Published in final edited form as:

Blood Coagul Fibrinolysis. 2015 October ; 26(7): 844–847. doi:10.1097/MBF.0000000000000365.

Plasma from chronic liver disease subjects exhibit differential ability to generate thrombin

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Abstract

Liver fibrosis in chronic liver disease (CLD) results in complex alterations in procoagulant and anticoagulant proteins. Although an elevated International Normalized Ratio (INR) is a prominent feature of progressive fibrosis, the utility of the INR to accurately reflect the net effect of these changes on the coagulation system is uncertain. In subjects with CLD, elevated INRs have been observed in both bleeding and thrombotic complications, suggesting limitations of the INR in characterizing the coagulation status. Unlike the INR, which is preferentially sensitive to the extrinsic pathway, the direct measurement of thrombin generation (TG) better captures the global coagulation cascade. We conducted a pilot study measuring the INR, chromogenic factor X (cFX) and TG in CLD subjects and compared them to control subjects and subjects on warfarin anticoagulation. We observed a large interquartile range (IQR) in TG among compensated CLD subjects across a narrow INR range, suggesting that the INR is a suboptimal surrogate measure of TG in CLD subjects.

Keywords

Thrombin generation; Factor X activity; Cirrhosis; Chronic liver disease; Coagulation

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Author's Contribution:

YZJ, SS, SCH, SAR, LAL, AA, CKD, SRE designed the study. YZJ, SS, SCH, SRE analyzed the data, wrote and edited the manuscript.

Conflict of Interest:

YZJ, SS, SCH, SAR, LAL AA, CKB and SRE have no conflict of interest to report.

Introduction

The liver is responsible for the synthesis and clearance of proteins involved in the coagulation cascade. Synthetic dysfunction of liver fibrosis results in complex alterations in procoagulant and anticoagulant proteins that are reflected by an elevated International Normalized Ratio (INR).[1] Alterations in hemostasis and coagulation proteins include a thrombocytopenic state, reduction of procoagulants factor II, V, VII, IX, X, XI, XII, and the natural inhibitors, antithrombin, protein C, protein S and dysfibrinogenemia.

However, the utility of the INR to accurately reflect the net effect of these changes on the coagulation system is less certain. The INR was originally developed to monitor the depth of warfarin anticoagulation and has been conveniently adapted to the chronic liver disease (CLD) population as a measure of coagulation function.[2] In the clinical setting, elevations in the INR resulting from liver fibrosis are frequently encountered in both bleeding and thrombotic settings that is exemplified by upper gastrointestinal bleeding and intra-abdominal thrombosis with progressive liver fibrosis. Taken together, this suggests limitations of the INR in characterizing the coagulation function in CLD subjects. Unlike the INR, which is preferentially sensitive to the extrinsic pathway, the measurement of thrombin generation (TG) is a superior method to capture the global coagulation cascade.[3] Therefore, our pilot study sought to characterize TG, chromogenic factor X (cFX) and the INR in CLD subjects and compared them to control subjects and subjects on warfarin anticoagulation.

Methods

We prospectively recruited medically stable subjects seen in the ambulatory setting at the University of Pittsburgh Medical Center (Montefiore/Presbyterian-Shadyside) from March 2013–May 2014. This study was approved by the institutional review board at the University of Pittsburgh. Thirty-four patients were recruited into the three study groups comprising of CLD (n=9), warfarin subjects (n=14), and control (n=11). Indications for warfarin anticoagulation were for fibrillation and venous thromboembolism. Peripheral blood were drawn from subjects into citrated blood collection tubes and centrifuged to obtain plasma. TG Assay was performed using the technothrombin TG assay (diaPharma, West Chester, OH) using RC low phospholipids reagent. Reactions assessed on a Synergy HT fluorometer (BioTek, Winooski, VT) and data analyzed using the Technothrombin TG assay software. The area under curve (AUC) plotted, representing TG was assessed. INR was performed using the Innovin reagent (Siemens, Tarrytown, NY) on the BCS-XP instrument (Siemens). cFX was performed using the cFX kit (diapharma) on a BCS-XP instrument using Standard Human Plasma (Siemens) as calibration standard. Statistical analysis was performed using SPSS Version 22. Spearman rho correlations were utilized to assess relationships between continuous variables.

Results

The INR demonstrated an inverse correlation with cFX in both warfarin ($\rho=-0.63$, $p=0.017$) and CLD ($\rho=-0.87$, $p=0.002$) subjects. In Figure 1, the respective cFX activity measured

within control, warfarin and CLD subjects ranged from 81–172% (median, $M=119\%$), 22–93% ($M=40\%$), and 27–80% ($M=44\%$). As validated in previous studies, warfarin subjects with therapeutic INR of 2.0–3.5 had cFX activity between 24–48% (mean, $\mu=35$), which corresponded to 20–35% of cFX activity observed in the control group ($\mu=126\%$).

The inverse relationship observed between the INR and cFX also extended to TG. The INR demonstrated an inverse correlation to TG in both warfarin ($\rho=-0.85$, $p<0.001$) and CLD ($\rho=-0.73$, $p=0.025$) subjects. The respective TG measured across control, warfarin subjects on therapeutic anticoagulation with INR 2.0–3.5 and CLD subjects ranged from 3000–4072 nmol/L x min ($M=3221$ nmol/L x min), 49–1487 nmol/L x min ($M=401$ nmol/L x min), and 210–2853 nmol/L x min ($M=1539$ nmol/L x min). As shown in Figure 2, warfarin subjects on therapeutic anticoagulation consistently demonstrates diminished levels <600 nmol/L x min. Compared to TG in control and warfarin subjects, TG in CLD subjects exhibited a large interquartile range (IQR) across the measured INR (CLD:1700 nmol/L x min vs. control:580 nmol/L x min vs. warfarin:290 nmol/L x min).

Lastly, we compared TG and cFX correlations within the same study groups by placing our Spearman rho correlations into a Hotelling's t test. In warfarin users, the correlation between INR and cFX was not significantly different from the correlation between INR and TG ($p=0.157$). Similarly, there was no difference in the INR correlation between the TG and cFX in the CLD patients. This finding was expected as the pairs of correlations were similarly strong and negative, as described above.

Discussion

Thrombin serves critical roles in hemostasis and thrombosis [3]. In patients with CLD, measuring plasma's ability to generate thrombin provides a better account of the net effects of elements involved in the forward reactions, reserve reactions, and natural inhibitors of coagulation. Therefore, TG may provide greater fidelity in characterizing coagulation status compared to the INR. The measurement of thrombin formation relies on the measurement of a fluorogenic substrate obtained via the activation of the coagulation cascade by tissue factor. The total area under the curve derived from this measurement over time gives the total thrombin generated, also known as the endogenous thrombin potential (ETP). Efforts to validate the use of TG in the clinical setting are ongoing with active research in numerous areas, including the hemophilia population. Unlike TG, the clinical application of cFX has been validated. The cFX assay represents a phospholipid-independent method of determining the activity of factor X. For example, the cFX assay is frequently used in monitoring warfarin anticoagulation in subjects with lupus anticoagulant, which interferes with the phospholipid-dependent INR testing. Other uses of cFX include guiding transition from direct thrombin inhibitors to warfarin and accurately monitoring warfarin therapy in dysfibrinogenemia where the interference of fibrin endpoint creates a falsely elevated INR. [4, 5]

In our pilot study, we observed a large IQR in TG among compensated CLD subjects across a narrow INR range. This observation contrasts against those IQR observed in control and warfarin subjects. This preliminary finding suggests that the INR is a suboptimal surrogate

measure of TG in CLD subjects. Given the wide variation in TG identified from our CLD subjects, we hypothesize that TG characterization in CLD subjects may allow for stratification of CLD subjects who are either at an increased thrombotic risk or increased bleeding risk.

We must note that almost three quarters of our CLD subjects had alcohol liver disease as their primary liver disease etiology. The limited data set in this preliminary evaluation precludes us from investigating differences across the various CLD etiologies. However, regardless of its etiologies, CLD is characterized by progressive stages of liver fibrosis and tissue remodelling leading to end-stage cirrhosis, we postulate that the stages of fibrotic remodelling may mirror the decrease in synthesis of coagulation factors where new investigational coagulation tests may be a superior measure than to the INR. This has clinical relevance since the INR is one parameter that is incorporated in to the Model for End-Stage Liver Disease (MELD) score which is a well validated predictor for survival. It remains to be determined whether the substitution of the TG for the INR within the MELD score may improve on predicting survival in the CLD population.

Acknowledgments

Funding source:

This work was supported in part by Thomas H. Nimick, Jr. Competitive Research Fund and the National Institutes of Health through Grant Numbers UL1RR024153 and UL1TR000005.

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Figure 1. Chromogenic Factor X (cFX) against the International Normalized Ratio (INR) across study groups of control, warfarin, and chronic liver disease (CLD) subjects.

Figure 2. Thrombin Generation (TG) against the International Normalized Ratio (INR) across study groups of control, warfarin, and chronic liver disease (CLD) subjects.