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Portal vein thrombosis and hemostatic alterations in patients with advanced liver diseases

Driever, Ellen

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Fibrin clot properties and thrombus composition in cirrhosis

Ellen G. Driever, Ton Lisman

Abstract

Patients with cirrhosis frequently acquire profound haemostatic alterations, which may affect thrombus quality and composition, factors that determine the susceptibility to embolization and fibrinolysis. In this narrative review, we describe *in vitro* studies on fibrin clot formation and quantitative and qualitative changes in fibrinogen in patients with cirrhosis, and describe recent findings on the composition of portal vein thrombi in patients with cirrhosis. Patients with mild cirrhosis have increased thrombin generation capacity and plasma fibrinogen levels, which may be balanced by delayed fibrin polymerisation and decreased Factor XIII (FXIII) levels. With progressing illness, fibrinogen plasma levels decrease, but thrombin generating capacity remains elevated. Fibrinogen is susceptible to post-translational protein modifications and is for example hypersialylated and carbonylated in patients with cirrhosis. Despite changes in thrombin generation, FXIII levels and the fibrinogen molecule, fibrin fibre thickness and density are normal in cirrhosis patients. Paradoxically, fibrin clot permeability in cirrhosis patients is decreased, possibly due to post-translational protein modifications. Most patients have normal fibrinolytic potential. We have recently demonstrated that portal vein thrombosis is likely a misnomer as the material that may obstruct the cirrhotic portal vein frequently consists of a thickened portal vein wall, rather than of true thrombus. Patients with cirrhosis often have thrombocytopenia and anaemia, which may also affect clot stability and composition, but the role of cellular components in clot quality in cirrhosis has not been extensively studied. Finally, we summarize abstracts on fibrin formation and clot quality that were presented at the ISTH 2022 meeting in London.

Introduction

Patients with liver diseases were historically considered at risk of bleeding complications, mainly due to derangements of routine laboratory measures such as prolongation of the prothrombin time (PT), the derived international normalized ratio (INR) and thrombocytopenia. These tests, however, are only sensitive to deficiencies or defects in procoagulant factors, and neglect the simultaneous decline in anticoagulant factors in patients with liver diseases. Indeed, as a result of a reduced capacity of the liver to produce both pro- and anticoagulant factors, patients with liver diseases are in a rebalanced haemostatic state.[1] Rebalanced haemostasis in liver disease has for example been demonstrated by a normal to increased thrombin generation capacity using thrombomodulin modified calibrated automated thrombinography, including the activation of anticoagulant pathways by the addition of thrombomodulin.[2–4]

The net effects of the complex haemostatic alterations in liver diseases have been extensively described.[5] However, there has not been much attention to the quality and composition of thrombi in these patients, but this aspect is of great importance since quality and composition of a thrombus affect mechanical properties[6], which determine the susceptibility to embolization and fibrinolysis.[7] For example, abnormally dense fibrin structures were shown in patients with deep vein thrombosis, coronary artery disease and stroke, and these dense fibrin clots are more resistant to degradation by plasmin and alter mechanical properties by increasing clot stiffness.[8] In this narrative review, we will describe *in vitro* studies on fibrin clot formation and quantitative and qualitative changes in fibrinogen in patients with liver diseases. In addition, we will discuss changes in coagulation and fibrinolysis and the composition and structure of portal vein thrombi in patients with cirrhosis. Finally, we will shine a light on future directions in this field and summarize new data from abstracts on the topic of fibrin clot quality and composition that were presented at the ISTH 2022 annual meeting in London.

Plasma fibrinogen concentration in patients with cirrhosis
Fibrinogen is a soluble 340-kD glycoprotein that is primarily synthesized by hepatocytes and is present in the blood of healthy individuals at concentrations between 1.5 - 4 g/L. Levels of fibrinogen are normal to increased in patients with stable liver disease.[9] Levels decrease with increasing severity of disease and may drop below 1.5 g/L in patients with acutely decompensated cirrhosis (AD) and acute-on-chronic liver failure (ACLF)[10]. ACLF is a condition that is characterised by critical illness, with disease complications such as ascites, gastrointestinal bleeding and hepatic encephalopathy, and is associated with multi-organ failure and increased mortality.[11] In the general population, decreased levels of fibrinogen do not necessarily induce a bleeding risk, but are in fact associated with both bleeding and thrombotic complications.[12] In hospitalised patients with liver disease, low fibrinogen levels were a risk factor for bleeding.[13] However, whether there is a causal relationship between hypofibrinogenemia and bleeding in liver disease is unclear. For example, in a study with critically ill patients with cirrhosis it was shown that administration of cryoprecipitate to

correct low (<1.5 g/L) fibrinogen levels did not affect survival or bleeding complications, which suggested that low fibrinogen levels was an additional marker of severity of illness, but is not itself a direct factor of bleeding complications in these patients.[14] Indeed, despite low levels of fibrinogen in patients with AD or ACLF, in vitro formed clots from plasma of these patients have normal to thrombogenic properties. For example, normal clot lysis times and decreased clot permeability (a function of clot pore size) were measured in *in vitro* formed clots from acutely ill cirrhosis patients, suggesting that other factors than plasma fibrinogen concentration determine these clot properties.[15] Only when fibrinogen levels are very low (< 0.5 U/dL, for example during liver transplant surgery) clot stability parameters in ex vivo experiments are severely impaired.[16]

Fibrin formation in patients with cirrhosis
Fibrinogen is converted by thrombin to fibrin, which forms the scaffold of a thrombus. Fibrinogen consists of 2 sets of 3 different polypeptide chains: 2-Aα, 2-Bβ, and 2-γ chains, which are held together by disulfide bridges.[17] It plays a central role in clot formation and stabilisation, and is converted to cross-linked fibrin in several steps[18]: 1) proteolytic cleavage of thrombin causes the release of fibrinopeptides and formation of fibrin monomers; 2) linear association of fibrin monomers results in double-stranded protofibrils; 3) association of protofibrils results in formation of fibrin fibers; 4) Factor XIII (FXIII) facilitates covalent cross-linking of polymerised fibrin.

The process of fibrin clot formation and stabilisation and potential changes in the process in patients with liver diseases are outlined in Figure 1, and described hereafter. In the first step, thrombin cleaves fibrinopeptides of the Aα and Bβ chains to produce fibrin monomers. Thrombin levels affect the fibrin clot, with higher levels of thrombin producing a dense network of relatively thin fibrin strands, resulting in less permeable clots and resistance to fibrinolysis.[19,20] Increased thrombin levels have shown to affect viscoelastic properties of a clot as assessed with thromboelastography (TEG), for example increased maximum amplitude (which represents the ultimate strength of a fibrin clot) and increased α-angle (which represents the speed of fibrin formation).[21,22] Patients with cirrhosis have normal to increased thrombin generation[23], which may suggest that levels of thrombin are not largely affecting the fibrin clot structure in patients with liver disease. Most stable cirrhosis patients show normal TEG parameters, and hypo- or hypercoagulable TEG profiles were correlated with platelet counts and plasma fibrinogen levels.[24]
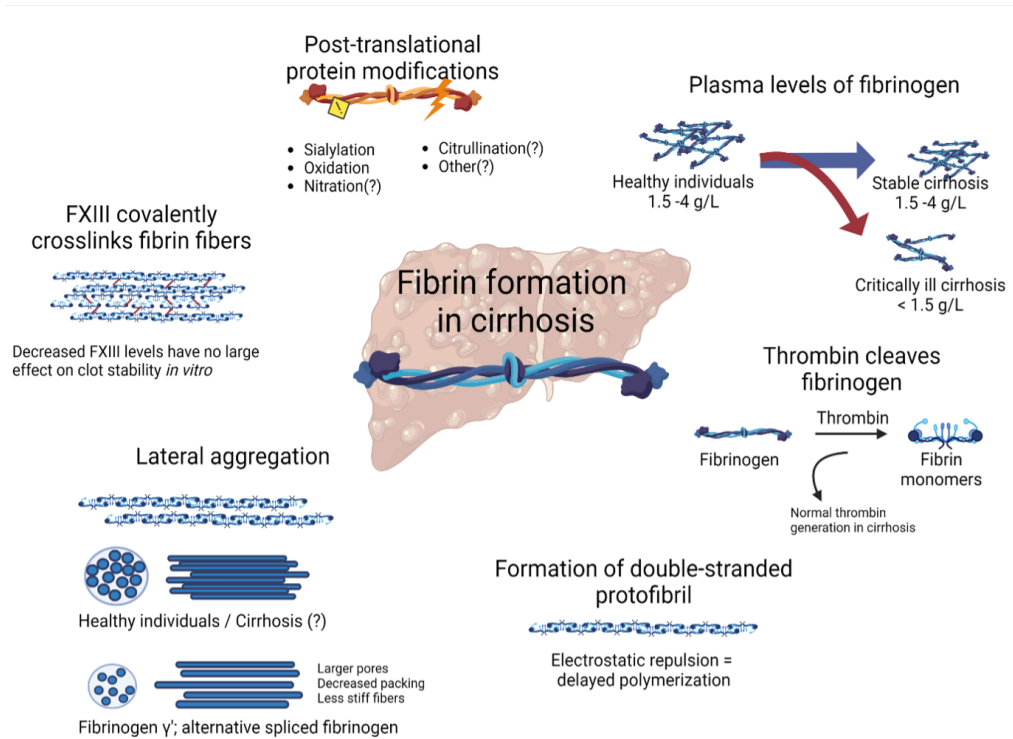
Figure 1. Fibrin formation and fibrinogen properties in patients with cirrhosis. Created with BioRender.

The second step of fibrin clot formation is the linear association of fibrin monomers, resulting in double-stranded protofibrils. Fibrin polymerisation assays have shown that this step is markedly delayed in patients with liver diseases, with 76% of cirrhosis patients and 86% of acute liver failure patients showing abnormal fibrin polymerisation rates.[25,26] This delay in polymerisation has been explained by increased sialylation of the fibrinogen molecule in patients with liver disease.[27] Sialylation is a post-translational protein modification, and is a form of glycosylation in which sialic acid is bound at the end of a sugar chain of the protein. It has been suggested that sialic acid inhibits polymerisation of fibrin by electrostatic repulsion between fibrin monomers, that impairs the fibrin monomers to interact with each other.[28] Sialic acid residues are neutralized by calcium, but with increased levels of sialic acid and normal calcium levels, the low affinity interaction between sialic acid and calcium is inadequate to neutralize the excessive charge repulsion between hypersialylated fibrin molecules, resulting in delayed fibrin polymerisation.[28]

In the third step, double-stranded protofibrils are connected to form fibrin fibers. This association can be affected by thrombin concentration and the presence of an alternatively spliced form of fibrinogen, fibrinogen γ'. This form of fibrinogen causes a partially impaired protofibril formation, again likely due to electrostatic repulsion.[29] Higher levels of fibrinogen γ' have been associated with decreased protofibril packing and less stiff fibrin clots.[30] A study conducted by our group showed normal levels of fibrinogen γ' in cirrhosis patients. In

addition, similar fibrin fibre density and fibre diameter between cirrhosis patients and healthy individuals were found, suggesting that the intrafibrillar structure is not altered in cirrhosis.[31]

Lastly, FXIII, a transglutaminase, is activated by thrombin to FXIIIa, which stabilizes the fibrin clot by forming covalent bonds between fibrin fibers. Thereby, it defines structure, stability, and effector functions of fibrin.[32] Patients with cirrhosis have decreased levels of FXIII[15], which would theoretically lead to less stable fibrin networks. *In vitro* experiments of fibrin clot structure with plasma from healthy controls and from patients with AD and ACLF showed very minor effects on clot parameters after the addition of exogenous FXIII concentrate, despite markedly decreased FXIII levels and reduced clot permeability in patients compared to controls.[15] Nevertheless, it was shown that FXIII does contribute to clot stability in these assays since complete inhibition of FXIIIa activity by T101 (a transglutaminase inhibitor) shortened clot lysis time and lowered permeability in pooled normal plasma. In addition, results of a clot retraction assay (in which isolated platelets and red blood cells (RBCs) from a healthy donor are mixed with patient plasma, and subsequently coagulation is initiated with tissue factor and calcium and the red cell extrusion is measured after 2 hours of clot formation[33] showed a slightly increased clot weight and a reduced percentage of extruded RBCs in patients compared to controls despite lower FXIII and fibrinogen levels. Again, full inhibition of FXIII in normal plasma decreased clot weight and increased RBC extrusion, showing that the assay is sensitive for FXIII. In this assay, clot weight is determined by the amount of RBCs that retain within the clot, which also determines the clot size.[34] It could be that only very small amounts of FXIII are necessary for clot protective effects in the performed assays in this study.[15]

Post-translational modifications of fibrinogen in cirrhosis
Acquired dysfibrinogenemia, a term used for altered functionality of the fibrinogen molecule, is common in patients with liver diseases.[35] Dysfibrinogenemia can be a result of post-translational protein modifications of fibrinogen, of which several are known to affect the function of fibrinogen and therefore affect clot formation and clot characteristics.[36] Fibrinogen in liver disease is altered compared to healthy individuals with increased sialylation (a form of glycosylation) and oxidation.[37–39]

Hypersialylation leads to delayed fibrin polymerisation rates due to electrostatic repulsion between fibrin monomers, as described in the previous section.[28] A recent study performed by our group showed that, despite delayed fibrin polymerisation rates in patients with cirrhosis, fibrin clot permeability was decreased, suggesting that although clot formation is delayed, once these clots are formed they are more thrombogenic compared to healthy controls.[31] Conversely, visual analysis of fibrin clots of glycosylated fibrinogen from healthy donors showed thinner, more branched fibrin bundles with a more porous network and decreased turbidity, suggesting that glycosylation results in a visually less thrombogenic clot structure.[40] Interestingly, when fibrin clots from cirrhotic patients where visualized with

electron microscopy, fibre thickness and density were similar compared with controls.[31] These findings suggest that sialic acid content of fibrin(ogen) in cirrhosis may affect polymerisation rates and decreased permeability, but that the structure of a matured clot may be affected by other mechanisms than sialylation of fibrinogen alone. For example, carbonylation and nitration of fibrinogen (which may be increased in patients with cirrhosis due to oxidative stress and subsequent production of reactive oxygen and -nitrogen species) have distinct effects on clot structure. Nitration of fibrinogen has for example been shown in hepatic fibrin deposits in acetaminophen-induced acute liver failure in mice, and was associated with delayed fibrin polymerisation rates and reduced clot turbidity.[41] Nitration of normal donor-fibrinogen has shown large bundles of thin fibrin fibers with large pores between the fibers in one study[42], but showed more thrombogenic clot properties in another study.[43] Carbonylation (a form of oxidation) of fibrinogen has been proposed to increase the thrombogenicity of fibrin clots, for example in the context of patients with acute myocardial infarction.(44) Nevertheless, conflicting reports on the effects of fibrinogen carbonylation on fibrin structure and function have appeared in literature. For example, one study demonstrated decreased fibrin polymerisation with thinner fibres and resistance to lysis in hypercarbonylated fibrinogen from patients with myocardial infarction[44], whereas another study showed enhanced polymerisation in a similar setting.[45] Interestingly, fibrinogen carbonyl content is increased in patients with cirrhosis, and was inversely related with clot permeability.[31] It could be that in patients with cirrhosis, multiple post-translational protein modifications have opposing effects on clot structure, resulting in a net neutral effect with a clot structure that is indistinguishable from controls when examined with electron microscopy or immunofluorescence techniques. Our observations on decreased permeability with normal clot structure may reflect the complex posttranslational changes in the fibrinogen molecule. For example, decreased permeability, which is normally accompanied by alterations in clot architecture, may in patients with cirrhosis be a result of increased hydrophobicity of the clot that results in enhanced retention of fluid within the clot.

Besides sialylation, nitration and oxidation of fibrinogen, other posttranslational modifications may also affect fibrinogen in patients with liver diseases. For example, citrullination of proteins by peptidyl arginine deiminase (PAD) enzymes[46], possibly modifies the fibrinogen molecule in patients with liver disease. PAD enzymes, specifically PAD4, play an important role in the formation of neutrophil extracellular traps (NETs), and NET formation have been demonstrated to play a role in progression and complications of liver disease.[47] The presence of citrullinated fibrinogen was shown in other pathologies, such as rheumatoid arthritis.[48] Citrullination of fibrinogen resulted in thinner fibrin fibers with increased fibre density and lower clot permeability.[49] Citrullination of fibrinogen and its potential consequences in liver diseases should be subject to future research. In addition, as fibrinogen is one of the most abundant plasma proteins and is very susceptible to modification, other post-translational protein modifications, such as inflammation-associated changes in fibrin fibre thickness[50], may also have effect on fibrin in patients with liver diseases. Future studies are required to

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study these changes and investigate the effect on clot properties.

Fibrinolysis and permeability of fibrin clots in cirrhosis
We have studied the stability of clots generated from plasma from patients with chronic liver diseases using two distinct assays, a plasma-based clot lysis test and a clot permeability assay. Both tests are sensitive for levels and function of key proteins involved in clot formation and breakdown.[51,52] Historically, patients with cirrhosis were classified as hyperfibrinolytic.[53] A hyperfibrinolytic state in cirrhosis patients has been linked to decreased levels of antiplasmin and thrombin activatable fibrinolysis inhibitor (TAFI), or increased levels of tPA and was described to potentially contribute to bleeding complications in these patients.[54,55] More recent studies used global assays of fibrinolysis, and found a normal fibrinolytic phenotype in patients with compensated or stably decompensated cirrhosis, which was explained by a simultaneous decline in pro- and antifibrinolytic factors.[9,56] Notably, in these studies individual patients had hypo- or hyperfibrinolytic profiles.[9,56] Others, however, have shown hyperfibrinolysis on a group level using similar methodology and apparently similar patients. The discrepancies may be explained by differences in methodology and by differences in selected patients.[54,56–59] For example, in patients with mild cirrhosis caused by non-alcoholic fatty liver disease or cholestatic liver disease, we demonstrated hypofibrinolytic profiles, which were not present in patients with cirrhosis related to alcohol or viral hepatitis.[9,60] Finally, although patients with stable cirrhosis may have hyperfibrinolytic features based on laboratory measures, hyperfibrinolysis-related bleeding complications in these patients are exceedingly rare.[58]

Whereas patients with relatively stable liver disease mostly have normal fibrinolytic phenotypes, a recent study from our group has shown that AD and ACLF patients, with higher disease severity and additional disease complications such as presence of ascites or development of hepatic encephalopathy, have a very variable fibrinolytic phenotypes.[61] Patients with AD were primarily hyperfibrinolytic and patients with ACLF were primarily hypofibrinolytic. Patients with ACLF and hypofibrinolysis often had sepsis. Indeed, sepsis in patients without underlying liver disease is often accompanied by a hypofibrinolytic state, which can be explained by high levels of plasminogen activator inhibitor-1 (PAI-1) in patients with sepsis.[61–63] Other co-morbidities such as diabetes mellitus type II or use of drugs such as anticoagulants or statins, also affect fibrinolysis.[64] How these factors affect fibrinolysis in patients with liver disease has not yet been established and should be subject to further research.

Clot permeability, another key measure of clot structure and function, is decreased in patients with cirrhosis when measured using an experimental set-up in which permeation is tested by measuring fluid permeation through the clot by the force of gravity. These thrombogenic clot properties were observed despite the delayed fibrinogen to fibrin conversion and was present even in patients with decreased plasma fibrinogen levels. Notably, decreased permeability

was observed despite unaltered fibrin fibre thickness or pore size within the clot.[31] To better understand these paradoxes, we recently reanalysed fibrin permeation and compared fibrin clot permeability assessed by the force of gravity, to permeability assessed by compressional force, where a clot is formed between two parallel plates of a rheometer, and fluid is pressed out of the fibrin network by lowering the upper plate.[65] We found that under the force of gravity, permeability is decreased with increasing severity of disease. In contrast, when permeability was assessed using rheometry, no differences in permeability were observed between patients and controls (unpublished data). Ongoing studies are addressing the reason for this discrepancy. We hypothesise that the studies under the force of gravity may show decreased permeability not because the clot is truly more thrombogenic, but because the increased negative charge in the clot retains water. In studies under compressional force, the electrostatic repulsion may not be strong enough to retain water in the fibrin network.

Clot composition in cirrhosis

The major determinant of clot stability and quality is fibrin, and effects of alterations in fibrinogen and fibrin have been extensively studied in patients with liver diseases as discussed in the previous sections. Other components of the thrombus, such as platelets and red blood cells, also contribute to clot stability and quality, but these have not been studied in patients with liver diseases yet. Patients with liver diseases often have thrombocytopenia, anaemia and changes in white blood cell counts and function,[66] and these alterations may affect thrombus composition and mechanical characteristics.[67,68] The composition of venous and arterial thrombi has been extensively studied in the general population[69], but data from patients with liver diseases are not available yet.

Venous thrombi consist mainly of RBCs and fibrin.[69] Upon clot contraction of venous thrombi, a phenomenon driven by activated platelets, RBCs undergo deformation and are displayed in a polyhedral shape.[70] Contraction of venous thrombi causes volume shrinkage of the thrombus, and determines the degree of vessel obstruction and the likelihood of thrombus mechanical rupture, which may lead to thrombotic embolization.[71,72] The majority of RBCs within a thrombus have a polyhedral shape, and are called polyhedrocytes.[70]

Recently, we studied the composition of portal vein thrombi in patients with cirrhosis who underwent liver transplantation[73], and observed RBCs in their biconcave shape instead of the polyhedral shape in those thrombi (see Figure 2).[71,74] This interesting and unexpected finding suggests that the portal vein thrombus in cirrhosis does not have the same features as other venous thrombi have. Future studies should investigate whether platelet activation differs in these thrombi or whether RBCs in cirrhotic portal vein thrombi are not able to deform into a polyhedral shape, for example due to changes in the lipid composition of the red cell membrane.[75,76]
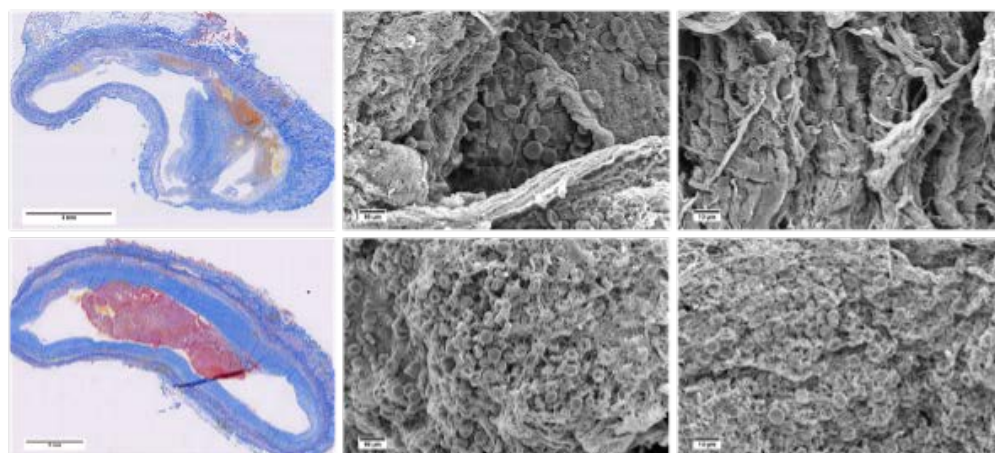
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Figure 2. Composition of non-malignant portal vein thrombi in patients with cirrhosis. Upper panel: MSB-stained section (left) of an extrahepatic portal vein thrombus which shows intimal hyperplasia with some haemorrhage, but no fibrin. The scanning electron microscopy images of the same thrombus sample (middle and right images of the upper panel) show collagen bundles, but no fibrin and few erythrocytes. Lower panel: MSB-stained section (left) of a portal vein thrombus which shows intimal hyperplasia with a fibrin-rich thrombus within the lumen of the vessel. The scanning electron microscopy images (middle and right images of the lower panel) show a fibrin mesh with erythrocytes entrapped. Note that most erythrocytes are in their biconcave shape and not in a polyhedral shape that is often observed in contracted blood clots or thrombi.

Moreover, in this study on the structure and composition of cirrhotic portal vein thrombi, we found that the thrombi lacked a fibrin-rich part in two-thirds of the cases, whereas all thrombi consisted of a thickened, fibrotic vessel wall (see Figure 2). In other words, portal vein thrombi often lack 'classical' thrombus components that are uniformly present in thrombi isolated from patients with deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke.[69,77] Rather, portal vein thrombi appear to consist of intimal fibrosis, in some cases overlaid by a fibrin-rich clot. These observations suggest that the term "portal vein thrombosis" may be a misnomer and that "portal vein stenosis" or "non-malignant portal vein occlusion" may be more appropriate. We propose two possible mechanisms by which portal vein intimal fibrosis develops: 1) an initial fibrin-rich thrombus organizes into a fibrotic structure that re-endothelializes over time; and/or 2) intimal fibrosis develops in the absence of overt initial fibrin formation, and is a result of for example portal hypertension and vascular endothelial cell stress. These findings may also explain why portal vein thrombi do not always recanalize after treatment with anticoagulant therapy. The current pharmacological treatment of portal vein thrombosis consists of low molecular weight heparins or direct oral anticoagulants, but this therapy does not always lead to recanalisation of the portal vein.[78] Treatment with anticoagulants seems most effective in more recently formed thrombi, which may consist of fibrin. A thrombus that only contains fibrotic tissue and no fibrin is not likely to respond to anticoagulants. Future studies should specifically focus on the pathophysiology and prevention and treatment of portal vein intimal hyperplasia.

Conclusions and future directions

In conclusion, the coagulation system of patients with compensated or stably decompensated cirrhosis is in a rebalanced state, characterized by normal to increased thrombin generation and plasma fibrinogen levels. Increased thrombin generation capacity and fibrinogen levels may be balanced by delayed fibrin formation and decreased FXIII levels compared to healthy subjects. Interestingly, despite changes in the fibrinogen molecule, FXIII levels, and thrombin generation, fibrin fibre density and thickness is normal in cirrhosis patients. Paradoxically, permeability of clots formed with plasma from cirrhosis patients is decreased, suggesting a more thrombogenic structure. These findings may be explained by posttranslational protein modifications of fibrinogen in liver disease, notably oxidation. Interestingly, when permeability was measured under compression, no differences were found between patients and controls, suggesting that the permeation under gravity assay overestimates thrombogenic capacity of clots from cirrhotic patients, possibly by retention of water as a result of hypersialylation.

It would be interesting to study the deformational changes of the fibrin clots under compressional forces in more detail, for example by analysing clot stiffness in between subsequent compression steps or by analysis with scanning electron microscopy. Such experiments would give more insights into the strength and stability of the fibrin fibers.
Fibrin clot quality in patients with AD or ACLF may be different from stable cirrhosis patients as fibrinogen and FXIII levels are substantially decreased in these patients. Indeed, fibrinolysis is increased in patients with AD and markedly impaired in patients with ACLF, which was ascribed to increased levels of PAI-1. Other parameters of fibrin clot formation and fibrin clot quality could also be altered in critically ill patients with cirrhosis. For example, systemic inflammation in these patients causes alterations in the hemostatic state,[79] may increase fibrinogen protein modifications and affect fibrin polymerisation rates and permeability of fibrin clots.

Future studies on thrombus quality and stability in patients with liver diseases should include effects of platelets and red blood cells. Patients with liver diseases are thrombocytopenic and often have anaemia, factors that affect clot characteristics.[66,68] In addition, our recent findings on portal vein thrombi composition and structure raised several questions. For example, future studies should investigate clot contraction and deformation of RBCs to polyhedrocytes in cirrhotic portal vein thrombosis and other thrombotic complications in cirrhotic patients. Further research is also required to gain more knowledge about the pathophysiology of portal vein thrombosis in cirrhosis, since we found that it consists of fibrin-rich thrombi in only one-third of the cases, whereas all cases had intimal thickening of the vessel wall. More research is required to provide more rational preventive and treatment strategies in cirrhosis patients with portal vein thrombosis.

Taken together, patients with cirrhosis have unique alterations in fibrinogen concentration, post-translational modification of the fibrinogen molecule, and decreases in FXIII concentration. Despite these extensive alterations, clot formation appears remarkably preserved, which may relate to prothrombotic post-translational modifications such as oxidation. These insights may help interpret findings of hypofibrinogenemia in cirrhosis and question liberal infusion of fibrinogen concentrate. Clinical studies are required to address in which situations fibrinogen concentrate may be beneficial in these patients.

ISTH London 2022 report

A number of abstracts on clot quality and composition were presented at the ISTH 2022 congress in London. In this section, we will briefly summarize studies on the topic of fibrin clot stability and composition.

The group of Dr. Luyendyk uses murine models to investigate intrahepatic microthrombosis in acetaminophen-induced acute liver injury. An abstract that was presented at the ISTH 2022 by Poole and co-workers describes the effects of acetaminophen overdose in mice with a mutation in the fibrinogen γ-chain, making fibrin(ogen) incapable of interacting with platelet integrin αIIbβ3 (*FibγΔ5*). It was previously shown that acetaminophen overdose causes accumulation of fibrinogen and platelets in the injured liver[80,81], but the mechanisms initiating platelet accumulation in the injured liver are not understood. Poole et al. [82] hypothesized that hepatic platelet accumulation in the acetaminophen-injured liver is mediated by fibrin(ogen) engagement of the platelet integrin αIIbβ3. Surprisingly, *FibγΔ5* mice had modestly higher platelet accumulation compared to wild-type mice after acetaminophen challenge. In addition, the *FibγΔ5* mice had enhanced hepatic accumulation of high molecular weight crosslinked γ-chain multimers of fibrin, but no γ-γ dimer formation. Fibrin clots from the mutant mice were denser and consisted of thinner fibers compared to the clots in wild-type mice. The effect was independent of platelet integrin αIIbβ3-fibrinogen interactions, as treatment of wild-type mice with an αIIbβ3 inhibitor had no effect on acetaminophen-induced hepatic necrosis. The aberrant fibrinogen cross-linking may exacerbate liver injury following acetaminophen-overdose.

More abstracts studied crosslinking of α- and γ-chains of fibrin. These crosslinking events increase clot stiffness and stabilize the final clot. In their study presented at the ISTH 2022, Feller et al. [83] describe a mouse model where α-α crosslinking was impaired by the introduction of 4 point-mutations in the fibrinogen α-chain. Clots made with plasma obtained from these mice rupture at lower stress and have reduced toughness compared with clots made of plasma from wild-type mice. In another abstract, this research group [84] described their study on the impact of impaired α-crosslinking on venous thromboembolism in the mouse model. Clotting time was delayed, clot firmness and clot contraction were reduced, and the mutant mice had higher risk of embolization. In a

previous study, the authors described that in the absence of γ-chain crosslinking[85], fibrin fibers rupture at lower stress and have reduced toughness, resulting in more frequent embolization of clots in a mouse model of vena cava thrombosis. Based on the results from these studies, the investigators conclude that deficiency in both α- and γ-chain crosslinking result in fibers that are more prone to rupture, and that α- and γ-crosslinking play complementary roles in generating key biochemical properties of fibrin clots for the prevention of embolism.

A study presented by Fish et al.[86] showed the effects of fibrinogen γ-chain knock-down in larval zebrafish and of a large deletion in the zebrafish fibrinogen γ-gene. Knock-down of fibrinogen γ-chain expression prevented laser-induced venous thrombosis, which was assessed by measuring the time until occlusion of the vessel. The authors also produced zebrafish with a deletion in the fibrinogen γ-gene, which showed no blood coagulation or thrombosis after laser-induced injury. The authors proposed that this model can serve as a stable knock-out background to study the effects of specific mutations of fibrinogen γ-chain expression.

Several studies described fibrin clot properties such as permeability and susceptibility to lysis in *in vitro* experimental set-ups. For example, Klajmon et al[87] described reduced clot permeability and prolonged clot lysis times in fibrin clots made with plasma from patients with genetically confirmed antithrombin deficiency. The findings suggest a mechanism by which antithrombin deficiency contributes to thrombosis risk. Another study, by Smith and Morrissey[88], described that fibrinogen that was incubated with a neutrophil-released enzyme (cathepsin G, for example released during inflammatory responses) causes cleavage of fibrinogen, which results in faster polymerisation rates of fibrin induced by thrombin, higher clot turbidity, weaker clots, and decreased lysis times compared to control. The authors conclude that release of cathepsin G from activated neutrophils may affect clot formation in the presence of an ongoing inflammatory response, with potential consequences for thrombosis.

Another interesting abstract was presented by Risman et al.[89] about clot contraction and its impact on fibrinolysis. By using a combination of mathematical modelling and experimental methodologies to characterize the process of external fibrinolysis, the authors aimed to understand how key structural changes mechanistically drive the decrease in fibrinolysis of contracted blood clots. In their modelling and experimental approach, the densification of fibrin was the most significant determinant of the rate of fibrinolysis, which the authors ascribed to reduced diffusion of tPA in the clot. These findings may potentially be used therapeutically to optimize timing and delivery of lytic agents.

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