Clinical and Applied Thrombosis/Hemostasis
2018, Vol. 24(2) 348-352

DOI: 10.1177/1076029616683045
journals.sagepub.com/home/cat
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# Thromboelastogram as a Tool to Predict Hypercoagulability in Children With Cystic Fibrosis

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**Abstract**

Increased thrombophilic tendency in patients with cystic fibrosis (CF) has recently been reported. The determinants of thrombosis in children with CF remain largely unknown. Our aim in this study was to evaluate the thromboelastography (TEG) profile of children with CF through ROTEM (whole blood rotation thromboelastometry). Nineteen patients with CF and 20 controls were included in the study. Whole blood count, prothrombin time, activated prothrombin time, fibrinogen, D-dimer levels, and ROTEM assays (INTEM, EXTEM) were performed. Clotting time, clot formation time (CFT), and maximum clot firmness (MCF) were determined by INTEM and EXTEM analysis. In INTEM assay, MCF ($P = .001$) value was significantly increased and CFT ($P = .031$) value was decreased in patients with CF compared with those of the control group. In the EXTEM assay, there was a similar significant increase in MCF ($P = .023$) value in patients with CF compared with that of the control group. There was a significant positive correlation between fibrinogen levels and MCF in EXTEM ($r = .72$) and INTEM ($r = .76$) assays, whereas there was a negative correlation with CFT in EXTEM ($r = -.61$) and INTEM ($r = -.67$). The results of our study indicated that TEG profiles in patients with CF were more hypercoagulable compared with those of the control group.

**Keywords**

cystic fibrosis, thromboelastogram, hypercoagulability

## Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder that is most commonly seen among the people of Northern Europe. It affects about 1 of 2500 to 3000 newborns, and about 1 in 25 people are carriers.[1] Cystic fibrosis is caused by defects in the CF gene, which encodes a cystic fibrosis transmembrane conductance regulator (CFTR) protein that functions as a chloride channel.[2] Mutations in the CFTR gene result in abnormalities of cyclic adenosine monophosphate–regulated chloride transport across epithelial cells on mucosal surfaces.

Cystic fibrosis can manifest as a coagulation disorder in some cases. Patients with CF are at risk of developing deficiencies of fat-soluble vitamins (A, D, E, and K) because of pancreatic insufficiency, hepatobiliary disease, or both.[3,4] Vitamin K is widely distributed in many foods (vitamin $K_1$ or phylloquinone) and is also produced by intestinal bacteria (vitamin $K_2$ or menaquinone). The factors in CF that predispose patients to vitamin K deficiency may include malabsorption, cholestatic or noncholestatic liver disease, and chronic antibiotic intake. Since the clotting factors (II, VII, IX, and X) are vitamin K–dependent, low levels of vitamin K can result in coagulation problems.[4]

Patients with CF have an increased risk of thrombosis due to central venous catheters, as well as acquired thrombophilia secondary to inflammation, deficiencies of anticoagulant proteins due to vitamin K deficiency, protein C, and protein S, antithrombin III deficiency, increased platelet activation, and liver dysfunction.[5,6] Central venous catheter–associated

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thrombosis commonly results in line occlusion but may develop into serious life-threatening conditions such as deep venous thrombosis, superior vena cava syndrome, or pulmonary embolism.

Thromboelastography has been used as a monitor of whole blood coagulation in a variety of clinical settings, including acute or previous venous thromboembolic events.[7] Thrombelastography is based on measurements of the viscoelastic properties of whole blood specimens and various activities that transpire in coagulation.[8] It is used in clinical settings to detect and quantify hypo- and hypercoagulability, fibrinolysis, clot strength, and anticoagulant drug effects.[9] The association of thromboelastogram with CF is unclear because of a lack of large cohort studies. In the present study, we aimed to test the hypercoagulability in children with CF using TEG data and correlate these parameters with those of the healthy group.

## Materials and Methods

### Patients

Within the study, the diagnosis of CF was based on a typical clinical profile with positive sweat test and/or identification of CFTR mutations. Patients were considered clinically stable when there was no evidence of exacerbations during the previous 2 months. The patients with preexisting hematological, coagulation disorders, and acute infectious processes, those not having regular visits, and those with liver dysfunction were excluded from the study. All the data were recorded at the time of stable clinical conditions for each regular visit of patients.

Control participants were randomly selected to participate in the study after their annual checkup, and these children were enrolled into the healthy control group. None of the control participants had personal or first-degree family history of bleeding or thrombosis, took any medications, or had any acute infections or chronic illnesses. No clinically evident thrombotic episodes were recorded during the study period. This study was approved by the institutional review board of the College of Medicine. Informed consent was obtained from parents of each patient before his or her inclusion in the study. The study was conducted according to the Declaration of Helsinki.

### Study Design

The blood samples from patients with CF were taken just before vitamin K dose (taken once a week). Using minimum stasis and a 19-gauge needle, blood samples were drawn into 4.5-mL vacutainers (Becton Dickinson) containing 3.2% trisodium citrate with a citrate/blood ratio of 1:9 for subsequent coagulation analyses. Complete blood count was measured using a Beckman Coulter LH750 machine (Kraemer Bluds-Brea, California). Prothrombin time (PT, seconds), activated partial thromboplastin time (aPTT, seconds), fibrinogen (mg/dL), and dimerized plasmin fragment D (D-dimer) tests were performed immediately on a Siemens BCS XP machine (Tem International, Marburg, Germany). Normal ranges for these tests at our laboratory are aPTT (26-36 seconds), PT (9.4-15 seconds), fibrinogen (200-400 mg/dL), and D-dimer (0.00-0.50 μg/mL).

Pulmonary function was assessed by forced expiratory volume 1 (FEV1), using a Vmax spirometer (Sensormedics, Milan, Italy). The best of 3 technically acceptable maneuvers was selected according to the American Thoracic Society guidelines.[10]

### Whole Blood Rotation Thromboelastometry (ROTEM) Analysis

Thrombelastography analysis was performed with the ROTEM Coagulation Analyzer model Gamma 2500 (Tem International, Munich, Germany). The blood was recalcified with 20 μL of 0.2 mol/L $CaCl_2$ (star-TEM; Pentapharm, Munich, Germany), and the activation of coagulation was performed through different agents:

INTEM: Contact pathway activation of the coagulation with 20 μL of contact activator (partial thromboplastin–phospholipid from rabbit brain extract and ellagic acid, in-TEM; Pentapharm).
EXTEM: Tissue factor pathway activation of the coagulation with 20 μL of tissue factor (TF, tissue thromboplastin from rabbit brain extract, ex-TEM; Pentapharm). The test starts automatically after the injection of the blood sample with an automated pipette, and calculated graphical results are obtained by the integrated computer of the device. All the TEG samples were analyzed within 30 to 90 minutes of blood collection in our study.

The mean parameters obtained were clotting time (CT, seconds), clot formation time (CFT, seconds), and maximum clot firmness (MCF, mm). Clotting time is the time from the beginning of the coagulation analysis until there is a 2 mm increase in amplitude that reflects the initiation phase of the clotting process. Clot formation time is the time for the amplitude of the thromboelastogram to increase from 2 to 20 mm and reflects the propagation phase of whole blood clot formation. Maximum clot firmness is the maximal amplitude reached during TEG and correlates with platelet count and functions with the concentration of fibrinogen[11] as well. A "hypercoagulable profile" was defined as a shorter CT, shorter CFT, and/or higher MCF than the corresponding values in healthy controls.[7,12] In addition, the laboratory values for fibrinogen, platelets, and hematocrit were determined in the study group.

### Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 15.0 (SPSS, Inc, Chicago, Illinois). The baseline characteristics of the study population were described by frequency and descriptive analysis. The Mann-Whitney *U* test was utilized to measure the difference in ROTEM parameters between patients and controls. The associations between ROTEM parameters and laboratory parameters were summarized by Spearman rank correlation coefficients. A probability value of less than .05 was considered as statistically significant.

**Table 1.** Complete Blood Count Characteristics of the Study Population.[a]

| Complete Blood Count | Cystic Fibrosis (n = 19) | Controls (n = 20) | *P* Value |
|---|---|---|---|
| Hemoglobin (g/dL) | 12.63 ± 1.30 | 12.78 ± 0.58 | .8 |
| White blood cell ($\times 10^3/\mu L$) | 11.42 ± 4.50 | 7.26 ± 1.46 | **<.001** |
| Platelet ($\times 10^3/\mu L$) | 357.36 ± 136.94 | 319.85 ± 87.83 | .244 |

[a]Boldface value indicates statistical significance.

**Table 2.** Values of ROTEM Parameters in the Study Population.[a]

| ROTEM Parameters | Cystic Fibrosis (n = 19) | Controls (n = 20) | *P* Value |
|---|---|---|---|
| EXTEM | | | |
| CT, s | 69.78 ± 8.76 | 72.8 ± 13.5 | .507 |
| CFT, s | 78.78 ± 23.07 | 88.4 ± 19.5 | .199 |
| MCF, mm | 67.47 ± 5.66 | 64.0 ± 3.6 | **.023** |
| INTEM | | | |
| CT, s | 172.63 ± 27.34 | 175.7 ± 38.9 | .699 |
| CFT, s | 62.21 ± 16.55 | 73.8 ± 16.0 | **.031** |
| MCF, mm | 66.89 ± 5.15 | 62.2 ± 3.6 | **.001** |

Abbreviations: CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness.
[a]Boldface values indicate statistical significance.

## Results

Thirty patients were included in the present study. Among these patients, 19 completed regular visits and were analyzed. The other patients were lost to follow-up because they did not go on visits during the period of the study. Patients did not present severe associated comorbidities; none had diabetes and 1 patient had a FEV1 below 60% at baseline.

Nineteen patients (7 girls and 12 boys; median age 8 years) with CF and 20 healthy children (8 girls and 12 boys; median age 8.2 years) were included in the study. There was no significant difference in terms of age and sex between the CF and control groups.

The results of laboratory parameters are presented in Table 1, and the result of ROTEM parameters are presented in Table 2. The mean monocytes and neutrophil counts were higher in the CF group than those of the control group ($P = .002$ and $P < .001$, respectively). The mean erythrocyte counts were higher in the control group than those of the patients ($P = .048$). The mean white blood cell count was higher in the CF group than that of the controls ($P < .001$). There was no difference between the 2 groups in the following parameters: PT, aPTT, fibrinogen levels, and platelet counts.

When TEM parameters were analyzed, it was seen that MCF on both EXTEM and INTEM analyses was significantly higher in the patients with CF than that of the controls ($P = .023$, and $P = .031$). Furthermore, CFT was shorter in the patient group than that of the controls on INTEM ($P = .031$). Fibrinogen showed a strong positive correlation with MCF on EXTEM and INTEM ($r = .72$, $P < .001$; $r = .76$, $P < .001$, respectively), though there was a negative correlation with CFT on EXTEM and INTEM ($r = -.61$, $P = .005$; $r = -.67$, $P = .02$; Table 3). No significant correlation was found between TEM parameters and PT and aPTT.

**Table 3.** Correlation Coefficients (*r*) Between ROTEM Parameters and Fibrinogen Levels.

| ROTEM Parameters | Fibrinogen Level, mg/dL | |
|---|---|---|
| | *r* | *P* |
| EXTEM | | |
| CFT, s | −.61 | .005 |
| MCF, mm | .72 | **<.001** |
| INTEM | | |
| CFT, s | −.67 | .02 |
| MCF, mm | .76 | **<.001** |

Abbreviations: CFT, clot formation time; MCF, maximum clot firmness.
[a]Boldface values indicate statistical significance.

## Discussion

Cystic fibrosis is a chronic inflammatory condition with a purported increased incidence of thrombophilia, although thromboembolic disease is rarely reported.[13,14] It has been reported that patients with CF have cytokine dysregulation and an excessive host inflammatory response. This excessive host inflammatory response exposes the vascular endothelium to unchecked chronic inflammation.[15,16] In addition, compared with healthier patients with CF, those with more progressive lung inflammation were reported to have increased circulating inflammatory cytokines and platelet activation.[6,17]

In CF children, several factors might increase not only the progressive inflammation but also the risk of thrombotic abnormalities. Those most relevant to CF are catheters, liver disease, diabetes, vitamin K deficiency, and immobility.[18-21] Vitamin K deficiency is associated with acquired protein S and protein C deficiencies, and low vitamin K levels are common in children with CF, even in those with pancreatic sufficiency.[13,21] None of our patients were receiving vitamin K supplements at the time. The supplements were taken at least 10 days before the study date. It is possible that low vitamin K levels were responsible for prolonged PT and aPTT, although all our patients had normal PT and aPTT.

There are some reports about the patients with CF having thrombotic episodes in the literature. But these episodes are possibly associated with line insertion.[13,17,22-24] In the literature involving patients with CF, Balfour-Lynn et al[13] found a thrombotic abnormality in 41 (20%) of 204 patients and Barker et al[5] identified a thrombophilic state in 53% of 66 patients. Besides, Munck et al[22] demonstrated a very high rate of thrombophilia and hypercoagulability status, corresponding to 50%; however, a very low frequency of catheter venous thrombosis was 6.6% in a CF cohort. It is generally accepted that antithrombin deficiency and homozygosity for factor V Leiden

represent the most severe risk factors for thrombosis in adults. The role of these abnormalities in childhood thrombosis has not been established.[24] But it is possible that children with CF are at greater risk of developing a thrombus than the general population. Patients with CF are at risk of developing disorders in bleeding because of pancreatic insufficiency, hepatobiliary disease, and antibiotic intake.[3,4] However, there are no reliable data in these patients about the bleeding episodes.

Hyponatremia, hypochloremia, and dehydration are well recognized in patients with CF. The abnormalities are multifactorial in origin, being explained by a combination of sweat losses together with gastrointestinal and respiratory illnesses.[4] Increased blood viscosity and hemoconcentration as a result of dehydration might contribute to increased coagulability and thrombogenesis.[25,26] However, during the study period, all children were examined for dehydration and other problems associated with diarrhea. Clinical findings, urine, and hematological and biochemical analyses were used for determining the hydration status of the children. The children with mild or moderate dehydration were excluded from the study.

The conventional laboratory coagulation analyses (PT, aPTT, platelet count, fibrinogen concentration) may be of limited use for the prediction and detection of coagulopathies. These tests neither convey any information about clot stability over time nor provide any information regarding hyperfibrinolysis. Thus, it is critically important to recognize that routine coagulation tests cannot detect clinically significant coagulation defects that contribute to bleeding, hypo- or hyperfibrinolysis, hypercoagulability, and platelet aggregation.[25] However, TEG is a sensitive method that is able to identify hypercoagulability, which is not detected by routine laboratory tests.[26,27] It provides an effective and convenient means of monitoring whole blood coagulation. Thrombelastography evaluates the elastic properties of whole blood and provides a global assessment of haemostatic function. It is recommended for and commonly used in assessing hemostasis during liver transplantation, obstetric procedures, and cardiac surgery. Thrombelastography is not only useful in cases of bleeding patients but also used to predict hypercoagulability.[28,29,30,31]

There is no criteria that will appear to predispose the patients with CF to a thrombophilic tendency. Other studies have reported CF-associated changes in the hemostatic system in a procoagulant direction based on the measurements of isolated components of the coagulation pathways.[13,14,22-24] No previous study has examined plasma coagulation by TEG in CF; however, we have previously demonstrated that CF displays hypercoagulability in whole blood by TEG in accordance with the results of this study. The results of the present study yielded significant hypercoagulability in children with CF, as detected by TEG. This hypercoagulability was diagnosed readily by the presence of significant shortening of CFT and increase of MCF on INTEM assays and evidenced by the increase of MCF on EXTEM assays. We have also demonstrated that CFT on EXTEM analysis was shorter in children with CF than that of the controls, but it was not statistically significant. With the present investigation, we identified the value of fibrinogen in CF-induced hypercoagulability. We therefore sought to correlate fibrinogen with those of ROTEM parameters. We observed that although MCF had a strong positive correlation and CFT had a strong negative correlation with plasma fibrinogen concentration. Maximum clot firmness measures the maximum clot strength, which is dependent on the fibrinogen level. Therefore, MCF appears to be the most important TEG parameter of identifying patients with hypercoagulopathic CF that makes TEG superior to other hemostatic test.

We recognize some limitations to this study. Firstly, the number of participants of the study group was small. Secondly, the association between ROTEM data and CF gene mutations could not be studied. In contrast to some authors and based on our data, we cannot recommend thrombophilic screening for all patients with CF. However, we can provide recommendations on laboratory screening when considering insertion of a vascular access device in patients with CF.

Finally, further and bigger studies are needed to recommend TEG as a valuable tool to evaluate the degree and risk of hypercoagulability in children with CF, especially with catheters.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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