



Hemostasis, Disorders of Coagulation and Transfusion in Cirrhosis

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Abstract

Coagulation disorders in liver diseases, especially cirrhosis occurs due to a complex play between procoagulant and anticoagulant factors. The understanding of bleeding and thrombosis in liver disease is fundamental to management and diagnosis of both these conditions that can occur in liver cirrhosis. In contrast to earlier teaching that considered cirrhosis to be an 'auto anticoagulated' state, the current approach to considering it as a state of 'rebalanced hemostasis' which can tip to either bleeding profile or thrombotic profile in the same patient has made way to considerable improvements in management and diagnosis of hemostatic and coagulation abnormalities in cirrhosis patients. The liver plays a central role in the four phases of clotting which includes platelet plug initiation and formation, coagulation cascade activity, clotting termination and clot removal by fibrinolysis. Multiple factors such as portal hypertension, decreased thrombopoietin production, endothelial dysfunction and thrombocytopenia and thrombocytopathy in cirrhosis pave way for coagulation abnormalities in this group of patients. Newer modalities like thromboelastography and thrombography have helped in making point of care reliable by improving assessment of coagulation processes globally and further studies and evidence point towards imparting a much better knowledge into this complex situation that is hemostasis/coagulation and liver disease.

Keywords: Thrombosis, Bleeding, Cirrhosis, Hemostasis, Thrombocytopenia, TEG

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Introduction

Coagulation abnormalities are the hallmark of end stage liver disease. Tests for coagulation have become an important part in staging and prognosis of liver disease; such as prothrombin time (PT) and International Normalized Ratio (INR) which have become an integral part of the Child-Turcotte-Pugh (CTP) classification and Model for End Stage Liver Disease (MELD) scoring systems. Coagulation profile is also important in acute liver failure and consideration for liver transplantation by King's College Criteria. Coagulation and homeostasis in humans is a complex area that remains poorly understood despite the growth new technologies for its assessment. Assessment of coagulation in patients of liver disease remains a difficulty because of unavailability of laboratory tests that accurately show changes in both procoagulant and anticoagulant systems. International Normalized Ratio, which is widely used as 'the' test for liver related coagulopathy and protein synthetic dysfunction, is a poor marker in acute and chronic liver disease states [1]. In-depth knowledge regarding the pathophysiology of hemostasis in liver disease is of utmost importance for holistic management of end stage liver disease related coagulopathy. End stage liver disease is characterized by features of decompensation such as conjugated hyperbilirubinemia, ascites development, gastrointestinal haemorrhage, hepatic encephalopathy, renal dysfunction, endogenous infections and coagulopathy. Coagulopathy in the setting of liver disease is defined as an INR > 1.5. Hemostatic defect in chronic liver disease is complex, involving opposing factors of primary hemostasis, coagulation, and fibrinolysis [2]. Even though causal relationship between coagulopathy of liver disease with bleeding is a widely accepted practice, sometimes, this is not completely balanced in the clinical scenario. A common practice is to treat or prevent bleeding with blood products and other agents such as fresh-frozen plasma, platelet concentrates, antifibrinolytic drugs, prothrombin complex concentrates and recombinant activated factor VII [3]. The ability of these agents and products to improve or correct the homeostatic abnormality has not been validated in randomized trials. Recent research has challenged this practice as well as the usefulness of conventional tests in assessment of bleeding risk as well as therapeutic interventions aimed at correcting abnormal haemostasis. The clotting process is a dynamic which involves phases of platelet plug initiation and formation, coagulation cascade activation, antithrombotic mechanisms that curb clotting progression thereby striking a balance and ultimately, clot fibrinolysis after stabilization. The liver plays a central role in each of these processes and in

the presence of hepatic dysfunction, the thin balance between procoagulation and anticoagulation leads to increased risk of bleeding and or thrombosis.

Hemostasis

Hemostasis is a cellular process where in the activated platelets function as primary effector of coagulation. This process involves primary homeostasis or the initial plugging of the vascular breach by activated platelets, activation and functioning of the coagulation cascade (intrinsic/extrinsic), fibrin mesh formation and clot stabilization by procoagulant proteins and once the repair is complete, fibrinolysis by plasma anticoagulant proteins. These advanced processes are classically triggered by exposure of tissue factor on the luminal side of the vascular endothelium. The phases in hemostasis (Figure 1) include a) Initiation in which, the tissue factor (TF), an integral component of the cellular membrane, is the primary starter for in vivo coagulation [5]. When a vascular injury occurs and blood makes contact with the subendothelium, an interaction between cellular TF and circulating factor VII is activated. The TF-VIIa complexes then activate factors IX and X. Factor Xa combines with factor Va on the cellular surface resulting in the production of small quantities of thrombin. The thrombin then participates in the activation of platelets and VIII factor in the next phase which is b) Amplification where in when the platelets along with the plasma components come into contact with extravascular tissues, they adhere to the subendothelial matrix and activate those sites where the TF is exposed. The thrombin generated in the first step amplifies the procoagulant signal by activating factors V, VIII and XI which are located on the platelet surface. These form the mediators that progress to the next phase, c) Spread, which is characterized by two principal phenomena. Firstly, the complex of Factor VIIIa, Factor IXa, calcium and phospholipids serves as a catalyst promoting the conversion of Factor Xa. Secondly, the prothrombinase complex, (Factor Xa, Factor Va, calcium and phospholipids) promotes the generation of large amounts of thrombin (thrombin burst or explosion) which are necessary for the formation of a clot resistant to fibrinolysis. The prothrombinase complex is 300,000 times more active than the factor Xa as a catalyst for prothrombin. Thrombin formed here activates the XIII factor and thrombin activatable fibrinolysis inhibitor (TAFI) substances which help achieve and maintain the stability of the clot [6].

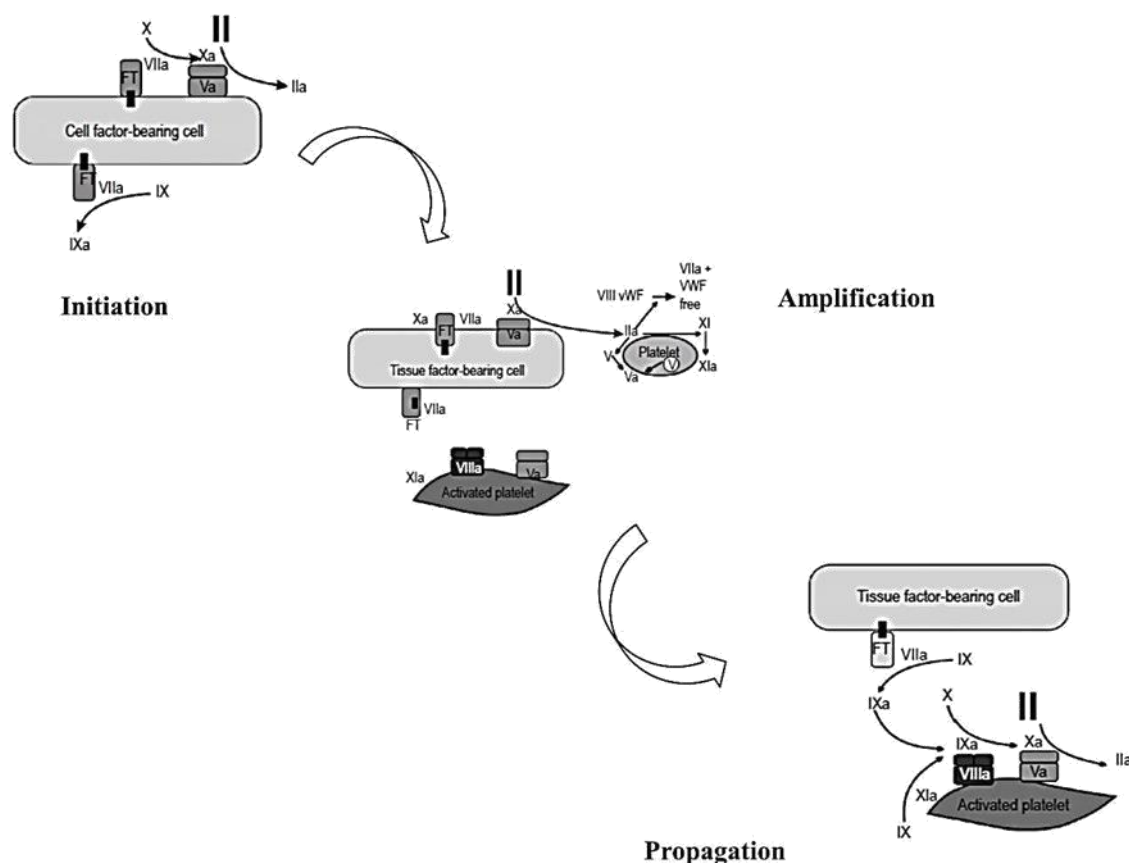


Figure 1. The three cellular phases of coagulation. Modified from : Hoffman M. A cell- based model of coagulation and the role of factor VIIa. Modified from - Blood Rev. 2003; 17 Suppl 1: S1 – 5.

In order to avoid excessive quantities of thrombin that is potentially harmful, the natural anticoagulants come into control play. These compounds are located in the vascular endothelium. The most important of them are tissue factor pathway inhibitors (TFPI), antithrombin and protein C. TFPI binds to the TF- factor VIII complex to prevent activation of factors IX and X. Antithrombin acts directly on thrombin but also acts on factors IXa and Xa. Circulating Protein C needs thrombomodulin by which it acts as a receptor during activation of thrombin and which joins with an endothelial receptor [endothelial protein C receptor (or EPCR)]. This results in the transformation of protein C into activated protein C, which in presence of cofactor protein S, inhibits factors V and VIII [7]. Available testing for coagulation poorly explains the changes that occur locally and is more concerned with systemic coagulopathic changes such as seen in disseminated intravascular coagulation

(DIC). A brief overview of the role of platelets, the coagulation cascade and clot formation and fibrinolysis in the normal environment is shown in Figure 2.

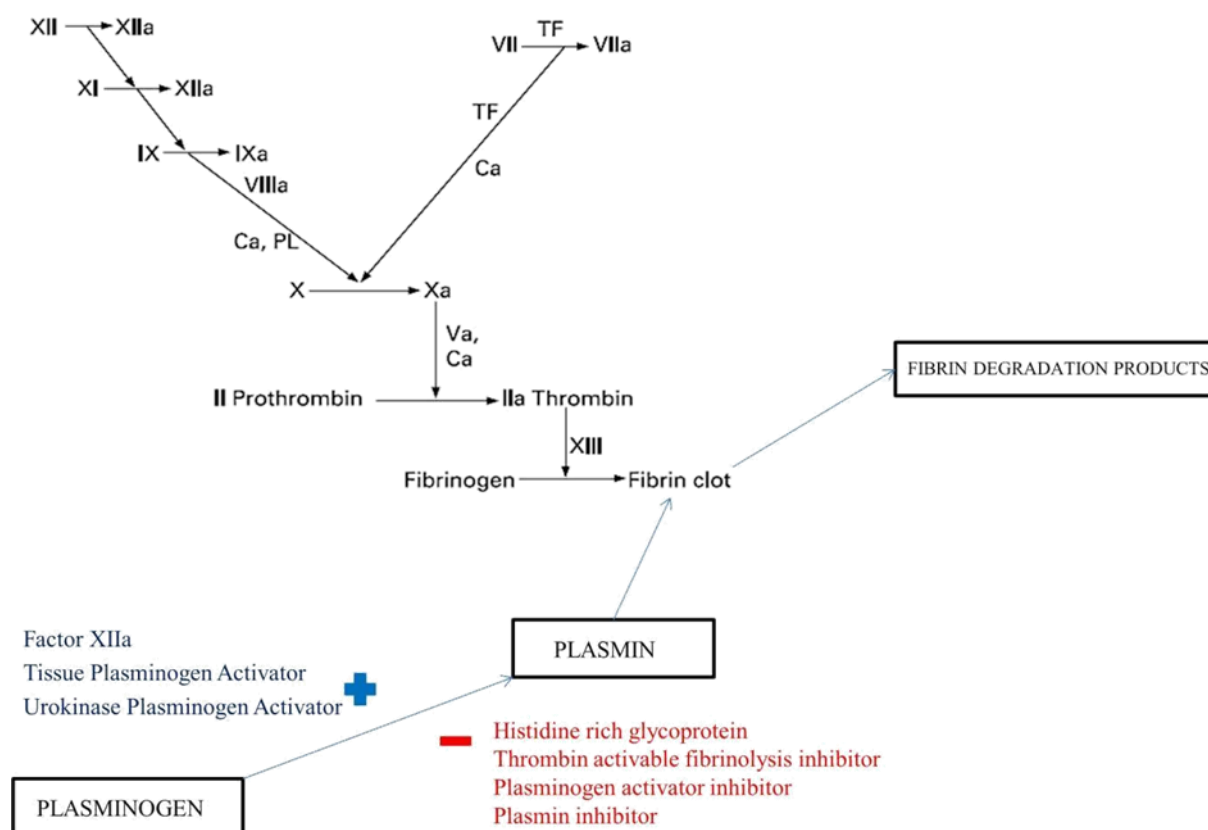


Figure 2. The coagulation cascade featuring intrinsic and extrinsic pathways and the common pathway of fibrin formation. (Blue cross represent activators and the red hyphenation represent inhibitors in the cascade).

Platelets and Liver Disease

In liver disease, all phases of hemostasis are deranged in the presence of synthetic dysfunction and portal hypertension (portosystemic shunting and endothelial dysfunction). Compensatory mechanisms come into play at this point and lead to rebalancing of the coagulation system whereby patients of end stage liver disease are less prone to spontaneous bleeding or clotting in the absence of specific trigger (such as acute variceal bleeding or systemic infections). Platelets are considered central to coagulopathy of liver disease and primary hemostatic derangement has been the focus of liver related bleeding/clotting diathesis [8]. In chronic liver disease, there is a decrease in number of

circulating platelets along with thrombocytopathy (platelet dysfunction). This is considered to be multifactorial, which includes pooling of platelets and sequestration in the spleen, presence of antiplatelet glycoprotein IIb-IIIa antibodies in cirrhosis patients, myelosuppression, shorter half-life and rapid turnover secondary to splenic destruction, increased platelet consumption secondary to sustained low grade DIC and lower levels of hepatic thrombopoietin production [9]. Qualitative defects in platelet function also prevail in cirrhosis patients. In the presence of endothelial injury, under normal circumstances, platelet adherence to endothelium is mediated by von Willebrand factor (vWF), a large molecular weight glycoprotein that gets activated when cleaved by endothelial derived protease ADAMTS13. In liver disease, in the presence of endothelial dysfunction, vWF levels are elevated in proportion to disease severity. Even though simulated studies of platelet function in cirrhosis patients have shown increased adherence of platelets to endothelial surfaces in the presence of exaggerated vWF levels, such compensatory mechanisms fail to modify injury in the presence of thrombocytopenia. It has been shown that the thrombin burst required for plug stability warrants a minimum of 50,000 to 60,000 platelets per cumm of blood. Other qualitative defects that are described includes acquired storage pool defects such as decreased adenosine triphosphate and serotonin in dense granules, altered transmembrane signal transduction, decreased thromboxane A2 synthesis, quantitatively decreased glycoprotein Ib, Ia and IIb3 receptors, decreased response to thrombin, adenosine monophosphate, arachidonic acid and collagen [10]. Platelet dysfunction is also dependent on lower haematocrit levels seen in patients of cirrhosis. The extrinsic factors like abnormal high density lipoprotein and apolipoprotein E levels and increased levels of endothelium derived nitric oxide and prostacyclin (which are platelet inhibitors) also add to platelet dysfunction in cirrhosis; this dysfunction is also augmented in the presence of concomitant renal dysfunction and sepsis or drug related additive effect. Even though platelet dysfunction plays a central role in coagulation abnormality of liver disease, there is a weak correlation between bleeding time, platelet count and bleeding risk in patients of cirrhosis [11]. The clinical relevance of thrombocytopenia and hypoaggregability in cirrhosis remains elusive. Platelet reduction in cirrhosis is mostly mild to moderate and there is no strong association with severe bleeding in patients with stable liver disease. Platelet function, as measured by ability for thrombin generation is mostly maintained in patients of stable cirrhosis in whom the platelet counts and haematocrit are well within normal range.

Coagulation Cascade and Liver Disease

Cirrhosis is characterized by reduction in synthesis of procoagulant proteins especially those that are vitamin K dependant such as II, VII, IX and X; as well as factor V and factor XI [13]. These factor abnormalities affect standard coagulation measures available in clinical practice, mainly PT and INR, thereby making these parameters substandard for defining coagulopathy in the presence of cirrhosis. Factor V and Factor VII have the shortest in vivo half-lives (12 and 4-6 h, respectively) and are the most decreased procoagulants in patients with liver disease. Vitamin K deficiency in chronic liver disease maybe due to poor nutrition or malabsorption caused by decreased bile production or biliary obstruction. Vitamin K deficiency is defined as low plasma vitamin-K1 levels measured by reverse-phase high performance liquid chromatography but do not correlate with the vitamin- K deficiency prevalence when PT prolongation is used as a surrogate marker, and therefore this parameter should not be assumed to be a reliable marker of vitamin K deficiency in patients with chronic liver disease. PT is dependent on Factor VII levels and since Factor VII has the shortest in vivo half-life, an isolated prolonged PT is common for patients with liver disease. Even though more than 6% of the normal activity level of a given coagulation factor is enough to prevent spontaneous bleeding, this single factor coagulation deficiency model may not be applicable to cirrhosis, in which almost every coagulation factor is deficient [14]. Currently, no significant correlation has been found between fibrinogen concentration, PT prolongation, factor V activity and bleeding tendency clinical markers such as melena, hematemesis, gastric ulcer bleeding, persistent skin bleeding after liver biopsy, menorrhagia, positive fecal occult blood test and blood transfusion requirements [15]. Factor VIII, which is synthesized in the liver and endothelial cells and vWF which is present in endothelial cells, platelets and megakaryocytes are also acute phase reactants and hence normal or elevated factor VIII and vWF levels may be found in cirrhotic patients caused by continued endothelial cell Factor VIII production despite decreased hepatic FVIII production and increased synthesis caused by acute disease or inflammation. Even in the presence of decreased procoagulant factors and abnormal PT/INR values, cirrhosis patients do not spontaneously bleed mostly due to the fact that there is a concomitant decrease in anticoagulant factors such as protein C, protein S and antithrombin III (attributed to decreased hepatocyte function, increased consumption due DIC or hyperfibrinolysis, and vitamin K deficiency) with additive effect from increased endothelial derived factor VIII. This promotes rebalancing of the hemostatic system in

patients of cirrhosis. Perturbation within this rebalanced system is what predisposes the cirrhotic patient to either bleeding or clotting. Progressive liver dysfunction leads to decrements in procoagulant protein activity and small precipitating events overcome the compensatory mechanisms. The tendency to either bleed or undergo clotting/thrombosis in a liver patient depends on the clinical situation in each patient. Infection in cirrhosis patients is associated with increased levels of endogenous heparinoids (due to infection related changes in nitric oxide metabolism, a reflection of endothelial dysfunction and associated changes in endothelial glycocalyx). Two specific glycosaminoglycans, heparan sulphate and dermatan sulphate, have anticoagulant properties similar to heparin. Increased levels of these proteins have been found in patients with cirrhosis, and even higher concentrations are seen in cirrhosis patients with active bleeding or infection [16]. Clot and fibrin degradation is determined by the endothelial release of tissue plasminogen activator (t-PA) and its subsequent inhibition by plasminogen activator inhibitor type-1 (PAI-1). To maintain a normal fibrinolytic balance, increases in plasma t-PA concentrations are associated with compensatory increases in plasma PAI-1 concentrations. PAI-1 forms a complex with t-PA to cause an overall reduction in free tPA activity. In patients with cirrhosis, plasma t-PA activity is up to seven times higher, and it is proportional to the severity of cirrhosis and may contribute to the risk of variceal hemorrhage by the removal of fibrin clot at the site of vascular injury. In lieu with the severity of liver dysfunction, evidence of fibrinolysis may be found in 30 to 60% of patients with chronic liver disease, and clinically evident hyperfibrinolysis has been estimated to occur in 5 to 10% of those with decompensated cirrhosis. Hyperfibrinolysis promotes clot dissolution and decrease platelet aggregation due to degradation of vWF and fibrinogen platelet receptors. Thrombin activatable fibrinolysis inhibitor or TAFI is a plasmatic proenzyme synthesized in the liver that, upon activation by thrombin or other enzymes (plasmin, trypsin, neutrophil elastase), inhibits fibrinolysis by removing carboxy-terminal lysine residues from partially degraded fibrin [17]. Reduction of plasmin formation by TAFI is an important regulator of physiological fibrinolysis and alterations in TAFI-activating mechanisms contribute to bleeding or thrombotic manifestations. Decreased plasma levels of TAFI have been reported in patients with acute or chronic hepatocellular diseases of different aetiology and the degree of TAFI levels is proportional to the severity of the disease. TAFI levels were found to be an independent predictor of mortality after correction for age, sex, aetiology of cirrhosis, and CTP class [18].

Bleeding and Hypercoagulation in Liver Disease

The clinically most relevant bleeding problem in patients with cirrhosis that is acute variceal bleeding, is considered mainly a consequence of local vascular abnormalities as well as increased splanchnic blood pressure. The role of deranged hemostasis in variceal bleeding is not yet proven. Other bleeding complications, including bruising, purpura, epistaxis, gingival bleeding, menorrhagia, and bleeding associated with invasive procedures may be related to defective hemostasis. The extent of coagulopathy as measured by the PT or INR does not appear predictive of bleeding complications in cirrhosis patients. In patients with acute liver failure, in whom coagulation parameters such as the PT are frequently more prolonged than in patients with cirrhosis, spontaneous bleeding complications are relatively uncommon, less than 5%. Invasive procedures (placement of indwelling venous catheters, intracranial pressure monitor) may result in serious bleeding. Complications of liver disease may aggravate the coagulopathy and thus contribute to the bleeding tendency. Bacterial infections are common in patients with cirrhosis and are associated with an increased bleeding risk. Cirrhosis patients with gastrointestinal bleeding are more prone to developing bacterial infections (66%) and prophylactic administration of antibiotics has been shown to reduce the bleeding risk. Renal failure in the form of hepatorenal syndrome and acute kidney injury frequently complicates liver disease, which also aggravates hemostatic abnormalities. Uremia is well known to be associated with disturbed platelet-vessel wall interaction and intrinsic platelet abnormalities, enhanced production of nitric oxide and anemia [19]. Even though laboratory evidence of hypocoagulation such as a prolonged PT/APTT has been long associated with cirrhosis patients, there has been an assumption that these patients are protected against thrombotic disease. On the contrary, the intravascular activation of coagulation may occur much more frequently. It has been shown that patients with liver disease do develop deep vein thrombosis and pulmonary embolism at rates between 0.5% and 1.9%. Large population-based studies have confirmed that patients with liver disease are at increased risk for development of venous thrombosis compared with healthy persons. Liver- related thrombosis events such as thrombosis of the portal and mesenteric veins, is common in patients with advanced cirrhosis. Change in portal hemodynamics (such as decreased portal flow) is one important factor that plays a major role in this thrombotic complication [20]. Inherited thrombophilias, in particular the prothrombin G20210A mutation, enhances the risk for portal vein thrombosis in patients with cirrhosis. Hence approaches to diagnosing liver related thrombosis must include a

thorough evaluation of underlying primary thrombophilic states [21]. Hepatic artery thrombosis which is more often seen after liver transplantation has been recently implicated secondary to hypercoagulation of liver disease rather than a surgical complication. Intrahepatic thrombus formation has been demonstrated in patients with cirrhosis and also in patients with acute liver failure. The presence of these microthrombi are believed to play a key role in progression of fibrosis as a result of local ischemia, a process referred to as parenchymal extinction. Additionally, the activation of hepatic stellate cells by thrombin may also contribute to the progression of disease. Attenuation of disease progression by anticoagulation or by genetic ablation of key players of coagulation has been shown to slow down fibrosis in animal models or acute and chronic liver disease. Patients with factor V Leiden mutation appear to have a faster progression of hepatitis and fibrosis in patients of hepatitis C–related liver disease with hemophilia has been suggested to be milder as compared with progression in patients without haemophilia. As a special scenario, the non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation of the metabolic syndrome. As such, afflicted patients suffer an increased burden of both thrombotic vascular disease and progressive liver disease. Patients with NAFLD may initially present with hemostatic alterations linked to general metabolic imbalance including high levels of plasminogen activator inhibitor-1 (a component of the metabolic syndrome), increased levels of vWF, Factor VII, soluble tissue factor and fibrinogen, as well as platelet hyperaggregability and endothelial dysfunction. Thus NAFLD is clearly associated with a prothrombotic state, which is reflected in a substantially increased incidence of thrombotic events in patients with NAFLD, rather than bleeding [22].

Rebalanced Hemostasis as A Clinical Concept (Figure 3)

Patients of liver disease, especially cirrhosis are prone to both bleeding and thrombotic risks. In a very simplistic model, the occurrence of bleeding complications has been explained by thrombocytopenia, thrombocyte dysfunction, decreased plasma levels of coagulation factors and decreased plasma levels of inhibitors of fibrinolysis. Thrombotic disease has been attributed to decreased plasma levels of the natural anticoagulants protein C and S and antithrombin. In patients with liver disease, in whom extremely complex alterations of hemostasis occur, reliability on the levels of individual coagulation factors, or on simplified tests of hemostasis such as the PT or APTT to predict the hemostatic status is arguable. These simple tests are extremely helpful in diagnosing and monitoring patients with isolated defects

in the hemostatic system, such as patients with hemophilia or patients with isolated thrombocytopenia. The defects in platelet number and function are accompanied by elevated levels of the platelet adhesive protein vWF. The vWF-cleaving protease ADAMTS13 is reduced in cirrhosis, which also promotes platelet function. ADAMTS13 not only cleaves newly synthesized ultra-large vWF multimers but is also responsible for regulation of thrombus growth by proteolysis of vWF within a growing thrombus. The ADAMTS13 deficiency of cirrhosis may result in enhanced thrombus formation by decreased proteolysis of vWF within a growing thrombus. The deficiencies in procoagulant proteins are accompanied by deficiencies in the natural anticoagulants protein C, S, and antithrombin. Although the PT and APTT suggest defective coagulation, these tests actually do not represent the balance between the pro- and anticoagulant proteins because the PT and APTT are not sensitive for deficiencies of the anticoagulants. It was demonstrated that the thrombin generation in patients with cirrhosis was actually not different from that of healthy volunteers in the presence of added thrombomodulin. This shows that the balanced coagulation potential of cirrhosis is not only explained by a reduction in both pro- and anticoagulants, but also by a marked resistance to the inhibitory action of thrombomodulin. The fibrinolytic system may also be in balance in patients with cirrhosis, due to the concomitant decrease of antifibrinolytics such as antiplasmin, thrombin activatable fibrinolysis inhibitor and plasminogen. Overall, the hemostasis is rebalanced due to simultaneous alterations in both pro- and antihemostatic processes, although routine laboratory tests of hemostasis would still suggest a hypocoagulable state [23]. This hemostatic balance is easily disturbed by complications of the disease including infections and renal failure. Although the hemostatic balance is preserved in patients with cirrhosis, in individual patients, the balance may fall toward either a hypo- or hypercoagulable status, explaining why both bleeding and thrombotic episodes occur. There is a possibility that detailed clinical assessment by standardized bleeding scores or thrombosis risk assessment be able to identify those cirrhosis patients who are at risk for either bleeding or thrombosis, but this has not been validated yet. In patients with liver disease, major surgical procedures, including liver transplantation may be performed without major bleeding complications, even in the absence of correction of underlying coagulopathy, which is definitely not possible in conditions of isolated coagulation disorder. This further proves that the hemostatic dysfunction in cirrhosis patients, is 'stable anomaly' rather than a simple clinical derangement [24]. Randomized controlled clinical trials

(RCT), which compared a policy of restrictive transfusions to that with a liberal transfusions in cirrhosis patients undergoing LT and in those patients of acute variceal bleed found that the former policy lead to a significant reduction in adverse effects.

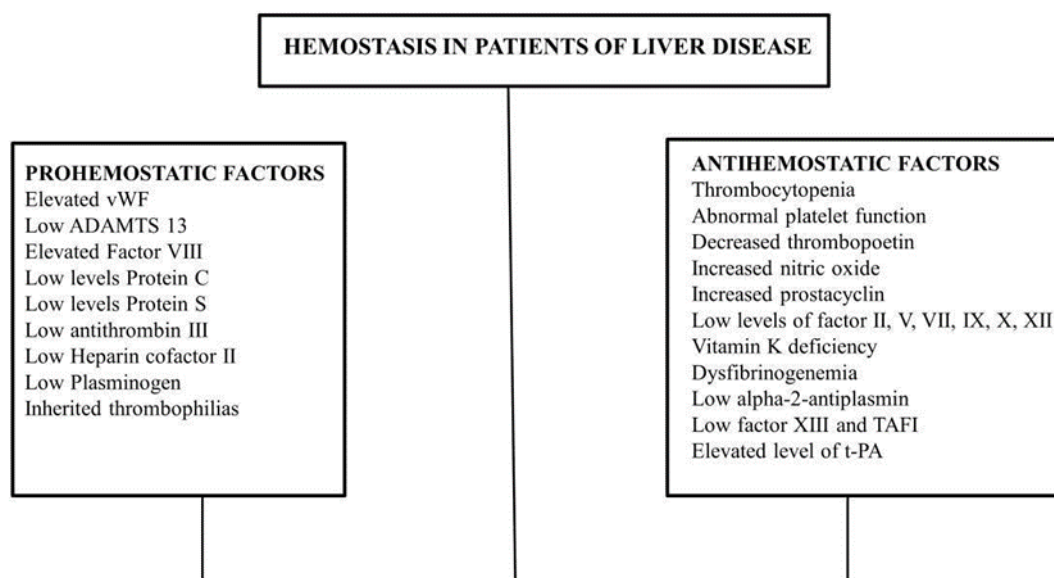


Figure 3. The pro and anti hemostatic factors at play in rebalanced hemostasis occurring in liver disease patients.

Hemostasis During Liver Transplantation

Dysfunctional hemostasis occurring during LT is divided as a correlate to the surgical phases. These include dissection or preanhepatic phase, anhepatic phase, post-reperfusion phase and early post-operative period [25]. In the initial surgical phase, there is dissection of adhesions in the abdominal cavity and transection of many collateral vessels. This first stage is usually characterized by minor blood loss, with the exception of patients who have intra-abdominal adhesions caused by previous upper abdominal surgery and patients who have significant portal hypertension, who generally have a higher bleeding tendency. Vascular injury and impaired clearance of activated coagulation factors caused by decreased hepatic blood flow may result in excessive activation of coagulation and consumptive coagulopathy, while surgical bleeding may deplete coagulation proteins and platelets inducing dilutional coagulopathy. The mild coagulation abnormalities occurring during the dissection phase are mainly related to the surgical technique, although the baseline hypocoagulable state and the aetiology of liver disease can also influence the blood product requirement.

In the anhepatic phase, no important surgical blood loss is usually seen because of appropriate clamping of vessels. But, significant haemostatic changes may occur nonetheless. Platelets and coagulation factors are depleted by surgical bleeding together with the absence of the hepatic synthetic and clearance function. The release of tissue thromboplastin and the absence of the hepatic clearance of activated coagulation factors may cause excessive activation of coagulation. There is gradual increase in thrombin-antithrombin-III complex and fibrin degradation products. The presence of clinically significant intravascular coagulation or thrombosis is usually rare in this phase. There is also a simultaneous decrease of α 2-antiplasmin and plasminogen activity along with the concomitant increase in fibrin and fibrinogen degradation products. There occurs no changes in PT and PTT. The reperfusion of the liver is a crucial point in this major surgery and usually leads to profound coagulation abnormalities. Indeed, diffuse and uncontrollable bleeding may occur in some patients within minutes after reperfusion. This effect is multifactorial and has been identified as DIC, hyperfibrinolysis, platelet activation, trapping of platelets in the graft, and the presence of heparin-like effect. The poor quality of the graft has been associated with signs of DIC after graft reperfusion. There occurs release and washout of plasminogen activators from the graft which is responsible for the increased levels of tPA at reperfusion heparin like substances are sourced from the residual heparin bound to the endothelium of the donor vessels, as the donor liver is perfused with heparin before clamping. Apart from this, there is also an endogenous source of heparinoids that are thought to occur from activation of macrophages following the ischemia reperfusion injury. Platelets also have a role in haemostatic abnormalities during this phase. After reperfusion, the platelet count decreases by 30% to 55% because of entrapment of platelets in the liver graft. Moreover, the release of t-PA from the graft results in proteolysis of key platelet receptors. In the postoperative period, thrombocytopenia is common, mainly due to platelet activation and consumption following graft reperfusion. Platelet count and coagulation factors usually increase steadily toward normal levels, if normal synthetic function of the liver is restored in the first few days after transplantation. The thrombopoietin levels increase significantly on the first day post-LT, followed by immature bone marrow megakaryocytes after 3 days and new circulating platelets after 5 days, with normalization of platelet count achieved after 14 days. Persistence of thrombocytopenia can be seen in some patients, which can be ascribed to persistent splenomegaly in some. In adult-to-adult living donor liver transplantation, persistently

elevated portal venous pressure and hypersplenism due to relatively small graft size have been associated with post-LT thrombocytopenia. Rarely, a small proportion of patients show excessive rise in thrombocyte count, post LT leading to thrombocytosis, which is usually transient and not associated with an increased risk of thrombotic complications [26]. Thromboelastography is a technique that can provide continuous observation and tracing of all the haemostatic functions that lead to clot formation and dissolution and is becoming a very useful tool during such major surgical interventions.

Common Laboratory Tests for Coagulation in Liver Disease

The basic clinical laboratory tests only give information on focal pathways of the coagulation system. This form a difficult scenario in clinical estimation of bleeding or clotting risk among cirrhosis patients as there is no comprehensive testing that show the complete coagulation picture in an individual patient. The INR is possibly the most commonly used and misunderstood laboratory test used in the evaluation of chronic liver disease patients. The original application of INR was for standardization of therapeutic anticoagulation with vitamin K antagonists (VKAs), and the calibration of the test is computed from healthy volunteers. The INR has been successful in improving the management of therapeutic anticoagulation in this scenario. The INR is simply a reflection of the PT ratio compared with controls by using a correction factor that is based on the specific thromboplastin used in the PT measurement. The common INR is not calibrated for use in liver disease, especially cirrhosis patients. This is most evident in the remarkably high interlaboratory variability in liver disease patients, depending on which thromboplastin reagent is purchased for the assay. Few experts have advised for the use of a 'Liver INR' which uses cirrhosis patients as controls and a standard thromboplastin reagent, but this calibration is economically and logistically difficult and widespread adoption is only a theoretical possibility [27]. The PT/INR measures the classic procoagulant extrinsic pathway, is inexpensive, quick and widely available. It also shows good correlation with the severity of the liver disease but has high interlaboratory variability and is not very good at predicting bleeding episodes. The activated partial thromboplastin time (APTT) measures the classic procoagulant intrinsic pathway and is also widely available and inexpensive. It can also detect congenital factor deficiencies but usually do not reflect on hepatic dysfunction; it has been usually found to be normal or nearly normal in liver diseases. Platelet count, even though widely available and quick and inexpensive, does not reflect platelet function in chronic liver disease. It is highly

reproducible and has some correlation with bleeding at low levels (below $50 \times 10^9/\text{cumm}$) but is also not predictive of bleeding at higher levels. Platelet function assays (measures primary hemostasis) are useful in providing generalized platelet function in the wake of normal thrombocyte counts. Most assays assume normal platelet and are not calibrated for thrombocytopenia in liver disease patients and hence not useful in this subgroup. Bleeding time assessment (measures mucosal and skin hemostasis) even though gives a better view of the whole hemostatic system, does not predict procedural bleeding and is time consuming and produces patient discomfort and not widely accepted. vWF complex levels which measures primary hemostasis reflects severity of liver disease and offers prognostic value in liver disease. Lower levels indicate the need for platelet transfusion, but are not generally validated in predicting bleeding episodes in cirrhosis patients and its complex relationship with ADAMTS13 makes inference difficult in cirrhosis patients [28]. Fibrinogen (measures fibrinolysis) levels of more than 100mg/dL suggest adequate fibrinogen for initiation of coagulation. Low levels are suggestive of hyperfibrinolysis but are also common in stable non bleeding cirrhosis patients. It is also an acute phase reactant and in the presence of systemic infection in cirrhosis or in inflammatory conditions (as cirrhosis is one such entity), the values may be erroneous. It is not predictive of DIC in cirrhosis due to this fact. Individual Factor level assays measure the procoagulant and anticoagulant system and can give a relative sense for factor deficiencies on either system. These levels are affected by acute clotting and other disease processes and there is no clear relationship to bleeding or clotting risks in cirrhosis patients; also shows significant laboratory variations and are expensive. Euglobulin lysis time is a validated measure of fibrinolysis and can be used as a measure of treatment efficacy in hyperfibrinolysis, but is not widely available [29].

Newer Modalities of Laboratory Testing of Coagulation Thromboelastography

Thromboelastography was originally invented in 1948 and the concept predates the introduction of APTT test. It was initially used for preclinical hemostasis measurement in research setting and has currently been developed for point of care use in emergency and surgical scenarios. There are two commercially available devices: TEG (Thromboelastograph; Haemoscope/Haemonetics, Niles, Ill) and ROTEM (Rotation Thromboelastometry; Pentapharm GmbH; TEM International, Munich, Germany). Thromboelastography is used to assess viscoelastic changes in clotting whole blood under low shear conditions after adding a specific coagulation activator. The tensile force between the cup and the immersed pin results

from the interaction between activated platelet glycoprotein IIb/IIIa receptors and polymerizing fibrin during endogenous thrombin generation and fibrin degradation by fibrinolysis. Thromboelastography is been used to assess hypo- and hypercoagulable states and to guide hemostatic therapies with fresh-frozen plasma, with platelet concentrates as well as with coagulation factor concentrates. Its methodology relies on a metal pin which is suspended by a torsion wire which is immersed in a non- anticoagulated whole blood in a metal cup. In case of TEG, the disposable cup moves back and forth through an arc of 4.75° around the fixed plastic pin. In case of ROTEM, the plastic pin rotates back and forth through an angle of 4.75° in the center of the plastic cup. Once blood starts to clot, fibrin strands are formed, increasing the torque between the pin and the cup. Dissociation of fibrin strands from the cup wall (clot retraction) or the degradation of fibrin by fibrinolysis decreases the torque. The change of torque is detected electronically in TEG and optically in ROTEM. The computer-processed signal from either thromboelastography is presented as a tracing of clot formation and clot dissolution. The initial torque is assumed to be zero (no clot) for the signal processing; thus, it is important to start a measurement immediately after a coagulation trigger is added to the sample. Otherwise, an already clotted sample produces a false baseline when the pin is immersed into the sample blood [30]. The normal TEG and ROTEM tracing with the corresponding variables is shown in Figure 4.

TEG Parameter	ROTEM Parameter	Definitions	Importance
R or Reaction Time (4 to 8 minutes)	CT or Clotting Time (INTEM 137 to 246 seconds, EXTEM 42 to 74 seconds)	Time to initiation of fibrin formation	Concentration of soluble clotting factors in plasma
K Time (1 to 4 minutes)	CFT or Clot Formation Time (INTEM 40 to 100 seconds, EXTEM 46 to 148 seconds)	Time for amplitude to increase from 2 to 20 mm	Clot kinetics measurement
Alpha Angle (47 to 74°)	Alpha Angle (INTEM 71 to 82°, EXTEM 63 to 81°)	Angle between tangent to 2mm amplitude and horizontal midline	Fibrin build up and cross linking rapidity
MA or Maximum Amplitude	MCF or Maximum Clot Firmness (INTEM 52 to 72mm, EXTEM 49 to 71 mm)	Maximum vertical width achieved by tracing equivalent to maximum clot strength	Platelet number and function and fibrinogen concentration
CL 30	LY 30	Percentage reduction in amplitude 30 minutes after MA	Clot stability and fibrinolysis

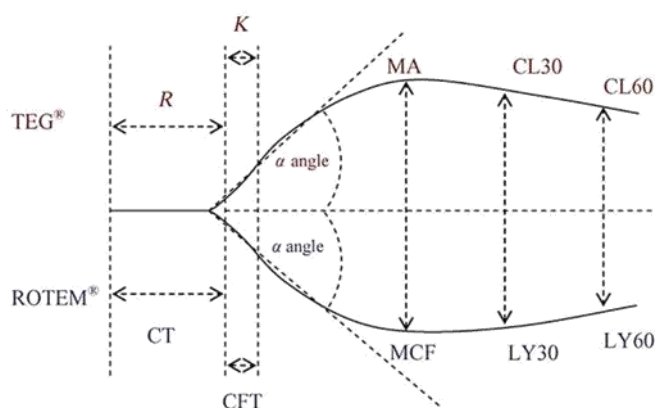
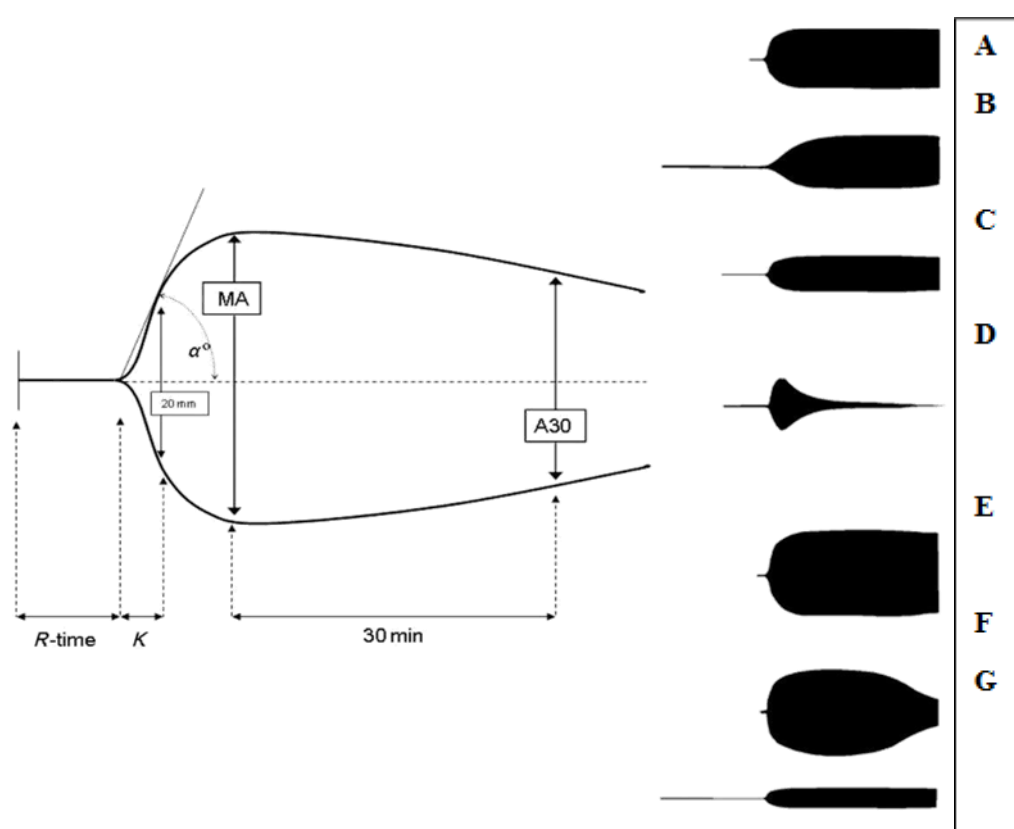


Figure 4. The normal TEG and ROTEM tracings with the corresponding variables and definitions of variables used in thromboelastography evaluation.

TEG and ROTEM systems are closely related but their results are not completely interchangeable. These differences can be attributed to technical aspects of testing such as the material surfaces of pin and cup (metallic versus plastic), plasma and native or recalcified citrated blood and most importantly, the use of different activators at various concentrations. Clinical reference values differ between the 2 systems and must be interpreted accordingly. The use of citrated whole blood is the standard for thromboelastographic systems today. Before initiation of the test, citrated samples are recalcified by adding 20 μ L of 0.2 mmol/L calcium chloride. The citrate may affect platelet GPIIb/IIIa and therefore influence thromboelastographic measurements which has to be considered in the interpretation of results. The main end point of TEG and ROTEM is the rate and stability of clot formation for clinical use. Clotting time and clot formation time are strongly dependent on the type of activators used. It has been shown that the concentration of tissue factor relevantly influences thromboelastographic parameters. Originally, celite was used as a contact activator on TEG, which was later replaced by kaolin (kaoTEG). A modification by addition of heparinise to neutralize the effects of heparin together with kaolin activation (hep-TEG) was recently introduced to monitor underlying blood coagulability in the presence of heparin. Another modification is the so-called rapid-TEG, where tissue factor is used as an activator in addition to kaolin. Rapid-TEG is increasingly used, as it gives fast results, but its information on coagulation and clot formation time is very limited. Both kao-TEG and rapid-TEG are insensitive to antiplatelet agents. Originally, TEG test required up to 60 minutes to complete. Currently, with the use of citrated blood and reecalcification at the time of TEG, the allowable time from blood draw to assay has increased to 2 hours and the time required for the assay has decreased to approximately 30 minutes. Five parameters are recorded in a standard TEG. The reaction (R, in minutes) represents the latency of clot formation from the beginning of the clotting reaction to the initial formation of fibrin (defined as clot amplitude of 2 mm); the R generally corresponds to INR and aPTT. The kinetic (K, in minutes) describes the time required for the initial fibrin formation (2 mm) to reach a specific clot firmness (defined as a clot amplitude of 20 mm); the α -angle (in degrees) corresponds to the kinetics of clot formation which reflects the rate of fibrin formation and crosslinking of platelets which is mostly affected by fibrinogen concentration and platelet count. The maximum amplitude (A, in mm) measures the maximum clot strength which is also most dependent on platelet count/function and fibrinogen concentration and finally, clot lysis at 30

minutes (Ly-30, in percent) that reflects clot dissolution within 30 minutes of reaching maximum amplitude which is a measure of fibrinolysis. In addition to these standard parameters, TEG can also detect the effects of endogenous and exogenous heparinoids, vitamin K antagonists (warfarin), and antiplatelet agents [31]. Thromboelastography is particularly sensitive to changes in fibrin polymerization and platelet count. It is most useful for early detection of trauma and surgery-related dilutional coagulopathy in which plasma fibrinogen and platelets fall rapidly. In addition, it is valuable in guiding the use of cryoprecipitate or purified fibrinogen concentrate and potentially platelet transfusion. Using thromboelastography in goal-orientated algorithms, clinicians may be able to optimize targeted transfusion therapies with specific coagulation factor(s) instead of empirically administering multiple components which could lead to adverse events. Since TEG can provide a global assessment of haemostatic function from initial clot formation to clot dissolution, serial TEG measurements can be suggestive of hypocoagulability which may be associated with early rebleeding in cirrhotic patients [32]. The normal TEG tracing in comparison with abnormal tracings is shown in Figure 5.



A	Normal
B	Anticoagulation/Hemophilia – Factor deficiency; R and K prolonged, MA and Angle decreased
C	Thrombocytopenia/Thrombocytopathy; R normal, K prolonged, MA decreased
D	Fibrinolysis; R normal, MA decreased, LY30 >7.5%
E	Hypercoagulation; R and K decreased, MA and Angle increased
F	Disseminated intravascular coagulation – Stage 1 – Hypercoagulation with secondary fibrinolysis
G	Disseminated intravascular coagulation – Stage 2 – Hypocoagulable state

Figure 5. The normal TEG tracings in comparison with abnormal tracings with reference to bleeding, fibrinolysis and thrombosis in patients of cirrhosis.

Thromboelastography In Liver Disease

The first use of TEG in patients with liver disease was to guide pro-hemostatic factor repletion in patients undergoing liver transplantation (LT), where in TEG-guided factor repletion led to a decrease in red blood cell and plasma infusion volumes. 1 unit of platelets decrease the R-time by 0.43 minutes, raise the angle by 1.5°, and raised the maximum amplitude of clot formation by 1.4 mm. The usual recommendations for correction of TEG parameters are 2 units of plasma for an R-time greater than 15 minutes, 10 units of platelets for a maximum amplitude less than 40 mm, and 6 units of cryoprecipitate for an a-angle less than 40–45°. TEG has also been used during LT to monitor and treat hyperfibrinolysis with ε-aminocaproic acid and aprotinin, also leading to a decrease in transfusion requirements. Intraoperative TEG has also been used to guide repletion of factor IX in a patient with hemophilia B undergoing LT for cirrhosis due to hepatitis C virus infection [3, 34]. The blood product transfusion protocol based on TEG and laboratory parameters are as shown in Figure 6.

Laboratory Based Transfusion Protocol	
Parameter	Management
Platelets < 50,000 to 75,000 cumm	Platelet concentrate or single donor platelets
Fibrinogen < 125mg/dL	10 units of cryoprecipitate for 70 kilogram person
Hematocrit < 30%	Packed red cells
International normalized ratio > 2	2 to 4 units of fresh frozen plasma

TEG Based Transfusion Protocol	
PARAMETER	MANAGEMENT
R 8 to 10 minutes	FF 0 to 2 units
R 10 to 12 minutes	FFP 2 to 4 units
R > 12 minutes	FFP 4 to 6 units
Angle < 600	Cryoprecipitate after FFP transfusion and repeat TEG
MA < 55 mm	Platelet or recombinant Factor VIIa after FFP transfusion and repeat TEG

Figure 6. Laboratory and TEG based transfusion protocol in coagulation disorders

Patients with stable cirrhosis often maintain normal global hemostasis as assessed by TEG, because of rebalance in hemostasis parameters as discussed above. Patients with cirrhosis secondary to cholestatic liver disease (primary biliary cirrhosis or primary sclerosing cholangitis) were found to be relatively hypercoagulable by TEG compared to patients with noncholestatic liver diseases or healthy controls. In contrast, INR and other standard laboratory tests of hemostasis is not different between patients with cholestatic versus noncholestatic liver diseases as seen in few studies. This may also explain why patients with cholestatic liver diseases have fewer bleeding complications and lower intraoperative transfusion requirements during LT compared to noncholestatic cirrhosis patients with similar degrees of portal hypertension. TEG is also useful in predicting complications of liver disease,

such as gastrointestinal bleeding and infection. TEG has been shown to be superior to INR or platelet count for estimating the risk of rebleeding from esophageal varices. TEG parameters become more hypocoagulable in patients of cirrhosis and infection compared to those without infection. Resolution of infection is associated with improvement in TEG parameters to preinfection levels, and persistence of infection is associated with continued deterioration of hemostasis as measured by TEG but not by standard laboratory tests of hemostasis. This change in TEG values in infection has been found to be secondary to endogenous heparinoid production in sepsis states, which resolves with due infection control. Comparable to patients with stable cirrhosis, patients with acute liver failure (ALF) generally have TEG parameters within normal limits. In this sub group of patients, the maximum amplitude of clot formation increases linearly with worsening inflammatory responses and increase in ammonia levels and encephalopathy grade [35].

Sonoclot And Platelet Function Analysis In Liver Disease

Sonoclot Coagulation and Platelet Function Analyzer (Sienco Inc., Arvada, CO, USA) was introduced by von Kaulla et al. in 1975. Sonoclot measurements are based on detection of viscoelastic changes in the whole blood sample that provide in vitro assessment of global coagulation. Sonoclot may also be useful in diagnosing systemic fibrinolysis, though it may not reflect localized clot breakdown by plasmin. Most conventional coagulation tests end when the first fibrin strands are developing, whereas viscoelastic coagulation tests begin at this point and continue throughout clot development, retraction, and lysis. The instrument provides information on the entire hemostatic process in the form of a qualitative graph known as sonoclot signature along with several quantitative measurements. These measurements include sonoclot activated clotting time (SONACT) which is the onset time in seconds till the beginning of fibrin formation, the rate of fibrin formation from fibrinogen [depicted by the gradient of primary slope (R1)] known as clot rate (CR) which is expressed in units per minute and the secondary slope (R2) which reflects fibrin polymerization and platelet-fibrin interaction. The R2 peak indicates completion of fibrin formation and has two variables, the time to peak (in minutes), which is an index of the rate of conversion of fibrinogen to fibrin and peak amplitude (expressed in units), which is an index of fibrinogen concentration. The downward slope (R3) after the peak is due to platelets induced contraction of the completed clot. In cases of low platelet counts and/or poor platelet function, a shallow R3 slope is obtained. Hence, the R3 slope gradient determines the number of available

platelets and the level of platelet function and is recorded as platelet function (PF) by the analyser [36]. In patients with accelerated fibrinolysis, the decrease in signal after the R3 slope can be clinically used as a measure of fibrinolysis. Sonoclot variables can be used to predict the PT-INR and APTT values as well as fibrinogen levels in liver disease patients. Sonoclot is immune to the levels of by-products of fibrin breakdown in plasma and is a better method for detection of hyperfibrinolysis. Hyperfibrinolysis can also be accurately assessed by TEG. As both TEG and sonoclot are based on similar principles, fibrinolysis as detected by sonoclot may be comparable to TEG fibrinolysis. No Sonoclot parameters show significant correlation with bleeding history in cirrhosis patients. TEG has certain disadvantages like increased failure rates of the test procedure. Some experts advocate that Sonoclot is more durable than TEG, requiring fewer repetitions. Also, Sonoclot is considered more specific as it represents the initial clot formation and reflects clotting factor defects whereas TEG reaction time (R) gives information about a more mature and developed fibrin clot. Sonoclot assay is quite an accurate tool, comparable to TEG for assessing global coagulopathic disorders in liver disease [37]. The normal SONOCLOT signature and variables are as shown in Figure 7.

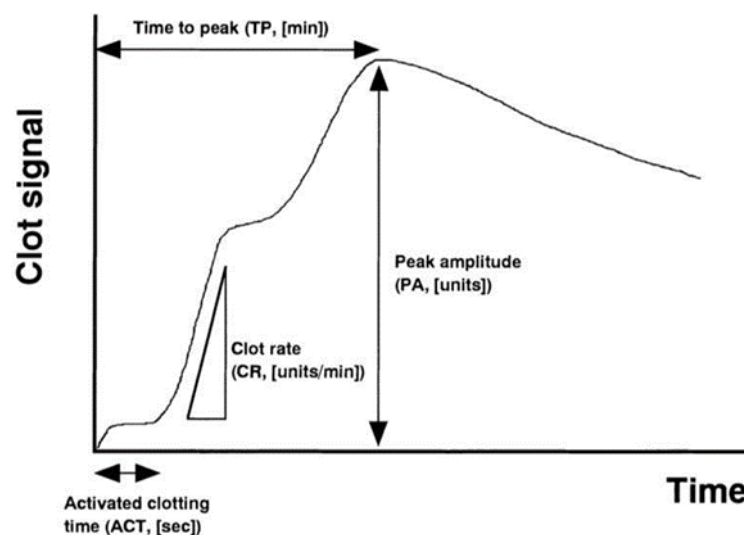


Figure 7. SONOCLOT Signature and corresponding variables. For interpretation of variables, please refer to text.

Thrombin Generation Tests

Thrombin concentration in a hemostatic plug or thrombus is technically impossible to measure. This can be indirectly achieved by measuring thrombin -products in samples from the blood in a wound-as does subsampling from clotting blood. This can be done in two ways—through use of reconstituted systems or plasma. Reconstituted systems use purified clotting factors to represent the physiologic situation. Reaction conditions are under tight control and can be varied at will. Thrombin generation in plasma (in platelet-poor or platelet-rich plasma, PPP or PRP) represents the function of the plasma proteins present, unmodified, near their physiologic concentrations. The thrombogram can be obtained through subsampling or through monitoring the conversion of a suitable substrate directly added to the clotting plasma. The former method is straightforward and time consuming; the latter allows automatic continuous measurement of many samples in parallel. In chromogenic assays, the generation of thrombin is monitored using a chromogenic substrate. Ordinarily thrombin is inactivated by antithrombin and α 2-macroglobulin and whilst thrombin bound to antithrombin is inactive [it binds to the active site of thrombin] thrombin that binds to α 2- macroglobulin retains activity to the chromogenic substrate as its binding is to the exosite of thrombin and so it can continue to cleave the chromogenic substrate. For these reasons a complex algorithm was employed to calculate free thrombin generation and to remove the contribution from thrombin bound to α 2-macroglobulin. This test required defibrination of the plasma and it is now recognised that this can have a significant effect upon the results obtained from thrombin generation assays. This paved way to fluorogenic assays in which the chromogenic substrate is replaced with a slow acting fluorogenic substrate enabled the continuous measurement of thrombin generation that required no prior defibrination of plasma. Furthermore the signal from the fluorogenic substrate was not quenched by turbidity and so thrombin generation assays could even be performed in platelet rich plasma. But, the use of a fluorogenic substrate results in addition problems as there is no direct correlation between thrombin activity and fluorescent signal intensity. To overcome this, the splitting of the fluorogenic substrate is compared to a constant known thrombin activity in a parallel non-clotting sample the so called calibrated automated thrombogram [CAT]. A calibrated automated thrombogram (CAT) method has been developed that continuously compares the signal from the experimental sample to that of a fixed known thrombin activity. This method allows visualizing the thrombin concentration in clotting PPP or PRP in parallel experiments

[38]. The three most important parameters are the lag time, the peak value, and the area under the curve or endogenous thrombin potential (ETP), which quantifies the enzymatic “work” that thrombin can do during its lifetime. Plasma clots at the end of the lag phase so the clotting time can also be calculated. The reaction mechanisms at lag phase are essentially different from those during the thrombin burst, one of the reasons that the clotting time does not represent thrombin generation. The clotting time represents the lag time. Several other techniques can be used to assess thrombin generation time, which is dependent on fibrinogen polymerization. Apart from the clotting time, they give little information on thrombin generation because fibrinogen is exhausted before 5% of all thrombin is formed. Studies of thrombin generation assays in various liver diseases, especially, in that of ALF patients have shown that the thrombin generation capacity of plasma was indistinguishable from that of healthy controls. Fibrinolytic capacity is profoundly impaired in patients with ALF and this is associated with decreased levels of the plasminogen and increased levels of plasminogen activator inhibitor type 1. Patients with ALF have a normal thrombin generating capacity and a decreased capacity to remove fibrin clots. This is in contrast with routine laboratory tests such as the PT/INR, which are by definition prolonged in patients with ALF and suggest a bleeding tendency. In patients of cirrhosis, in the presence of a raised INR, thrombin generation parameters are consistent with a hypercoagulable profile which again confirms that the PT or INR should not be used to assess bleeding risk in these patients. Thrombin generation tests are at present mostly part of research tools and are not commercially available [39].

Procoagulant Microparticle Assay

Microparticles (MPs) are present in low numbers in the blood of healthy individuals and are increased in various diseases. MPs are fragments shed from the cell membrane of most stimulated or apoptotic cells. There is growing evidence that MPs are physiologically important. Increased production of MPs has been associated to various physiological and pathophysiological conditions such as cell adhesion, apoptosis, immune response, vascular function, vascular remodelling and angiogenesis, haemostasis and thrombosis, cardiovascular diseases, cancer, infections, and normal and pathological pregnancy. MPs contribute to thrombus formation and the development of embolic diseases. MPs could serve as a prognostic marker in various disease conditions. MPs have a diameter ranging from 0.05 to 1 μm and are rich in phosphatidylserine (PS). Normally, the particles serve as important

signalling structures between cells. MPs are defined by their size and the antigens from the parental cell located on their surface. The parental cell may mainly be platelets, but also erythrocytes, granulocytes, monocytes, lymphocytes and endothelial cell derived MPs. Platelet derived MPs are generated under high shear stress in atherosclerotic arteries. Monocyte derived MPs are thought to be the source of MPs bearing tissue factor. MPs promote coagulation cascade and participate in thrombin generation. Many different methods are used in analysis of MPs including flow cytometry, capture assays, MP activity assay and MP TF assay. In patient of cirrhosis, MP procoagulant assay is highly experimental and not validated as of yet [40].

Clinical Aspects of Coagulopathy in Liver Disease Bleeding

The various important clinical aspects of coagulation dysfunction in patients of liver disease, especially cirrhosis is given briefly as follows [41].

Variceal bleeding

Gastrointestinal bleeding is mainly haemodynamic in its mechanisms and is related to portal hypertension. Risk factors for developing variceal bleeding include advanced Child-Pugh class, large varices and the presence of red wale markings. High levels of D-dimer and t- PA have been described as significant laboratory markers of risk of variceal bleeding, independently of other laboratory markers and severity of liver disease. In decompensated cirrhotic patients, variceal bleeding is also the main coagulopathy-related problem, representing 50% of all hemorrhagic events. Even though variceal bleeding is portal pressure related, the platelet plug sign suggests a possible role of hemostatic system in re-bleeding risk. The treatment is primarily by endoscopic or by measures to decrease portal hypertension through drug therapy. The role of procoagulants is currently still not established.

Intracerebral Haemorrhage

The prevalence of spontaneous intracerebral hemorrhage in hospitalized cirrhotic patients, is low (0.8% for all aetiologies, 80.3% in virus-related and 1.8% in alcohol-related groups), and it is reported not to be related to severity of liver cirrhosis (CTP) or prolonged PT. Alcohol-related cirrhotic patients have the shortest time interval between diagnosis of liver disease and hospitalization due to brain haemorrhage and heavy drinkers have a significantly higher risk

of hemorrhagic stroke than non-heavy drinkers. Rarely, cerebral metastasis bleeding may be the initial manifestation of hepatocellular carcinoma in cirrhotic patients.

Portal hypertensive gastropathy

It is primarily a mechanically driven pathology associated with high portal pressures, as suggested by response to TIPS or beta blocker therapy. Portal decompression by either mechanical or pharmacological means is usually the management.

Gastric vascular ectasia

It is a microvascular disorder and the relationship to hemostatic defects is not well explored in this condition. The management is sometimes difficult with sometimes being refractory to all measures and there is no definitive therapy and is often treated with endoscopic cauterization procedures.

Abdominal Paracentesis/ Pleurocentesis

Common laboratory indices of coagulation are of no value in predicting bleeding episodes related to these interventional procedures. No prophylactic measures need to be undertaken to prevent bleeding related to these procedures as they may result in excessive and wasteful transfusion which may adversely affect the patient at average risk. Paracentesis has a 0.2% incidence of severe haemorrhage, with a death rate of 0.016%. Paracentesis-related bleeding risk has not been found to be associated with a PT prolonged up to twice the midpoint of normal (highest noted at INR of 8.8) or with a platelet count of even at 19,000 per cumm.

Percutaneous Liver Biopsy

Major bleeding in this situation is not well-predicted by conventional coagulation indices. The presence of hyperfibrinolysis can be a marker for delayed massive bleeding in patients of cirrhosis. Sonoclot and TEG can be helpful in this situation. The incidence of biopsy associated bleeding in patients with histological diagnosis of cirrhosis is 0.7%; neither PT nor prolonged APTT and thrombocytopenia are reliable predictors of biopsy related bleeding. Other risk factors in cirrhosis patients for bleeding due to interventional procedures include those without were lower albumin and presence of large varices.

Generalized Mucosal Bleeding

The presence of generalized bleeding from mucosal surfaces especially gums or gastrointestinal tract can be indicative of the presence of a fibrinolytic disorder.

Contusions and Cutaneous Hematomas

In cirrhosis patients, these can lead to compartment syndromes and represent significant blood loss. The development of severe hematomas suggests the presence of a net bleeding diathesis and warrants careful investigation in this group of patients. Tests of global coagulation assessment are more favourable in this situation than the conventional coagulation parameters.

Tooth Extractions

These are frequently performed in patients undergoing liver transplantation and is associated with delayed bleeding in a significant proportion of patients. The presence of high fibrinolytic activity in oral cavity suggests a role for antifibrinolytic therapy in this setting.

Central Venous Cannulation.

Bleeding complications during central venous cannulation in cirrhotic patients are rare and can be safely performed in patients with liver disease and abnormal coagulation tests, even if the INR is 1.5 or more. Although the occurrence of severe bleeding complications is very low for both for subclavian and internal jugular routes of access, in order to prevent minor bleedings and vascular complications, cannulations should to be performed by experienced personnel with ultrasound guidance.

Other conditions that lead to clinically relevant bleeding in cirrhosis patients include tracheostomy procedures in decompensated patients, which maybe a necessity at one point. But the bleeding risk does not appear to be prohibitive to this procedure even in this cohort of patients. The use of extracorporeal perfusion circuits such as dialysis circuits and artificial liver support devices where in prophylaxis for circuit thrombosis may be complicated by bleeding. The management in this scenario is complex but includes citration and re-calcification of the blood passing through the circuits. Intracranial bleeding or subdural hematoma may present as encephalopathy in decompensated cirrhosis patients. The suspicion for the same should be high in case of recent minor or major trauma, focal neurological deficits or failure to improve with full-fledged conventional anticoma measures within 48 to

72 hours of therapy. The bleeding risk in patients with ALF was traditionally estimated to be higher than that of cirrhosis, which was thought to be related to the increased severity of hemostatic alterations. Currently bleeding is an uncommon cause of death in ALF patients. Spontaneous and clinically significant bleeding in ALF is rare in current practice. Routine prophylactic correction of coagulation is currently discouraged in ALF because of uncertain benefit and the potential harm of volume expansion with plasma which may worsen intracranial hypertension or products such as recombinant Factor VIIa (rFVIIa) which carry thrombotic risks. The risk of potentially fatal intracranial bleeding with placement of an ICP monitor, varies with the depth of the monitor device, but ranges from 3% to 10%. In this setting, guidelines recommend cautious use of prophylactic rFVIIa which is evidence based and not well validated [42].

Thrombosis

The incidence of arterial thrombosis, including coronary artery disease and ischemic stroke is thought to be lower in cirrhosis compared to the general population. A well- established exception is NASH-related cirrhosis where the incidence of arterial thrombosis is increased. Recent studies have indicated that venous thrombosis does occur in patients with cirrhosis, even in those patients that receive thromboprophylactic measures including pharmacological thromboprophylaxis. Liver disease is even associated with an increased risk of venous thrombosis compared to individuals without liver disease [43]. Patients with cirrhosis frequently develop portal vein thrombosis (PVT) during the course of their disease. Approximately 15% of patients will have an overt PVT at the time of liver transplantation, and the incidence of occult PVT is even higher. Patients with PVT and cirrhosis have a more severe clinical course than those without. PVT is associated with an aggravated portal hypertensive bleeding, ascites, encephalopathy, hyperdynamic circulation, and may lead to intestinal ischemia in the event of extension of thrombosis. Risk factors for PVT include the prothrombin 20210A mutation and decreased portal flow. PVT may recur following liver transplantation and, depending on the location of the thrombus, PVT may adversely affect survival after transplantation [44]. Thrombosis of the hepatic artery (HAT) may occur both early and late after liver transplantation. Early HAT frequently occurs when routine laboratory values have not completely normalized and thus paradoxically indicate a hypocoagulable state. Mechanical factors along with postoperative hypercoagulability are contributory to this condition. Preoperative PVT was recently identified as a strong predictor of a postoperative

HAT, which may indicate that a relative hypercoagulable state may be involved in the pathogenesis of HAT. Thrombosis as a contributor of progression of fibrosis in patients of liver disease as shown in both animal models and human explants, where in microthrombi in the liver vasculature has been observed in fibrotic areas. These observations have led to the hypothesis that coagulation activation within the liver vasculature occurs in response to the fibrotic process and contribute to progression of fibrosis by inducing local ischemia; a process known as ‘parenchymal extinction’. The activation of stellate cells by coagulation proteases such as thrombin and factor Xa also play a role. The presence of factor V Leiden leads to an accelerated progression of fibrosis in patients of liver disease. Conversely, treatment with anticoagulant drugs has been shown to attenuate progression of fibrosis in animal models [45].

Management Considerations of Bleeding in Liver Disease (Table 1)

Patients with cirrhosis will experience the development of varices at a rate of about 8% per year after the onset of cirrhosis. Once formed, risk factors for bleeding are predominantly related to hemodynamic and mechanical parameters such as hepatic vein– portal pressure gradient, varix size, their appearance and the severity of the underlying liver disease. The presence of the platelet plug (nipple sign) as a high-risk marker for variceal bleeding hints at some transient role of primary hemostasis in acute bleeding. There are no specific recommendations on coagulation parameters for prophylactic esophageal variceal band ligation. In those with severe coagulopathy that exclude safe variceal band ligation, then nonselective beta-blockers alone would be the preferred therapy for primary bleeding prophylaxis. In the setting of acute variceal bleeding, there is little consensus regarding coagulation management during the immediate bleeding episode. Blood resuscitation should aim for a target haemoglobin in the 7–8 g/dL range. Overtransfusion of red cells or large volumes of plasma should be avoided because of increase in re bleeding rates due to resultant increases in portal pressures [46]. Optimal platelet counts remain uncertain, although on the basis of adequate thrombin production, levels exceeding $50 \times 10^9/\text{cumm}$ are recommended. A fibrinogen level above 100–150 mg/dL is sometimes recommended by using cryoprecipitate transfusion but remains to be validated in controlled trials. Fresh frozen plasma (FFP) is a difficult transfusional product because of the large volume needed to actually replenish coagulation factors (approximately 20–40 mL/kg). This dose usually requires several liters of FFP to “correct” the INR and thus may worsen portal pressure,

precipitate anasarca, and expose the patient to events such as transfusion-related acute lung injury, cardiogenic pulmonary edema, and iatrogenic increases in portal pressures leading to rebleeding risks [47]. Under controlled trials, it was recommended that recombinant activated factor VII cannot be recommended for general use. An algorithm based on TEG for blood product transfusions and the corresponding blood product dosing is shown in Figure 8 and Figure 9.

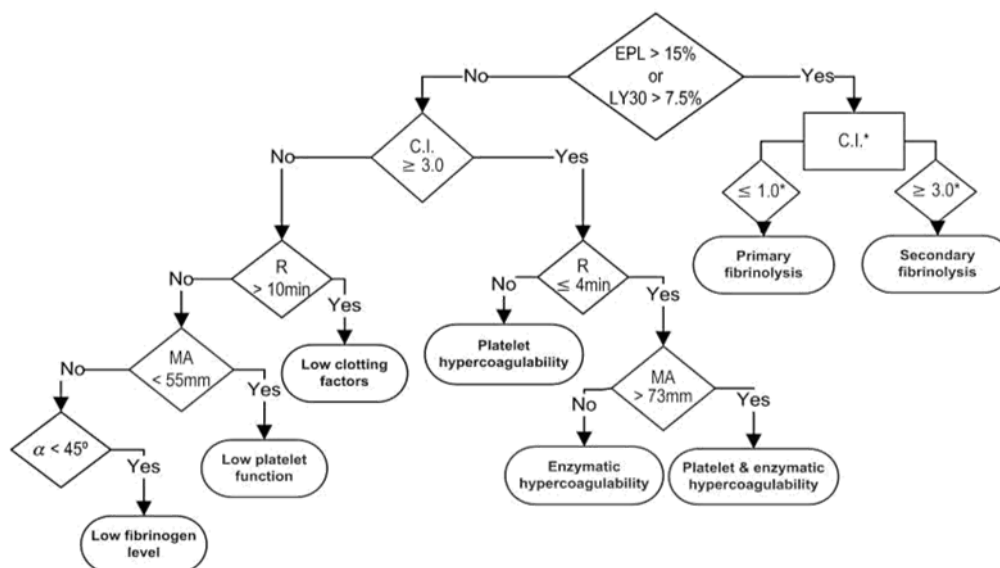


Figure 8. TEG based transfusion algorithm and interpretation in bleeding, fibrinolysis and hypercoagulable states associated with liver diseases.

There are no good measures of bleeding risk before invasive procedures that are currently available to the practicing clinician. Practically, one can recommend that truly elective procedures should be delayed during acute events (acute infection, severe acute alcoholic hepatitis, and uraemia) that might upset the rebalanced hemostatic system in cirrhosis patients. Decisions to proceed with elective or semielective procedures should not be solely based on the INR and platelet counts but on the severity of comorbidities of the patient, the urgency of the procedure, the accessibility of the procedural site to mechanical hemostasis maneuvers, and the ability to detect bleeding at the site early in the hemorrhage. If the policy of the treating unit is for prophylactic transfusion, then in cirrhosis patients the INR should not be used as a sole measure of bleeding risk, and physiologically it would be more appropriate to achieve a platelet count greater than $50\text{--}60 \times 10^9/\text{L}$ for high-risk procedures by using platelet transfusions [48]. The MELD is a validated tool for predicting surgical

mortality in cirrhosis patients. But, coagulation and bleeding complications are rare causes of mortality in the cirrhosis patient undergoing elective surgery. Using thromboelastography in the operating room could be helpful in targeting transfusion practice. The surgeon should be aware that persistent postoperative wound, mucosal and/or puncture site bleeding could be a sign of hyperfibrinolysis, which warrants more specific therapy. The use of desmopressin in compensated cirrhotics showed that it shortened the bleeding time and partial thromboplastin time with increases in factor VIII and vWF, but it was not efficacious in controlling variceal bleeding or reducing blood loss in patients undergoing liver transplantation or liver resection. This indicated that normalization of bleeding time does not necessarily diminish bleeding risk and bleeding time is not a reliable prognostic factor of significant bleeding [49]. The combination of desmopressin and terlipressin increased the rebleeding rate compared with terlipressin alone in a study. So the concomitant use of these two agents together should be cautious [50]. The different modalities of microvascular bleeding management in cirrhosis patients is shown in Table 1.

Table 1. Available modalities for management of microvascular bleeding related to coagulopathy in patients of liver disease

AVAILABLE MODALITIES AND CHARACTERISTICS FOR BLEEDING MANAGEMENT IN LIVER DISEASE
Packed Red Cells – improvement in anemia and platelet function, life saving, correction of hypoxemia; trasnfusion related side effects in over correction; target hemoglobin in variceal bleeding 7 to 8 g/dL
Fresh Frozen Plasma – helps in replenishment of both pro- and anticoagulants; transfusion-related side effects, trasnfusion related acute lung injury, fluid overload and exacerbation of portalhypertension leading to rebleeding
Platelet concentrate - improves primary hemostasis; provides stage for adequate thrombin production with levels exceeding 50,000 per microliter; transfusion-related side effects, adversely affects outcome of liver transplantation
Recombinant Factor Vila - small volume product, encouraging data from uncontrolled studies and case reports; expensive and no proven effect in randomized trials in patients with liver disease, theoretical risk of thrombosis exists
1-desamino-8-D-arginine vasopressin (DDAVP) – helps in laboratory improvement of primary hemostasis, relative lack of side effects, easy administration; efficacy in patients with liver disease is not proven
Factor concentrates - small volume product, no trasnfusional overload, only repletes part of the coagulation factors; no benefit on global abnormal hemostatic parameters, no data from controlled studies yet, theoretical risk of thrombosis exists
Thrombopoietin receptor agonists – are effective in increasing endogenous platelet count, no transfusion-related side Effects; but no data from controlled studies on efficacy, theoretical risk of thrombosis exists
Antibiotics – helpful in reduction of variceal bleeding, improves coagulation status in patients with active infection, improvement in systemic hemodynamics; side effect profile and bacterial resistance or overgrowth

Many studies have evaluated the role of Factor VII in the control of bleeding during variceal hemorrhage, liver transplantation, and partial hepatectomy. None of these reported a demonstrable efficacy of factor therapy on the control bleeding within 24 hours, failure to prevent clinically significant rebleeding or death within 5 days of first dosing. However, significant changes in the composite endpoint within upper gastrointestinal hemorrhage in

patients with Child-Turcotte-Pugh class B and C cirrhosis, higher survival, and reduced transfusion requirements at the time of transplant were noted. Antifibrinolytic agents such as Aprotinin and Tranexamic acid have been shown to reduce blood loss during liver transplantation. The use of prothrombin complex concentrates in patients with liver disease is also very limited. A major advantage of these products as compared to plasma is the small volume in which the concentrates are administered, thus avoiding the fluid overload and increase in portal hypertension that may be associated with plasma infusion [51]. prothrombin complex concentrates have been associated with an increased risk for thrombotic events, which should be monitored carefully in future clinical studies. A novel strategy to improve platelet function in patients with hepatitis C is the administration of a thrombopoietin analog (Eltrombopag) which has been shown to substantially increase the platelet count in these patients. With Eltrombopag, the chances of hypercorrection of the platelet count exists and in some patients, a theoretical risk of thrombosis occurs, especially in light of the highly elevated vWF levels [52].

Parameter	Inference	Intervention
R > 10 min	Decreased coagulation factors	FFP 10–20 ml/kg (if FFP is without clinical efficacy, consider cryoprecipitate 3–5 ml/kg)
Angle < 52	Hypofibrinogenemia Poor thrombin generation (thrombocytopenia/ thrombocytopathy)	Functional Fibrinogen analysis
MA < 49 mm MAff < 14 mm	Decreased fibrinogen	FFP 20–30 ml/kg Fibrinogen konc. 25–50 mg/kg Cryoprecipitate 5 ml/kg
MA < 49 mm MAff > 14 mm	Decreased platelets	Platelets 5–10 ml/kg
Ly30 > 8%	Primary hyperfibrinolysis	Tranexamic acid 1–2 g IV (adults); 10–20 mg/kg IV (children)
Ly30 > 8% and Angle and/or MA increased	Reactive hyperfibrinolysis	Tranexamic acid contraindicated
Difference in R > 2min between st-TEG and Hep-TEG	Heparinization	Protamine sulphate or FFP 20–30 ml/kg

Figure 9. TEG based inference and interventional modalities in coagulation disorders.

Management Considerations in Thrombotic Events in Liver Disease

Cirrhotic patients may need thromboprophylaxis or anticoagulation in certain clinical scenarios, especially those involving an additional thrombotic risk. Antifactor Xa activity negatively correlates with the severity of the liver disease, and a positive correlation is seen with antithrombin-III levels and antifactor-Xa value. Antithrombin-III itself negatively correlates with the severity of liver disease [53]. The prophylactic use of low molecular weight heparin (LMWH) in cirrhotic patients is safe. A decreased anti-Xa value in cirrhotic patients and a negative correlation with liver function challenge the unconditional use of anti-Xa assays in LMWH monitoring in cirrhotic patients and reveals a potential limitation of anti-Xa analysis in these patients. Low levels of antithrombin, because of reduced hepatic synthesis, are the most likely cause of this phenomenon. Cirrhotic patients have also been found to have an increased response to LMWH, which correlates with the severity of liver disease, despite reduced antithrombin and anti-Xa activity levels. The factor Xa inhibitors Rivaroxaban and Apixaban are metabolized in the liver and they are contraindicated in severe hepatic diseases because their metabolic inactivation is impaired [54]. Metabolic conversion of the prodrug Dabigatran etexilate to Dabigatran, a thrombin inhibitor, is completed in the liver. Idraparinux, an antithrombin dependent FXa inhibitor, has no hepatic clearance, but its long half-life, approximately 80 hours and the lack of antidote do represent major problems if bleeding occurs. These drugs must be use with caution or are contraindicated in the presence of renal failure. In cirrhotic patients with acute variceal bleeding and PVT, anticoagulation with LMWH administered after hemostasis is achieved (by band ligation, repeated band ligation or injection sclerotherapy combined with argon plasma coagulation) has been reported to achieve complete recanalization of the portal vein within 2-11 days, with no significant increase in rebleeding incidence [55]. Thromboembolism has been reported to behigher between cirrhotic patients who do not receive thromboprophylaxis, and thromboprophylaxis with LMWH has not shown any increase the relative risk of bleeding post hepatectomy in cases of cirrhosis with hepatocellular carcinoma. In a randomized non-blinded single-center controlled trial which evaluated the safety and efficacy of Enoxaparin versus no treatment, in preventing PVT in patients with advanced cirrhosis, none of the patients receiving enoxaparin developed PVT, compared 10 of 36 controls at 96 weeks of follow up. The Enoxaparin treated group also showed a trend toward amelioration in renal function biomarkers, liver function test and higher survival rates. The evaluation of safety and

efficacy of LMWH to treat non-neoplastic PVT in cirrhotic patients who were suitable for liver transplantation was undertaken in a study, once PVT was documented with ultrasound and computed tomography. It was found that at six months, complete recanalization occurred in 33.3%, partial recanalization in 50% and 16.7% of patient had no response to therapy. Among those patients who had achieved partial recanalization, 65% reached complete recanalization after continuing anticoagulation for 7 to 17 months [56]. Conglomerated results obtained from published series on anticoagulation therapy for PVT thrombosis in cirrhotic patients have shown that anticoagulation is effective in achieving portal vein repermeation and does not increase the risk of major complications. Acute or subacute PVT can be treated with therapeutic anticoagulation (LMWH) and may prolong survival in these patients. It should be noted that esophageal varices need to be treated aggressively endoscopically before anticoagulation [57]. The currently available vitamin K antagonists have a very narrow therapeutic window in cirrhosis patients and are especially problematic in patients with baseline elevated INR. Those patients with chronic PVT and cavernous transformation are less likely to benefit from anticoagulation and premature discontinuation of anticoagulation (like in before transplant) is likely to result in thrombus recurrence and predispose to hepatic artery thrombosis. In patients with deep vein thrombosis or pulmonary embolus associated with cirrhosis, consideration must be given for medical prophylaxis in all hospitalized cirrhosis patients as with any medical inpatient. The medical therapy for acute venous thromboembolism should be with LMWH in therapeutic doses similar to PVT treatment unless contraindicated and the presence of nonbleeding esophageal varices should not preclude prophylaxis [58].

In summary, medical knowledge has only recently begun to expand in the area of hemostasis in liver disease. In patients with advanced liver disease, there is a lack of accurate, reliable, and clinically available testing methods to properly assess the true state of hemostasis because some patients with chronic liver disease are predisposed for bleeding, some for hypercoagulation, and some in an apparent stable balance. Patients of liver disease, especially cirrhosis, are not “auto-anticoagulated” and conventional parameters of bleeding do not correlate well with the risk of future bleeding episodes. Patients of cirrhosis are like a double-edged sword with both bleeding and thrombotic events capable of complicating the natural history. Newer modalities of hemostasis evaluation which enables the clinician to assess hemostatic pathway globally, as in thromboelastography and thrombograms could soon

become the point of care in managing this difficult patient group. Efforts at correction of abnormal hemostatic parameters prior to invasive procedures are without a scientific basis and in fact may pose unwarranted risk with little or no benefit. Increasing experience with a restrictive transfusion policy in liver transplant surgery suggests that an on-demand transfusion strategy is safe and substantially reduces blood product use. This policy reduces transfusion-related complications also. Patient and situation specific therapeutic management in the light of efficacy and safety need to be sensibly applied in this sub group of patients so as to improve outcomes.

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