The use of thrombin generation assays (TGA) as prognostic tools in patients with hepatic disease

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INTRODUCTION

- The current tests, including PT/INR used to assess bleeding problems in patients with hepatic disease, do not reflect overall thrombin generation and therefore are insensitive to hypercoagulable states.
- The Thrombin Generation Assay (TGA), which measures the amount of thrombin generated, performed in whole blood (WB) is the most physiologic, but to date, has been technically challenging.
- Florida Hospital Center for Thrombosis Research has developed a technique for measuring thrombin generation in whole blood. This test has not yet been studied clinically, but is anticipated to be applicable to the study of hemostatic abnormalities.
- The primary purpose of this study was to compare TGA with the coagulation parameters conventionally used to evaluate the hemostatic status and hepatic function of patients with mild, moderate and advanced liver cirrhosis. The MELD score (routinely calculated for all patients with cirrhosis) was used to classify the patients with liver cirrhosis. A MELD score range of 6–9 indicates mild cirrhosis, a score in the range of 10–14 indicates moderate cirrhosis, and above 15 classifies the patients as advanced cirrhosis. We analyzed blood samples from 16 patients with cirrhosis and 5 patients with non-cirrhotic liver disease.

METHODS

- Whole blood (WB), autologous washed red blood cells (WBCs) to the hematocrit of the original blood.
- Each sample had its own calibrator, comprised of 20 μl of PPP or WB and 80 μl of blood were pipetted in quadruplicate in transparent round-bottom 96-well microtiter plates. Each sample had in triplicate, calibration of 20 μl thrombin and 80 μl of PPP or WB calibrator in duplicate. WB calibrator was prepared by mixing heat-inactivated PPP and autologous washed red blood cells (WBCs) to the hematocrit of the original blood sample. CaCl2 was added to trigger coagulation. The thrombin generated specifically cleaves the fibrinogen substrates (Z-TRA AMC HO), Bachiom BDD, PNA, included in each reaction mixture. The reaction was followed for 60 min. (B) The results are obtained in fluorescent units (FIU) transformed into a curve known as a thrombogram (right). Lag time (LT): peak thrombin (PTh), time to peak (TTP), and endogenous thrombin potential (ETP) were measured.

RESULTS

- Figure 1. (A) To measure the thrombin generation capacity of whole blood (WB). 20 μl of buffer (PBS, saline buffer pH 7.38, containing 6 μg heparin, water- and 80 μl of blood were pipetted in quadruplicate in transparent round-bottom 96-well microtiter plates. Each sample had in triplicate, calibration of 20 μl thrombin and 80 μl of PPP or WB calibrator in duplicate. WB calibrator was prepared by mixing heat-inactivated PPP and autologous washed red blood cells (WBCs) to the hematocrit of the original blood sample. CaCl2 was added to trigger coagulation. The thrombin generated specifically cleaves the fibrinogen substrates (Z-TRA AMC HO), Bachiom BDD, PNA, included in each reaction mixture. The reaction was followed for 60 min. (B) The results are obtained in fluorescent units (FIU) transformed into a curve known as a thrombogram (right). Lag time (LT): peak thrombin (PTh), time to peak (TTP), and endogenous thrombin potential (ETP) were measured.

- Figure 2. (A) A statistically significant difference was found between control and patient groups for PT and INR and coagulation factors II and X. PT and INR were significantly higher in patients with liver disease than in controls (p < 0.05). Thrombin generation (BTG), performed in whole blood, is insensitive to hypercoagulable states.

- Figure 3. Results shown for TGA in whole blood. The TGA parameters, peak thrombin (A) and ETP (B) showed negative correlation to MELD score (p < 0.05). MELD score was positively correlated with PT and INR (p < 0.05). When the groups of patients with non-cirrhosis, mild, moderate and advanced cirrhosis were compared with each other, there were statistically significant differences in INR and PT values of moderate versus mild cirrhosis, and moderate versus non-cirrhosis (p < 0.01). Even though the TGA parameters were not significantly different among the patients (most likely because of the relatively low numbers of patients), there was a very clear trend towards diminishing thrombin generation potential with increasing hepatic dysfunction (A and B), and this was mirrored when the PT was clearly by the INR (C).

- Figure 4. thrombogram in the 4 groups of patients with liver disease: non-cirrhosis, mild, moderate and advanced cirrhosis. The maximum amount of thrombin produced, and total amount of thrombin generated is higher in the group of patients with non-cirrhotic liver disease. PT and INR values in patients with liver disease. TGA parameters, peak thrombin and ETP, correlated with decreased levels of coagulation factors II, V, VII and X, and prolongations of the PT and INR in patients with liver disease.

- Table 1. (A) Patient demographics, clinical and routine test results. (B) Coagulation Variables. (C) TGA variables

- Table 2. A statistically significant difference was found between control and patient groups for PT and INR, and coagulation factors II and X. PT and INR were significantly higher in patients with liver disease than in controls (p < 0.05). Even though the TGA parameters were not significantly different among the patients (most likely because of the relatively low numbers of patients), there was a very clear trend towards diminishing thrombin generation potential with increasing hepatic dysfunction (A and B), and this was mirrored when the PT was clearly by the INR (C).

CONCLUSIONS

- The TGA in WB and plasma appears to better distinguish between the clinical grades of cirrhosis in the patients with liver disease. TGA in WB showed superior performance. The TGA parameters, PT and ETP, clearly show a difference between each group (figure 3A and 3B). On the other hand, the INR (figure 3C) was not able to distinguish between the patients without cirrhosis and those with mild cirrhosis, or between patients with moderate and advanced cirrhosis. Thus, the TGA might be a better parameter to include in the MELD score than the INR.

- Decreased amount of thrombin generation as reflected by TGA parameters, peak thrombin and ETP, correlated with decreased levels of coagulation factors II, V, VII and X, and prolongations of the PT and INR in patients with liver disease.

- The MELD score directly correlated with PT and INR and inversely correlated with coagulation factors V, VIII and X and the TGA parameter, peak thrombin.

- TGA in whole blood is the most physiological approach and may be a better prognostic clinical tool in patients with hepatic disease. TGA correlates closely with conventional methods of hemostatic assessment and may correlate more closely than these methods with the clinical stage of liver cirrhosis.

- Caution should be used when interpreting our results due to the relatively small numbers of patients studied. More studies, especially with patients with the more severe grades of hepatic dysfunction, are needed to fully evaluate the potential benefits of this new assay.

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REFERENCES

