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Fibrinogen function is impaired in whole blood from patients with cyanotic congenital heart disease

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ABSTRACT

Background: Patients with cyanotic congenital heart disease (CCHD) have haemostatic abnormities associated with bleeding and thrombo-embolic events. The haemostatic abnormalities are not fully understood, but recent studies indicate that elevated haematocrit and fibrinogen function may be of importance.
The aim of this study was to characterise the haemostatic profile and examine the potential role of haematocrit on clot formation and strength in CCHD patients. Furthermore to examine whether CCHD patients with history of haemoptysis have diminished fibrinogen function compared to those without haemoptysis.
Methods: In a prospective study 75 adult CCHD patients had haematocrit, platelet count, and plasma fibrinogen concentration examined. Furthermore thrombelastography(TEG) as well as TEG Functional Fibrinogen(TEG FF) assay evaluating fibrinogen function(FLEV) was performed. Data were compared with historical data regarding previous haemoptysis in CCHD patients.
Results: Haematocrit was 57 ± 8% and platelet counts in the lower normal range. TEG revealed a hypocoagulable condition with impaired clot formation. TEG values were correlated to haematocrit, indicating that elevated haematocrit causes impaired clot formation and strength. Despite high levels of plasma fibrinogen, TEG FF demonstrated that FLEV was diminished and negatively correlated to haematocrit. Furthermore CCHD patients with previous history of haemoptysis had significantly lower FLEV compared to CCHD patients without haemoptysis.
Conclusion: Patients with CCHD are hypocoagulable mainly due to impaired fibrinogen function. Despite a low platelet count, platelet function does not seem to be severely affected in CCHD patients. Haemostasis, and especially fibrinogen function, is negatively affected by elevated haematocrit, and fibrinogen function is diminished in CCHD patients with haemoptysis.

1. Introduction

Patients with cyanotic congenital heart disease (CCHD) often demonstrate haemostatic abnormalities, associated with a high incidence of both thrombo-embolic events and bleeding complications [1]. The pathogenesis of the thrombo-embolic complications has been associated to hyperviscosity and pro-thrombotic conditions such as dilated pulmonary arteries, slow blood flow and arrhythmias [1–4]. Regarding bleeding complications in CCHD, which most often present as haemoptysis, previous studies have reported that this may be related to reduced count, survival and function of the platelets, as well as additional defects in the haemostasis [5–10]. The exact nature of the haemostatic abnormalities in patients with CCHD have been difficult to characterise, partly due to small patient sample sizes but also the use of conventional coagulation analysis, such as prothrombin time (PT) and activated partial thromboplastin time (APTT). These analysis are performed on plasma alone and do not reflect the interaction of the different coagulation proteins with the cellular elements, i.e. the platelets and red blood cells.

Recent studies have employed a whole blood (WB) viscoelastical haemostatic assay thrombelastography (TEG), to characterise haemo stasis in CCHD patients [11,12]. TEG is a WB global, dynamic analysis that reflects the different phases of the haemostatic process including the initiation, amplification and the propagation phases resulting in clot formation as well as clot degradation [13].

Using TEG in an animal model has recently demonstrated that elevated haematocrit values affect thrombus formation negatively and increase clot development time [14]. Similarly Cui et al. used TEG with different coagulation stimulating agents and platelet inhibitors

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in CCHD children undergoing complex heart surgery. They found that clot strength was negatively correlated to elevated haematocrit and concluded that the diminished clot strength was mainly due to impaired fibrinogen function and not platelet function, despite of low platelet count [11]. These findings indicate that the elevated haematocrit and impaired fibrinogen function could be of importance in the haemostatic abnormities, also observed in adult CCHD patients.

The aim of the present study was to characterise the haemostatic profile of adult CCHD patients, especially focusing on the fibrinogen and platelet function, evaluated by TEG and TEG functional fibrinogen (TEG FF). Furthermore, examine the potential role of haematocrit on clot formation and clot strength. Finally to compare blood sample results with the prevalence of haemoptysis in CCHD patients, in order to examine whether diminished fibrinogen function is a predictor of increased risk of bleeding in CCHD patients.

2. Methods

2.1. Study design

This was a prospective multicenter study, divided in two parts. The first part was a descriptive study characterizing the haemostatic profile in CCHD patients, aiming specific to examine the correlation between haematocrit and coagulability, with specific focus on the fibrinogen and platelet function.

The second part of the study evaluated whether CCHD patients who previously had experienced haemoptysis had a difference in the haemostatic profile than patients without previously haemoptysis.

2.2. Patients

Between October 2009 and December 2010 clinically stable adult CCHD patients followed at the University Hospitals in Lund and Stockholm, Sweden, as well as the University Hospital Skejby and Rigshospitalet in Denmark were invited to participate in the study. If the patients received anti-coagulation/thrombotic medicine, the medicine was paused one week before participating in the study. All patients who participated in the study were referred for examinations at Rigshospitalet.

2.3. Blood samples

Blood was drawn from an antecubital vein into Vacutainer tubes containing EDTA for the whole blood count and citrate for the conventional coagulation parameters, standard TEG and TEG FF.

2.4. Blood count and conventional coagulation parameters

The whole blood count and the conventional plasma coagulation parameters were analyzed at Rigshospitalets institutional laboratory according to their standards, using commercially available assays. Haemoglobin, haematocrit, and platelets were measured on the SYSMEX XE 2100 system. Fibrinogen (p-fibrinogen) was measured by the Clauss method on the ACL TOP system.

2.5. Standard TEG

Standard TEG reflects the different phases of the haemostasis. In the standard TEG assay WB was added to a kaolin vial, which was carefully mixed by inverting the tube five times before loading the activated blood into the TEG cup containing Calcium Chloride (CaCl2) for re-calcification. The following parameters were recorded from each sample; clotting time R, clot formation Angle, and maximum strength of the clot MA.

2.6. TEG FF

The Functional Fibrinogen reagent activates the extrinsic pathway using tissue factor and inhibits platelet aggregation using a platelet inhibitor that binds to the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptors. In this way the platelets ability to interact with fibrin and von Willebrand factor is fully inhibited, thereby excluding their contribution to clot strength (MAp), measuring only the functional fibrinogen contribution (MAf), to clot strength. In the TEG FF assay WB was added to a Functional Fibrinogen (FF) reagent vial, the vial was carefully mixed by inversion five times before loading the activated blood into the TEG cup containing CaCl2.

The following parameters were recorded from each sample when paired with standard TEG; the value of angle due to platelet function (ANGp) and fibrinogen function (ANGf), maximum strength of the clot due to fibrinogen function (MAf) and platelet contractility (MAp) and estimated functional fibrinogen level (FLEV).

Both standard and TEG FF were performed in accordance with the manufacturer's instructions and based on WB, why the results were not affected by diminished plasma levels due to increased haematocrit. All samples were analyzed 30 minutes after the blood sample was taken. One person analyzed all samples. The haemostatic process was recorded by use of a TEG coagulation analyzer (5000 series TEG analyzer; Haemoscope Corporation, Chicago, Illinois, USA).

2.7. Medical history

Data regarding previous haemoptysis was obtained through a questionnaire concerning medical history and medical records. Haemoptysis was defined as visual expectoration of blood from the bronchi, larynx, trachea, or lungs. Bloodstained sputum was not defined as haemoptysis.

2.8. Statistics

All statistical analysis was conducted with PASW statistics 18.0. All normally distributed variables were expressed as means ± standard deviation (SD) otherwise as medians [25 and 75 percentile]. Correlations between variables were analyzed by Pearson's coefficient of correlation (r).

The blood sample values of CCHD patients with and without previous history of haemoptysis were analyzed using an unpaired *t*-test. A p-value less than 0.05 were considered statistically significant.

2.9. Ethics

The study was conducted according to the most recent amendments to the Declaration of Helsinki and in adherence to good clinical practice guidelines. The Danish Ethical Committee had approved the protocol. Written informed consent was obtained from all patients.

3. Results

Seventy-five out of 80 patients accepted to participate in the study. There was a slight overweight of female patients (57%), mean age was 41 years (range of 18–78 years) (Table 1). Mean oxygen saturation at the time of inclusion was 83% (range of 57–94%) falling during exercise to a mean of 67% (range of 42–90%) (Table 1).

The conventional blood samples showed that the 75 patients had a mean haematocrit of 57 ± 8%, platelet counts in the lower normal range ($157 \pm 59 \times 10^9$/L) and p-fibrinogen were in the higher normal range (Table 2). The standard TEG results showed, when compared

Table 1
Demographic of cyanotic congenital heart disease (CCHD) patients.

N = 75	CCHD patients
Demographic	
Age, years	41 ± 13
Sex	
Female, N (%)	43 (57)
Saturation	
Rest, %	83 ± 6
Exercise, %	67 ± 10
Diagnosis	
Eisenmenger syndrome, N (%)	56 (76)
Ventricular septal defect, N	40
Atrial septal defect, N	8
Atrioventricular septal defect, N	4
Persistent ductus arteriosus, N	4
Univentricular heart, N (%)	13 (16)
Pulmonary atresia, ventricular septal defect and aortopulmonary collateral arteries (MAPCA), N (%)major	5 (7)
Pulmonary arteriovenous malformation, N (%)	1 (1)
Pulmonary hypertension	
CCHD patients with pulmonary hypertension, N (%)	67 (89)
Medication	
Patients receiving anti-coagulation/thrombotic medicine, N (%)	24 (32) [a]
Aspirin, N (%)	11 (15)
Clopidogrel, N (%)	1 (1)
Warfarin, N (%)	12 (16)
Marcoumar, N (%)	3 (4)

The results are shown as mean ± standard deviation or actual number and (%).
[a] Some of the patients received more than one anti-coagulation/thrombotic medication.