General Discussion
The concept of a rebalanced hemostasis in patients with liver disease is increasingly gaining acceptance. This balance is perceived to be more unstable than in individuals with intact liver function, with the risk of both bleeding and thrombotic complications. However, it is still unclear which patients are at risk for bleeding or thrombotic complications and how we can predict those risks. Furthermore, the hemostatic system in patients with liver disease is still incompletely understood, especially in the increasing patient population with a fatty liver disease. Due to the increase in patients with NAFLD and aging of the patient population, thrombotic complications are likely to be increasingly diagnosed in patients with liver disease. However, little research has been done on the efficacy and safety of the available anticoagulant drugs in patients with liver disease. Therefore, guidelines on the anticoagulant drug of choice and dosing for the various indications are still lacking. The aim of this thesis was to gain a better understanding of the hemostatic system in patients with (fatty) liver disease and to study the efficacy and monitoring of anticoagulant drugs in plasma from patients with liver disease. Results of this thesis will contribute to better strategies to prevent or treat thrombosis in patients with liver disease in the future. In this chapter, the results of this thesis are discussed in the context of the current literature on the hemostasis in liver disease and the prevention or treatment of thrombotic complications.

**Hemostatic changes in liver disease**

As outlined in chapter 2, there are changes in all phases of the hemostatic system in patients with liver disease. Most important alterations of the primary hemostasis in patients with liver disease include a reduced platelet count [1], elevated levels of von Willebrand factor (VWF) [2], and low levels of ADAMTS13 [3]. In secondary hemostasis, most procoagulant and anticoagulant proteins are reduced. Finally, in the fibrinolytic system, levels of plasminogen, plasmin inhibitor, thrombin activatable fibrinolysis inhibitor (TAFI), and factor XIII are frequently reduced [4,5], and levels of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) are frequently elevated [6] in patients with chronic liver disease.

If we focus on the secondary hemostasis in patients with liver disease, it is well known that levels of coagulation factors V, VII, IX, X, XI, and prothrombin are reduced, because the liver is the site of synthesis of these proteins [7]. In contrast, levels of factor VIII are often elevated [8]. The decreased production of procoagulant proteins is mostly counterbalanced by decreased production of anticoagulant proteins, such as protein C and antithrombin [9]. Also protein S levels are reduced in patients with cirrhosis [10,11]. Tissue factor pathway inhibitor (TFPI) is not produced by the liver, but the microvascular endothelium is thought to be the principal source of TFPI [12]. It has recently been established that protein S acts as a cofactor for TFPI in the down regulation of thrombin generation [13]. As a result, both acquired and congenital protein S deficiencies are associated with a concomitant TFPI deficiency [14]. In chapter 3, we studied protein S and TFPI plasma levels in patients with cirrhosis, and found that patients with cirrhosis have an acquired protein S deficiency, which is, however, not accompanied by a decrease in TFPI plasma levels. This indicates that the decrease in TFPI levels in conjunction with acquired protein S deficiency as described previously [14] does not occur in patients with chronic liver diseases. A possible explanation might be that TFPI release in patients with cirrhosis is substantially increased due to continuous activation of the endothelium, but that this increased TFPI release is masked, in part, by the protein S deficiency. Indeed, in both chronic and acute liver diseases, continuous activation of the endothelium is common, resulting in increased plasma levels of endothelial-derived proteins,
such as VWF [2,15]. The combination of increased TFPI release, due to continuous endothelial cell activation, and protein S deficiency may thus result in normal TFPI levels. Despite normal TFPI plasma levels, patients with cirrhosis did show a reduced activity of the TFPI-protein S system when compared to healthy controls. Recently, studies have proposed that the increase in factor VIII in combination with decreased protein C is responsible for a hypercoagulable state contributing to thrombotic complications in patients with cirrhosis [16]. However, in the study of chapter 3 we have shown that the impaired activity of the TFPI-protein S system in patients with cirrhosis may also contribute to this hypercoagulable state increasing the risk of thrombotic complications in these patients.

**Laboratory tests of hemostasis in patients with liver disease**

Since patients with liver disease can suffer from both thrombotic and bleeding complications, there is a frequent clinical need for predicting the risk of bleeding or thrombosis in individual patients with liver disease. However, current available clinical tests fail to accurately predict those risks in patients with liver disease.

The standard coagulation profile processed in most clinical laboratories includes the prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT). In fact, many consider the PT the test of choice for diagnosing either inherited or acquired coagulopathies, in addition to the monitoring of vitamin K antagonists. However, these conventional coagulation tests fail to assess all the changes in the coagulation in patients with a liver disease. This can be explained by two considerations. First, the PT and APTT only measure the first 5% of thrombin being generated, since this is enough for plasma to start clotting [17]. Second, in the PT and APTT the anticoagulant factor protein C, whose activation requires thrombomodulin, cannot be properly activated [18]. Thrombomodulin is a protein located on the endothelial cells, and plasma and reagents needed to perform the PT or APTT do not contain sufficient amounts of this protein. The PT and APTT are, therefore, sensitive to changes in the procoagulant proteins, but not to changes in the anticoagulant proteins. Since in patients with liver disease both pro- and anticoagulant proteins are decreased, these conventional coagulation tests cannot reliably predict bleeding risk in these patients. Indeed, multiple studies have shown that the PT is a poor predictor of bleeding following liver biopsy as well as other surgical procedures, and that the PT does not correlate with severity of gastrointestinal bleeding in patients with cirrhosis [19-21].

To better resemble the hemostatic system in patients with chronic liver disease, other tests have been recently studied. These include thrombin generation, thromboelastography (TEG), and thromboelastometry. The TEG uses whole blood to measure speed and strength of clot formation continuously. It can, thus, theoretically analyze all components of the hemostatic system [22]. Both TEG and rotational thromboelastometry have been routinely used to guide transfusion of platelet concentrates, factor repletion, and fibrinolytic therapy during liver transplantation [23]. In fact, a recent randomized controlled trial reported a significant lower use of blood products without an increase in bleeding complications by using TEG-guided transfusion strategy compared to transfusion guided by INR and platelet count [24]. However, most parameters of the test are not standardized yet and TEG thresholds remain unclear. Furthermore, to date no studies have examined the use of TEG in predicting bleeding or thrombosis in nonsurgical patients with liver disease. Other drawbacks of TEG include pre-
analytic and analytic variables that impact test reliability and reproducibility, and the various methods to perform the test which results poorly correlate [25-27].

Another test that has been increasingly used to reassess the hemostatic capacity of patients with liver disease is the thrombin generation test. The thrombin generation assay evaluates thrombin generation (resulting from the action of the procoagulants) and decay (resulting from the action of the anticoagulants). In the assay, coagulation of test plasma is activated by small amounts of tissue factor and phospholipids, and the formation of thrombin is continuously monitored by means of a thrombin-specific fluorogenic substrate. There are several parameters that can be derived from the thrombin generation curve, but the endogenous thrombin potential (ETP) is the most used, which refers to the total amount of thrombin generated during the test. When thrombomodulin is added to the test plasma, the thrombin generation test is sensitive to all anticoagulant proteins in the plasma and, thus, measures the true balance between the pro- and anticoagulant factors. Indeed, thrombin generation testing in the presence of thrombomodulin has demonstrated normal or even superior thrombin generation in patients with liver disease [28-32]. Some studies have also shown that thrombin generation testing is useful in identifying patients with an increased risk of venous thrombosis or portal vein thrombosis [33-36]. However, the thrombin generation test is not widely available, the test is not standardized yet, and currently the test is too complicated for routine use in diagnostic laboratories.

The Thrombodynamics assay is a newly developed plasma-based global hemostasis assay, which continuously monitors clot growth in non-stirred plasma. Whereas in other coagulation tests (such as the PT or thrombin generation test) clotting is activated by homogenously dissolved tissue factor, in the Thrombodynamics test clotting is activated by a surface with immobilized tissue factor [37,38]. Therefore, this spatial clot growth assay better resembles in vivo clot formation. The spatial clot growth assay has been successfully used to assess the hemostatic system in various indications [37-40], including in patients with hemophilia [41]. In chapter 4, we also show preserved clot formation in plasma from patients with cirrhosis using the novel Thrombodynamics assay.

Advantages of this assay include the stability of the assay (provided a strict blood processing protocol is followed) and the easy use and clear methods of the assay. Furthermore, the test provides clear reference values for the various parameters of clot growth that can be measured. The Thrombodynamics assay may hold promise in the prediction of bleeding or thrombotic risk in patients with a liver disease. However, currently the test is for research use only and should not be used in diagnostic procedures. Future studies to assess the clinical value of this novel test in predicting hemostatic abnormalities in patients with liver disease are needed.

Finally, it should be noticed that it may also be possible that detailed clinical assessment, for example by standardized bleeding scores or thrombosis risk assessment, could be able to identify those patients who are at risk for either bleeding or thrombosis [42]. However, data on the use of such clinical scores in patients with liver disease are scarce. Nevertheless, one recent study evaluated the Padua Predictor Score (PPS) as a risk-stratification tool for the development of venous thromboembolism (VTE) in patients with chronic liver disease [43].
The PPS was significantly associated with risk of VTE and, thus, appears to be an effective assessment tool in patients with chronic liver disease. However, this was a retrospective study and prospective studies would be needed to confirm this finding.

**Bleeding risk in surgical patients**

Patients with a liver disease are not as prone to bleeding complications as clinicians formerly thought. As mentioned in the previous section, routine coagulations tests cannot be reliably used to assess a bleeding tendency in these patients. Furthermore, many patients can currently undergo a liver transplantation with minimal or even without transfusion of blood products [44-47]. Such a major surgical procedure would never be possible in a patient with a true coagulopathy, such as in hemophilia, without correction of the coagulopathy with factor concentrates or blood product transfusion. There is no evidence for the benefits of prophylactic blood product transfusion in perioperative medicine in patients with liver disease. Despite the lack of benefits, prophylactic use of blood products prior to smaller invasive procedures in these patients is still common practice. However, the side effects, such as risk of infection, risk of transfusion related acute lung injury, and transfusion associated circulatory overload with an increasing risk of bleeding, are increasingly acknowledged. In chapter 2 we, therefore, argue against routinely correcting a prolonged PT or INR with transfusion of blood products prior to procedures in patients with liver disease. Instead, only those patients with significant bleeding should be treated. Moreover, risk factors for bleeding, such as portal hypertension, endothelial dysfunction, bacterial infection, and renal failure, should also be addressed when preventing (re)bleeding.

Previously, patients following liver surgery were also perceived to be in a hypocoagulable state due to a prolonged PT or APTT [48-50]. However, we now know that these conventional coagulation tests are not reliable for the assessment of the overall hemostatic status in these patients. In fact, in chapter 5 we showed a hypercoagulability of plasma from patients following liver resection using the thrombin generation test in the presence of thrombomodulin. This hypercoagulable state following liver resection is also clinically evidenced by studies showing that the occurrence of venous thromboembolism following liver surgery is common [51] and, in fact, the risk increases with the extent of heptectomy [52,53]. Furthermore, several recent studies also found a normal to hypercoagulable state following liver surgery using thromboelastography [50,54,55]. However, in chapter 5 we also provide a mechanistic explanation for the hypercoagulable state following liver resection. Specifically, the hypercoagulable state was associated to a profound thrombomodulin resistance, meaning that thrombin generation only slightly decreased by the addition of thrombomodulin in plasma from patients following liver resection compared to a substantial decrease in plasma from healthy controls.

This thrombomodulin resistance was likely attributable to decreased levels of protein C and elevated levels of factor VIII. Combined with the decreased levels of antithrombin, the net effect is an increased thrombin generation when tested in the presence of thrombomodulin following liver resection. This decrease in coagulation factors might, in part, be explained by the decreased synthetic capacity of the liver remnant. Consumption of coagulation proteins as a result of surgical damage may also play a role. This might explain why in patients following pancreatic resection in our study also certain coagulation proteins decreased. Furthermore, this consumption theory may be supported by the finding that the risk of venous
thromboembolism increases with the extent of the heptectomy [52,53], and that extended operative time forms a risk factor for venous thromboembolism [51]. Finally, hemodilution may also contribute to the decrease in coagulation factors following liver resection.

The results of chapter 5 on the hypercoagulable state following liver resection in combination with the discussed literature in the previous section, has important clinical implications in the management of patients following liver surgery. First, clinicians should be aware of the limitations of the use of conventional coagulation tests to guide hemostatic management. A prolonged PT or APTT following liver surgery should not be routinely corrected by transfusion of blood products. Second, patients following liver surgery should not be withheld from thrombosis prophylaxis. In fact, more aggressive anticoagulation may even be necessary in the post-operative period when certain risk factors for venous thromboembolism are present. Examples of such risk factors might be a history of venous thromboembolism, the presence of cancer, an extended operation time, or a prolonged length of stay.

**Hemostasis in NAFLD**

Nonalcoholic fatty liver disease (NAFLD) is, nowadays, considered to be the most frequent chronic liver disorder in Western countries [56,57]. It represents a histopathological spectrum ranging from simple hepatic steatosis to steatohepatitis (NASH) with increasing risk for progression to advanced fibrosis and cirrhosis [58-60]. NAFLD is considered the hepatic manifestation of the metabolic syndrome, and is associated with an increased risk of cardiovascular disease (CVD) [61]. Furthermore, rates of venous thromboembolism and portal vein thrombosis (PVT) appear also increased in patients with NAFLD [62,63]. Multiple processes probably contribute to this increased risk of thrombosis in NAFLD, which have been mentioned in chapter 6. Some recent studies have also suggested a role for a hypercoagulable state in the increased risk of thrombosis in patients with NAFLD. However, in chapter 7, we show that, except for some pro-thrombotic features, the overall hemostatic status is comparable between patients with NAFLD and controls. Therefore, our study suggests a limited role for hyperactive hemostasis in the increased thrombotic risk in NAFLD. In this section the results found in chapter 7 in combination with the current literature on the hemostatic alterations in NAFLD will be further discussed.

**Alterations in primary hemostasis**

Previous studies have suggested an increase in platelet activation in patients with the metabolic syndrome [64,65] and in patients with NAFLD [66-69], which might contribute to their increased risk of thrombosis. However, the results of these studies are inconsistent and most of these studies are limited by their relative small sample size. Furthermore, most of these studies use indirect markers of platelet activation, such as the mean platelet volume. In chapter 7, we show that NAFLD is not associated with an increase in platelet activation, which was measured using a direct flow cytometric assay of platelet activation status. Additionally, NAFLD was not associated with major changes in pivotal proteins in primary hemostasis. We, therefore, suggest a limited role for liver disease-induced platelet activation in the perceived increased risk of thrombosis in patients with NAFLD.
Alterations in coagulation & fibrinolysis

Levels of various individual pro-thrombotic factors appear to be increased in patients with NAFLD [70-76]. Although results of these studies on different factors have been inconsistent, an increase in PAI-1, fibrinogen, and factor VIII and a decrease in antithrombin are most frequently reported. In chapter 7, we also observed increased fibrinogen levels in patients with non-cirrhotic NAFLD, although the difference with controls did not reach statistical significance. However, levels of fibrinogen are mostly decreased in patients with cirrhosis [77], which we also observed in patients with alcoholic related cirrhosis. Nevertheless, patients with NASH-related cirrhosis had normal fibrinogen levels, which might be attributed to a relative increase in fibrinogen production due to the fatty liver disease.

Furthermore, we observed increased levels of factor VIII and decreased levels of antithrombin in patients with cirrhosis, which is in line with previous studies [7,28,30]. Finally, in chapter 7 we also observed increased levels of PAI-1 in patients with NAFLD. In fact, PAI-1 levels appeared to increase with increasing severity of the disease and increasing severity of steatosis. Also Verrijken et al. found significant elevated levels of PAI-1 in patients with NASH, which correlated with underlying histological parameters [72]. In fact, increased levels of PAI-1 and resultant reduction in fibrinolysis have been extensively reported as a risk factor for venous thrombosis [78,79]. However, levels of PAI-1 are not independently associated with risk of myocardial infarction, and PAI-1 levels appear to reflect other cardiovascular risk factors [80].

In chapter 7, we observed hypofibrinolysis using the clot lysis assay in patients with non-cirrhotic NAFLD. This hypofibrinolytic status might contribute to thrombosis risk in these patients since a hypofibrinolytic state as determined with the clot lysis assay has been demonstrated to form a risk factor for both venous and arterial thrombosis in the general population [81-83].

More important than to study individual hemostatic proteins, is to study if these changes lead to a hypercoagulability in patients with NAFLD which might explain their increased risk of thrombosis. We performed both TEG and thrombin generation testing in patients with NAFLD in chapter 7. TEG test results were comparable between non-cirrhotic NAFLD patients and controls. However, TEG revealed moderate hypocoagulability in cirrhotic patients. Furthermore, thrombin generation was also normal in patients with NAFLD. This contrasts with Tripodi et al., who concluded that NAFLD is characterized by a procoagulant-imbalance, as shown by an increase in ETP-ratio (with-to-without thrombomodulin) in these patients [73]. We also observed an increase in a similar ratio in patients with NASH-related cirrhosis. However, such ratios only represent the resistance of the plasma to the anticoagulant action of thrombomodulin, which is increased due to decreased levels of protein C and increased levels of FVIII in patients with liver disease. Furthermore, in patients with ASH-related cirrhosis in our study and in patients with alcoholic/viral cirrhosis in the study of Tripodi et al. the ratio was also increased. An increase in the ETP-ratio in patients with NAFLD, therefore, does not explain their increased risk of thrombosis compared to patient with alcoholic/viral cirrhosis.

In chapter 7 we, therefore, argue against the use of this ratio to classify if patient samples are normo- or hypercoagulable, and instead believe that the thrombin generation performed in the presence of thrombomodulin is the most accurate laboratory measure of the coagulant potential of a patients’ plasma.
Besides hypofibrinolysis, we did find another pro-thrombotic feature of patients with NAFLD, namely a pro-thrombotic structure of the fibrin clot. This was observed by decreased fibrin clot permeability and increased clot density in patients with NAFLD. Indeed, previous studies have shown decreased fibrin clot permeability to be associated with thrombotic diseases [84-86].

Finally, it should be mentioned that we found a higher variability of several hemostatic test results in patients compared to controls. This might suggest that individual patients have a more thrombogenic hemostatic profile. In chapter 7, we could, however, not identify any characteristics of individual patients to explain the more extreme values in individual patients.

**Conclusion: hemostasis in NAFLD**

Despite a slight hypofibrinolysis and pro-thrombotic structure of fibrin clots in patients with NAFLD, in chapter 7 we show that the overall hemostatic status is comparable between patients with NAFLD and healthy individuals. This suggests that the role for hemostasis in the increased risk of thrombosis in patients with NAFLD and NASH-related cirrhosis is probably limited.

**Thrombotic complications in patients with liver disease**

Various thrombotic complications do occur frequently in patients with liver disease. For instance, PVT is a common complication of chronic liver disease and is perceived to be associated with clinical deterioration [87]. However, recent studies question whether PVT truly affects cirrhosis outcome [88,89]. A recent systematic review and meta-analysis of multiple studies on anticoagulation for treatment of PVT in cirrhosis concluded that anticoagulant therapy could lead to a relatively high rate of portal vein recanalization [90]. However, further randomized controlled trials are warranted to confirm the risk-to-benefit ratio of anticoagulation in such patients, especially concerning anticoagulant-related bleeding. Recent case studies and one retrospective study have also reported both the efficacy and safety of new oral anticoagulants as a treatment of PVT [91-94]. Currently, there are no strategies available to prevent the development of PVT in cirrhosis. Nevertheless, one recent randomized trial reported that enoxaparin prevents PVT in patients with cirrhosis and, in addition, appears to delay hepatic decompensation [95].

Another frequent occurring thrombotic complication in patients with chronic liver disease is venous thrombosis (which includes deep vein thrombosis (DVT) and pulmonary embolism (PE)). In fact, the estimated incidence of venous thrombosis in cirrhotic patients is 0.5% to 6.3%, compared with 0.5% to 0.9% in non-cirrhotic patients, and the incidence appears to increase with increasing severity of cirrhosis [96]. Current guidelines on the treatment of venous thrombosis in the general population recommend the use of new oral anticoagulants (e.g. Dabigatran, Rivaroxaban, Apixaban, or Edoxaban) over vitamin K antagonist (VKA) therapy [97]. Furthermore, initial parenteral anticoagulation (typically with low molecular weight heparin (LMWH)) is given before Dabigatran and Edoxaban, is not given before Rivaroxaban or Apixaban, and is overlapped with vitamin K antagonist therapy. Except in cancer-associated thrombosis the guidelines suggest LMWH therapy over VKAs or new oral anticoagulant drugs. Primary prevention of venous thrombosis in the general population
is achieved by LMWH, fondaparinux, low-dose unfractionated heparin, or by new oral anticoagulant drugs [98]. There is mounting evidence that thromboprophylactic treatment with LMWH is safe in patients with chronic liver disease [96].

Finally, arterial thrombotic events also occur in patients with liver disease and, in fact, patients with NAFLD have been repeatedly shown to have an increased risk for arterial disease (see chapter 6). For primary prevention of cardiovascular disease in the general population with an age of >50 years, low dose aspirin therapy is suggested. For secondary prevention of cardiovascular events in patients with established coronary artery disease, monotherapy with aspirin or clopidogrel is recommended. Finally, following acute coronary syndromes with percutaneous coronary intervention and stent placement, dual antiplatelet therapy with aspirin in combination with ticagrelor, clopidogrel, or prasugrel is recommended [99]. Secondary prevention of arterial events should probably not be withheld from patients with liver disease, but the risk of bleeding complications may be increased [100]. This thesis further focuses on the use of anticoagulant drugs in patients with liver disease, the use of anti-platelet agents in patients with cirrhosis has been extensively reviewed elsewhere [100].

**Different anticoagulant drugs in patients with liver disease**

Table 1 shows the different anticoagulant drugs with their mechanisms of action, advantages or disadvantages, and in vitro potency (as studied in chapter 8) in patients with liver disease.

**Heparins**

Heparins act by binding to antithrombin and enhancing its effect to inhibit factor Xa and/or thrombin. They can be used for the prevention or treatment of both venous thrombosis and PVT. Currently, three classes of heparins are available: unfractionated heparin, LMWH, and fondaparinux. Several studies reported that heparins are efficacious and safe in patients with cirrhosis and PVT or venous thrombosis [95,101-105]. Most studies used LMWH, rather than unfractionated heparin or fondaparinux. Furthermore, studies suggest that the use of LMWH may be more favorable in terms of bleeding complications than unfractionated heparin for the indication of thromboprophylaxis in cirrhotic patients [105,106]. Nevertheless, there are important drawbacks in using heparins in both the general population and, specifically, in patients with liver disease. For instance, the long-term use of these drugs may be limited by the mode of administration (i.v. for unfractionated heparin or s.c. for LMWH and fondaparinux) as well as the concern for heparin-induced thrombocytopenia (HIT). The mode of administration may be associated with both poor compliance and skin reactions. In patients with renal failure LMWH accumulation is known to occur, which may require dose adjustments in patients with cirrhosis and decreased renal function. Furthermore, heparin requires antithrombin to exert its anticoagulant effect and antithrombin levels are frequently decreased in patients with liver disease, which theoretically leads to an unpredictable anticoagulant effect. Additionally, monitoring issues of heparins occur in patients with liver disease, which will be discussed further on. Recently, Senzolo et al. showed an increased anticoagulant response to LMWH in plasma from patients with cirrhosis [107]. In chapter 8, we also show a modestly increased anticoagulant response to both unfractionated heparin and LMWH in plasma from patients with cirrhosis, which was, however, only significant in the absence of thrombomodulin. In contrast, we observed a reduced response to fondaparinux in patients with, especially, advanced cirrhosis (e.g. Child-Pugh C). Dose adjustments based on both altered pharmacokinetics and altered drug potency might, thus, be required for...
these drugs in patients with cirrhosis. However, the limited data showing that LMWH is safe to use in patients with cirrhosis contradicts that dose adjustments for this drug are needed.

**Vitamin K antagonists**
VKAs act by decreasing vitamin K-dependent procoagulant factors II, VII, IX, X, and anticoagulant proteins C and S, with a reduction of hemostatic potential as a net result. A major advantage of VKA therapy is the oral mode of administration. However, the major concern with the use of VKAs in patients with liver disease is the monitoring of the drug by INR levels, which are already abnormal in patients with liver disease. The target INR for VKAs in patients with cirrhosis is, therefore, unclear. In addition, the use of VKAs in patients with cirrhosis has been associated with an increased risk of bleeding complications [108,109]. A recent retrospective study demonstrated significantly better recanalization rates of PVT in patients treated with warfarin therapy (with a target INR range of 2.0-3.0) compared to untreated patients. However, the study did report on bleeding events [110]. Given the reported increased bleeding risk and unclear target INR, the use of VKAs in patients with cirrhosis is likely associated with an unfavorable risk-to-benefit ratio.

**New-generation oral anticoagulant drugs**
The new-generation oral anticoagulant drugs include the direct factor Xa inhibitors Rivaroxaban, Apixaban, and Edoxaban and the direct thrombin inhibitor Dabigatran. Major advantages of these drugs include the oral mode of administration, the action independent of antithrombin, and the lack of need for monitoring. Although, the lack of need for monitoring can also be interpreted as a disadvantage of the drug as it may increase the risk for non-compliance. Previously, one of the major concerns for these drugs was the lack of specific antidotes to reverse the anticoagulant effect in emergency situations. However, Idarucizumab is currently being used in the clinics as an antidote against Dabigatran, and Andexanet alfa is currently being studied in a phase IV trial as a reversal agent against factor Xa inhibitors [111]. Due to favorable efficacy and safety, new guidelines currently recommend the use of these newer drugs over VKAs and LMWH as long-term anticoagulant therapy in venous thrombosis in the general population (in the absence of cancer) [97]. The direct factor Xa and thrombin inhibitors may also be applicable in the prevention and treatment of thrombotic complications in patients with liver disease. Reports of success of these new-generation oral anticoagulant drugs in patients with cirrhosis are emerging [91,93,94], however larger clinical trials on efficacy and safety of these drugs in cirrhotic patients are lacking. We studied the efficacy of three of the new oral anticoagulant drugs in plasma from patients with cirrhosis in chapter 8 and the appendix to chapter 8. Addition of the direct thrombin inhibitor Dabigatran resulted in an increased response in plasma from patients with cirrhosis. The enhanced effect of Dabigatran was proportional to the severity of the disease. In contrast, the in vitro anticoagulant potency of both factor Xa inhibitors Rivaroxaban and Apixaban was substantially reduced in patients with moderate and advanced cirrhosis. Thus, the new oral anticoagulants may work differently in liver disease patients compared to patients with intact liver function, which is a potential caveat. Drug-specific dose adjustments may be required for these drugs in patients with liver disease. Since these drugs are cleared by the liver and kidneys, the pharmacokinetics may also potentially be altered in patients with liver disease. Dose adjustments should, thus, ideally take both the altered drug potency and the altered pharmacokinetics into account. Furthermore, since the in vitro anticoagulant potency of Dabigatran is substantially increased in patients with cirrhosis, clinicians may be cautious in
using this drug in patients with liver disease. The results of chapter 8 (and appendix) suggest that anticoagulant treatment with direct factor Xa inhibitors will likely not result in over-anticoagulation, with a potentially increased bleeding risk, provided drug levels remain in the target range. Future clinical trials should, therefore, focus on the efficacy and safety of the direct factor Xa inhibitors in patients with liver disease. Finally, monitoring of these new drugs in patients with liver disease may be advisable, which will be discussed later on.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Pros</th>
<th>Cons</th>
<th>In vitro potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated heparin</td>
<td>AT-dependent inhibition of factor Xa and thrombin</td>
<td>- Costs</td>
<td>- I.v. administration</td>
<td>Modestly increased</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fully reversible with protamine</td>
<td>- Risk of HIT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Dependent on AT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Monitoring issues with APTT and anti-Xa assay</td>
<td></td>
</tr>
<tr>
<td>LMWH</td>
<td>AT-dependent inhibition of factor Xa and (to a lesser extent) thrombin</td>
<td>- Reduced risk for HIT compared to other heparins</td>
<td>- S.c. administration</td>
<td>Modestly increased</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Synthetic drug</td>
<td>- Dependent on AT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Monitoring issues with anti-Xa assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Accumulation in renal failure</td>
<td></td>
</tr>
<tr>
<td>Fondaparinux</td>
<td>AT-dependent inhibition of factor Xa</td>
<td>- Reduced risk for HIT compared to other heparins</td>
<td>- Monitoring issues in patients with already elevated INR</td>
<td>Decreased, especially in advanced cirrhosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Synthetic drug</td>
<td>- Bleeding risk</td>
<td></td>
</tr>
<tr>
<td>Vitamin K antagonists</td>
<td>Reduce functional levels of vitamin K-dependent proteins</td>
<td>- Costs</td>
<td>Monitoring issues in patients with already elevated INR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Oral mode of administration</td>
<td>- Monitoring issues in patients with already elevated INR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Bleeding risk</td>
<td></td>
</tr>
<tr>
<td>Rivaroxaban, Apixaban, Edoxaban</td>
<td>Direct factor Xa inhibitor</td>
<td>- Lack of AT dependence</td>
<td>- Lack of experience</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Mode of administration</td>
<td>- GI bleeding risk</td>
<td>Rivaroxaban and Apixaban: decreased in moderate and advanced cirrhosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Wider therapeutic window than VKAs</td>
<td>- Accumulation in renal and liver disease</td>
<td></td>
</tr>
<tr>
<td>Dabigatran</td>
<td>Direct thrombin inhibitor</td>
<td>- Lack of AT dependence</td>
<td>- Lack of experience</td>
<td>Substantially increased, proportional to severity of disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Mode of administration</td>
<td>- GI bleeding risk</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Wider therapeutic window than VKAs</td>
<td>- Accumulation in renal and liver disease</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Anticoagulant drugs in the prevention or treatment of venous thrombosis and portal vein thrombosis in patients with liver disease. Shown are their mechanisms of action, advantages or disadvantages, and in vitro potency (as studied in chapter 8) in patients with liver disease. AT: antithrombin, HIT: heparin-induced thrombocytopenia, APTT: activated partial thromboplastin time, LMWH: low molecular weight heparin, VKA: vitamin K antagonist, GI: gastro-intestinal.

Prevention or treatment of thrombosis in patients with liver disease

There are multiple indications for antithrombotic treatment in patients with liver disease. However, due to the lack of clinical data, the anticoagulant drug of choice and dosing regimens are in most instances unclear. In this section, some guidance on how to approach prevention or treatment of thrombosis in patients with liver disease will be provided. In addition, it is critical to evaluate each patient individually to best determine which benefits and burdens are most prominent in the setting of prevention or treatment of thrombosis. Finally, before starting thrombosis prophylaxis or treatment in patients with liver disease, pharmacologic (i.e. non-selective beta-blockers) or endoscopic variceal bleeding prophylaxis should be considered in case of (known) esophageal varices.
Portal vein thrombosis
To date, only one study reported the efficacy and safety of prevention of PVT with LMWH in patients with cirrhosis [95]. Since insufficient data on this topic exists, the prevention of PVT with anticoagulant therapy should, at present, only be considered in the context of clinical trials.

In general, evidence indicates that anticoagulant therapy for PVT in carefully selected patients with cirrhosis is safe and effective. However, conservative dosing, in particular in patients with advanced disease, and in those patients with risk factors for bleeding, such as renal failure and severe thrombocytopenia, may be suggested. Furthermore, certain patients may benefit more from anticoagulant treatment of PVT, such as liver transplantation candidates compared to patients not suitable for liver transplantation and with an otherwise poor prognosis [88]. In addition, patients being considered for anticoagulation should undergo evaluation for esophageal varices before initiation of anticoagulation. At present, LMWH may be the anticoagulant drug of choice, since the available clinical data suggest LMWH to be safe and effective in patients with liver disease. VKAs are discouraged given the high rates of bleeding and the lack of suitable target INR. In the future, the new oral anticoagulants may be applicable in the treatment of PVT, however clinical data on this are currently lacking.

Venous thrombosis
Thrombosis prophylaxis should not be withheld in patients with a liver disease when risk factors for thrombotic complications are present, even in the presence of abnormal routine tests of hemostasis. Risk factors include hospitalization and immobilization, surgery, and the presence of (hepatocellular) cancer. Dosing regimens may be altered in patients with risk factors for bleeding, such as renal failure or profound thrombocytopenia. Mechanical thromboprophylaxis should be considered in those patients with clear contraindications for anticoagulant treatment. For the same reasons as for treatment of PVT, in prevention of venous thrombosis LMWH is also currently the drug of choice.

While studies on prevention of venous thrombosis and prevention or treatment of PVT exist, no current study examined the efficacy or safety of anticoagulants for the treatment of venous thrombosis in cirrhotic patients. Clinicians are, therefore, left to extrapolate therapeutic choice and regimen for venous thrombosis in cirrhosis patients from trials and guidelines in medical patients [97]. This would imply that treatment of venous thrombosis should be initiated with LMWH, and may be continued with VKAs or new-generation oral anticoagulant drugs. However, the use of VKAs should be performed with great care, since dosing regimens are unclear. Furthermore, the use of new oral anticoagulant drugs has not been studied in clinical trials in patients with cirrhosis. As mentioned before, due to the unpredictable effect of Dabigatran observed in chapter 8, the use of direct factor Xa inhibitors may be more promising in patients with cirrhosis. It may, therefore, be suggested to use either prolonged LMWH administration or direct factor Xa inhibitors (with initial LMWH for Edoxaban) in the treatment of venous thrombosis in patients with liver disease. Future studies on the efficacy and safety of these anticoagulant drugs for treatment of venous thrombosis in patients with cirrhosis are urgently needed.
**Antithrombotic therapy to reduce progression of liver disease**

Increasing evidence from animal models of liver disease suggests that antithrombotic therapy reduces progression of liver disease [112-119]. Furthermore, a single randomized clinical trial showed that LMWH therapy delays decompensation in patients with cirrhosis [95]. There may be two hypotheses that could explain the involvement of the coagulation cascade in the rate of liver fibrogenesis: the formation of thrombi resulting in tissue ischemia (also referred to as parenchymal extinction) and the activation of disease-promoting cells by coagulation proteases [120]. If antithrombotic therapy truly prevents progression of liver disease and substantially delays decompensation, this will profoundly impact the clinical management of patients with (early) cirrhosis. However, future clinical studies on the benefits and safety of antithrombotic treatment in different patient groups are required before this may be considered in clinical practice.

**Monitoring of anticoagulant drugs**

Due to the extensive hemostatic changes in patients with cirrhosis, careful monitoring of anticoagulant therapy may be required. For monitoring of the different heparins in the general population the anti-Xa assay and/or the APTT (for unfractionated heparin) can be used. However, recent data suggest that plasma levels of LMWH are substantially underestimated by the anti-Xa assay in patients with cirrhosis [104,107,121]. This is most probably related to the reduced antithrombin levels in these patients. Indeed, in chapter 9 we show that the anti-Xa assay not only underestimates the LMWH mass, but the test also underestimates the plasma levels of other antithrombin-dependent drugs (i.e. unfractionated heparin and fondaparinux) in plasma from patients with cirrhosis. Furthermore, when exogenous antithrombin was added to the anti-Xa assay, the reduced recovery of LMWH in patients compared to controls was fully blunted. Based on these data, we strongly suggest that anti-Xa levels should not be relied upon for monitoring heparins (that exert their effect through antithrombin) in patients with cirrhosis, unless exogenous antithrombin is added to the test. However, this modification of the test is not readily available in many routine diagnostic laboratories. Since the use of LMWH appears safe in patients with cirrhosis, monitoring of LMWH should maybe not be performed at all, except in certain situations, for example in patients with severe renal failure in which the anti-Xa with the addition of exogenous antithrombin may be used. In contrast to the underestimation of levels of heparins with the anti-Xa assay, we show in chapter 9 that the APTT overestimates levels of unfractionated heparin in plasma from patients with cirrhosis. This test, therefore, also seems unsuitable for heparin monitoring in patients with cirrhosis. Since the different anticoagulant drugs may also have an altered anticoagulant potency in patients with cirrhosis (as outlined before), the ideal monitoring test for patients with cirrhosis should take both drug levels and drug effect into account.

Monitoring of the new oral anticoagulant drugs may be required in patients with liver disease, since these drugs are cleared by the liver and kidneys and the anticoagulant potency of these drugs may be altered. The direct factor Xa and thrombin inhibitors may be monitored through the respective anti-Xa and anti-IIa assays, as in chapter 9 comparable anti-Xa and anti-IIa levels were observed after the addition of Rivaroxaban and Dabigatran, respectively, in plasma from patients and controls. For monitoring of Dabigatran the thrombin time (TT), ecarin clotting time (ECT), or APTT may also be used [122]. However, the TT and ECT are not widely available, and the APTT is mostly already prolonged in patients with cirrhosis.
**Future perspectives**

The results of this thesis have contributed to our understanding of the hemostatic system and the prevention or treatment of thrombosis in patients with liver disease. However, as mentioned throughout this last chapter, there is still a lot of research to be done in order to provide better strategies to deal with hemostatic abnormalities in patients with liver disease. Therefore, future studies should include (but should not be restricted to) the following topics:

- Prospective studies to assess the clinical value of promising laboratory tests (thrombin generation testing, TEG, or Thromboelastography) in the prediction of bleeding or thrombosis risk in patients with liver disease.

- Randomized controlled studies assessing efficacy and safety of restrictive transfusion strategies during invasive procedures in patients with liver disease.

- Randomized controlled studies on reduction of bleeding complications during invasive procedures in patients with small volume prohemostatics, such as prothrombin complex concentrate.

- Randomized studies on efficacy and safety of prophylaxis or treatment of venous thrombosis and portal vein thrombosis in patients with liver disease, specifically with the use of LMWH or new-generation oral anticoagulants.

- Prospective studies on anticoagulation in patients with NAFLD with thrombotic disease.

- Studies on the use of TEG or thrombin generation to monitor anticoagulant therapy in patients with chronic liver disease.

- Finally, prospective clinical studies on the benefits and safety of antithrombotic therapy to slow down progression of liver disease.
References


109. Levi M, Hovingh GK, Cannegieter SC, Vermeulen M, Buller HR, Rosendaal FR. Bleeding in patients receiving vitamin K antagonists who would have been excluded from trials on which the indication for anticoagulation was based. Blood 2008 May 1;111(9):4471-4476.


