

To clot or not to clot? A comparison between standard coagulation tests and rotational thromboelastometry in patients with End-Stage Liver Disease

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ABSTRACT

Introduction: Haemostatic alterations are common in patients with End-Stage Liver Disease (ESLD) and vary among haemorrhagic and prothrombotic states. Defining the haemostatic profile of such patients undergoing liver transplantation (LT) remains a challenge for the anaesthesiologist. The aim of the present study is to evaluate coagulation in patients with ESLD by comparing standard coagulation tests (SCT) with rotational thrombo-elastometry.

Methods: We retrospectively analyzed 56 patients that underwent LT between May and October 2013. Preoperative data were collected: age, etiology, severity of liver disease (Model for End-Stage Liver Disease – MELD and MELD-sodium – MELD-Na) and paraclinical results. SCT and rotational thromboelastometry (ROTEM) were assessed 1-3 hours prior to surgery.

Results: The mean age in the study group was 53.5 years (SD 11.7). MELD score had a median value of 17.0 (range 7-32) and MELD-Na of 22.0 (range 8 - 35). Fibrinogen polymerization (FibTEM MCF) strongly correlated with the severity of liver disease, assessed by MELD score (correlation coefficient -0.330, $p=0.05$) and MELD-Na score (correlation coefficient -0.353, $p=0.035$), and low cholesterol levels (correlation coefficient 0.443, $p=0.011$). When compared with SCT, FibTEM MCF strongly correlated with fibrinogen values (correlation coefficient 0.796, $p<0.001$), but no statistical correlation was found between InTEM CT and InTEM CFT and INR ($p=0.525$), aPTT ($p=0.108$) and PT ($p=0.406$) values. Platelet count correlated with spleen size (correlation coefficient -0.551, $p=0.009$), but did not correlate with either ExTEM CT ($p=0.345$) or ExTEM CFT ($p=0.254$).

Conclusion: In patients with ESLD, SCT are imprecise in establishing the haemostatic profile. The severity of liver disease strongly correlates with fibrinogen production and polymerization. Abnormal SCT values (high INR value, prolonged PT/aPTT time) do not correlate with TEM findings.

Key words: standard coagulation tests, rotational thromboelastometry, liver cirrhosis, coagulopathy, liver transplantation

INTRODUCTION

Patients with ESLD have been considered to have a bleeding tendency especially in the setting of major surgery such as LT. For this reason, large amounts of fresh frozen plasma are transfused in order to correct abnormal laboratory results such as an elevated international normalized ratio (INR) or activated partial thromboplastin time (aPTT). In the last decade the coagulation system in such patients has been redefined (1). In this new approach, the entire haemostatic system is rebalanced: for each alteration in an anti-haemostatic driver there is an opposite alteration in pro-haemostatic factors that counterbalances the entire system (2). Low platelet count is counterbalanced by an increase in von Willebrand factor and a down-regulation of ADAMTS13 levels (3), there is a decrease in both pro-coagulant and anti-coagulant factors (like protein C, S, antithrombin III), the low level of α 2-antiplasmin associate low levels of plasminogen. This new equilibrium is fragile and different factors such as infection, variceal bleeding or surgery can produce a disbalance in the haemostatic system and patients can shift towards thrombosis (4) or severe bleeding.

The question remains whether patients with ESLD have a bleeding or a thrombotic tendency. This question can't be answered by SCT such as INR and aPTT or by platelet count and fibrinogen concentration. Although INR represents a key point in the assessment of ESLD, as part of MELD score and Child-Pugh score, it remains a poor predictor of blood loss during LT (5). At the same time, overcorrection of SCT may itself promote bleeding by increasing portal hypertension (6). The mirage of SCT still remains in clinical practice today but the main indications for such tests have been forgotten: aPTT was developed as a diagnostic test for patients exhibiting features consistent with hemophilia and INR for monitoring warfarin anticoagulation. Such tests have received from clinicians a prospective value and the question that they were supposed to answer shifted from „why does this patient bleed?“ to „will this patient bleed“ (7). The disadvantages of such tests stretch beyond their predictive value for bleeding: there are multiple factors that create a high incidence of false positive results (specific factor deficiency, hyperfibrinolysis, presence of heparin, lupic anticoagulant), no assessment of hyperfibrinolysis and delayed availability (8).

In recent years viscoelastic testing has gained importance in haemostatic monitoring (9) and guidance of blood transfusion. Such tests have the advantage of

presenting a more detailed picture of the entire haemostatic process when compared with SCT in terms of platelet function, activation of the coagulation cascade, fibrin formation and polymerization and clot breakdown. Rotational thromboelastometry has a high specificity to detect hypo-, normo- and hyper- coagulability in non-cardiac surgery (10).

AIM OF STUDY

The aim of the present study was to assess coagulation in patients with ESLD by comparing SCT with rotational thromboelastometry, to evaluate the incidence of severity of coagulation defects. The secondary objective was to evaluate whether severity scores for ESLD correlate with the severity of coagulopathy.

METHODS

The ethical approval for this study was granted by the Ethical Committee of Fundeni Clinical Institute, Bucharest, Romania, in accordance with the principles of the Declaration of Helsinki. We prospectively included 56 patients with ESLD who had undergone LT at Fundeni Clinical Institute between May – October 2013. Exclusion criteria consisted of: acute liver failure as the main indication for LT, emergency re-transplantation and incomplete data recall. Epidemiological variables, co-morbidities, severity of liver disease, para-clinical results were recorded.

Coagulation assessment

Haemostasis was assessed, using a single blood sample, by SCT and thromboelastometry prior to surgery. Although subsequent assays were performed during surgery and postoperative period they were not included in the study. Blood was collected from the superficial venous system of the forearm 1-6 hours prior to surgery for haemostatic assessment. The same blood sample was divided into two identical test tubes (citrate 4.5 ml) and one additional tube for platelet count. SCT (INR, aPTT and PT – prothrombin time) were performed on automatic equipment provided by Fundeni Clinical Institute central laboratory. Fibrinogen concentration was determined using Clauss method. Thromboelastometric measures were performed on a ROTEM® device (Tem Innovations® GmbH, Germany) and included 4 standard assays: InTEM, ExTEM, FibTEM and ApTEM. ROTEM results were recorded after a 10 minute interval and at the end of the test. A graphic

Table 1 - Normal values for SCT and ROTEM assay as provided by the manufacturer (11)

Standard coagulation tests	INR 0.9-1.27 aPTT (sec) 22-32 PT (sec) 10.4-14.4 Fibrinogen (mg/dl) 200-400			
ROTEM	CT (s)	CFT (s)	MCF (mm)	ML (%ML)
InTEM	100-240	30-110	50-72	0-15
ExTEM	38-79	34-159	50-72	0-15
FibTEM	-	-	9-25	-
ApTEM	40-80	34-160	50-72	-

Abbreviations: CT – clotting time, CFT – clot formation time, MCF – maximum clot firmness, ML- maximum lysis

representation of the results was printed for further interpretation. Normal values for both SCT and ROTEM are listed in *table 1*.

Statistical analysis

Statistical analysis was performed using SPSS v19.0 (IBM, Armonk, NY). Data are presented as mean ± standard deviation of the mean, median (min, max), and otherwise percentage. Data distribution was examined in order to insure the proper statistical examination. Categorical variables were analyzed with Chi-square test and quantitative data were analyzed with independent samples t-test. Mann-Whitney test was used when the analyzed data did not follow a normal distribution. All P values are two-tailed. Statistical significance was considered at a p-value <0.05.

RESULTS

Of 56 patients two patients were excluded because of acute liver failure (one Wilson disease and one acute graft dysfunction). The remaining 54 patients were included in the study and statistically analyzed. The mean age in the study group was 53.5 ± 11.78 years and the median MELD score and MELD-Na score were 17 ± 6.6, 22 ± 6.8 respectively. Viral hepatitis was the main indication for LT (31.5%) and we observed a high incidence of hepatocarcinoma (33.3%). Demographic and laboratory data are presented in *table 2*.

Spleen size was a good predictor for platelet count (r=-0.511, p=0.009). MELD and MELD-Na score were strongly correlated with the severity of coagulopathy as assessed by SCT: INR (r=0.858, p=0.0001), PT (r=0.546, p=0.0001) – *figure 1*, aPTT (r=0.648, p=0.0001), Fibrinogen (r=-0.389, p=0.01). The severity of cholestasis correlates with the severity of coagulopathy: we observed an increase in INR (r=0.516, p=0.0001), PT

Table 2 - Demographic and paraclinical results

Age (years)	53.5 ± 11.78
Sex	
Male	59.3% (n=32)
Female	40.7% (n=22)
Etiology of ESLD	
Viral	31.5% (n=17)
hepatocarcinoma	33.3% (n=18)
Toxic	18.5% (n=10)
Other	16.7% (n=9)
MELD	17 ± 6.6
MELD-Na	22 ± 6.8
Cholesterol (mg/dl)	88 ± 48.1
Total Bilirubin (mg/dl)	3 (0.3 – 39.9)
Albumin (g/dl)	2.9 ± 0.71
Sodium (mmol/l)	135 ± 7.25
Spleen size (mm)	160 ± 30.9
INR	1.54 ± 0.51
PT (sec)	16.7 ± 5.83
Fibrinogen (mg/dl)	166 ± 88.6
Platelet count (/ul)	66500 (25000-336000)

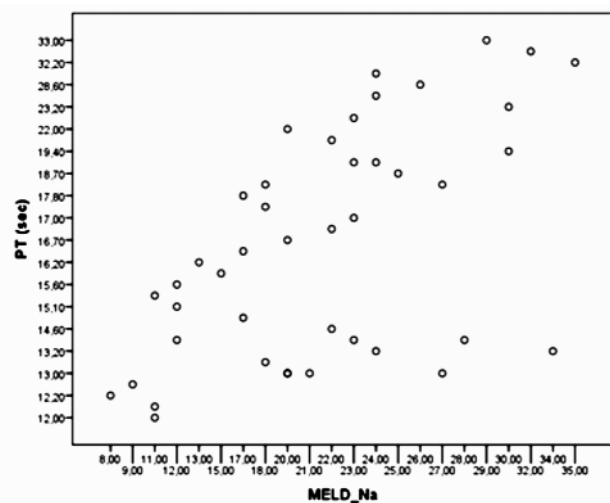


Figure 1 - Correlation between MELD-Na score and PT

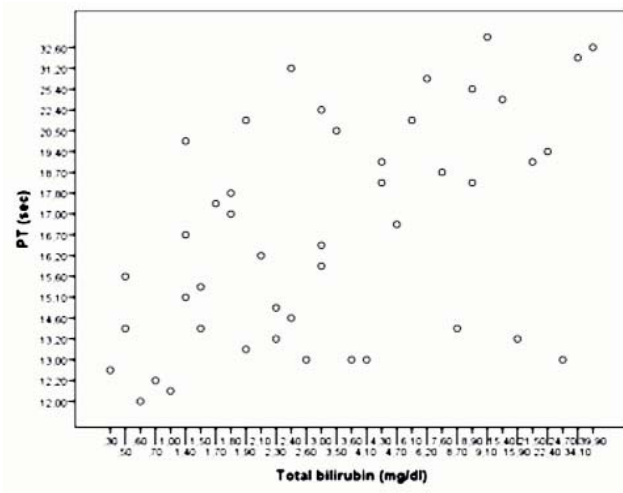


Figure 2 - Correlation between bilirubin levels and PT

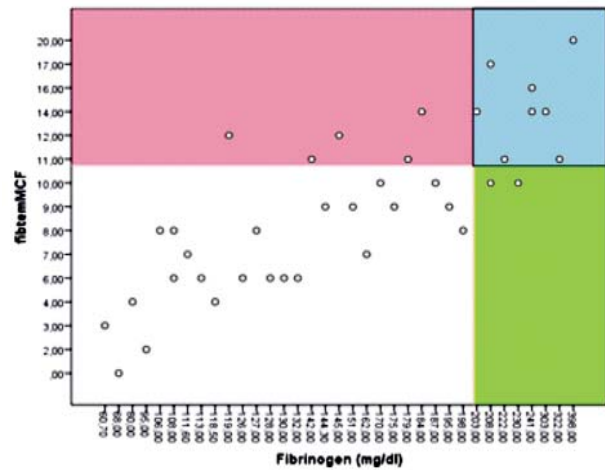


Figure 3 - Correlation between Fibrinogen concentration (determined by Clauss method) and FibTEM MCF

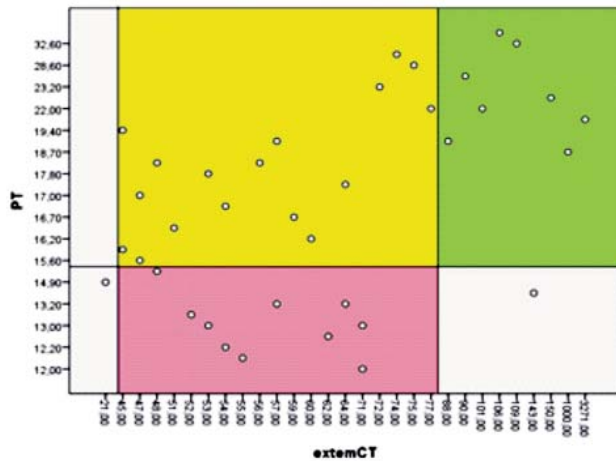


Figure 4 - Comparison between PT and ExTEM CT

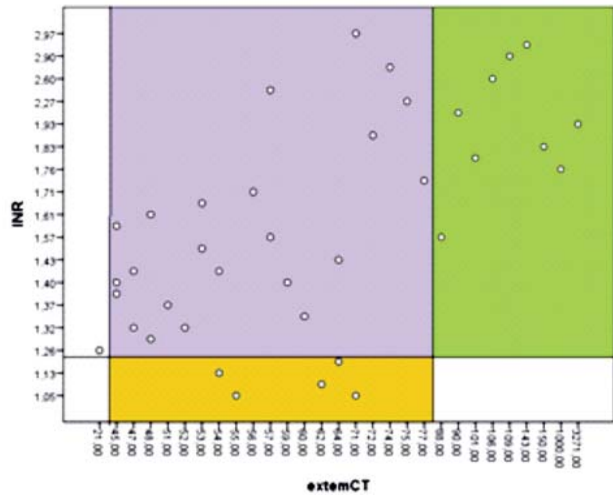


Figure 5 - Comparison between INR and ExTEM CT

($r=0.498$, $p=0.001$) and aPTT ($r=0.431$, $p=0.004$) proportionally with the increase in bilirubin levels - figure 2.

Low cholesterol levels correlated with an increase in INR ($r=-0.571$, $p=0.001$) and PT ($r=-0.314$, $p=0.05$) and a decrease in fibrinogen levels ($r=0.358$, $p=0.023$). Hypoalbuminemia correlated with higher aPTT values ($r=-0.536$, $p=0.001$) but not with INR ($p=0.166$) and fibrinogen levels ($p=0.056$). ROTEM 10 minutes results correlated with final assays at 60 minutes: FibTEM A10 with FibTEM MCF ($r=0.985$, $p=0.0001$) and ExTEM A10 with ExTEM CFT ($r=0.511$, $p=0.001$).

Comparison of SCT with ROTEM assay.

FibTEM MCF correlates with Fibrinogen levels (Clauss method): $r=0.796$, $p=0.001$. In the study group

5 pts (9.25%) had fibrinogen levels below 200 mg/dL although normal FibTEM MCF and 2 pts (3.7%) had normal fibrinogen levels with low fibrin polymerization (FibTEM MCF) – figure 3.

ExTEM CT does not correlate with either PT ($p=0.629$) – figure 4 or INR ($p=0.557$) – figure 5. Seventeen pts (31.5%) had a normal ExTEM CT value despite an increase in PT, 10 pts (18.5%) had both a normal PT and ExTEM CT and only 8 pts (14.8%) had a coagulopathy documented by an increase in both PT and ExTEM CT. We observed an increased INR in 49 pts (90%) but only 10 pts (18.5%) had an increase in ExTEM CT.

InTEM CT does not correlate with aPTT ($p=0.179$) – figure 6. Of all patients, only two (3.7%) had both an increased InTEM CT and aPTT, and two (3.7%) had an

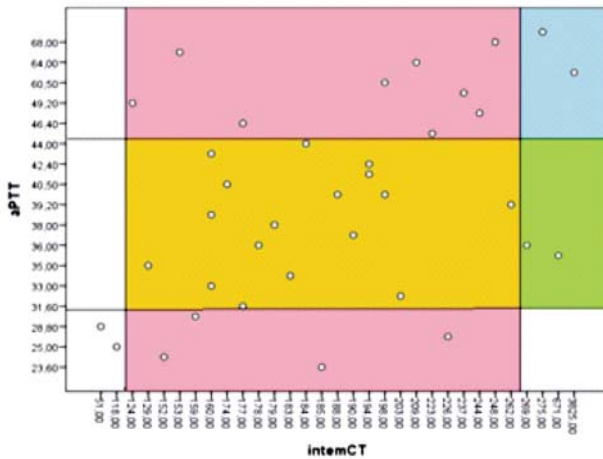


Figure 6 - Comparison between aPTT and InTEM CT

increased InTEM CT and normal aPTT. Seventeen pts (31.5%) had both a normal ExTEM assay and aPTT.

Hyperfibrinolysis was documented in 5 pts (9.25%) by an increased ExTEM CT that was significantly lower in ApTEM CT or ML > 15%.

When we compared ROTEM assays with ESLD severity scores we found a significant correlation between FibTEM MCF and MELD ($r=-0.330$, $p=0.04$) and MELD-Na ($r=-0.335$, $p=0.035$). Neither InTEM CT nor ExTEM CT correlated with MELD ($p=0.745$) and MELD-Na ($p=0.755$) scores.

DISCUSSIONS

In the present study we found a positive correlation between SCT and severity of ESLD scores but not between ROTEM assay and the same scores. This can be explained by the fact that INR remains an important part of ESLD assessment and organ allocation. SCT overestimate the severity of cirrhotic coagulopathy. Over 50% patients with prolonged aPTT, PT or INR results had normal ROTEM assay. Thus, an aggressive correction of SCT to near normal values is not necessary and may have negative effects on patient outcome [promoting bleeding by increasing portal pressure, transfusion-related adverse effects, thrombotic events (12)]. However, fibrinogen polymerization, determined by FibTEM MCF, correlates with fibrinogen concentration determined by Clauss method. This finding is consistent with the fact that protein synthesis, is an important marker of ESLD and overall outcome.

In their study Blasi et al. (13) did not find a positive correlation between ROTEM variables and PT or PT ratio but MCF ExTEM correlated with Child and MELD scores. This can be explained by a higher MELD score in

their study group and a lower incidence of prothrombotic illness such as hepatocarcinoma and cholestatic biliary disease.

Our results are in agreement with those of Tripodi et al. (14) that found overt coagulopathy in only 27% of patients. In their study MCF and CFT were correlated with fibrinogen concentration, platelet count and antithrombin concentration. When they correlated severity of liver disease (using the Child-Pugh score) they found MCF as a suitable prognostic index. The comparison was not performed in our study due to the lack of clinical usefulness of Child-Pugh score for organ allocation and severity assessment in the MELD era. This can be explained by the relative low severity of illness in their study when compared with ours: although different scoring systems were used, the majority of the patients included by Tripoldi (43/51 pts) were classified as Child-Pugh A or B.

Thromboelastometry is a very useful tool in coagulation assessment and guiding transfusion (15). The importance of ROTEM is partially based on its ability to provide fast results (16) in both numeric values and graphic representation. Rouillet et al. (17) also found that thromboelastometry can accurately detect thrombocytopenia and hypofibrinogenemia in cirrhotic patients. In their study clot amplitude (A10) at 10 minutes of ExTEM strongly correlated with platelet count and FibTEM A10 with fibrinogen. A threshold of 29 mm in ExTEM A10 had a sensitivity of 79% and a specificity of 60% for detecting platelet count < 50000/ul. Their relative low specificity and sensitivity can be explained by the fact that ROTEM analyses clot initiation and firmness (qualitative properties of platelets) rather than quantity (dimension of the clot). Nevertheless, their results are in agreement with ours and showed a good correlation between 10 minutes results and final ROTEM assay, stating that ROTEM is an accurate and rapid test.

Although not analyzed in the present study ROTEM represents a point-of-care tool for guiding blood transfusion. Recent studies (18,19,20) focused on establishing different protocols aimed towards fast interpretation and goal-directed trans-fusion therapy (21) in LT, without reaching any definitive results. Larger multicentre randomized studies are needed in order to develop such protocols.

CONCLUSION

In conclusion, SCT remain important tools for ESLD assessment and organ allocation but are unreliable in correctly assessing the severity of coagulopathy in

cirrhotic patients. Unnecessary transfusion has to be avoided when it is, dictated by the fear of bleeding in cirrhotic patients with prolonged SCT but with no clinical signs of bleeding. By contrast, rotational thromboelastometry offers a fast and reliable tool for both diagnosis and evidence-based treatment of such patients undergoing liver transplantation. Fast visualization of clot quality (by both numeric variables and graphics) at 10 minute interval offers reliable results for individual administration of blood products and clotting factors. The role of ROTEM in lowering transfusion requirements is still under debate and needs to be proven.

Acknowledgement

This paper was co-financed from the European Social Fund, through the Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/159/1.5/S/138907 "Excellence in scientific interdisciplinary research, doctoral and postdoctoral, in the economic, social and medical fields - EXCELIS", coordinator The Bucharest University of Economic Studies.

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