Chronic Liver Failure and Hemostasis

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Abstract
Hepatic insufficiency is a pathology that conditions synthesis and metabolism of various biomolecules. Alterations of hemostasis is one of its first systemic consequences. Because of this and the size of the risks, it is not uncommon for clotting tests to be indispensable for formulating prognostic scales in patients with liver disease.

Knowledge about hemostasis has advanced in the last decade, and the classic cascade of activation of coagulation factors has been perfected until it has become the now-current cellular model that considers the valuable and indispensable participation of the endothelium and platelets. Thanks to this, it is possible to understand that the risk of bleeding in patients who have hepatic insufficiency is not only due to deficiencies in production of coagulation factors, and that for this reason administration of vitamin K is questionable. Even more relevant, is the fact that, thanks to this knowledge, we can understand the sometimes contradictory and potentially life-threatening complication of thrombosis in these patients.

Keywords
Blood coagulation, liver failure, thrombosis, vitamin K.

INTRODUCTION
The liver is a vital organ that performs various metabolic processes ranging from transformation and elimination of toxins to storage and production of biomolecules that are indispensable for life. Liver failure, or hepatic insufficiency, occurs when liver is unable to carry out its functions of synthesis and metabolism. Initially, liver failure was classified as either acute or chronic, but a new state of acute-on-chronic liver failure (ACLF), characterized by acute exacerbation of chronic liver damage, has been recently recognized. (1, 2)

Drug-induced injury is the main cause of liver failure, but it is subdivided according to the type of damage into intrinsic or dose-dependent damage idiosyncratic, regardless of dose, damage. (3-5). The best example of intrinsic damage is acetaminophen related liver failure. Chronic liver failure due to alcohol is the most frequently occurring type, followed by those caused by infectious agents. Of these, 75% to 80% are associated with the hepatitis C virus (HCV). Some experts consider that the proinflammatory states associated with steatosis, such as obesity or diabetes, should also be considered as factors for the development of chronic liver failure. Autoimmune diseases deserve particular mention because they can manifest as acute liver failure but can also cause chronic deterioration (Table 1). (6, 7)

PHYSIOLOGY OF HEMOSTASIS
Hemostasis is a complex process that involves the stimulation of coagulation factors on the surface of activated platelets to achieve vascular tissue repair. (8) In general, hemostasis is divided into primary and secondary, the for-
In recent decades, understanding of hemostasis has improved. One of the most relevant changes is that platelets are no longer thought to be the sole element forming the hemostatic plug during primary hemostasis. Now it is known that they are more than 300 substances contained in intracellular granules (α granules) among which growth factors necessary for tissue regeneration should be highlighted. (10)

Cellular Coagulation Model

Traditionally, coagulation was understood as a cascade of ordered activation of factors, but current model proposed by Hoffman and Monroe considers interactions between factors and cellular elements. (11) The cellular model of coagulation starts from the basal state in which factors X and VII are inactive in the bloodstream. Tissue factor (TF) carrier cells including monocytes and cells from the endothelium and subendothelium and monocytes are already present before noxious stimuli cause TF to be released into the bloodstream which begins the process of coagulation. This consists of three phases:

1. Phase 1 (initiation): TF binds factor VII to transform and activate factors IX and X. Factor Xa is combined with Factor Va present on cell surfaces thereby transforming small amounts of prothrombin (Factor II) into thrombin (Factor IIa).
2. Phase 2 (amplification): Thrombin maintains production of Factor Xa through intervention in the intrinsic pathway (Factors XI and IX), and, more importantly, through releasing plasma transporter and activating Factor VIII. Factor VIIIa is a very efficient Factor X catalyst. Overall, these pathways maintain and substantially increase thrombin production and create an environment conducive to recruiting and activating more platelets.
3. Phase 3 (propagation): Once platelets are activated, they release chemoattractants such as adenosine diphosphate (ADP) and thromboxane A2 and change their physical form to one which facilitates creation of the hemostatic plug. This form expresses negatively charged phospholipids such as phosphatidyserine on the platelet cell surface. In conjunction with calcium, these phospholipids serve as a template for the formation of tenase complexes (Factors VIIIa and IXa) and prothrombinase complexes (factors Xa and Va), which have the capacity to generate large amounts of thrombin. Finally, thrombin conditions the polymerization of fibrinogen fragments in fibrin fibers that stabilize the initial clot. In addition, thrombin activates Factor XIII which stabilizes fibrin and provides greater resistance to the lysis process (Figure 1). (9, 12, 13)

PHYSIOPATHOLOGY OF HEMOSTASIS DURING HEPATIC INSUFFICIENCY

Alterations in Primary Hemostasis

From the early stages of liver disease massive accumulations of lipids develop in hepatocytes and induce the release of various inflammatory mediators, among which are interleukin (IL) and tumor necrosis factors (TNF) 1 and 6. Expression of Toll-like receptor 4 is stimulated, (14, 15) and these pathways trigger dysregulation of the endothelium by activating asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase (eNOS). (16, 17) Animal models have shown that decreased activity of eNOS is a fundamental part of the pathophysiology of portal hypertension. (18, 19)

In addition to serving as cellular elements in the hemostatic plug, platelets possess intracellular granules whose elements are capable of modifying inflammatory and immunological processes within the liver. (10) The α-granules contain growth factors whose targets are the Akt pathway (also known as the PI3K-Akt pathway) and extracellular signal-regulated kinase (ERK) 1 and 2 pathways all of which are transcendental for hepatocytes (20) to the degree that recent research suggests that platelet transfusion may improve liver regeneration. (20, 21) The dense granules contain adenine nucleotides that inactivate the stellar cells of the sinusoidal space and, consequently, slow profibrotic mechanisms. (21, 22) Thrombocytopenia is one of the most frequently occurring manifestations of liver disease. Approximately 76%

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NSAIDs: non-steroidal anti-inflammatory drugs

Table 1. Classification of agents that can cause liver failure
to 85% of patients develop thrombocytopenia, defined as less than 150,000 thrombocytes (platelets)/μL of blood, although 13% of these patients have less than 50,000 thrombocytes/μL. (23) It should be remembered that the main stimulus for formation of platelets is thrombopoietin (TPO), a hormone produced primarily by hepatocytes. (24) TPO binding with its c-Mpl receptor promotes activation of cascades of the Janus 2 kinase (JAK2) and tyrosine kinase 2 (TYK2) and synergizes with other stimulants such as IL-3, IL-11 and stem-cell factor resulting in the proliferation and maturation of megakaryocytes. (24, 25) Panasiuk et al. have shown decreased production of TPO in patients with liver cirrhosis and, consequently, decreased numbers of platelets. They also found a direct association between the platelet count and hepatocyte growth factor (HGF) (p <0.01). (26) In this context, clinical trials began for TPO mimetics for treatment of liver failure, especially for patients with chronic viral hepatitis. McHutchison et al. evaluated the addition of eltrombopag, a TPO receptor agonist. The four week trial was conducted with HCV carriers who could not be treated with antivirals due to severe thrombocytopenia. Dosages of 30, 50 and 75 mg were compared to controls who received placebos. The best response was observed in the group treated with 75 mg doses of eltrombopag, 95% of whose platelet counts increased to acceptable levels. (27) Afdhal et al. obtained similar favorable results in the Endothelin Antagonist Used To Decrease Cardiac Events (ENABLE) 1 and 2 test cohorts which treated patients with bosentan and which had nine weeks of follow-up. (28) Afdhal et al. had reported the benefits of using eltrombopag for elevated platelet counts before carrying out invasive procedures in an earlier study called Early Levosimendan Vs. Usual Care In Advanced Chronic Heart Failure (ELEVATE). It compared levosimendan with usual care in advanced chronic heart failure using 75 mg doses for 14 days before each procedure. This reduced requirements for transfusions by 72% over those of patients who received placebos, although case patients experienced more portal vein thrombosis than did controls. (29).

The pathophysiology of thrombocytopenia includes increased destruction of platelets mediated by antibodies or due to hypersplenism. The two mechanisms can be

![Cellular model of coagulation](image)

**Figure 1.** Cellular model of coagulation. F: factor.
differentiated because hypersplenism is accompanied by moderate thrombocytopenia accompanied by a decrease in leukocytes due to splenic sequestration. (30, 31) Two large series point to traditional or laparoscopic splenectomies as therapeutic measures that help elevate platelet numbers to over 100,000/μL while also increasing the leukocyte counts of patients with liver cirrhosis. (32, 33) In animal models of hepatic fibrosis, splenectomies have been shown to reduce the levels of transforming growth factor β (TGF-β) making it is possible to limit and even reduce the areas of cirrhosis while also changing the immunological profile by increases of cytotoxic T cells. (34, 35)

**Alterations in Secondary Hemostasis**

Most procoagulant factors including fibrinogen, prothrombin and factors V, VII, IX, X, XI and XII are synthesized in the liver, so their serum levels depend on the degree of liver damage. (36, 37) Factors V and VIII are the first to decrease because they have the shortest half-lives (12 and 4-6 hours, respectively). (38) It is common for cirrhotic patients to suffer moderate reductions of factor VII which can be identified through the significant relationship between factor VII levels and prothrombin time (PT) (r = 0.76, p <0.001). (39)

Ruling out congenital defects is important for evaluating coagulation tests in patients with liver disease. The most frequent congenital defect is factor VII deficiency (one case per 500,000 births). (40) Factor VII deficiencies have been described in a number of clinical entities including pregnancy, heart disease and obstructive pulmonary disease. A small number of cases have been reported in cirrhotic patients. (41)

**THROMBOSIS AND HEPATIC INSUFFICIENCY**

Thrombin’s molecular pathways and their ability to generate states of microthrombosis and consequent ischemic states in the sinusoids have all been mentioned. Expression of pro-fibrogenic factors results from these processes and in turn accelerates and perpetuates damage to the parenchyma of the liver. (42) It is not uncommon for patients with hepatic insufficiency, even when they are prone to hemorrhaging, to suffer from highly localized thrombophilia in the splanchnic venous system. Clinical, this condition is expressed as Budd-Chiari Syndrome (SBC), mesenteric thrombosis (one case per million people) and extrahepatic portal vein thrombosis (four cases per million people). (43) It should be remembered that up to 40% of patients with liver cirrhosis suffer from portal vein thrombosis. (19, 44-46) Some experts suggest that reduction of portal vein flow velocity is a predictor of thrombotic events, (45) so that, once detected, an antithrombotic strategy should be initiated. Among the possibilities are anticoagulant therapy, angioplasty, stenting, intrahepatic transjugular shunts and even liver transplantation. (47, 48)

**von Willebrand Factor (vWF)**

vWF is a glycoprotein stored in Weibel-Palade bodies inside endothelial cells. Once secreted, vWF favors platelet adhesion and binds to factor VIII to prevent its early degradation. (49) As liver damage progresses, coagulation factors decrease, but, contrary to what had been previously thought, there is also parallel elevation of VWF and factor VIII. (50) The underlying mechanism seems to be very high levels of endothelial activity at the site of vWF synthesis where the cells involved in hepatic fibrosis processes are located that will lead to the reduction of proteins C and S (anticoagulant factors) and ADAMTS13, (A Disintegrin And Metalloproteinase With Thrombospondin Type 1 Motif # 13). (44) This protease is synthesized by stellate cells and is responsible for dividing vWF into small multimers capable of binding factor VIII. (51, 52) Decreases of ADAMTS13 activity to less than 10% caused by mutations, autoantibodies or by reduced production due to liver failure results in the production of ultra-long vWF multimers (UL-FvW) that cannot bind to factor VIII. This leads to increased amounts of factor VIII in its free and active form. (52, 53) A group of researchers from the University of Nara in Japan have shown that, the activity of ADAMTS13 decreases and serum levels of UL-FvW increase in patients with hepatic impairment due to alcohol. More importantly, these events correlate with the degree of severity of liver damage and might be useful as prognostic markers. (53, 54)

**Proteins C and S**

Protein C is one of the main anticoagulants produced in the liver and is a zymogen that is activated in the presence of the thrombin-thrombomodulin complex on endothelial surfaces. Activated protein C (APC) inhibits factors Va and VIIIa, two of the main cofactors of coagulation. (55)

The S protein is encoded by the PROS1 gene and is produced by hepatocytes, endothelial cells, megakaryocytes and osteoblasts. It is fundamental to coagulation but is also key to atherosclerosis, angiogenesis and the progression of neoplastic cells. (56) Dependent on vitamin K, it acts by stimulating the production of APC to regulate coagulation. (57)

Liver damage results in decreased activation of these proteins, or even complete absence of activation, which must be taken into account in diagnosing patients who have suffered thrombosis. Other concomitant clinical situations must also be ruled out. These include resistance to APC, hereditary...
coagulation defects including deficiencies of proteins C and S, Factor V Leiden, Factor II, mutations in the methylene tetrahydrofolate reductase (MTHF) gene, hyperhomocysteinemia, and antiphospholipid syndrome. (57, 58) Neoplasms must also be considered because there are homologs of the S protein in some hepatic tumors. (59, 60)

**EVALUATION OF HEMOSTASIS IN PATIENTS WITH HEPATOPATHY**

Hemostasis equilibrates the great weight of procoagulant and anticoagulant factors. When any of these are deficient or over abundant, the balance will move in favor of the dominant side. In patients with liver disease, this balance is precarious due to decreases of factors on both sides: the smallest increase or decrease of any of element can cause erratic movements of the balance needle until it finds and marks the dominant site (Figure 2). (23, 36, 61) This should be taken into account in assessment of the hemostatic state of patients with liver disease at the beginning of routine tests such as complete blood counts and the basic coagulation tests of thrombin time, prothrombin time (PT), international normalized ratio (INR) and partial thromboplastin time (PTT). These tests only evaluate procoagulant factors, (23, 36) so evaluation should be complemented with determinations of coagulation factors unless there is a strong suspicion of a congenital defect. (40, 61)

**Figure 2A. Hemostatic balance in healthy people. Figure 2B. Hemostatic balance in patients with liver disease**

It is important to say that PT and PTT are poor predictors of bleeding in liver patients: measurements within the usual range do not guarantee protection against bleeding, and high measurements are not synonymous with uncontrollable bleeding requiring corrective or preventive therapeutic measures. (61, 62) The search for a reliable method for determining the real hemostatic state of patients with liver failure continues. Two of the most promising possibilities are thrombin generation testing and thromboelastography although both are far from becoming routine tests. (61, 62)

**Vitamin K**

Vitamin K (from the German word koagulationsvitamin, vitamin coagulant) is composed of a group of fat-soluble molecules derived from 2-methyl-1,4-naphthoquinone. (63) Phytomenadione, known as vitamin K1, is of vegetable origin while Menaquinone, known as vitamin K2, is of bacterial origin. The water-soluble forms are menadione and menadiol which are known as vitamin K3 and vitamin K4, respectively. (63, 64)

When it is ingested orally, vitamin K is absorbed in the intestine, passes to the lymph system and then into the bloodstream where 75% to 90% of it is transported to the liver by various high-density lipoproteins, mainly apolipoprotein E (ApoE) (Figure 3). This complex not only serves as transport but also facilitates binding and internalization from the Disse space to the interior of the hepatocyte. (65) Rapid uptake of phytomenadione in the liver is evidenced by excretion of its metabolites in bile salts (30% - 40%). Other excretion routes are urine (15%) and stool (65, 66).

In hemostasis, vitamin K catalyzes carboxylation of hepatic synthesis coagulation factors VII, IX, X and prothrombin by forming calcium binding sites in the glutamic acid residues on the sides of the protein chain. Proteins C and S which are natural anticoagulants also require vitamin K for their activity (66, 67). Because of this, it is easy to understand that patients with significant chronic liver disease do not respond adequately to vitamin K supplementation and coagulation disorders become difficult to control.

**Administration of vitamin K**

Vitamin K deficiencies in newborns are the most frequent reason for administration of the substance even though this rare alteration occurs in only 0.1% of all live newborns. It is a potentially lethal condition but can be prevented with oral administration of 2 mg of the vitamin in all newborns. (65, 68, 69) The second most frequent therapeutic indications are anticoagulation regimens with vitamin K antagonists for active bleeding and high risk of bleeding for which the vitamin is used as an antidote. Usually vitamin K1 is slowly administered intravenously or intramuscularly in a period of not less than 30 seconds at an average dose of 10-20 mg in conjunction with fresh frozen plasma or prothrombin concentrates. (70, 71) Usually administration requires no more than two hours allows to reduce INR to usual levels, but in severe cases a similar second dose can be administered eight to twelve hours later. (66, 72)
Vitamin K is frequently prescribed by some specialists to reverse alterations of hemostasis in patients with liver damage under the hypothesis that there is a deficiency of coagulation factors dependent on vitamin K. (73) Nevertheless, it is important to point out that most hemorrhages are not caused by deficiencies of secondary hemostasis, but rather are caused by rupture of esophageal varices or gastric ulcers secondary to portal hypertension due to localized thrombophilia. (74) According to the systematic review by Martí-Carvajal et al., there is no benefit from the administration of vitamin K in patients with hepatic insufficiency and hemorrhaging. This statement is based on series of cases or observational studies and still lacks the support of randomized clinical trials. (75)

Final Considerations

Understanding the latest scientific evidence about the bleeding-coagulation process allows us to better understand why patients can develop hemostatic disorders from early stages of liver disease. For this reason it is essential to assess secondary hemostasis through TP or INR which are also necessary for the usual prognostic scales for Hepato-pathologies.

Nevertheless, it is now known that fibrinolysis is the main risk factor associated with persistent hemorrhaging. In the event of hemorrhaging, appropriate transfusion support is equally or more important because it has been shown to have a positive impact on short-term prognosis. Transfusion support is indicated prophylactically for patients without bleeding who are going to undergo liver biopsies or other invasive procedures as well as for therapeutic purposes such as treatment of gastrointestinal bleeding. In the latter case, greater pathophysiological support than empirical administration of vitamin K is needed. Before starting transfusion support, it is important to consider complications inherent in transfusion such as formation of alloantibodies as well as hemodynamic alterations typical of patients with liver failure such as Transfusion-Related Acute Lung Injury (TRALI) and volume overload. It is important to remember that the transfusion of plasma and platelets are the main causes of TRALI. (76, 77). Mention was also made of the apparent usefulness of TPO mimetic drugs for reducing thrombocytopenia and avoiding the use of transfusion support, but, since the evidence is still limited, widespread use cannot yet be recommended.

References


69. Shearer MJ, Fu X, Booth SL. Vitamin K nutrition, metabolism, and requirements: current concepts and future


