COAGULATION IN CHILDREN WITH LIVER DISEASE

Maria Magnusson

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"Där bor små fina älvor bak mossig jättesten.
De kommer fram och dansar ibland i månens sken,
Och tomtebarnen tycker, att inte någonting
kan vara lika vackert som älvorna i ring.

Så lätt och späd är älvan, men tomten tung och tjock,
Det märktes häromafton, de skulle gunga bock.
Fast älvorna var åtta och barnen bara två
Det ville inte lyckas att väga jämt ändå."

From Tomtebobarnen by Elsa Beskow, 1910
© Bonnier Carlsen

To Kalle and Jens
&
To the children with liver disease
ABSTRACT

About 100 new children in Sweden require care at a tertiary pediatric ward each year for severe liver disease. These children are at risk for both severe gastrointestinal bleeding, which may be life threatening, and intra- or extrahepatic thrombosis. Both pro- and anticoagulant factors are synthesized in the liver and their levels decrease as liver failure progresses. Coagulation factors are thus used as prognostic tests. The balance between pro- and anticoagulant mechanisms, although maintained in liver disease, seems to be instable and can easily tip towards either bleeding or thrombosis. The coagulation system in children undergoes age-specific changes and the etiology and/or pathogenesis of pediatric liver diseases is different than in adults.

The aims of this thesis were to improve the treatment and the analysis of coagulation defects in pediatric liver disease and also to improve the prognostic evaluation in liver disease.

In Study 1 children with liver disease were treated with recombinant FVIIa due to life threatening bleeding or as prophylaxis prior to invasive procedures. In the first group, the bleeding decreased in 50% of the evaluated occasions and a combination of rFVIIa and octreotide in gastrointestinal hemorrhage was advantageous. In the second group, rFVIIa was useful as prophylactic treatment before various diagnostic and therapeutic procedures.

In Study 2 the thrombin generation test was evaluated in children with liver disease, with and without increased bleeding risk. The results were compared to age-matched healthy controls. This assay did not provide additional information compared to routine coagulation tests.

In Study 3 the correlation between bile acids and coagulation factors was investigated. In children with markedly elevated levels of bile acids, i.e. above 200 µmol/L, the levels of coagulation factors increased with rising levels of bile acids, despite a worse clinical outcome. In an in vitro study, no interference between bile acids and coagulation factor concentrations was detected.

In Study 4 the Owren method for analyzing INR in patients with liver disease was assessed. Plasma samples from adult patients with liver disease were analyzed at eight laboratories. The coefficient of variance between the laboratories was 5.3%, which is low. Additionally, the ISI_{VKA} and ISI_{liver} were determined and the difference between them was below 10%. These results show that the previously reported high interlaboratory variability regarding INR in patients with liver disease does not constitute a problem when Owren-based reagents are used.

Conclusion: rFVIIa is beneficial for selected patients with severe bleeding and as prophylactic treatment. However, with current knowledge regarding coagulation in liver disease, new treatment strategies aiming to maintain the hemostatic balance in critical situations need to be studied. The thrombin generation test did not provide more information than routine tests. A modified method might be more successful. Coagulation factors may be questionable as prognostic markers in patients with highly elevated bile acids. The mechanisms behind the effect of high bile acids on the coagulation system are very important targets of further studies. Finally, Owren-based reagents for measurement of INR in patients with liver disease provide a solution to the problem with high interlaboratory variability seen internationally.

This thesis adds important information regarding several aspects of the coagulation in children with liver disease and highlights directions for future research.

Keywords: FVIIa, thrombin generation, cholestasis, bile acids, prognosis, INR, ISI, Owren
LIST OF PUBLICATIONS


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¹ Maria Magnusson’s maiden name is Pettersson
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<thead>
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<th>Abbreviation</th>
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<td>AASLD</td>
<td>American Association for the Study of Liver Disease</td>
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<tr>
<td>ADAMTS-13</td>
<td>A disintegrin and metalloproteinase with a thrombospondin type-1 motifs, 13</td>
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<td>ALF</td>
<td>Acute liver failure</td>
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<td>ALF</td>
<td>Alanin aminotransferase</td>
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<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>APC</td>
<td>Activated protein C</td>
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<td>APTT</td>
<td>Activated partial thromboplastin time</td>
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<td>ARDS</td>
<td>Acute respiratory distress syndrome</td>
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<td>AST</td>
<td>Aspartate aminotransferase</td>
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<td>CAT</td>
<td>Calibrated Automated Thrombogram</td>
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<td>CRASH-2</td>
<td>Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>CTP</td>
<td>Child, Turcotte and Pugh score</td>
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<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
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<td>ESLD</td>
<td>End-stage liver disease</td>
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<td>ETP</td>
<td>Endogenous thrombin potential</td>
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<td>EQUALIS</td>
<td>External Quality Assurance of Laboratory medicine</td>
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<tr>
<td>FII</td>
<td>Factor II, prothrombin</td>
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<td>FIIa</td>
<td>Activated factor II, thrombin</td>
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<td>FV, FVa</td>
<td>Factor V, activated Factor V</td>
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<td>Factor XIII</td>
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<td>FFP</td>
<td>Fresh frozen plasma</td>
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<td>FSBA</td>
<td>Total fasting serum levels of bile acids</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>GT</td>
<td>γ-glutamyltransferase</td>
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<td>INR</td>
<td>International Normalized Ratio</td>
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<td>ISI</td>
<td>International Sensitivity Index</td>
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<td>ISI_liver</td>
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<td>ISI_{VKA}</td>
<td>International Sensitivity Index for vitamin K antagonist treatment</td>
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<td>LMWH</td>
<td>Low molecular weight heparin</td>
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<td>LT</td>
<td>Lag time</td>
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<td>MELD</td>
<td>Model of End-Stage Liver Disease score</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
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<td>PCC</td>
<td>Prothrombin complex concentrate</td>
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<td>Pediatric End-Stage Liver Disease score</td>
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<td>PT</td>
<td>Prothrombin time</td>
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<td>PVT</td>
<td>Portal vein thrombosis</td>
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<td>RBC</td>
<td>Red blood cell transfusion</td>
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<td>RCT</td>
<td>Randomized controlled trial</td>
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<td>rFVIIa</td>
<td>Recombinant activated Factor VII</td>
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<td>TACO</td>
<td>Transfusion-associated circulatory overload</td>
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<td>TAFI</td>
<td>Thrombin-activatable fibrinolysis inhibitor</td>
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<td>TF</td>
<td>Tissue factor</td>
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<td>TGT</td>
<td>Thrombin generation test</td>
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<td>TM</td>
<td>Thrombomodulin</td>
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<td>t-PA</td>
<td>tissue-plasminogen activator</td>
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<td>TPO</td>
<td>Thrombopoetin</td>
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<td>TRALI</td>
<td>Transfusion related acute lung injury</td>
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<td>TTP</td>
<td>Time to peak</td>
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<td>tx</td>
<td>Transplantation</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>VKA</td>
<td>Vitamin K antagonist</td>
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<tr>
<td>vWF</td>
<td>Von Willebrand factor</td>
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1 BACKGROUND

The liver is the largest solid organ in the body and has several important functions including metabolism of nutrients, synthesis of proteins and detoxification. Bile, which is important for the absorption of fat and fat-soluble vitamins, is also produced in the liver [1].

The micro-anatomy of the liver is shown in Figure 1. The liver consists predominantly of cords of hepatocytes. The walls of adjacent hepatocytes form bile canaliculi which empty into intrahepatic bile ducts formed by cholangiocytes. Blood enters the liver via the portal vein and the hepatic artery and flows slowly through the sinusoids, which are lined by endothelial cells, to the central vein.


In the fetus, liver structures develop from cells of different origins. The hepatocytes and the cholangiocytes are derived from the liver bud, which consists of endodermal epithelium of the foregut. The cells lining the sinusoids are derived from mesodermal mesenchymal cells. The extrahepatic bile ducts, which drain the intrahepatic bile ducts, are formed from the intestine [1, 2].
1.1 PEDIATRIC LIVER DISEASE

Hepatic health problems are uncommon in children. Still, about 100 new pediatric cases in Sweden require care at a tertiary pediatric ward each year due to liver disease. There are numerous different liver diseases that may affect neonates, infants and children. The etiologic and pathogenic spectrum varies to a great extent with age at presentation [3]. Many of the diseases have a genetic component; these diseases may be subclassified as chromosomal, single gene (Mendelian) and complex. The complex diseases are multifactorial and caused by interplay between several genes and the environment. The clinical picture, thus, differs between patients [4].

According to etiology and/or pathogenesis, pediatric liver disease can be subdivided as follows. Selected examples are given for each group.

**Abnormal development:** Micro- and macroanatomical anomalies in the structure of the hepatobiliary system caused during the fetal or early neonatal period, for example: biliary atresia, Alagille’s syndrome, congenital liver fibrosis, choledochal cyst.

**Infections:** Diseases caused by bacteria, viruses or parasites, for example: hepatitis B, hepatitis C, cytomegalovirus, Epstein-Barr virus, liver abscess.

**Inborn errors of metabolism:** Defects in the synthesis, turnover, breakdown and elimination of amino acids, proteins, carbohydrates and lipids, causing acute or chronic intoxication by some intermediary product or leading to a defective or absent function. These patients may have hepatic and/or extrahepatic symptoms. Examples of metabolic diseases with hepatic presentation: tyrosinemia, Wilson’s disease, progressive familial intrahepatic cholestasis (PFIC), Aagenaes syndrome, glycogen storage disease, non-alcoholic steatohepatitis (NASH).

**Immune-mediated disorders:** Diseases caused by an inappropriately targeted reaction of the immune system, for example: autoimmune hepatitis, sclerosing cholangitis, neonatal systemic lupus erythematosus, graft versus host disease.

**Xenobiotic-induced liver injury:** Liver damage caused by pharmaceutical, chemical, herbal or nutritional agents, for example: Reye’s syndrome, acetaminophen/
paracetamol-induced damage, venoocclusive disease (VOD), total parenteral nutrition associated cholestasis.

**Vascular disorders:** Symptoms caused by changes in blood flow in intra- and/or extrahepatic vessels or focal changes in the blood flow, for example portal vein thrombosis, portal vein stenosis, porto–systemic shunt, as well as sickle cell disease, and congestive heart failure.

**Neoplasms:** Benign or malignant tumors (primary or metastases) for example: hepatoblastoma, hepatocellular carcinoma, adenoma.

1.1.1 **Cholestasis**
This condition implies an impaired canalicular flow of bile, owing either to reduced synthesis of bile or an obstruction in the biliary tree [5]. The immediate consequence of cholestasis is the intracellular accumulation of lipophilic substances in the hepatocytes. This will cause deranged or altered hepatocytic functions. Soon, the cholestasis will also lead to decreased intraluminal gut concentration of bile compounds including bile acids, causing fat malabsorption [6]. Newborn children are especially prone to develop cholestasis and this syndrome in a neonate is referred to as neonatal cholestasis. The most common causes of neonatal cholestasis are biliary atresia, α1-antitrypsin deficiency and PFIC [7]. However, several of the different diagnoses listed above can cause this condition and in the final stage of their course most hepatic diseases show cholestatic features [8].

1.1.2 **Acute liver failure**
This clinical condition is defined by the following criteria: (1) no evidence of chronic liver disease (2) biochemical evidence of acute liver injury (3) hepatic-based coagulopathy that is not corrected by parenteral administration of vitamin K. In addition hepatic encephalopathy must be present if INR 1.5-1.9, but is not required if INR is equal to or greater than INR 2.0 [9]. The most common causes of acute liver failure in children are metabolic diseases, autoimmune disease and infectious diseases. Survival rates, without liver transplant, are reported to be 41-94%, depending on etiology [10].
1.1.3 End stage liver disease

Chronic liver disease of various etiologies can progress into liver failure and end-stage liver disease. Cirrhosis is the common final pathway of all chronic liver diseases. It is caused by an abnormal regeneration which replaces injured hepatic tissue by high amounts of fibrous tissue. This progressive fibrosis disrupts the normal microcirculation of the liver and causes decreased hepatocellular function and portal hypertension [3, 11]. Hepatocellular insufficiency might lead to secondary failure of other organs such as the kidneys, lungs, the gastrointestinal tract and finally the central nervous system.

1.1.4 Portal hypertension

Portal hypertension is defined as a pathological increase in the pressure of the portal venous system [12]. Apart from cirrhosis, this can also be caused by extrahepatic portal vein obstruction including portal vein thrombosis. Portal hypertension can lead to the development of ascites, splenomegali and formation of oesophageal and rectal varices [13]. Rupture of these varices causes severe gastrointestinal bleeding, which may be life threatening [14].

1.1.5 Routine methods for assessment of liver disease

(Selected examples below are tests of importance in this thesis)

**Laboratory test:** assays for the evaluation of: a) synthetic function (albumin, INR), b) hepatocyte integrity (ALT, AST) and c) markers of cholestasis (GT, ALP, Bilirubin, total and conjugated, FSBA).

**Imaging techniques:** Ultrasound, CT-scan, scintigraphy, elastography, MRI.

**Procedures:** Liver biopsy, endoscopy.

1.1.6 Treatment

The therapeutic alternatives for liver diseases include nutritional support, pharmacological treatment, surgical procedures and in selected cases liver transplantation [3, 10, 13, 15-17].
1.2 THE COAGULATION PROCESS
After an injury to the wall of a blood vessel, the body responds in a three-step process: primary hemostasis, secondary hemostasis (Figure 2) and the tertiary hemostasis (Figure 3).

1.2.1 Primary hemostasis
Platelets in the circulation are slowed down at the site of the injury in the vessel wall, as a response to interaction with plasma proteins including von Willebrand factor (vWF) bound to subendothelial collagen. Subsequently, the platelets adhere to the subendothelial collagen and are activated. The activation includes; a conformational change important for the spread on subendothelial surfaces, release of granulae and exposure of receptors on the platelet surface. This promotes formation of platelet aggregates preventing further blood loss, and is important for the cell-based coagulation cascade (see below) [18, 19]. The primary hemostasis, thus, results in the formation of a platelet plug.

1.2.2 Secondary hemostasis: The cell based model
(Figure 2)
The initiation phase: When an injury occurs at the vessel wall, activated Factor VII (FVIIa) binds to exposed tissue factor (TF). This complex activates Factor X (FX), which together with activated Factor V (FVa) activates a small amount of thrombin (FII). Though small, the quantity of thrombin formed during this phase starts the amplification phase.

The amplification phase: The thrombin formed leads to further activation of the platelets that adhere to the collagen at the site of the injury. In addition, thrombin activates FXI to FXIa and the cofactor FV to FVa and uncouples the cofactor FVIII from its carrier protein vWF.

The propagation phase: Complexes ofFIXa – FVIIIa and FXa – FVa are formed on the activated platelet and a large amount of thrombin is generated that can cleave fibrinogen to form a fibrin network, a clot, which is stabilized by FXIII, antiplasmin and thrombin-activatable fibrinolysis inhibitor (TAFI) [20, 21]. The secondary hemostasis thus results in stabilization of the platelet plug.
1.2.3 Anti-coagulant mechanisms
The tissue factor pathway inhibitor (TFPI) inhibits the initiation phase of the coagulation system by binding and deactivating FXa associated to the TF-FVIIa complex, and then prevents further activation of FX [22]. Antithrombin inhibits the function of thrombin, FIXa, FXa and FXIa. The anticoagulant effect of antithrombin is, however, dependent on binding to heparin sulfate on the endothelium, and thus localized to the vessel wall [23]. Thrombin is also inactivated by α2-macroglobulin and heparin cofactor II [24]. The protein C system inactivates the cofactors FV and FVIII. Protein C is activated by thrombomodulin (TM) at the endothelial cell surface. The activated protein C (APC) that is formed floats into the circulation. APC forms a complex with its cofactor Protein S to inhibit FV at a phospholipid surface. The inhibitory effect on FVIII also requires FV, as an additional cofactor [25].

1.2.4 Tertiary hemostasis: The fibrinolytic system
(Figure 3)
Plasminogen is predominantly activated by tissue-plasminogen activator (t-PA) in the circulatory system. Both plasminogen and t-PA have a high affinity to fibrin. Thus, the active form of plasminogen, plasmin, is localized to the surface of the fibrin clot. The plasmin degrades the clot and when this process is completed the free plasmin is inactivated by antiplasmin. FXIII cross-links a small amount of antiplasmin to fibrin.

Figure 2: Overview of the cell based model, reproduced with kind permission from M. Hoffman, previously published in Journal of Thrombosis and Haemostasis 2012;10:1478-1485.
which prevents early degradation of the clot. Plasminogen activator inhibitor-1 (PAI-1) regulates the activation of plasminogen by binding to t-PA [26].

![Diagram of the fibrinolytic system](image)

**Figure 3:** Overview of the fibrinolytic system, reproduced with kind permission from B. Wiman, previously published in MFR informerar, 1987.

1.3 COAGULATION ASSAYS

**Blood sampling and preparation:** The blood samples are preferably obtained with a conventional straight needle with a light tourniquet. Collection of samples from a central venous line infers a risk of contamination with heparin. Coagulation samples are collected into citrate tubes with the current recommended concentration of citrate, 0.105-0.109 mol/L (3.2%). Most coagulation assays are performed on platelet-poor plasma (PPP). To prepare PPP, single or double centrifugation steps are required within 30 minutes. PPP is divided into aliquots and snap frozen at -70 to -80°C until analysis. Platelet rich plasma is used in assays evaluating the effect from the platelets and needs to be analyzed, in general, within 2 hours [27-29].

**Platelet function tests:** Various methods are used for evaluation of platelet function for example, template bleeding time, platelet aggregometry, platelet function analyzer (PFA-100™), flow cytometry and Verify now [30].

**Routine coagulation assays:** Automated assays are available for prothrombin time /INR, APTT, fibrinogen, antithrombin and D-dimer, with results provided by the hospital laboratory within 2 hours.
Specific coagulation assays: Generally, the pro-coagulant factors: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, vWF and the anticoagulant proteins: protein C and protein S are analyzed at special coagulation laboratories.

Global coagulation assays: There are several new global tests evaluating the thrombin generation or the clot formation and aimed to provide an overall assessment of the hemostatic capacity, for example, thrombin generation (TGT), thrombelastography, aPTT waveform analysis, Clot formation and Lysis assay and Overall Hemostatic Potential [31, 32].

Selected examples below are tests of importance in this thesis

1.3.1 Template bleeding time
This method is used for the evaluation of the platelet function. A standardized incision is made with a disposable template device and the time for the blood flow to cessation is measured. Except for platelet function, the result is dependent on platelet count, level of vWF and vascular pattern. This test has a low reproducibility and is highly dependent on operator technique [33].

1.3.2 Prothrombin time/INR
This test is widely used to monitor treatment with vitamin K antagonists. There are two different methods to measure the prothrombin time (PT): the Quick method and the Owren method. The result of the Quick method is dependent on the levels of FII, FVII, FX, which are vitamin K dependent, and on FV and fibrinogen [34]. The Owren method is only dependent on the levels of FII, FVII and FX since depleted bovine plasma containing FV and fibrinogen is added to the sample prior to the analysis [35].

The result of the PT-assay can be reported in seconds or as percent of the PT activity in normal plasma (PT %). However, several different reagents and instruments are in use for measurement of PT, causing differences in results from different laboratories. To reduce these differences, the INR system was developed for reporting of PT results. Within this calibration system a correction factor, the international sensitivity index (ISI) is used. The ISI is obtained from a procedure where plasma from patients treated with vitamin K antagonists and from normal controls is analyzed with a reference reagent from WHO and with the reagent in use at the specific laboratory. The ISI and
the measured prothrombin time with the local reagent can then be used in the formula:

\[ \text{INR} = (\text{patient PT} / \text{mean normal PT})^{\text{ISI}} \]

to obtain the results in INR [36].

1.3.3 Activated partial thromboplastin time (APTT)

This is a screening test to detect reduced levels of FXI, FIX, FVIII, FX, FV, FII and fibrinogen. The test is activated by contact activation and reduced levels of FXII can also lead to a prolonged APTT. However, FXII deficiency is not associated with an increased bleeding tendency. Other causes of prolonged APTT, without bleeding symptoms, are low levels of high molecular weight kininogen and prekallikrein or circulating lupus anticoagulants and/or antiphospholipid antibodies [27].

1.3.4 The thrombin generation test

The first thrombin generation method was developed by McFarlane et al. in 1953 [37]. However, the method was considered too complicated and time-consuming to be a part of clinical coagulation testing. However, after several modifications by Hemker et al., it is now increasingly being used [38-40]. This test measures the continuous generation of thrombin in plasma. The parameters obtained from the analysis are, endogenous thrombin potential (ETP), peak height, Lag time (LT), and time to peak (TTP) (Figure 4). ETP is the area under the curve which reflects the amount of thrombin generated and is given in nanomolar thrombin per minute. Peak height corresponds to the maximum of thrombin generation and is reported in nanomolar thrombin. Lag time is the time in minutes until the thrombin generation starts. TTP is the time in minutes until the peak occurs [41].
There are different TGT methods. The fluorogenic Calibrated Automated Thrombogram (CAT) method according to Hemker (Thrombinscope BV, Maastricht, The Netherlands) has been used in most publications concerning thrombin generation. With this method, as each sample is run, calibration curves are created simultaneously using a thrombin calibrator, in order to correct for the activity of the α2-macroglobulin complex, inner filter effect and substrate consumption. Both platelet-poor plasma and platelet-rich plasma can be used for this assay as well as reagents of different tissue factor concentrations [42, 43]. Efforts are being made to deal with standardization issues regarding this method [28, 41, 44].

**Figure 4:** The different parameters of the thrombin generation in two patients with liver disease.
1.4 COAGULATION IN CHILDREN: DEVELOPMENTAL HEMOSTASIS

The coagulation system in children undergoes age-specific changes and differs from that in adults. During infancy and childhood the coagulation system seems to function well, providing a reduced risk of thrombosis without an increased risk of bleeding [45, 46]. The incidence of thrombotic events, however, is increasing due to better survival of children with severe diseases, to the use of central venous lines and to improved imaging techniques [47]. The risk of thrombosis is highest in the neonatal period and in adolescents. Studies in clinically stable children who had undergone a liver transplantation, receiving a liver from an adult donor, show that the liver graft produces coagulation factors and inhibitors according to the pediatric profile. Thus the liver itself does not regulate the plasma levels of hemostatic proteins [48]. Age-specific reference ranges for different coagulation assays in healthy children have mainly been provided by three groups: Andrew et al., Monagle et al. and recently Appel et al. [46, 49-51]. There are a few more studies that provide reference ranges in fetuses and neonates [24]. The reference ranges are specific for each combination of instrument and reagent [24, 51].

1.4.1 Primary and secondary hemostasis

**Platelets:** The platelet counts in children are similar to those in adults. Neonatal platelets are hyporesponsive compared to adult platelets. For example, they have lower expression of adhesion receptors and a weaker response to thrombin [52].

**vWF and FVIII:** The levels of vWF are high from birth until 6 months of age. The lowest levels are documented at one year of age after which they rise to adult levels. A reduced cleavage of vWF in infants, due to lower levels of ADAMTS-13, might explain the higher levels this age-group [51, 53]. The levels of FVIII were elevated in the neonatal period in one study [46]. In another study the mean FVIII value in the neonates was the same as in adults. However, a skewed distribution towards very high levels was noted [49]. In both these studies, the levels subsequently decreased and a nadir was reached between 1 month and 1 year of life, before increasing to adult levels [46, 49, 50]. A third study showed instead a gradual increase the entire childhood. However, the youngest children enrolled in the study were between 1-6 months of age [51].
**Vitamin K dependent coagulation factors and FV:** The studies demonstrate low levels of vitamin K dependent coagulation factor in the neonatal period [46, 49]. Although levels increase during childhood, adult levels are not reached until the age of 16 years. The changes in the concentration of FV during childhood show a somewhat different pattern, with levels in the age group 11-16 years, decreasing to the low levels seen in the newborns [46, 49, 51].

**Fibrinogen:** Fibrinogen is elevated in the neonatal period, decreases in children aged 1 month to 1 year, and subsequently increases with age [46]. The age at which adult levels are reached differs between the studies and is reported as 1 year, 6-10 years and 18 years, respectively [46, 50, 51].

**Specific anticoagulant proteins:** Proteins S and C are low in the neonatal period and increase during the first year. The levels of protein C nevertheless remain lower than adult levels until 16 years of age in all three studies [46, 50, 51]. The published results on the levels of protein S between the ages of 1 and 16 years are contradictory, being reported as significantly elevated [46], not significantly different [50] and significantly lower [51] compared to the levels in adults. The levels of antithrombin are low in neonates; however, from 1 month until 16 years of age the levels are increased by 10% compared to adults in two studies [45, 46]. However, the levels of antithrombin were low or equal to the levels of adults throughout childhood in the third study [51]. The low levels of antithrombin in the neonatal period might be of physiologic importance since antithrombin has anti-angiogenic properties. Conversely, another important inhibitor of thrombin, A-2 macroglobulin, is present at high levels in neonates [24].

**INR:** The Quick method has been used to obtain the reference ranges regarding INR in children. INR is elevated in the youngest children and might be slightly elevated throughout childhood [46].

**APTT:** APTT is increased in the neonatal period, probably due to increased levels of the contact activators including FXII, FXI, high molecular weight protein and kininogen [24]. Transient antiphospholipid antibodies are also more common in children, which can prolong the test results [54].
TGT: A few reports on reference ranges in children have been published, showing that ETP increases with age [46, 55, 56]. However, several different reagents and instruments are used, and these influence the results. The CAT method is the method that has come closest to standardization. This method was used in a study by Tripodi et al., who found that neonates have the same results on the TGT as adults if thrombomodulin was added to the tests for both groups [57]. The plasma levels of thrombomodulin are increased in the neonatal period [24].

1.4.2 Tertiary hemostasis

Neonates have a reduced fibrinolysis due to low levels of plasminogen and PAI-1. Low levels of t-PA are also seen [58].
1.5 COAGULATION IN LIVER DISEASE

1.5.1 Bleeding symptoms

Bleeding symptoms are common in children with chronic liver disease, especially from the mucosal membranes, including nosebleeds and gastrointestinal hemorrhage [13, 59-61]. Life-threatening bleeding episodes may also occur in other sites, such as intracranially [62]. Varicose veins develop secondary to portal hypertension and about 40% of children with biliary atresia, without liver transplant, have had bleeding from esophageal varices by the age of 5 years [13]. These bleeding episodes can be life-threatening and a mortality of 50% within 4 months has been described in children with recurrent variceal bleeding in combination with biliary atresia if bilirubin was above 10 mg/dL [59].

The most important factor for the development of a bleeding from varicose veins is the increase in portal pressure [14]. There is also an association between bacterial infections and episodes of bleeding from varicose veins in patients with cirrhosis [63]. A heparin-like effect secondary to the infection has been suggested as the mechanism [64]. Randomized trials have shown that antibiotic prophylaxis prevents rebleeding [65, 66].

Patients with liver disease often undergo various invasive procedures, of which liver biopsy and central line placement are the most common in pediatric patients. In a recent evaluation of risk factors for bleeding with ultrasound-guided liver biopsy, a drop in hemoglobin count of more than 2.0 g/dL was recorded in 1.5% of the 275 biopsies performed [67]. In the study, prophylactic treatment was given to all patients with INR >1.7 and/or platelet count below 70×10⁹ cells/L in the form of platelet concentrates, prothrombin complex concentrates (PCC) and/or plasma. From other studies, major complication rates of 1-4.6% have been reported in pediatric patients undergoing liver biopsy [68-70]. Also in these studies, the patients were given pretreatment to correct of coagulopathies and platelet counts. Increased complication rates have been shown in infants, children with acute liver failure, focal lesion, malignancy, previous bone marrow transplantation, and those treated with low molecular weight heparin (LMWH) [67, 71-73].
1.5.2 Thrombotic symptoms

The most common thrombotic events in children with liver disease are portal vein thromboses (PVT) which can cause portal hypertension and also complicate a liver transplantation. The prevalence of PVT in children undergoing liver transplantation is 3.7-10% and the condition may be associated with a higher post-transplant mortality [74, 75]. The prevalence of PVT in cirrhotic adult patients without malignancy is 10-20% [76]. A reduced portal flow velocity has been shown to be important for the development of PVT in patients with cirrhosis [77]. The incidence of venous thromboembolism in adult chronic liver disease is reported to be 0.5-6.3% as compared to 4-15% in hospitalized patients with medical disorders [78]. Corresponding studies regarding the incidence of VTE in children with liver disease are lacking.

Several studies in both animal models and in patients with liver disease have shown an increased progression to cirrhosis in patients with hypercoagulability. This association has mostly been described in patients with chronic viral hepatitis and thrombotic risk factors including FV Leiden, reduced levels of protein C, antithrombin and fibrinogen and/or increased levels of FVIII or homocysteine [79]. Conversely, patients with hemophilia and chronic hepatitis C have a slower progression of liver fibrosis [80]. The most predominant theory behind this increased hepatic fibrinogenesis is the ”direct stellate activation”. This model focuses on thrombin- or FXa-mediated activation of hepatic stellate cells leading to secretion of extracellular matrix proteins and fibrogenesis. Increased thrombin generation in the circulation in patients with cirrhosis and hypercoagulopathy might enhance this activation [81]. The potential use of anticoagulant treatment to reduce fibrinogenesis has been investigated in several animal studies with encouraging results [79]. One study of 34 patients (age 14-70 years) with chronic hepatitis B who were treated with heparin/LMWH for 3 weeks showed a treatment-related reduction in collagen fibrils in liver biopsies [82]. A study evaluating use of vitamin K antagonists to prevent fibrosis in patients with hepatitis C is ongoing [83].
Veno-occlusive disease (VOD): This severe condition is also called sinusoidal obstructive syndrome or SOS. It is a xenobiotic-induced liver injury predominantly occurring after hematopoietic stem-cell transplantation. The condition is more common in children than adults and causes jaundice, painful hepatomegaly, ascites, weight gain within 30 days after transplantation [84]. The pathogenesis includes a toxic effect by chemotherapy on the sinusoids of the liver, causing swelling and hypoxia. Disruption of the sinusoidal endothelial lining leads to accumulation of cell debris in the venous veins. Furthermore, fibrin deposition within sinusoids and veins causes microthrombosis and fibrosis. Subsequently, the congestion of the liver causes portal hypertension [85]. In patients who develop a secondary multi-organ failure, mortality is more than 85% [86]. An upregulation of thrombomodulin, TFPI, PAI-1 P-selectin and soluble tissue factor is documented in VOD. The association with hereditary prothrombotic factors it not clarified, but prothrombin gene 20210G-A-mutation and Factor V Leiden may be associated [85].

1.5.3 Coagulation mechanisms associated with bleeding

Primary hemostasis:

Reduced platelet count/platelet dysfunction: Thrombocytopenia (platelet count below 150 x10^9/L occurs frequently in liver disease [18, 87]. The most common explanation has been a sequestration of platelets in the spleen secondary to portal hypertension [88]. However, there is no correlation between platelet count, portal pressure and the spleen size. Kinetic studies using radiolabelled platelets in kinetic studies show that platelets seem to be destroyed in the spleen instead of just being pooled [87]. Increased destruction of platelets has also been attributed to platelet associated antibodies, especially in hepatitis C, and to endotoxemia with or without disseminated intravascular coagulation [18]. Other causes of thrombocytopenia are decreased platelet production due to bone marrow suppression and changed metabolism of the platelet growth factor thrombopoietin (TPO) which is synthesized in the liver [18]. Reduced levels of TPO have been shown in children with cirrhosis and thrombocytopenia [89]. However, in general, the results from different studies concerning TPO in liver disease are contradictory and the laboratory assays are not
standardized. A large trial to evaluate use of a TPO-receptor agonist (eltrombopag) before procedures in cirrhotic patients with thrombocytopenia showed a reduced need for platelet transfusions but had to be closed due to an increased incidence of portal vein thrombosis [90]. Platelet dysfunction is common in liver cirrhosis according to platelet aggregation tests and template bleeding time [91, 92]. Both these tests are influenced by the number of platelets.

**Secondary hemostasis:**

**Reduced levels of specific coagulation factors:** Most of the coagulation factors are produced in the liver and the levels of FII, FV, FVII, FIX, FX and FXI fall as parenchymal cell damage increases [93]. In patients with concomitant vitamin K deficiency a further reduction of the vitamin K dependent factors (FII, FVII, FIX and FX) is seen secondary to impaired $\gamma$-carboxylation, especially in cholestatic liver disease [94].

**Decrease in fibrinogen:** Fibrinogen is also synthesized in the liver and the levels are often reduced in patients with moderate to severe liver disease [93]. Dysfunctional forms of fibrinogen have been described in liver disease [95].

**Elevated INR:** Prothrombin time is reported according to INR and is traditionally used to evaluate of the risk of bleeding as well as for prognostic purposes in liver disease. PT/INR is also widely used to monitor VKA treatment and the INR system to report the prothrombin time was developed specifically for this group of patient. Several studies have shown that when the Quick method is used on plasma from patients with liver disease, the results in INR are highly variable [96-99]. To overcome this problem an alternative calibration has been proposed where plasma from patients with liver disease is used to obtain an ISI specific for this patient group, ISI_{liver} [100, 101]. However, this system has never been taken to clinical practice [102].

**APTT:** This test can be normal or prolonged in liver disease. A prolongation may reflect reduced plasma levels of coagulation factors or circulating antiphospholipid antibodies or lupus anticoagulants [103].
**Tertiary hemostasis:**

**Hyperfibrinolysis:** Thrombin-activatable fibrinolysis inhibitor (TAFI), alpha2-antiplasmin and FXIII are synthesized in the liver and their levels are reduced in liver cirrhosis. Tissue-plasminogen activator (t-PA) is often elevated due to decreased clearance in the liver [104].

1.5.4 Coagulation mechanisms associated with thrombosis

**Primary and secondary hemostasis:**

**Platelet dysfunction:** Recent data from flow cytometry, platelet-monocyte aggregate analysis and measurement of soluble P-selectin is consistent with hyperactivation of platelets [18]. In a mouse model, P-selectin mediated platelet aggregation in liver sinusoids was shown to contribute to a large extent to development of liver injury in cholestasis [105].

**Increased levels of specific coagulation factors:** The levels of von Willebrand factor (vWF) and FVIII are often elevated in liver disease [93]. vWF is synthesized by endothelial cells and megakaryocytes. It is stored in endothelial cells and platelets and circulates in plasma with FVIII as a carrier protein [106]. Both vWF and FVIII are acute-inflammatory proteins [107]. Increased levels of vWF in liver cirrhosis have been linked to endothelial perturbation induced by endotoxemia and to an increased vWF expression in the vasculature of portal areas and sinusoidal endothelial cells in the liver [108, 109]. Reduced clearance of vWF secondary to decreased activity of the cleavage protease ADAMTS-13, has also been discussed as a possible cause [110, 111]. vWF was recently shown to correlate with the portal pressure [112]. The very high vWF levels in liver disease may compensate for both a reduced function of the vWF molecule as well as for thrombocytopenia and platelet function defects in the primary hemostasis [111]. FVIII is expressed in sinusoidal endothelial cells in the liver, and also in kidney, spleen and lungs. The elevated levels of FVIII in liver disease seem to be related to prolonged half-life secondary to the increased levels of vWF and reduced clearance in the liver [109]. Further, increased release of FVIII from storage sites and enhanced secretion of FVIII from FVIII-producing cells might contribute to the elevated levels [110].
**Increased levels of fibrinogen:** Fibrinogen is an acute-phase protein elevated in inflammatory conditions and can thus be increased in patients with liver disease in an inflammation state [113, 114]. A study in human hepatoma cells has demonstrated that activation of the FXR receptor can induce fibrinogen expression. Since bile acids are known to activate these receptors this could suggest a link between bile acids and fibrinogen synthesis [115].

**Reduced levels of specific anticoagulant proteins:** The levels of antithrombin, protein S and protein C are reduced in liver disease as a result of diminished hepatocellular synthesis. Protein S and protein C are also vitamin K dependent and require vitamin K to undergo γ-carboxylation, necessary for proper physiologic function [107].

**Tertiary hemostasis:**

**Hypofibrinolysis:** The level of plasminogen is low in liver disease due to reduced synthesis in the liver. Plasminogen activator inhibitor 1 (PAI-1) acts as an acute-phase protein and can be elevated in acute liver failure [116, 117].

1.5.5 Global assays in liver disease

**Thrombin generation test (TGT):** Tripodi et al. evaluated thrombin generation with the CAT method in adult patients with cirrhosis and showed that the ETP was lower in cirrhotic patients than in controls. However, when thrombomodulin, which activates the protein C system, was added to the assay (TGT-thrombomodulin) there was no significant difference between the groups. This study was performed on platelet–free plasma with a tissue factor concentration of 1 pmol/L [118]. Adult patients with acute liver failure (ALF) have been evaluated with a similar protocol with corresponding results [117]. Further, another study enrolling patients with AFL showed higher ETP with TGT-thrombomodulin, than in controls, indicating a hypercoagulable state [119]. When platelet-rich plasma from patients with cirrhosis was analyzed with a TGT-thrombomodulin assay, ETP correlated with the platelet count in the patients and the ETP in the patients was lower than in the controls. The platelet count is thus important for the result of the TGT in these patients [120]. Neither treatment with fresh frozen plasma (FFP) corresponding to 10 ml/kg nor transfusion of a standard dose of platelets in patients with liver disease affect the TGT result [121]. In summary, the results of the TGT in acute or chronic liver disease are highly dependent on whether
thrombomodulin is added to the test and if platelet-rich or platelet-poor plasma has been used.

**Thrombelastography**: In this assay, the level of fibrinogen and the platelet count are strongly associated with the measured clot strength [122, 123]. The assay is fast and it is used together with standardized protocols to guide the clinician in the use of coagulation factor concentrates, transfusions and antifibrinolytic therapy during liver transplantations [124, 125]. In a study, enrolling patients with acute liver failure heterogeneous results of the assay were obtained [119]. Hypercoagulability has been detected by thromboelastography in adult patients with primary biliary cirrhosis and primary sclerosing cholangitis [126].
1.5.6 The concept of a balanced coagulation system
The coagulation defects in liver disease, can thus affect both pro- and anticoagulant mechanisms. Historically, most focus was directed to prolonged prothrombin time /reduction in INR and the bleeding risk. During the recent years, however, evidence has been evolving supporting that the balance between pro- and anticoagulant mechanisms is maintained in liver disease, even though the levels differ from those seen in healthy individuals [127, 128]. As early as 1981, Ewe et al. reported that the duration of the bleeding after liver biopsies did not correlate with the prothrombin time, the platelet count or whole blood clot time. In the study, 200 adult patients with various liver diseases, including 21 cirrhotic patients, were enrolled. The liver biopsies were performed in the context of a laparoscopic procedure during which postoperative bleeding could be inspected visually [129]. De Boer et al. reported in 2005 that several transplant centers performed up to 30% of the liver transplantations without any blood transfusions [130]. This was supported by the thrombin generation tests performed by Tripodi et al. the same year, showing normal thrombin generation in patients with cirrhosis after addition of thrombomodulin [118]. However, the balance between the pro- and anticoagulant systems seems to be instable and can easily tip towards either bleeding or thrombosis [131].
1.6 COAGULATION FACTORS AS PROGNOSTIC MARKERS

**Coagulation tests:** Coagulation analyses are used as liver function tests and prognostic markers, since the levels of the coagulation factors, in general, decrease as the synthetic function of the liver deteriorates. FV is used in France as a prognostic marker in liver disease [132]. FV has a relatively long half-life, around 12 hours, to compare with FVII which has a half-life of only 5-6 hours [133]. Thus FVII has an advantage as a prognostic marker in acute liver failure [134]. The level of FVII has a large impact on the INR result and INR is the coagulation test most frequently used for prognostic purposes. INR is also included in the King’s College criteria for acute fulminant liver failure [135].

**Scoring systems:** The most well established scoring systems for prognostic evaluation in liver disease are the Child-Turcotte-Pugh (CTP)-, MELD- and PELD scores which all include INR. The CTP was mainly intended for cirrhotic patients undergoing surgery but is also used for liver graft allocation before liver transplantation [136]. The CTP classification is based on INR, bilirubin, albumin and also the clinical parameters, degree of encephalopathy and ascites. The result is given as CTP class A, B or C of which class C reflects the most severe condition. The CTP score is not used in children.

Since the evaluation of the clinical parameters in the score can diverge between different physicians a new scoring system, the model of end stage liver disease (MELD) was developed in 2000 [137]. This is used for patients with chronic liver disease, above 12 years of age and patients with MELD above 15 are prioritized for liver transplantation [138]. MELD is calculated according to:

\[
MELD\ \text{Score} = 10 \times (0.957 \times \log_e(\text{creatinine\ mg/dL}) + 0.378 \times \log_e(\text{bilirubin mg/dL}) + 1.120 \times \log_e(\text{INR}) + 0.643).
\]

McDiarmid et al. developed the Pediatric end stage liver disease score (PELD) for children below 12 years of age [139]. PELD score is calculated as:

\[
PELD\ \text{Score} = 10 \times (0.480 \times \log_e(\text{bilirubin\ mg/dL})+1.857 \times \log_e(\text{INR}) -0.687 \times \log_e(\text{albumin\ g/dL})+0.436 \text{ if } < 1\ \text{ yrs}+ 0.667 \text{ if growth failure (<2\ SD)}).
\]

The average PELD score at time of liver transplantation was 11.5 for those patients where the PELD result was utilized to determine liver allocation, in the USA 2003-2004 [140].
The difference in INR between different laboratories using the Quick method, has led to concomitant differences in MELD scores between different hospitals [96, 97]. The effect of interlaboratory variation in INR on the PELD score has not been evaluated.
1.7 PRODUCTS WITH EFFECT ON HEMOSTASIS

**Pro-hemostatic treatment used in liver disease**

1.7.1 Vitamin K1

(Fytonenadion, Konakion Novum, Roche, Basel, Switzerland). Vitamin K is necessary for the \(\gamma\)-carboxylation of vitamin K dependent coagulation factors including FII, FVII, FIX and FX as well as the anti-coagulant factors Protein C and Protein S [141]. It is used to treat vitamin K deficiency and is given to newborns worldwide to prevent vitamin K deficiency bleeding (VKDB) [142]. Another indication is to reverse vitamin K antagonist treatment. The levels of the vitamin K dependent factors increase more rapidly after the treatment in children as than in adults and an effect on INR is often obtained within one hour in infants [142, 143]. Vitamin K can be administered orally, intramuscularly or intravenously. In patients whose vitamin K deficiency is associated with cholestasis or whose gut mucosa is affected, the intestinal absorption is reduced and intravenous or intramuscular treatment may be necessary [144].

1.7.2 Desmopressin

(Octostim, Ferring Pharmaceuticals, Saint-Prex, Switzerland) is a synthetic vasopressin analogue which selectively increases release of endogenous vWF from the endothelium. This leads to a secondary increase in FVIII levels [145]. Desmopressin can also improve the platelet adhesion by enhancing expression of glycoprotein Ib on the platelet membrane [146]. The main indications are mild von Willebrand’s disease, mild hemophilia type A and platelet function defects. It is also used as prophylaxis prior to liver biopsy in patients with platelet defects, as indicated by a prolonged template bleeding time [147, 148]. Desmopressin was shown to be effective in cirrhotic patients undergoing dental extraction [149]. However, it has not been shown to be efficient in the treatment of bleeding from varicose veins [150]. Desmopressin activates the fibrinolytic system through an increase of t-PA and is often combined with tranexamic acid to reduce this effect [151]. Further, desmopressin, acting as antidiuretic hormone, may lead to hyponatremia and fluid retention, especially in young children, thus fluid overload needs to be prevented [152].
1.7.3 Tranexamic acid
(Cyklokapron, Meda, Solna, Sweden) inhibits the fibrinolytic system by inhibiting the conversion of plasminogen to plasmin [153]. Tranexamic acid is used in hyperfibrinolytic conditions and in several different bleeding disorders including hemophilia, platelet defects and von Willebrands disease to prevent premature dissolution of the clot. Tranexamic acid reduces blood loss and the need for transfusion in elective surgery including major pediatric surgery [154, 155]. It has been used increasingly to counteract massive bleeding in recent years after the CRASH-2 study showed that it reduced mortality when used in adult trauma patients [156]. Randomized clinical trials are lacking concerning anti-fibrinolytic agents in upper gastrointestinal bleeding in patients with liver disease [157]. A Cochrane review on six randomized trial regarding tranexamic acid in liver transplantation states that no effect is seen from tranexamic acid on mortality, graft failure, blood loss or blood products used, compared to the control groups [158].

1.7.4 Fibrinogen concentrate
(Riastap, CSL Behring, Marburg, Germany) is derived from plasma and used to increase the fibrinogen levels in patients with congenital or acquired fibrinogen deficiency [159]. It is increasingly used in the surgical setting, including liver transplantation, to reduce the need for transfusion. Thromboelastography-guided administration is often used [160, 161]. The blood product cryoprecipitate, containing fibrinogen, is used in countries where this concentrate is unavailable.

1.7.5 Recombinant activated Factor VII (rFVIIa)
(NovoSeven, Novo Nordisk A/S, Bagsvaerd, Denmark) is recombinant coagulation factor VII in its active form. It has a localized effect in the activation of FX on the platelet [20]. It is often referred to as a “bypassing agent” since it bypasses most of the coagulation cascade including the FVIIIa-FIXa complex and acts at the end of the cascade. rFVIIa reduces the INR level since it has a major impact on the INR assay. The best way to monitor the hemostatic effect of the treatment is still unknown [20, 162]. rFVIIa has a shorter half-life in children than in adults [163]. rFVIIa was originally developed for patients with hemophilia and acquired inhibitors against exogenously administered FVIII or FIX [164]. Other indications are Factor VII deficiency and a severe form of platelet function disorder, Glanzman thrombastenia [165, 166]. It has, however, been tried in a variety of patient groups outside the
original indication (“off-label use”) to prevent or control active bleeding. The risk of arterial thromboembolic events is increased in elderly patients receiving rFVII on an “off-label” basis [167].

**Prophylactic treatment with rFVIIa in liver disease (Table 1):** Six studies have been published regarding prophylactic treatment with rFVIIa in patients undergoing hepatic procedures. The first was performed by Jeffers et al. in 2002 as rFVIIa was evaluated in laparoscopic liver biopsy in patients with cirrhosis and coagulopathy. The majority responded with a normalization of prothrombin time and 74% achieved hemostasis within 10 minutes after the biopsy [168]. Two placebo controlled randomized trials in patients undergoing partial hepatectomy did not show any effect on transfusion requirements [169, 170]. Further, three studies evaluated rFVIIa in liver transplantation [171-173]. Efficacy was shown in two of them i.e. a reduction in the number of patients receiving RBC transfusion and a reduction in blood loss, respectively [171, 173].

**Therapeutic trials with rFVIIa in liver disease (Table 1):** Two double blind, placebo controlled trials have been performed in patients with cirrhosis and upper gastrointestinal hemorrhage [174, 175]. The results of the first study showed no overall benefit from rFVIIa; however, a subgroup analysis of patients with CTP class B or C it showed that the number of patients who failed to control bleeding was reduced in the group given rFVIIa [174]. Thus, a second study in patients with CTP class B or C was performed, but no difference was confirmed regarding the primary composite endpoint (failure to control 24-hour bleeding, or failure to prevent rebleeding or death at day 5) between the patients receiving rFVIIa compared to those receiving placebo.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Patients Number+ diagnosis</th>
<th>Indication</th>
<th>rFVIIa Doses</th>
<th>Results</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeffers 2002</td>
<td>Double-blind RCT No placebo</td>
<td>66 patients Cirrhosis</td>
<td>Laparoscopic liver biopsy</td>
<td>5-120 µg/kg</td>
<td>Effect on INR and in 74% on visible bleeding</td>
<td>[168]</td>
</tr>
<tr>
<td>Lodge 2005</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>204 patients Liver tumor</td>
<td>Partial hepatectomy</td>
<td>20-80 µg/kg</td>
<td>No effect on transfusion requirement/blood loss</td>
<td>[169]</td>
</tr>
<tr>
<td>Shao 2005</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>235 patients Liver tumor + cirrhosis</td>
<td>Partial hepatectomy</td>
<td>50-100 µg/kg x 1-4</td>
<td>No effect on transfusion requirement</td>
<td>[170]</td>
</tr>
<tr>
<td>Lodge 2005</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>209 patients ESLD² + cirrhosis</td>
<td>Liver tx³</td>
<td>60-120 µg/kg x 3</td>
<td>Effect on number of patients requiring transfusion</td>
<td>[171]</td>
</tr>
<tr>
<td>Planinsic 2005</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>87 patients ESLD</td>
<td>Liver tx</td>
<td>20-80 µg/kg</td>
<td>No effect on transfusion requirement</td>
<td>[172]</td>
</tr>
<tr>
<td>Pugliese 2007</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>20 patients ESLD</td>
<td>Liver tx</td>
<td>40 µg/kg</td>
<td>Reduction in blood loss</td>
<td>[173]</td>
</tr>
<tr>
<td>Bosch 2004</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>245 patients Cirrhosis</td>
<td>Upper GI³-bleeding</td>
<td>100 µg/kg</td>
<td>Effect in subgroup CTP B-C on failure to control bleeding</td>
<td>[174]</td>
</tr>
<tr>
<td>Bosch 2008</td>
<td>Double-blind, placebo-controlled RCT</td>
<td>265 patients Cirrhosis CTP B-C</td>
<td>Upper GI-bleeding</td>
<td>200 µg/kg + 100 µg/kg x1-4</td>
<td>No effect</td>
<td>[175]</td>
</tr>
</tbody>
</table>

² ESLD = end-stage liver disease
³ Tx = transplantation
⁴ GI = gastrointestinal
rFVIIa in pediatric patients

There are no randomized controlled trials in pediatric patients with liver diseases. Most of the published data on off-label rFVIIa use in children are case reports or reports from single centers or pooled data from registers [60, 176]. Alten et al. have reported the experience from the Childrens Hospital of Alabama 1999-2006, where 135 patients were treated with 997 doses of rFVIIa [177]. The median dose was 88 µg/kg (27-160 µg/kg) and the patients had various conditions including DIC/sepsis, surgical bleeding, procedure prophylaxis, trauma, intracranial bleed etc. Thirty-six patients had hepatic dysfunction, 12 patients received rFVIIa on indication liver failure, 16 as procedure prophylaxis and 4 due to gastrointestinal bleeding. However data concerning these patients’ response to treatment are not presented in detail or separately discussed in the paper. Overall, it was stated that rFVIIa is associated with a significant decrease in blood transfusions when given to treat bleeding and that it may be effective as prophylaxis before invasive procedures. The authors express concern regarding the use of rFVIIa in children with disseminated intravascular coagulation due to high mortality in this group. Three patients developed thromboembolic complications.

One large registry study from the Haemostasis Registry in Australia and New Zealand included 388 pediatric patients during 2003-2009, half of whom received rFVIIa in cardiac surgery [176]. Other clinical contexts were medical, other surgery than cardiac, hematology/oncology, trauma or intracranial hemorrhage. Only 2.8% received rFVIIa in association with liver disease. The median doses used were 114 µg/kg (range 7-2250 µg/kg). The rate of thromboembolic events was 5.4%. The overall subjective estimate was that 82% showed a response in terms of bleeding and a significant reduction in the volume of transfused blood products 24 hours after treatment was shown. There was no association between the dose of rFVIIa and response or thromboembolic event.

In the published case reports regarding rFVIIa in children with liver disease outcome data have shown a response rate between 54-100%, with a dose range of 26-300 µg/kg [60, 178-183].
1.7.6 Prothrombin complex concentrates (PCC)
(Ocplex, Octapharma, Lachen, Switzerland and Confidex, CSL Behring, Marburg, Germany). The PCCs in use in Sweden contain the vitamin K-dependent coagulation factors FII, FVII, FIX, FX and inhibitors protein S and protein C and are referred to as 4-factor PCC. Other PCC-products may contain a different combination of factors and inhibitors. PCCs available in the US contain very little or no FVII (3-factor PCC) [184]. PCC products are derived from plasma and are mostly used to reverse vitamin K antagonist treatment [185]. The other indications are bleeding or prophylaxis prior to surgery in patients with acquired or congenital deficiency of vitamin K dependent factors. There is limited information concerning its use in patients with liver disease. In an uncontrolled non-randomized trial, Lorenz et al. showed good efficacy of PCC in combination with endoscopic treatment in three patients with gastrointestinal bleeding and in 19 patients as prophylaxis prior to different invasive procedures. The product used did not contain protein S and additional treatment with antithrombin was used in five patients [186]. Data regarding treatment with PCC in children without congenital coagulation deficiencies are very limited and studies in pediatric patients with liver disease are lacking [187, 188].

1.7.7 Octreotide
(Sandostatin, Novartis, Basel, Switzerland) is not a hemostatic agent. It reduces portal pressure by lowering splanchnic vascular tone. A significant effect against variceal bleeding from has been proven in adults [189]. Octreotide has been evaluated in children with acute gastrointestinal bleeding and has shown good efficacy and safety. In a retrospective trial by Eroglu et al., the response rate was 71%; however, 52% of the children experienced rebleeding. It is thus recommended to consider a combination of pharmacologic treatment and endoscopic intervention [190, 191]. There are other similar products on the market such as terlipressin but octreotide is the one most often used in the pediatric setting.
Anti-coagulant treatment

1.7.8 Antithrombin
(Atenativ, Octapharma, Lachen, Switzerland, and Antithrombin III, Baxter, Chicago, USA) is used to elevate the levels of the anticoagulant protein antithrombin in patients with congenital or acquired antithrombin deficiency. This product is increasingly used in infants and children with complex conditions, often in the intensive care setting, without congenital antithrombin deficiency [192]. The evidence for this treatment is still limited, though. Hardikar et al. evaluated the use of antithrombin concentrate in combination with fresh frozen plasma (FFP) and heparin postoperatively after liver transplantation in children. The purpose was to counter-balance the hypercoagulable condition that has been demonstrated in children during the early post-liver-transplant phase. A reduction in both thrombotic events and major bleeding was noted compared to historic controls [193].

1.7.9 Low-molecular-weight heparin (LMWH)
(Fragmin, Pfizer, New York, USA) potentiates the inhibitory effect of antithrombin on FXa and thrombin. It is routinely used as anticoagulant treatment in thromboembolic conditions in children [194]. The use of LMWH in cirrhotic patients with portal vein thrombosis or VTE seems safe and effective [195, 196]. Cirrhotic patients show an increased response to LMWH as measured by TGT [197]. Treatment with LMWH the day before percutaneous liver biopsy is a risk factor for bleeding complications in children [67]. The potential role of LMWH as an anti-fibrotic agent needs to be studied further [82].

1.7.10 Defibrotide
(Gentium SpA, Villa Guardia, Italy) is a polydeoxyribonucleotide which acts at the endothelium. Different studies have shown several effects on the endothelium, including increased t-PA expression, reduced levels of PAI-1, inhibitory effect on tissue factor and a reduced expression of vWF [84]. The product is not yet approved and clinical trials are ongoing. A recent large open-label randomized controlled trial regarding prophylactic treatment with defibrotide in pediatric hemopoietic stem cell transplantation showed a reduction in the incidence of VOD without any increase in bleeding complications [86]. Clinical reports, small trials and a phase II trial on treatment effects for severe VOD are also promising [84, 85]. Whether this substance
can have an impact in other types of liver disease or reduce fibrosis remains to be studied.

**Transfusion therapy**

1.7.11 Fresh frozen plasma (FFP)

FFP contains the coagulation factors and inhibitors that normally occur in plasma. FVIII is, however, a heat labile protein and the levels are reduced by 24% within 24 hours after the plasma has been thawed [198]. In a large retrospective epidemiologic study assessing the use of FFP in hospitalized children in the US, FFP was given in 2.85% of the admissions [199]. That pediatric patients benefit from treatment with FFP has only been proven in the context of heart surgery, where patients who received FFP had a shorter stay in the intensive care unit compared to patients who received whole blood [200]. Randomized controlled trials in children regarding either massive hemorrhage or liver disease are missing. A retrospective analysis of 243 children undergoing liver transplantation at a single center showed that perioperative transfusion of FFP and red blood cell concentrates (RBC) were independent risk factors for patient and graft survival one year after the surgery [201].

A volume of 1 ml fresh frozen plasma/ kg will theoretically increase the level of, for example the vitamin K dependent factors by 1 U/dL or 1% of normal adult level and 10-15 ml FFP/kg is often administered in children [202]. The response to this treatment is, however, unpredictable, especially if the patient is bleeding. If the goal of the transfusion is to correct a coagulopathy much larger volumes might be needed [203]. Giving large volumes of plasma may, on the other hand, worsen the portal hypertension of a patient who has varicose veins and thus further, increase the bleeding, or provoke a bleeding in a stable patient [204]. Another complication that can occur when blood products are given at a high infusion rate or in large volumes is transfusion-associated circulatory overload (TACO). This condition has been described in adult patients and includes acute onset of dyspnea, hypertension, tachypnea and tachycardia, congestive heart and increase in brain-type natriuretic peptide. The condition is associated with high mortality [205]. In the expert pediatric opinion on the report from the Baveno V Consensus Workshop, the advice is to use plasma in acute variceal hemorrhage as general supportive care, but not with the goal of correcting clotting abnormalities [206]. There are several other adverse events associated with plasma transfusions, diagnosed in both adults and children. One is transfusion related acute lung injury (TRALI). In
TRALI the acute lung injury or acute respiratory distress syndrome (ARDS) occurs within 6 hours after transfusion [207]. The risk of transfusion-transmitted infections including hepatitis B, hepatitis C and HIV is minimal nowadays, but there is a potential risk from emerging infections [208].

1.7.12 Platelet transfusion
Platelet transfusion is used in patients with low platelet count and/or platelet defects, as prophylaxis prior to invasive procedures and in bleeding states. The British Committee for Standards in Haematology as well as the American Association for the Study of Liver disease (AASLD) have recommended a platelet count above 50-60 x 10⁹ prior to liver biopsy [209, 210]. These recommendations are mainly based on expert opinions and are also applied in pediatric literature [211]. However, a standard platelet dose in adult cirrhotic patients with thrombocytopenia did not increase the TGT result and the effect on thromboelastography was limited [212]. In patients with massive bleeding, higher ratios of platelets to red blood cell concentrates (RBC) have been recommended the past few years based on studies in trauma patients [213]. Similar guidelines are being discussed for pediatric patients [214]. An association between platelet transfusions during liver transplantation and increased mortality has been shown in adult, but not in pediatric patients [201, 215]. Data regarding the use of platelets in patients with liver disease are thus contradictory. Both TRALI and bacterial infections have been documented in patients who received platelet transfusions [207, 215].

1.7.13 Red Blood Cell transfusions (RBC)
An adequate hematocrit is important for oxygenation but also to drive the platelets towards the vessel wall, to be able to respond to a injury [216]. Overtreatment with blood transfusions may lead to volume overload and increased bleeding. A restrictive transfusion policy is regarded as safe and superior to a more liberal one and is recommended also in pediatric patients with bleeding from varices [206, 217]. The expert pediatric opinion on the report from the Baveno V Consensus Workshop recommends a target hemoglobin level between 7 and 8 g/dL [206]. An association between RBC transfusions and increased morbidity and mortality in adult patients undergoing liver transplantation has been described [218].
2  AIM

The overall objectives of the first part of the thesis were to improve the treatment and the analysis of coagulation defects in pediatric liver disease. The specific aims of the separate studies were to:

1. investigate the clinical and biochemical effects of rFVIIa in the treatment of children with liver disease suffering from life threatening bleeding or as prophylaxis before invasive procedures.

2. evaluate the value of thrombin generation test in addition to routine coagulation assays in children with liver disease compared to normal controls.

   evaluate the difference regarding thrombin generation test and routine coagulation tests between patients with standard bleeding risk versus those with increased risk.

   evaluate the effect on thrombin generation test of pro-hemostatic treatment in connection with liver disease.

The overall objectives of the second part of the thesis were to improve the prognostic evaluation in liver disease. The specific aims of the separate studies were to:

3. investigate if there is a correlation between fasting serum bile acids and coagulation factor levels in children with chronic liver disease.

   investigate whether increased levels of bile acids could influence the measurement of coagulation factor levels.

4. assess the Owren-based INR reagents that are used in Scandinavia for analyzing INR values in patients with liver disease.

   determine the international sensitivity index (ISI) for these reagents in patients with liver disease and in patients on vitamin K antagonist treatment.
3 METHOD

3.1 ETHICS
The Ethics committees of the Karolinska Institute approved all the study protocols. The Ethics committee of Linköping University approved the protocols concerning enrollment of the patients at Linköping University Hospital in Study 4. Informed consent was obtained from all patients and the children who served as normal healthy controls and/or their parents.

3.2 PATIENTS (ALL STUDIES)
Patients at the tertiary center for pediatric hepatology at Karolinska University Hospital were enrolled in study 1-3. In study 4 we recruited adult patients, instead of children, due to the required larger sample volume. These patients were included at the Departments of Gastroenterology & Hepatology at Karolinska University Hospital and Linköping University Hospital.

**Age:** The mean age in years and age-range of the patients in the different studies were:
- Study 1, 6.8(0.2-15.9)
- Study 2, 13.4(1.2-20.5)
- Study 3, 7.7(0.2-18.8)
- Study 4, 57.8(16.6-80.0)

**Diagnoses:** In Study 1-3 the predominant groups of liver diseases were: abnormal development, inborn errors of metabolism and immune mediated liver diseases. In Study 4 the predominant groups of liver diseases were: infection and xenobiotic – induced liver disease (alcohol induced liver cirrhosis).

**Prognostic scoring systems:** The PELD scores in Study 3 were in median 7 (range 1-11:22). In Study 4 the MELD score was in median 16 (range 8-40) and the number of patients with CTP class A was 15, class B 16 and class C 30 patients.

**Normal healthy controls:** The children who served as healthy normal controls in Study 2 were patients planned for ear, nose or throat surgery at the ENT department of the same hospital. In study 4 anonymous plasma samples from healthy controls and redundant plasma from patients on oral anticoagulation were used for the ISI-determination. These samples were collected at Linköping University Hospital for the purpose of calibrating hospital laboratory instruments.
3.3 METHODS (ALL STUDIES)

**Laboratory assays:**

**Setting:** In Study 1-3 the laboratory assays were performed at Karolinska University Laboratory in study 1-3. The different hospital laboratories involved in Study 4 are shown in Table 2.

**Blood collection:** Blood was collected into citrated vacutainer tubes and centrifuged at 2000x g for 15 minutes prior to all coagulation assays in study 1 and 3-4. In study 2, the blood samples were double centrifuged at 2000x g for 15 minutes prior to the specific coagulation assays and the thrombin generation assay. The citrate concentration in the tubes was 0.129 mol/L trisodium citrate in study 1, 3 and the assessment study of study 4. The additional samples taken for the ISI project of Study 4 as well as in study 2, were collected in vacutainer tubes with 0.105 mol/L, due to an overall change in the Swedish laboratory standard in order to adhere to WHO guidelines. The difference in INR between samples with these two citrate concentrations is below 2% according to an evaluation performed by the External Quality Assurance of Laboratory medicine (EQUALIS) in Sweden (unpublished data).

**Routine coagulation tests:** The prothrombin time method according to Owren was used in all studies. INR was not established at Swedish laboratories at the time of sample collection in Study 3. Instead, the results were given as prothrombin time in percent of normal activity (PT%) and to obtain the results in INR the data were recalculated according to the formula \( \text{INR} = (1/\text{PT%} + 0.018)/0.028 \) [219]. The reagent SPA (Diagnostica Stago, Paris, France) was used in study 1 and 3 and SPA+ (Diagnostica Stago) was used in study 2 and for the analyses performed at the Karolinska University Laboratory in Study 4. In fact, both mean SPA and SPA+ were evaluated in the assessment study, Study 4, but only the results obtained with SPA+ are given in the manuscript since the mean difference between the results was 1.1% and SPA is no longer in use. Fibrinogen was analyzed according to the Clauss method with two different reagents in Study 4 plus an additional reagent in Study 2. However, the reference ranges were equivalent and differences between reagents with this method are low [220]. APTT was included the study protocol in Study 2.
**Specific coagulation assays:** FII, FV, FVII and FX were analyzed in Study 2-4, FIX was analyzed in Study 2 and 3. Chromogenic methods were used for FII, FVII, and FX in the clinical part of Study 3, however, these were replaced by one-step clotting assays when the other studies were performed. The reference ranges remained unchanged despite the change in methods. The anticoagulant factors protein C, free protein S, and antithrombin were measured in Study 2. Detailed information concerning the reagents and instruments used is provided in each manuscript. Fluorogenic computerized thrombin generation test (CAT) according to Hemker was performed in Study 2.

**Liver function tests:** Baseline liver function tests results obtained according to hospital routine are provided in each manuscript of Study 2-4. These include serum concentrations of ALT, bilirubin, albumin and fasting bile acids in Study 2-3 and bilirubin and albumin in study 4.
3.4 STUDY 1-2

3.4.1 Patients and methods:

**Study design:** Study 1 was a prospective, open, uncontrolled clinical trial and Study 2 was a prospective cross-sectional observational study.

In study 1 children with liver disease received rFVIIa on indication life threatening bleeding and failure of conventional therapy or prior to invasive procedures as prophylaxis.

In study 2 patients with liver disease with or without increased bleeding risk were enrolled. Increased bleeding risk was defined by at least one of following; INR >1.4, APTT >44 sec., fibrinogen <1.5g/L or overt bleeding. Thrombin generation assay, routine coagulation tests and specific pro- and anticoagulant factors were analyzed. For patients undergoing liver biopsy with or without pro-hemostatic treatment follow-up analyses were performed.

**Bleeding:** Seven patients in Study 1 received rFVIIa on 22 occasions on indication life threatening bleeding including, gastrointestinal bleeding, epitaxis and bleeding into cholecystostomy. On five occasions rFVIIa was given before different procedures related to bleeding. Three of the patients in the “increased bleeding risk” group had overt bleeding from the gastrointestinal tract.

**Liver biopsy:** Six patients in Study 1 and 74 patients in Study 2 underwent percutaneous liver biopsy on clinical indication, under general anesthesia. In the transplanted patients the biopsy was carried out under real-time ultrasound guidance. In Study 1, transjugular biopsy technique was used in two patients.

**Pro-hemostatic treatment:** In Study 1 rFVIIa was given as intravenous bolus doses of 34-163 µg/kg, with 1-3 doses/patient on indication bleeding or as prophylaxis prior to invasive procedures.

In Study 2, three patients received single rFVIIa doses of 71-90 µg/kg on indication elevated INR prior to liver biopsy. Desmopressin 0.3 µg/kg was administered to nine patients due to prolonged template bleeding time prior to the invasive procedure. In nine patients, additional treatment with tranexamic acid was given.
Follow-up evaluation after invasive procedure and/or treatment:

**Clinical outcome:** In Study 1 the vital signs and serious events within 48 hours were registered as well as thromboembolic complications, transplantation and death within 2 weeks. The effect on bleeding from treatment was based on visual inspection (if the bleeding source was visible), changes in hemoglobin and need for blood transfusions. In Study 1 and 2 bleeding symptoms after liver biopsy were recorded. In Study 2 signs of bleeding post liver biopsy were stated as tachycardia, reduction in blood pressure, reduction in hemoglobin of more than 20 g/L and the need for blood transfusion.

**Laboratory outcome:** In both studies hemoglobin was checked 24 hours after the biopsy and as needed if there were clinical signs of bleeding. INR was analyzed before, 1, 4, 12 and 24 hours after treatment with rFVIIa in Study 1. In Study 2, samples were obtained before and 4 hours after treatment for thrombin generation test including ETP, LT, peak height and TTP.
3.5 STUDY 3-4

3.5.1 Patients and Methods

**Study design:** Both studies were prospective cross-sectional observational studies with an additional laboratory study each.

In Study 3 the patients were divided into two groups, those with FSBA below and above 200µmol/L, respectively, and the correlation between FSBA and the coagulation factors was analyzed in each group. In the laboratory study, increasing amounts of bile acids were added to normal plasma and FII, FV, FVII and FX were measured in the different bile dilutions.

In Study 4 plasma from patients with liver disease was sent to eight different hospital laboratories and analyzed for Owren based INR with the local INR reagent to determine the interlaboratory variance. In the laboratory study the ISI\textsubscript{Liver} and the ISI\textsubscript{VKA} and were determined according to the WHO protocol for the reagents included in the assessment study. The difference between these ISI values was determined.

**Owren based reagents:** Nycotest PT, (Medinor, Stockholm, Sweden), Owrens PT (Medirox) and SPA+ (Diagnostica Stago) were used in both the assessment study and the ISI study (Table 2).

**Table 2:** The hospital laboratories involved in Study 4.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Hospital</th>
<th>Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab1</td>
<td>Karolinska</td>
<td>SPA+, Diagnostica Stago</td>
</tr>
<tr>
<td>Lab2</td>
<td>Malmö</td>
<td>SPA+, Diagnostica Stago</td>
</tr>
<tr>
<td>Lab3</td>
<td>Sahlgrenska</td>
<td>SPA+, Diagnostica Stago</td>
</tr>
<tr>
<td>Lab4</td>
<td>Linköping</td>
<td>Owrens PT, Medirox</td>
</tr>
<tr>
<td>Lab5</td>
<td>St Göran</td>
<td>Owrens PT, Medirox</td>
</tr>
<tr>
<td>Lab6</td>
<td>Örebro</td>
<td>Nycotest PT, Medinor</td>
</tr>
<tr>
<td>Lab7</td>
<td>Falun</td>
<td>Nycotest PT, Medinor</td>
</tr>
<tr>
<td>Lab8</td>
<td>Växjö</td>
<td>Nycotest PT, Medinor</td>
</tr>
</tbody>
</table>

**ISI determination:** The WHO protocol was used to determine the ISI\textsubscript{VKA} for each reagent [36]. Within this protocol plasma from 60 Warfarin treated patients and 20 normal controls are analyzed with the reference thromboplastin RBT/05 with an ISI of 1.15 (NIBSC, Potters Bar, England [221]) and the manual tilt-tube technique. The
ISI\textsubscript{\text{ Liver}} was determined with the same procedure, except for the plasma, which in this setting, was plasma from the included patient with liver disease patients and 20 normal controls. This alternative calibration procedure for patients with liver disease was first described by Bellest et al. and Tripodi et al. [100, 101]. These analyses were performed at Linköping University Hospital.

3.6 STATISTICS (ALL STUDIES)
Study 1 was a descriptive study. All results from Study 2-4 were entered into the statistics software Statistica, release 10 (StatSoft Scandinavia, Uppsala, Sweden). Mann-Whitney’s test was used in all these studies for comparison between groups since the distributions were skewed. A $P$ value of <0.05 was considered statistically significant. Spearman’s rank correlation was used to obtain correlation coefficients. The difference in parameters before and after liver biopsy in Study 2 was calculated and compared to the difference in patients undergoing the same procedure without treatment or bleeding within the same age group. Coefficients of variation were calculated as CV= (SD/mean x 100), in both the in vitro study in Study 3 and in the assessment study in Study 4. Calculations of ISI and CV of slope were done using Excel (Microsoft) and Sigma plot 10 (Systat software, Port Richmond, CA, USA).
4 RESULTS

4.1 STUDY 1-2:

4.1.1 Baseline laboratory tests:
The median INR prior to treatment in Study 1 was 1.88 (range 1.04-6.0) in the group with life threatening bleeding and 1.52 (range 1.08-2.85) in the prophylaxis group.
The patients with liver disease in Study 2 had higher levels of vWF and FVIII and shorter LT and TTP compared to normal controls. The 71 liver patients with standard risk of bleeding had an ETP similar to normal controls. The 11 patients with an increased bleeding risk had a reduced ETP, compared to standard risk patients, concordant with their results of the routine coagulation tests. These patients had also reduced levels of procoagulant factors (II, VII, IX, X and for adolescents also V and fibrinogen) and concomitant lower levels of anticoagulant factors such as antithrombin and protein C. Thus the thrombin generation assay did not provide additional information regarding the balance between pro- and anticoagulant factors in children with liver disease compared to routine coagulation tests.
The ETP increased with increasing age in both the patients with liver disease and the normal controls (Figure 5).

Figure 5: The correlation between the age and ETP in all patients in Study 2.
4.1.2 Effect of pro-hemostatic treatment

**Bleeding:** rFVIIa was administered on 22 occasions in Study 1. The bleeding decreased in ten of these cases and remained unchanged in seven and increased in two. The treatment could not be evaluated on three occasions. In the two patients who had an increased bleeding, the preceding treatment with Octreotide was interrupted before the treatment with rFVIIa was initiated. A combination of Octreotide and rFVIIa was successful on eight of fourteen occasions. Additional treatment with fresh frozen plasma was given on three occasions.

**Invasive procedures:** rFVIIa was given before different therapeutic invasive procedures, related to bleeding, on five occasions in Study 1. The procedures were dilatation of a portal vein stenosis on two occasions and endoscopic sclerotherapy or ligation on three occasions. The last event was combined with Port-á-Cath placement and hip arthrocentesis. On all these occasions the bleeding stopped with the combination of rFVIIa and the procedure.

rFVIIa was also given on nine occasions as prophylaxis prior to liver biopsy in Study 1 and on three occasions in Study 2. These patients had all an expected increased bleeding risk, however, none of them experienced any bleeding complication. Additional treatment with desmopressin was given in two cases and fresh frozen plasma in one case in Study 1. In Study 2 the rFVIIa treatment was combined with tranexamic acid in two patients. Another eight patients in Study 2 underwent liver biopsy after prophylactic treatment with desmopressin, which was combined with tranexamic acid on seven occasions.

4.1.3 Laboratory evaluation:

The patients in Study 1 responded to the rFVIIa treatment with a clinically relevant reduction in INR in samples collected within 1-4 hours after rFVIIa was given. The effect was transient and on ten of the treatment occasions INR values above baseline were noted at follow-up 12-24 hours after the treatment. In Study 2 the rFVIIa treated patients had a significant decrease in LT and TTP compared to the untreated patients, however, the effect on ETP and peak height was inconclusive. In the patients who received desmopressin the reduction in peak height and the increased TTP after the biopsy were not as pronounced as in the normal controls. The ETP and LT were not significantly influenced by desmopressin treatment.
4.1.4 Serious events:
One 8-year-old girl in Study 1 had a portal vein thrombosis detected on ultrasonography after three doses of rFVIIa, however, this could not be verified on CT-scan 6 hours later or at liver transplantation 2 days later. This patient and two other patients in the bleeding group of Study 1, who received multiple doses of rFVIIa and had very severe liver disease, developed multiorgan failure and died within 1-14 days after their last dose of rFVIIa. In Study 2 neither thrombosis nor multiorgan failure was seen after pro-hemostatic treatment.
None of the patients who underwent liver biopsy experienced any bleeding complication with a decrease in hemoglobin exceeding 20 g/L.
4.2 STUDY 3

4.2.1 Clinical data

In the 12 children with FSBA above 200 µmol/L there was a significant positive correlation between FSBA and FV, FVII and prothrombin time (Figure 6). There was a significant negative correlation between FSBA and INR (Figure 7). Conversely, in the 33 patients with FSBA under 200 µmol/l, significant negative correlations between FSBA and FVII and FIX were seen. The patients with bile acids above 200 µmol/l had a significantly worse outcome than patients with lower levels of bile acids.

Figure 6-7: The relationship between FSBA and the levels of FVII and INR, respectively.
4.2.2 Laboratory study:
We observed no interference between bile acids and coagulation factors in the laboratory study (Table 3.).

**Table 3:** Serum bile acid levels and coagulation factor levels after in vitro addition of increasing amounts of bile acids.

<table>
<thead>
<tr>
<th></th>
<th>Taurocholic acid and/or normal plasma</th>
<th>Glycocholic acid and/or normal plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-bile acids (μmol/l)</td>
<td>FII (kIE/L)</td>
</tr>
<tr>
<td>Sample 1</td>
<td>386</td>
<td>0.96</td>
</tr>
<tr>
<td>Sample 2</td>
<td>193</td>
<td>0.90</td>
</tr>
<tr>
<td>Sample 3</td>
<td>69</td>
<td>0.90</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>3.6</td>
<td>0.85</td>
</tr>
<tr>
<td>Sample 1</td>
<td>467</td>
<td>0.93</td>
</tr>
<tr>
<td>Sample 2</td>
<td>198</td>
<td>0.90</td>
</tr>
<tr>
<td>Sample 3</td>
<td>62</td>
<td>0.84</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>3.8</td>
<td>0.90</td>
</tr>
</tbody>
</table>
4.3 STUDY 4

4.3.1 Assessment study
The mean difference between the highest and the lowest reported INR was 0.31 (range 0.13-0.86) for all 20 patients (Figure 8). The coefficient of variance for the Owren based INR methods at the hospital laboratories was 5.3% in average, which is low compared to previous studies [96, 97]

![Figure 8: The reported INR for each patient from each laboratory.](image)

4.3.2 ISI-study
All Owren based reagents had a difference between $\text{ISI}_{\text{VKA}}$ and $\text{ISI}_{\text{Liver}}$ below 10% (Table 4).

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>Instrument</th>
<th>$\text{ISI}_{\text{VKA}}$</th>
<th>$\text{ISI}_{\text{Liver}}$</th>
<th>% Diff $\text{ISI}_{\text{VKA-liver}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owren-method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPA+</td>
<td>ACL Top</td>
<td>1.01</td>
<td>1.00</td>
<td>0.7</td>
</tr>
<tr>
<td>Owrens PT</td>
<td>ACL Top</td>
<td>1.23</td>
<td>1.14</td>
<td>7.1</td>
</tr>
<tr>
<td>Nycotest PT</td>
<td>ACL Top</td>
<td>1.08</td>
<td>1.05</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 4: The obtained ISI for each reagent.

5 The percentage difference between $\text{ISI}_{\text{VKA}}$ and $\text{ISI}_{\text{Liver}}$. 
5 DISCUSSION AND FUTURE PERSPECTIVES

5.1 STUDY 1-2

5.1.1 Balance in liver disease and in children
The balance between pro- and anticoagulant factors is maintained in patients with liver disease. However, this balance seems to be unstable due to the lower levels of both the pro- and anticoagulant factors [131]. Although it is the bleeding symptoms that predominate in the clinical setting, the association between fibrosis and hypercoagulation, as well as the occurrence of thromboembolic events in adult patients are increasingly discussed [222-224]. Factors that may tip the balance in either direction include infections, inflammatory conditions, hereditary bleeding or prothrombotic disorders, massive gastrointestinal bleeding, volume overload and renal failure [63, 64, 79, 206, 222, 225, 226]. The coagulation system of healthy children also maintains a different balance compared to that of healthy adults. Higher levels of vWF, FVIII and lower levels of vitamin K-dependent factors and inhibitors are also seen in infants and neonates [46]. The physiological plasma concentrations of coagulation factors change during infancy and childhood before reaching the levels of adults [45, 46]. The risk of thrombosis is lower in children than in adults, without an increase in bleeding risk [58, 227]. On the other hand, bleeding and thrombosis do occur children with disease states including: infections, inflammatory states, malignancies, hereditary bleeding or prothrombotic disorders, renal failure and liver disease [47, 58, 176, 177, 228]. The etiologies of the pediatric liver diseases are different from those in adults [3]. It is, thus necessary to perform studies regarding coagulation specifically in children with liver disease.

5.1.2 Treatment of overt bleeding
Important means of treating bleeding episodes from varicose veins are the use of antibiotics against infections that aggravate the bleeding, and octreotide to reduce portal flow [65, 66, 191]. Octreotide is known to be effective in about 70% of the patients, but non-responders require additional treatment [190]. We could show in Study 1 that the addition of rFVIIa to the treatment with octreotide may be successful in these patients and that rFVIIa may help stabilize the patient and give us extra time to perform diagnostic or therapeutic procedures.
Large randomized controlled trials in adults have not proven any effect of treatment with rFVIIa in terms of stopping bleeding from varicose veins [174, 175]. On the other hand, in children clinical reports and small studies, including ours, have been more promising [60, 176-183]. Thus, specific large randomized trials for children with liver disease and severe gastrointestinal bleeding symptoms are needed.

5.1.3 Prophylactic treatment

As evidence accumulates regarding the balance between pro- and anticoagulant mechanisms, the general practice of routinely giving preoperative treatment in order to correct coagulopathy prior to invasive procedures is being questioned [131]. However, with the lack of controlled trials evaluating preoperative treatment against no treatment, most recent published recommendations are still advocating platelet count above 50-60 x10^9 [209, 210] and INR below 1.5 [211, 229, 230], both in adults and children. We evaluated rFVIIa as prophylactic treatment in patients with an elevated INR above 1.4 prior to invasive procedures and none of the patients experienced any bleeding complication. rFVIIa can, thus be used in these children with liver disease when prophylaxis is considered indicated. An advantage with the use of rFVIIa might be the product’s half-life which is short, and particularly short in children [163]. Thus, the duration of an expected tip towards a more hypercoagulable condition induced by the treatment, is theoretically very limited.

5.1.4 Balance and future treatment strategies

The incidence of multiorgan failure and the high mortality rate in our study are primarily considered to have been caused by the severe condition of the children receiving this treatment. Alten et al. have had similar experience [177]. Nonetheless, the potential risk of adverse events needs to be considered. Multiple doses of rFVIIa should be avoided, if possible, and special attention must be paid to concomitant low levels of anticoagulant factors. In many publications the advice is to avoid hemostatic treatments in patients with liver disease and to administer pro-hemostatic agents only if a bleeding episode necessitates “rescue therapy” [204, 231, 232]. However, some of the children with severe liver failure are in such a severe clinical condition and their hemostatic balance is so unstable that it could be deleterious to perform an endoscopic procedure under general anesthesia to stop a variceal bleeding. In these patients, with depleted levels of both pro- and anticoagulant factors, with an obvious bleeding tendency and concomitant slow portal flow, a pragmatic way to prevent severe bleeding
or thrombus formation would be substitution with both pro- and anticoagulant factors in risk situations and prior to invasive procedures. This regimen would theoretically maintain the balance, but at a higher level and closer to or within age-specific ranges. Hemostatic treatments for this purpose would include a combination of fibrinogen concentrate, antithrombin, 4-factor PCC including protein S and protein C and possibly platelets. Routine coagulation tests (including fibrinogen, antithrombin, INR and platelet count) and perhaps TGT could be used to guide this treatment. The use of factor concentrates instead of plasma would decrease the risk of volume overload and the risk of transmitting infections. If this treatment fails and variceal bleeding leads to medical emergency, both octreotide and tranexamic acid, as well as rFVIIa should be considered. Although expensive, this treatment strategy might save the lives of patients with a severe condition. However, this proposed treatment strategy still needs to be evaluated in clinical trials. A combination of a modified bleeding risk score and coagulation tests might be useful to select the right patients.

5.1.5 Thrombin generation

In view of the uncertainty regarding which laboratory assays are best at helping the clinician find patients at high risk for bleeding or thrombosis or both, and personalize treatment on that basis, we evaluated TGT in Study 2. However, this commercially available assay did not provide other information than routine coagulation tests. The patients with an increased bleeding risk according to routine coagulation tests had reduced levels of the procoagulant factors (II, VII, IX, X and for adolescents also V and fibrinogen), as well as the anticoagulant proteins (antithrombin, protein S and C). The TGT showed a reduction in ETP, as expected from the routine tests; however, it did not provide additional information regarding the suspected counteracting effect of the anticoagulant proteins. TGT with the addition of thrombomodulin might provide more information concerning the balance of the coagulation system, due to the activation of the protein C system [118]. However, TGT with thrombomodulin has not been shown to predict bleeding or thrombotic events in the clinical setting [117]. This might be explained by the fact that the bleeding occurs irrespective of the coagulation status, but it may also be that the assay is not sensitive enough to detect a disturbed hemostatic balance. Study 2 also shows that many of the patients in this cohort, with a clinical need for liver biopsy, have normal coagulation tests as compared to healthy children, except for the increase in the levels of vWF and FVIII. These patients were regarded as “standard risk group” in this study. Healthy children generally have a lower risk of thrombosis than
adults, without an increased bleeding risk [227]. These differences are probably of
importance in children with compensated liver disease, as well. The levels of ETP
increased with increasing age in the patients in the standard risk group, similarly to the
healthy controls.

5.1.6 Studies in children – limitations

Researchers attempting coagulation studies in the pediatric population encounter
several difficulties. It is often difficult to obtain samples from children and small
needles including butterfly needles are frequently used, which increase the risk of pre-
analytical errors[27]. This was the reason we chose the 5 pM tissue factor concentration
in Study 2, instead of the 1 pM reagent. The 1 pM reagent is known to be more
sensitive both to coagulation defects and to pre-analytical errors [28, 229]. We also
avoided TGT with platelet-rich plasma and the platelet function assays in this study for
several reasons, including: difficulties interpreting the results owing to the interference
cauased by thrombocytopenia, the requirement for large sample volumes and sensitivity
to pre-analytical conditions [52]. In this thesis only template bleeding time was used to
evaluate the platelet function, despite the assay’s poor reproducibility. However, only a
few, experienced and well trained nurses of the staff at our department are certified to
perform this test, which helps limit this problem. Development of better platelet
function assays is warranted as this would enable us to perform important studies
regarding the platelet function in children with liver disease. Improved assays would
also make it possible to assess the use of treatment with desmopressin in patients with
liver disease. There is also a need for assays validated in children for the evaluation of
the fibrinolytic system and endothelial function.

Although age-specific reference ranges for coagulation assays used on children are of
clinical and scientific importance such data are scarce. We enrolled healthy children as
controls in Study 2 because at that time, there were no reference ranges for the
combination of instruments and reagents we intended to use. The collection of blood
samples from healthy children is time consuming, and costly in terms of both money
and personnel, and there are also ethical considerations. It is thus unrealistic to expect
each laboratory to develop its own reference ranges for children. It will probably be
necessary to offer incentives similar to those aimed to encourage pharmaceutical
companies to perform clinical trials in children. Collaboration between manufactures of
laboratory equipment and large pediatric centers could be a way to provide age-specific
reference ranges as new assays are developed. Companies that offer such collaboration should be favored when laboratory equipment is being procured.
5.2 STUDY 3-4

5.2.1 Severe cholestasis and coagulation
Coagulation factor levels are thought to decrease and INR, PELD, MELD and CTP scores to increase as liver function deteriorates, irrespective of the etiology. This is the rationale behind using these analyses and scores for assessment of liver function, prognosis and liver allocation. However, there are reports showing that the PELD score underestimates mortality in children with liver disease, and previous studies have indicated a hypercoaguable state in patients with cholestatic liver disease of adulthood, including primary biliary cirrhosis and sclerosing cholangitis [126, 230, 233]. In Study 3, we, thus wanted to investigate the levels of coagulation factors in children with cholestasis. We chose the patients with the highest fasting bile acid concentrations (i.e. those in whom bile acids exceeded 200 µmol/L) since in clinical practice, their condition is most severe and they are the patients most likely to be evaluated as candidates for liver transplant. It has been demonstrated that levels between 10-100 µmol/L do not differentiate between hepatocellular and cholestatic liver damage [234]. We could show that the patients in the high FSBA group had a worse outcome due to liver failure, and although they were regarded as being more seriously ill, their INR was not worse (i.e. not higher) than would have been expected. On the contrary, this study shows that when the FSBA are above 200 µmol/L the coagulation factor levels do not reflect the severity of the liver disease since increasing levels of bile acids are correlated to increasing levels of coagulation factors and decreasing INR.

5.2.2 In vitro evaluation
To exclude that the bile acids had a direct effect on the performance of the coagulation factor assays that could explain these results, we added tauro- and glycocholic acids to samples with normal plasma and measured the INR and coagulation factor levels. The bile acids chosen were the ones which occur most abundantly in plasma and the levels used were relevant for this patient group. The bile acids did not have any detectable effect on pro-coagulants in vitro in this study.
5.2.3 Mechanism and implication

Bile acids can activate the nuclear farnesoid X receptor in human hepatoma cells and activation of these receptors induces an increased fibrinogen expression [115]. This potential link between bile acids and the expression of coagulation factors needs to be further explored. We have initiated a study where the mRNA expression of such factors in liver biopsies before and after bile diversion is analyzed. Bile diversion is a well established method to decrease the serum concentrations of bile acids in patients with progressive familial intrahepatic cholestasis [235].

It is also important to evaluate if the increase in procoagulant factors in patients with high levels of bile acids is counteracted by a similar increase in the levels of the anticoagulant factors including antithrombin, protein C and protein S, or if a hypercoaguable condition is present, as described in adults with cholestasis [126, 233]. Thus, there might be an increased need for anticoagulant prophylaxis in conditions associated with an increased risk of thrombosis in children with highly elevated levels of bile acids. However, our study, revealed increased risk of both bleeding and thrombosis in the high FSBA group compared to the low FSBA group. Although the number of patients was small and the differences not statistically significant, this would indicate an unstable coagulation system in these patients. It is essential to further explore these issues since a majority of the pediatric patients evaluated for liver transplantation in fact have a cholestatic condition [8].

5.2.4 INR in liver disease

The use of INR as a coagulation screening test as well as for prognostic purposes in patients with liver disease highlights the need for a robust method with a low variability. The INR according to the Quick method has not been able to fulfill these requirements [102]. The INR system according to both Quick and Owren was developed for patients on VKA treatment with the purpose of evaluating the treatment effects on FII, FVII and FX [34, 35]. The result of the Quick assay is dependent on these factors, but also on FV and fibrinogen. The last two factors are, assumed to be normal in the VKA treated patients [70]. In patients with liver disease, however, the levels of all of these factors – including FV and fibrinogen – are often reduced, though fibrinogen levels can sometimes be elevated. This discrepancy between the coagulation defects seen in patients undergoing VKA treatment and in patients with liver disease might explain the larger interlaboratory variance in samples from patients with liver disease compared to those from VKA treated patients analyzed with this method. In
Scandinavia, the Owren method is used which only depends on the levels of FII, FVII and FX. These factors are similarly affected by VKA treatment and by liver disease. In the assessment study of Study 4, we showed that the Owren method had a low interlaboratory variability, when samples from patients with liver disease were sent to eight Swedish hospital laboratories. In the ISI study of Study 4, we could show that the calibration system, developed for patients on VKA treatment, could also be used for patients with liver disease.

A limitation of the study was that the samples were collected in tubes with different citrate concentrations in the assessment part of the study and during the inclusion of additional patients for the ISI study. However, the difference in INR between samples collected at these two concentrations is minimal.

5.2.5 The Owren method and scoring systems

Several scoring systems – including the PELD, MELD and CTP scores – have been developed in countries that use the Quick method [136, 137]. There might be a concern that FV and fibrinogen could be of importance for the evaluation of patients with liver disease and that these scores might be inappropriate if these two factors are excluded from the assay, as they are in the Owren method. However, it can be argued that INR has been used for several years as a liver function test and prognostic factor in Scandinavia and that the Quick method’s high interlaboratory variability is a bigger problem. A study comparing the prognostic value of PELD and MELD in centers using the Quick method and centers using the Owren method would be worthwhile to further evaluate this issue.
6 CONCLUSIONS

1. rFVIIa is beneficial in some patients in the short term management of life threatening bleeding in severe liver disease.
   
rFVIIa can be used as prophylaxis before various diagnostic and therapeutic procedures in children with liver disease and coagulopathy.

2. Measurement of thrombin generation with current assay does not provide additional information regarding the hemostatic function in children with liver disease compared to routine coagulation tests.

3. Positive correlations were found between fasting serum bile acids and coagulation factors in patients with bile acids levels exceeding 200µmol/L, despite a worse clinical outcome. Coagulation factors may be questionable as prognostic markers in patients with markedly elevated bile acids.
   
   Increased levels of bile acids did not influence the measurement of coagulation factor levels.

4. Interlaboratory variation in INR analyses in chronic liver disease was low using Owren-based methods.
   
The differences between ISI\textsuperscript{VKA} and ISI\textsubscript{liver} for the studied Owren reagents were below 10%. ISI\textsuperscript{VKA} for Owren reagents can be used in the INR calibration when analyzing plasma from patients with liver disease.
7 DIRECTIONS FOR FUTURE RESEARCH

To improve the treatment and the analysis of coagulation defects in pediatric liver disease;

- Explore differences in coagulation mechanisms in children compared to adults, with or without liver disease, including the clot formation.
- Further development and clinical assessment of global as well as platelet function assays.
- Evaluation of TGT with thrombomodulin in the clinical setting including the predictive value of this assay regarding bleeding and thrombosis.
- Develop a modified bleeding risk score for children with liver disease.
- Clinical multicenter trials regarding combined treatment with pro- and anticoagulant coagulation factor concentrates in children with severe liver disease.

To improve prognostic evaluation in liver disease

- Investigate the mechanisms by which bile acids can affect the levels of coagulation factors, possibly in animal models.
- Investigate the impact of bile diversion on the mRNA expression of coagulation factors in liver tissue.
- Investigate the outcome of patients with high bile acids (including patients with PFIC, without bile diversion) in a PELD based liver allocation system.
- Compare the prognostic value of PELD and MELD in centers for pediatric hepatology that use the Quick method versus the Owren method, or run the two methods in parallel in a multicenter study.
8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Levern är ett av kroppens största organ. Den har stor betydelse för ämnesomsättning, avgiftning av blodet och produktion av olika proteiner, bland annat de som behövs för att kunna levra blodet.

Varje år insjuknar ungefär 100 barn i Sverige i allvarlig leversjukdom. Åldern vid insjuknandet kan variera från nyföddhetsperioden till 18 års ålder. Ungefär 10-15 av dessa behöver genomgå levertransplantation, medan många kan räddas till livet med annan behandling.

Denna avhandling är inriktad på leverns förmåga att tillverka blodlevringsfaktorer. Tillverkningen av dessa minskar ofta vid leversjukdom och flera barn med leversjukdom drabbas av svåra blödningar. Blödningarna förekommer framför allt i näsa och mag-tarmkanal och kan vara livshotande och svårbehandlade.

Studie 1

I den första studien studerades effekten av en ny typ av behandling mot de här blödningarna, nämligen konstgjort framställd blodlevringsfaktor VII. Vi såg att preparatet är värdefullt som akutbehandling och ger en möjlighet att kunna gå vidare med mer specifika behandlingar mot blödningskällan.

Flera av barnen behöver genomgå olika typer av ingrepp även i lugnt skede. Ett sådant vanligt ingrepp är att man med nål tar ett litet vävnadsprov från levern och analyserar i mikroskop för att kunna se vilken typ av leversjukdom barnet lider av. Detta ingrepp är förknippat med blödningsrisk och man vågar därför inte alltid göra detta på barn med påverkan på blodlevringssystemet. Vi har på grund av detta provat att ge faktor VII preparatet till just dessa barn innan ingreppet och sett att vi på så sätt kunnat undvika blödningskomplikationer. Detta har förbättrat diagnostiken för dessa barn.

Studie 2

Alla barn med leversjukdom blöder inte, en del kan till och med drabbas av motsatsen, dvs. få blodproppar i kärlen som omger levern. Detta kan bland annat påverka möjligheten att genomgå levertransplantation. Inte bara blodlevringsfaktorerna bildas i levern utan även de faktorer som behövs för att hindra blodlevring, så kallade hämmare. Hos friska personer råder en balans mellan blodlevringsfaktorer och hämmare. På senare år har forskning indikerat att det finns en liknande balans vid leversjukdom, men att den är mycket skörare än hos friska och att systemen lättare tippar om barnet t.ex. får en infektion. Det skulle vara värdefullt att finna bra analysmetoder som kan ge bra information om balanssituationen för att tidigt kunna ge rätt behandling innan en livshotande blödning eller blodpropp bildats. Vi har undersökt en ny typ av
analysmetod (trombingenerering) men den kunde tyvärr inte ge mer information än vanliga rutinprover.

Studie 3
Barn med leversjukdom har ofta ett tillstånd som innebär att gallflödet är nedsatt. Detta kallas gallstas, eller kolestas, och kan inträffa vid flera olika typer av leversjukdom. Vid gallstas ansamlas gallsyror som skadar levercellerna. Vi har studerat om extremt höga nivåer av gallsyror kan påverka nivåerna av blodlevringsfaktorer i blodet. Trots att dessa barn kan vara mycket sjuka har vi visat att de har oväntat höga nivåer av blodlevringsfaktorer. Vad detta beror på är oklart, men det skulle kunna bero på att gallsyror stimulerar levern att öka produktionen av blodleveringsfaktorer. Våra fynd kan ha betydelse för bedömning av blodlevringsförmågan hos dessa barn men också för bedömning inför en levertransplantation och behöver studeras vidare.

Studie 4
Analyser av blodlevringsfaktorer används för att bedöma barnets prognos och om levertransplantation behövs. Internationella studier har dock visat att den vanligaste analysen som använts för det, INR, kan visa mycket olika resultat vid olika laboratorier för samma patient. I Skandinavien används en annan metod för denna analys och vi har i vår forskning kunnat visa att denna ger betydligt mer samstämmiga resultat än de metoder som används i USA och stora delar av Europa. Övergång till den Skandinaviska metoden kan således innebära en lösning på detta problem.

Slutsats
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10 REFERENCES


