

## 2016 Liver Transplantation: Global view

## Coagulopathy and transfusion therapy in pediatric liver transplantation

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### Abstract

Bleeding and coagulopathy are critical issues complicating pediatric liver transplantation and contributing to morbidity and mortality in the cirrhotic child. The complexity of coagulopathy in the pediatric patient is illustrated by the interaction between three basic models. The first model, "developmental hemostasis", demonstrates how a different balance between pro- and anticoagulation factors leads to a normal hemostatic capacity in the pediatric patient at various ages. The second, the "cell based model of coagulation", takes into account the interaction between plasma proteins and cells. In the last, the concept of "rebalanced coagulation" highlights how the reduction of both pro- and anticoagulation factors leads to a normal, although unstable, coagulation profile. This new concept has led to the development of novel techniques used to analyze the coagulation capacity of whole blood for all patients. For example, viscoelastic methodologies are increasingly used on adult patients to test hemostatic capacity and to guide transfusion protocols. However, results are often confounding or have limited impact on morbidity and mortality. Moreover, data from pediatric patients remain inadequate. In addition, several interventions have been proposed to limit blood loss during transplantation, including the use of antifibrinolytic drugs and surgical techniques, such as the piggyback and lowering the central venous pressure during the hepatic dissection phase. The rationale for the use of these interventions is quite solid and has led to their incorporation into clinical practice; yet few of them have been rigorously tested in adults, let alone in children. Finally, the postoperative period in pediatric cohorts of patients has been characterized by an enhanced risk of hepatic vessel thrombosis. Thrombosis in fact remains the primary cause of early graft failure and re-transplantation within the first 30

d following surgery, and it occurs despite prolongation of standard coagulation assays. Data, however, are currently lacking regarding the use of anti-aggregation/anticoagulation therapies and how to best monitor for thrombosis in the early postoperative period in pediatric patients. Therefore, further studies are necessary to elucidate the interaction between the development of the coagulation system and cirrhosis in children. Moreover, strategies to optimize blood transfusion and anticoagulation must be tested specifically in pediatric patients. In conclusion, data from the adult world can be translated with difficulty into the pediatric field as indication for transplantation, baseline pathologies and levels of pro- and anticoagulation factors are not comparable between the two populations.

**Key words:** Children; Coagulation; Thrombosis; Liver disease; Transfusion; Transplantation; Point of care coagulation

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**Core tip:** In the last two decades, extensive investigation of abnormalities in hemostasis in adult cirrhotic patients and improvements in both surgical and anesthetic management has enhanced outcome following liver transplantation. Unfortunately, such knowledge cannot be directly applied to pediatric patients, as major differences exist between adults and children undergoing liver transplantation. In this review, we discuss the pattern of hemostatic abnormalities in children with end-stage liver disease, point-of-care coagulation monitoring, and clinical strategies designed to reduce bleeding and thrombosis in pediatric liver transplantation. In conclusion, we propose a prioritized research agenda for this pediatric subspecialty.

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## INTRODUCTION

Pediatric liver transplantation (PLTx) is the treatment of choice for children suffering from end-stage liver disease<sup>[1,2]</sup>. The reported 5-year survival rate after PLTx is over 85%<sup>[3-5]</sup>. In the last two decades, a growing body of research has provided insight into the donor and recipient factors affecting graft and patient survival<sup>[6-13]</sup>, and incorporation of these findings into clinical practice has improved graft and patient survival following PLTx. Red blood cell (RBC) transfusion, for example, is one factor which has a negative impact on outcome following liver transplantation in adult patients<sup>[14-17]</sup>. A similar result was recently reported

for PLTx<sup>[18]</sup>. One of the critical challenges in liver transplantation is the maintenance of hemostasis perioperatively<sup>[19,20]</sup> as liver disease already leads to decreases in the hemostatic reserve<sup>[21]</sup>. Furthermore, conventional coagulation tests are inappropriate for clinical decision-making<sup>[22,23]</sup>. Finally, even though general rules valid in transfusion medicine can be applied to pediatric patients<sup>[24]</sup>, children have a number of important differences compared to adults, especially with respect to the hemostatic system<sup>[25-31]</sup>. Here, we review the pattern of hemostatic abnormalities observed in pediatric patients with end-stage liver disease, point-of-care coagulation (POC) monitoring during PLTx, and the transfusional and non-transfusional agents clinically useful to prevent or reduce bleeding and thrombosis in PLTx. The review ends with a discussion concerning future areas of investigation for PLTx.

## HEMOSTASIS DEFECTS IN PEDIATRIC LIVER DISEASE

### *Normal hemostatic mechanism in the pediatric population*

“Developmental hemostasis” and the “cell based model of coagulation” are the two basic concepts required to evaluate hemostatic disorders in children with coagulopathy. “Developmental hemostasis” was coined in the late 1980s by Andrew *et al.*<sup>[32]</sup> to describe hemostasis as an evolving process that is age dependent, from fetal to geriatric periods. Accordingly, the functional levels of proteins change in a predictable way with age. Vitamin K-dependent factors, the contact factors and eight inhibitors [antithrombin III (AT-III), heparin cofactor II, a<sub>2</sub>-antiplasmin, tissue factor pathway inhibitor (TFPI), alpha-1-antitrypsin, C1 esterase inhibitor, protein C (PC) and protein S (PS)] are at minimum levels at birth but gradually increase approaching adult levels by 6 mo of life. Conversely, factor VIII and von Willebrand factor (vWF, the main platelet-vessel wall adhesive protein) are the only two procoagulant proteins that exhibit markedly elevated levels at birth when compared to adult values. Moreover, the protein levels of alpha-2-macroglobulin (a<sub>2</sub>M), a thrombin inhibitor of secondary importance in adults, are extremely high in neonates and often reach twice those measured in adults<sup>[25,32-34]</sup>. Both quantitative and qualitative differences of the coagulation protein have been reported. Post-translational modifications (PTMs) may in particular affect the structure of hemostatic proteins and alter their function. For example, mean fibrinogen values are comparable between neonates and adults, but results indicate that neonatal fibrinogen is dysfunctional, and it is this form which persists until 1 year of age<sup>[34]</sup>.

However, despite differences in the quantity and quality of hemostatic proteins relative to the adult (Table 1), hemostasis is intact in neonates and young

**Table 1 Hemostatic differences in the pediatric population**

Parameters	Defects	Activity	Time to normalization
Procoagulant factors			
Factors II, VII, IX, X (vitamin K-dependent)	↓ quantity	↓	6 mo
Fibrinogen	Dysfunctional	↓	1 yr
Platelet	Hyporesponsiveness	↓	2-4 wk
vWF	↑ quantity/ Hyperfunction	↑	/
Factor VIII	↑ quantity	↑	/
Anticoagulant factors			
AT-III	↓ quantity/ dysfunctional	↓	6 mo
Heparin Cofactor II	↓ quantity	↓	6 mo
TFPI	↓ quantity	↓	6 mo
α2-antiplasmin	↓ quantity	↓	6 mo
α1-antitrypsin	↓ quantity	↓	6 mo
C 1 esterase inhibitor	↓ quantity	↓	6 mo
Protein C	↓ quantity	↓	6 mo
Protein S	↓ quantity	↓	6 mo
α2-macroglobulin	↑ quantity	↑	6 mo

infants. However, this “restored balance” can only be demonstrated using an assay, such as endogenous potential thrombin generation (EPT)<sup>[35]</sup>, which reflects the interaction of pro- and anticoagulants. Consequently, traditional tests, such as prothrombin time (PT) and activated partial prothrombin time (APTT), might be inadequate to assess coagulation capacity in neonates<sup>[27,36]</sup>.

This discrepancy may be explained by the complexity of the hemostatic system. It is not a simple cascade model with an “intrinsic” and an “extrinsic” component<sup>[37,38]</sup>, but rather an interaction between the blood vessel wall, coagulation proteins within the plasma and cellular components of the blood which are predominantly platelets. Hoffman and Monroe in 2000<sup>[39]</sup> proposed a new concept of hemostasis that integrates both the humoral cascade and cellular factors, which is now widely accepted as representative of actual hemostasis *in vivo*. These investigators conceptualized a cell-based model of coagulation in which the three overlapping phases of coagulation (initiation, amplification, and propagation) occur on different cell surfaces. The initiation phase takes place on tissue factor-bearing cells such as fibroblasts. If the procoagulant stimulus is strong enough to produce adequate levels of the factors Xa, IXa and thrombin, then the amplification phase is triggered. Small amounts of thrombin generated on TF-bearing cells amplify the initial procoagulant signal by enhancing adhesion and activation of platelets and activating factors V, VIII and XI. In the final stage, the propagation phase, the active proteases combine with their cofactors on the surface of platelets producing thrombin on a large scale which results in the polymerization of fibrin.

According to this model, TF-bearing cells and

platelets have specialized procoagulant functions, while vascular endothelial cells have specialized anticoagulant features. The endothelial cells express the thrombomodulin (TM) which binds the thrombin released into the circulation from the site of injury, and converts it from a procoagulant to an antithrombotic. Thrombin in complex with TM activates Protein C on the endothelial cell surface, and this activated Protein C (APC) forms a complex with Protein S. The APC/Protein S complex cleaves and inactivates any factor Va and factor VIIIa that has been activated on endothelial cell surfaces and thus, prevents further formation of additional procoagulant enzymes where an intact endothelium is present. In addition to TM, endothelial cells also express other important antithrombotic surface proteins, such as antithrombin III (ATIII) and tissue factor pathway inhibitor (TFPI). These plasma protease inhibitors prevent inappropriate intravascular coagulation by inhibiting active proteases which have been released from the cell surface into the fluid phase.

This cell-based model may explain some aspects of pediatric hemostasis that a protein-centric model does not. For example, while platelet number and volume are similar between neonates and adult, neonatal platelets clearly exhibit hyporesponsiveness during the first 2 to 4 wk after birth<sup>[40]</sup>. However, in most *in vivo* assays used to detect platelet function, platelet dysfunction is not observed in neonates. This result is probably due to the fact that vWF plays a more prominent role in neonatal hemostasis<sup>[41]</sup>, so that a higher concentration of circulating vWF and a greater percentage of large vWF multimers, the molecules most effective in promoting platelet vessel wall adhesiveness, are present. This model also potentially provides an explanation for the regulation of the plasma levels of hemostatic proteins. Lisman *et al*<sup>[30]</sup> reported that children transplanted with an adult liver graft maintain a pediatric hemostatic profile after transplantation despite receiving the adult graft. This result indicates that the liver graft does not control the plasma levels of hemostatic proteins. Instead, regulatory mechanisms may involve the hormonal system<sup>[42,43]</sup> or alternatively, an extra-hepatic sensor which might be present on the vascular endothelium.

Why does “developmental hemostasis” even exist? For this answer, we have to look at hemostasis as a system working within a network of physiological systems, such as wound repair, inflammation and angiogenesis. Recently, ATIII has also been shown to possess potent anti-angiogenic properties<sup>[44]</sup>. Interestingly, the levels of ATIII are low in the neonate, a time at which angiogenesis is extremely active. It is therefore possible that the levels of ATIII modulate angiogenesis, making AT replacement therapy potentially deleterious during neonatal life<sup>[45]</sup>.

**Table 2 Liver transplantation phases and coagulation status**

Liver transplantation phases	Coagulation status
Pre-existing coagulation disorders	Developmental hemostasis
	Rebalanced hemostasis in chronic disease
	Unpredictable effect in acute liver failure
Liver transplantation surgery	Coagulopathy of surgical trauma
	Pre-anhepatic phase
	Anhepatic phase
	Reperfusion phase
After liver transplantation surgery	Ischemic-reperfusion syndrome
	Heparin-like effect
	tPa release
	Tendency to thrombotic state (days 1-14 after liver transplantation)
	Recovery of coagulation activity in 1 mo

## COAGULOPATHY IN PEDIATRIC LIVER DISEASE AND DURING LIVER TRANSPLANTATION

The mantra of pediatricians that “children are not little adults” is especially true for pediatric patients with end-stage liver disease because of differences in the natural history of their disease, responses to medical therapy and overall nutritional status<sup>[31]</sup>. Although research focused on coagulopathy in the context of PLTx is lacking, data from adult patients indicates that derangements of the hemostatic system occur in three phases: pre-operatively (pre-existing), intraoperatively, or postoperatively (Table 2).

### **Pre-existing coagulation disorders**

The first variable to consider is age of the pediatric patient, which ranges widely from the newborn to the adolescent. As discussed above, the entire coagulation system is normally subject to substantial changes in the early years of life<sup>[45]</sup>, especially in the first months, and it is unclear how these changes influence the development of coagulopathy in liver disease. While no scientific studies have specifically addressed this topic, patient weight, a proxy of age in the pediatric population, has been related to blood loss in some case series<sup>[46-48]</sup>, yielding a threshold value of 10 kg<sup>[49]</sup>. This finding however was not confirmed in a more recent study<sup>[50]</sup>. The relevance of other factors, such as indication for PLTx or technical difficulty of the surgery, was also not defined. What is certain, is that our current knowledge and ability to monitor and interpret the hemostatic system in young children, especially in the newborn, is limited<sup>[27,36]</sup>.

The second major variable to consider is the heterogeneity in the indications for PLTx. There are four major indications: cholestatic cirrhotic disease, acute liver failure, metabolic disease and cancer. The clinical features of the pediatric patient with chronic

and acute liver failure are completely different, and it remains unknown as to whether or not alterations in hemostasis influence outcome in these patients<sup>[22,51-54]</sup>.

Indication for PLTx in 30%-50% of cases is cholestatic disease, such as biliary atresia or familial intrahepatic cholestasis<sup>[1,7,8,10]</sup>. These patients exhibit a normal hemostatic profile<sup>[55]</sup>. It remains uncertain however as to whether one of the features of their disease, as in their adult counterparts, is a hypercoagulable state<sup>[56]</sup>. It is a reasonable possibility as these pediatric patients have a higher incidence of hepatic vessel thrombosis following transplantation.

Acute liver failure<sup>[51]</sup> is the indication for PLTx in about 10% to 15% of cases, but it is associated with a higher mortality rate<sup>[57-59]</sup>. In two studies, coagulation impairment during acute liver failure in adult patients has been evaluated<sup>[60,61]</sup>. Patients were assessed simultaneously using the PT test and the thromboelastogram (TEG). In both studies, the two methods did not generate comparable results. PT was elevated in most patients with acute liver failure, but TEG results were very different; in most cases, coagulation appeared to be normal, but when altered (either hypo- or hypercoagulability), coagulation status was not predictable with classical laboratory tests. Similar evaluations have not yet been performed on pediatric patients so it remains unclear as to how these results and methodologies can be applied to different patient populations.

Exceptions to this argument are metabolic disease and cancer. In these cases, patients do not experience classic “global” liver failure from a functional point of view. Deficient synthesis for metabolic disease is mostly associated with specific metabolic pathways, but such changes do not affect the hemostatic system. Although data is lacking, it can be inferred that alterations of coagulation during PLTx in patients with metabolic disease and cancer can be entirely attributed to the surgery and not to a pre-existing coagulopathy.

The last variable to consider is the concept of rebalanced coagulation in liver disease.

The liver is the site where procoagulation, anticoagulation and fibrinolytic factors are produced. As liver failure progresses, production of these factors is generally reduced<sup>[21,22,62-67]</sup> which subsequently drives the establishment of a “rebalanced system”. This rebalanced system includes multiple alterations in the clotting cascade, clot lysis, and the number and function of platelets (Table 3).

However, rebalanced hemostasis is typically less stable with the possibility of a rapid switch from a hypo- to a hypercoagulable state along with clinical complications such as renal failure, infection and/or trauma. Furthermore, coagulopathy, as detected by PT/PTT, does not protect from thrombotic events. Indeed, cirrhotic patients exhibit an incidence of peripheral deep vein thrombosis and pulmonary embolism that is two-fold higher than in controls (0.5%-1.0%)<sup>[68,69]</sup>.

**Table 3** Factors implicated in rebalancing

System	Increases	Decreases
Clotting cascade	Factor VIII	Procoagulation factors V, VII, IX, X, XI, prothrombin Vitamin K-dependent procoagulation factors II, VII, IX, X Vitamin K-dependent anticoagulation factors protein C and protein S; anticoagulant proteins synthesized by the liver such as protein Z, protein Z-dependent protease inhibitor, antithrombin, heparin cofactor II, and alpha-2-macroglobulin Fibrinogen and dysfibrinogenemia
Clot lysis	Tissue plasminogen activator (tPA) (due to enhanced release by the activated endothelium and/or by reduced hepatic clearance) Levels of plasminogen activator inhibitor (PAI-1)	Plasminogen, antiplasmin (alpha-2 plasmin inhibitor or alpha-2 PI), thrombin-activatable fibrinolysis inhibitor (TAFI), and factor XIII
Platelet	Plasma von Willebrand factor (vWF; main platelet vessel wall adhesive protein)	Thrombocytopenia and thrombocytopeny (usually from hypersplenism, altered levels of thrombopoietin metabolism, antiplatelet antibodies and defective platelet aggregation) ADAMTS-13 (vWF cleaving protease)

The incidence of portal vein thrombosis in children in end-stage liver disease has been reported to be about 10%<sup>[70]</sup>.

PT and APTT fail to detect this rebalanced state because these tests are insensitive to plasma levels of anticoagulant proteins (protein C pathway, antithrombin and tissue factor pathway inhibitor) and cannot take into account the role of the endothelium and cells in the hemostatic process<sup>[22]</sup>. By contrast, global assays, such as EPT, which test for both procoagulant and anticoagulant components, will detect this rebalanced state<sup>[65,66]</sup>.

A recent study<sup>[71]</sup> performed on children and adolescents with chronic liver disease seems to contradict the concept of "rebalanced hemostasis" observed in cirrhotic adults: EPT data was found to be in agreement with routine coagulation tests in this pediatric cohort. However, the reference values for TF and TM concentrations chosen to evaluate EPT were not based on those used in other studies involving adult patients<sup>[65,66]</sup>. This conflicting result represents only one of the many pitfalls and dilemmas emerging from the research agenda to evaluate hemostasis in neonates and children<sup>[27]</sup>. To our knowledge, no other significant studies have undertaken the issue of hemostasis impairment in pediatric chronic liver disease. However, several studies promoted by the Biliary Atresia Research Consortium (BARC) and the Cholestatic Liver Disease Consortium (CLIC) are currently in progress.

### Coagulation disorders during liver transplantation

Liver transplant surgery is academically divided into three phases<sup>[72]</sup>: pre-anhepatic, anhepatic and post-reperfusion phases.

The pre-anhepatic phase is characterized by the presence of pre-existing coagulopathy superimposed with other factors, including coagulopathy due to the trauma of surgery, intraoperative bleeding related to surgical challenge, such as lysis of adhesions in the case of reoperations, bleeding due to the development of collateral circulation and portal hypertension,

increased capillary fragility, and dilution coagulopathy secondary to fluid replacement<sup>[72,73]</sup>.

The anhepatic phase includes by definition procedures from the occlusion of hepatic vasculature to revascularization of the transplanted-liver. During this phase, the production of coagulation factors and hepatic clearance is reduced. Hyperfibrinolysis may be the major problem during this phase due to lack of tissue plasminogen activator (tPA) clearance while levels of PAI-1 remain relatively unchanged<sup>[74]</sup>.

Reperfusion is the most delicate of the three phases because several imbalances, as a part of a larger ischemia/reperfusion injury (IRI) syndrome, may affect both anticoagulant and procoagulant pathways. Thrombocytopenia is readily apparent, mostly due to the trapping of platelets in the liver sinusoids, but platelet activation is also occurring<sup>[23]</sup>. Additional complications originate from the so-called heparin-like effect (HLE) due to release of heparinoids from the endothelium of the donor tissue<sup>[75]</sup> and an enhanced release of t-PA causing hyperfibrinolysis<sup>[74]</sup>.

In a recent retrospective study, analysis of a series of TEG (native and heparinase) collected during each surgical phase of liver transplantation was performed and demonstrated that alterations in coagulation were occurring during liver transplantation<sup>[76]</sup>. Apart from a transient period of HLE, a significant number of patients presented with hypercoagulable TEGs during the procedure. In addition, a prospective study using trans-esophageal monitoring detected incidental intracardiac thromboemboli in 1.9% of the patients during liver transplantation<sup>[77]</sup>. Thus, hypercoagulability during liver transplantation deserves a closer look as thromboembolic events are associated with high morbidity and mortality rates<sup>[78]</sup>.

Only one study describing the alterations of coagulation during liver transplantation in pediatric patients was found<sup>[55]</sup>. The study was well conducted but included only 8 children. TEG data was collected for these pediatric patients in each phase of surgery. Coagulation changes were found to be similar to

those recorded in adults<sup>[76]</sup> but less severe. Authors speculated that the reason for this difference was the preponderance of cholestatic disease in children compared to hepatocellular disease in adults.

In another pediatric study<sup>[79]</sup>, platelet function during PLTx was evaluated using aggregometry, with the assumption that platelet function could be implicated in the development of the IRI syndrome. Analysis of platelet function revealed that a reduction in aggregation occurred after surgery began, reaching a nadir during anhepatic and post-reperfusion phases. A slow normalization followed in the first 6 d after surgery. Interestingly, ADP triggered platelet aggregation levels were characterized by a strong linear correlation with markers of liver injury and IRI.

In summary, a tendency towards a prothrombotic state with an initial worsening of platelet function during the dissection and anhepatic phase of surgery has been observed. During the reperfusion phase, the heparin-like effect predominates but appears to be generally a transient effect. At this stage, there is, on average, a slight deterioration of coagulation and platelet functions, in terms of strength of the clot.

### **Coagulation disorders after liver transplantation**

Early surgical complications after liver transplantation include primary nonfunction (PNF) of the graft, bleeding, hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT)<sup>[1,4,5,11,80,81]</sup>. PNF is a rare but catastrophic event of unknown etiology that is most likely related to IRI syndrome. It is characterized by high transaminase levels, coagulopathy and progression to multiple organ failure<sup>[1]</sup>. HAT occurs in 5%-18% of pediatric recipients, which is three to four times more frequent than in adult transplant patients<sup>[4,5,80,82]</sup>. HAT occurs most often within the first 30 d after transplantation and leads to massive graft failure in early onset cases. PVT occurs in 5%-10% of recipients<sup>[4,5,80,82]</sup> and may lead to progressive portal hypertensive complications. It is more frequent in children transplanted for biliary atresia, because of pre-existing portal vein hypoplasia, which requires a complex surgical anastomosis<sup>[5]</sup>.

Avoidance of vascular complications is critical in liver transplantation, especially with today's paucity of liver donors. Etiology of this apparent prothrombotic state after PLTx has been explored in the last year. Lisman *et al.*<sup>[30]</sup> showed that the hemostatic profile of the pediatric group receiving a left split from an adult donor liver was remarkably different from the adult group receiving the right split for several months after liver transplantation. In a recent paper, Chen *et al.*<sup>[81]</sup> monitored the biochemical markers of coagulation impairment (PT, APTT, thrombin time, fibrinogen and platelet count) for the first 7 d after surgery in 20 children undergoing liver transplant from a living donor. Post-operative tests did not detect a deficit in coagulation function. Mimuro *et al.*<sup>[83]</sup> studied the

coagulation and fibrinolysis system in 63 pediatric patients following liver transplantation by measuring PAI-1, TM, ADAMTS-13, soluble E-selectin, protein C and plasminogen activity, fibrin and PT. Blood samples were obtained from day 0 to day 28 after liver transplantation in order to develop a complete postoperative profile. Results showed a rapid recovery of coagulation activity from day 1 with a full recovery of the coagulation and fibrinolysis system to normal levels by days 21-28. Soluble fibrin levels, a marker of the thrombogenic state, increased significantly on day 1 and then gradually decreased, normalizing by day 14. These data indicate that the prothrombotic state may continue for a timeframe of up to 14 d after liver transplantation, and that appropriate antithrombotic therapy may therefore be required during this period. Therapy to counteract these imbalances has been tested. Hardikar *et al.*<sup>[80]</sup> treated 41 children after PLTx with a standardized hemostatic replacement therapy protocol to reduce hemorrhagic and thrombotic complications. Plasmatic antithrombin levels were measured daily, and replacement therapy was given to maintain levels between 70% and 100%. Fresh frozen plasma (FFP; 15 mL/kg) was given daily to supplement native protein C and protein S levels and ceased once native protein C levels were maintained/stabilized. Intravenous unfractionated heparin was started 24 h postoperatively at 10 UI/kg per hour, and monitored to maintain Anti-factor Xa levels between 0.1 and 0.3 UI/mL. As a result, both thrombotic and bleeding complications were reduced. While the use of FFP in the period after PLTx is questionable because of impact on graft survival<sup>[18]</sup>, this study raises the importance of the development of clinical guidelines addressing perioperative management of coagulation for this particular pediatric group.

In summary, analysis of the key data available in the literature indicates that the post-operative period after PLTx is not characterized by significant bleeding disorders, but rather by prothrombotic activity which can last for up to at least 14 d following surgery.

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## **POINT-OF-CARE COAGULATION MONITORING DURING PEDIATRIC LIVER TRANSPLANTATION**

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POC devices for coagulation have been developed in order to provide the clinician with immediate and reliable results near the patients without the need for a central laboratory. Long turnaround times of standard coagulation tests from a central laboratory (about 45-60 min) stimulated POC device development. The main goal is to avoid blind transfusion of blood product in massively bleeding patients. Blind massive transfusion is still advocated in resuscitation algorithms<sup>[84]</sup> when POC devices are not available. A determined amount of blood products is often

used in these cases, maintaining a defined ratio between platelets, red blood cells and plasma<sup>[85,86]</sup>. The second goal is to spare blood products in order to reduce costs and to avoid inappropriate use of them. Growing evidence indicates that transfusion *per se* is an independent risk factor for mortality, respiratory failure, sepsis and graft survival<sup>[16,18,87,88]</sup>. Moreover, every blood product may have a detrimental effect on morbidity and mortality, at least in liver transplant patients<sup>[16]</sup>.

Three families of POC devices developed for monitoring coagulation are commercially available. The first family is made of POC, such as the CoaguChek XS (Roche Diagnostics, West Sussex, United Kingdom) and i-STAT (Abbott Point of Care, Abbott Park, IL, United States), that gives as an output the PT/INR ratio. Although fast and reliable<sup>[89]</sup>, they are useless in the setting of cirrhotic patients as the standard coagulation tests are sufficient. PT and APTT do not predict bleeding or thrombotic events and are not useful in determining the type of blood product to be transfused. This reflects the fact that these tests are not sensitive to the reduction of antithrombotic factors<sup>[21,22]</sup> and give no insight into the process of the generation of thrombin<sup>[65]</sup>, interaction between platelets and fibrinogen or fibrinolysis. The role of these POC devices in the treatment of the cirrhotic patient is thus limited to risk stratification for post-transplant morbidity and mortality<sup>[90]</sup>.

The second family of devices is made up of a large group of POCs that have been developed to study the functional activity of the platelets. Platelets lead the process of clot formation as they initiate the plug process adhering to the vascular injuries, activate the aggregation process and are eventually responsible for the propagation phase. The number and function of platelets is decreased in cirrhotic patients. However, POCs for coagulation function have been developed to assess platelet function impairment mostly due to anti-aggregation therapies, and they are therefore mainly useful in two therapeutic scenarios<sup>[91]</sup>: stable patients to achieve an anti-aggregation profile in order to reduce their atherothrombotic risk; and bleeding patients to rule out a possible pharmacologic impairment of platelet function<sup>[92]</sup>.

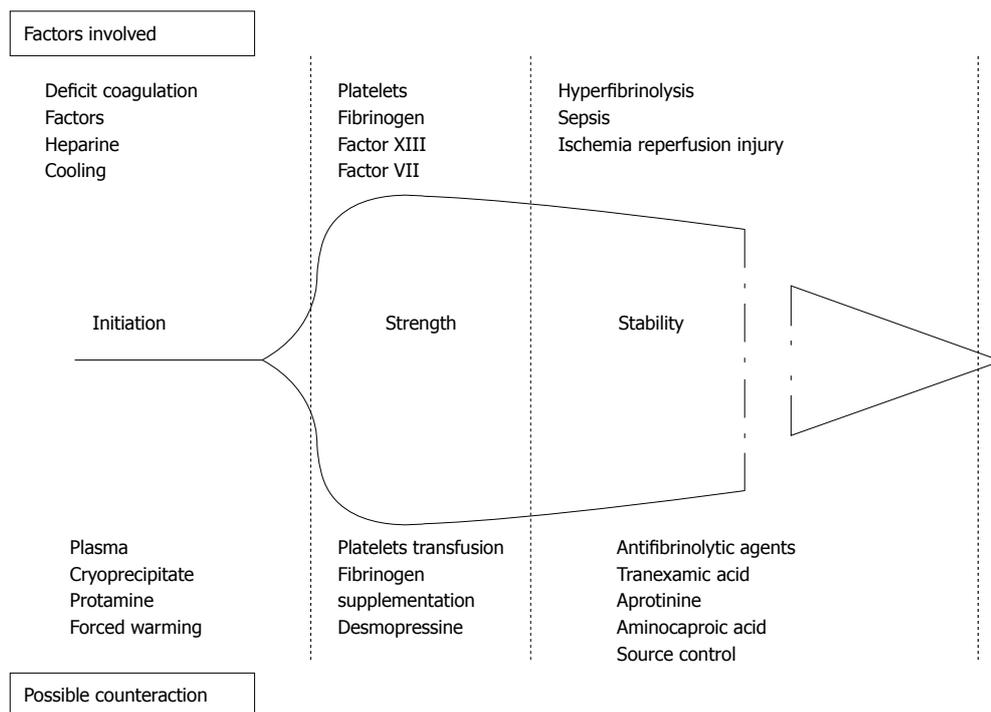
Among the POCs marketed to evaluate platelet function, only three have been implemented in the pediatric population. The Verify Now Rapid platelet Function analyzer (Accumetrics, San Diego, CA, United States) is a POC that measures the degree of aggregation of platelets in whole blood over fibrinogen-coated polystyrene microparticles in the presence of a platelet agonist. The agonists specifically test for inhibition due to the aspirin-like effect (agents active over the arachidonic acid pathway), P2Y<sub>12</sub> inhibitors and GP II b/III a inhibitors. The main indications for the use of this device are to evaluate the efficacy of anti-aggregation therapies<sup>[93]</sup> and to guide the intervention in order to counteract the platelet inhibitors in the case

of bleeding. Two POCs measure platelet function under high shear stress conditions in whole blood. These tests have the advantage of assessing the ability of the platelet to initiate the process of primary hemostasis in whole blood. The high shear stress condition closely simulates that of flow in blood vessels. The first, the Platelet Function Analyzer-100 (PFA-100; Siemens Healthcare Diagnostics, Deerfield, IL, United States) measures the time a plug takes to occlude the aperture of a capillary. Platelet aggregation is enhanced by the capillary membrane, which is coated with either collagen/epinephrine or collagen/ADP, and by the high shear stress condition. PFA-100 has been investigated in the pediatric population, and reference values from healthy subjects are available<sup>[94]</sup>. This test is sensitive to a multitude of different factors: platelet number, hematocrit, drug and dietary effects, platelet receptor defects, vWF deficit, release and granule defect, and anti-aggregation therapies<sup>[95]</sup>. However, this global test of hemostasis is not able to discriminate between different types of primary defects in hemostasis<sup>[95]</sup>, and its main indication is in monitoring aspirin and desmopressin therapy<sup>[96]</sup>. Although tested in the pediatric population, mainly in cardiac patients, neither PFA-100 nor Verify Now was entered into the guidelines of antithrombotic therapy for pediatric patients in 2012<sup>[97]</sup>.

The Cone and Platelet Analyzer (CPA) is the second device that analyzes primary hemostasis in whole blood under high shear conditions<sup>[98]</sup>. It has been used to test platelet function in newborns<sup>[99]</sup> and in the cardiac pediatric patients<sup>[100]</sup>, but the available data for pediatric patients remain limited. These POCs in fact have little utility in the setting of a transplant or when the patient is bleeding as they are designed largely to evaluate platelet function which is mainly a characteristic of cirrhotic coagulopathy. Their clinical utility for pediatric as well as adult patients can be related to their ability to monitor anti-aggregation therapy in those patients needing long term therapy following a thrombotic event. Their utility in the setting of PLTx is potentially more appropriate for the postoperative period in order to reduce the incidence of HAT/PVT while maintaining a targeted anti-aggregation profile.

Devices that measure the viscoelastic properties of a clot in whole blood make up the third group of POCs. Described for the first time in the 1940s by Hartert<sup>[101]</sup>, these devices assess clot formation and dissolution kinetics. They measure the force transmitted to a pin that is immersed in the blood by the rotation of the cup in the case of the thromboelastography (TEG; Haemonetics Corporation, Braintree, MA, United States) or by the rotation of the pin itself in the case of the rotational thromboelastography (ROTEM; Tem International GmbH, Munich, Germany).

These tests have been extensively studied as monitor devices to guide transfusion during surgery and in particular, in trauma, cardiac or liver transplant



**Figure 1 Standard thromboelastographic trace and liver transplant.** A thromboelastographic trace superimposed with the surgical phases, affected factors, and counteractive measures.

patients. The standard tests have been modified with the inclusion of a particular activator or inhibitor to detect specific drug effects on coagulation, as in the case of the platelet mapping panel for TEG, or to define a specific factor effect such as FIBTEM for functional fibrinogen levels. These tests were entered into the guidelines of the European Society of Anaesthesiology<sup>[102]</sup> for application with massively bleeding patients in the perioperative period and into the Task Force for Advanced Bleeding Care in Trauma in 2007<sup>[84,103]</sup>. TEG and ROTEM generate basically the same information on clot kinetics through a slightly different technique. However, the two outputs are not directly interchangeable. TEG and ROTEM are particularly appealing because of their ability to obtain information at each stage of clot formation and dissolution. The tests collect information far beyond the initiation of clot information, which is typically evaluated in standard coagulation testing with PT and APTT. TEG and ROTEM provide information about the strength of the clot and fibrinolysis. These two data seem to be of the utmost importance because a reduction in clot strength is an ominous sign in a bleeding patient, and it is strongly and directly correlated with mortality<sup>[104,105]</sup>.

Hyperfibrinolysis is a well-known complication in the setting of liver failure. There is strong evidence that by counteracting it with antifibrinolytic drugs, such as aprotinine or tranexamic acid, at least blood product transfusions can be reduced<sup>[106]</sup>. Moreover, a reduction in mortality was observed in massive bleeding trauma treated with tranexamic acid in the CRASH-2 trial<sup>[107]</sup>. These results can potentially be applied to cirrhotic

patients with massive bleeding, where consumption coagulopathy is viewed as a common feature and in PLTx patients experiencing HAT because a hypercoagulative state can be detected<sup>[82]</sup>. In Figure 1, a standard thromboelastographic trace is shown, along with the factors that can alter the trace and the possible action used to counteract the disorder.

TEG/ROTEM tests and their clinical application have been extensively discussed in several reviews in recent years<sup>[108]</sup>. Enthusiasm for these techniques must be balanced against weak evidence for their utility and the need for better studies to sustain their wide spread use in clinical practice. To date, TEG/ROTEM tests have been included in three reviews from the Cochrane Collaboration. The first review demonstrated that a thromboelastographic guided algorithm reduced the amount of blood product transfused but had little, if any, impact on mortality and morbidity<sup>[109]</sup>. The second review failed to detect a reduction in blood product transfusion with a TEG guided transfusion algorithm, but the number of studies was limited and of poor quality<sup>[106]</sup>. In the most recent study, thromboelastographic technique failed to identify trauma induced coagulopathy. However, the analysis was performed on the data from a small number of poor quality trials so that the authors suggested that a greater research effort be put forth in this field<sup>[110]</sup>.

TEG/ROTEM tests share some drawbacks that have slowed their entry into routine clinical practice. The first is that these test are perceived as awkward to use by doctors. Trained personnel are needed, as well as daily calibration and a standardized technique.

**Table 4** Reference parameters for Thromboelastogram in healthy and pathologic pediatric populations

Ref.	Type of patient	Age	n	R, min	k, min	Alpha, 0	MA, mm	Lys30, %	Note
Rajwal <i>et al</i> <sup>[113]</sup>	Healthy	1-15 yr	14	17.4 ± 3.6	7.6 ± 1.4	31.2 ± 4.8	52.6 ± 6.4	0.8 ± 1.0	Native blood
Pivalizza <i>et al</i> <sup>[114]</sup>	Healthy	< 13 mo	25	10.1 ± 3.1	2.4 ± 0.5	74.2 ± 3.5	70.2 ± 6.1	3.4 ± 2.9	Recalcified blood Celite as activator
		13-24 mo	33	9.8 ± 3.3	2.5 ± 0.6	73.2 ± 3.1	70.2 ± 4.7	2.1 ± 1.3	
		25-48 mo	24	10.5 ± 3.0	2.7 ± 0.7	71.2 ± 4.2	68.4 ± 5.2	2.1 ± 1.4	
		49 mo-9 yr	29	9.6 ± 2.5	2.8 ± 0.6	72.9 ± 4.2	70.5 ± 3.3	2.0 ± 1.1	
Edwards <i>et al</i> <sup>[112]</sup>	Healthy	Newborn	59	5.3 ± 1.3	1.6 ± 0.4	67.2 ± 4.5	61.8 ± 4.6	0.7 ± 0.7	Cord blood, recalcified blood
Chan <i>et al</i> <sup>[29]</sup>	Healthy	8.7 ± 2.6 yr	44	8.7 ± 2.6	2.1 ± 0.7	61.6 ± 7.1	59.6 ± 4.2	0.2 ± 1.3	Recalcified blood
		< 1 yr	24	7.7 (4.5-11.6)	1.8 (1.2-2.3)	66.5 (58.8-73.4)	67.2 (60.7-73.2)	3.8 (0.3-8.4)	Mean and
		1-5 yr	24	8.3 (5.7-10.9)	2.0 (1.4-3.3)	63.6 (53.8-70.3)	65.2 (57.6-71.3)	3.0 (0.2-7.8)	2.5%-97.5%
		6-10 yr	26	7.8 (5.3-11.0)	2.0 (1.4-2.8)	63.9 (54.3-70.7)	65.0 (57.3-72.8)	3.3 (0.2-6.2)	
Brenn <i>et al</i> <sup>[115]</sup>	Cerebral palsy	15 ± 3 yr	15	4.8 ± 1.0	1.6 ± 0.6	68 ± 8	65 ± 8		Patients undergoing spinal fusion surgery
	Idiopathic scoliosis	14 ± 1.5 yr	15	5.0 ± 0.6	1.3 ± 0.4	71 ± 5	70 ± 4		
Kang <i>et al</i> <sup>[55]</sup>	Cirrhotic undergoing liver transplant	9 mo-7 yr	8	9.6 ± 6.2			46.9 ± 11.5		Baseline
				7.4 ± 2.8			47.3 ± 7.9		Anhepatic phase
				10.1 ± 2.4			46.1 ± 9.7		30' after reperfusion
				9.2 ± 3.2			49.9 ± 8.8		90' after reperfusion

R: Reaction time for the time from placement of blood in the cup until the clot formation; k: Coagulation time, the time between TEG trace elevation from 2 to 20 mm; Alpha: Alpha angle, the slope of the TEG trace describing the kinetics of clot formation; MA: Maximum amplitude as an indicator of clot stability and firmness; Lys30: Clot lysis expressed as an amplitude reduction at 30 min after MA. TEG: Thromboelastogram.

This last issue is of utmost importance because without standardized technique, it is difficult to directly compare data across studies. For example, reference values vary between cohorts of patients with differences in age<sup>[29,111]</sup>, pathology, type of activator used (kaolin vs tissue factor), and the use of native or citrated whole blood, or plasma. Moreover, pre-analytical factors, such as the type of phlebotomy, the mean time before starting the assay, the methods in which the different factors are added to the blood sample, or even the way they are mixed, may profoundly influence the results of the test. The need for standardization is illustrated by the fact that in the last few years, three societies were founded with this focus: the TEG-ROTEM Working Group, the Working Party for the Standardization of Thromboelastography, and a subcommittee of the International Society on Thrombosis and Hemostasis.

Experience with these two methodologies on pediatric patients is increasing, but they were applied to pathologic patients long before reference values were defined for different age ranges. One of the first reports of thromboelastographic parameters in children were from patients undergoing liver transplantation in 1989<sup>[55]</sup> while the first report on reference values from healthy individuals was in 2007<sup>[29]</sup>. One of the most significant findings was with neonates. These tests revealed a nearly normal pattern of clotting kinetics despite profound alterations detected in standard coagulation assays<sup>[112]</sup>. This result seems to reinforce the concept of restored coagulation in neonates, where concomitant reduction of pro- and anticoagulants has occurred.

Restored balance in neonates was confirmed *in vitro* with the ETP test, which includes thrombomodulin<sup>[35]</sup>, but not by conventional coagulation tests, such as PT or APTT<sup>[27,36]</sup>.

In Tables 4 and 5, the thromboelastometric parameters published for the different pediatric populations considering the underlying pathology/disease and the technical characteristics of the exam are shown.

## MEASURES TO REDUCE BLEEDING AND TRANSFUSION

Strong data indicate that the use of blood products during liver transplantation in adults is associated with morbidity and mortality<sup>[14-17,87]</sup>. Our group found similar results with PLTx exploring both intraoperative and the post-transplant phase<sup>[18]</sup>. In our study, perioperative transfusion of FFP and RBCs was found to be an independent risk factor for predicting one-year patient and graft survival. The effect on one-year survival was dose-related. All of these studies were retrospective, and the main criticism is that the transfusion requirements may be considered as a surrogate marker for sicker patients. Prospective randomized trials analyzing different transfusion strategies are needed, but they are very difficult to design as levels of hemoglobin and coagulation factors are highly variable during surgery. The mechanism linking transfusion and poor outcomes after liver transplantation is unknown. Transfusion related immunomodulation, transfusion

**Table 5** Reference parameters for rotational thromboelastogram in healthy and pathologic pediatric populations

Ref.	Type of patient	Age	n	CT, s	CFT, s	MCF, mm	CLI60, %	Note		
Strauss <i>et al</i> <sup>[28]</sup>	Pre-term	Newborn	47	185 (108-357)	80 (52-183)	57 (42-66)		Median (MIN-MAX)		
	Full term		184	194 (98-588)	76 (34-208)	60 (39-71)				
Oswald <i>et al</i> <sup>[111]</sup>	Healthy	0-3 mo	51	184 (105-285)	44 (27-88)	66 (54-73)		InTEM median (2.5%-97.5%)		
		4-12 mo	55	172 (76-239)	60 (37-100)	63 (52-73)				
		13-24 mo	54	161 (99-207)	61 (42-112)	64 (50-72)				
		2-5 yr	70	170 (99-239)	60 (40-94)	63 (53-73)				
		6-10 yr	79	168 (97-212)	64 (48-93)	62 (53-69)				
		11-16 yr	50	171 (128-206)	68 (45-106)	62 (54-71)				
		0-3 mo	51	48 (38-65)	57 (30-105)	62 (54-74)	87 (71-94)		ExTEM median (2.5%-97.5%)	
		4-12 mo	55	53 (37-77)	72 (44-146)	60 (46-71)	86 (71-95)			
		13-24 mo	54	55 (37-73)	75 (46-139)	60 (46-72)	88 (77-94)			
		2-5 yr	70	56 (46-97)	72 (41-109)	61 (52-70)	86 (74-93)			
Oasthaus <i>et al</i> <sup>[116]</sup>	Normal	211 ± 116 d	17	177 ± 28	60 ± 21	64 ± 6		InTEM (mean ± SD)		
		Acyanotic	134 ± 61 d	17	178 ± 41	70 ± 16	61 ± 4			
		Cyanotic	135 ± 132 d	17	194 ± 43	105 ± 68	56 ± 6			
		Normal	211 ± 116 d	17	51 ± 6	71 ± 25	62 ± 6		94 ± 2	ExTEM (mean ± SD)
		Acyanotic	134 ± 61 d	17	50 ± 5	88 ± 22	59 ± 6		93 ± 2	
Haizinger <i>et al</i> <sup>[117]</sup>	Cyanotic	135 ± 132 d	17	68 ± 40	141 ± 99	54 ± 9	91 ± 4	InTEM (mean ± SD)		
		ASA I	0-1 mo	6	179 ± 17	56 ± 23	68 ± 7		91 ± 2	
		ASA III-IV cardiac		17	332 ± 207	127 ± 184	62 ± 10		93 ± 3	
		ASA I	1-3 mo	6	166 ± 25	45 ± 9	69 ± 3		89 ± 2	
		ASA III-IV cardiac		6	257 ± 95	78 ± 47	61 ± 6		91 ± 2	
		ASA I	3-6 mo	6	183 ± 22	49 ± 22	67 ± 7		90 ± 3	
		ASA III-IV cardiac		6	187 ± 29	53 ± 11	69 ± 4		94 ± 3	
		ASA I	6-12 mo	6	172 ± 11	60 ± 17	63 ± 8		89 ± 2	
		ASA III-IV cardiac		6	196 ± 55	61 ± 11	66 ± 3		92 ± 4	
		ASA I	0-1 mo	6	35 ± 12	65 ± 31	65 ± 9		91 ± 2	ExTEM (mean ± SD)
		ASA III-IV cardiac		17	55 ± 62	119 ± 119	54 ± 10		92 ± 4	
		ASA I	1-3 mo	6	35 ± 9	65 ± 12	64 ± 2		90 ± 4	
		ASA III-IV cardiac		6	35 ± 7	98 ± 43	54 ± 7		91 ± 2	
		ASA I	3-6 mo	6	33 ± 9	75 ± 33	65 ± 9		89 ± 3	
		ASA III-IV cardiac		6	34 ± 15	79 ± 29	63 ± 5		94 ± 4	
	ASA I	6-12 mo	6	45 ± 19	96 ± 39	59 ± 9	89 ± 2			
		ASA III-IV cardiac		6	36 ± 11	85 ± 17	58 ± 5		92 ± 3	

CT: Clotting time, "r" time in TEG; CFT: Clot formation time, "k" time in TEG; MCF: Maximum clot firmness, MA in TEG; CLI60: Final clot lysis index at 60 min = LY A60 in TEG; INTEM and EXTEM are two different activated baseline analyses. ROTEM: Rotational thromboelastogram; TEG: Thromboelastogram.

associated circulatory overload, transfusion associated acute lung injury, hemolytic transfusion reactions, acute non-hemolytic transfusion reactions, transfusion-associated graft versus host disease and transfusion transmitted infection have all been proposed as possible mechanisms<sup>[118-121]</sup>.

A significant decrease in blood loss and blood product requirement has been observed during liver transplantation over the past 10 years, even if a wide range of blood product transfusion rates still exist between organ transplantation centers<sup>[86]</sup>. This decrease can be explained by improvements in surgical and anesthetic techniques, and by a better understanding of the rebalanced hemostatic system in cirrhotic adult patients<sup>[21,22]</sup>. Pediatric studies in this field are often observational studies or case reports. Based on data from adult patients, the strategies to reduce bleeding and transfusion should be analyzed in three different phases: pre-operative, intraoperative, and postoperative (Table 6).

## PRE-OPERATIVE MANAGEMENT

In some pediatric and adult series, the value of pre-operative hemoglobin was found to be one of the factors most strongly correlated to the need for transfusion during surgery<sup>[122,123]</sup>. It has been shown that platelet activation, as well as RBCs, have an active role in the generation of thrombin<sup>[21]</sup>. It therefore seems reasonable to treat children with erythropoietin, supplemental iron and folic acid when feasible, in order to achieve higher hemoglobin levels before surgery<sup>[124,125]</sup>. Although portal hypertension and hypersplenism can slow the rise of the pre-operative mass of red cells, bone marrow normally responds to stimulation with erythropoietin. The time available before transplantation appears to be the determining factor since this type of therapy requires several weeks to be effective. In adults, the transjugular intrahepatic portal-systemic shunt (TIPS) may be considered to optimize the pre-operative state of the patient. TIPS

**Table 6 Measures to reduce bleeding complications and transfusions during liver transplantation**

Surgical phase	Procedure
Pre-operative	Erythropoietin Supplemental iron and folic acid
Intraoperative	Low CVP (fluid restriction, phlebotomy, vasopressors, Pringle maneuver) Acute intraoperative hemodilution Low transfusional trigger Drugs (rFVIIa, antifibrinolytics) Blood salvage Surgical technique TEG/ROTEM
Postoperative	Low transfusional trigger Minimize blood sampling Erythropoietin Supplemental iron and folic acid

CVP: Central venous pressure; rFVIIa: Activated recombinant factor VII; TEG: Thromboelastogram; ROTEM: Rotational thromboelastogram.

is mainly used as therapy for upper gastrointestinal bleeding but also less frequently as a treatment to reduce portal hypertension in patients with pronounced varicose veins<sup>[126]</sup>. Some authors have speculated that this second effect could reduce bleeding during surgery, especially during hepatectomy<sup>[84]</sup>. A recent study indicated that TIPS is a feasible and effective technique also in children with ascites or gastrointestinal bleeding who are unresponsive to medical and endoscopic treatment<sup>[127]</sup>.

Given the lack of scientific studies of high quality, none of these strategies has been rigorously validated statistically, although they have already entered into clinical medical practice.

## INTRAOPERATIVE MANAGEMENT

### Blood volume management

Different strategies in the management of blood volume can influence the amount of blood loss in the course of liver transplantation, especially during the dissection phase of surgery. Some studies have reported a lower amount of blood loss when strategies to reduce central venous pressure (CVP) are utilized<sup>[122,128-130]</sup>. These strategies are realized mostly through intraoperative fluid restriction, phlebotomy, vasodilators and the Pringle maneuver. The rationale is that low CVP should favor the venous return from the liver, thereby reducing the portal venous pressure and blood loss from surgical tranche. In addition, in case of injury to the vena cava, this strategy would facilitate surgical repair maneuvers, although with a possible increased risk of venous air embolism. All of these studies are prospective, nonrandomized, underpowered, and on adults. For these reasons, they cannot be considered conclusive, although almost all reported a reduction of blood loss when a policy of CVP reduction was implemented. The only pediatric

study<sup>[131]</sup> reporting a reduction of blood loss with low CVP management is of limited scientific value because bleeding was not defined as the primary outcome. However, the possible effects of such a strategy cause concern. A low CVP strategy may reduce cardiac output and, ultimately, oxygen delivery to peripheral tissues which could lead to secondary damage, especially to the kidney, and impact mortality, as reported by Schroeder *et al.*<sup>[132]</sup>.

Based on the data available, it is not currently possible to state any clear indications for or against strategies for lowering the CVP during liver dissection, for either pediatric or adult patients.

### Acute intraoperative hemodilution and transfusion trigger

Another strategy for the reduction of perioperative blood transfusions in the course of liver transplant is acute intraoperative hemodilution<sup>[125]</sup>. It is generally performed by the removal of a certain amount of the circulating blood mass of the patient, up to 30%, with immediate reinfusion of the same amount of crystalloid or colloid solutions. The blood collected is then reinfused as needed, based on blood loss or after implantation of the liver. So far, there is no precise information concerning the target hemoglobin or hematocrit to reach or effect on outcome.

Some studies in PLTx and in the craniosynostosis repair field have reported a safe transfusion trigger as up to 8 g/L of hemoglobin and a 25% hematocrit<sup>[123,133]</sup>. In the pediatric critical care area, it has been reported that in a stable child, a transfusion trigger of 7 g/L of hemoglobin appears to be safe<sup>[134]</sup>.

Further research is necessary to define the transfusion trigger based on absolute values of hemoglobin rather than on other pathophysiological parameters<sup>[24]</sup>.

### Pharmacological strategies

Drugs that have been used during liver transplantation surgery to reduce blood losses are essentially activated recombinant factor VII (rFVIIa) and antifibrinolytics.

### Activated recombinant factor VII

rFVIIa acts by directly binding to tissue factor (TF). This complex activates the common pathway of coagulation in turn by activating factor X, which precipitates the conversion of prothrombin to thrombin to form a hemostatic clot. In addition, rFVIIa binds the activated platelets at the site of tissue damage<sup>[135]</sup>. There are two case series which describe the use of rFVIIa within the liver transplant. In the first<sup>[125]</sup>, two cases are described as part of overall strategies for saving blood components in patients who were Jehovah's Witnesses. In this case, the drug, which has a half-life of 2-3 h, was administered prophylactically before skin incision, in order to reduce bleeding during the liver dissection phase. In the second series<sup>[136]</sup>, the rFVIIa was used as a treatment for post-reperfusion

coagulopathy with apparently good results in terms of clinical and laboratory data. However, antifibrinolytic drugs were simultaneously used, so they could be an important source of bias. Systematic reviews<sup>[137,138]</sup> of the use of rFVIIa in the adult population (including liver transplantation) failed to show a benefit in terms of the number of blood transfusions, and it was associated with a significant increase in the incidence of arterial thrombotic events. For this reason, rFVIIa is recommended only as rescue therapy for uncontrolled bleeding<sup>[102]</sup>.

### **Antifibrinolytics**

As discussed in the previous section, hyperfibrinolysis may be enhanced during anhepatic and post-reperfusion phases. There are two families of antifibrinolytics: serine protease inhibitors (aprotinin) and lysine analogs (tranexamic acid and epsilon aminocaproic acid). Aprotinin is a non-specific Kunitz-type protease inhibitor. In addition to plasmin, it also inhibits trypsin, kallikrein, elastase, urokinase and thrombin. Lysine analogs competitively inhibit the activation of plasminogen to plasmin, preventing plasmin from degrading fibrin<sup>[135]</sup>.

Several systematic reviews have shown that all antifibrinolytic drugs safely reduce blood loss and transfusion, in both adult liver transplantations<sup>[106,139,140]</sup> and in major pediatric surgeries<sup>[141-143]</sup>. No randomized controlled trials have been performed in the PLTx field. Aprotinin is no longer available, having been withdrawn from the market due to safety concerns<sup>[144]</sup>. With regard to the use of antifibrinolytic drugs, it is important to understand whether they should be used extensively prophylactically or only in a documented hyperfibrinolysis state, because pediatric patients show an increased prothrombotic state<sup>[4,5,80,82]</sup>.

Other pharmacological methods, such as fibrinogen and prothrombin complex concentrates (PCC), have not been investigated in PLTx. Fibrinogen use in adult patients is recommended by the European Society of Anaesthesiology according to FIBTEM tracing<sup>[102]</sup>. A randomized controlled trial (the PROTON trial) studying the effect of PCC on RBC transfusion requirements in adult liver transplantation is currently in progress<sup>[145]</sup>. Use of protamine at the time of reperfusion to reverse the HLE<sup>[72]</sup> does not seem useful because HLE is usually a temporary phenomenon unless graft function is poor<sup>[75,146]</sup>.

### **Blood salvage**

The use of the cell saver for blood salvage within the surgical field is now considered standard in the management strategies of liver transplantation in patients who are Jehovah's Witnesses<sup>[124]</sup>. With the cell saver technique, shed blood is suctioned from the surgical field, centrifuged, washed, mixed with an additive/anticoagulant solution and then reinfused as required<sup>[147]</sup>.

A few case reports suggest that disseminated intravascular coagulopathy, acute respiratory distress syndrome and renal failure can arise from the re-introduction of fat microemboli, denatured protein, free hemoglobin, cell fragments, and platelet-leukocyte aggregates into the blood stream. More carefully designed studies have failed to show a significant increase in these complications. The washing phase after centrifugation eliminates these products as well as heparin, plasma elastases and soluble cytokines such as TNF-alpha. By now, the safety of this procedure has been well demonstrated in adults<sup>[148,149]</sup>. In the pediatric field, there are some studies which have evaluated the effectiveness of the cell saver, especially in cardiac surgery and surgery for scoliosis<sup>[150,151]</sup>. Traditionally, patients undergoing bowel resection and cancer surgery are not considered as suitable candidates for the use of intraoperative blood scavenging because of the fear of retransfusing bowel flora and exfoliated cancer cells. At present, the discussion is still open as to whether these are contraindications for the use of the cell saver<sup>[102,147]</sup>. The use of these techniques in liver transplantation still remains controversial<sup>[152,153]</sup>. Moreover, these techniques are relatively expensive and require sophisticated equipment and trained personnel<sup>[20]</sup>.

### **Surgical techniques**

There are essentially two surgical techniques used to decrease blood loss: the application of a veno-venous bypass and the piggyback technique<sup>[20,154]</sup>. In the first, the bypass allows decompression of the splanchnic venous congestion allowing less loss and enhancing hemodynamic stability. In the second technique, a direct anastomosis between the retrohepatic vena cava of the donor with the inferior vena cava of the recipient is performed with tangential clamping. There are several variants of this technique, but all enable a less challenging retroperitoneal dissection and avoid a vascular anastomosis between the graft and the recipient cava. A recent Cochrane review<sup>[154]</sup> has shown no significant difference in postoperative mortality and morbidity between the two techniques. The warm ischemic time was significantly shorter in the piggyback method. No scientific studies investigate this issue in pediatrics. One last issue discussed in the literature is the possible difference in bleeding between transplants from living or deceased donors. Two case series compared the intraoperative bleeding trend of liver transplants from living and deceased donors. In the first study of 157 pediatric patients<sup>[155]</sup>, a significant increase in blood transfusions was observed in liver transplants from living donor relative to deceased donors. The second study ( $n = 46$  pediatric cases)<sup>[156]</sup> confirmed the trend although statistical significance was not achieved because of sample size. These data should be further validated because of limitations in

terms of number and homogeneity in the cohorts.

## POSTOPERATIVE MANAGEMENT

The main strategy to decrease transfusion requirements without increasing adverse outcomes in a pediatric intensive care unit is to maintain a hemoglobin threshold of 7 g/dL for red cell transfusion<sup>[134]</sup>. Another feasible strategy to reduce the possibility of transfusion in the postoperative period is to minimize the amount of blood sampling. In addition, the early use of erythropoietin, early supplementation with iron, multivitamins and folic acid have been described in some case series<sup>[124,125]</sup>.

## UP-TO-DATE CLINICAL PRACTICE GUIDELINES

In the previous paragraphs, we briefly went through the possible clinical strategies aimed at reducing the use and need of blood products during PLTx. Those strategies have been extrapolated from different population settings, such as healthy individuals, adult or pediatric cardiac patients, or from very small and biased trials. Even though some evidence exists, standardized protocols have not yet been established, so that local clinical practice currently prevails. However, the European Society of Anaesthesiology<sup>[102]</sup> has published some clinical guidelines for the treatment of perioperative bleeding in patients in 2013.

The recommendations suggested for pediatric surgery patients to reduce blood loss and transfusion requirements are the following: (1) perioperative antifibrinolytic therapy (2A); (2) TEG/ROTEM or timely detection of coagulation defects, including dilutional coagulopathy and hyperfibrinolysis (2C); (3) critical hemoglobin threshold of 8 mg/dL for RBC transfusion (2C); (4) transfusion of PLTs concentrates if PLTs count < 50000/ $\mu$ L (2C); (5) fibrinogen concentrate (30-50 mg/kg) or cryoprecipitate (5 mL/kg) may be used to increase plasma fibrinogen concentrations above trigger values of 1.5-2.0 g/L or FIBTEM MCF > 7 mm (2C); (6) no clear recommendation can be made regarding the indication for FFP transfusion, PCC, or FXIII; and (7) recommendation against the use of rFVIIa (1C) and routine use of desmopressin in the absence of hemophilia (2C).

Other recommendations from adult liver transplant patients that may be translated into the pediatric cirrhotic field but are waiting for pediatric derived evidence are the following: (1) use of a viscoelastic test to evaluate the coagulation profile and recommendation against the use of standard laboratory tests, such as PT/APTT for cirrhotic patients (C); (2) fluid restriction, phlebotomy, vasopressors, transfusion protocols (C) and low central venous pressure (B) may be associated with low transfusion rates during liver transplantation; (3)

antifibrinolytic drugs for treatment of fibrinolysis (evident from microvascular oozing or TEG/ROTEM clot lysis measurements) but not for routine prophylaxis (1C); and (4) recommendation against the routine use of rFVIIa (1A) and against the use of FFP for preprocedural correction of mild to moderately elevated INR (1C).

## SUMMARY AND FUTURE DIRECTIONS

The first international conference on Coagulopathy of Liver Disease was held in 1995 in Charlottesville, VA, United States. This event stimulated the creation of a stable multidisciplinary group with a focus on coagulation disorders in liver disease where the two fields of hematology and hepatology converge<sup>[22]</sup>. Unfortunately, new knowledge in this area cannot be simply applied to pediatric patients as emphasized by Lisman *et al.*<sup>[30]</sup>. Few pediatric data have been published, and clinical algorithms used in adult practice must be specifically tested in pediatric settings before they can be applied to neonates and children for many reasons. First of all, hemostasis is still in development in children, and they suffer from different liver disease pathologies than adults. Thus, the age of onset and etiology of the end-stage liver disease may influence the hemostatic system in unpredictable ways. For example, hemostasis in healthy neonates is already considered to be rebalanced<sup>[35,118]</sup>. Second, laboratory tests for the evaluation of coagulation require standardization, including maintenance of sample integrity, equipment/analyzers, reagents and age dependent reference levels, in order to avoid erroneous or conflicting results<sup>[27]</sup>. Third, the impact of the use of the blood products on morbidity and mortality of children with end-stage liver disease must be further investigated both before and after liver transplantation. Lastly, more randomized controlled trials analyzing the medical strategies to reduce bleeding and thrombotic complications should be performed specifically in PLTx patients. In conclusion, a multidisciplinary effort involving hematologists, hepatologists, pediatricians, surgeons, and anesthesiologists is needed to fill the gap.

## RESEARCH AGENDA

The end-stage liver disease responsible for the observed age and disease related changes in coagulation proteins must be elucidated because it may have important biological ramifications. The concept of rebalanced hemostasis should be evaluated in cirrhotic pediatric patients.

New laboratory assays to evaluate the coagulation system need to be validated in children, in light of our understanding of developmental hemostasis.

The development of pediatric specific algorithms to guide clinical management of bleeding disorders and transfusion therapy during liver transplantation requires specific clinical outcome studies in children.

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