5)	2.0 (1.1–4.2)
Student t test (p)	0.61	0.51	0.93	0.93	0.20	0.38
Syndecan-1 level (≤16 years)						
≥Median ($n = 23$)	64.4 (58.6–129.7)	97.0 (73.6–109.5)	5.6 (4.4–6.8)	180.0 (71.6–435.3)	5.5 (4.1–8.3)	3.9 (2.5–5.5)
<Median ($n = 22$)	38.8 (26.7–43.2)	104.9 (80.3–117.9)	4.8 (3.0–5.7)	84.4 (45.5–121.6)	4.3 (3.7–5.8)	1.7 (1.1–2.6)
Student t test (p)	NA	0.87	0.04	**0.009**	0.04	**<0.001**
Syndecan-1 level (>16 years)						
≥Median ($n = 12$)	68.9 (49.9–138.5)	117.6 (89.6–124.5)	4.1 (3.3–6.1)	148.5 (120.2–316.8)	5.2 (4.2–6.6)	3.3 (1.5–9.3)
<Median ($n = 11$)	24.6 (18.9–32.8)	114.1 (80.2–137.0)	3.7 (3.0–4.2)	80.5 (67.8–110.8))	4.6 (4.0–5.8)	1.3 (0.9–2.0)
Student t test (p)	NA	0.64	0.25	0.03	0.32	**0.01**

Data are presented as median (IQR) or mean (range) if $n < 3$. Comparisons between groups were performed after logarithm transformation. p is *bolded* if <0.01

sTM Soluble thrombomodulin, *sCD40L* Soluble CD40 ligand, *tPA* Tissue-type plasminogen activator, *HcDNAf* Histone-complexed DNA fragments, *VKA* Vitamin K antagonist, *ANOVA* analysis of variance, *TE* thromboembolic event

clinical parameters (weight, height, systolic blood pressure, oxygen saturation, resting heart rate), and biochemical parameters (TEG, TEG FF, Multiplate, other biomarkers, and all biochemical parameters) were investigated (data of correlations between biomarkers are listed in Table 4). All biomarkers correlated with several other biomarkers except for protein C. In particular, syndecan-1 and hcDNAf strongly correlated with all other biomarkers except for protein C. Syndecan-1, sCD40L, tPA, and hcDNAf did not correlate with other parameters investigated other than the biomarkers, whereas sTM also correlated to age at Fontan surgery ($r = -0.33$, $p = 0.003$) and Multiplate ASPItest ($r = -0.33$, $p = 0.004$). Separate analyses of correlation in the groups with high and low syndecan-1 levels were performed. Only the group with high syndecan-1 levels correlated with the other biomarkers (except protein C and sCD40L [Table 5]).

In univariate logistic regression analyses with TE as the dependent variable and TEG, TEG FF, Multiplate, biomarkers and biochemical parameters (homocysteine, antithrombin, D-dimer, protein S, protein C, INR, APTT, p-fibrinogen, fecal alfa-1-antitrypsin, F5 gene, and F2 gene) as the independent variables, none of the parameters were associated with TE.

Table 4 Correlations between biomarkers in 76 Fontan patients

Biomarkers *r* (*p*)	Syndecan-1	Protein C	tPA	sCD40L	sTM	HcDNAf
Syndecan-1		NS	0.53 (<0.001)	0.38 (0.001)	0.53 (<0.001)	0.70 (<0.001)
Protein C	NS		NS	NS	NS	NS
tPA	0.53 (<0.001)	NS		NS	0.31 (0.005)	0.41 (<0.001)
sCD40L	0.38 (0.001)	NS	NS		NS	0.38 (<0.001)
sTM	0.53 (<0.001)	NS	0.31 (0.005)	NS		0.30 (0.009)
HcDNAf	0.70 (<0.001)	NS	0.41 (<0.001)	0.38 (<0.001)	0.30 (0.009)	

tPA Tissue-type plasminogen activator, *sCD40L* Soluble CD40 ligand, *sTM* Soluble thrombomodulin, *HcDNAf* Histone-complexed DNA fragments, *NS* Not significant, *r* Pearson's correlation coefficient

Table 5 Correlation between Fontan patients stratified according to high versus low syndecan-1 level

	High syndecan-1 level	Low syndecan-1 level
Syndecan-1		
Protein C	NS	NS
tPA	0.58 (<0.001)	NS
sCD40L	NS	NS
sTM	0.63 (<0.001)	NS
HcDNAf	0.72 (<0.001)	NS

tPA Tissue-type plasminogen activator, *sCD40L* Soluble CD40 ligand, *sTM* Soluble thrombomodulin, *HcDNAf* Histone-complexed DNA fragments, *NS* not significant, *r* Pearson's correlation coefficient

Syndecan-1 $\geq$ median vs. $<$ median, respectively

Data presented as *r* (*p*)

Discussion

In this study, a population-based prevalence of TE of 8.1 % was found in 210 Fontan patients after a mean follow-up after Fontan completion of 8.4 years. In assessment of the etiology of TE in Fontan patients, we found a normal global hemostasis, normal platelet activation/function, and normal clot strength. In a subset of patients, evidence of some endothelial activation/damage, including glycocalyx degradation and fibrinolysis as assessed by biomarkers, was found; however, no correlations between biomarkers and clinical data were found. No significant differences in hemostasis were found between Fontan groups stratified according to antithrombotic therapy, previous TE, and glycocalyx degradation.

When evaluating the prevalence of TE in Fontan patients, one must distinguish between clinical and silent events. Balling et al. [2] performed a cross-sectional study of 52 Fontan patients and found silent thrombus in 17 (33 %) patients on transesophageal echocardiography (TEE), of which only one was identified on TTE. Similarly, Varma et al. [34] found silent pulmonary emboli in 5 of 30 (17 %) Fontan patients using combined ventilation-perfusion scans and confirmatory computed tomography (CT) angiography. In the present study, routine follow-up included annual clinical evaluation and TTE; therefore, most likely our prevalence does not include all silent TE. Mean time from Fontan surgery to TE was 5.0 $\pm$ 5.5 years, which is similar to Rosenthal et al. [30] who reported a prevalence of clinical TE of 11.4 % in 70 Fontan patients and mean time from Fontan surgery to TE of 6.1 $\pm$ 5.0 years. Approximately 13 years after surgery, we found clustering TEs (four cases). Apart from that the TE risk appears to be constant, consistent with previous studies [6, 30]. Khairy et al. [17] found that cumulative hazard for thromboembolic death increased steadily 15 years after Fontan surgery. The prevalence of TE in Fontan patients is higher than after any other cardiac surgery in children except patients with prosthetic valves [11]. However, establishing the true prevalence of TE is difficult because longer duration of follow-up and improved diagnostic studies will contribute to increase the prevalence.

Despite antithrombotic treatment, TEs are common in Fontan patients [6].The etiology of thrombus formation in Fontan patients has been extensively investigated. Several coagulation factor abnormalities have been reported [7, 12, 26, 33], and it is likely that the pathophysiology of TE in Fontan patients is multifactorial [11]. To ensure that patients did not suffer from general coagulopathies, simple screening of proteins S and C, APTT, INR, and fibrinogen was conducted, and normal results were found. Furthermore, a normal concentration of D-dimer indicated that persistent microthrombus formation is not a problem. Previous studies have confirmed that TEG and Multiplate can identify patients at increased risk of TE [4, 19]. We performed several whole-blood assays of hemostasis and analyzed endothelial, glycocalyx, platelet, and fibrinolysis function in a large Fontan cohort. Overall, global hemostasis appeared normal as assessed by TEG. This is