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Regular Article

Increased thrombin generation in splanchnic vein thrombosis is related to the presence of liver cirrhosis and not to the thrombotic event

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ABSTRACT

Introduction: In recent years there have been increasing evidence associating liver disease with hypercoagulability, rather than bleeding. The aim of the study was to evaluate the haemostatic potential in patients with liver disease.

Patients and methods: We measured thrombin generation in the presence and absence of thrombomodulin in patients with portal vein thrombosis (PVT, n = 47), Budd-Chiari syndrome (BCS, n = 15) and cirrhosis (n = 24) and compared the results to those obtained from healthy controls (n = 21). Fifteen patients with PVT and 10 patients with BCS were treated with warfarin and were compared to an equal number of patients with atrial fibrillation matched for prothrombin time-international normalized ratio. We assessed resistance to thrombomodulin by using ratios [marker measured in the presence/absence of thrombomodulin].

Results: There were no differences in thrombin generation between patients on warfarin treatment and their controls. Cirrhotic patients generated more thrombin in the presence of thrombomodulin and exhibited thrombomodulin resistance compared to controls [$p = 0.006$ for endogenous thrombin potential (ETP) and $p < 0.001$ for peak thrombin and both ratios ETP and peak] and patients with non-cirrhotic PVT ($p = 0.001$, $p = 0.006$, $p < 0.001$, $p < 0.001$ for ETP, peak, ratio ETP, ratio peak, respectively). The patients with cirrhotic PVT exhibited higher ETP ($p = 0.044$) and peak ($p = 0.02$) in the presence of thrombomodulin than controls, as well as thrombomodulin resistance (ETP and peak ratios: $p = 0.001$).

Conclusions: Hypercoagulability and thrombomodulin resistance in patients with cirrhosis were independent of the presence of splanchnic vein thrombosis. The hypercoagulability in patients with cirrhotic PVT could have implications for considering longer or more intensive treatment with anticoagulants in this group.

Introduction

The liver plays a central role in maintaining the haemostatic balance by synthesizing several procoagulant, anticoagulant and fibrinolytic proteins [1]. Impaired liver function, as in chronic liver disease (CLD), results in thrombocytopenia, low levels of many procoagulant and anticoagulant factors and changes in fibrinolysis [2–5] and is reflected by the Child-Pugh (CP) class [6,7].

CLD has previously been considered a prototype for bleeding disorders, mainly due to abnormal values of coagulation tests such as prothrombin time-international normalized ratio (PT-INR) and activated partial thromboplastin time [2,7]. However, abnormal coagulation

Abbreviations: CLD, chronic liver disease; CP, Child Pugh; PT-INR, prothrombin time-international normalized ratio; VTE, venous thromboembolism; RR, relative risk; ETP, endogenous thrombin potential; PC, protein C; FVIII, factor VIII; BCS, Budd-Chiari syndrome; NC-PVT, non-cirrhotic portal vein thrombosis; C-PVT, cirrhotic portal vein thrombosis; AF, atrial fibrillation; PPP, platelet poor plasma; CAT, calibrated automated assay; nM, nanomolar; ttpeak, time to peak; IU/mL, international units/milliliter; hsCRP, high sensitivity CRP; TM, thrombomodulin.

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tests do not necessarily correspond to or predict the risk of bleeding manifestations or even the degree of coagulopathy, as they provide inadequate information on the complex mechanisms that regulate the coagulation cascade [8]. It is thus desirable to find new haemostatic tests that better estimate the degree of coagulation imbalance in patients with CLD.

The dominance of bleeding complications in CLD has been challenged [9–11]. More recent data [10] indicate that hypercoagulability might be frequently associated with CLD and cirrhosis, meaning that these patients are not 'naturally anticoagulated'; on the contrary they have an increased risk for thromboembolic complications. In a large, nationwide, case–control, Danish study patients with liver disease had an increased relative risk (RR) of VTE, both in cirrhotic (RR 1.74) and non-cirrhotic liver disease (RR 1.87) [12]. Furthermore, VTE is the main reason behind 1%-1.8% of hospital admissions for patients with cirrhosis [13,14].

The complex resulting from the binding of the protein thrombomodulin to thrombin activates the natural anticoagulant protein C (PC). Activated PC binds to its co-factor protein S and subsequently inhibits the activated forms of the procoagulants factor VIII (FVIII) and factor V, thus inhibiting thrombin generation. Thrombomodulin inhibits thrombin generation even in vitro, which is evident by the lower results of thrombin generation assays when thrombomodulin is added to the plasma sample [2]. Thrombin generation assays are coagulation tests that provide a global measure of haemostatic potential [15]. The effect of thrombomodulin, i.e. inhibiting thrombin generation, is more evident in plasma from healthy individuals than in plasma from patients with CLD, indicating partial resistance to thrombomodulin [7]. Resistance to thrombomodulin as expressed by the ratio [thrombin generation marker measured in the presence/in the absence of thrombomodulin] is a marker for hypercoagulability in vitro and has been described in patients with liver disease as a result of high levels of FVIII and low levels of PC in those patients [2]. Tripodi et al. showed that thrombin generation in cirrhotic patients, assessed as endogenous thrombin potential (ETP) in the presence of thrombomodulin was nearly identical to thrombin generation in controls, in contrast to the belief that patients with CLD are at increased bleeding risk [9]. Thrombin generation assays provide therefore a more accurate picture of the haemostatic potential in patients with hepatic disease, in contrast to traditional coagulation tests.

The Budd-Chiari syndrome (BCS) is a rare condition characterized by obstruction of the hepatic venous outflow tract mainly due to thrombosis of the hepatic veins [16]. The etiology of BCS has been associated with myeloproliferative diseases [17] and hereditary thrombophilic conditions, such as the presence of factor V Leiden [18].

Non-cirrhotic portal vein thrombosis (NC-PVT) is the main cause of presinusoidal portal hypertension in adults and children in the Western world [19]. NC-PVT can either be idiopathic, mediated by inherited or acquired prothrombotic conditions or associated with local conditions, for example inflammation and abdominal surgery. NC-PVT is often caused by a combination of local and systemic factors [20,21]. Cirrhotic-PVT (C-PVT) is a common complication during the course of cirrhosis, occurring mainly during the advanced stages of the disease [22].

In this report, we study thrombin generation, as well as the resistance to thrombomodulin in patients with cirrhosis, C-PVT, NC-PVT, BCS and in controls in order to investigate the haemostatic potential in vitro in patients with various forms of CLD. The ratio [thrombin generation marker measured in the presence/in the absence of thrombomodulin] is a marker of hypercoagulability in vitro as it expresses the grade of increased FVIII and decreased PC in the plasma of patients with CLD [9]. The hypothesis behind this study is that findings supporting hypercoagulability in vitro (high thrombin generation and thrombomodulin resistance) could be linked not only to cirrhosis but even to in vivo hypercoagulability in these patients, in this case the presence of splanchnic thrombosis.

Patients and Methods

Patients

This study was performed in collaboration with the Swedish Internal Medicine Liver Club, SILK, a nationwide network of university hepatologists. Forty-seven PVT patients (36 with NC-PVT and 11 with C-PVT) diagnosed 1995–2009, 15 patients with BCS diagnosed 1988–2009 and 24 patients with cirrhosis diagnosed 2002–2010 were included. The basic characteristics of the patients are shown in Table 1.

The causes of cirrhosis in patients with C-PVT were: hepatitis C virus (n = 4), alcoholic liver disease (n = 2), cryptogenic (n = 2), autoimmune hepatitis (n = 2), hepatitis B virus (n = 1) and for the patients with cirrhosis and no PVT: alcoholic liver disease (n = 10), non-alcoholic fatty liver disease (n = 4), hepatitis C virus (n = 4), autoimmune hepatitis (n = 3), hemochromatosis (n = 1), cryptogenic (n = 1) and primary sclerosing cholangitis (n = 1).

Controls for Thrombin Generation Measurement

We measured thrombin generation in 21 healthy volunteers [8 male and 13 female, median age (range): 57 years (29–69)]. The term 'control group' in the text refers to this group, unless otherwise specified.

Fifteen patients with PVT (NC-PVT n = 13, C-PVT n = 2) and 10 patients with BCS were under treatment with warfarin at the time of the blood sampling. We therefore investigated an equal number of otherwise healthy patients with atrial fibrillation (AF) on warfarin as prophylaxis against stroke. These AF patients were age-, gender- and PT-INR matched to the PVT patients treated with warfarin. The AF patients who served as controls for the BCS patients were gender- and PT-INR -matched, as their median age was very young (32 years) and most of the patients in the AF cohort were older. The median (range) PT-INR (both patients and controls) was 2.9 (1.9-3.5).

Blood Sampling and Handling

The median time between diagnosis and sampling was 46 months (1–164) for PVT, 78 months (1–246) for BCS and 13 months (0–96) for cirrhotic patients. Blood samples were collected after an overnight fast from antecubital veins in sodium 1/10 volume of 0.13 mmol/L citrate, EDTA and serum tubes (Becton Dickinson, Meylan, France). The samples were centrifuged within two hours at 2500 g for 10 minutes at room temperature and stored at −70 °C pending analyses.

The citrate samples used for analysis of thrombin generation were centrifuged once more at 2500 g for 10 minutes at room temperature following thawing.

Thrombin Generation Measurement by the Calibrated Automated Thrombogram

Thrombin generation was measured by the Calibrated Automated Thrombogram (CAT®) as described in the Thrombogram Guide by Thrombinoscope BV (Maastricht, the Netherlands) [23].

The samples used were platelet poor plasma (PPP) obtained from whole blood as described above. The frozen plasma was thawed by immersion into a water bath at 37 °C immediately prior to analysis. Each sample was assessed with and without thrombomodulin in duplicate. All thrombin generation analyses were performed by the first author in order to avoid variability in the performance of the experiments.

Briefly, each experiment used two sets of readings, one from a well in which thrombin generation takes place (thrombin generation well) and a second one from a well to which a calibrator has been added. To each well, 80 microliter (μl) of plasma was added. The thrombin generation wells received 20 μl of buffer, containing the trigger but no Ca^{2+}, and the calibrator wells 20 μl of the α2macroglobulin-thrombin