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## 2.4.2 The liver and coagulation

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### Introduction

The liver plays a major role in haemostasis, as most of the coagulation factors, anticoagulant proteins and components of the fibrinolytic system are synthesized by hepatic parenchymal cells. Additionally, the reticuloendothelial system of the liver helps to regulate coagulation and fibrinolysis by clearing these coagulation factors from the circulation. Finally, because the liver is a highly vascularized organ with vital venous systems draining through the parenchyma, liver diseases can affect abdominal blood flow and predispose patients to significant bleeding problems.

The aetiology of impaired haemostasis resulting from abnormal liver function is often multifactorial and may include impaired coagulation factor synthesis, synthesis of dysfunctional coagulation factors, increased consumption of coagulation factors, altered clearance of activated coagulation factors and quantitative and qualitative platelet disorders. In this chapter, we will review the normal physiology of haemostasis, describe the role of the liver in the haemostatic system and discuss the coagulation abnormalities that may occur in patients with liver disease and during liver transplantation.

### Physiology of haemostasis

Normal haemostatic balance is dependent on a complex interplay between procoagulant, anticoagulant and fibrinolytic proteins. Initiation of coagulation begins when tissue factor (TF) is exposed after an injury to the vessel wall (Fig. 1). TF forms a

**Fig. 1** Tissue factor (TF) is the major initiator of coagulation. Tissue factor-bearing cells include stimulated monocytes, endothelial cells and vascular smooth muscle cells. Exposure of TF to blood is rapidly followed by the formation of a complex between TF and factor VIIa that activates both factor IX and X, leading to generation of thrombin. Thrombin also activates factor V, VIII, XI and platelets.

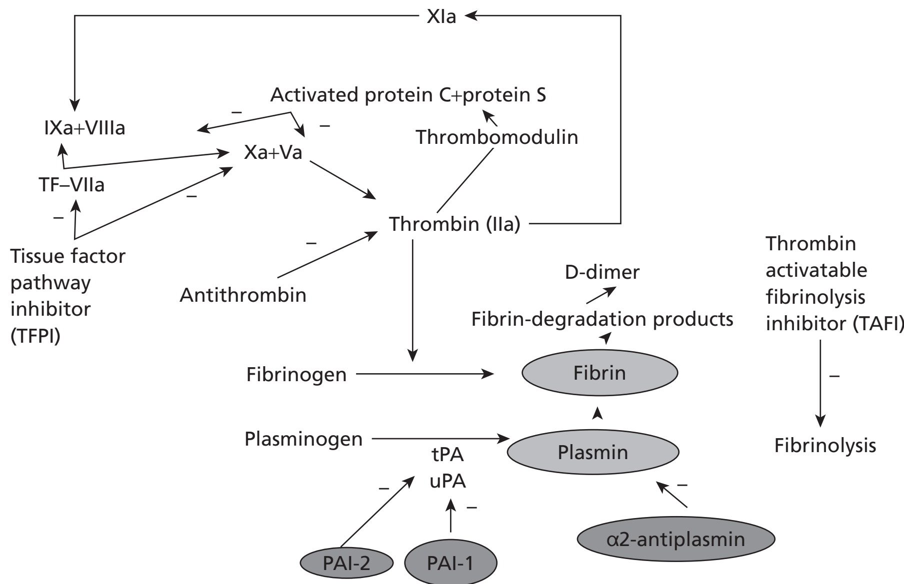


**Fig. 2** The fibrinolytic and natural anticoagulant pathways.

complex with activated factor VII (factor VIIa) present in the plasma. The TF/factor VIIa complex converts factor X to factor Xa, which, in turn, along with a cofactor, factor Va, converts prothrombin (factor II) to thrombin (factor IIa) [1]. Although this process generates a small amount of thrombin, this thrombin serves to prime the coagulation cascade by increasing the enzymatic activity of factor VIIa, making it 100-fold more active. In addition, thrombin activates factor V, VIII, XI and platelets, which form the infrastructure to amplify the enzymatic reactions of the coagulation cascade. Ultimately, thrombin is formed directly by the TF/VIIa complex activating factor X to Xa or indirectly by converting factor IX to factor IXa, which in turn complexes with its cofactor, factor VIIIa, to convert factor X to factor Xa. The large amount of thrombin that is generated cleaves fibrinogen to fibrin monomers, which in turn spontaneously polymerize and are cross-linked by factor XIIIa (which itself is activated by thrombin) to produce a stable clot. At this time, thrombin-activatable fibrinolysis inhibitor (TAFI) becomes fully active and serves to diminish the incorporation and activation of plasminogen, leading to delayed clot lysis (Fig. 2).

TF-dependent generation of thrombin is rapidly inhibited by TF pathway inhibitor (TFPI), which binds TF/VIIa/Xa forming the quaternary complex TFPI/Xa/TF/VIIa, which is internalized by the TF-bearing cell. The main endogenous anticoagulant system is the protein C-dependent system. Protein C and its cofactor protein S are both vitamin K-dependent factors synthesized by the liver. Protein C binds to an endothelial cell protein C receptor (ECPR) and is activated by thrombin bound to thrombomodulin, another endothelial cell membrane-based protein [2]. The activated protein C complex inactivates factors VIIIa and Va (Fig. 2). Another important endogenous anticoagulant system involves antithrombin (AT), also primarily produced in the liver, which inactivates thrombin (IIa), factors Xa, IXa, XIa and XIIa. The anticoagulant activity of AT can be increased by up to 1000-fold in the presence of heparin. Another vitamin K-dependent anticoagulant is protein Z, whose structure is similar to coagulation factors VII, IX, X, protein C and protein S. However, in contrast to these other vitamin K-dependent factors, protein Z is not a serine protease and plays an important role in inhibiting coagulation by serving as a cofactor for the inactivation of factor Xa by forming a complex with the plasma protein Z-dependent protease inhibitor. The role of alterations in the protein Z levels has been evaluated in different disease states, with conflicting findings [3]. Finally, to prevent excess clotting, fibrin is digested by the fibrinolytic system, the major components of which are plasminogen and tissue-type plasminogen activator (tPA) (Fig. 2). Both these proteins are incorporated into polymerizing fibrin, where they interact to generate plasmin, which in turn acts on fibrin to dissolve the preformed clot. Plasminogen binds to fibrin at specific lysine binding sites and to tPA. The binding of plasminogen to tPA converts the proenzyme plasminogen to active proteolytic plasmin. Plasmin cleaves polymerized fibrin strands at multiple sites and releases fibrin degradation products (FDPs) [3].

The fibrinolytic system is regulated by three serine proteinase inhibitors: alpha2-antiplasmin ($\alpha_2$-PI), plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) (Fig. 2). $\alpha_2$-PI is secreted by the liver, is present within platelets and serves to immediately inactivate free plasmin, whereas PAI-1 is the most important and most rapidly acting physiological inhibitor of both tPA and urokinase-type plasminogen activator (uPA). PAI-2, originally purified from human placenta, inhibits both two-chain tPA and two-chain uPA with comparable efficiency, but it is less effective towards single-chain tPA [4].

## Role of the liver in the haemostatic system

The liver is the primary site of synthesis of most of the clotting factors and the proteins involved in the fibrinolytic system. These include all the vitamin K-dependent coagulation proteins (factors II, VII, IX, X, protein C, protein S and protein Z), as well as factor V, XIII, fibrinogen, antithrombin, $\alpha_2$-PI and plasminogen. The notable exceptions are von Willebrand factor (VWF), tPA, thrombomodulin, TPFI and uPA. The VWF, tPA, thrombomodulin and TFPI are synthesized in endothelial cells, while uPA is expressed by endothelial cells, macrophages, renal epithelial cells and some tumour cells [4].

Vitamin K, a fat-soluble vitamin, is required to achieve proper levels of procoagulant factors (II, VII, IX and X) and anticoagulant factors (proteins C, S and Z). These factors require vitamin K as a cofactor for post-ribosomal modification to render them physiologically active. All the vitamin K-dependent factors have in their amino-terminal several glutamic acid residues that must be converted to gamma-carboxyglutamic acid residues. This process is crucial to allow these proteins to bind calcium ions to form bridges to phospholipid surfaces, which are essential for the formation of activation complexes [5]. Finally, the liver plays a vital role in the regulation of anticoagulation. Removal of activated clotting and fibrinolytic factors, especially tPA, is mediated through the hepatic reticuloendothelial system [6].

## Haemostatic abnormalities in liver disease

Various haemostatic abnormalities can occur in patients with liver disease and, in general, the severity of these abnormalities is dependent on the degree of hepatic dysfunction (Table 1).

**Table 1** Hemostatic changes in patients with liver disease.

| Haemostatic abnormality | Mechanism |
| --- | --- |
| Hypocoagulability | Decreased synthesis of coagulation factors (except VIII and VWF)<br>Hypofibrinogenaemia (endstage liver failure)<br>Vitamin K deficiency (II, VII, IX, X)<br>Decreased clearance of degraded coagulation factors |
| Hypercoagulability | Decreased synthesis of natural anticoagulant proteins antithrombin (AT), proteins C, S and Z<br>Decreased clearance of activated coagulation factors |
| Dysfibrinogenaemia | Synthesis of abnormal fibrinogen |
| Hyperfibrinolysis | Increased levels of circulating tPA activity due to impaired hepatic clearance<br>Decreased synthesis of fibrinolytic inhibitors (PAI-1 and α2-antiplasmin)<br>Decreased thrombin-activatable fibrinolytic inhibitor (TAFI) |
| Quantitative and qualitative platelet defects: | Decreased bone marrow production (due to decreased thrombopoietin)<br>Splenic sequestration |
| Thrombocytopenia | Immune-mediated platelet destruction<br>Folate and vitamin B12 deficiencies |
| Thrombocytopathies | Direct effect of ethanol<br>Non-specific platelet aggregation abnormalities |

### Coagulation defects in acute liver disease

Liver disease can cause both quantitative and qualitative abnormalities in coagulation factors. Commonly, the vitamin K-dependent factors decrease first, starting with factor VII and protein C owing to their short half-life (6 h), followed by reductions in factor II and X levels. Factor V levels are decreased in both acute and chronic liver disease [7]. Factor IX levels are usually only modestly reduced until advanced stages of liver disease. In contrast, VWF (synthesized by the endothelial cells) and factor VIII levels may be normal even in the presence of advanced liver disease because there is an increased production of factor VIII by the sinusoidal endothelial cells when the liver is damaged, combined with decreased clearance of the VWF/factor VIII complex.

Fibrinogen levels are rarely decreased and may even be elevated because of abnormal non-functional fibrinogen (dysfibrinogenaemia) related to defective polymerization. A decrease in fibrinogen levels may indicate the presence of disseminated intravascular coagulation (DIC) or progression to fulminant hepatitis with hepatic failure.

In a study of patients with significant liver injury and associated coagulopathy, factors II, V, VII and X were reduced to a similar degree and were significantly lower than factors IX and XI [8]. Factor VIII, however, was increased as well as interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF$\alpha$), thrombin–antithrombin (TAT) and soluble TF levels [9]. Of note, all the decreased factor levels were directly activated by TF. In patients with acute fulminant hepatic failure, the haemostatic alterations are attributed to quantitative and qualitative platelet defects, impaired synthesis and clearance of the coagulation factors and related inhibitory proteins and enhanced fibrinolysis. DIC may also play a role; however, DIC is difficult to distinguish from changes resulting from impaired hepatic synthesis and clearance alone [10]. In a study of 42 patients with acute fulminant hepatic failure, the activities of plasminogen and its inhibitor $\alpha_2$-PI were reduced while tPA activity was normal. PAI-1, however, was greatly increased, indicating a shift towards inhibition of fibrinolysis in these patients. TAT complex levels and D-dimer, a fragment of cross-linked

fibrin in plasma, were also significantly increased, indicating activation of coagulation and fibrinolysis respectively. Thus, gross abnormalities of the fibrinolytic system occur in fulminant liver failure but, because inhibitory activity appears to be present in adequate quantities, this limits the incidence of bleeding due to fibrinolysis [11].

## Coagulation defects in chronic liver disease

In patients with liver cirrhosis, most coagulation factors and inhibitors of the coagulation and fibrinolytic systems are markedly reduced because of impaired protein synthesis, except for factor VIII and fibrinogen levels, which may be normal or increased. Possible explanations for the increased factor VIII levels are the increased hepatic biosynthesis of VWF and decreased expression of low-density lipoprotein receptor-related protein, both of which modulate the level of factor VIII in plasma, rather than increase factor VIII synthesis [12]. Because fibrinogen is an acute-phase reactant, its synthesis tends to be preserved in patients with stable cirrhosis.

The deficiencies in vitamin K-dependent factors in cirrhosis may occur by several mechanisms, including reduced hepatic synthesis and reduced absorption of bile salts required for absorption of vitamin K-dependent factors, which may occur in the setting of cholestatic liver disease. Other contributing factors include poor oral intake and treatment with antibiotics that destroy the intestinal bacteria that synthesize vitamin K. As with acute liver disease, the reductions in coagulation factors parallel the degree of progression of liver disease.

In addition to impaired synthesis of clotting factors, excessive fibrinolysis, DIC, thrombocytopenia and platelet dysfunction account for the diverse spectrum of haemostatic defects in chronic or endstage liver disease [13] (Table 1).

The abnormalities of the fibrinolytic system are complex and result from impaired synthesis and altered clearance of the fibrinolytic factors. One of the most striking mechanisms is an imbalance between tPA and its specific inhibitor (PAI-1), which results in an increase in free tPA in the plasma and a reduction in $\alpha_2$-PI. TAFI plays an important regulatory role in fibrinolysis. TAFI is a procarboxypeptidase synthesized by the liver and, upon activation by thrombin or plasmin, TAFI is converted into TAFIa. TAFIa inhibits fibrinolysis by removing C-terminal lysines from partially degraded fibrin, causing a decrease in the cofactor function of fibrin in the plasminogen activation catalysed by tPA, resulting in decreased plasmin generation [14,15]. Thrombin is the most likely activator of TAFI and, when thrombin is complexed to thrombomodulin, TAFI activation is increased by more than 1000-fold [16]. The levels of TAFI are markedly reduced in cirrhotic patients and correlate with the severity of disease [16,17].

In addition to the reduced hepatic synthesis of clotting factors, cirrhotic patients also have a significant deficit of natural anticoagulants, particularly protein C and antithrombin [18]. Activated protein C (APC), is the main anticoagulant that, in combination with its cofactor protein S, downregulates thrombin generation *in vivo* by inhibiting the action of the cofactors factors Va and VIIIa. AT complexed to endothelial heparin-like substances inhibits thrombin (factor IIa) directly through the formation of an equimolar complex and indirectly through the inhibition of the serine proteases (factors IX, X, XI and XII). Thrombin formation is also downregulated by the TFPI, which specifically inhibits the complex of TF and VIIa [1].

Recent studies have indicated that standard coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) may not reflect the true coagulation status of patients with liver cirrhosis. This is because these tests do not take into account the activation of the primary endogenous anticoagulant protein C, levels of which are considerably reduced in cirrhosis. Tripodi *et al.* [19] reported impairment of thrombin generation measured without thrombomodulin, consistent with the reduced levels of procoagulant factors typically seen in cirrhosis. However, when the test was modified by adding thrombomodulin, patients generated as much thrombin as control subjects. The authors concluded that thrombin generation is normal in cirrhosis and that the reduction in procoagulant factors in these patients is compensated for by the reduction in anticoagulant factors, thus leaving the coagulation balance unaltered. Furthermore, their findings suggest that measurement of thrombin generation in the presence of thrombomodulin may be more suitable for the evaluation of bleeding risk [19].

## Thrombocytopenia

Patients with liver disease may develop quantitative (thrombocytopenia) and/or qualitative platelet abnormalities (thrombocytopathies) such as impaired platelet adhesion and aggregation. The aetiology of thrombocytopenia in these patients is often attributed to splenic sequestration (hypersplenism), but may also occur as a result of platelet destruction mediated by platelet-associated immunoglobulins (PAIgG) [20] and impaired hepatic synthesis and/or increased degradation of thrombopoietin (TPO) by platelets sequestered in the congested spleen [21].

Mild to moderate thrombocytopenia occurs in 16–52% of patients with acute hepatitis with or without cirrhosis. Severe thrombocytopenia can occur as a consequence of aplastic anaemia, a rare complication of acute hepatitis [22]. Idiopathic immune thrombocytopenia (ITP) has also been reported in patients with hepatitis C [23]. Cocaine may cause thrombotic thrombocytopenic purpura associated with toxic acute hepatitis [24]. Ethanol directly suppresses platelet formation and decreases the lifespan of platelets, both of which contribute to thrombocytopenia commonly seen with alcohol-related liver disease [25]. Other aetiologies including medications, folate and vitamin B12 deficiencies, severe infections and DIC should also be considered in evaluating thrombocytopenia in patients with liver disease within the appropriate clinical setting.

Thrombocytopenia is a more common feature of chronic liver disease and has been reported in 49–64% of cirrhotic

patients [26]. The primary mechanism for thrombocytopenia is thought to be due to hypersplenism secondary to portal hypertension, which results in increased platelet sequestration and destruction. There is also increased destruction of platelets by immunological mechanisms that result from increased PAIgG. Increased levels of PAIgG have been reported in 55–88% of patients with chronic liver disease [20,27]. The elevated levels of PAIgG correlate inversely with the platelet count in some [20,28], but not all studies [27]. Recently, diminished protein synthesis in the liver has been reported to cause inadequate synthesis of TPO [29]. TPO is a glycoprotein produced primarily in the liver that acts to increase the basal production rate of megakaryocytes and platelets. TPO levels are significantly lower in cirrhotic patients with thrombocytopenia than in those with normal platelet counts. Serum TPO levels correlate inversely with the severity of liver disease [30,31]. Finally, platelet counts post liver transplantation also correlate with TPO levels, regardless of splenic size [32].

Intravascular activation and increased consumption of platelets in the diseased liver as a result of low-grade DIC is also a possible but controversial mechanism of thrombocytopenia. In patients with alcohol-induced cirrhosis, the thrombocytopenia may result from folate deficiency, direct toxic effects of ethanol on megakaryocytopoiesis [33,34] and increased platelet activation [35].

### Platelet function abnormalities

In patients with chronic liver disease, impaired platelet aggregation with different agonists including adenosine diphosphate (ADP), thrombin, epinephrine and ristocetin has been described [36]. The abnormal platelet aggregation is thought to be caused by circulating platelet inhibitors (fibrin degradation products and D-dimers), plasmin degradation of platelet receptors, dysfibrinogenaemia and excess nitric oxide synthesis [13,37]. Conversely, hyper-responsiveness rather than a defective platelet/VWF interaction is observed in cirrhosis, which may compensate for other haemostatic problems; this appears to be mediated primarily by increased VWF levels [38]. The platelet function defects may account for the prolongation of the bleeding time in 40% of patients with cirrhosis and correlates with disease severity [39,40]. Erythropoietin is the primary stimulus to erythrocyte production and also induces megakaryocyte formation. Treatment with erythropoietin significantly increases platelet counts and platelet function in patients with alcoholic liver cirrhosis [41].

### Disseminated intravascular coagulation

Low-grade DIC is commonly found in patients with endstage liver disease (ESLD). This syndrome is typically characterized by thrombocytopenia, prolongation of the PT and aPTT, decreased fibrinogen and elevated levels of fibrin degradation products (FDPs). Additionally, elevated levels of prothrombin activation fragment F1 + 2, fibrinopeptide A, D-dimer and TAT complexes are also observed in varying degrees [42–44]. The frequency and severity of DIC tends to correlate with the stage of liver disease [13,42,44,45].

The aetiology of DIC in chronic liver disease is multifactorial and includes release of procoagulants from injured hepatocytes, impaired clearance of activated clotting factors, decreased synthesis of coagulation inhibitors and endotoxin entry into the portal circulation [13].

The diagnosis of DIC in patients with chronic or endstage liver disease is often difficult and challenging, as the coagulation defects in both disorders are quite similar. Typically, an elevated D-dimer is more specific for DIC as it indicates activation of both coagulation and fibrinolysis, whereas high levels of fibrinogen degradation products (FDP) or dysfunctional fibrinogen are more common in ESLD [46]. Importantly, decreasing levels of factor VIII and fibrinogen with an increased D-dimer level on serial testing is more characteristic of DIC.

Clinically significant DIC is uncommon in patients with liver disease, usually complicates severe bacterial infections or severe sepsis and can also develop in patients with peritoneovenous shunts [47].

## Haemostatic changes in liver transplant

Complex coagulation disorders may occur during liver transplantation including preoperative coagulation abnormalities due to the underlying liver disease and haemostatic changes related to the transplantation, all of which may contribute to severe bleeding. Prior to the anhepatic phase, there are usually no serious haemostatic alterations. Bleeding during transplantation is greatly influenced by the activation of the fibrinolytic system, which occurs during the anhepatic and reperfusion phases. The hyperfibrinolysis is mediated by an intense release of tPA and a lack of hepatic clearance during the neohepatic period. A second fibrinolytic burst results from release of tPA by the endothelial cells of the revascularized graft [48]. Conversely, PAI-1 decreases during the anhepatic period and increases during the neohepatic period [49]. A preserved capacity to generate thrombin and less fibrinolytic activation during the anhepatic phase occurs in primary biliary cirrhosis compared with other types of cirrhosis [50].

Factors that influence the risk of bleeding during liver surgery also include the presence of cirrhosis, portal hypertension, high levels of central venous pressure, renal dysfunction and the length of graft preservation [49]. A hypercoagulable state has occasionally been reported in some patients with neoplasm or Budd–Chiari syndrome [45,51]. Platelet count decreases during liver transplantation with a nadir at the time of reperfusion, and may worsen in the case of a damaged organ graft. It has been suggested that the transplanted liver has a major role in the thrombocytopenia with intrahepatic platelet sequestration, local thrombin generation on the damaged graft endothelium,

**Table 2** Laboratory features of coagulopathy of liver disease.

| Laboratory abnormality | Haemostatic defect |
|---|---|
| Prolonged PT and normal aPTT | PT corrects with mixing studies → factor VII deficiency |
| Prolonged PT and aPTT | PT and aPTT correct with mixing studies → factors I, II, V, X deficiencies |
| Prolonged thrombin time (TT) and reptilase time (RT) | Dysfibrinogenaemia |
| Shortened euglobulin lysis time | Decreased PAI-1, decreased $\alpha_2$-PI, increased tPA (during liver transplant) |
| Thrombocytopenia (verified by manual interpretation of the peripheral blood smear) | Platelet sequestration<br>Immune-mediated thrombocytopenia<br>Decreased synthesis of thrombopoietin |
| Prolonged bleeding time/abnormal platelet aggregation studies | Thrombocytopathies |
| Marked increase in D-dimer, low fibrinogen and normal factor VIII | Possible DIC (in the proper clinical setting) |

platelet extravasation and increased phagocytosis by the Kupffer cells as potential mechanisms. Platelet function abnormalities have also been described after revascularization, whereby hypothermia enhances splanchnic platelet dysfunction and prolongs coagulation reaction time by reducing enzymatic activity [52]. Release of exogenous heparin from the harvested graft after donor heparinization or endogenous heparin-like substances from the damaged ischaemic graft endothelium may also play a role in the coagulopathy at reperfusion [53]. Haemodilution secondary to fluid replacement and the preservation solution from the donor liver can additionally reduce plasma levels of coagulation factors at reperfusion [54].

## Laboratory testing for coagulation defects in liver disease

Initial standard screening tests in patients with liver disease should include a PT, aPTT, complete blood count with examination of the peripheral blood smear and a fibrinogen level. In selected patients, additional testing should include a D-dimer test, thrombin time (TT), reptilase time (RT), euglobulin clot lysis time and a bleeding time (Table 2).

Often, the clotting tests remain normal until clotting factor levels fall to less than 30–40% of normal. In mild liver disease, the PT is prolonged, but the aPTT is usually normal. As the liver disease progresses, both PT and aPTT levels are prolonged, although in compensated cirrhosis, the high factor VIII level may blunt the prolongation of the aPTT. It is important to note, however, that international normalized ratio (INR) values may not be accurately reflective of the coagulopathy in patients with ESLD [55].

Fibrinogen levels are either normal or increased in patients with stable chronic liver disease.

In decompensated cirrhosis or DIC, severe hypofibrinogenaemia (< 100 mg/dL) is present, resulting in marked prolongation of the PT, aPTT and TT. Functional abnormalities of fibrinogen or dysfibrinogenaemias are diagnosed by a prolongation of the TT and RT.

## Therapy for haemostatic abnormalities in liver disease

The management of haemostatic abnormalities in patients with liver disease is often difficult and challenging. Therapy is directed at correction of haemostatic defects in patients who are actively bleeding or who require surgery or other invasive procedures. Additionally, therapy should be targeted to the type of procedure and site and severity of bleeding. The bleeding risk appears to be higher in patients with multiple haemostatic defects, renal failure or a previous history of bleeding.

### Vitamin K

Vitamin K deficiency in patients with severe acute liver disease may be treated with one dose of 10 mg of vitamin K administered intravenously (i.v.) [56]. In patients whose prolonged PT does not completely correct with vitamin K therapy, impaired hepatic synthesis of coagulation factors from parenchymal liver disease must be suspected as the cause of the coagulopathy. Other patient subgroups in whom vitamin K may be useful include patients with primary biliary cirrhosis and those with chronic liver disease who are receiving broad-spectrum antibiotics and have poor nutritional intake [57].

### Plasma

Fresh frozen plasma (FFP) is prepared from units of whole blood and from plasmapheresis. Plasma contains all the coagulation

factors. Transfusion of FFP is the main therapy for patients with liver disease and coagulopathy who are actively bleeding [58]. However, the response to FFP is unpredictable. The use of FFP for the correction of moderate to severe coagulopathy prior to invasive procedures, e.g. percutaneous liver biopsy, is controversial and more studies are needed [59]. In general, it is recommended that, if the PT is prolonged by < 4 seconds, percutaneous biopsy can be undertaken safely. If the PT is prolonged by 4–6 seconds, FFP transfusion may allow the PT to decrease to the desired range. However, if the PT is prolonged by > 6 seconds, other biopsy techniques may need to be considered [60]. The recommended dose of FFP is 10–15 mL/kg. In the majority of patients with chronic liver disease, repeated transfusions of FFP every 12 h may be required for complete correction of the PT. There is currently no consensus on the volume of FFP or type of infusion regimens required to prevent or treat bleeding [13,59]. If FFP is given, repeat coagulation tests should be performed as soon as the infusion is completed to guide further management [59]. Potential adverse effects of FFP include volume overload, transmission of bloodborne infections, transfusion-related acute lung injury (TRALI) and allergic, febrile or haemolytic reactions.

## Cryoprecipitate

Cryoprecipitate is prepared from FFP and is rich in fibrinogen, factor VIII, VWF, factor XIII and fibronectin. Each bag of cryoprecipitate contains 80–100 IU/mL factor VIII and at least 140 mg of fibrinogen [59]. Cryoprecipitate is indicated in patients with severe coagulopathy and hypofibrinogenaemia (< 100 mg/dL) or dysfibrinogenaemia.

## DDAVP

Deamino-8-D-arginine vasopressin (desmopressin acetate or DDAVP) is a synthetic analogue of antidiuretic hormone, which raises the plasma levels of factor VIII and VWF and enhances platelet adhesion to the vessel wall. The agent is usually administered at a dose of 0.3 μg/kg by i.v. infusion over 20–30 min. In patients with liver cirrhosis, DDAVP may be used to shorten or normalize the prolonged bleeding time in those who need invasive procedures [61]. However, in two randomized trials, DDAVP did not reduce intraoperative blood loss and transfusion requirements in patients undergoing hepatectomy or control bleeding in cirrhotic patients with acute variceal bleeding [62,63].

## Platelets

Platelet transfusions are indicated in patients with liver disease who are actively bleeding and have a platelet count below 50 000/μL or known history of platelet dysfunction. Prophylactic platelet transfusions may be necessary before invasive procedures (e.g. percutaneous liver biopsy) in patients with platelet counts < 50 000/μL [64]. A 1-h post-transfusion platelet count is commonly used to determine the efficacy of platelet transfusion and to guide subsequent therapy. Patients with marked splenomegaly, however, may not respond with an increase in platelet count after transfusion because of increased sequestration of the transfused platelets. Failure to increase the platelet count after transfusion may also be observed in patients with DIC, severe infection and alloimmunization due to platelet-specific and/or human leukocyte antigen (HLA) antibodies [13].

## Antifibrinolytic agents

Epsilon aminocaproic acid, tranexamic acid and aprotinin are antifibrinolytic agents that inhibit plasmin generation and have been demonstrated to decrease bleeding associated with fibrinolysis in chronic liver disease [65] and intraoperative blood loss and transfusion requirements during liver transplantation [66–68]. These agents are contraindicated in patients with DIC and must only be used in selected patients with bleeding caused by excessive fibrinolysis.

## Recombinant factor VIIa (rFVIIa)

Recombinant activated factor VII (rFVIIa) is a genetically engineered concentrate of human coagulation factor VIIa, which is structurally similar to native human plasma-derived factor VIIa. By enhancing thrombin generation on activated platelets, rFVIIa promotes the formation of a stable fibrin clot that is resistant to premature lysis [69]. This agent is Food and Drug Administration (FDA) approved for the treatment of haemophilia A and B patients with inhibitors against factors VIII and IX. Limited studies have shown correction of coagulopathy and decreased bleeding with the use of rFVIIa in patients with acute and chronic hepatic failure [70]. In these studies, rFVIIa was administered at doses ranging from 5 to 80 mg/kg i.v. for at least two doses [71–73]. Further studies are needed to identify the optimal dosing and confirm the safety, efficacy and cost–benefit of rFVIIa in patients with liver disease. Similarly, rFVIIa has been used to control bleeding associated with coagulopathy in patients undergoing orthotopic liver transplantation, including Jehovah's Witness patients [74]. Caution should be undertaken in administering rFVIIa to patients with DIC, coronary artery disease and severe sepsis because of their higher risk of thrombosis [75,76].

## Thrombopoietin

TPO is a relatively lineage-specific cytokine that stimulates megakaryocyte growth and maturation *in vitro* and is a potent *in vivo* thrombopoietic growth factor. This cytokine may be potentially useful for reducing bleeding in patients with thrombocytopenia due to liver disease or preparing these patients for liver transplantation [77], although it is not FDA approved to date.

## Conclusions

The liver plays a crucial role in haemostasis as it is responsible for the synthesis of most of the clotting and fibrinolytic proteins and the clearance of these coagulation factors from the circulation. Acute and chronic liver diseases are associated with a spectrum of haemostatic defects, and their severity tends to parallel the degree of hepatic injury. The aetiology of impaired haemostasis due to liver disease is multifactorial and includes impaired synthesis of coagulation factors, vitamin K deficiency, altered clearance of activated coagulation factors, excessive fibrinolysis, DIC and quantitative and qualitative platelet disorders. Standard laboratory testing should be supplemented with more specific tests of activation of the coagulation and fibrinolytic systems. The management of symptomatic haemostatic changes in patients with liver disease requires a multidisciplinary approach directed at correction of the haemostatic defects in patients who are actively bleeding or who require surgery or other invasive procedures.

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## 2.4.3 Function and metabolism of collagen and other extracellular matrix proteins

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### Introduction

The extracellular matrix (ECM) occupies a small percentage of the volume of the normal liver, yet plays a disproportionately important role in liver function in health and disease. ECM proteins are both signalling molecules and architectural elements of the liver and are responsible for maintaining the differentiated state of normal hepatocytes and non-parenchymal cells. In liver fibrosis and cirrhosis, there are changes in the distribution, quantity and relative proportions of collagens and other ECM proteins (Fig. 1); these result in altered cell phenotypes, architectural distortion with abnormal blood flow, impaired diffusion and altered cell signalling.

### ECM proteins in the normal liver

The normal liver encompasses physically separate regions with different matrix and cellular compositions. The matrix in the capsule and surrounding the bile ducts and central venous region is similar to that in other epithelial organs, with an organized basement membrane of collagen IV, laminin, entactin and perlecan. The interstitium of the portal space contains the fibrillar collagens I, III and V as well as collagen VI and fibronectin [1].

The space of Disse, which lies in between sinusoidal endothelial cells (SEC) and hepatocytes, has a matrix unique in the liver and the body. This narrow space, less than 1 μM wide, lacks the continuous laminin, perlecan and entactin that are found in most basement membranes [2]. Although collagen IV is present, it is in discrete discontinuous deposits not associated with laminin or perlecan. Fibronectin is abundant, applied closely to the microvilli of hepatocytes. Collagens III and VI are also found in the space of Disse, collagen III in discontinuous deposits and collagen VI arranged relatively homogeneously, increasing from the portal to the central region [3]. Structure is provided by a continuous network of thick collagen I cables, which extend into the lobular areas from the adjacent portal tracts [1]. This unusual matrix is essential for maintaining the differentiation of hepatocytes and SEC as well as other non-parenchymal cells [4]. Gradients of matrix material are found in the sinusoids and may have functional relevance, resulting in phenotypical differences between cells in the periportal vs. central regions [1,5].

### ECM proteins in the fibrotic liver

Fibrosis results in a nearly 10-fold increase in the expression of matrix proteins in the liver. The most impressive change is the capillarization of the sinusoids, in which the sparse, atypical matrix of the space of Disse is replaced by a complete and continuous basement membrane, with accompanying loss of fenestration of the sinusoidal endothelium. This process begins with an increase in cellular isoforms of fibronectin in the space of Disse, followed by increases in collagens I, III and IV and the appearance of laminin. As fibrosis progresses, portal to central gradients are lost, and the new matrix becomes continuous [6] (see also Chapters 4.1 and 6.1). This results in loss of the differentiated phenotype of the resident cells of the space of Disse, in particular SEC, hepatocytes and hepatic stellate cells (HSC). Additionally, the increased ECM impairs the normal exchange of soluble proteins and fluids between sinusoidal blood and adjacent liver cells.

When fibrosis advances to cirrhosis, the normal architecture of the liver is lost with the formation of increasingly dense fibrous septae of fibronectin and collagens I, III, V and VI. These bands of matrix may become progressively stabilized (for example through collagen cross-linking) and protease resistant, impeding remodelling and the resolution of cirrhosis.

### Structure and key features of specific ECM proteins

The ECM and its individual components are multifunctional. They have architectural and barrier functions, regulate growth factors and are themselves signalling molecules (Table 1). There are multiple complex interactions between different matrix components, making it difficult to understand the function of individual proteins. A variety of related mesenchymal cells in the liver synthesize normal and pathological matrix, suggesting that fibrosis is best understood as a generalized process rather than one limited to specific ECM molecules or specific fibrogenic cells such as HSC (see also Chapters 6.1 and 6.2).

**Fig. 1** (*opposite*) Distribution of major matrix components in normal and fibrotic liver**.** A liver acinus is shown graphically to demonstrate changes in extracellular matrix (ECM) distribution in normal and diseased liver. Note that continuity between portal, parenchymal and central structures is not shown due to controversy regarding their relative anatomy. The size of the space of Disse compared with the sinusoidal space is greatly exaggerated, particularly in the normal liver. The interrelationship between different ECM components is not well understood and is shown only schematically. The organized basement membrane shown contains collagens VIII, XIX, XV, XIV and XVIII in addition to collagen IV, laminin, perlecan, and entactin. Proteoglycans other than perlecan are not shown. PV, portal vein; CV, central vein; HA, hepatic artery; SC, hepatic stellate cell; EC, sinusoidal endothelial cell; PF, portal fibroblast; SD, space of Disse; BD, bile duct; H, hepatocyte; TS, thick, organized septa; S, sinusoid; P, portal tract.