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Comparison of standard fibrinogen measurement methods with fibrin clot firmness assessed by thromboelastometry in patients with cirrhosis

Dragica Vucelic [a], Rada Jesic [b,c], Snezana Jovicic [d,e], Maja Zivotic [b,f], Nikica Grubor [a,b], Goran Trajkovic [b,g], Ivana Canic [e], Ivo Elezovic [b,h], Aleksandra Antovic [i,*]

[a] Clinic of Digestive Surgery, Clinical Center of Serbia, Belgrade, Serbia
[b] Faculty of Medicine, University of Belgrade, Serbia
[c] Clinic of Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia
[d] Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[e] Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia
[f] Institute of Pathology, Belgrade, Serbia
[g] Institute of Medical Statistics and Informatics, Belgrade, Serbia
[h] Clinic of Hematology, Clinical Center of Serbia, Belgrade, Serbia
[i] Karolinska Institute, Department of Medicine Solna, Rheumatology Unit and Karolinska University Hospital, Rheumatology Clinic, Stockholm, Sweden

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ABSTRACT

Background: The Clauss fibrinogen method and thrombin clotting time (TCT) are still routinely used in patients with cirrhosis to define fibrinogen concentration and clotting potential. The thromboelastometric functional fibrinogen FIBTEM assay evaluates the strength of fibrin-based clots in whole blood, providing information on both quantitative deficit and fibrin polymerization disorders.
Objective: To compare these three methods of assessing fibrinogen in patients with cirrhosis of different aetiologies, characterized by impairment in fibrinogen concentration as well as functional aberrance.
Methods: Sixty patients with alcoholic and 24 patients with cholestatic cirrhosis were included (Child-Pugh score (CPs)A, n = 24; B, n = 32; C, n = 28). All parameters were compared with those from a control group. Maximum clot firmness (MCF) in the FIBTEM test was assessed in regard to its relevance in detection of qualitative fibrinogen disorders in comparison with results obtained by standard measurement methods, i.e. the Clauss fibrinogen method and TCT.
Results: With increased cirrhosis severity, fibrinogen and FIBTEM-MCF levels significantly declined ($p = 0.002$), while TCT was significantly prolonged ($p = 0.002$). In all CPs groups, fibrinogen strongly correlated with FIBTEM-MCF ($r = 0.77$, $r = 0.72$, $r = 0.74$; $p < 0.001$), while cross-correlations of other assays were highly variable. The prevalence of decreased FIBTEM-MCF values (<9 mm) was significantly higher in advanced CPs categories ($p = 0.027$), whereby the highest prevalence was detected in patients with CPsC (10/16; 62.5%). Nine of the 16 patients with decreased FIBTEM-MCF values had also decreased fibrinogen levels, while in the remaining 7 patients fibrinogen levels were within the reference range, indicating the possible presence of qualitatively altered fibrinogen that could be detected by FIBTEM-MCF.
Conclusions: FIBTEM-MCF may be considered as a reliable alternative to standard plasma fibrinogen measurement in cirrhotic patients, especially in evaluating fibrin polymerization disorders in these patients. Further studies are needed to evaluate the usefulness of this assay in predicting bleeding complications in cirrhotic patients as well as monitoring replacement treatment.

Introduction

Alterations in fibrinogen metabolism contribute to the development of coagulation disorders in cirrhotic patients [1]. Since plasma fibrinogen is an acute-phase reactant, its levels remain normal or are increased in stable chronic liver disease. It seems that hypofibrinogenaemia occurs predominantly in patients with advanced cirrhosis. Decreased fibrinogen levels in such patients may be a consequence of impaired synthesis, accelerated clearance and consumption due to intravascular coagulation [1–5]. In chronic liver disease, fibrinogen molecules are often functionally aberrant (dysfibrinogenaemia) [6,7]. Dysfibrinogenaemia is not dependent on the fibrinogen concentration, which may vary from reduced to increased [8], but appears to be the result of re-expression of

* Corresponding author at: Karolinska Institute, Department of Medicine Solna, Rheumatology Unit, Karolinska University Hospital, Rheumatology Clinic, 171 76 Stockholm, Sweden. Tel.: +46 734294448.
E-mail address: aleksandra.antovic@ki.se (A. Antovic).

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fetal genes coding for fetal fibrinogen production in liver cell regeneration [9]. The content of sialic acid is increased in aberrant fibrinogen molecules from patients with liver disease, as in newborn infants, and raised serum and liver sialyltransferase activity has been described in association with hepatocellular regeneration [10,11]. These fibrinogen molecules are characterized by prolonged clotting time, normal release of fibrinopeptide A, and delayed polymerization of fibrin monomers. The mechanism by which sialic acid affects polymerization is unclear [9].

In routine coagulopathy screening, the Clauss fibrinogen assay or measurement of thrombin clotting time (TCT) are widely performed for detection of fibrinogen levels and abnormalities [12]. In contrast to these plasma-based coagulation tests reflecting time to clotting, the use of viscoelastic tests in whole blood, such as thromboelastometry or thromboelastography, enables recognition of overall disturbances in haemostasis [13]. Using whole blood, these methods cover most aspects of haemostasis, i.e. cells, coagulation and fibrinolytic components, reflecting overall clot elasticity. Thromboelastometry and thromboelastography have been extensively explored as unique gross tests of clot strength perfectly suited to assess haemostatic changes during liver transplantation [14–17]. It has been suggested that these techniques may be appropriate laboratory tools to investigate the coagulopathy associated with cirrhosis [18–20].

We aimed in this study to compare standard fibrinogen measurement methods with thromboelastometric FIBTEM assay and its derivate maximum clot firmness (MCF) in patients with increasing severity of cirrhosis.

Materials and Methods

Study was approved by the Institutional Review Board of the Faculty of Medicine, University of Belgrade and the Local Medical Ethics Committee. This was a cross-sectional single-centre study involving patients with alcoholic cirrhosis, patients with cholestatic cirrhosis and a control group. All patients were admitted to the Clinic of Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, between March 2011 and June 2013. Written informed consent was obtained from the majority of the patients prior to inclusion in the study. In the cases presented with quantitative disturbances of consciousness close relatives, who were completely informed about the aim of the study, provided consent.

The control group comprised 50 healthy volunteers (17 males and 33 females), selected among the medical staff. The mean age was 36 ± 8 (SD) years (range 20–58 years). None of the volunteers had a history of haematological, hepatic or other disorders, or any known treatment which could influence haemostasis.

Sixty patients (57 males and 3 females) with cirrhosis of an alcoholic aetiology were included. The mean age was 54 ± 11 years (range 29–79 years). Additionally, a group of 24 patients (5 males and 19 females), aged 54 ± 14 years (range 22–83 years) with cholestatic cirrhosis was included. Among them, 16 patients (15 females and 1 male) were diagnosed with primary biliary cirrhosis (PBC). The remaining 8 patients (4 males and 4 females) had primary sclerosing cholangitis (PSC). Exclusion criteria were: history of hereditary bleeding disorders, use of medication that affects haemostasis, known hereditary thrombophilia factors, hepatocellular carcinoma/extrahepatic malignant disease, viral hepatitis and postoperative states.

Diagnosis of cirrhosis was based on anamnesis, clinical features, laboratory tests, upper digestive endoscopy, imaging diagnostics, and, whenever possible, liver histology. The degree of liver failure was assessed according to Child-Turcotte-Pugh (a.k.a. Child-Pugh) classification, based on the levels of serum bilirubin and albumin, and prothrombin time, and the degree of ascites and encephalopathy. A total score of 5–6 is considered as grade A (well-compensated disease), 7–9 as grade B (significant functional compromise) and 10–15 as grade C (decompensated disease) [21,22].

Blood samples were taken at admission.

Blood Sampling and Plasma Preparation

Peripheral venous blood was collected into BD Vacutainer® Plus citrate tubes containing sodium citrate solution at 0.109 mol/L, pH 7.4 (Becton Dickinson, Plymouth, UK). For standard coagulation tests, within 60 minutes of sampling, platelet-poor plasma was obtained by centrifugation of citrated blood at 2000 ×*g* for 15 minutes at room temperature. Plasma samples were divided into aliquots of 0.5 mL and deep frozen at −70 °C until the assays were performed.

Serum samples for analyses of albumin and bilirubin were collected into BD Vacutainer® plastic serum tubes (Becton Dickinson, Plymouth, UK) and centrifuged at 1500 ×*g* for 10 minutes at room temperature.

Samples for full blood count determination were collected using BD Vacutainer® Plastic K_2EDTA tubes containing 3.6 mg K_2EDTA (Becton Dickinson, Plymouth, UK) and analysed on a Coulter® HmX Haematology Analyzer (Beckman Coulter, Inc., Fullerton, CA, USA).

For thromboelastometric examination, peripheral venous blood was collected into BD Vacutainer® Plus citrate tubes containing sodium citrate solution 0.109 mol/L, pH 7.4 (Becton Dickinson, BD-Plymouth, UK).

Measurements

Conventional Coagulation Tests and Biochemical Parameters

Assays were performed using a BCS® XP automated coagulation analyzer (Siemens Healthcare Diagnostics GmbH, Marburg, Germany). Prothrombin time (PT), activated partial thromboplastin time (APTT), TCT and fibrinogen were measured by clotting method using Thromborel® S, Pathromtin® SL, BC Thrombin® and Multifibren® U reagents, respectively (Siemens Healthcare Diagnostics GmbH, Marburg, Germany). Coagulation factors were assayed by using a PT-based clotting assay with Thromborel® S reagent for factor (F) II, FV, FVII and FX, as well as an APTT-based clotting assay with Pathromtin® SL reagent for FVIII, FIX, FXI, and FXII. Berichrom® FXIII reagent (Siemens Healthcare Diagnostics GmbH, Marburg, Germany) was used for spectrophotometric determination of FXIII activity. Von Willebrand factor antigen (vWF Ag) was measured using immunoturbidimetric assay (vWFAg®; Siemens Healthcare Diagnostics GmbH, Marburg, Germany). Antithrombin (AT) and protein C (PC) were determined by chromogenic assays (Berichrom® Antithrombin III and Berichrom® Protein C, respectively). Concentrations of albumin and bilirubin were determined by standard spectrophotometric methods and commercial reagents (Beckman Coulter, Inc., Brea, CA, USA). Reference values for parameters determined with Siemens reagents (coagulation factors, PT, APTT, TCT, AT, PC) are reported by Siemens, and the values for full blood count, albumin and bilirubin were provided by Beckman Coulter. All the reference values were validated on the local reference population, during the method validation process in the Center for Medical Biochemistry, Clinical Center of Serbia, where all these analysis were performed.

Rotational Thromboelastometry

For thromboelastometric examination, recalcified citrated blood (300 μL) was used, within one hour after venipuncture. A ROTEM® computerised analyser and a set of standard reagents (TEM International GmbH, Munich, Germany) were employed according to the manufacturer's recommendations. The tissue factor-triggered ROTEM® FIBTEM test was performed with addition of cytochalasin D, a strong inhibitor of platelet cytoskeleton. Graphical presentation of clot firmness against the time displayed on the ROTEM® screen defines the fibrin part of the clot only [17,23]. Maximum clot firmness in the FIBTEM test represents the maximum amplitude that the clot achieves during the measurement regardless of time. MCF is achieved after formation of a stable fibrin clot and it is a reflection of the absolute strength and stability of the clot. Reference ranges for the FIBTEM-MCF test were previously determined in a multicentre investigation [24]. Reference values were