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Assessment of thrombin generation using thromboelastometry in cirrhotic patients with hepatocellular carcinoma undergoing liver transplantation

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ABSTRACT

Due to the mixed etiology of liver disease, cirrhotic patients with hepatocellular carcinoma may have either a pro-thrombotic or a bleeding tendency. Haemostatic assessment and blood management represent key issues during liver transplantation (LT) having a significant impact on survival and postoperative outcome. Our aim was to assess haemostatic profile in liver transplant recipients with hepatocellular carcinoma (HCC) and to evaluate its impact on perioperative anaesthetic management. We prospectively included 122 patients that underwent liver transplantation between January and December 2013. Exclusion criteria were incomplete data recall, preoperative anticoagulation treatment, acute liver failure and emergency re-transplantation. Coagulation was assessed using standard coagulation tests and rotational thromboelastometry (ROTEM®) assay performed preoperative and 15 minutes after reperfusion of the liver. We recorded intraoperative blood loss, transfusion requirements, Post Anaesthesia Care Unit length of stay and incidence of postoperative renal, pulmonary, thrombotic and hemorrhagic complications. After we applied the exclusion criteria, 80 patients were included in the final analysis: 24 patients in the HCC groupand 56 in the non-HCC group. Patients with HCC had increased preoperative thrombin generation demonstrated by a shorter clot formation time (142 vs. 381 sec, p= 0.01, 95% CI[41.9, 292.4]) and a shorter time to maximum velocity of clot formation (153 vs. 209, p=0.027, 95% CI [6.59, 105.3]). Intraoperative findings showed no differences in blood loss between the two groups, but lower fresh frozen plasma requirements (11.5 vs. 16.6 units, p =0.031) was observed in the HCC group. Intraoperative ROTEM® variables were similar in both groups measured after reperfusion of the graft. No statistically significant differences in post-operative outcome were observed between groups. In conclusion, patients in HCC group have increased preoperative thrombin generation as demonstrated by ROTEM derived parameters. During major abdominal surgery, like LT, and increased blood loss thrombin generation is similar in patients with and without HCC, demonstrating that haemostatic reserve is limited in HCC group.

Key words: liver transplantation, thromboelastometry, thrombin generation potential, hepato-cellular carcinoma

INTRODUCTION

Liver transplantation (LT) represents the only definitive treatment for patients with End-Stage Liver Disease (ESLD) (1). Intraoperative blood loss and transfusion represent important prognostic factors (2) in liver transplant recipients. Evaluation of haemostasis remains a crucial issue in perioperative anaesthetic management. Standard coagulation tests, although used on a great scale in cirrhotic patients, are not able to fully assess haemostasis or to guide perioperative blood transfusion (3). Moreover, they tend to overestimate bleeding tendency and increase transfusion requirements promoting both thrombotic complications and hypervolemia (4). Rotational thromboelastometry has shown to be a more precisepoint-of-care tool, for the assessment of coagulation and guiding transfusion (5).

Hepatocellular carcinoma (HCC) represents a common indication for LT in Romania. With an increased incidence of patients with HCC, anaesthesiologists must take into account a more complex view of haemostasis and thrombosis. National guidelines, in accordance with international protocols, should be developed in order to improve perioperative management and overall outcome.

The aim of our study was to compare perioperative haemostatic changes in liver transplant recipients with HCC and cirrhotic patients without HCC. Secondary endpoint was the comparison between the two study groups in terms of blood loss, transfusion and postoperative outcome.

METHODS

Ethical approval for the present study was granted by Fundeni Clinical Institute Ethical Committee (chairman Prof Mihai Voiculescu), in accordance with principles of the Declaration of Helsinki. We prospectively included 122 patients who underwent LT during one year period (January – December 2013) at Fundeni Clinical Institute. Exclusion criteria consisted of incomplete data recall, preoperative anticoagulation treatment, acute liver failure and emergency re-transplantation. At the time of LT, the patients were included in one of two groups: patients diagnosed with hepatocellular carcinoma (HCC group) and patients without hepatocellular carcinoma (non-HCC group). Hepatocellular carcinoma was diagnosed preoperatory by imagistic investigations (magnetic resonance imagining or contrast computer tomography) and serum levels of tumoral markers (alpha-fetoprotein). Histopathological confirmation of the diagnosis was performed in all cases after LT.

Data analysis

Clinical and laboratory data were collected during the preoperative, intraoperative and early postoperative period. Demographic variables, etiology of ESLD, severity of ESLD (assessed by Model for End-Stage Liver Disease score – MELD and MELD-sodium – MELD-Na score), incidence of portal vein thrombosis, standard coagulation tests (prothrombin time – PT, activated thromboplastin time – aPTT, international normalized ratio – INR, fibrinogen – Clauss method and platelet count), rotational thromboelastometry (ROTEM®, Tem Innovations GmbH, Germany) parameters were recorded. Intraoperative variables included: type of LT (deceased donor LT – DDLT or living-donor LT - LDLT), blood loss and transfusion requirements, duration of the anhepatic phase, incidence of postreperfusion syndrome. Standard coagulation tests and ROTEM® were performed before surgery and 15 minutes after the neohepatic phase. Postoperative incidence of renal (Acute Kidney Injury), pulmonary, hemorrhagic and thrombotic complications, transfusion requirements, hepatic artery resistance index (HARI) and Post Anaesthesia Care Unit length of stay (PACU LoS) were recorded. HARI was measured using Doppler echography as: (peak systolic velocity - end diastolic velocity)/ peak systolic velocity. Normal range for HARI was considered between 0.55 and 0.8.

ROTEM ® assay

ROTEM® assay was performed prior to LT (1-3 hours) and 15 minutes into the neohepatic phase, using 4,5 ml blood collected from the peripheral venous system of the forearm on citrate test tubes. The test consisted of whole blood analysis of haemostasis. Four thromboelastometric tests were performed on each blood sample: ExTEM, ApTEM, FibTEM and InTEM. Results were recorded after 10 minutes running time and at the end of the assay (one hour). A graphic representation of the results was printed for further interpretation. Thromboelastometric parameters included in the final analysis were: clotting time (CT), clot formation time (CFT), maximum lysis (ML), maximum clot firmness (MCF), maximum clot firmness at 10 minutes (A10), alpha angle, thrombin potential index (TPI), maximum velocity of clot formation (MaxV), time to MaxV (MaxVt) and area under the curve (AUC).

Statistical analysis

Statistical analyses were performed using SPSS 19.0 (IBM, Armonk, NY). Data distribution was examined in

order to insure the proper statistical analyses. Data is presented as mean ± standard deviation, median (range) or percentage. Categorical variables were analyzed with Chi-square test and quantitative data were analyzed with independent samples t-test (for normally distributed variables) or with Mann-Whitney test. Statistical significance was considered at a p value <0.05 (95% confidence interval).

RESULTS

After we applied the exclusion criteria, 80 patients were included in the final analysis: 24 patients in the HCC group and 56 in the non-HCC group. The mean age was 51.54±11.29 years, the median MELD score was 16.5 (7-34) and the median MELD-Na score was 21 (8-35). Preoperative data are presented in *table 1*.

Inter-group analysis demonstrated a significant difference between HCC and non-HCC groups regarding age (T-test, p=0.003) and MELD score (T-test, p=0.041). No difference was observed regarding MELD-Na, a score considered more precise to evaluate severity of ESLD. Standard coagulation tests showed no statistical difference in the two groups, except for a low difference in INR value, 1.41 in the HCC group and 1.57 in the non-HCC group (T-test, p=0.015).

Preoperative ROTEM® assay showed a higher incidence of hyperfibrinolysis (ExTEM and ApTEM ML) in the non-HCC group (24% vs. 5.7%, p=0.038), a shorter CFT in the HCC group (197 vs 364 sec, p= 0.01) and a shorter MaxVt (153 vs. 209 sec, p=0.027).

Patients in the HCC group required more fresh frozen plasma (FFP) transfusion during surgery (T-test, p=0.031), although no differences in either blood loss or other blood compounds transfusion was observed. Intraoperative ROTEM® assay showed no significant differences between the groups, except for ApTEM MCF (22.3 mm in the non-HCC group and 39.2 in the HCC group, p=0.002). Intraoperative data are presented in *table 2*.

Table 1 - Preoperative demographic data comparing patients with HCC and patients without HCC

	All patients (N=80)	HCC group (N=24)	Non-HCC group (N=56)	P value
Age (years)	51.54±11.29	56.29±5.53	49.5 ± 12.08	0.003*
MELD score	16.5 (7-34)	15 (8-24)	17 (7-34)	0.041*
MELD-Na score	21 (8-35)	21 (11-30)	20.5 (8-35)	0.271
INR	1.51 (0.98-3.97)	1.41 (1.05-1.93)	1.57 (0.98-3.97)	0.015*
aPTT (sec)	39.4 (22.0-70.9)	36.0 (23.6-60.7)	40.3 (22.0-70.9)	0.219
PT (sec)	16.5 (12.0-33.0)	16.0 (12.1-31.2)	16.7 (12.0-33.0)	0.721
Fibrinogen (mg/dl)	160 (60-478)	184 (60-393)	144 (68-478)	0.286
Platelet count (/ul)	67500 (17000-336000)	63500 (30000-188000)	72500 (17000-336000)	0.404
Portal vein thrombosis (%)	15% (n=12)	25% (n=6)	10% (n=6)	0.252

MELD - Model for End-Stage Liver Disease score, MELD-Na- Model for End-Stage Liver Disease –sodium score, INR- international normalized ratio, PT- prothrombin time, aPTT- activated thromboplastin time * refers to a significant p value<0.05

Table 2 - Comparison of intraoperative data between patients with HCC and patients without HCC

	All patients (N=80)	HCC group (N=24)	Non-HCC group (N=56)	P value
Blood loss (ml)	3500 (500-23000)	3400 (500-15500)	3500 (500-23000)	0.354
Transfusion (U)				
FFP	10 (0-38)	10 (0-34)	15 (3-38)	0.031*
PRBc	4 (0-27)	4 (0-27)	4 (0-19)	0.662
Cryo	1 (0-17)	1 (0-16)	1 (0-17)	0.609
PLT	1 (0-18)	1 (0-13)	1 (0-18)	0.708
Duration of anhepatic phase (min)	46.96 ± 17.59	42.3 ± 19.61	48.70 ± 16.66	0.232
Postreperfusion syndrome (%)	73.7% (n=59)	83.3% (n=20)	69.6% (n=39)	0.328
INR1.5 (1.16-2.12)	1.49 (1.19-1.88)	1.54 (1.16-2.12)	0.375	
aPTT (sec)	48.6 (26.1-150.1)	46.2 (30.0-72.1)	51 (26.1-150.1)	0.126
PT (sec)	15.7 (12.9-26.4)	15.7 (12.9-26.0)	15.8 (12.9-26.3)	0.746
Fibrinogen (mg/dl)	154 (90-354)	165 (101-354)	152 (90-294)	0.446
Platelets (/ul)	53500 (15000-173000)	46000 (29000-173000)	61000 (15000-154000)	0.442

FFP – fresh frozen plasma, PRBc – packed red blood cells, Cryo – cryoprecipitate, PLT – platelet apheresis, INR- international normalized ratio, PT- prothrombin time, aPTT- activated thromboplastin time * refers to a significant p value <0.05

Table 3 - Comparison in short term outcome between with HCC and patients without HCC

	All patients (N=80)	HCC group (N=24)	Non-HCC group (N=56)	P value
PACU LoS (days)	7 (3-30)	7 (4-19)	7 (3-30)	0.607
Renal complications (%)	46.2 % (n=37)	41.7% (n=10)	48.2% (n=27)	
Pulmonary complications (%)	61.2% (n=49)	70.8% (n=17)	57.1% (n=32)	0.364
Thrombotic complications (%)	2.5% (n=2)	8.3% (n=2)	0% (n=0)	0.071
Hemorrhagic complications (%)	3.8% (n=3)	8.3% (n=2)	1.8% (n=1)	0.177
Postoperative transfusion requirements (%)	73.7% (n=59)	75% (n=18)	73.2% (n=41)	0.051
Delayed graft function (%)	53.7% (n=43)	62.5% (n=15)	50% (n=28)	0.343
Abnormal HARI (%)	25% (n=20)	20.8% (n=5)	26.8% (n=15)	0.517

PACU LoS-Post Anaesthesia Care Unit length of stay, HARI - hepatic artery resistance index (HARI)

No difference in short term outcome was observed between the two groups (*table 3*). Although they did not reach statistical significance, all thrombotic complications were observed in the HCC group.

DISCUSSION

The incidence of hepatocellular carcinoma remains high (30%) among liver transplant recipients in Romania. HCC patients are younger and have lower severity of liver disease score (MELD score) at the time of LT. Our results show that patients with HCC have an increased thrombin generation as demonstrated by derived thromboelastometric parameters such as CFT and MaxVt. This translates into a shorter duration in maximum thrombin generation time than in non-HCC patients. Also, a lower incidence in hyperfibirnolysis may denote a more stable clot with increased firmness. In the setting of LT and increased blood loss, no differences were observed between the two groups, denoting that haemostatic resources are limited in patients with HCC.

In our study the incidence of HCC among liver transplant recipients is within the range reported in Europe and in the United States (6), between 26% and 34%. The development and implementation of Milan criteria lead to similar survival benefits among patients with and without HCC (7).

Recent studies (8) demonstrated increased procoagulant activities and increased thrombin generation in patients with cancer. At the same time, patients with ESLD may develop either a pro-thrombotic or a hemorrhagic state (9). Patients with HCC that undergo LT associate liver cirrhosis in over 90% of cases (10). The question that we ask is: where do cirrhotic patients with HCC stand - does their haemostatic balance lean towards hyper-coagulation or hemorrhage? Unfortunately, no clinical trials have been reported in order to answer that question. To the author knowledge, this study is the first to address this critical issue. Our results demonstrate that HCC patients have increased thrombin generation when compared with non-HCC cirrhotic patients. This may be due to the presence of pro-thrombotic activity in patients with HCC. Another possible explanation resides in the fact that patients with HCC have milder severity scores for ESLD when compared with cirrhotic patients without HCC.

It has been well documented that intraoperative transfusion requirements have a detrimental effect on postoperative outcome and survival after LT (11,12). Different studies have tried to identify reversible risk factors for massive intraoperative blood loss and transfusion during liver transplantation reaching conflicting results (13,14). In our study group, there were no significant differences in regard to PRBc, cryoprecipitate and platelet transfusion. Patients with HCC had lower FFP transfusion requirements probably due to a lower incidence of hypo-coagulability as demonstrated by ROTEM® assay. Published data support the idea that transfusion-free transplantation in HCC may have a benefic effect on recurrence-free survival (15), with some studies (16) even augmenting the benefits of autologous blood transfusion during surgery.

Rotational thromboelastometry has gained increasing acceptance as a point-of-care device in establishing haemostatic profile (17) and guiding transfusion during LT (18). Both thromboelastometry and thromboelastography proved to be exact tools in detecting hyper-coagulability (19,20). The incidence of thrombotic complications remains low after LT (2.5% in our study). Although we could not demonstrate a significant correlation between postoperative thrombotic complications and HCC, all observed cases (n=2) were in the HCC group. Larger clinical trials are required in order to clarify this issue. Recent studies (21) identify HCC as a

potential risk factor for deep venous thrombosis and indicate thromboprophylactic anticoagulation.

No difference between short-term outcomes has been observed between groups. We did not observe any statistical differences in median PACU LoS and incidence of postoperative complications. As mentioned, all thrombotic complications were observed in the HCC group, but the relative small number of patients diagnosed with thrombotic complications prevented us from reaching any definitive conclusion.

In conclusion, patients with liver cirrhosis and HCC have increased thrombin generation as demonstrated by preoperative ROTEM assay. This may be due to the relative lower severity of liver disease in patients with HCC when compared to patients with ESLD without HCC. In the setting of major surgery and blood loss, thrombin generation is similar in the two groups, demonstrating that haemostatic reserve in the HCC group is limited.

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