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ORIGINAL ARTICLE

Enhanced thrombin generation in patients with cirrhosis-induced coagulopathy

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Summary. *Background:* Prothrombin time (PT) and the international normalized ratio (INR) are still routinely measured in patients with liver cirrhosis to 'assess' their bleeding risk despite the lack of correlation with the two. Thrombin generation (TG) assays are global assays of coagulation that are showing promise in assessing bleeding and thrombosis risks. *Aim:* To study the relationship between the INR and TG profiles in cirrhosis-induced coagulopathy. *Methods:* Seventy-three patients with cirrhosis were studied. All TG parameters were compared with those from a normal control group. Contact activation was prevented using corn trypsin inhibitor. TG was also assayed in the presence of Protac®. The endogenous thrombin potential (ETP) ratio was derived by dividing the ETP with Protac® by the ETP without Protac®. *Results:* The INR (mean 1.7) did not correlate with the ETP and the velocity of TG ($P > 0.05$). There was no difference between the lag time and ETP of the two groups ($P > 0.05$). The velocity of TG was increased in cirrhosis (67.95 ± 34.8 vs. 45.05 ± 25.9 nM min^{-1}; $P = 0.016$) especially in patients with INRs between 1.21 and 2.0. Both the ETP with Protac® and the ETP ratio were increased in cirrhosis (mean 1074 ± 461.4 vs. 818 ± 357.9 nM min, $P = 0.004$ and 0.80 ± 0.21 vs. 0.44 ± 0.15, $P \leq 0.0001$, respectively). *Conclusion:* Despite a raised INR, TG parameters are consistent with a hypercoagulable profile in cirrhosis-related coagulopathy. This confirms that the PT or INR should not be used to assess bleeding risk in these patients, and other parameters, such as TG, need to be explored as clinical markers of coagulopathy.

Keywords: hypercoagulable, INR, liver coagulopathy, protein C, thrombin generation.

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Introduction

Since its inception by Armand Quick [1], prothrombin time (PT) has been noted to be prolonged in patients with liver disorders. For the past 70 years physicians worldwide have been using this test [or its derivative, the international normalized ratio (INR)] to assess the bleeding risk of patients with liver disorders. The value of this test in predicting bleeding in these patients has been questioned as no association has been found between bleeding outcomes and PT or INR values [2,3]. Despite this, guidelines still advocate the use of blood products to correct the INR prior to invasive procedures [4] and a recent multidisciplinary symposium on the subject showed that a significant number of physicians still concur with this practice [5]. This practice not only can delay these procedures, but also exposes patients to transfusion-related complications.

Thrombin generation (TG) assays are global tests of coagulation that measure the dynamics of thrombin production using small amounts of tissue factor as a trigger in order to achieve a full interplay of all coagulation factors. Thrombin is one of the most crucial coagulation enzymes in the coagulation cascade. It cleaves the soluble fibrinogen to the clot forming fibrin. In view of this, these assays could be superior in assessing bleeding risk when compared with the traditional coagulation tests such as the PT and activated partial thromboplastin time, which are completely 'unphysiological'. Thrombin generation tests have already been shown to be useful in identifying patients at an increased risk of thrombosis [6–8] and those with bleeding tendencies [9]. Specifically in liver disorders, Tripodi *et al.* [10] reported that patients with cirrhosis had normal thrombin generation in the presence of thrombomodulin. This publication highlighted the limitations of the PT, as this test is only sensitive to variations of coagulation factors (F)I, II, V, VII and X, most of which are usually reduced in cirrhosis but not to other factors such as FVIII which is increased, and antithrombin (AT) and protein C (PC) which are reduced.

The aim of the present study was to check the utility of a thrombin generation assay, the calibrated automated thrombogram (CAT), in patients with cirrhosis and especially those with coagulopathy as judged by a high INR. We wanted to verify the findings from the above publication [10] which might have been influenced by contact activation, a phenomenon that results in a spurious increase in thrombin production as a result of the activation of FXII, or by bias owing to some patients having a normal PT. We also postulated that even if the endogenous thrombin potential (ETP), which measures the amount of thrombin produced in a given time, was normal, there could be defects in other stages of the thrombin production loop as recently observed in patients with sepsis [11].

Subjects and methods

Blood samples from 73 unselected adult patients with cirrhosis were studied as part of service development with a view to introducing thrombin generation into routine clinical practice, as approved by our local research ethics committee and National Research Ethics Service guidelines. The diagnosis of cirrhosis was based on clinical, laboratory and ultrasound evidence. The patients were not using medications known to affect blood coagulation, and were not known to have hemostatic disorders other than liver disease. Severity of cirrhosis was estimated according to model for end-stage liver disease (MELD) classification [12]. Thirty-eight healthy subjects were also recruited after providing written informed consent. These individuals were not taking any medications, including oral contraception, which could have affected their coagulation, and were not known to suffer from bleeding or thrombotic disorders.

'*Unmodified*' *thrombin generation*

Blood was drawn from an antecubital vein with minimal stasis and collected in Sarstedt Monovette® blood collection tubes (Nuembrecht, Germany) containing 0.106 M sodium citrate (final dilution 1:10) and 20 μg mL^{-1} corn trypsin inhibitor (CTI) (Cambridge BioSciences, Peterborough, UK) to inhibit contact factor activation. Platelet-poor plasma (PPP) was prepared by double centrifugation at 2000 × *g* for 12 min at 4 °C and then a further centrifugation step at 13 000 × g for 2 min in order to eliminate platelet fragments. Samples were stored in aliquots at −45 °C until tested. Thrombin generation was assessed using the CAT method as published by Hemker *et al.* [13]. Both the patient group and the control group's CTI samples were tested at 1 pM TF. The TG parameters lag time (LT), time to peak thrombin (ttPeak), peak thrombin (peak), endogenous thrombin potential (ETP) and start tail time (STT) were generated by dedicated software version 3.0.0.29 (Thrombinoscope™ B.V., Maastricht, the Netherlands). STT corresponds to the time from the start of the reaction to when the TG curve touches zero at the end of the reaction. We calculated the maximum velocity of thrombin generation by dividing the peak thrombin by the difference between the ttPeak and the LT. The rate of thrombin inhibition was derived from the formula

$$\frac{\text{Peak thrombin (nM)}}{\text{STT - ttPeak (min)}}$$

The Protac®-modified TG assay

A sample of blood was taken in standard Vacutainer (Becton Dickinson, Plymouth, UK) tubes containing 0.109 M buffered trisodium citrate for the Protac® TG studies. Protac® (Pentapharm, Basel, Switzerland) is a snake venom extract that activates protein C (PC). This assay was used as previously described [14]. The concentration of Protac® used was 0.3 U mL^{-1} and tests were carried out at 5 pM TF. Of note is that this assay has been validated in patients with defects in the protein C anticoagulant pathway and has been shown to be sensitive to deficiencies of PC, PS and other prothrombotic states such as factor (F)V Leiden [14–16]. When evaluating this assay, we had demonstrated that this does not require contact inactivation. This was done by testing the same patients' blood with and without CTI. No variations were noted in the TG parameters in these two groups [14]. As the third centrifugation step (as outlined above) can be cumbersome, we tested whether there were any differences between double and triple centrifugation of plasma samples when tested at 5 pM TF. No differences were observed (data not shown). So for this assay which was devised to test specifically the impact of the protein C pathway, we utilized standard double centrifugation at 2000 × *g* for 12 min. The patient and normal group samples were processed in exactly the same way. The ETP ratio was derived by dividing the ETP with Protac by the ETP without Protac.

All the TF trigger reagents were obtained from Thrombinoscope™ B.V. and contain a 4 μM L^{-1} final concentration of phospholipids. Next, 80 μL of plasma are pipetted in a 96-well plate (Immulon 2HB, U bottom plate; Thermo Lab Systems, Franklin, MA, USA) to which 20 μL of trigger is added. The reaction is initiated after automated dispensing of 20 μL of substrate reagent containing 0.1 M $CaCl_2$ and 2.5 mM fluorogenic substrate Z-Gly-Gly-Arg-AMC.HCl (BACHEM, Switzerland). Fluorescence is read using a Fluoroskan Ascent fluorimeter (Thermolab Systems, Helsinki, Finland). The same batch of reagents was used throughout the study to minimize interassay variation. PPP samples were tested in duplicate together with one calibrator (Thrombin Calibrator reagent Thrombinoscope™ B.V.) well running in parallel.

Routine coagulation tests

Blood samples were taken in standard Vacutainer (Becton Dickinson, Plymouth, UK) tubes containing 0.109 M buffered trisodium citrate and using 23-g butterfly needles. Prothrombin times were performed using PT Fibrinogen HS Plus thromboplastin reagent (Instrumentation Laboratory, Warrington, UK) with an international sensitivity index (ISI) of 1.15

according to manufacturer's instructions using an ACL TOP automated coagulometer (Instrumentation Laboratory) as per routine practice. PT ratios were calculated for each patient from the geometric mean PT (GMPT) calculated from 23 normal individuals. INRs were calculated using the GMPT and the manufacturer's ISI. FVIII was assayed using a standard one-stage clotting assay and antithrombin (AT) activity assays by an in-house chromogenic assay on a ACL 300R. Protein C activity was tested using the HaemosIL chromogenic protein C assay and free Protein S using the HaemosIL Free Protein S assay (both bought from Instrumentation Laboratory). FII and FV were analyzed by a one-stage clotting PT-based assay on an ACL 3000 (IL). Tissue factor pathway inhibitor (TFPI) activity was assayed using the Actichrome TFPI activity assay (American Diagnostics, Stamford, USA). Fibrinogen was measured using the recommended Clauss method [17]. Liver function tests and other analyzes were done as part of routine patient care.

Statistics

All groups of results were tested for normality. Where the distribution was normal Pearson's correlation test was used. In the case of non-Gaussian distributions a non-parametric Spearman's correlation was performed. A P-value of < 0.05 was considered to be statistically significant. The normal ranges were calculated by the mean $\pm$ 2 SD if not otherwise specified. A hierarchical multiple regression analysis was carried out to identify independent predictors of the various thrombin generation parameters. The LT showed a skewed distribution and was log transformed for normalization purposes in this model. GraphPad Prism version 4.0.3 (GraphPad Software Inc., La Jolla, CA, USA) and SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) were used for analyzes.

Results

From a total of 73 patients, 47 were men and 26 women, with a mean age of 53.6 years (range 26–79 years). These were all non-bleeding patients. Cirrhosis was related to alcohol (ALD) in 33 patients, hepatitis C virus(HCV) in 15, hepatitis B virus (HBV) in 1, primary biliary cirrhosis(PBC) or primary sclerosing cholangitis (PSC) in 10 and cryptogenic cirrhosis in 10 patients. Three patients had HCV and ALD and another patient had HBV and ALD. The mean MELD value was 12.1 $\pm$ 8.8.

The control group consisted of 38 people, 17 males and 21 females. The mean age was 38.4 years (range 24–64 years).

Demographic, clinical and laboratory features of patients are shown in Table 1a, b.

The INR range was 0.9–5.3 (mean INR 1.7).

Thrombin generation

No correlation was found between the INR and the ETP or velocity of TG ($P \geq 0.05$). There was no difference between the LT of the normal individuals and the patients (Table 2). The maximum velocity of thrombin formation was significantly increased in the patients with cirrhosis (difference between the means -18.71 ± 7.603 nM min^{-1}; 95% CI -33.83 to -3.595; $P = 0.016$) (Table 2).

No difference between the mean ETP of the patient group and the controls was detected (Table 2). Indeed only 8.2% of patients had an ETP lower than the lower limit of normal (i.e. 682 nM min). The rate of thrombin inhibition was higher in the controls than in the patients albeit not significant (mean 9.97 vs. 8.85 nM min^{-1}; $P = 0.27$).

Table 1 (a) Patient demographics and routine test results. (b) Coagulation variables

Variable	Result mean $\pm$ SD	Controls and normal ranges
(a)		
Age (years)*	53.6 $\pm$ 12.4	38.4 $\pm$ 9.8
Gender		
Male	47(64.4%)	17 (44.7%)
Female	26 (35.6%)	21 (55.3%)
Etiology of cirrhosis		
ALD	33 (45.2%)	N/A
HCV	15 (20.6%)	
HBV	1 (1.4%)	
PBC, PSC	10 (13.7%)	
Cryptogenic	10 (13.7%)	
HCV and ALD	3 (4.1%)	
HBV and ALD	1 (1.4%)	
AST (IU L^{-1})*	75.0 $\pm$ 52.1	< 31
ALT (IU L^{-1})*	58.7 $\pm$ 56.9	< 31
ALP (IU L^{-1})*	166.5 $\pm$ 185.6	35–129
WBC*	7200 $\pm$ 4000	3500–11000
PLT*	138100 $\pm$ 92000	140000–400000
INR*	1.7 $\pm$ 0.7	0.9–1.2
Bilirubin (mg dL^{-1})*	5.0 $\pm$ 8.5	< 1.2
Albumin (g L^{-1})*	34.1 $\pm$ 7.1	35–50
Creatinine (mg dL^{-1})*	1.0 $\pm$ 0.5	0.6–1.0
MELD*	12.1 $\pm$ 8.8	N/A

Variable	Result Mean $\pm$ SD	Reference ranges Mean $\pm$ SD
(b)		
PT (s)	20.1 $\pm$ 6.5	13.5 $\pm$ 0.9
APTT (s)	40.9 $\pm$ 10.4	31.1 $\pm$ 2.7
Antithrombin (IU dL^{-1})	60.5 $\pm$ 23.2	98.6 $\pm$ 8.6
Protein C (IU dL^{-1})	46.7 $\pm$ 30.9	99.3 $\pm$ 19.8
Free protein S (IU dL^{-1})	93.9 $\pm$ 35.1	98.5 $\pm$ 15.1
TFPI (U mL^{-1})	0.99 $\pm$ 0.65	1.0 $\pm$ 0.20
Fibrinogen (g L^{-1})	3.3 $\pm$ 3.4	2.9 $\pm$ 0.4
FII (IU dL^{-1})	60.2 $\pm$ 32.2	101.1 $\pm$ 14.0
FV (IU dL^{-1})	78.4 $\pm$ 39.8	92.7 $\pm$ 18.1
FVIII (IU dL^{-1})	184.1 $\pm$ 73	113.9 $\pm$ 33.0
FXII (IU dL^{-1})	76.3 $\pm$ 36.4	107.8 $\pm$ 42.3

*Mean $\pm$ SD.

ALD, related to alcohol; HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; WBC, white blood cell count; PLT, platelet count; INR, international normalized ratio; MELD, model of end-stage liver disease; PT, prothrombin time; APTT, activated partial thromboplastin time; TFPI, tissue factor pathway inhibitor.

Table 2 Mean (SD) of the individual thrombin generation (TG) parameters

TG Parameters	Controls	Cirrhosis	*P*
LT (s)	4.45 (0.95)	6.31 (5.13)	0.66
Peak (nM)	183 (72.1)	184.2 (72.39)	0.94
Velocity (nM min^{-1})	45.05 (25.9)	67.95 (34.8)	0.0019
ETP (nM min)	1484 (401)	1331 (414.7)	0.094

LT, lag time; ETP, endogenous thrombin potential.

As not all patients with cirrhosis had an abnormal INR, we hypothesized that the above results might be as a result of skewing owing to patients with 'normal' hemostasis. Hence, we selected those samples with a high INR (INR > 1.2; $n = 49$) and repeated the analyzes. The mean INR was 1.9 (95% CI 1.7–2.1). Again no correlation was detected between the INR and the various TG parameters. The maximal velocity was increased in cirrhosis compared with a healthy population (mean 74.4 ± 34.1 vs. 45.1 ± 25.9 nM min^{-1}, respectively; $P = 0.002$). No statistical differences were detected between controls and this cohort of patients in their ETP and the LT.

We also subdivided the cirrhosis group into those with an INR between 1.21 and 2.0 ($n = 38$) and those greater than 2.0 ($n = 11$) and compared their maximal TG velocity with the controls to check for different trends depending on the degree of coagulopathy. Figure 1 shows that the velocity was increased in the group with INR of 2.0 or less when compared with the controls (mean 79.7 ± 32.9 vs. 45.1 ± 25.9 nM min^{-1}, respectively; $P \leq 0.001$). The cohort with the higher INRs had velocity similar to the normal group ($P \geq 0.05$).

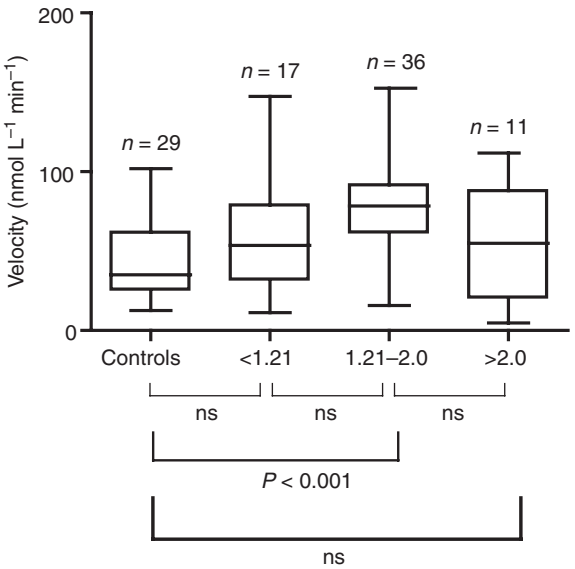

Fig. 1. Maximal thrombin generation (TG) velocity depending on the international normalized ratio (INR) of patients with cirrhosis and compared with the controls. The velocity was increased in the group with INRs between 1.21 and 2.0, and was similar to the controls in the group with INR > 2.0 and those patients with cirrhosis but normal INR (group marked INR < 1.21).

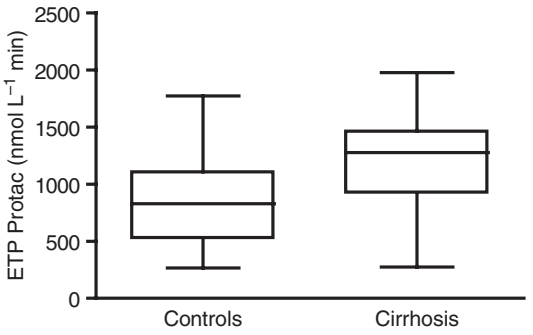

Fig. 2. The endogenous thrombin potential (ETP) after the addition of Protac®. The ETP in the patients with cirrhosis was increased with a difference between the means of 255.4 ± 86.91 nM min ($P = 0.004$).

The Protac®-modified TG assay

Figure 2 shows that upon the addition of Protac®, there was a significant difference in the ETP of the patients with cirrhosis when compared with the controls (mean 1074 ± 461.4 vs. 818 ± 357.9 nM min, respectively; $P = 0.004$). The ETP ratio was significantly higher in the patients with liver disease than the normal individuals (mean 0.72 ± 0.26 vs. 0.40 ± 0.16, respectively; $P \leq 0.0001$) (Fig. 3).

Again, we selected those patients with an abnormal INR, and repeated the analyzes. The ETP ratio was even more significantly increased in the cirrhosis group (mean 0.80 ± 0.21 vs. 0.40 ± 0.16; $P \leq 0.0001$). When patients were subdivided depending on the degree of coagulopathy, the ETP ratio was significantly increased in those patients with INR > 1.2 when compared with the controls (see Fig. 4).

Finally, we also checked whether there were any differences in TG parameters by etiology but no significant differences were found.

Routine coagulation tests

The mean FVIII was raised in the liver cohort (184.1 IU dL^{-1}; 95% CI 165.9–202.3 IU dL^{-1}), AT was reduced (mean

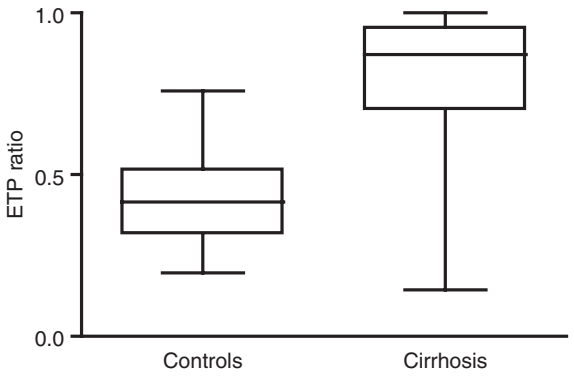

Fig. 3. The endogenous thrombin potential (ETP) ratio between healthy controls and patients with liver cirrhosis. A significant increase in the ETP ratio is shown in the patient cohort ($P \leq 0.0001$).

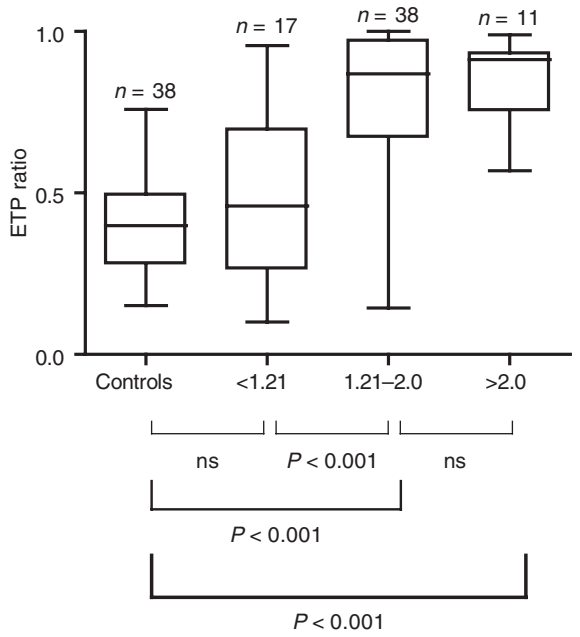

Fig. 4. Endogenous thrombin potential (ETP) ratio depending on the international normalized ratio (INR) of patients with cirrhosis and compared with the controls. A significant difference was found between the controls and the patients with INRs greater than 1.2.

60.5 IU dL^{-1}; 95% CI 54.4–66.6 IU dL^{-1}), FV was normal (mean 78.4 IU dL^{-1}; 95% CI 68.5–88.3 IU dL^{-1}) as was the fibrinogen (mean 3.3 g dL^{-1}; 95% CI 2.5–4.2 g dL^{-1}) and FXII (mean 76.3 IU dL^{-1}; 95% CI 66.6–86.1) (Table 1b). PC activity was reduced at 46.7 IU dL^{-1} (95% CI 39–54.4) but free PS was normal at a mean of 93.9 IU dL^{-1} as was TFPI activity (mean 0.99 U mL^{-1} 95% CI 0.83–1.16). The FVIII and fibrinogen did not correlate with the various TG parameters. There was a positive correlation between both AT and LT (Spearman's rho 0.53; $P < 0.0001$) and a negative correlation between AT and the ETP ratio ($r = -0.53$; $P < 0.0001$). A negative correlation was also observed between FV and ETP with Protac and the ETP ratio ($r = -0.31$, $P = 0.014$; and -0.36, $P = 0.003$, respectively). No correlation was present between the ETP and TG velocity at 1 pM and the PC or FII. However, a moderate negative correlation was found between PC and ETP with Protac and the ETP ratio($r = -0.40$, $P = 0.0012$ and $r = -0.55$, $P \leq 0.0001$).

In the multiple regression analysis we studied the effect of all the coagulation parameters tested on the LT, velocity, peak, ETP and also the ETP Protac® and ETP ratio. Only TFPI activity was an independent variable on the LT ($\beta = 0.624$, $P = 0.002$). No individual factor was shown to have an independent effect on velocity, peak thrombin, ETP, ETP Protac® and ETP ratio.

Discussion

Coagulation in patients with liver disease is complex and varies in each individual. Not only are there individual pro- and anti-coagulant and fibrinolytic factors but also fixed cellular components such as endothelial cells and circulating ones such as platelets, tissue factor bearing white cells and also red cells. Flow is also very important, as are genetic predispositions towards thrombosis or bleeding. It is clear that the PT test not only assesses a small fraction of all these factors but is only affected by pro-coagulant factors that decrease in cirrhosis. So it is not surprising that the PT has never been shown to correlate with outcomes related to bleeding in patients with cirrhosis undergoing an invasive procedure.

The present study shows that patients with cirrhosis and a high INR actually have a hypercoagulable thrombin generation profile in plasma with an increased maximum velocity of TG, decreased rate of thrombin inhibition, higher ETP after the addition of Protac® and higher ETP ratios. To our knowledge, this is the first time this has been shown. Splitting this cohort of patients by different INR groups has allowed us to identify the most 'hypercoagulable' group which is that with INRs between 1.21 and 2.0.

We used a low TF concentration and contact inhibition in our thrombin generation assay to mimic more closely the *in vivo* milieu. Looking at the whole thrombin generation dynamics, we found that the initial stage of thrombin formation (represented by the LT) is normal in patients with cirrhosis. Antithrombin levels showed a positive correlation with the LT indicating another important mechanism in the balance of hemostasis in similar patients. However, as in previous studies [18] TFPI was shown to be an independent variable on the LT. The maximum velocity index was increased in cirrhosis when compared with the normal cohort, however, we could not identify a specific reason for this. When the PC anticoagulant pathway was activated with Protac®, the ETP was increased when compared with normal. The 'resistance' pattern to Protac®, as reflected by the ETP ratio, showed the patients to be more resistant to this snake venom and formed more thrombin. This is the same profile exhibited by patients with inherited thrombophilia such as PC/PS deficiency and FV Leiden who are well known to be at a greater risk of thrombosis development [14–16]. This increased Protac® resistance can be explained not only by the reduction in PC activity levels in cirrhosis but also by the reduction in FV and the increased FVIII which also lead to activated protein C resistance. FV not only acts as a cofactor to FXa in the production of thrombin but when inactivated, it also acts as a cofactor to activated protein C in the inactivation of FVIIIa. Hence, it has both procoagulant and anticoagulant properties in hemostasis. So, overall, as judged by the velocity of TG and the Protac resistance, the coagulation system in plasma of patients with cirrhosis is procoagulant. These findings are in-keeping with the recent findings by other groups that patients with liver disease are not protected against thrombosis despite the raised INR [19,20], and have an increased thrombotic risk as compared with age-matched controls [20]. Additionally, Agarwal *et al.* [21] recently showed that the coagulopathy of liver disease did not prevent continuous renal replacement therapy circuits from clotting.

Our study confirms and extends the findings of Tripodi *et al.* [10]. We tried to optimize the TG assay conditions and test a larger number of patients in order to verify their findings. Several groups have shown that when using low TF concentrations, contact activation can significantly increase thrombin generation [22,23]. This is especially important when comparing a group with inherently low FXII levels like patients with cirrhosis to a normal cohort with higher FXII. This spuriously increases the thrombin generation in the normal controls. This could explain the differences in the ETP at 1 pM TF between these two studies. In fact, in our study the mean patient ETP with CTI was equivalent to normal whereas using the same TF concentration, Tripodi *et al.* found that the mean ETP was lower in the cirrhosis group. One could argue that another possible explanation is the difference between the trigger reagents used in the two studies. Albeit both at 1 pM, the TF in our study was Innovin® whereas in the other study Recombiplastin® with a lower phospholipid concentration (4 vs. 0.5 μmol L^{-1}) was used. However, in both studies the patient group was compared with a normal control group studied in exactly the same way and hence this does not readily explain the differences outlined.

The use of the Protac® in our study instead of soluble recombinant thrombomodulin (rTM) to activate PC has already been shown to be suitable in other patient cohorts [14–16]. The two reagents activate endogenous PC and in both cases, an empirical concentration of 'activator' is used. Also, TM is a transmembrane protein cofactor expressed on endothelial cells. It is as yet not possible to emulate this environment using the CAT. However, our results are very similar to those using soluble rTM and we show that thrombin generation is not only normal but increased in patients with cirrhosis when the protein C pathway is activated with Protac® [10]. We feel that it is also important to note the increased 'resistance' pattern to Protac® that we demonstrated in patients with liver disease, as this is similar to that seen in patients with defects in their PC pathway leading to a prothrombotic state.

Finally, we have demonstrated that the majority of patients with INRs greater than 1.2 are resistant to Protac® but those with INRs between 1.21 and 2.0 also demonstrate increased thrombin generation velocities making them possibly more hypercoagulable. Further studies are required to look specifically at a larger patient group with INRs greater than 2.5 in order to ascertain whether this phenomenon is largely confined to those patients with mild to moderate coagulopathy.

Some limitations in our study need to be addressed. First, we did not use platelet-rich plasma in our thrombin generation tests. Cirrhosis usually leads to thrombocytopenia which results in lower thrombin generation [24]. However, the main aim of our study was to compare the coagulopathy indicated by the PT which is a PPP-based test with thrombin generation. In order to compare like-with-like, we used PPP in the TG assays as well. This means that in clinical practice, these patients might still require platelet support, depending on their platelet count. Another limitation is the age and gender differences between the patients and the controls. Age has been shown to correlate positively with TG [25,26]. Our control group was slightly younger than the patient group and this might have influenced our results. However, we do not believe that this was a major confounder as both groups were adults and the mean age difference was only 15.2 years. Studies have shown that the main age-related differences in TG are in newborns/childhood vs. adults. No one has actually looked at particular differences per decade and these would be expected to be small as judged by the correlation coefficients of these variables in these studies [25]. Also, if age was a major confounder, one would have expected all patients groups to have increased velocity of TG and not just the ones with INR 1.21–2.0. With regards to gender, thrombin generation has been shown to be higher in females than in males and after the addition of Protac® or rTM females show more activated protein C resistance [14,25]. Since our control group included more females than males whereas the patient group had more males than females, it would have been expected that both the velocity and the ETP ratio would have been lower in the patient group and not higher.

In conclusion, we demonstrated that overall, as judged by the CAT, the coagulation balance in patients with cirrhosis-induced coagulopathy favours thrombosis. This not only provides a pathophysiological mechanism underlying the increased risk of thromboembolism in epidemiological studies [20], but also adds more evidence against the long respected dogma of 'autoanticoagulation' in similar patients. It also supports the view that the PT/INR test should not be used to assess bleeding risk and that in the majority of cases, prophylactic transfusion of clotting factors, such as fresh frozen plasma, is not warranted and could be hazardous by increasing the tendency to thrombosis and delaying some clinically important diagnostic and therapeutic procedures. The data also suggests that a prolonged PT *per se* should not deter physicians from considering appropriate chemical thromboprophylaxis when similar patients are hospitalized. Our results justify the need for new studies to generate new guidelines for the prophylactic and therapeutic use of blood products in cirrhosis. Finally, measuring thrombin generation could be the way forward in assessing hemostasis and to guide the use of hemostatic agents in similar patients and also merits evaluation using outcome studies. It would be interesting to see whether the newly devised INR (liver) system [27,28] could also result in a better test to assess bleeding risk in people with liver disease.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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