Research Article

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von Willebrand factor and procoagulant imbalance predict outcome in patients with cirrhosis and thrombocytopenia

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Background & Aims: Several lines of evidence suggest that the hemostatic disorders of cirrhosis may have a significant clinical impact. We investigated the independent predictive value of components of the hemostatic system on the occurrence of ascites, variceal bleeding (VB), and survival.

Methods: One hundred and two patients with thrombocytopenia (Child-Pugh class A/B/C: 34/34/34) were enrolled. Platelet counts, factors (F) II, V, VII, and VIII, antithrombin, protein C (PC), FVIII-to-PC ratio as an index of procoagulant imbalance, von Willebrand factor antigen (vWF-Ag), and model for end-stage liver disease (MELD) were evaluated. Two multivariate analyses were performed: one excluding (model 1) and one including MELD (model 2).

Results: Higher vWF-Ag levels and FVIII-to-PC ratios were the most prominent hemostatic disorders in patients with cirrhosis. Increased levels of vWF-Ag and FVIII, and higher FVIII-to-PC ratios independently predicted the presence of ascites and varices at baseline. Independent predictors of ascites and VB during follow-up were vWF-Ag (model 1/2: $p = 0.001/p = 0.009$ and $p = 0.008/p = 0.01$, respectively) and FVIII-to-PC ratio (model 1/2: $p = 0.003/p = 0.02$ and $p = 0.01/p = 0.03$, respectively). vWF-Ag (model 1/2: $p = 0.007/p = 0.002$), FVIII-to-PC ratio (model 1/2: $p = 0.001/p = 0.01$), and MELD ($p = 0.02$) independently predicted mortality. Patient groups with significantly higher probability of new-onset ascites, VB, and mortality were identified by certain cut-offs of vWF-Ag (213%, 466%, and 321%, respectively) and FVIII-to-PC ratio (1.99, 3.29, and 2.36, respectively). vWF-Ag and FVIII-to-PC ratio equaled MELD in mortality prediction.

Conclusions: Advanced cirrhosis is characterized by increased thrombotic potential. vWF-Ag and FVIII-to-PC ratio independently predict new-onset ascites, VB, and mortality. Targeting hypercoagulability could improve the outcome of patients with cirrhosis.

Lay summary: Higher von Willebrand factor antigen (vWF-Ag) levels and factor VIII-to-protein C (FVIII-to-PC) ratio are the prominent hemostatic disorders in patients with cirrhosis. vWF-Ag and FVIII-to-PC ratio independently predict new-onset ascites, variceal bleeding, and mortality in these patients.

Introduction

Low platelet counts and decreased levels of most procoagulant factors have been recognized for many years as the main hemostatic abnormalities in patients with cirrhosis. Depressed synthesis of extrinsic pathway factors, particularly factor VII, accounts for prolongation of prothrombin time in cirrhosis. The combination of thrombocytopenia with a prolonged prothrombin time or its derivative, the international normalized ratio, has long been suggestive of a hypocoagulable state, which predisposes patients with cirrhosis to increased bleeding risk [1]. However, the coagulation disorder as measured by routine laboratory tests does not appear to fully reflect the underlying hemostatic changes [2]. Indeed, abnormal coagulation tests in cirrhosis neither are predictive of bleeding complications [3] nor protect from venous thromboembolism [4–6]. Further, recent data documented that international normalized ratio is not itself a significant predictor of decompensation and mortality in patients with cirrhosis [7,8] despite the fact that it is included in common prognostic indices (Child-Pugh, model for end-stage liver disease [MELD]) [9,10].

Laboratory experiments performed in the past decade have demonstrated that the hemostatic system in patients with cirrhosis may not actually be in a hypocoagulable state. First, defects in platelet number are accompanied by substantially elevated levels of the platelet adhesive protein von Willebrand factor (vWF) [11,12]. The elevated levels of vWF in cirrhosis may be a consequence of endothelial perturbation taking into account that vWF antigen (vWF-Ag) is released by activated endothelial cells

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Abbreviations: MELD, Model for End-stage Liver Disease; vWF-Ag, von Willebrand factor antigen; PC, protein C; AT, antithrombin; FVIII, factor VIII; PH, portal hypertension; VB, variceal bleeding; AUC, area under the curve.

[13]. The induction of vWF-Ag synthesis in the cirrhotic liver is another possibility as vWF immunostaining is negative in the sinusoidal endothelial cells in the normal liver [14] but positive in cirrhotic liver tissues [15,16], presumably due to capillarization of the hepatic sinusoids [17]. Second, the prolongation of the coagulation times reflects the activity of only a portion of procoagulant factors and does not consider the concomitant decrease in anticoagulant factors [3]. It is now well-established that, despite the decreased levels of most coagulation factors, *in vitro* thrombin generation is maintained in patients with cirrhosis in the presence of thrombomodulin [18] or Protac® [19,20]. Even more counterintuitive is the increasing evidence that plasma from patients with cirrhosis may possess a procoagulant *vs.* anticoagulant imbalance, detected by thrombin generation tests, which appears to be related to two combined abnormalities: reduced levels of the most powerful naturally occurring anticoagulants antithrombin (AT) and protein C (PC) and increased levels of the most powerful procoagulant factor VIII (FVIII) [21]. Anticoagulant proteins decrease with liver disease severity due to impaired synthesis [1] while the considerable increase in FVIII levels in advanced cirrhosis has been attributed to decreased clearance from the circulation [22]. Since activated factor FVIII is inhibited *in vivo* by activated PC, the ratio of FVIII-to-PC is currently considered as an index of procoagulant imbalance [21].

From a clinical point of view, we have recently demonstrated a significant association of increased levels of thrombin-antithrombin complexes, as a marker of thrombotic potential in cirrhosis, with portal hypertension (PH)-related events, such as new-onset ascites and variceal bleeding (VB), and survival [7]. In addition, it was recently reported that prophylaxis with enoxaparin in patients with advanced cirrhosis reduced the risk of decompensation and improved survival without bleeding complications [8]. Further, vWF-Ag levels were shown to correlate with the degree of PH, as measured by hepatic venous pressure gradient, and predicted survival free of PH-related events and liver transplantation [23,24]. On the other hand, no data exist on the association of procoagulant imbalance with certain clinical endpoints in patients with advanced cirrhosis.

The aims of the present study were to investigate the impact and independent predictive value of major components of the hemostatic profile of cirrhosis, including platelet count, factors related to pro-and anticoagulant activity, FVIII-to-PC ratio, and vWF-Ag on the occurrence of major decompensating events, such as ascites and VB, and survival in patients with cirrhosis.

Patients and methods

Patients

One hundred and two adult patients with cirrhosis and thrombocytopenia defined by platelet count $<150 \times 10^9$/L [25] referred to the outpatient clinics between September 2010 and May 2013 were recruited after approval of the institutional review board and informed consent. Liver cirrhosis was proven either histologically or by unequivocal clinical and radiological findings. Severity of cirrhosis was evaluated by Pugh's modification of the Child classification [9] and MELD [10]. The patients were prospectively enrolled for each class of Child-Pugh until an equal distribution of severity of liver disease was attained. Criteria for exclusion at the time of blood sampling were: a) previously or ongoing use of therapy known to interfere with blood coagulation and platelet function (including concentrates of coagulation factors and fresh frozen plasma), b) known hemostatic disorders other than cirrhosis, c) portal, splenomesenteric or peripheral vein thrombosis by ultrasound evaluation and angio-computed tomography, d) portal hypertensive bleeding for at least 3 months before enrollment, e) ongoing bacterial infection, f) hepatocellular carcinoma or other intrahepatic or extrahepatic malignancy, g) abstinence from alcohol for less than 6 months, h) diabetes mellitus, i) hypertension, k) serum creatinine levels >1.5 mg/dl. All our patients abstained form alcohol throughout their follow-up and treated with indicated prophylactic as well therapeutic regimens. Patients were followed prospectively at least every 3 months at the outpatient clinic. PH-related events, such as ascites and varices at baseline, development of new-onset ascites and first episode of VB in patients with no history of VB during follow-up, portal vein thrombosis, hepatocellular carcinoma, liver transplantation, and death were recorded. As portal vein thrombosis is *per se* associated with development of ascites [26] and VB [6], only cases of new-onset ascites and VB not related to this complication were evaluated. Primary prophylaxis for VB in patients with varices on entry included propranolol, endoscopic band ligation or combination of the two. For survival analysis, the censoring was performed not only for death but also for hepatocellular carcinoma diagnosis and transplantation.

Methods

Blood collection and plasma preparation

Fasting whole blood samples (15 ml) were drawn from each patient at enrollment by clear venipuncture and collected in vacuum tubes (Becton and Dickinson, Meylan, France) containing 109 mmol/L trisodium citrate as anticoagulant in the proportion of 1:9 parts of anticoagulant/blood. Platelet-poor plasma was prepared by double centrifugation at 2000 g for 10 min. Plasma was aliquoted in plastic tubes, snap-frozen and stored at −80 °C to allow batch analysis in a blinded fashion. All measurements were performed on the same day and after all patients had been enrolled.

Procoagulant factors

Factors II, V, VII, and VIII activities were measured using commercially available reagents on British comparative thromboplastin (BCT) (Siemens, Marburg, Germany) and the results were expressed as percentage of normal pooled plasma arbitrarily set at 100% of normal. FVIII activity was measured by a one stage assay using Dade®Actin® FSL activated partial thromboplastin time reagent (Siemens Healthcare Diagnostics Inc. Newark USA).

Anticoagulant factors

Antithrombin (AT) and protein C (PC) activities were detected using the Berichrom AT and PC kits, respectively (Siemens Healthcare Diagnostics Inc. Newark USA) and the results were expressed as percentage of normal pooled plasma arbitrarily set at 100% of normal.

Procoagulance imbalance index

The ratio of factor VIII (FVIII)-to-PC was taken as an index of the procoagulant imbalance (the greater the ratio the higher the procoagulant imbalance).

vWF-Ag assay

vWF-Ag levels were determined by a turbidimetric assay on a Siemens BCS XP system applying appropriate reagents. Normal ranges of vWF-Ag were 70–120%.

Other routine laboratory tests

Other laboratory examinations, including platelet count, international normalized ratio, serum creatinine, and liver function tests were done as part of routine patient care.

Statistical analysis

Statistical analyses were performed using the SPSS 19.0 statistical package (SPSS Inc., Chicago, IL). Continuous variables are expressed as means ± standard deviation and compared by the unpaired Student's *t* test. Univariate linear regression analysis was performed to evaluate the effect of liver disease severity on coagulation measurements and to identify a relation of coagulation measurements with PH-related events and survival. The following variables at baseline were considered for univariate analysis: platelet count, factors II, V, VII, and VIII, AT, PC, FVIII-to-PC ratio, vWF-Ag, and MELD score. Continuous variables showing significance in univariate analyses were used in a multivariate logistic regression analysis (forward stepwise method) to assess the independence of predictive factors. Two multivariate models were employed: one including (model 1) and one excluding (model 2) MELD score. Receiver operator characteristic curves were used to define the cut-off points for vWF-Ag and FVIII-to-PC ratio as predictors of new-onset ascites, VB, and death. The value with the best sensitivity and