Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis

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Summary

There is increasing recognition that thrombotic complications may occur in patients with cirrhosis, and literature on antithrombotic treatment in these patients is rapidly emerging. Due to extensive haemostatic changes in patients with cirrhosis, careful monitoring of anticoagulant therapy may be required. Recent data suggest that plasma levels of low molecular weight heparin (LMWH) are substantially underestimated by the anti-activated factor X (anti-Xa) assay in patients with cirrhosis. We studied the in vitro recovery of antithrombin (AT)-dependent and -independent anticoagulant drugs in plasma from 26 patients with cirrhosis and 30 healthy controls and found substantially reduced anti-Xa levels when AT-dependent anticoagulant drugs were added to the plasma of patients with cirrhosis. LMWH (0.2 U/ml) had the poorest recovery in plasma from patients with cirrhosis (0.13 ± 0.06 U/ml, compared to 0.23 ± 0.03 U/ml in controls, P < 0.0001), followed by unfractionated heparin and fondaparinux. In contrast, the recovery of rivaroxaban and dabigatran was identical between patients and controls. These data suggest that the anti-Xa assay cannot be used to monitor AT-dependent anticoagulant drugs in patients with cirrhosis, as it substantially underestimates drug levels. The direct factor Xa and IIa inhibitors, however, may be monitored through the respective anti-Xa and anti-IIa assays in patients with cirrhosis.
Introduction

In patients with chronic liver disease the haemostatic system is considered to be in a ‘rebalanced’ status, due to a concomitant decrease in pro- and anti-haemostatic systems [1]. However, the relatively high incidence of thrombotic events and bleeding complications in these patients suggests that this balance is less stable than that in healthy individuals and that it can be easily tipped over to a hyper- or a hypocoagulable state [2]. In fact, treatment of thrombotic complications is frequently required, as patients with liver disease can suffer from deep vein thrombosis, pulmonary embolism or portal vein thrombosis [3,4]. Furthermore, patients may require anticoagulation because of concomitant cardiovascular disease[3]. Nowadays, there is increasing recognition of the various thrombotic complications that may occur in patients with chronic liver disease and therefore the increased use of anticoagulant therapy in these patients may be expected [5]. Due to the limited clinical experience, the anticoagulant of choice for the various indications is still unclear.

Vitamin K antagonists and/or heparins are widely used in the prevention or treatment of thrombosis, but both drug classes have drawbacks in patients with liver diseases. Vitamin K antagonist therapy requires monitoring by the international normalized ratio (INR). However, as the INR is frequently already prolonged in patients with cirrhosis in the absence of anticoagulant therapy, the target INR is unclear. In addition, there is major inter-laboratory variability in the INR measurement in patients with cirrhosis, making the test results inherently unreliable in these patients [6].

A problem with monitoring unfractionated heparin (UFH) is that the activated partial thromboplastin time (APTT), which is instrumental in dosing this agent, is already prolonged in many patients with chronic liver disease, and therefore APTT target ranges for these patients are unclear.

Low molecular weight heparin (LMWH) and fondaparinux do not require laboratory monitoring in the general population, except in patients with extreme obesity and with renal dysfunction. Patients with cirrhosis may also require laboratory monitoring of these agents, for example because of concomitant renal failure. LMWH can be monitored with anti-activated factor X (anti-Xa) assays, but these appear to be unreliable in patients with cirrhosis. It has been shown that, after the administration of a standard prophylactic or therapeutic dose of LMWH to patients with cirrhosis, anti-Xa levels fall below the recommended ranges for optimal anticoagulant control [7]. However, the decreased anti-Xa levels in these patients appear to be a laboratory anomaly and not a true indication of anticoagulant effect [8,9]. In fact, anti-Xa values have been shown to correlate positively with AT levels [7], which are reduced in patients with cirrhosis. Despite reduced anti-Xa values, LMWH was shown to be safe and effective in patients with cirrhosis [7,10,11]. Whether similar monitoring problems also occur with fondaparinux has not yet been assessed.

New antithrombotic agents, such as the direct factor Xa inhibitor rivaroxaban and the direct thrombin inhibitor dabigatran, have theoretical advantages over heparins: fewer (fatal) bleeding events, rapid onset of action, fewer drug-drug interactions, and oral mode of administration [12]. In addition, both agents do not require laboratory monitoring in the general population. Although patients with liver disease have been excluded from clinical trials on the new oral anticoagulants, they have theoretical advantages over currently used drugs, in
particular the oral mode of administration. Since LMWH is currently used for months or years to treat or prevent portal vein thrombosis, it is conceivable that the new oral anticoagulants will be considered in the future to avoid prolonged subcutaneous LMWH administration [5]. However, the current package insert for rivaroxaban indicates that it is contra-indicated for patients with cirrhosis, and other theoretical disadvantages of the new oral anticoagulants require attention [5]. Monitoring of these drugs may be required in patients with liver disease due to the possible altered clearance and the increased extravascular volume of patients with cirrhosis. No clinical studies on the efficacy and safety of these agents in cirrhotic patients have been performed yet. Furthermore, laboratory methodologies for monitoring these new drugs are still in development, and validation in patients with chronic liver disease will be required.

Thus, monitoring of anticoagulant drugs may be required in patients with cirrhosis, however, this appears to be difficult, due to the haemostatic changes associated with the underlying liver disease. The aim of this study was to determine whether different anticoagulant drugs could be reliably monitored using the anti-Xa or anti-activated factor II (anti-IIa) tests in plasma from patients with cirrhosis.

**Patients and methods**

**Patients**

Twenty-six adult patients with liver cirrhosis, who were seen on an out-patient basis or were admitted to the hospital, were included in the study. Patients were classified according to the Child-Pugh classification [13]. Ten patients were classified as Child A, ten as Child B, and six patients as Child C cirrhosis. Exclusion criteria were a documented history of congenital coagulation disorders, presence of active infection (<2 weeks), presence of acute liver failure, use of anticoagulant drugs in the past 10 d, pregnancy, human immunodeficiency virus (HIV) positivity and recent (<7 d) transfusion with blood products. The control group consisted of 30 adult healthy volunteers working at our institution. Exclusion criteria for the control group were a documented history of congenital coagulation disorders, documented history of hepatic disease, recent viral infection (<2 weeks), use of anticoagulant drugs in the past 10 d, pregnancy and HIV positivity.

This study protocol was approved by the local medical ethical committee and informed consent was obtained from each subject before inclusion in the study.

**Plasma samples**

Blood samples were drawn by venepuncture and collected into vacuum tubes containing 3.8% trisodium citrate as an anticoagulant, at a blood to anticoagulant ratio of 9:1. Platelet poor plasma was prepared by double centrifugation at 2000 g and 10 000 g respectively for 10 min. Plasma was snap-frozen and stored at -80°C until use.

**Addition of anticoagulants to plasma samples**

The following anticoagulants were added to plasma samples of cirrhotic patients and control. The indicated concentrations represent final concentrations in plasma.

1. UFH (Leo Pharma, Ballerup, Denmark), 0.3 U/ml
2. LMWH (Clexane; Sanofi-Aventis BV, Gouda, the Netherlands), 0.2 U/ml
3. Fondaparinux (Arixtra; GlaxoSmithKline BV, Zeist, the Netherlands), 0.5 µg/ml
4. Dabigatran (Alsachim, Illkirch Graffenstaden, France), 0.3 µg/ml
5. Rivaroxaban (Alsachim, Illkirch Graffenstaden France), 100 ng/ml

**Anti-Xa/anti-IIa assay and APTT**

Anti-Xa and anti-IIa values were all measured using the ACL 500 TOP analyser (Instrumentation Laboratory, Breda, the Netherlands). Anti-Xa values were measured in the plasma samples after the addition of UFH, LMWH, or fondaparinux with the Biophen Heparin (LRT) kit (Hyphen Biomed, Neuville Sur Oise, France), the Biophen heparin calibrator (for UFH and LMWH) or the Biophen arixtra calibrator (for fondaparinux), both purchased from Hyphen Biomed. No exogenous antithrombin is added in these assays. We repeated anti-Xa measurements in a limited set of plasma samples (10 patients, 10 controls), which we spiked with LMWH using the same kit, to which was added exogenous antithrombin (Hyphen Biomed, 75 lg/ml, final concentration) to assess the effect of low endogenous antithrombin levels on the outcome of the assay. Anti-Xa values, after the addition of rivaroxaban, were measured using the Biophen DiXal kit (Hyphen Biomed). A calibration curve was constructed by adding rivaroxaban to pooled normal plasma (obtained by combining plasma from >200 individuals). Anti-IIa values in the plasma samples were measured after addition of dabigatran using the Hemoclot thrombin inhibitors kit (Hyphen Biomed). A calibration curve was constructed by adding dabigatran to pooled normal plasma.

UFH activity was estimated by determination of the APTT on the ACL 500 TOP analyser, using reagents (HemosIL SynthASil) and protocols from the manufacturer (Instrumentation Laboratory, Breda, The Netherlands).

**Routine coagulation laboratory tests**

The INR was assessed with commercially available methods on an automated coagulation analyser (ACL 500 TOP) with reagents (Recombiplastin 2G) and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands). Levels of factor (F) VIII, FII, FX and antithrombin (AT) were measured on an automated coagulation analyser (ACL 500 TOP) with reagents and protocols from the manufacturer (Recombiplastin 2G for FII and FX, HemosIL SynthASil for FVIII, and Liquid Antithrombin reagent for AT, all from Instrumentation Laboratory).

**Statistical analysis**

Data are expressed as mean ± standard deviation (SD), medians (with ranges), or numbers (with percentages) as appropriate. Means of two groups were compared by Student’s t-test or Mann-Whitney U test as appropriate. Multiple groups were compared using One-way analysis of variance (ANOVA) (with the Bonferroni post-test) or Kruskal-Wallis H test (with Dunn’s post-test) as appropriate. Spearman’s correlation coefficient was used to assess correlation between continuous variables. P values of 0.05 or less were considered statistically significant. GraphPad Prism (San Diego, USA) and IBM SPSS Statistics 20 (New York, USA) was used for all analyses.
Results

**Patient characteristics**

The main characteristics of the study population are reported in Table 1. Twenty-six patients with cirrhosis (14 males and 12 females) were included, and they were categorized according to the severity of liver disease as expressed by the Child Pugh score (10 Child A, 10 Child B and 6 Child C patients). The most common aetiology of liver disease was alcoholic, especially in the Child class C patients. None of the patients used pro- or anti-coagulant drugs. Thirty healthy subjects (14 males and 16 females) were included as normal controls.

The baseline INR and plasma levels of FVIII, FII, FX, and AT are shown in Table 2. Patients with cirrhosis showed a statistically significant prolongation of the INR and APTT, and a decrease in all measured coagulation proteins, except for FVIII (which was increased), as compared to controls. The reduction in levels of plasmatic factors was proportional to the severity of liver disease.

<table>
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<tr>
<th>Characteristics</th>
<th>Cirrhotic patients</th>
<th>P-value</th>
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<tbody>
<tr>
<td></td>
<td>Child A n=10</td>
<td>Child B n=10</td>
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<tr>
<td>MELD score</td>
<td>8.0 [6.0-10.0]</td>
<td>11.5 [8.0-19.0]</td>
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<tr>
<td>Age (yrs)</td>
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<td>50.5 [12.5]</td>
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<tr>
<td>Sex (male)</td>
<td>4 (40)</td>
<td>6 (60)</td>
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<tr>
<td>BMI</td>
<td>25.4 [4.0]</td>
<td>28.4 [6.0]</td>
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<tr>
<td>Smoking (number)</td>
<td>4 (40)</td>
<td>1 (10)</td>
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<tr>
<td>Alcohol (U per week)</td>
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<td>0 [0-7]</td>
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<td>Etiology of liver disease</td>
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<tr>
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<td>1 (10)</td>
</tr>
<tr>
<td>HCV</td>
<td>1 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>NASH</td>
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<td>1 (10)</td>
</tr>
<tr>
<td>PBC</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>PSC</td>
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<td>2 (20)</td>
</tr>
<tr>
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<tr>
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<td>2 (20)</td>
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<tr>
<td>Diabetes Mellitus</td>
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<td>1 (10)</td>
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<td>Hemoglobin (mmol/L)</td>
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<td>7.2 [0.9]</td>
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<tr>
<td>Leukocytes (10^9/L)</td>
<td>6.5 [4.5]</td>
<td>5.1 [2.1]</td>
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Table 1. Demographic and clinical characteristics of the study population.
HCV: Hepatitis C virus, NASH: Non-alcoholic steatohepatitis, PBC: Primary biliary cirrhosis, PSC: Primary sclerosing cholangitis.
Data are expressed as number (%), mean [SD], or median [range].
Table 2. Coagulation parameters in cirrhotic patients and normal controls.

<table>
<thead>
<tr>
<th>Coagulation Parameter</th>
<th>Cirrhotic Patients</th>
<th>Normal Controls</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Child A</td>
<td>Child B</td>
<td>Child C</td>
</tr>
<tr>
<td>INR</td>
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<td>1.2 [1.0-2.0]</td>
<td>1.5 [1.4-1.7]</td>
</tr>
</tbody>
</table>

Data are expressed as mean [SD], or median [range].

Figure 1. (A) Mean anti-Xa activity in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis after addition of 0.3 U/ml unfractionated heparin (UFH). Error bars indicate the standard error of the mean (SEM). (B) Correlation between antithrombin (AT) levels and anti-Xa values after addition of 0.3 U/ml UFH in plasma from patients with cirrhosis.

*P < 0.001 compared to controls.

Figure 2. (A) Mean activated partial thromboplastin time (APTT) ratios after the addition of 0.3 U/ml unfractionated heparin (UFH) in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis. Error bars indicate the standard error of the mean (SEM). (B) Correlation between antithrombin (AT) levels and APTT ratios in plasma from patients with cirrhosis.

APTT ratios were defined as (APTT in presence of UFH/APTT in absence of UFH). *P < 0.01 compared to controls.

Recovery of anticoagulant drugs assessed by anti-Xa and anti-IIa activity

UFH. When 0.3 U/ml UFH was added to the plasma of the controls, the mean anti-Xa level was 0.29 ± 0.04 U/ml. In contrast, plasma from patients with cirrhosis spiked with the same amount of heparin resulted in a mean anti-Xa level of only 0.21 ± 0.05 U/ml. The reduced recovery
in the plasma of cirrhotic patients was statistically significant (P < 0.0001) and correlated with the severity of liver disease (Figure 1A). Furthermore, we observed a significant positive correlation between AT levels and anti-Xa values in plasma from cirrhotic patients (r = 0.68, P < 0.0001; Figure 1B).

When UFH activity was determined by the APTT, we observed a significantly greater prolongation of the APTT in plasma from patients compared to controls. The APTT in patients increased from 38.7 ± 4.9 to 104.6 ± 49.1 s after addition of 0.3 U/ml of UFH and from 33.8 ± 3.8 to 63.8 ± 15.0 s in controls, resulting in APTT ratios (APTT with UFH/APTT without UFH) of 2.6 ± 1.0 and 1.9 ± 0.3, respectively (P = 0.0009). The increased effect of heparin on the APTT became more pronounced with increasing severity of liver disease (Figure 2A). Interestingly, the response to heparin (expressed as the APTT ratio) negatively correlated with the AT levels (r = -0.73, P < 0.0001; Figure 2B).

Low molecular weight heparin. When 0.2 U/ml LMWH was added to the plasma of the controls, the mean anti-Xa level was 0.23 ± 0.03 U/ml (mean ± SD). In contrast, when plasma from patients with cirrhosis was spiked with the same amount of LMWH, the mean anti-Xa level detected was only 0.13 ± 0.06 U/ml. The reduced recovery in plasma from cirrhotic patients, compared to normal controls was statistically significant (P < 0.0001) and correlated with the severity of liver disease (Figure 3A).

Furthermore, we observed a significant positive correlation between AT levels and anti-Xa values in plasma from cirrhotic patients (r = 0.71, P = 0.0002; Figure 3B).

To assess whether the reduced recovery in patients was due to the decreased AT levels, we tested the effect of addition of exogenous AT (75 µg/ml, final concentration) to the plasma in 10 patients and 10 controls. When anti-Xa levels were set at 100% in controls, the anti-Xa level in the absence of exogenously added AT in patients was only 64 ± 10%, P < 0.0001. In contrast, when exogenously added antithrombin was present, the recovery in patients was 97 ± 8%, P = 0.56).

Figure 3. (A) Mean anti-Xa activity in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis after addition of 0.2 U/ml low molecular weight heparin (LMWH). Error bars indicate the standard error of the mean (SEM). (B) Correlation between antithrombin (AT) levels and anti-Xa values after addition of 0.2 U/ml LMWH in plasma from patients with cirrhosis. *P < 0.05; **P < 0.001.
Fondaparinux. When 0.5 µg/ml fondaparinux was added to the plasma of the controls, mean anti-Xa levels were 0.59 ± 0.04 µg/ml. Mean anti-Xa levels were significantly lower in plasma from patients with cirrhosis (0.50 ± 0.07 µg/ml, P < 0.0001). The reduced recovery in cirrhosis plasma correlated with the severity of liver disease (Figure 4A).

Again, we observed a significant positive correlation between AT levels and anti-Xa values in plasma from cirrhotic patients (r = 0.86, P < 0.0001; Figure 4B).

Rivaroxaban. When 100 ng/ml rivaroxaban was added to the plasma samples, mean anti-Xa levels were comparable between patients and normal controls (85.0 ± 14.2 vs. 88.0 ± 11.4 ng/ml; P = 0.38) (Figure 5).

Dabigatran. After the addition of 0.3 µg/ml dabigatran, anti-IIa levels were 0.33 ± 0.01 µg/ml and 0.31 ± 0.02 µg/ml in plasma from patients and normal controls, respectively. The observed anti-IIa levels were slightly higher in plasma from patients compared to controls (P = 0.0003). However, when separating patients according to the Child-Pugh classification, the anti-IIa levels were only significantly higher in the patients with Child C cirrhosis (0.33 ± 0.01 µg/ml; P < 0.05), compared to controls (Figure 6).
Figure 6. Mean anti-IIa activity in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis after addition of 0.3 lg/ml dabigatran. Shown are means. Error bars indicate the standard error of the mean (SEM).

*P < 0.05 compared to controls.

Discussion

We observed a reduced recovery of AT-dependent anticoagulants assessed by anti-Xa levels when drugs were added to the plasma of patients with cirrhosis, compared to the recovery in plasma from healthy individuals. The addition of LMWH led to the most pronounced underestimation of drug levels, followed by UFH and fondaparinux. In contrast, comparable anti-Xa and anti-IIa levels were measured in plasma from patients and controls after the in vitro addition of direct FXa (rivaroxaban) and direct FIIa inhibitors (dabigatran).

We [8] and others [7,9] have previously demonstrated that the anti-Xa assay underestimates the LMWH mass present in plasma from patients with cirrhosis. In addition, this current study showed that the anti-Xa assay also underestimates the masses of other AT-dependent anticoagulant drugs (UFH and fondaparinux) in plasma from patients with cirrhosis.

The reduced recovery of heparins correlated with the severity of liver disease, as assessed by the Child-Pugh score. Accordingly, and as a potential explanation for this phenomenon, a positive correlation between AT levels and anti-Xa values was observed. The reduced recovery of AT-dependent anticoagulants thus appears to be a direct consequence of the acquired AT deficiency of patients with liver disease [14]. Indeed, when exogenous AT was added to the anti-Xa assay, the reduced recovery of the LMWH in patients compared to controls was fully blunted. Furthermore, other studies have also shown decreased anti-Xa values in patients with AT deficiency treated with UFH or LMWH [15-17]. In addition, in neonates, who have reduced plasma levels of AT, the anti-Xa assays have also been shown to be unreliable for this reason [18].

We observed a more pronounced increase in the APTT after the addition of UFH in plasma from patients with cirrhosis in comparison to the controls, suggesting an enhanced anticoagulant effect of UFH in cirrhosis. In clinical practice, the APTT would thus suggest that dose reductions are required in patients with cirrhosis. In contrast, recovery of UFH in the anti-Xa assay is reduced in cirrhosis, which, in clinical practice, may lead to dose escalations to reach a desired anti-Xa level. Importantly, both tests are used in clinical practice to monitor UFH.

Based on the data of this study, we strongly suggest that anti-Xa levels should not be relied upon for monitoring heparins (that exert their effect through AT) in patients with cirrhosis, unless an anti-Xa test with exogenous antithrombin is available. However, many clinical laboratories...
may not offer such a modified anti-Xa test. Novel monitoring methods (such as, for example, thrombin generation tests) may provide better monitoring options in patients with cirrhosis, but unfortunately such methods are not yet available in routine diagnostic laboratories. Indeed, in vitro studies using thrombin generation tests have demonstrated that LMWH has a more profound anticoagulant effect in plasma from patients with cirrhosis as compared to plasma from healthy controls [9]. Furthermore, we have also recently demonstrated that the anticoagulant potency of clinically approved drugs differs substantially between patients with cirrhosis and healthy individuals, using thrombin generation tests [19]. Whereas dabigatran and, to a lesser extent, heparin and LMWH are more potent in plasma from patients with cirrhosis, fondaparinux and rivaroxaban showed a decreased anticoagulant effect. Thus, although anti-Xa levels underestimate drug levels in cirrhotic patients treated with UFH or LMWH (which may prompt dose escalations), thrombin generation tests suggest that UFH or LMWH are slightly more potent in patients with cirrhosis compared to individuals with intact liver function. In other words, dose escalations instigated by a low anti-Xa level will potentially lead to a substantial bleeding risk.

Although our study shows that heparin monitoring in patients with cirrhosis may be improved by using anti-Xa assays to which exogenous AT is added, further studies on the performance of such assays in patients with cirrhosis are required. The direct FXa and FIIa inhibitors, however, may be monitored through the respective anti-Xa and anti-IIa assays, as comparable anti-Xa and anti-IIa levels were observed after the addition of rivaroxaban and dabigatran, respectively, in plasma from patients and controls.

Due to the limited clinical experience, the anticoagulant of choice and the dosages for the various indications is still unclear. Nevertheless, (theoretical) advantages and disadvantages of the available drugs in patients with cirrhosis have been recognized, which may facilitate a rational choice for a drug in a specific clinical situation [5]. The results of the present study will assist clinicians and clinical laboratories in interpreting anti-Xa or anti-IIa test results when applied in patients with cirrhosis treated with established or new anticoagulant drugs.

In conclusion, routine coagulation assays underestimate levels of antithrombin-dependent drugs, but not of direct anticoagulant drugs in plasma from patients with cirrhosis. This finding has practical consequences for monitoring heparins in these patients. Clinical (dose-finding) studies on the monitoring, efficacy and safety of heparins are urgently required to improve antithrombotic therapy in the patients with cirrhosis.
References


Hemostasis and Anticoagulant Therapy in Liver Diseases
APPENDIX TO CHAPTER

Issues With Monitoring of Unfractionated Heparin in Cirrhosis

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To the Editor

Fuentes et al report a retrospective analysis on bleeding events and monitoring of unfractionated heparin in patients with cirrhosis compared with matched controls [1]. We would like to comment on a number of issues related to the results described in this publication.

First, this study suggests that patients with cirrhosis are at greater risk for bleeding compared with matched controls when treated with unfractionated heparin. Patients with cirrhosis frequently have substantial alterations in their hemostatic system. The net result of these changes is considered to be a rebalanced hemostatic status, which is much more fragile to disruption compared with the hemostatic balance in healthy individuals [2]. As a consequence, it is of utmost importance to avoid excessive anticoagulation in patients with such a delicate hemostatic balance to avoid bleeding [3]. It may thus not come as a surprise that patients with cirrhosis seem to be at a greater risk for anticoagulant-associated bleeding as compared with matched controls. The findings by Fuentes et al, however, contrast with several recent studies, which have suggested that low-molecular-weight and unfractionated heparin to have a favorable safety profile in patients with cirrhosis [4–9]. Their study concluded that patients with cirrhosis have an increased anticoagulant-associated bleeding risk on basis of an increase in administered blood products. This conclusion may not be justified, as it is unclear whether these products were actually administered to actively bleeding patients. It is well known that a substantial proportion of blood products in the cirrhotic population are administered prophylactically [10]. As patients with cirrhosis often have abnormal routine hemostatic test results that are suggestive of a bleeding tendency (such as a prolonged prothrombin time or a decreased platelet count), prophylactic administration of fresh frozen plasma or platelet concentrates is still common practice, despite increasing awareness that these abnormal routine coagulation tests do not predict a bleeding risk [11].

Second, we wonder why these patients all received unfractionated (instead of low-molecular weight) heparin. Low-molecular-weight heparin is the drug of choice for at least some of the indications listed (e.g., treatment of acute venous thromboembolism of the leg or acute pulmonary embolism [12]). In addition, given the favorable published data on low-molecular-weight heparin in patients with cirrhosis, this drug may be preferable over unfractionated heparin, which has a less established safety profile in patients with cirrhosis. We do acknowledge that concomitant renal failure, in which accumulation of low-molecular-weight heparin is known to occur, may lead to the decision to use unfractionated and not low-molecular-weight heparin.

Third, the authors note discrepancies between activated partial thromboplastin time (APTT) and anti-Xa monitoring of low-molecular-weight heparin. We would like to comment that both tests fail to accurately measure drug levels in patients with cirrhosis, as we have recently shown [13]. With in vitro experiments in which we added a known concentration of unfractionated heparin to plasma from healthy individuals or plasma from patients with cirrhosis, we demonstrated that the anti-Xa tests underestimate drug levels whereas the APTT gives an overestimation. Monitoring of heparin by APTT or anti-Xa tests is indirect. These tests do not assess drug levels directly but rather estimate drug levels based on the anticoagulant action of the drug. Heparins exert an anticoagulant effect by enhancing the anticoagulant
effect of the endogenous anticoagulant antithrombin, and it is the heparin-enhanced antithrombin effect that is assessed by the APTT and anti-Xa assays. The decreased antithrombin levels in plasma from patients with cirrhosis lead to falsely elevated APTT and falsely decreased anti-Xa levels in heparin-treated patients, and both tests thus seem unsuitable for heparin monitoring in patients with cirrhosis [13].

Although the anti-Xa and APTT tests are designed to get an estimate of drug levels in plasma, the clinically relevant parameter is the “true” anticoagulant effect, which may, for example, be estimated by thrombin generation testing. We have investigated the functional effects of unfractionated and low-molecular-weight heparin in plasma from healthy individuals and plasma from patients with cirrhosis using thrombin generation testing [14]. When a known concentration of (low-molecular-weight) heparin was added to plasma, thrombin generation was inhibited to a larger extent in patients with cirrhosis compared with the decrease in healthy individuals, indicating that the anticoagulant potency of heparins is not necessarily equal in plasma from patients with cirrhosis and healthy individuals. The differences in anticoagulant potency, however, were not present when thrombin generation was tested in the presence of thrombomodulin, which allows activation of the endogenous anticoagulant protein C system. In other words, depending on the exact experimental conditions, heparins exert an equal to enhanced anticoagulant activity in plasma from patients with cirrhosis. This enhanced drug potency contrasts with the anti-Xa assay, which at equal drug levels gives a much lower value in patients with cirrhosis and thus suggests inadequate anticoagulation. The problems with monitoring of heparins and the altered potency of heparins in plasma from patients with cirrhosis are related to the antithrombin deficiency that is common in these patients, which is understandable because heparin exerts its anticoagulant effect by enhancing the anticoagulant action of endogenous antithrombin.

Finally, we agree with the authors that studies on safety and efficacy of heparin anticoagulation specifically in patients with cirrhosis are warranted. These studies should also take improvements in laboratory monitoring in consideration. Ideally, heparin monitoring tests for patients with cirrhosis should take both drug levels and drug effect into account. Given the drawbacks of heparin use in patients with cirrhosis, there may be opportunities for new (antithrombin-independent) oral anticoagulants [3]. Recently, the successful use of rivaroxaban and apixaban in the treatment of cirrhotic portal vein thrombosis has been reported [15,16]. Although monitoring of these drugs in cirrhosis seems to be much more accurate compared with monitoring of heparins, prospective studies on efficacy and safety are essential before such drugs can be implemented routinely in hepatology clinics.
References
