Pathophysiology and management of hemostatic alterations in cirrhosis and liver transplantation
Arshad, Freeha

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CHAPTER 4

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

Wilma Potze, Freeha Arshad, Jelle Adelmeijer, Hans Blokzijl, Arie P. van den Berg, Robert J Porte, Ton Lisman

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ABSTRACT

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 hemostatic 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-Xa assay in patients with cirrhosis. We studied the \textit{in vitro} recovery of antithrombin (AT)-dependent and –independent anticoagulant drugs in plasma from twenty-six patients with cirrhosis and thirty 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 (UFH) and fondaparinux. In contrast, the recovery of rivaroxaban and dabigatran was identical between patients and controls. These data suggest that anti-Xa assay cannot be used in the monitoring of 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.
ROUTINE COAGULATION ASSAYS UNDERESTIMATE LEVELS OF ANTI-XA-DEPENDENT BUT NOT DIRECT ANTICOAGULANT DRUGS IN CIRRHOSIS

INTRODUCTION

In patients with chronic liver disease the hemostatic system is considered to be in a ‘re-balanced’ status, due to a concomitant decrease in pro- and antihemostatic systems (1). However, the relatively high incidence of thrombotic events and bleeding complications in these patients suggests that this balance is more unstable as compared to the balance in healthy individuals and 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 an 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 prevention or treatment of thrombosis, but both drugs 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 prolonged in patients with cirrhosis already in the absence of anticoagulant therapy, the target INR is unclear. In addition, there is major lab-to-lab variability in the measurement of INR in patients with cirrhosis, making the test results inherently unreliable in these patients (6).

A problem with monitoring of 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.

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-Xa assays, but such assays appear unreliable in patients with cirrhosis. It has been shown that anti-Xa levels after a standard prophylactic or therapeutic dose of LMWH administered to patients with cirrhosis 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 has 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 to 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, currently rivaroxaban is contra-indicated for patients with cirrhosis according to the package insert, 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 hemostatic changes associated with the underlying liver disease. The aim of this study was to determine whether different anticoagulant drugs can be reliably monitored using the anti-Xa or anti-IIa tests in plasma from patients with cirrhosis.

**METHODS**

**Patients**

Twenty-six adult patients with liver cirrhosis, who were seen as out-patients or were admitted to the hospital, were included in this study. Patients were classified according to the Child-Pugh classification (13). Ten patients were classified as Child A, 10 as Child B, and 6 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 days, pregnancy, HIV positivity, and recent (<7 days) transfusion with blood products.

The control group consisted of thirty 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 days, 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 2000g and 10,000g 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 mentioned concentrations represent final concentrations in plasma.

- UFH (Leo Pharma, Ballerup, Denmark), 0.3 U/ml
- The LMWH Clexane (Sanofi-Aventis BV, Gouda, the Netherlands), 0.2 U/ml
- Fondaparinux (Arixtra) (GlaxoSmithKline BV, Zeist, the Netherlands), 0.5 µg/ml
- Dabigatran (Alsachim, Illkirch Graffenstaden, France), 0.3 µg/ml
- 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 (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), using 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 we now added exogenous antithrombin (Hyphen Biomed, 75 µg/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, 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 analyzer (ACL 500 TOP) with reagents (Recombiplastin 2G) and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands). Levels of factor (F) VIII, II, and X, and antithrombin (AT) were measured on an automated coagulation analyzer (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 analyses

Data are expressed as mean ± standard deviation (SD), medians (with interquartile range (IQR)), 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 ANOVA (with the Bonferroni posttest) or Kruskal-Wallis H test (with Dunn’s posttest) 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 etiology of liver disease was alcoholic, especially in the Child class C patients. None of the patients used pro- or anticoagulant drugs. Thirty healthy subjects (14 males and 16 females) were included as normal controls.

The baseline INR and plasma levels of factors 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 factor VIII (which was increased), as compared to controls. The reduction in levels of plasmatic factors was proportional to the severity of liver disease.
Routine coagulation assays underestimate levels of anti-Xa-dependent but not of direct anticoagulant drugs in cirrhosis

Table 1. Demographic and clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Child A</th>
<th>Child B</th>
<th>Child C</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>MELD score</td>
<td>8.0 [6.0-10.0]</td>
<td>11.5 [8.0-19.0]</td>
<td>16.5 [12.0-19.0]</td>
<td>0.0006</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>56.0 [14.2]</td>
<td>50.5 [12.5]</td>
<td>59.0 [7.4]</td>
<td>0.367</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>4 (67)</td>
<td>0.682</td>
</tr>
<tr>
<td>BMI</td>
<td>25.4 [4.0]</td>
<td>28.4 [6.0]</td>
<td>28.4 [4.2]</td>
<td>0.337</td>
</tr>
<tr>
<td>Smoking (number)</td>
<td>4 (40)</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>0.368</td>
</tr>
<tr>
<td>Alcohol (U per week)</td>
<td>0 [0-1]</td>
<td>0 [0-7]</td>
<td>0 [0-0]</td>
<td>0.731</td>
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Etiology of liver disease

<table>
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<th>p-value</th>
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<tr>
<td>Alcoholic</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td>6 (100)</td>
<td>0.001</td>
</tr>
<tr>
<td>HCV</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>NASH</td>
<td>1 (10)</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>0.769</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>PBC</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>PSC</td>
<td>2 (20)</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>0.639</td>
</tr>
<tr>
<td>Auto-immune</td>
<td>3 (30)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>0.403</td>
</tr>
<tr>
<td>Alcoholic + NASH</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>1.000</td>
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<tr>
<td>Unknown</td>
<td>0 (0)</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>0.323</td>
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Co-morbidity

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<th>Child A</th>
<th>Child B</th>
<th>Child C</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>3 (30)</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>0.391</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>0.769</td>
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</table>

Laboratory variables

<table>
<thead>
<tr>
<th>Laboratory variables</th>
<th>Child A</th>
<th>Child B</th>
<th>Child C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bilirubin (µmol/L)</td>
<td>15 [5-35]</td>
<td>40 [18-61]</td>
<td>93 [63-131]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>36 [28-44]</td>
<td>33 [27-63]</td>
<td>26 [25-29]</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum creatinin (µmol/L)</td>
<td>69 [23]</td>
<td>72 [32]</td>
<td>75 [17]</td>
<td>0.920</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>8.2 [1.4]</td>
<td>7.2 [0.9]</td>
<td>6.2 [0.8]</td>
<td>0.005</td>
</tr>
<tr>
<td>Leukocytes (10⁹/L)</td>
<td>6.5 [4.5]</td>
<td>5.1 [2.1]</td>
<td>5.4 [2.1]</td>
<td>0.591</td>
</tr>
<tr>
<td>Platelets (10⁹/L)</td>
<td>112 [16-258]</td>
<td>86 [28-471]</td>
<td>73 [44-114]</td>
<td>0.482</td>
</tr>
</tbody>
</table>

Data are expressed as number (%), mean [SD], or median [IQR]. HCV= hepatitis C virus; NASH= non-alcoholic steatohepatitis; PBC= primary biliary cirrhosis; PSC= primary sclerosing cholangitis.
CHAPTER 4

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, mean anti-Xa levels were 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 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; Fig.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 sec to 104.6 ± 49.1 sec after addition of 0.3 U/ml

**Table 2.** Coagulation parameters in cirrhotic patients and normal controls

<table>
<thead>
<tr>
<th>Coagulation parameters</th>
<th>Cirrhotic patients</th>
<th>Healthy controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Child A</td>
<td>Child B</td>
<td>Child C</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 [0.9-1.2]</td>
<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 [IQR]. INR= international normalized ratio; APTT= activated partial thromboplastin time; AT= antithrombin.

**Figure 1.** A. 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 UFH. Shown are means. Error bars indicate the standard error of the mean (SEM). B. Correlation between AT levels and anti-Xa values after addition of 0.3 U/ml UFH in plasma from patients with cirrhosis. *p < 0.001.
Routine coagulation assays underestimate levels of anti-Xa-dependent but not of direct anticoagulant drugs in cirrhosis.

When 0.2 U/ml LMWH was added to the plasma of the controls, mean anti-Xa levels were 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 = 0.66, 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 ug/ml, final concentration) to the plasma in 10 patients and 10 controls. When anti-Xa levels were set at 100% in controls, anti-Xa levels in the absence of exogenously added AT in patients were only 64 ± 10%, p<0.0001. In contrast, when exogenously added antithrombin was present, the recovery in patients was 97 ± 8%, p=0.56.

**Low molecular weight heparin**

When 0.2 U/ml LMWH was added to the plasma of the controls, mean anti-Xa levels were 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).

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**Figure 2.** A. Activated partial thromboplastin time (APTT) ratios after the addition of 0.3 U/ml UFH in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis. Shown are means. Error bars indicate the standard error of the mean (SEM). B. Correlation between 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.
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 significant 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).

**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 significant 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).
Routine coagulation assays underestimate levels of anti-Xa-dependent but not of direct anticoagulant drugs in cirrhosis

**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 ng/ml versus 88.0 ± 11.4 ng/ml; p=0.38) (Figure 5).

![Figure 5. Anti-Xa activity in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis after addition of 100 ng/ml rivaroxaban. Shown are means. Error bars indicate the standard error of the mean (SEM).](image)

**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 (Fig. 6).

![Figure 6. Anti-IIa activity in plasma from healthy controls and patients with Child A, Child B, and Child C cirrhosis after addition of 0.3 µg/ml dabigatran. Shown are means. Error bars indicate the standard error of the mean (SEM). P<0.005.](image)
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 factor Xa (rivaroxaban) and direct factor IIa 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, in the current paper we show 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, 16). 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 (17).

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 not to rely on anti-Xa levels for monitoring of 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 pa-
tients 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 (18). 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 factor Xa and IIa inhibitors, however, may likely 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 respectively rivaroxaban and dabigatran 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 study presented in this manuscript 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, but not of direct anticoagulant drugs in plasma from patients with cirrhosis. This finding has practical consequences for monitoring of heparins in these patients. Clinical (dose-finding) studies on monitoring, efficacy, and safety of heparins are urgently required to improve antithrombotic therapy in the patients with cirrhosis.
REFERENCES

Part B.
Blood loss and prevention of blood loss and RBC transfusion during liver transplantation