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Platelets as modulators of liver diseases

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Abstract

Platelets are key players in thrombosis and hemostasis. Alterations in platelet count and function are common in liver disease, and may contribute to bleeding or thrombotic complications in liver diseases and during liver surgery. In addition to their hemostatic function, platelets may modulate liver diseases by mechanisms that are incompletely understood. Here we will present clinical evidence for a role of platelets in progression of chronic and acute liver diseases including cirrhosis, acute liver failure, and hepatocellular carcinoma, and will present clinical evidence that platelets promote liver regeneration following partial liver resection. Subsequently, we will summarize studies in experimental animal models that support these clinical observations, but will also highlight studies that are in contrast with clinical observations. The combined results of clinical and experimental studies suggest that platelets may be a therapeutic target in treatment of liver injury and repair, but the gaps in our understanding of mechanisms involved in platelet-mediated modulation of liver diseases call for caution in clinical application of these findings.

Keywords

Platelet; liver; cirrhosis; regeneration; inflammation

Introduction

Platelets in liver disease – alterations and consequences for hemostasis

Chronic and acute liver diseases are frequently accompanied by complex alterations in the hemostatic system¹. These changes include decreased plasma levels of proteins involved in coagulation and fibrinolysis that are synthesized by hepatocytes. Causes and consequences of the changes in plasmatic coagulation and fibrinolysis have been reviewed elsewhere²⁻⁴. In addition, thrombocytopenia and alterations in platelet function are common^{5,6}.

Thrombocytopenia develops as a result of decreased platelet production, increased platelet turnover, increased consumption, and/or increased splenic sequestration. Platelet production may be decreased due to decreased production of thrombopoietin, which appears to

contribute significantly to thrombocytopenia in patients with cirrhosis⁷, but this mechanism does not appear to explain the thrombocytopenia of acute liver failure (ALF)⁸. In addition, platelet production may be compromised as a result of direct toxic effects of alcohol or the hepatitis C virus, for example, on the bone marrow^{9,10}. The development of auto-antibodies targeting platelets decreases platelet half-life and may contribute to thrombocytopenia in patients with cirrhosis¹¹. Platelet consumption may occur by localized or disseminated activation of hemostasis¹²⁻¹⁴ and multiple processes including generation of thrombin, exposure of fibrotic tissue (i.e., collagens), and activation of endothelial cells which may result in (continuous) platelet activation and subsequent decreases in the circulating platelet count. Finally, splenomegaly, which occurs as a consequence of portal hypertension may result in a dramatic increase in the proportion of the total platelet mass that is sequestered within the spleen¹⁵.

Thrombocytopenia is common in patients with cirrhosis and in patients with ALF, but rarely becomes severe. Platelet counts <50,000/ μ l are rare in both cirrhosis and ALF, even in critically ill patients^{3,16,17}. Besides a decreased platelet count, platelet function appears altered in patients with liver disease, although these functional defects are poorly understood. It has long been generally accepted that platelet function in cirrhosis was compromised by a combination of intrinsic defects and inhibition of platelet function by plasma-derived factors⁶. More recent studies, however, suggest *ex vivo* platelet function to be increased in patients with cirrhosis and provide evidence for increased *in vivo* platelet activation. Some of these studies point to technical flaws in earlier studies^{18,19}, but technical issues of these recent studies have also been criticized^{20,21}. Additional recent studies suggest platelet function to be unaltered in cirrhosis^{22,23}, which clearly indicates the need for additional studies to solve the controversy of platelet functionality in patients with liver diseases. Regardless of the functional status of platelets, it has been demonstrated using *in vitro* studies that the highly elevated plasma levels of von Willebrand factor (VWF) and the low levels of the VWF-cleaving protease a disintegrin-like metalloproteinase with thrombospondin motif type 1 member 13 (ADAMTS13) compensates for the thrombocytopenia of liver disease²⁴⁻²⁶. Overall, therefore, the primary hemostatic system appears much more preserved than suggested by the platelet count alone, and this 'rebalance' in primary hemostasis is in line with the concept that the entire hemostatic system in patients with liver diseases is in a 'rebalanced' status due to simultaneous changes in both pro- and antihemostatic processes^{2,3}. Indeed, the bleeding risk of patients with liver diseases appears low, and is in some²⁷, but not all²⁸, studies unrelated to the circulating platelet count. We and others have therefore advocated a restrictive use of prophylactic platelet transfusions in patients with liver diseases undergoing invasive procedures given the uncertain efficacy and notable side effects^{27,29}. Of note, administration of platelet concentrate has been shown to have very little effect on global hemostatic capacity³⁰. Taken together, the primary hemostatic system of patients with cirrhosis has both pro- and antithrombotic features, which in individual patients may contribute to bleeding or thrombosis.

Platelets and liver surgery – alterations and consequences for hemostasis

In surgical procedures involving the liver, notably partial liver resections and liver transplantation, substantial perioperative changes in hemostatic components may occur³¹. These changes may occur in patients that had a normal preoperative hemostatic status, such as in the non-cirrhotic patient undergoing partial hepatectomy for metastatic disease or the patient with a disorder such as familial amyloid polyneuropathy requiring a liver transplant. Perioperative hemostatic changes may also occur in patients that had cirrhosis-related hemostatic changes preoperatively. During the intraoperative and early post-operative phase, platelet numbers usually decline^{32,33}, likely due to consumption and hemodilution. Platelet counts normalize following surgery, but in contrast to patients undergoing major surgical procedures not involving the liver, post-operative thrombocytosis does not occur^{34,35}, which may relate to defective platelet production, potentially due to decreased thrombopoietin production as a result of transiently decreased liver function, following liver surgery³⁶.

In non-cirrhotic patients undergoing partial hepatectomy, we have demonstrated a profound and sustained increase in plasma VWF levels with substantially decreased ADAMTS13 levels³⁷, which may relate to ongoing inflammation-associated VWF release with concomitant ADAMTS13 consumption^{38,39}. This VWF/ADAMTS13 unbalance resulted in an increased capacity to support platelet adhesion and aggregation under conditions of flow. A similar VWF/ADAMTS13 unbalance is present in patients with cirrhosis²⁴, and we have demonstrated exacerbation of this unbalance following liver transplantation⁴⁰. In light of the (near) normal post-operative platelet counts in patients undergoing liver surgery, we speculate that the VWF/ADAMTS13 unbalance may contribute to post-operative thrombotic complications. Thus, whereas platelet transfusions may be indicated in the intraoperative phase when active bleeding is evident, post operatively the primary hemostatic system appears hyperfunctional, and antihemostatic interventions may be helpful in diminishing thrombotic risks⁴¹.

Extrahemostatic effects of platelets

Beyond a well-appreciated role in hemostasis and thrombosis, platelets are known to contribute to the pathogenesis of a broad spectrum of diseases. Platelets have well-appreciated roles in physiological and pathological processes including inflammation, tissue repair, angiogenesis, and tumor growth⁴². Upon activation, platelets release a plethora of stored mediators capable of modifying cellular responses, particularly inflammation, at the site of activation⁴³. These mediators can have direct effects on various cell types including endothelium and inflammatory cells⁴⁴. Mediators released by platelets include arachidonic acid metabolites, cytokines, proteases and growth factors⁴⁵. Moreover, activated platelets express an array of cellular adhesion molecules driving both local cell activation processes as well as reciprocal activation of platelet processes such as aggregation. Collectively, and depending on context, appropriately timed release of platelet-derived mediators can have powerful effects on both disease progression and tissue repair through effects on numerous cell types, including parenchymal cells, vascular endothelium, and a spectrum of inflammatory cells. Thus, while the hemostatic function of platelets is well-recognized, strong evidence indicates that anti-platelet therapies could have profound disease-modifying effect by modulating extrahemostatic platelet functions. In this review, we will focus on the

role of both hemostatic and extrahemostatic platelet functions in liver injury, disease, and regeneration.

Clinical evidence for a role of platelets in modulation of liver diseases

Platelets have been implicated in progression of liver diseases and in liver regeneration following liver surgery in humans, mostly by retrospective epidemiological studies, which will be discussed in detail below. These studies are not only difficult to interpret due to their retrospective nature, but also as a result of the complex changes in platelet count and function in many of the cohorts that have been studied. In addition, part of the evidence relies on association with aspirin use. Although aspirin is well known for its platelet-inhibitory properties, it interferes with other processes which may explain the relation between aspirin use and modulation of liver diseases. We will first outline the clinical evidence for a contribution of platelets in modulation of liver diseases and then provide evidence from experimental models which, in part, mechanistically explain the clinical correlations.

Platelets in progression of chronic liver diseases

Four recent studies on aspirin use suggest that platelets may be related to progression of chronic liver diseases. The first report retrospectively studied 188 patients that had received a liver transplant for hepatitis C⁴⁶. A substantial proportion of patients transplanted for hepatitis C have clinically significant disease recurrence, with cirrhosis in the graft within 5 years in 25-35% of patients. Some of the patients in this multicenter cohort were on long-term aspirin treatment for prevention of hepatic artery thrombosis. In a multivariable analysis, aspirin use was associated with a 35% decreased risk of having stage F2 fibrosis on biopsy after a median follow up of 2.7 years. A second study analyzed data from the National Institutes of Health - American Association of Retired Persons (NIH-AARP) and Health Study Cohort representing the general population in the United States⁴⁷. Among over 300,000 individuals, 428 died of chronic liver disease (excluding hepatocellular carcinoma [HCC]) during follow up. In this cohort, aspirin users had a 50% reduced risk of death due to chronic liver disease. A third study was a cross-sectional analysis of the Third National Health and Nutrition Examination Survey (NHANES III), which contains a representative sample of the general population in the United States. In 11,416 adults who underwent ultrasonography for assessment of non-alcoholic fatty liver disease (NAFLD), 2889 individuals were identified as having NAFLD, whereas the remainder served as controls. In multivariable analysis, aspirin use was shown to be associated with a decreased risk of NAFLD, primarily among men and older individuals⁴⁸. Also in the NHANES III study, aspirin use was associated with a decreased fibrosis score as assessed with non-invasive indices in 1856 individuals with suspected chronic liver disease⁴⁹. Importantly, ibuprofen (which lacks antiplatelet activity) was not associated with decreased fibrosis.

A population-based cohort in the Netherlands measured plasma levels of VWF in 1228 individuals. VWF levels were associated with liver fibrosis as assessed by liver stiffness measurements using transient elastography after 10 years of follow-up, and associations

remained after adjustment for potential confounders (https://repub.eur.nl/pub/79702/160218_Plompen-Elisabeth-Petrus-Cornelia.pdf, page 107).

In aggregate, these studies suggest a link between platelet function and progression of chronic liver disease. Potential mechanisms underlying these associations will be discussed later in this review. Importantly, many alternative explanations for these findings deserve attention. For example, aspirin has many platelet-independent effects⁵⁰ that potentially could affect progression of chronic liver disease⁵¹.

Platelets in progression of ALF

The ALF Study Group in the United States analyzed changes in the platelet count over time in 1598 patients during the course of their illness⁵². It was demonstrated that those patients with a poor outcome (death or requirement for liver transplantation) develop a more profound thrombocytopenia in the first week after admission. In addition, it was shown that platelet counts in the first week of admission were lower in patients who developed the systemic inflammatory response syndrome, patients that required vasopressors or renal replacement therapy, and in patients that developed hepatic encephalopathy⁵². The development of thrombocytopenia likely involves platelet activation, for example by thrombin or inflammatory stimuli derived from activated leucocytes or endothelial cells⁵³. Importantly, thrombocytopenia was accompanied by an increase in platelet-derived tissue factor-positive microparticles, and the extent of microparticle release was proportional to the severity of the systemic inflammatory response syndrome⁵⁴. Activated platelets and the procoagulant microparticles have been proposed to drive progression of disease by causing intrahepatic microischemia and by enhancing inflammatory responses (see section on ‘potential mechanisms’). Importantly, in patients with sepsis, platelets have been proposed to contribute to disease progression and end-organ failure through similar mechanisms⁵⁵. Additional analyses of the ALF study group data have demonstrated that patients that receive platelet transfusions have a worse outcome than those who do not⁵⁶, again suggesting that platelets (or platelet-derived microparticles) may drive progression of ALF. Finally, a small study has shown decreased ADAMTS13 plasma levels in patients with a poor outcome²⁵, which may again indicate that excessive (intrahepatic) platelet thrombus formation by insufficient regulation of VWF could drive disease progression.

Platelets in progression of hepatocellular carcinoma

Platelets have been implicated in growth and metastasis of many different cancer types⁵⁷⁻⁵⁹. Thrombocytosis is a poor prognostic sign in cancer⁶⁰, and platelets have not only been suggested to fuel tumor growth directly⁶¹, but thrombopoietin-dependent platelet production is enhanced by the tumor⁶². Multiple studies have demonstrated aspirin use to be associated with a reduced risk to develop cancer, reduced cancer-related mortality, and reduced risk of cancer metastasis⁶³⁻⁶⁶. Importantly, these studies were meta-analyses of large randomized trials of aspirin use for prevention or treatment of cardiovascular events, increasing the reliability of the conclusion.

A single study has assessed the relation between aspirin use and diagnosis of HCC in the NIH-AARP and Health Study Cohort. Among more than 300,000 individuals, 250 had a

confirmed diagnosis of HCC, and aspirin use was associated with a 37% decreased risk of developing HCC⁴⁷.

In patients diagnosed with HCC it has been demonstrated that tumor size and platelet count are positively correlated⁶⁷. Moreover, thrombocytosis is associated with an increased risk of developing distant metastases⁶⁸. Confusingly, however, thrombocytopenia is associated with post-treatment HCC recurrence and death⁶⁹.

In patients that underwent a partial hepatectomy for (metastatic) malignancies, it was demonstrated that preoperative high levels of serotonin within platelets decreased the risk of post-operative morbidity, suggesting beneficial effects of platelet serotonin on liver regeneration. However, patients with high serotonin levels had an increased risk of cancer recurrence⁷⁰.

Platelets and liver regeneration

Multiple studies have demonstrated that a low platelet count following a partial liver resection or living donor liver transplantation in humans is associated with poor outcome, including postoperative liver dysfunction and mortality, which might be linked to defective regeneration⁷¹⁻⁷⁵. In addition, it has been recently demonstrated that platelet transfusion in living donor transplant recipients is associated with better liver regeneration, as assessed by graft volume measurements by computed tomography⁷⁴. In the same study it was demonstrated that in those patients that do not receive intraoperative platelets transfusions, the intraoperative platelet count is positively associated with graft regeneration. Moreover, a small uncontrolled study in humans suggested that platelet transfusion improves liver function in patients with established cirrhosis⁷⁶.

Clinical studies have suggested that release of growth factors from platelets are crucial in stimulating liver regeneration following partial liver resection in humans^{33,77}. It has been demonstrated that platelets accumulate in the liver remnant early after resection in mice⁷⁸ and humans³³, suggesting that platelets are recruited to the liver to deliver mitogenic cargo. Studies showing that low plasma levels of vascular endothelial growth factor and low platelet levels of serotonin are associated with an increased risk of post-operative liver dysfunction suggest that platelet-derived growth factors are important for liver regeneration in humans^{33,77}. In addition, high plasma levels of thrombospondin, an inhibitor of liver regeneration stored in platelet alpha granules, were associated with an increased risk of post-operative liver dysfunction⁷⁹. These studies suggested consumption of growth factors in the first post-operative days following liver resection. Other studies, however, failed to provide evidence for a role of platelet-derived growth factors in liver regeneration in humans, and pointed towards similar changes in platelet growth factor content following major abdominal surgery without subsequent requirement for liver regeneration^{80,81}. Technical concerns in both series of studies were present, which makes it difficult to draw definitive conclusions.

Experimental evidence in support of clinical data

Platelets in progression of chronic liver diseases

In agreement with clinical studies, studies in experimental rodent models of chronic liver injury have shown that administration of anti-platelet drugs reduces the severity of liver injury and fibrosis in experimental settings of chronic liver disease. Several studies indicate that platelets contribute to chronic liver injury in transgenic mice expressing hepatitis B proteins in liver, a model of immune-mediated liver damage resembling features of viral hepatitis^{82,83}. Notably, in this model of immune-mediated liver damage, administration of the antiplatelet drugs aspirin and clopidogrel reduced liver injury as well as fibrosis, and remarkably, reduced the incidence of HCC and improved survival^{84,85}. Antiplatelet drugs also reduced the severity of liver pathology in an experimental rat model of non-alcoholic fatty liver disease⁸⁶. Similarly, aspirin reduced fibrosis in a genetic model of sclerosing cholangitis (i.e., *Mdr2*^{-/-} mice)⁸⁷.

Platelets in progression of ALF

A pathologic role for platelets in liver damage has been suggested in several experimental models of acute liver injury induced by T cell activation, endotoxemia, cholestasis, apoptosis, and drug-induced liver injury^{53,83,88-91}. Overdose of the analgesic acetaminophen (paracetamol) is a leading cause of ALF⁹², and because of this, acetaminophen overdose is a widely utilized model of acute liver injury in rodents. acetaminophen overdose in mice produces a dose-dependent hepatotoxicity resembling the clinical scenario, and there is strong congruence between many of the mechanisms of acetaminophen hepatotoxicity in mice and observations in cases of ALF caused by acetaminophen overdose⁹³. Indeed, experimental acetaminophen overdose in mice has been utilized to determine mechanisms whereby platelets contribute to the progression of liver damage. Experimental acetaminophen overdose in mice is associated with activation of the extrinsic pathway of coagulation and elevation of plasma thrombin-antithrombin (TAT) levels and fibrin deposition in the injured liver⁹⁴⁻⁹⁶. In agreement with observations in patients with ALF, a rapid and persistent thrombocytopenia is evident in mice with acetaminophen -induced liver injury, which was accompanied by accumulation of platelets within the injured liver⁵³. Antibody-mediated thrombocytopenia reduced plasma TAT levels, suggesting a role for platelets in driving coagulation after acetaminophen overdose liver⁵³. Moreover, direct thrombin inhibition attenuated thrombocytopenia and hepatic platelet accumulation⁵³, implying that thrombin proteolytic activity drives platelet activation after acetaminophen overdose. This could occur through protease activated receptors on platelets or engagement of platelets with fibrin deposited in the liver. Notably, mice lacking PAR-4, which mediates thrombin signaling in mouse platelets, had reduced hepatic platelet accumulation after acetaminophen overdose⁵³. Of importance, antibody-mediated platelet depletion significantly reduced acetaminophen -induced liver injury in mice, as did PAR-4 deficiency, indicating that platelets contribute to the progression of liver injury in this experimental setting⁵³. Another study found that aspirin reduced liver injury after acetaminophen overdose, although clopidogrel had no effect on mortality at a high acetaminophen dose, suggesting the effect of aspirin could be platelet-independent⁵¹.

Platelets in progression of HCC

Platelets have been demonstrated to contribute to the growth and metastasis of different types of cancer in animal models⁹⁷. In addition, platelets have been shown to stimulate growth of various types of cancer cells in cell culture models. Several studies have shown that platelets or platelet lysates stimulate growth of cultured HCC cell lines⁹⁸⁻¹⁰⁰. In addition, platelets and platelet lysates have been demonstrated to promote cell invasion in matrigel assays¹⁰¹. Little is known on how platelets stimulate HCC cell growth and invasion. As platelet lysates also stimulate cell growth and proliferation, it has been suggested that release of mitogens from platelet granules are involved, but no direct experimental evidence has been provided. It has been demonstrated that serotonin stimulates HCC growth *in vitro*¹⁰² and *in vivo*¹⁰³, and since platelets carry >95% of circulating serotonin, it appears plausible that serotonin release from platelets acts in stimulating HCC growth. A preliminary study published in abstract form demonstrated that platelet-mediated stimulation of proliferation of HepG2 cells was attenuated by antagonists of the FcγRIIa and TGF-β1 receptors¹⁰⁰, and TGFβ has indeed been implicated in HCC progression¹⁰⁴. Platelet lysate has been demonstrated to stimulate proliferation of 4 different hepatoma cell lines. In addition, platelets inhibited apoptosis of these cell lines which may have contributed to the net stimulatory effects of platelet lysate on cell growth⁹⁸.

In mouse models of HCC, platelet inhibitors such as aspirin and clopidogrel were demonstrated to decrease tumor development and growth, and to improve survival^{84,105-107}. In a model of spontaneous HCC development in mice infected with hepatitis B, it was shown that antiplatelet therapy with aspirin and clopidogrel decreased liver injury, HCC development, and mortality⁸⁴. Inhibition of platelet-mediated recruitment of CD8 positive T cells directed to the hepatitis B virus was the main mechanism explaining the inhibitory effect of antiplatelet therapy. Importantly, in the same study, carbon tetrachloride-induced HCC formation was not inhibited by antiplatelet therapy indicating that the specific immunological mechanism underlying hepatitis B-induced HCC development was indeed the target of antiplatelet therapy. In an orthotopic HCC model, in which HepG2 cells were subcutaneously injected in immune deficient mice, antiplatelet clopidogrel therapy decreased tumor growth, which was accompanied by a decrease in platelet infiltration in the tumor, and better tumor differentiation¹⁰⁷. Platelet-tumor cell interaction was suggested to drive HCC progression in this paper. Another study demonstrated that aspirin inhibited tumor growth in a similar model, but this study concluded that direct, platelet-independent, apoptotic effects of aspirin were responsible for inhibition of tumor growth¹⁰⁶.

Platelets could potentially contribute to HCC by driving coagulation. In models of tumor growth and angiogenesis, components of the coagulation cascade including tissue factor, thrombin, and PAR-1 contribute to various cancer-related processes including tumor growth and angiogenesis^{108,109}. Although this connection is possible, studies coupling a hemostatic function of platelets with these processes in models of liver cancer are lacking.

Platelets and liver regeneration

It has been well established that platelets are vital for liver regeneration after a partial hepatectomy in rodent models^{78,110,111}. Significantly, liver regeneration after partial

hepatectomy was substantially delayed in mice that were treated with drugs that inhibit platelet function, or in mice with thrombocytopenia induced by chemotherapeutic drugs or platelet-depleting antibodies¹¹⁰. Conversely, mice in which the platelet count was increased by treatment with thrombopoietin receptor agonists or by administration of platelet concentrates showed accelerated liver regeneration^{111,112}. Interestingly, the presence of platelets in the first two hours after partial hepatectomy was sufficient to fully stimulate liver regeneration⁷⁸, which takes around 5-7 days in mice. Platelets accumulate in the remnant liver within 15 minutes after partial hepatectomy in mice, and have largely disappeared after 1 hour, suggesting that platelets stimulate liver regeneration by delivery of a mitogenic stimulus in the very early stages of liver regeneration. Notably, platelet deposition in the remnant liver has also been demonstrated in humans undergoing partial hepatectomy³³. Platelet accumulation in the liver remnant is dependent on VWF⁷⁸, which may mean that platelet accumulation is governed by activation of endothelial cells and exposure of endothelial-derived VWF within the liver. Alternatively, it may be that release of VWF by platelets contributes to platelet accumulation.

Platelets have been shown to simulate proliferation of cultured hepatocytes^{99,113,114}. Some studies have demonstrated that release of platelet granule content, including serotonin, hepatocyte growth factor, or insulin like growth factor are responsible for platelet-mediated hepatocytes proliferation^{113,114}. We have recently demonstrated that platelet RNA transfer partly drives proliferation of culture hepatocytes⁹⁹. It has however not been demonstrated yet that release of platelet granule content or platelet RNA transfer occurs *in vivo*.

Experimental evidence potentially contradicting clinical data

Platelets in progression of chronic liver diseases

Despite indication by many clinical studies that antiplatelet drugs are beneficial in patients with liver disease, experimental studies have not provided a uniform consensus on the role of platelets. The basis for this discrepancy is likely multi-factorial. For instance, the potency and efficacy of pharmacologic antiplatelet strategies varies. Thus, while literature categorically assigns these as “antiplatelet” drugs [or interventions], the mechanism and degree to which platelets are inhibited may impact on the observed experimental result. It is also plausible that the precise impact of platelets on liver disease pathogenesis depends on the experimental challenge, as in the case of immune (i.e., HBV)- vs. chemical (CCl₄)-mediated chronic liver damage. For example, despite aspirin and clopirogrel reducing fibrosis and cancer in experimental HBV, neither drug affected these pathologies in a model of fibrosis driven by chronic challenge with the hepatotoxicant CCl₄⁸⁴. In fact, several studies in the CCl₄ model suggest a protective role for platelets in liver fibrosis¹¹⁵⁻¹¹⁷. Thus, while several studies suggest that platelets contribute to liver fibrosis, their role is not yet crystal clear. In part, as with clinical studies, protective effects of drugs such as aspirin could potentially be attributed to non-platelet effects.

Experimental cholestasis models are an excellent example of how driving liver damage by a mechanism distinct from chemical- or immune-mediated hepatocellular injury can change the perceived role of platelets. Moreover, published studies in cholestasis models provide evidence in support of the concept that both timing and strength of platelet-directed

interventions can impact experimental results. Experimental ligation of the common bile duct causes both acute liver damage and fibrosis if uncorrected. In this setting of liver damage, depletion of platelets (~90%) with anti-GpIb antibody attenuates neutrophil accumulation and liver injury¹¹⁸. In a genetic model of complete thrombocytopenia, this protection from liver injury was retained, but surprisingly, liver fibrosis was dramatically increased¹¹⁷. Similarly, a near complete thrombocytopenia increased cholestatic liver injury in a model of chemical-induced bile duct injury, whereas more modest thrombocytopenia or administration of clopidogrel reduced injury¹¹⁹. These studies highlight the potentially different contribution of platelets in acute and chronic liver damage, as well as the potential difference between partial and complete thrombocytopenia.

In another model, chronic exposure of mice to the bile duct toxicant alpha-naphthylisothiocyanate (ANIT) elicits bile duct hyperplasia and fibrosis. Various pathways driving platelet function have been inhibited in this model, each implying a protective function of platelets, but with each platelet-directed intervention producing slightly different effects on liver histology. For example, $\text{Fib}\gamma^{-5}$ mice, in which fibrinogen cannot support platelet aggregation by engaging $\alpha_{\text{IIb}}\beta_3$, developed much more severe hepatocellular injury and fibrosis when challenged with ANIT¹²⁰. Mice lacking PAR-4, which mediates thrombin signaling in mouse platelets, also developed increased fibrosis, but the increase in hepatocellular damage was minimal compared to $\text{Fib}\gamma^{-5}$ mice¹²⁰. Finally, whereas clopidogrel increased peribiliary fibrosis, it had no effect on hepatocellular injury in ANIT-challenged mice, and had no additional effect on liver pathology in ANIT-challenged PAR-4-deficient mice¹²¹. Collectively, these studies indicate that the effect of generalized “anti-platelet” interventions can ultimately depend on the specific platelet activation pathways or element of platelet function being modified. Notably, aspirin treatment reduced biliary fibrosis in $\text{Mdr}2^{-/-}$ mice⁸⁷, which develop peri-biliary fibrosis much like ANIT-challenged mice. As noted earlier, aspirin may have non-platelet effects, but perhaps these different results could also be anchored in the mechanism whereby each drug affects platelet activation.

Importantly, clinical studies addressing the effect of anti-platelet drugs on liver disease in patients with cholestatic liver diseases, such as primary biliary cirrhosis and primary sclerosing cholangitis, have not been performed. Interestingly, however, there are a few studies that suggest that platelet function may indeed be different in patients with cholestatic liver disease compared to non-cholestatic liver disease^{122,123}. This observation, combined with somewhat surprising evidence in animal studies, provides a strong case for deciphering the basis for a different role of platelets in this type of liver disease.

Platelets in progression of ALF

Platelets are the sink for peripheral serotonin and upon activation release serotonin from intracellular granules. Platelet-derived serotonin is among the potential mechanisms whereby platelets could contribute to liver regeneration and repair. Interestingly, mice lacking peripheral serotonin developed modestly increased acetaminophen hepatotoxicity and serotonin deficiency worsened survival at a high dose of acetaminophen¹²⁴. It is worth noting that while platelets appear to exacerbate early hepatotoxicity in the acetaminophen

model, their role in repair and restoration of hepatic function has not been addressed experimentally. In fact, in other models of ALF, thrombocytosis improves survival. For example, in a surgical model of ALF induced by 90% hepatectomy in mice or rats, thrombocytosis improved survival, implying a protective role for platelets^{125,126}.

Platelets in progression of HCC

The experimental evidence linking platelets to progression of HCC is scarce but appears consistent. There are, however, inconsistencies in the clinical observations with both thrombocytosis and thrombocytopenia being identified as prognostically unfavorable factors⁶⁷⁻⁶⁹. It may be that the relation between thrombocytosis and outcome directly reflects stimulatory effects of platelets on tumor growth and/or metastasis, whereas the relation between thrombocytopenia and outcome is a reflection of the severity of underlying disease (i.e., cirrhosis). Experimental studies might be able to clarify this apparent paradox.

Platelets and liver regeneration

Based on extensive work in animal models and a number of recent clinical studies, the concept that release of platelet granule content (serotonin, growth factors) drives liver regeneration appears plausible and has been proposed by multiple groups^{77,110,114,127}. However, no direct definitive evidence that platelet granule content indeed drives liver regeneration has been provided, as we have recently reviewed¹²⁸. Studies whose conclusions were presented as conclusive evidence have unexplored alternative explanations. For example, the conclusion that platelet serotonin drives liver regeneration is not yet justified, as the delay in liver regeneration in mice that lack platelet serotonin is not only compatible with a scenario in which serotonin directly exerts mitogenic activity, but also with an scenario in which the role of serotonin on liver regeneration is indirect. Serotonin is well known as a potent platelet activator, and serotonin deficiency in platelets is associated with decreased functional platelet responses¹²⁹. Platelet activation is required for platelet-mediated liver regeneration, as P2Y₁₂ inhibition also delays liver regeneration¹¹⁰. Therefore, reduced platelet activation rather than defective mitogenic activity may explain the delayed liver regeneration in mice lacking platelet serotonin. Similarly, it has been shown that platelet serotonin levels decrease following a partial hepatectomy in humans⁷⁷. Combined with the observation that platelet serotonin content was associated with outcome, it was concluded that platelet serotonin is important for liver regeneration in humans. This study, however, did not address whether serotonin consumption was directly related to the partial hepatectomy or simply the consequence of major (abdominal) surgery, and inferred effects on liver regeneration based on complications of surgery.

Thus, more direct evidence for a role of specific platelet granule molecules is eagerly awaited. In addition, at least two alternative mechanisms that may underlie platelet-mediated liver regeneration deserve further experimental study. First, the role of platelet RNA transfer, which we recently demonstrated to drive hepatocyte proliferation *in vitro*, now requires exploration *in vivo*⁹⁹. Secondly, the possibility that platelets stimulate liver regeneration indirectly should be considered. It has been well established that inflammatory cells are important in liver regeneration¹³⁰⁻¹³². As platelets facilitate influx of inflammatory cells in

models of liver injury and repair¹³³, it may be that platelets drive liver regeneration by stimulating inflammatory cell recruitment.

Mechanistic insights into the role of platelets in liver disease pathogenesis

Increasing clinical and experimental evidence is consistent with a role for platelets in liver disease progression. Although the dogma is that platelets accelerate disease progression, there is also some experimental evidence suggesting the exact opposite. Part of the beneficial effects of platelets on the injured liver may reflect the role of platelets in liver regeneration. In this section, we will summarize potential mechanisms by which platelets exert beneficial and detrimental effects on the injured liver.

How platelets modulate liver injury

The mechanisms whereby platelets contribute to progression of injury in acute and chronic liver disease are not entirely understood. Occam's razor, a problem-solving principle that states that among competing hypotheses, that with the fewest assumptions should be selected, would suggest that one likely possibility is the formation of microthrombi within the liver microvasculature. In principle, both in acute and chronic liver disease, this could disrupt perfusion of the hepatic sinusoids, leading to hypoxia and cellular necrosis. Strong evidence indicates that antihemostatic agents reduce liver fibrosis in experimental settings¹³⁴, but direct evidence showing that formation of platelet and/or fibrin-containing thrombi is the pathological mediator of liver fibrosis is lacking. An alternative explanation for the efficacy of antihemostatic agents in reducing disease progression regards signaling effects of coagulation proteases such as factor Xa and thrombin¹³⁴. Given the role of platelets in supporting coagulation reactions, antiplatelet agents may delay disease progression by dampening Xa and/or thrombin generation.

Another mechanism whereby platelets could drive liver injury and fibrosis is by exacerbating the local inflammatory response. As noted previously, the release of pre-stored inflammatory mediators could drive activation of inflammatory processes that ultimately lead to tissue damage. Recent studies suggest that platelet-derived inflammatory mediators and growth factors contribute to chronic liver damage⁸⁷, but no direct evidence that intrahepatic release of pathogenic molecules by platelets drives disease progression was provided. In another study, cxcl4 (platelet factor 4)-deficient mice had reduced liver fibrosis after chronic challenge with carbon tetrachloride or thioacetamide, again suggesting a platelet-derived mediator could exacerbate fibrosis¹³⁵. Confusingly, there is also evidence that platelets deliver antifibrotic mediators, notably hepatocyte growth factor¹¹⁷.

Expression of a large number of adhesion molecules by platelets makes it plausible that they contribute to liver injury through recruitment and activation of inflammatory cells. For example, depletion of platelets reduces accumulation of both leukocytes and lymphocytes to the injured liver^{83,118} depending on the experimental context, although it is unclear whether this represents a direct interaction of platelets with these cells or a failure of chemotaxis to the liver. Other lines of evidence suggest that interactions between platelets and other non-parenchymal cells such as sinusoidal endothelial cells or liver macrophages can impact the progression of liver damage¹³⁶⁻¹³⁸. However, it is challenging to definitively nail down the

mechanisms whereby platelets either drive or protect against liver damage, particularly given the variable effect of various platelet-directed interventions on experimental liver disease. Approaches including Cre-LoxP targeted deletion of specific genes in platelets¹³⁹ or depletion and adoptive transfer of platelets in thrombocytopenic mice with modified function may offer more opportunity to definitively identify mechanisms whereby platelets contribute to injury.

The mechanistic basis for the apparent protective function of platelets in cholestatic liver injury is not known. It is plausible that the same battery of platelet-derived mediators have distinct functions depending on the nature of the disease process being studied. In part, this may depend on the differential role of other cell types with which platelets interact in the course of disease. For example, in cases where macrophage activity exacerbates injury, amplification of proinflammatory macrophage function by platelets could worsen injury. Under conditions where monocyte/macrophage activity is altered (e.g., cholestasis¹⁴⁰), the role of platelets could shift. Likewise, wherein the function of inflammatory cells is to repair the injured tissue, platelet-mediated inflammatory function or engagement of provisional fibrin(ogen) matrix could contribute to repair processes.

How platelets modulate HCC

Published data are scarce and inconsistent, and many potential mechanisms for platelet-mediated stimulation of development, growth, and metastasis of HCC require additional experimental study. It appears plausible that platelets stimulate growth and metastasis of HCC by mechanisms that have been identified for other tumor types, which include direct mitogenic effects of platelets⁶¹, stimulation of angiogenesis¹⁴¹, increasing survival of circulating tumor cells, and effects on cancer cell intra- and extravasation⁵⁹. Our understanding of the role of platelets in development of HCC is complicated as these tumors generally develop within cirrhotic livers. In cirrhosis, not only platelet number and function is altered, but also the composition of platelet granules^{142,143}, which may have consequences for the stimulatory effects of platelets on tumor growth and angiogenesis.

How platelets stimulate liver regeneration

Although there is increasing experimental and clinical data showing that platelets drive liver regeneration after partial hepatectomy, mechanisms involved are incompletely understood. Whereas some investigators advocate for the release of proteins or small molecules stored within platelet granules as drivers of regeneration^{77,110,114,127}, solid experimental evidence in support of this mechanism is lacking. We feel that based on current literature three plausible hypotheses exist, which require verification by experimental studies. These three potential mechanisms involve transfer of platelet RNA, and platelet-mediated influx of inflammatory cells, in addition to the widely accepted granule release mechanism.

Conclusion

Here, we have reviewed clinical and experimental evidence for a role of platelets in liver injury and repair. Table 1 summarizes our findings. We have stressed the limitations of both clinical and experimental studies and have indicated apparent paradoxes in both clinical and

experimental observations. Nevertheless, there is increasing evidence that platelets may be an interesting therapeutic target in the treatment of liver diseases. However, it is not yet time to start clinical studies on modulation of platelet functions to decrease progression of fibrosis/cirrhosis and ALF, to treat or prevent HCC in patients with cirrhosis, and to stimulate liver regeneration in patients at risk for post-resection liver failure due to inadequate regeneration. Before we can embark on such studies, we need to better understand mechanisms involved not only to identify the best therapeutic strategies, but also to be aware of potential detrimental side effects of platelet-modulating therapies.

Abbreviations

ALF	acute liver failure
VWF	von Willebrand factor
ADAMTS13	a disintegrin-like metalloproteinase with thrombospondin motif type 1 member 13
NAFLD	non-alcoholic fatty liver disease
HCC	hepatocellular carcinoma
ANIT	alpha-naphthylisothiocyanate
TAT	thrombin-antithrombin
PAR	protease activated receptor

References

1. Lisman T, Leebeek FW, de Groot PG. Haemostatic abnormalities in patients with liver disease. *J Hepatol.* 2002; 37:280–7. [PubMed: 12127437]
2. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease: evidence and clinical consequences. *Blood.* 2010; 116:878–85. [PubMed: 20400681]
3. Lisman T, Stravitz RT. Rebalanced Hemostasis in Patients with Acute Liver Failure. *Semin Thromb Hemost.* 2015; 41:468–73. [PubMed: 26049071]
4. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med.* 2011; 365:147–56. [PubMed: 21751907]
5. Violi F, Basili S, Raparelli V, Chowdary P, Gatt A, Burroughs AK. Patients with Liver Cirrhosis Suffer from Primary Haemostatic Defects? Fact or Fiction? *J Hepatol.* 2011; 55:1415–27. [PubMed: 21718668]
6. Witters P, Freson K, Verslype C, et al. Review article: blood platelet number and function in chronic liver disease and cirrhosis. *Aliment Pharmacol Ther.* 2008; 27:1017–29. [PubMed: 18331464]
7. Peck-Radosavljevic M, Wichlas M, Zacherl J, et al. Thrombopoietin induces rapid resolution of thrombocytopenia after orthotopic liver transplantation through increased platelet production. *Blood.* 2000; 95:795–801. [PubMed: 10648388]
8. Schiødt FV, Balko J, Schilsky M, et al. Thrombopoietin in acute liver failure. *Hepatology.* 2003; 37:558–61. [PubMed: 12601353]
9. Levine RF, Spivak JL, Meagher RC, Sieber F. Effect of ethanol on thrombopoiesis. *Br J Haematol.* 1986; 62:345–54. [PubMed: 3947552]

10. Garcia-Suarez J, Burgaleta C, Hernanz N, Albarran F, Tobaruela P, Alvarez-Mon M. HCV-associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy. *Br J Haematol.* 2000; 110:98–103. [PubMed: 10930984]
11. Kajihara M, Kato S, Okazaki Y, et al. A role of autoantibody-mediated platelet destruction in thrombocytopenia in patients with cirrhosis. *Hepatology.* 2003; 37:1267–76. [PubMed: 12774004]
12. Bakker CM, Knot EA, Stibbe J, Wilson JH. Disseminated intravascular coagulation in liver cirrhosis. *J Hepatol.* 1992; 15:330–5. [PubMed: 1447499]
13. Kondo R, Yano H, Nakashima O, Tanikawa K, Nomura Y, Kage M. Accumulation of platelets in the liver may be an important contributory factor to thrombocytopenia and liver fibrosis in chronic hepatitis C. *J Gastroenterol.* 2013; 48:526–34. [PubMed: 22911171]
14. Ikura Y, Ohsawa M, Okada M, Iwai Y, Wakasa K. The significance of platelet consumption in the development of thrombocytopenia in patients with cirrhosis. *Am J Med Sci.* 2013; 346:199–203. [PubMed: 23979210]
15. Aster RH. Splenic platelet pooling as a cause of “hypersplenic” thrombocytopenia. *Trans Assoc Am Physicians.* 1965; 78:362–73. [PubMed: 5864981]
16. Boyer TD, Habib S. Big spleens and hypersplenism: fix it or forget it? *Liver Int.* 2015; 35:1492–8. [PubMed: 25312770]
17. Drolz A, Horvatits T, Roedl K, et al. Coagulation parameters and major bleeding in critically ill patients with cirrhosis. *Hepatology.* 2016; 64:556–68. [PubMed: 27124745]
18. Raparelli V, Basili S, Carnevale R, et al. Low-grade endotoxemia and platelet activation in cirrhosis. *Hepatology.* 2017; 65:571–81. [PubMed: 27641757]
19. Basili S, Raparelli V, Riggio O, et al. NADPH oxidase-mediated platelet isoprostane overproduction in cirrhotic patients: implication for platelet activation. *Liver Int.* 2011; 31:1533–40. [PubMed: 22093328]
20. Caldwell S, Lisman T. The cirrhotic platelet: Shedding light on an enigma. *Hepatology.* 2017; 65:407–10. [PubMed: 28012255]
21. Lisman T, Porte RJ. Pitfalls in assessing platelet activation status in patients with liver disease. *Liver Int.* 2012; 32:1027. [PubMed: 22212705]
22. Lisman T, Adelmeijer J, de Groot PG, Janssen HL, Leebeek FW. No evidence for an intrinsic platelet defect in patients with liver cirrhosis—studies under flow conditions. *J Thromb Haemost.* 2006; 4:2070–2. [PubMed: 16836657]
23. Potze W, Siddiqui MS, Boyett SL, et al. Preserved hemostatic status in patients with non-alcoholic fatty liver disease. *J Hepatol.* 2016; 65:980–7. [PubMed: 27302378]
24. Lisman T, Bongers TN, Adelmeijer J, et al. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatology.* 2006; 44:53–61. [PubMed: 16799972]
25. Hugenholtz GC, Adelmeijer J, Meijers JC, Porte RJ, Stravitz RT, Lisman T. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: implications for hemostasis and clinical outcome. *Hepatology.* 2013; 58:752–61. [PubMed: 23468040]
26. Uemura M, Fujimura Y, Matsumoto M, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. *Thromb Haemost.* 2008; 99:1019–29. [PubMed: 18521503]
27. Napolitano G, Iacobellis A, Merla A, et al. Bleeding after invasive procedures is rare and unpredicted by platelet counts in cirrhotic patients with thrombocytopenia. *Eur J Intern Med.* 2017; 38:79–82. [PubMed: 27989373]
28. Giannini EG, Greco A, Marengo S, Andorno E, Valente U, Savarino V. Incidence of bleeding following invasive procedures in patients with thrombocytopenia and advanced liver disease. *Clin Gastroenterol Hepatol.* 2010; 8:899–902. [PubMed: 20601131]
29. Weeder PD, Porte RJ, Lisman T. Hemostasis in liver disease: implications of new concepts for perioperative management. *Transfus Med Rev.* 2014; 28:107–13. [PubMed: 24721432]
30. Tripodi A, Primignani M, Chantarangkul V, et al. Global hemostasis tests in patients with cirrhosis before and after prophylactic platelet transfusion. *Liver Int.* 2013; 33:362–7. [PubMed: 23231699]
31. Bos S, Bernal W, Porte RJ, Lisman T. Hemostatic complications in hepatobiliary surgery. *Semin Thromb Haemost.* 2017 in press.

32. Pereboom IT, Lisman T, Porte RJ. Platelets in liver transplantation: friend or foe? *Liver Transpl.* 2008; 14:923–31. [PubMed: 18581510]
33. Starlinger P, Haegele S, Offensperger F, et al. The Profile of Platelet alpha-Granule Released Molecules Affects Postoperative Liver Regeneration. *Hepatology.* 2016; 63:1675–88. [PubMed: 26528955]
34. Nijsten MW, ten Duis HJ, Zijlstra JG, et al. Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Crit Care Med.* 2000; 28:3843–6. [PubMed: 11153624]
35. Trotter JF, Gillespie BW, Terrault NA, et al. Laboratory test results after living liver donation in the adult-to-adult living donor liver transplantation cohort study. *Liver Transpl.* 2011; 17:409–17. [PubMed: 21445924]
36. Haegele S, Offensperger F, Pereyra D, et al. Deficiency in thrombopoietin induction after liver surgery is associated with postoperative liver dysfunction. *PLoS One.* 2015; 10:e0116985. [PubMed: 25611592]
37. Groeneveld DJ, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Balance between von Willebrand factor and ADAMTS13 following major partial hepatectomy. *Br J Surg.* 2016; 103:735–43. [PubMed: 27005894]
38. Reiter RA, Knobl P, Varadi K, Turecek PL. Changes in von Willebrand factor-cleaving protease (ADAMTS13) activity after infusion of desmopressin. *Blood.* 2003; 101:946–8. [PubMed: 12393734]
39. Schwameis M, Schorzenhofer C, Assinger A, Steiner MM, Jilma B. VWF excess and ADAMTS13 deficiency: a unifying pathomechanism linking inflammation to thrombosis in DIC, malaria, and TTP. *Thromb Haemost.* 2015; 113:708–18. [PubMed: 25503977]
40. Pereboom IT, Adelmeijer J, van Leeuwen Y, Hendriks HG, Porte RJ, Lisman T. Development of a severe von Willebrand factor/ADAMTS13 dysbalance during orthotopic liver transplantation. *Am J Transplant.* 2009; 9:1189–96. [PubMed: 19422343]
41. Arshad F, Lisman T, Porte RJ. Hypercoagulability as a contributor to thrombotic complications in the liver transplant recipient. *Liver Int.* 2013; 33:820–7. [PubMed: 23490221]
42. Weyrich AS. Platelets: more than a sack of glue. *Hematology Am Soc Hematol Educ Program.* 2014; 2014:400–3. [PubMed: 25696885]
43. Gremmel T, Frelinger AL 3rd, Michelson AD. Platelet Physiology. *Semin Thromb Hemost.* 2016; 42:191–204. [PubMed: 26926581]
44. Thachil J. Platelets in Inflammatory Disorders: A Pathophysiological and Clinical Perspective. *Semin Thromb Hemost.* 2015; 41:572–81. [PubMed: 26276938]
45. Smyth SS, McEver RP, Weyrich AS, et al. Platelet functions beyond hemostasis. *J Thromb Haemost.* 2009; 7:1759–66. [PubMed: 19691483]
46. Poujol-Robert A, Boelle PY, Conti F, et al. Aspirin may reduce liver fibrosis progression: Evidence from a multicenter retrospective study of recurrent hepatitis C after liver transplantation. *Clin Res Hepatol Gastroenterol.* 2014; 38:570–6. [PubMed: 25130796]
47. Sahasrabudhe VV, Gunja MZ, Graubard BI, et al. Nonsteroidal anti-inflammatory drug use, chronic liver disease, and hepatocellular carcinoma. *J Natl Cancer Inst.* 2012; 104:1808–14. [PubMed: 23197492]
48. Shen H, Shahzad G, Jawairia M, Bostick RM, Mustacchia P. Association between aspirin use and the prevalence of nonalcoholic fatty liver disease: a cross-sectional study from the Third National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther.* 2014; 40:1066–73. [PubMed: 25176122]
49. Jiang ZG, Feldbrugge L, Tapper EB, et al. Aspirin use is associated with lower indices of liver fibrosis among adults in the United States. *Aliment Pharmacol Ther.* 2016; 43:734–43. [PubMed: 26749582]
50. Botting RM. Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology. *Pharmacol Rep.* 2010; 62:518–25. [PubMed: 20631416]
51. Imaeda AB, Watanabe A, Sohail MA, et al. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. *J Clin Invest.* 2009; 119:305–14. [PubMed: 19164858]

52. Stravitz RT, Ellerbe C, Durkalski V, et al. Thrombocytopenia is Associated with Multi-organ System Failure in Patients With Acute Liver Failure. *Clin Gastroenterol Hepatol*. 2016; 14:613–20. [PubMed: 26453953]
53. Miyakawa K, Joshi N, Sullivan BP, et al. Platelets and protease-activated receptor-4 contribute to acetaminophen-induced liver injury in mice. *Blood*. 2015; 126:1835–43. [PubMed: 26179083]
54. Stravitz RT, Bowling R, Bradford RL, et al. Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. *Hepatology*. 2013; 58:304–13. [PubMed: 23389887]
55. Levi M. Platelets in Critical Illness. *Semin Thromb Hemost*. 2016; 42:252–7. [PubMed: 26926582]
56. Stravitz RT, Ellerbe C, Durkalski V, Schilski ML, Lee WM. Spontaneous Bleeding Complications in Acute Liver Failure (ALF). *Hepatology*. 2013; 58:347A.
57. Erpenbeck L, Schon MP. Deadly allies: the fatal interplay between platelets and metastasizing cancer cells. *Blood*. 2010; 115:3427–36. [PubMed: 20194899]
58. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. *Blood*. 2015; 126:582–8. [PubMed: 26109205]
59. Sharma D, Brummel-Ziedins KE, Bouchard BA, Holmes CE. Platelets in Tumor Progression: A Host Factor that Offers Multiple Potential Targets in the Treatment of Cancer. *J Cell Physiol*. 2014; 229:1005–15. [PubMed: 24374897]
60. Lin RJ, Afshar-Kharghan V, Schafer AI. Paraneoplastic thrombocytosis: the secrets of tumor self-promotion. *Blood*. 2014; 124:184–7. [PubMed: 24868077]
61. Cho MS, Bottsford-Miller J, Vasquez HG, et al. Platelets increase the proliferation of ovarian cancer cells. *Blood*. 2012; 120:4869–72. [PubMed: 22966171]
62. Stone RL, Nick AM, McNeish IA, et al. Paraneoplastic thrombocytosis in ovarian cancer. *N Engl J Med*. 2012; 366:610–8. [PubMed: 22335738]
63. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet*. 2011; 377:31–41. [PubMed: 21144578]
64. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet*. 2012; 379:1591–601. [PubMed: 22440947]
65. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol*. 2012; 13:518–27. [PubMed: 22440112]
66. Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. *Lancet*. 2012; 379:1602–12. [PubMed: 22440946]
67. Carr BI, Guerra V. Hepatocellular carcinoma size: platelets, gamma-glutamyl transpeptidase, and alkaline phosphatase. *Oncology*. 2013; 85:153–9. [PubMed: 23988857]
68. Lee CH, Lin YJ, Lin CC, et al. Pretreatment platelet count early predicts extrahepatic metastasis of human hepatoma. *Liver Int*. 2015; 35:2327–36. [PubMed: 25752212]
69. Pang Q, Qu K, Zhang JY, et al. The Prognostic Value of Platelet Count in Patients With Hepatocellular Carcinoma: A Systematic Review and Meta-Analysis. *Medicine (Baltimore)*. 2015; 94:e1431. [PubMed: 26376382]
70. Pereyra D, Padickakudy R, Haeghele S, et al. Preoperative intra-platelet serotonin levels are associated with early disease recurrence after liver resection in humans. *J Hepatol*. 2017; 66:s439.
71. Margonis GA, Amini N, Buettner S, et al. Impact of early postoperative platelet count on volumetric liver gain and perioperative outcomes after major liver resection. *Br J Surg*. 2016; 103:899–907. [PubMed: 26961709]
72. Wang HQ, Yang J, Yang JY, Wang WT, Yan LN. Low immediate postoperative platelet count is associated with hepatic insufficiency after hepatectomy. *World J Gastroenterol*. 2014; 20:11871–7. [PubMed: 25206294]
73. Alkozai EM, Nijsten MW, de Jong KP, et al. Immediate postoperative low platelet count is associated with delayed liver function recovery after partial liver resection. *Ann Surg*. 2010; 251:300–6. [PubMed: 19779326]

74. Han S, Park HW, Song JH, et al. Association Between Intraoperative Platelet Transfusion and Early Graft Regeneration in Living Donor Liver Transplantation. *Ann Surg.* 2016; 264:1065–72. [PubMed: 26720430]
75. Li L, Wang H, Yang J, et al. Immediate Postoperative Low Platelet Counts After Living Donor Liver Transplantation Predict Early Allograft Dysfunction. *Medicine (Baltimore).* 2015; 94:e1373. [PubMed: 26313775]
76. Maruyama T, Murata S, Takahashi K, et al. Platelet transfusion improves liver function in patients with chronic liver disease and cirrhosis. *Tohoku J Exp Med.* 2013; 229:213–20. [PubMed: 23459612]
77. Starlinger P, Assinger A, Haegerle S, et al. Evidence for serotonin as a relevant inducer of liver regeneration after liver resection in humans. *Hepatology.* 2014; 60:257–66. [PubMed: 24277679]
78. Kirschbaum M, Jenne CN, Veldhuis ZJ, et al. Transient von Willebrand factor-mediated platelet influx stimulates liver regeneration after partial hepatectomy in mice. *Liver Int.* :2017. in press.
79. Starlinger P, Haegerle S, Wanek D, et al. Plasma thrombospondin 1 as a predictor of postoperative liver dysfunction. *Br J Surg.* 2015; 102:826–36. [PubMed: 25871570]
80. Kirschbaum M, Adelmeijer J, Alkozai EM, Porte RJ, Lisman T. Evidence against a role for platelet-derived molecules in liver regeneration after partial hepatectomy in humans. *J Clin Transl Res.* 2016; 2:97–106.
81. Alkozai EM, van Faassen M, Kema IP, Porte RJ, Lisman T. Evidence against a role of serotonin in liver regeneration in humans. *Hepatology.* 2015; 62:983. [PubMed: 25546821]
82. Lang PA, Contaldo C, Georgiev P, et al. Aggravation of viral hepatitis by platelet-derived serotonin. *Nat Med.* 2008; 14:756–61. [PubMed: 18516052]
83. Iannacone M, Sitia G, Isogawa M, et al. Platelets mediate cytotoxic T lymphocyte-induced liver damage. *Nat Med.* 2005; 11:1167–9. [PubMed: 16258538]
84. Sitia G, Aiolfi R, Di Lucia P, et al. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. *Proc Natl Acad Sci U S A.* 2012; 109:E2165–72. [PubMed: 22753481]
85. Sitia G, Iannacone M, Guidotti LG. Anti-platelet therapy in the prevention of hepatitis B virus-associated hepatocellular carcinoma. *J Hepatol.* 2013; 59:1135–8. [PubMed: 23742914]
86. Fujita K, Nozaki Y, Wada K, et al. Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease. *Gut.* 2008; 57:1583–91. [PubMed: 18596193]
87. Yoshida S, Ikenaga N, Liu SB, et al. Extrahepatic platelet-derived growth factor-beta, delivered by platelets, promotes activation of hepatic stellate cells and biliary fibrosis in mice. *Gastroenterology.* 2014; 147:1378–92. [PubMed: 25173753]
88. Khandoga A, Hanschen M, Kessler JS, Krombach F. CD4+ T cells contribute to postischemic liver injury in mice by interacting with sinusoidal endothelium and platelets. *Hepatology.* 2006; 43:306–15. [PubMed: 16440342]
89. Massaguer A, Perez-Del-Pulgar S, Engel P, Serratos J, Bosch J, Pizcueta P. Concanavalin-A-induced liver injury is severely impaired in mice deficient in P-selectin. *J Leukoc Biol.* 2002; 72:262–70. [PubMed: 12149416]
90. Pearson JM, Schultze AE, Jean PA, Roth RA. Platelet participation in liver injury from gram-negative bacterial lipopolysaccharide in the rat. *Shock.* 1995; 4:178–86. [PubMed: 8574752]
91. Hisakura K, Murata S, Takahashi K, et al. Platelets prevent acute hepatitis induced by anti-fas antibody. *J Gastroenterol Hepatol.* 2011; 26:348–55. [PubMed: 21261726]
92. Lee WM. Acetaminophen and the U.S. Acute Liver Failure Study Group: lowering the risks of hepatic failure. *Hepatology.* 2004; 40:6–9. [PubMed: 15239078]
93. Ramachandran A, Jaeschke H. Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. *J Clin Transl Res.* 2017; 3:157–69. [PubMed: 28670625]
94. Kopeck AK, Joshi N, Cline-Fedewa H, et al. Fibrin(ogen) drives repair after acetaminophen-induced liver injury via leukocyte alphaMbeta2 integrin-dependent upregulation of Mmp12. *J Hepatol.* 2017; 66:787–97. [PubMed: 27965156]
95. Ganey PE, Luyendyk JP, Newport SW, et al. Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. *Hepatology.* 2007; 46:1177–86. [PubMed: 17654741]

96. Sullivan BP, Kopec AK, Joshi N, et al. Hepatocyte tissue factor activates the coagulation cascade in mice. *Blood*. 2013; 121:1868–74. [PubMed: 23305736]
97. Goubran HA, Stakiw J, Radosevic M, Burnouf T. Platelet-cancer interactions. *Semin Thromb Hemost*. 2014; 40:296–305. [PubMed: 24590421]
98. Carr BI, Cavallini A, D'Alessandro R, et al. Platelet extracts induce growth, migration and invasion in human hepatocellular carcinoma in vitro. *BMC Cancer*. 2014; 14:43. 2407-14-43. [PubMed: 24468103]
99. Kirschbaum M, Karimian G, Adelmeijer J, Giepmans BN, Porte RJ, Lisman T. Horizontal RNA transfer mediates platelet-induced hepatocyte proliferation. *Blood*. 2015; 126:798–806. [PubMed: 26056167]
100. He A, Liang M, Liu G, et al. The Role of Fc γ RIIa and TGF- β 1/KLF6 Pathway in Platelet's Promoting Hepatocellular Carcinoma Cells Growth. *Blood*. 2014; 124:1429.
101. Bihari C, Rastogi A, Shasthry SM, et al. Platelets contribute to growth and metastasis in hepatocellular carcinoma. *APMIS*. 2016; 124:776–86. [PubMed: 27457354]
102. Liang C, Chen W, Zhi X, et al. Serotonin promotes the proliferation of serum-deprived hepatocellular carcinoma cells via upregulation of FOXO3a. *Mol Cancer*. 2013; 12:14. [PubMed: 23418729]
103. Soll C, Jang JH, Riener MO, et al. Serotonin promotes tumor growth in human hepatocellular cancer. *Hepatology*. 2010; 51:1244–54. [PubMed: 20099302]
104. Fabregat I, Moreno-Caceres J, Sanchez A, et al. TGF-beta signalling and liver disease. *FEBS J*. 2016; 283:2219–32. [PubMed: 26807763]
105. Lu L, Sun HC, Zhang W, et al. Aspirin minimized the pro-metastasis effect of sorafenib and improved survival by up-regulating HTATIP2 in hepatocellular carcinoma. *PLoS One*. 2013; 8:e65023. [PubMed: 23741443]
106. Hossain MA, Kim DH, Jang JY, et al. Aspirin induces apoptosis in vitro and inhibits tumor growth of human hepatocellular carcinoma cells in a nude mouse xenograft model. *Int J Oncol*. 2012; 40:1298–304. [PubMed: 22179060]
107. Zhang R, Guo H, Xu J, et al. Activated platelets inhibit hepatocellular carcinoma cell differentiation and promote tumor progression via platelet-tumor cell binding. *Oncotarget*. 2016; 7:60609–22. [PubMed: 27542264]
108. Milsom C, Rak J. Tissue factor and cancer. *Pathophysiol Haemost Thromb*. 2008; 36:160–76. [PubMed: 19176989]
109. Ruf W, Mueller BM. Thrombin generation and the pathogenesis of cancer. *Semin Thromb Hemost*. 2006; 32(Suppl 1):61–8. [PubMed: 16673267]
110. Lesurtel M, Graf R, Aleil B, et al. Platelet-derived serotonin mediates liver regeneration. *Science*. 2006; 312:104–7. [PubMed: 16601191]
111. Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R. Platelets promote liver regeneration in early period after hepatectomy in mice. *World J Surg*. 2007; 31:808–16. [PubMed: 17354025]
112. Matsuo R, Nakano Y, Ohkohchi N. Platelet administration via the portal vein promotes liver regeneration in rats after 70% hepatectomy. *Ann Surg*. 2011; 253:759–63. [PubMed: 21475016]
113. Balasubramanian S, Paulose CS. Induction of DNA synthesis in primary cultures of rat hepatocytes by serotonin: possible involvement of serotonin S2 receptor. *Hepatology*. 1998; 27:62–6. [PubMed: 9425918]
114. Matsuo R, Ohkohchi N, Murata S, et al. Platelets Strongly Induce Hepatocyte Proliferation with IGF-I and HGF In Vitro. *J Surg Res*. 2008; 145:279–86. [PubMed: 17688880]
115. Takahashi K, Murata S, Fukunaga K, Ohkohchi N. Human platelets inhibit liver fibrosis in severe combined immunodeficiency mice. *World J Gastroenterol*. 2013; 19:5250–60. [PubMed: 23983427]
116. Watanabe M, Murata S, Hashimoto I, et al. Platelets contribute to the reduction of liver fibrosis in mice. *J Gastroenterol Hepatol*. 2009; 24:78–89. [PubMed: 18624898]
117. Kodama T, Takehara T, Hikita H, et al. Thrombocytopenia exacerbates cholestasis-induced liver fibrosis in mice. *Gastroenterology*. 2010; 138:2487–98. [PubMed: 20206174]

118. Laschke MW, Dold S, Menger MD, Jeppsson B, Thorlacius H. Platelet-dependent accumulation of leukocytes in sinusoids mediates hepatocellular damage in bile duct ligation-induced cholestasis. *Br J Pharmacol*. 2008; 153:148–56. [PubMed: 18026126]
119. Sullivan BP, Wang R, Tawfik O, Luyendyk JP. Protective and damaging effects of platelets in acute cholestatic liver injury revealed by depletion and inhibition strategies. *Toxicol Sci*. 2010; 115:286–94. [PubMed: 20133375]
120. Joshi N, Kopec AK, O'Brien KM, et al. Coagulation-driven platelet activation reduces cholestatic liver injury and fibrosis in mice. *J Thromb Haemost*. 2015; 13:57–71. [PubMed: 25353084]
121. Joshi N, Kopec AK, Ray JL, Luyendyk JP. Inhibition of PAR-4 and P2Y12 receptor-mediated platelet activation produces distinct hepatic pathologies in experimental xenobiotic-induced cholestatic liver disease. *Toxicology*. 2016; 365:9–16. [PubMed: 27475285]
122. Pihusch R, Rank A, Gohring P, Pihusch M, Hiller E, Beuers U. Platelet function rather than plasmatic coagulation explains hypercoagulable state in cholestatic liver disease. *J Hepatol*. 2002; 37:548–55. [PubMed: 12399218]
123. Ben-Ari Z, Panagou M, Patch D, et al. Hypercoagulability in patients with primary biliary cirrhosis and primary sclerosing cholangitis evaluated by thrombelastography. *J Hepatol*. 1997; 26:554–9. [PubMed: 9075662]
124. Zhang J, Song S, Pang Q, et al. Serotonin deficiency exacerbates acetaminophen-induced liver toxicity in mice. *Sci Rep*. 2015; 5:8098. [PubMed: 25631548]
125. Myronovych A, Murata S, Chiba M, et al. Role of platelets on liver regeneration after 90% hepatectomy in mice. *J Hepatol*. 2008; 49:363–72. [PubMed: 18602717]
126. Lopez ML, Kielsing CO, Uribe Cruz C, et al. Platelet increases survival in a model of 90% hepatectomy in rats. *Liver Int*. 2014; 34:1049–56. [PubMed: 24119092]
127. Meyer J, Lejmi E, Fontana P, Morel P, Gonelle-Gispert C, Buhler L. A focus on the role of platelets in liver regeneration: do platelet-endothelial cell interactions initiate the regenerative process? *J Hepatol*. 2015; 63:1263–71. [PubMed: 26169159]
128. Lisman T, Porte RJ. Mechanisms of platelet-mediated liver regeneration. *Blood*. 2016; 128:625–9. [PubMed: 27297793]
129. Walther DJ, Peter JU, Winter S, et al. Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell*. 2003; 115:851–62. [PubMed: 14697203]
130. Selzner N, Selzner M, Odermatt B, Tian Y, Van Rooijen N, Clavien PA. ICAM-1 triggers liver regeneration through leukocyte recruitment and Kupffer cell-dependent release of TNF-alpha/IL-6 in mice. *Gastroenterology*. 2003; 124:692–700. [PubMed: 12612908]
131. Hosoya S, Ikejima K, Takeda K, et al. Innate immune responses involving natural killer and natural killer T cells promote liver regeneration after partial hepatectomy in mice. *Am J Physiol Gastrointest Liver Physiol*. 2013; 304:G293–9. [PubMed: 23086918]
132. Rao R, Graffeo CS, Gulati R, et al. Interleukin 17-producing gammadeltaT cells promote hepatic regeneration in mice. *Gastroenterology*. 2014; 147:473–84.e2. [PubMed: 24801349]
133. Slaba I, Wang J, Kolaczowska E, McDonald B, Lee WY, Kubes P. Imaging the dynamic platelet-neutrophil response in sterile liver injury and repair in mice. *Hepatology*. 2015; 62:1593–605. [PubMed: 26202541]
134. Anstee QM, Dhar A, Thursz MR. The role of hypercoagulability in liver fibrogenesis. *Clin Res Hepatol Gastroenterol*. 2011; 35:526–33. [PubMed: 21570930]
135. Zaldivar MM, Pauels K, von Hundelshausen P, et al. CXC chemokine ligand 4 (Cxcl4) is a platelet-derived mediator of experimental liver fibrosis. *Hepatology*. 2010; 51:1345–53. [PubMed: 20162727]
136. Poisson J, Lemoine S, Boulanger C, et al. Liver sinusoidal endothelial cells: Physiology and role in liver diseases. *J Hepatol*. 2017; 66:212–27. [PubMed: 27423426]
137. Tamura T, Kondo T, Pak S, et al. Interaction between Kupffer cells and platelets in the early period of hepatic ischemia-reperfusion injury—an in vivo study. *J Surg Res*. 2012; 178:443–51. [PubMed: 22480836]

138. Sindram D, Porte RJ, Hoffman MR, Bentley RC, Clavien PA. Synergism between platelets and leukocytes in inducing endothelial cell apoptosis in the cold ischemic rat liver: a Kupffer cell-mediated injury. *FASEB J.* 2001; 15:1230–2. [PubMed: 11344097]
139. Tiedt R, Schomber T, Hao-Shen H, Skoda RC. Pf4-Cre transgenic mice allow the generation of lineage-restricted gene knockouts for studying megakaryocyte and platelet function in vivo. *Blood.* 2007; 109:1503–6. [PubMed: 17032923]
140. Sung JJ, Go MY. Reversible Kupffer cell suppression in biliary obstruction is caused by hydrophobic bile acids. *J Hepatol.* 1999; 30:413–8. [PubMed: 10190723]
141. Sabrkhany S, Griffioen AW, Oude Egbrink MG. The role of blood platelets in tumor angiogenesis. *Biochim Biophys Acta.* 2011; 1815:189–96. [PubMed: 21167916]
142. Alkozai EM, Porte RJ, Adelmeijer J, et al. Levels of angiogenic proteins in plasma and platelets are not different between patients with hepatitis B/C-related cirrhosis and patients with cirrhosis and hepatocellular carcinoma. *Platelets.* 2014:1–6. [PubMed: 23469931]
143. Laffi G, Marra F, Gresele P, et al. Evidence for a storage pool defect in platelets from cirrhotic patients with defective aggregation. *Gastroenterology.* 1992; 103:641–6. [PubMed: 1386051]

Table 1

Evidence in support of a role of platelets in progression of chronic and acute liver failure, HCC, and liver regeneration, with limitations in available data and potential mechanisms involved.

Disease	Clinical evidence	Experimental evidence	Caveats	Potential mechanisms
Chronic liver disease (fibrosis/cirrhosis)	* Aspirin use associated with decreased disease burden or progression ⁴⁶⁻⁴⁹ * Elevated VWF levels associated with fibrosis in the general population	* Injury and fibrosis decreased by anti-platelet drugs in rodent models of fibrosis ⁸²⁻⁸⁷	* Platelets also show protective effects in rodent models of fibrosis ^{115-117, 120}	* Formation of microthrombi within the liver ¹³⁵ * Exacerbation of local inflammation ⁸⁷
Acute liver failure	* More profound decrease in platelet count over time associated with worse disease outcome ⁵² * Increased platelet microparticle content in plasma associated with worse disease outcome ⁵⁴	* Platelet depletion reduces liver injury in acetaminophen-induced ALF in mice ⁵³	* Thrombocytosis improves survival in rodent surgical models of ALF ^{125,126}	* Formation of microthrombi within the liver ^{3,25} * Exacerbation of local inflammation
HCC	* Aspirin use associated with decreased risk of development of HCC ⁴⁷ * Thrombocytosis associated with risk of developing metastases ⁶⁸	* Anti-platelet drugs decrease tumor development, tumor growth, and death in mouse models of HCC ⁸⁴	* Clinically, both thrombocytopenia and thrombocytosis appear related to unfavorable outcome ^{68,69}	* Stimulation of cancer cell proliferation ⁹⁸ * Increased angiogenesis ¹⁴² * Protection of circulating tumor cells ⁵⁹
Liver regeneration	* Low platelet count associated with poor outcome of liver resection and speed of regeneration ⁷¹⁻⁷⁵ * Platelet and plasma levels of known stimulators of liver regeneration decreased in patients with poor outcome of liver resection ^{33,77}	* Platelet depletion and antiplatelet drugs delay liver regeneration in rodents ^{78, 110, 111}	* Clinical studies on growth factors contained within platelets and outcome of liver resection are inconsistent ^{33,77,80,81}	* Release of growth factors from platelet granules ^{33, 77, 110, 114, 127} * Transfer of platelet RNA ⁹⁹ * Modulation of inflammatory cell influx ^{128, 133}