Thrombosis Research 158 (2017) 113–120

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

# The utility of thromboelastography and thrombin generation in assessing the prothrombotic state of adults with sickle cell disease

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ARTICLE INFO

*Keywords:*
Sickle cell disease
Blood transfusion
Hypercoagulability
Thrombin
Hydroxyurea

ABSTRACT

*Introduction:* Previous studies have suggested a chronic hypercoagulable state in SCD, and that thrombosis also plays a role in the pathophysiology of sickle cell vaso-occlusive pain crises (VOC). Studies looking at thrombin generation have produced conflicting results. In this study we aimed to assess and compare whole blood thromboelastography (TEG) and plasma Calibrated Automated Thrombogram (CAT) in SCD versus healthy controls and in four different SCD subgroups.

*Materials and methods:* In this prospective observational study, TEG and 1 pM TF activated CAT assays were performed in citrated blood samples from 77 adult (18–66 years old) SCD patients (HbSS and HbSB) and 22 healthy (HbAA) ethnically-matched controls.

*Results and conclusions:* SCD was associated with a prothrombotic state in all TEG parameters. CAT results showed that the upslope of the CAT in SCD displayed a hypercoagulable state with shorter time to peak and higher velocity index, but the downslope was also faster leading to an overall lower endogenous thrombin potential (ETP) compared to healthy controls. TEG subgroup analyses showed that during VOC the prothrombotic state is greater compared to patients on disease ameliorating therapy. CAT did not display statistically significant differences between the SCD subgroups. This study shows that the prothrombotic state in SCD is best displayed with TEG, and suggests the hypercoagulable changes of SCD rely at least in part in the cellular components of blood, which can only be detected in whole blood assays.

## 1. Introduction

Sickle cell disease (SCD) is one of the most common inherited red cell disorders, with the genetic mutation in SCD resulting in the production of an abnormal haemoglobin (HbS) [1]. HbS polymerises under conditions of stress and hypoxia resulting in the formation of sickle-shaped red blood cells which are removed early from the circulation resulting in a chronic haemolytic anaemia and intermittent vaso-occlusive pain crises (VOC) [1–3]. There is an increased risk of arterial and venous thromboembolism (VTE) in SCD [4–7]. The VTE incidence among SCD patients is greater than in patients with common hereditary thrombophilia's, with VTE affecting between 11% and nearly one quarter of adults with SCD. This acquired thrombophilic state appears to be a risk factor for premature death in SCD [7,8].

The pathophysiology of VOC is thought to be multifactorial, not only due to polymerisation of HbSS but also involvement of endothelial, platelet and white cell activation [9,10]. Hydroxycarbamide (HC) therapy and regular blood transfusion therapy have both shown to decrease the frequency of VOC [11–14].

SCD is associated with chronic activation of coagulation, even in the steady state (i.e. non-crises) [5,15]. Previous studies have shown hypercoagulable changes in most of the haemostatic systems [3,16,17]. Platelet activation is increased [10] and markers of thrombin generation such as thrombin-antithrombin (TAT) complexes, prothrombin fragment 1.2, fibrinopeptide A and Factor VIII levels are elevated [15]. Fibrinolysis is also activated as evidenced by elevated levels of D-dimers and plasmin-antiplasmin (PAP) complexes. Some studies have shown increased levels of circulating tissue factor (TF) however results have not been consistent [16]. Reduced levels and activity of the physiological anticoagulants protein S and protein C are observed [3,4,18].

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http://dx.doi.org/10.1016/j.thromres.2017.08.020
Received 3 July 2017; Received in revised form 30 August 2017; Accepted 31 August 2017
Available online 06 September 2017

Normal membrane phospholipid asymmetry is lost, probably due to repeated cycles of sickling and this [5,19] results in erythrocytes expressing phosphatidylserine at their cell membrane which likely contributing to thrombin generation [20,21]. It is suggested that treatment with regular transfusion and HC reduce haemostatic activation [12,13,22].

Thrombin plays a central role in haemostasis and the thrombin generation assay is a reliable indicator of the coagulation activation [23] in platelet poor plasma. Endogenous thrombin potential (ETP) is generally considered as the most important parameter derived from thrombin generation tests (TGT) as it measures thrombin generation in real time. However former studies performed using TGT, such as the calibrated automated thrombogram (CAT), to assess the prothrombotic state in SCD patients showed conflicting results [4,20,24–27]. Methods of these studies have varied with subgroups defined differently, controls not always race-matched and analytic conditions and sample preparation differing between the studies. It has been suggested that the prothrombotic changes of SCD reside in the cellular elements [5,16,28] and as these are removed prior to performing TGT, this may not be the optimal methodology to show thrombotic abnormalities. Indeed, although many haemostatic parameters are altered in SCD there has not been an assay to adequately assess the global prothrombotic state (both cellular and plasma) in individual SCD patients [15,17,18].

Thromboelastography (TEG) is a near patient, rapid global assay performed on whole blood samples that provides a detailed view of clot formation ex vivo. We hypothesized TEG could be useful in assessing the complex hypercoagulable state in SCD patients. A study by Yee et al. [18] assessed TEG in children with SCD and showed that children with HbSS displayed significant differences compared with healthy controls. However the study lacked power due to the small sample size and heterogeneity. It is well recognized that children are haemostatically different from adults [29]. A recent study by Whelihan et al. [30] used plasma based and whole blood thrombin generation and in 25 “steady state” SCD patients, but not in the multiple different clinical scenarios in adults with SCD. Furthermore they did not use a TEG assay. Indeed, a side-by-side study of TEG and CAT in different subgroups of SCD patients has never been performed.

Our primary aim in this study was to assess the utility of TEG to investigate the prothrombotic state in adults with SCD compared to healthy race-matched controls. Our secondary objective was to compare TEG and CAT results of the four different SCD subgroups i.e. steady state (clinically well) without the use of disease ameliorating treatment, during sickle cell crisis (VOC), patients on long-term regular exchange transfusion program (EBT) and on hydroxycarbamide (HC) therapy. We hypothesized SCD is associated with a prothrombotic state that is demonstrable by TEG and CAT; and that state would be more pronounced during VOC and partially or fully ameliorated by EBT and HC treatment.

## 2. Material and methods

### 2.1. Study design & ethics

This prospective observational exploratory study of patients with sickle cell disease attending Guy's & St Thomas' Sickle cell Unit between April and July 2016 was approved by the National Research Ethics Service Committee Cambridge East and the London NHS Research Ethics Committee (reference 16/EE/0114).

### 2.2. Study population

We included patients with HbSS and $HbSB^0$-thalassemia genotype as they show a similar clinical course [2,4]. Patients with HbSC and $HbSB^+$ patients were excluded. The SCD genotype had previously been confirmed by high performance liquid chromatography. Other exclusions included pregnancy, an inherited bleeding disorder, anticoagulant therapy or severe liver disease with liver transaminases $> 5\times$ upper limit of normal.

This study was designed as a pilot study; no sample size calculation was performed. Study subjects having incomplete data were excluded from analysis.

We aimed to include SCD patients in four clinical scenarios:

1) SCD patients in steady state not using disease ameliorating therapy (nor HC nor EBT),
2) SCD patients in steady state on a regular exchange blood transfusion program (EBT),
3) SCD patients in steady state on stable dose hydroxycarbamide (HC) therapy for at least one month SCD patients experiencing sickle cell pain crisis (VOC) and
4) SCD patients experiencing sickle cell pain crisis (VOC).

Groups 1, 2 and 3 are all in steady state however different in therapy. To simplify we will refer to group 1 as steady state (without use of therapy), group 2 as EBT and group 3 as HC. Patients in groups 1 to 3 were excluded if they had a VOC in the previous 6 weeks. Additionally, patients in the steady state and HC groups were excluded if they had received blood products within the previous 12 weeks. Blood was obtained from patients in the VOC group within 36 h of admission to hospital.

Healthy ethnically-matched controls were recruited from the hospital staff. Groups were age (10 years range) and ethnicity matched since there are racial differences in coagulation parameters [5]. Furthermore all healthy controls were tested for sickle cell trait (HbAS) since recent studies have shown HbAS subjects display alteration in coagulation parameters [24].

### 2.3. Blood sampling

After informed consent was obtained, venous blood was drawn into two 0.102 M trisodium citrate tubes (Greiner, Gloucestershire, UK). In healthy controls an additional EDTA tube (Greiner, Gloucestershire, UK) was taken to obtain a full blood count (FBC) and for haemoglobinopathy testing. Tests on the EDTA samples were performed in the same laboratory where the FBCs for SCD patients are assessed to minimize interlaboratory variability. All EBT patients were bled immediately prior to transfusion.

Samples for TEG and ETP were taken to the Thrombosis and Vascular Biology group laboratory for analysis and kept at room temperature until processed. The 0.102 M trisodium citrate samples for ETP were centrifuged at 2000*g* for 15 min at room temperature within 3 h of being obtained. Citrate samples were further processed to obtain platelet free plasma, by transferring the plasma into a clean tube and respun at 2000*g* for 15 min. The platelet free plasma (PFP) was then aliquoted and frozen at − 80 °C until a later date. Blood was processed within 3 h of venepuncture for the TEG.

### 2.4. Full blood count and haemoglobinopathy testing

Full blood counts were assessed using the UniCel DxH 800 Coulter Cellular Analysis System manufactured by Beckman Coulter, High Wycombe, UK. To assess the presence of sickle cell trait or disease the Bio Rad HPLC Variant II Dual-Program was used. Sickle carriers were confirmed by the sickle solubility test using kit S-test from Micorgen Bioproducts Limited, Camberley, UK.

### 2.5. Thromboelastography

TEG was performed using TEG® 5000 Thromboelastograph ® Analyser (Haemonetics UK Ltd., Coventry, UK). The 0.102 M trisodium citrate samples were tested around approximately 2.5 h of venepuncture to reduce variability. We set the time at 2.5 h because of