Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Patients with liver cirrhosis suffer from primary haemostatic defects? Fact or fiction?

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Patients with cirrhosis can have abnormalities in laboratory tests reflecting changes in primary haemostasis, including bleeding time, platelet function tests, markers of platelet activation, and platelet count. Such changes have been considered particularly relevant in the bleeding complications that occur in cirrhosis.

However, several studies have shown that routine diagnostic tests, such as platelet count, bleeding time, PFA-100, thromboelastography are not clinically useful to stratify bleeding risk in patients with cirrhosis. Moreover, treatments used to increase platelet count or to modulate platelet function could potentially do harm. Consequently the optimal management of bleeding complications is still a matter of discussion.

Moreover, in the last two decades there has been an increased recognition that not only bleeding but also thrombosis complicates the clinical course of cirrhosis. Thus, we performed a literature search looking at publications studying both qualitative and quantitative aspects of platelet function to verify which primary haemostasis defects occur in cirrhosis. In addition, we evaluated the contribution of qualitative and quantitative aspects of platelet function to the clinical outcome in cirrhosis and their therapeutic management according to the data available in the literature.

From the detailed analysis of the literature, it appears clear that primary haemostasis may not be defective in cirrhosis, and a low platelet count should not necessarily be considered as an automatic index of an increased risk of bleeding. Conversely, caution should be observed in patients with severe thrombocytopenia where its correction is advised if bleeding occurs and before invasive diagnostic and therapeutic procedures.

Introduction

Complex haemostatic changes coexist in liver cirrhosis (LC) [1–4]. These changes include defects in primary haemostasis, abnormalities of the clotting system due to impaired synthesis of pro-coagulant and anti-coagulant factors, low-grade coagulation activation, and hyperfibrinolysis. Patients with cirrhosis can have abnormalities in laboratory tests reflecting changes in primary haemostasis, including bleeding time (BT), platelet aggregation tests, and platelet count. Laboratory tests of platelet function consistently show that primary haemostasis is impaired in cirrhosis as a consequence of reduced platelet function and/or low platelet count [5]. Such changes have been considered particularly relevant in the bleeding complications that occur in cirrhosis, so that the clinical effect of drugs that improve platelet function [6–9], or number of platelets [10] has been investigated in cirrhosis.

However, in the last two decades, there has been an increased recognition that not only bleeding but also thrombosis complicates the clinical course of cirrhosis. Portal vein thrombosis is a frequent finding in cirrhosis (without concomitant hepatocellular carcinoma) occurring in about 10–20% of LC [11–14]. Moreover, the occurrence of thrombosis in other vascular territories, such as peripheral veins, is increasingly being reported. Patients with cirrhosis are prone to deep vein thrombosis and embolic disease [15–17], as evidenced by epidemiological data [18]. Thus, a review of primary haemostasis within the newly accepted paradigm of preserved haemostasis in cirrhosis, with normal thrombin formation [19], or increased thrombin generation [20], and the coexistence of bleeding and thrombotic complications in cirrhosis [3], and its possible role in these abnormalities is pertinent.

We performed a literature search looking at publications studying both qualitative and quantitative aspects of platelet function to verify which primary hemostasis defects occur in cirrhosis, and if so, how they contribute to clinical outcome.

Primary haemostasis: physiology

Haemostasis is the process that maintains the integrity of the circulatory system after vascular damage. Platelets are recruited to the site of injury where they become a major component of the developing thrombus. When pathologic processes overwhelm the regulatory mechanisms of haemostasis either thrombosis or bleeding can occur.

The endothelium is crucial in providing a defense against thrombus formation: it contains thromboregulators able to inhibit platelet activation such as nitric oxide, [21,22], prostacyclin, [23] and the endothelial ectoadenosine diphosphatase

Keywords: Liver disease; Thrombocytopenia; Thrombocytopathy; Bleeding; Platelets.

Received 28 February 2011; received in revised form 20 June 2011; accepted 21 June 2011

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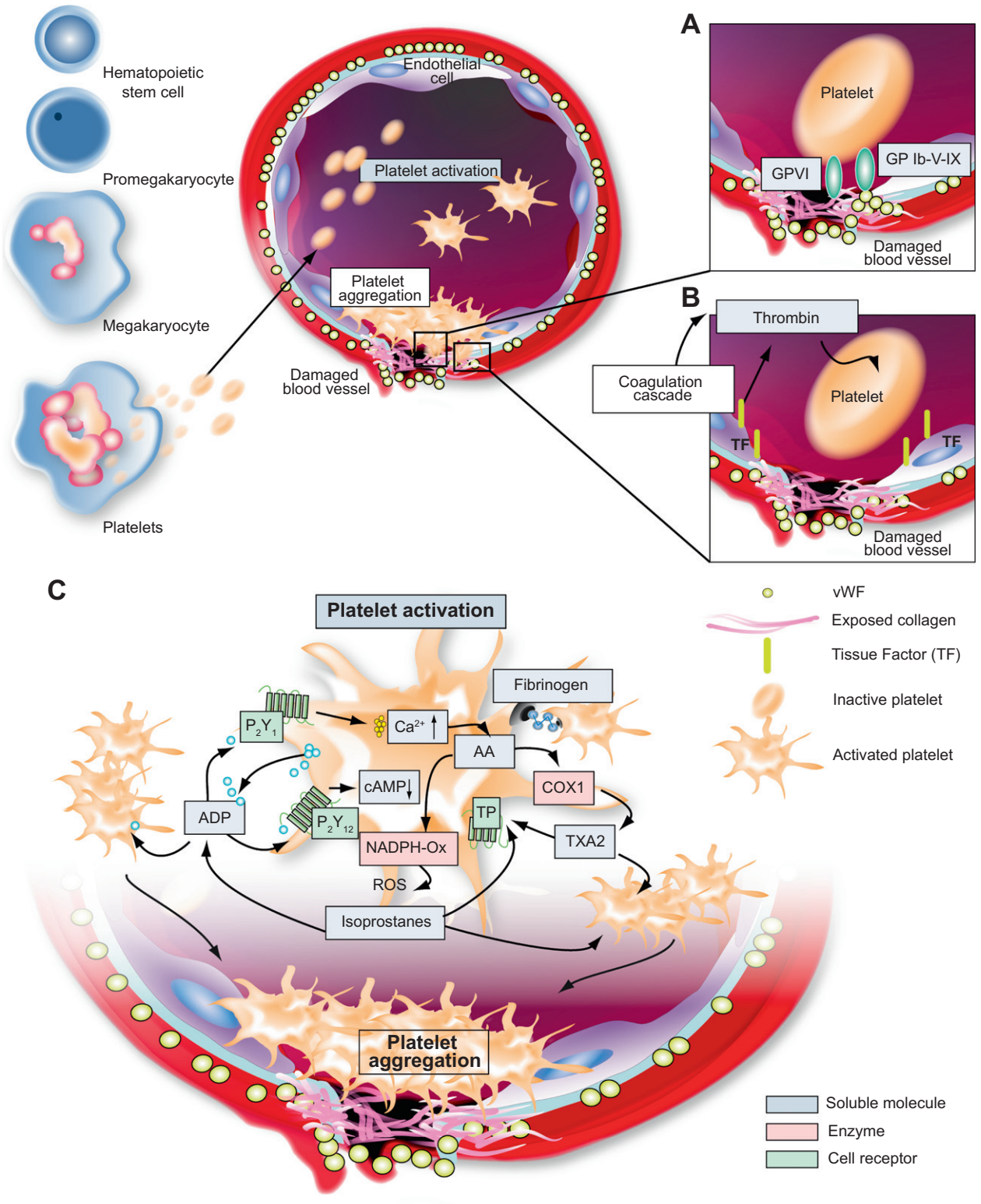

Fig. 1. Primary haemostasis: physiology. Platelets, anucleated cells derived from megakaryocytes, at the side of vessel wall injury, adhere to exposed collagen or vWf. Adhesion and activation is initiated by two distinct pathways acting in parallel or separately: (A) exposure of sub-endothelial collagen initiates platelet activation via Gp VI binding to the collagen and Gp Ib-V-IX exposure of collagen triggers the adhesion and activation of platelets; (B) TF initiates the generation of thrombin. (C) After adhesion to endothelium, in the activation phase, thrombin derived by coagulation cascade and platelets-derived mediators, such as ADP, TXA_2 and isoprostanes, activate several pathways resulting in glycoprotein IIb/IIIa activation and in turn platelet aggregation. AA, arachidonic acid; ADP, adenosine diphosphate; COX1, cyclo-oxygenase 1; GP, glycoprotein; NADPH-Ox, NADPH oxidase; TF, tissue factor; TP, thromboxane receptor; TXA_2, thromboxane A_2; vWf, Von Willebrand factor.

(ecto-ADPase) pathway [24]. When the endothelium is disrupted, collagen triggers the adhesion and activation of platelets, whereas tissue factor (TF) initiates the generation of thrombin, which not only converts fibrinogen to fibrin but also activates platelets. The initial platelet adhesion is followed by activation which is characterized by these phases: (i) platelet granule release reaction that facilitates further platelet activation and platelet recruitment, (ii) cytoskeletal rearrangements (necessary for shape change including spreading, pseudopodia formation, and clot retraction), (iii) mobilization of arachidonic acid to amplify intracellular signaling, (iv) glycoprotein (Gp) IIb/IIIa expression on the surface of the platelet for aggregation mediated by the receptors and fibrinogen and (v) exposure of procoagulant phospholipids to facilitate coagulation.

Recent studies of thrombus formation in genetically altered mice [25,26] show two distinct pathways acting in parallel or separately, for platelet activation. In the first pathway, exposure of sub-endothelial collagen initiates platelet activation via Gp VI binding to the collagen and Gp Ib-V-IX binding to collagen-bound von Willebrand factor (vWf). In the second one, TF initiates platelet activation independently of vWf [27] and GpVI [26]. It forms a complex with factor VIIa, initiating a proteolytic cascade that generates thrombin. Thrombin thereby activates platelets [28] through PAR4 receptors causing them to release adenosine diphosphate (ADP), serotonin, and thromboxane (Tx) A_2. The consequences of platelet activation triggered by these pathways are identical [25,26].

Platelet activation and aggregation

Thrombus formation is a dynamic process in which some platelets adhere to and others separate from the developing thrombus [27]. Activation of platelets bound to the injured vessel wall causes a conformational transition in GpIIb/IIIa that increases its affinity for fibrinogen and vWf [29]. At low shear rates fibrinogen is the predominant ligand, whereas vWf plays an important role at higher shear rates [30,31]. Neither vWf nor fibrinogen is required for platelet accumulation [32]. Propagation of platelet activation depends upon interaction of several agonists with receptors expressed on platelets themselves. Three outside-in signals of particular relevance are mediated by ADP, thrombin and TxA_2 [33] and responsible for platelet activation and aggregation.

Platelets express at least two ADP receptors, $P2Y_1$ and $P2Y_{12}$. The activation of $P2Y_{12}$ inhibits adenylate cyclase causing a decrease in the cyclic AMP (cAMP) level and the activation of $P2Y_1$ causes an increase in the intracellular Ca^{2+} level. The $P2Y_{12}$ receptor is the major receptor able to amplify and sustain platelet activation in response to ADP, by facilitating the release of intracellular calcium stores by decreasing cAMP levels in the platelet.

Platelets produce two eicosanoids, namely TXA_2 and isoprostanes with pro-aggregating properties. TXA_2 derives from enzymatic oxidation of arachidonic acid by COX1 while isoprostanes derive from non-enzymatic oxidation of arachidonic acid by reactive oxidant species (ROS)-generated nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase [34]. While TXA_2 serves for the initial phase of platelet activation, isoprostanes are implicated in the propagation of platelet aggregation along with ROS and ADP release from activated platelets (Fig. 1).

Global tests for primary haemostasis and bleeding

Bleeding time

The skin bleeding time (BT) (Table 1) is an easy and frequently used global test for primary haemostasis [35,36]. Because the BT is prolonged in congenital and acquired platelet defects, it has been common reasoning for years that this test could provide a screening for hemorrhagic tendencies in other platelet disorders.

The Simplate® template device is the technique most commonly used to measure BT with an upper limit of normal being between 9 and 10 minutes [36]. However, this test may be sensitive to a variety of variables that may produce false-positive and false-negative results. Thus, it is not only influenced by the platelet count and function, but may also be affected by the packed cell volume, blood urea concentration, platelet volume, and the nature of skin connective tissue. In haematological (bone marrow) causes of thrombocytopenia (TCP) there is a direct correlation between BT and platelet count $<100 \times 10^9$/L [37].

On this basis, BT has been used to measure primary haemostasis in cirrhosis [38,39]. However, in 100 patients with cirrhosis, Blake *et al.* [38] showed that only 42% had BT prolongation, which could be explained only in part by a concomitant TCP. In fact, there was only a weak correlation between BT and platelet count. A prolonged BT in LC patients with platelet counts greater than 100×10^9/L, a level typically regarded within "safe limits" for invasive procedures, was observed in some patients, and conversely there was a normal BT in some patients with platelet count $<100 \times 10^9$/L. Liver failure itself is associated with prolongation of BT. A prospective study conducted in seventy LC patients demonstrated a progressive prolongation of BT from Child-Pugh class A to class C patients [40]. Nevertheless, the clinical value of BT prolongation as risk factor for bleeding in liver cirrhosis remains uncertain. Boberg *et al.* [41] reported that a prolonged BT was associated with a 5-fold increase risk of hemoglobin reduction after liver biopsy. Two retrospective studies [39,42] showed a significant association between BT and a previous history of gastrointestinal-haemorrhage, but a prospective study failed to confirm this association [43]. Thus, in patients with cirrhosis without previous bleeding, only variceal size and severe liver failure, but not BT, significantly predicted bleeding events during one-year follow-up [43]. It is therefore possible that the prolongation of BT will have a different impact on provoked (liver biopsy) or spontaneous (gastrointestinal bleeding) bleeding but literature data are not sufficient to support such hypothesis.

The poor association of BT as risk factor for bleeding in cirrhosis is supported by interventional studies with drugs that increase platelet vascular adherence and activation [6–9]. Thus, treatment with desmopressin (DDAVP), a synthetic peptide homologous to human vasopressin that is usually employed in congenital bleeding disorders such as von Willebrand's disease, was able to shorten the prolonged BT in patients with cirrhosis [6,7]. This is probably due to the increase in von Willebrand factor (vWF) and FVIII seen after administration of DDAVP which possibly compensates for the thrombocytopenia. However, de Franchis *et al.* [8] showed that in LC patients with active variceal hemorrhage, on treatment with terlipressin, recurrence of bleeding occurred more frequently in patients who received desmopressin compared to those who did not (54% *vs.* 27%, respectively). In addition, desmopressin did not reduce the transfusion requirement in patients

Table 1. Tests for assessing primary hemostasis.

Platelet tests	Description	Pro	Con
	Haemostasis global tests		
In vivo bleeding time assessment	The time it takes for a standardized skin wound to stop bleeding The bleeding time measures the ability of platelets to arrest bleeding and, therefore, is a measure of both platelet number and function	Physiological (platelet-vessel interaction) Cheap No specialised laboratory required Not influenced by blood sampling	Poorly reproducible Invasive Insensitive Time consuming No correlation with bleeding tendency
Thromboelastography	Blood clots in a backward and forward rotating sample cup around a suspended pin. Because of clot formation, the cup's motions become limited. These movements are mapped and different variables (depending on coagulation, fibrinolysis and platelet function) can be calculated	Global haemostasis Automated Point of care test Rapid and Simple to perform Commonly used for helping define thresholds of transfusion	Measures Clot properties only Not sensitive to platelet function
PFA-100	The test attempts to mimic *in vivo* shear-dependent platelet function. It measures the closure time (CT), by platelets, of an aperture in a membrane coated with either collagen/ADP or collagen/epinephrine	Simple to perform Rapid Small volumes Citrated blood up to 4 h	Sensitive to many variables (haematocrit, drug, dietary effects) Can give false negative results
	Platelet Function Assessment		
Aggregometry	Tests the *in vitro* ability of platelets to stick to one another, i.e platelet aggregation in response to stimulation by a panel of exogenous agonists [collagen, thrombin, adenosine diphosphate (ADP), etc.] - Platelet Rich Plasma (PRP) Method (optical light turbidity is a measure of aggregation) - Whole Blood Method (electrical impedance measurement)	Valuable for identifying and diagnosing platelet function defects which are inherited or secondary to drugs	Specialised laboratory required Test platelets under un-physiological conditions (Low shear conditions and in free solution within PRP) Performed within 2 h of blood sampling
Flow cytometric quantification of membrane molecule expression [fluorescent-activated cell sorting (FACS) analysis]	Evaluates the presence of stimulation-dependent antigens [e.g. CD62P (P-selectin)] or platelet-leucocyte complexes. Measure of platelet activation	Whole blood test Flexible Wide variety of tests available Only small quantities of blood are required May help predict patients at risk of thrombosis	Expensive instrument and specialised laboratory required Never evaluated for assessing bleeding risk
Measurement of Soluble activation markers	To measure platelet-release products within platelet-poor plasma by radioimmunoassays (RIAs) and/or enzyme-linked immunosorbent assays (ELISAs) - Soluble P-selectin - PF4 and β-TG - Soluble CD40L	Simple, systemic measure of platelet activation may help predict patients at risk of thrombosis	Prone to artefact Careful handling and blood processing Cannot predict risk of bleeding
Platelet Adhesion test under flow conditions	Assays that try to simulate the *in vivo* platelet function *in vitro*, under flow conditions to study shear-induced platelet activation	Simulate physiological condition Small volumes of citrated blood up to 4 h from sampling	Expensive instrument and specialised laboratory required Sensitive to many variables that influence platelet function (platelet count, haematocrit, drug, and diet)
Molecular mechanism detection of platelet function	The concentrations of second messengers [e.g. calcium, cyclic adenosine monophosphate (cAMP)], and the release of platelet granules containing proaggregatory molecules [serotonin, adenosine triphosphate (ATP), PF4, bTG]	Molecular insight	Expensive instrument and specialised laboratory required

undergoing hepatectomy [9]. The limited effect of desmopressin in increasing the size of the vWF complexes [44], also suggests a reduced or ineffective action of this drug in cirrhosis.

As platelet activation is not diminished but can be increased in cirrhosis, it is possible that BT prolongation in these patients results more from changes in vasoreactivity and/or arterial

dysfunction which are well documented in cirrhosis [45], than from platelet number or function. Enhanced platelet activation could also explain the normal BT found in some patients with cirrhosis despite low platelet counts [7,38].

Platelet function assay (PFA)-100®

The PFA-100 (Table 1) test attempts to mimic *in vivo* shear-dependent platelet function. It measures the closure time (CT), by platelets, of an aperture in a membrane coated with either collagen/ADP or collagen/epinephrine. While it may substitute for skin bleeding-time testing in the assessment of suspected von Willebrand's disease and qualitative platelet disorders [46], it appears to be of limited value in other settings compared to healthy controls. Prolonged CT occurs in patients with end stage liver disease [47] as well as in stable cirrhotic patients [48,49]. Nevertheless, the prognostic value of abnormal PFA-100 in predicting bleeding complications in LC patients has never been investigated [50].

Thromboelastography (TEG®)

Recently, other global *in vitro* laboratory tests such as Thrombelastography (TEG) have been developed to explore both platelet and clotting function. TEG gives information on clotting factor activity, platelet function, and fibrinolysis. TEG abnormalities indicating hypercoagulability have been reported in LC [51,52]. Recently, PlateletMapping using TEG technology has been suggested as a potentially useful and novel approach to evaluate platelet function in patients with cirrhosis [53,54] (Table 1). These tests require further evaluation to verify their utility in various clinical situations.

Thrombocytopenia and cirrhosis

Thrombocytopenia is defined as any decrease in platelet count below the lower normal limit, which is usually around 140 x 10^9/L. TCP is a common finding in cirrhosis and reported in as many as 76% of patients [55].

However, thrombocytopenia has not been associated with an increased risk of bleeding from esophageal varices or other sites, although it is correlated with blood loss during surgery [56]. Moderate TCP (50–75 $\times$ 10^9/L) is observed in approximately 13% of patients with cirrhosis. Severe TCP, defined as platelet count less than 50 $\times$ 10^9/L occurs in only 1% of patients [57]. This threshold is often used as a cut-off when managing patients with cirrhosis, as a contraindication for elective invasive procedures for example liver biopsy, paracentesis, thoracentesis, because of an assumed increased risk of bleeding [58–61]. Often platelet transfusions are used prophylactically to cover similar procedures.

Thus, as in any critically ill patient [62], severe TCP could impact the routine care of liver cirrhosis patients [63], potentially

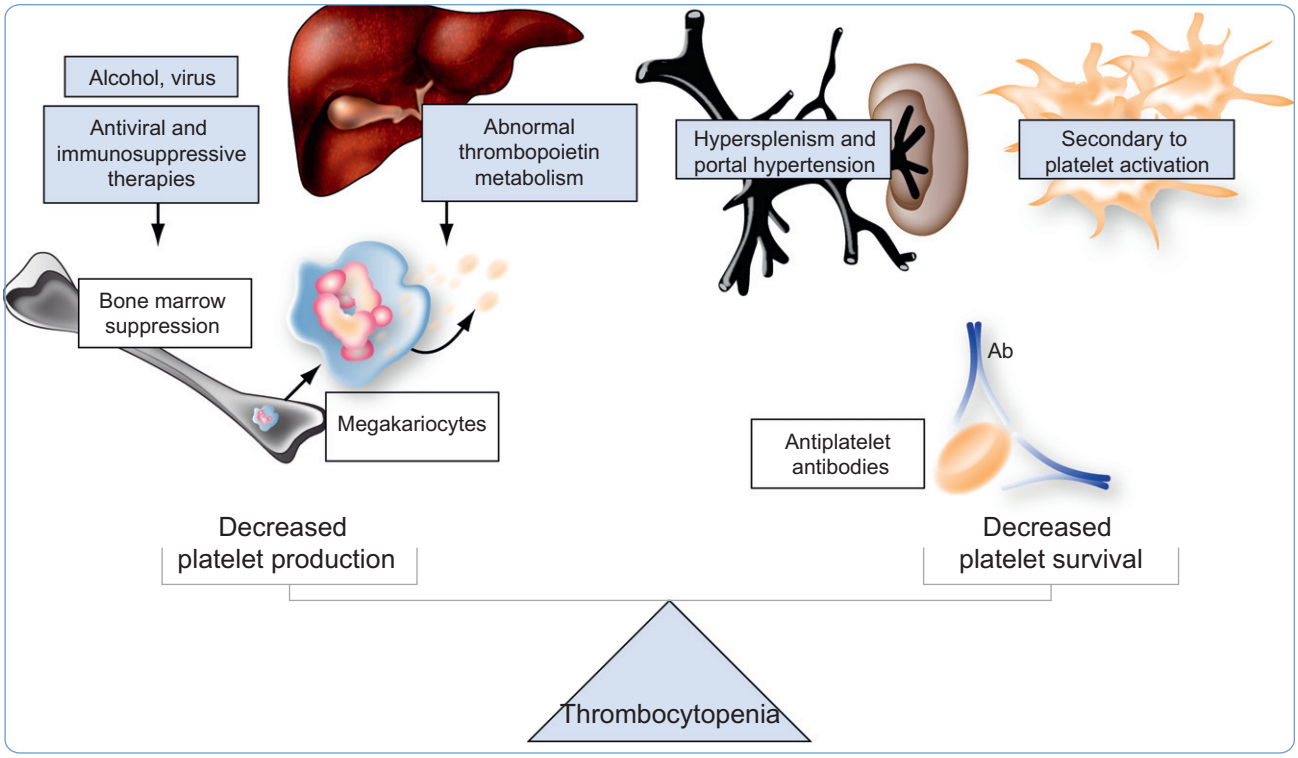

Fig. 2. Mechanisms of thrombocytopenia in liver cirrhosis. Thrombocytopenia in liver cirrhosis is traditionally believed to be the result from an imbalance between (1) platelet production and (2) platelet survival. Factors that decrease platelet production: (a) direct bone marrow suppression caused by the underlying etiology o liver disease and (b) the inadequate thrombocytopoiesis for abnormal thrombopoietin production or activity. Factors decreasing platelet survival include: (a) enhanced splenic and splanchnic sequestration secondary to portal hypertension (b) autoantibodies directed against platelet surface antigens that produce an augmented removal of platelets by the splenic and hepatic reticulo-endothelial systems (c) increased platelet consumption as a result of platelet activation.

postponing or interfering with diagnostic and therapeutic procedures including liver biopsy, antiviral therapy, and medically indicated or elective surgery.

Accordingly, Tripodi *et al.* [64] documented that thrombocytopenia limits thrombin generation in LC patients so potentially predisposing to bleeding tendency. Thus, platelet transfusion might be useful only in patients with low platelet counts during acute bleeding or before undergoing surgery or liver biopsy.

Mechanisms of thrombocytopenia

The pathogenesis of TCP in cirrhosis is still not fully understood. Multiple factors have been proposed for the pathogenesis of TCP in advanced liver cirrhosis. Traditionally TCP is believed to result from an imbalance between platelet production and platelet survival (Fig. 2).

Decreased platelet production

Bone marrow suppression

Suppression of platelet production in the bone marrow can be caused by the underlying etiology of the liver disease [65–67]. Thus, in chronically infected hepatitis C (HCV) patients, either HCV itself or interferon treatments [68] seem to be responsible for bone marrow suppression and eventually TCP [69]. Alcohol is another factor causing TCP via direct inhibition of megakaryocyte maturation and ultimately platelet formation [70,71]. Cirrhotic patients might also have dietary deficiencies like in the case of vitamin B_{12}, folic acid and iron that could also contribute towards thrombocytopenia.

Altered thrombopoietin metabolism

Liver cells produce thrombopoietin (TPO), an important cytokine affecting megakaryocytes ploid amount, growth, and size [72]. Low *TPO*-mRNA expression was detected in the liver of patients with advanced liver disease and could be responsible for a deficient hepatic thrombopoietin production [73,74]. Accordingly, patients with LC and TCP revealed significantly lower reticulated platelet levels than LC patients without TCP [72,75]. Moreover, after orthotopic liver transplantation an increase of TPO was observed [76,77].

However, the clinical impact of these data is confused by the divergent results on serum TPO levels, including normal, decreased or increased in LC [72,74–83]. This issue may be consequent to an inadequate standardization of TPO laboratory assays that should be solved in the future [84].

Decreased platelet survival

Hypersplenism and portal hypertension

The original theory by Aster *et al.* [85] suggested that in LC, TCP could exclusively be explained by an increased pooling of platelets in the enlarged spleen because of portal hypertension. Thus, kinetic radiolabelled platelet studies showed shorter platelet survival time [86]. Additionally, partial splenic embolization demonstrated an increase in platelet count suggesting a contribution of splenic sequestration in TCP [87,88]. Despite these findings, a direct correlation between portal pressure or spleen size and platelet count was never firmly demonstrated.

Gastroesophageal varices (GEV), a direct consequence of portal hypertension, appear to be associated with a wide range of TCP (ranging from 68 to 160×10^9/L) as showed by several cross-sectional studies [89–93] performed in decompensated and compensated LC patients [94,95]. Thus, Giannini *et al.* [55] proposed platelet count/spleen diameter ratio as a non-invasive predictor of GEV; the diagnostic accuracy of this ratio for GEV was 86% with a negative predictive value of 87% [96]. However, normalization of portal pressure with transjugular portosystemic shunts (TIPS) could not consistently demonstrate a benefit in terms of platelet count [97,98].

Platelet-associated antibodies

Increased levels of immunoglobulin G (IgG) bound to platelets, suggest the presence of autoantibodies reactive with platelets in patients with chronic liver disease [79,99]. Autoantibodies directed against platelet surface antigens can enhance removal of platelets by the splenic and hepatic reticulo-endothelial systems. This is clearly the case in certain patients with hepatitis C [100]. Nevertheless, the role of these antiplatelet antibodies in TCP in other causes of cirrhosis is still unclear because of their nonspecific binding to platelet surface [100,101].

Platelet consumption

Platelet activation, with ensuing platelet consumption, could be implicated in TCP of LC (see paragraph: Thrombocytopathy). Low-grade disseminated intravascular coagulation may play a role as suggested by the concomitant increase of prothrombin fragment 1+2 (F1+2) and D-dimer in advanced LC [4]. Endotoxemia may be implicated as LC patients treated with non-absorbable antibiotics showed a significant and simultaneous reduction of endotoxemia, F1+2, and D-dimer plasma levels [4]. Furthermore, endotoxemia *per se* could reduce peripheral blood counts either directly or indirectly though the release of cytokines [102,103]. Thus, Kalambokis *et al.* showed that intestinal decontamination lowered endotoxaemia and raised peripheral blood counts by inhibiting cytokines and enhancing the production of nitric oxide (NO), a potent vasodilator and anti-aggregating molecule [104].

Co-existing conditions and bleeding risk in LC patients

Anemia may complicate the clinical course of cirrhosis and could theoretically predispose to bleeding by impairing platelet function. Thus, red cells activate platelet COX1 [105], inactivate NO [106] and greatly contribute to vessel repair by favoring platelet attachment to damaged vasculature [107]. However, the impact of anemia on bleeding complication of cirrhotic patients needs to be further investigated along with exploring the cost/benefit of red cell transfusion in case of cirrhosis with associated anemia [107]. It has been shown that a restrictive transfusion approach (target hemoglobin level: 7.0 and 9.0 g/dl) was as effective as

Table 2. The advantages and disadvantages of strategies available to prevent bleeding in patients with liver cirrhosis: focus on platelet count and function.

Strategies	Pro	Con
Platelet transfusion	Improves primary haemostasis Restore thrombin generation to normal (if platelet count rises to more than 50,000 per microliter)	Transfusion-related side effects Negative impact on liver transplantation outcome Fluid overload and Exacerbation of portal hypertension
Red cell transfusion	Improves platelet function	Transfusion-related side effects Negative impact on liver transplantation outcome Fluid overload and Exacerbation of portal hypertension
1-desamino-8-D-arginine vasopressin (DDAVP)	Laboratory improvement of primary haemostasis Well tolerated Simple to administrate	No benefits in clinical trials
Thrombopoietin Receptor agonists	Increases endogenous platelet count No transfusion-related side effects	No clinical efficacy Safety issues (risk of thrombosis, rebounded thrombocytopenia)

and possibly superior to a more liberal transfusion policy [108], accompanied by reduction of transfusion-related side effects.

A restrictive transfusion policy should also be adopted during liver transplantation procedures. Indeed, it has been shown that transfusion of red cell concentrates, as well as the amount of transfused blood product, could be associated with a reduced graft and patient survival [109].

Patients with cirrhosis have increased risk to develop sepsis and sepsis-related complications [110,111]. Endotoxemia-related sepsis could promote platelet aggregation, microvascular obstruction, and tissue injury [112] and eventually platelet exhaustion with subsequent enhanced risk of bleeding [113]. Of note, prophylactic antibiotic therapy can reduce the early rebleeding after a first bleeding episode and permits a better control of active bleeding [114].

Treatment of thrombocytopenia in liver cirrhosis

Thrombocytopenia may have a negative impact on clinical management of patients. Therapeutic platelet transfusions are unequivocally indicated for patients with active bleeding associated with thrombocytopenia [115]. There is consensus that the platelet count should not be allowed to fall below 50×10^9/L in patients with acute bleeding.

According to the guidelines for the general use of platelet transfusions [115], a threshold of 10×10^9/L is as safe as higher levels for patients without additional risk factors such as sepsis, concurrent use of antibiotics or other abnormalities of haemostasis.

However, in patients with advanced liver disease there is a lack of consensus regarding the degree of thrombocytopenia that may be associated with an increased risk of bleeding [116,117]. Thus, specific guidelines in LC are lacking to indicate the platelet cut-off below which procedures (as well as liver transplantation) should be delayed and/or platelet transfusions or platelet-stimulating agents should be administrated.

Several therapeutic options (Table 2) are currently available to raise platelet count to a safe level for invasive procedures or in case of active bleeding.

Therapeutic options

Platelet transfusion (PT)

Platelet transfusion (PT) is the standard-of-care in general to temporarily increase platelet counts prior to invasive procedures [115]. Current recommendations for PT [115] concerning liver biopsy, lumbar puncture, epidural anaesthesia, or similar procedures in patients with chronic and stable thrombocytopenia suggest that the platelet count should be raised to at least 50×10^9/L.

Recently, the American Association for the Study of Liver Disease guidelines [118] recommend that platelet transfusion before liver biopsy, transcutaneously or transvenously, should be considered when levels of platelet count are less than 50–60 $\times 10^9$/L (Class 1, Level C i.e. without evidence from randomized studies). Thus, in LC randomized controlled studies, assessing efficacy and safety of restrictive PT strategies in thrombocytopenic patients during invasive procedures such as liver biopsy, are needed [119]. Moreover, PT seems to be inappropriate for long-term management partially due to potential allo-immunization and also because shortened allogeneic platelet survival due to sequestration in enlarged spleens. In addition, in liver transplant patients, platelet transfusions have been associated with increased post-operative mortality, as a result of an increased risk of acute lung injury [120]. Additionally, there are no data to support benefit of PT strategies in subjects with TCP and variceal bleeding [121].

Splenectomy or partial splenectomy

Splenectomy or partial splenectomy by embolization can partially reverse TCP in patients with liver disease [122]. However, these procedures are not routinely recommended for their immunological consequence (impaired immunity and bacterial vaccination) and the non-negligible associated mortality and morbidity [123].

TPO targeting agents

Recently, the role of TPO targeting agents (Table 3) has also been tested. Currently, only two TPO mimetics, Romiplostin

Table 3. Agents targeting thrombopoietin (TPO) pathway.

Agents	Mechanism of action	Administration route	Pro	Con
First-generation thrombopoietins (Recombinant human TPO-rhTPO- and pegylated recombinant human megakaryocyte growth and development factor -PEG-rHuMGDF)	TPO receptor agonist	Subcutaneous	Increase megakaryopoiesis and thrombopoiesis	Immunogenic issues (development of antibodies against PEG-rHuMGDF, which cross-reacted with and neutralized endogenous TPO, producing thrombocytopenia) Significantly primed platelet activation (Risk of thrombosis) Use abandoned in 1998
Second-generation thrombopoietins (TPO peptide mimetic -romiplostim- and TPO nonpeptide mimetic -eltrombopag-)	TPO receptor agonist	Subcutaneous: peptide mimetic Oral: non peptide mimetic	Dose-dependent increase in platelet counts Management of hepatitis C virus-related thrombocytopenia Well tolerated, (Mild headache-most common side effect) Non immunogenic TPO	Potential long-term complications (risk of thrombosis, rebound worsening of thrombocytopenia upon discontinuation)
Cytokines with potent thrombopoietic activity (Recombinant human interleukin-11 -rhIL-11-)	IL-11 mimetic	Subcutaneous	Increase in platelet counts	Toxicity High Cost

and Eltrombopag, have been approved for the treatment of idiopathic thrombocytopenic purpura [124]. They seem to be potentially useful before invasive procedure in TCP patients. Nevertheless, the higher incidence of portal axis thrombosis in patients with advanced liver disease, observed in a recent trial, prompted the FDA to advise against use of TPO mimetics in LC patients (ClinicalTrials.gov Identifier: NCT00678587).

Thrombocytopathy in liver cirrhosis

The belief that cirrhosis is associated with impaired platelet activation has been based on the existence of changes of laboratory tests exploring platelet aggregation. Thus, decreased agonist-induced platelet aggregation (PA) by common agonists such as thrombin, collagen, ADP, epinephrine, and arachidonic acid has been detected in LC [125–128]. Intra- and extra-platelet mechanisms including multiple defects in signal transduction or storage pool defect and membrane-related defects caused by enhanced high-density lipoprotein (HDL) apolipoprotein E content were all considered to account for platelet dysfunction [125, 128] (Table 4).

However, more recent data have questioned this hypothesis. For instance, the urinary excretion of 11-deydro-thromboxane (Tx) B_2, a stable metabolite of TxA_2, was increased in cirrhosis suggesting that platelets could be activated [129]. Although this interpretation cannot be fully supported by the data of 11-deydro-TxB2 urinary excretion as it only partly reflects the activation of platelet COX1 [130], other studies also seem to support the existence of enhanced platelet activation in cirrhosis. Soluble P-selectin (sPs), which is an *in vivo* marker of platelet activation is consistently elevated in plasma particularly in cases of severe liver disease and correlated with markers of hepatic protein synthesis and low platelet counts [131–133].

More recent flow cytometry analysis of platelet activation, as well as platelet-monocyte aggregates, is consistent with hyperactivation of platelets in cirrhosis [134,135].

The discrepancy between the early studies of platelet aggregation suggesting platelet hypo-aggregability secondary to impairment of platelet activation, and more recent studies showing platelet over-secretion of P-selectin, suggesting increased platelet COX1 activation, is not easy to explain. One issue is that aggregation tests in cirrhosis are intrinsically difficult because the reduced platelet count makes them difficult to interpret since these tests are dependent on platelet count.

The biological plausibility of platelet activation in cirrhosis does not have a clear-cut mechanism either. Thrombin is the key player in the clotting cascade and through platelet receptors

Table 4. Platelet function tests in patients with liver cirrhosis. (See below-mentioned references for further information.)

[Reference]	Subjects (n)	Platelet function test	Results
Ingerberg S *et al.,* 1985 [144]	20 LC	PA	Reduced
Laffi G *et al.,* 1988 [125]	24 LC	PA	Reduced
		Thromboxane Metabolites	Increased
Desai K *et al.,* 1989 [128]	30 LC	PA	Reduced
Laffi G *et al.,* 1992 [126]	31 LC	PA	Reduced
		BTG/PF4	Increased
Laffi G *et al.,* 1993 [127]	12 LC	PA	Reduced
		Mean platelet volume	Increased
Laffi G *et al.,*1996 [145]	9 LC	PA	Reduced
		P-selectin stimulated expression	Reduced
		Thromboxane Metabolites	Increased
Ferro D *et al.,* 1996 [45]	32 LC	vWF Antigen and vWF ristocetin cofactor activity	Increased
		vWF Multimers	No difference
Davì G *et al.,* 1998 [129]	44 LC	Thromboxane Metabolites	Increased
Panasiuk A *et al.,* 2001 [133]	27 LC	sPs and BTG/PF4	Increased
		Mean platelet volume	Reduced
Ferroni P *et al.,* 2001 [132]	39 CH HCV	sPs and vWF Antigen	Increased
Pihusch R *et al.,* 2002 [49]	25 CH 65 LC	Platelet Function Assay-100	Increased (HCV and alcoholic LC); Normal in PBC/PS
		PMP expression	No difference
		vWF Antigen	Increased
Ogasawara F *et al.,* 2005 [134]	9 LC HCV 20 AFLD	sPs and PMP	Increased
Lisman T *et al.,* 2006 [138]	54 LC	vWF Antigen and vWF ristocetin cofactor activity	Increased
		vWF Collagen Binding Capacity and vWF Multimers	Reduced
Vardareli E *et al.,* 2007 [131]	40 LC	sPs	Increased if platelet count $<100x10^{-3}/\mu l$ Decreased in PTS with portal vein thrombosis
Sayed D *et al.,* 2010 [135]	60 LC	PMP	Increased (in patients without bleeding complications and in patients with splenomegaly)
Ozhan H *et al.,* 2010 [146]	70 NAFLD	Mean platelet volume	Increased
Ercin CN *et al.,* 2010 [147]	50 NAFLD	sPs	No difference
Kilciler G *et al.,* 2010 [148]	60 NAFLD	Mean platelet volume	No difference

AFLD, alcoholic fatty liver disease; BTG, beta-thromboglobulin; CH, Chronic hepatitis; LC, Liver Cirrhosis; MPV, mean platelet volume, NAFLD, non alcoholic fatty liver disease; PA, platelet aggregation; PBC, primary biliary cirrhosis; PF4, platelet factor 4; PMP, platelet micro-particles; PSC, primary sclerosing cholangitis; sPs, soluble P selectin; vWF, von Willebrand Factor.

PAR1 and 4 acts as a potent platelet activator. It is possible that an enhanced *in vivo* formation of thrombin may account in part for platelet activation. This is suggested by the correlation between plasma levels of F1+2 and the urinary excretion of 11-dehydro-TxB_2 [129]. The evidence that thrombin generation in cirrhosis is normal or even increased [4,19,20] would "allow" for sufficient thrombin despite the relatively low prothrombin levels found in patients with cirrhosis.

Changes in vWF may also play a role. vWF is a large, multimeric protein with a crucial role in primary haemostasis, since platelet–vWF interaction is one of the first steps in platelet adhesion [136]. Accordingly, severe bleeding tendency has been associated with vWF deficiency [137]. We have shown that higher molecular weight multimers (HMWMs) of vWF are present in cirrhosis. These HMWMs are more active in binding platelets [44]. These data were confirmed by Lisman *et al.* [138] who demonstrated that in cirrhosis elevated vWF plasma levels resulted in a substantially elevated platelet deposition on collagen in a vWF-dependent, flow-driven, platelet adhesion assay. The increased adhesion induced by plasma from patients with cirrhosis was observed with both normal and patients' platelets, and was independent of platelet count. This indicates that the increase in vWF might in part compensate for the quantitative platelet defects described in these patients.

Conclusions

Recent data from the literature indicate that stable patients with LC may seldom have defects in primary haemostasis that predisposes them to bleeding. The prolongation of BT is not related directly to platelet count unless a severe thrombocytopenia defined arbitrarily as 50 x 10^9/L or less is present.

The increased platelet activation by chronic inflammation including increased endotoxemia, coupled with increased levels of vWF may improve primary haemostasis. Similar compensatory mechanisms need further elucidation.

To date the clinical impact of platelet hyperactivity in LC patients has never been studied and needs to be clarified. An important aspect is whether platelet hyper-function has some relationship with the thrombotic outcomes that may complicate the clinical course of cirrhosis. Also, experimental data demonstrate that platelet hyperactivity might be implicated in the progression of liver disease [139,140], and fibrosis [141].

Accordingly, platelet activation is necessary to accumulate virus-specific cytotoxic T lymphocytes and determine organ damage in mouse models of acute viral hepatitis and antiplatelet treatment seems capable of modulating it [140,142].

If a relationship with clinical outcomes is proven, then antiplatelet or anticoagulant treatment may have a place in the management of compensated cirrhosis [143] but this hypothesis needs to be carefully and cautiously explored.

In conclusion, our review indicates that platelet function or primary haemostasis may not be defective in cirrhosis, and a low platelet count should not necessarily be considered as an automatic index of an increased risk of bleeding.

It is likely that treatments used to increase platelet count in the chronic state could potentially harm the patient. Thus, the recent report that LC patients with thrombocytopenia experienced thrombosis of the portal venous system during treatment with a thrombopoietin receptor agonist warrants further evaluation. Conversely, caution should be observed in patients with severe low platelet count ($<50 \times 10^9$/L) where correction of platelet count is advised if bleeding occurs and before invasive diagnostic and therapeutic procedures.

Key Points

- Patients with cirrhosis can have abnormalities in laboratory tests reflecting changes in primary haemostasis.
- There is not universally accepted platelet function assay in cirrhosis - this is needed in order to establish evidence based clinical guidelines.
- Such changes have been considered particularly relevant in the bleeding and thrombotic complications that occur in cirrhosis.
- Routine diagnostic tests, such as platelet count, bleeding time, PFA-100, thrombelastography are not clinically useful to stratify bleeding risk in LC patients.
- Data from a randomized study suggests a reduced or ineffective action of desmopressin (DDVAP) administration in cirrhosis to prevent variceal re-bleeding.
- The platelet count threshold for platelet transfusion prior to invasive procedures does not have an evidence base and needs further study.
- The role of platelet transfusion for bleeding in cirrhosis does not have a universally accepted protocol - this requires further study.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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