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Regular Article

Reduced clot-stability during the first 6 hours after aneurysmal subarachnoid haemorrhage – a prospective case-control study

Carl C. Larsen [a,*], Benny Sørensen [b], Jørn D. Nielsen [c], Jens Astrup [d]

[a] Department of Neurosurgery, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark
[b] Haemostasis Research Unit, Centre for Haemostasis & Thrombosis, St Thomas' Hospital , Westminster Bridge Road, London SE1 7EH, UK
[c] Thrombosis & Haemostasis Unit, University Hospital of Copenhagen, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark
[d] Department of Neurosurgery, Glostrup University Hospital, Nordre Ringvej 57, DK-2600 Glostrup, Denmark

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ABSTRACT

Introduction: Early rebleeding is an important cause of death and disability following aneurysmal subarachnoid haemorrhage (SAH). Recent studies have shown that 50-90% of the rebleedings occurred within the first 6 hours after the primary bleeding. The mechanism leading to rebleeding remains to be established. In the present prospective case-control study we hypothesize that patients with SAH develop a coagulopathy characterized by reduced clot stability during the early period after the initial bleeding.
Methods: Patients with aneurysmal SAH was studied with a dynamic clot lysis assay and markers of fibrinolysis and clot stabilizers in blood samples taken within and after 6 hours after onset of bleeding. Results were compared with blood samples from age and gender matched healthy controls.
Results: 36 patients were enrolled, 26 patients had blood samples collected within 6 hours after the initial bleeding whereas 10 patients had blood samples taken later than 6 hours after the initial bleeding. Patients demonstrated significantly reduced clot stability during the first 6 hours after initial bleeding. Fibrinolytic activity was increased during the first 6 hours along with the inhibitors of fibrinolysis whereas the modulators of fibrinolysis were reduced or inactivated.
Conclusion: During the first 6 hours after SAH patients exhibit reduced clot-stability. Probably a consequence of activated fibrinolysis in combination with reduced or inactivated factor XIII and thrombin-activable fibrinolysis inhibitor.

Introduction

Early rebleeding is an important cause of death and disability following aneurysmal subarachnoid haemorrhage (SAH) [1]. Recent reports have shown that the risk of rebleeding is as high as 10-15% within the first 6 hours after the initial bleed [1]. The clinical importance is illustrated by a randomized clinical study in which the rate of rebleeding was reduced from 10.8% to 2.4% by the use of antifibrinolytic treatment by tranexamic acid (TXA) [2] without observed side-effects. Outcome studies have not yet been conducted to indicate a possible effect on overall outcome of this dramatic effect on rebleeding. However, the results suggest that SAH may be associated with a coagulopathy where the haemostatic capacity is distorted towards reduced clot stability. Understanding the pathophysiological mechanisms of SAH-induced acute coagulopathy could potentially contribute to optimize timing and type of clot-stabilizing treatment modalities.

A series of studies have measured markers of haemostasis in blood samples from patients with SAH. Since antifibrinolytic therapy has reduced the rate of rebleeding, it has been hypothesized that patients may suffer from hyperfibrinolysis. However, studies aimed at measuring markers of fibrinolysis such as levels of plasmin-antiplasmin complexes (PAP), α2-antiplasmin and D-dimer has not been able to demonstrate abnormalities in SAH patients [3–7]. A potential limitation of these studies may be that blood samples were collected as late as 36 hours after the initial bleed. Also, it may be argued that biochemical markers of coagulation and fibrinolysis lack sensitivity and that more global functional tests such as thrombelastography or dynamic clot lysis assays may be useful. In a rat animal model of SAH, we recently reported that thrombelastography immediately following SAH showed signs of hypercoagulation rather than hyperfibrinolysis [8]. Another recent study has shown that increased intracranial pressure is associated with acute hypercoagulation as a result of increased tissue factor exposure [9].

During recent years, a new concept of acute traumatic coagulopathy has been proposed in which initial increased thrombin generation

Abbreviations: AUC, area under the clot lysis curve; CT, computed tomography; ELISA, enzyme-linked immunosorbent assays; MRI, magnetic resonance imaging; PAI-1, plasminogen activator inhibitor 1; PAP, plasmin-antiplasmin; SAH, subarachnoid haemorrhage; t-PA, tissue plasminogen activator; TAFI, thrombin-activatable fibrinolysis inhibitor; TAT, thrombin-antithrombin; TXA, tranexamic acid.

* Corresponding author. Tel.: +45 35 45 35 45; fax: +45 35 45 53 55.
E-mail addresses: larsen-cc@hotmail.com (C.C. Larsen), benny.sorensen@kcl.ac.uk (B. Sørensen), jorn.dalsgaard.nielsen@rh.regionh.dk (J.D. Nielsen), jens.astrup@dadlnet.dk (J. Astrup).

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leads to excessive stimulation of thrombomodulin and thereby activation of protein C [10]. Activated protein C (aPC) subsequently inactivates coagulation factor V and VIII, both being essential for sustained propagation of thrombin generation. Furthermore, aPC inhibits plasminogen activator inhibitor 1 (PAI-1), thus potentially facilitating fibrinolysis. Thrombin activates key mediators of clot stability, such as thrombin activatable fibrinolysis inhibitor (TAFI) as well as factor XIII (FXIII). Thus, despite initial increased thrombin generation, patients may develop a condition characterized by inappropriate early shutdown of thrombin generation resulting in insufficient activation of TAFI and FXIII together with lack of inhibition of fibrinolysis.

In the present prospective case-control study we hypothesize that patients with SAH develop a coagulopathy characterized by reduced clot stability. The objectives of the study were to measure dynamic clot stability as well as markers of fibrinolysis and clot stabilizers in blood samples taken from patients with SAH within and after 6 hours after onset of bleeding and compare results with blood samples from age and gender matched healthy controls.

Methods

Patients and healthy controls

A total of 54 patients were enrolled of which 36 cases were demonstrated to have SAH as a result of a ruptured aneurism. Remaining 18 patients did not reveal an aneurysm by angiography and was thus excluded. One patient was excluded due to antithrombotic treatment with warfarin. In addition, 21 healthy age and gender matched volunteers constituted the control group. All patients were admitted at Glostrup University Hospital, Denmark, between August 2008 and February 2010, whereas the control group was recruited from hospital staff and relatives of the staff. Local ethics committee approved the research protocol and granted a waiver of consent for the blood sampling as a minimal-risk intervention (H-1-2010-030). Information about the time of onset of SAH was collected by interview of the patient and/or relatives to the patient focusing on classical symptoms i.e. sudden headache, nausea, vomiting or altered level of consciousness. If patients were found unconscious, the time of the finding was recorded as the onset time. The clinical status of each patient was classified according to Glasgow Coma Score (GCS) at arrival to the hospital emergency unit. The diagnosis of aneurysmal SAH was confirmed with computed tomography (CT), CT angiography, conventional angiography, or magnetic resonance imaging (MRI) angiography. In one case where the angiographic procedure could not be performed, the patient was considered to have an aneurysm since the CT scan showed a typical aneurysmal pattern of haemorrhage.

The severity of the SAH was graded according to Fisher Grade and modified by Claassen [11].

Rebleeding was recorded as a sudden definitive worsening of the clinical state (e.g. drop in Glasgow Coma Scale (GCS)) of the patient and/or an increased amount of blood on the CT scan after the SAH diagnosis was establish.

The time of blood sampling was recorded and all patients received antifibrinolytic therapy with TXA (1000 mg as i.v.bolus) immediately after blood sampling. For each patient the time interval between onset of SAH and blood sampling was defined as the bleeding-blood sampling interval. The patients were classified in two groups: patients who had a bleeding-blood sampling interval less than 6 hours (SAH<6 hrs group) and patients with a bleeding-blood sampling interval longer than 6 hours (SAH>6 hrs group). Clinical characteristics of the patients and controls are presented in Table 1.

Table 1
Patient characteristics.

	SAH <6t	SAH >6t	Control
Number of patients	26	10	21
Mean age (year ± SD)	60 ± 12	59 ± 16	56 ± 19
Male/Female ratio	8/19	5/5	9/12
GCS on arrival at primary hospital			
3-8	9 (35%)	1 (10%)	
9-13	7 (27%)	1 (10%)	
14-15	9 (35%)	8 (80%)	
Intubated	1 (4%)		
Median time from bleeding onset to blood sampling			
	2 h. 17 min.	25 h. 45 min.	
Modified Fischer Grade on admission			
1	5	3	
2	1	0	
3	12	5	
4	8	2	

Blood Sampling and Analysis

Blood samples were drawn in blood tubes containing citrate 3.2% (Vacuette, Greiner Bio One, Frickenhausen, Germany). Platelet poor plasma was prepared by centrifugation at 2200 G for 10 minutes and stored at -80 °C until analysis.

The dynamic clot lysis assay was the primary endpoint. The test was performed using a flat bottomed 96-well microplate (NUNC). Citrated plasma (50 μl) was mixed with an equal volume of reaction solution consisting of 50 mM $CaCl_2$ (Sigma-Aldrich), 0.2% bovine serum albumin (Sigma-Aldrich), 8 μM Phospholid-TGT (0.5 mM, Rossix, Mölndal, Sweden), tissue factor (Innovin, Dade-Behring, Marburg GmbH, Germany) diluted 1:3000, and 60 U/ml t-PA (60 KU/ml, Calbiochem) all diluted in a buffer (HEPES 20 mM, NaCl 150 mM, pH = 7.4). The reaction was kept at 37 °C and changes in turbidity were measured at 1-min intervals at 405 nm using a Victor microplate reader (Perkin Elmer, Turku, Finland). Primary endpoint variables were area under the clot lysis curve (AUC) as well as the relative clot lysis following 60 minutes of measurement (CL%60), as defined by 100% - (amplitude at 60 min divided by maximum amplitude) × 100. Additional clotting variables included maximum rate of clot formation (MaxVel) and maximum clotting amplitude (MCA). All variables were derived by signal processing of the turbidity raw data using DyCoDerivAn Gold (AvordusoL Risskov, Denmark) [12]. Evaluation of analytical performance showed a coefficient of variation of 4%. Fig. 1 shows typical tracings of the clot-lysis assay from patients and healthy controls. The clot stability assay is extremely sensitive to premature fibrinogen depletion, hence a very conservative approach was adapted and samples were discarded on the smallest suspicion of premature coagulation.

D-dimer was determined by standard latex-enhanced immunoturbidimetry (ACL TOP, Instrumentation Laboratories, Barcelona, Spain). Fibrinogen was determined by standard clot analysis (ACL TOP, Instrumentation Laboratories, Barcelona, Spain). Commercially available enzyme-linked immunosorbent assays (ELISA) were used for measurement of: PAI-1 (Haemochrom Diagnostica, Essen, Germany), t-PA (Haemochrom Diagnostica, Essen, Germany), PAP complex (Haemochrom Diagnostica, Essen, Germany), and FXIII (Technoclone, Vienna, Austria). TAFI (Haemochrom Diagnostica, Essen, Germany) results are shown as a percentage of normal pooled plasma supplied with the test kit.

Statistical Analysis

All values are expressed as the mean ± the standard error of the mean (mean ± sem). Data were analyzed with GraphPad Prism, version 5 (GraphPad Software, Inc, La Jolla, CA, USA). Continuous variables were compared among groups by the one way ANOVA with Tukey post test or Kruskal-Wallis test with Dunn's post test. A p-value of >0.05 was considered significant. Following a pilot study