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References:
1- Halloran, et al. Manuscript in preparation, 2021
2- Akalin, et al. Clinical Validation of an Immune Quiescence Gene Expression Signature in Kidney Transplantation. Kidney360 September 2021, 10.34067/KID.0005062021; DOI: https://doi.org/10.34067/KID.0005062021
3- Park S, et al. Combining Blood Gene Expression and Cell Free DNA to Diagnose Subclinical Rejection in Kidney Transplant Recipients. Clin J Am Soc Nephrol. 2021 Oct;16(10):1539-1551. doi: 10.2215/CJN.05530421. PMID: 34620649; PMCID: PMC8499014.

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Impact of Preemptive Fibrinogen Concentrate on Transfusion Requirements in Liver Transplantation: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial

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Trial Registration: The trial was registered in the European Clinical Trials Database (EudraCT, number 2010-024584) and at ClinicalTrials.gov (number NCT01539057).

We hypothesized that preemptive fibrinogen administration to obtain an initial plasma level of 2.9 g/L would reduce transfusion requirements in liver transplantation. A randomized, multicenter, hemoglobin-stratified, double-blind, fibrinogen-versus-saline–controlled trial was conducted. The primary end point was the percentage of patients requiring red blood cells. We evaluated 51 patients allocated to fibrinogen and 48 allocated to saline; the primary end point was assessed using data for 92 patients because the electronic record forms were offline for three patients in the fibrinogen group and four in the saline group. We injected a median of 3.54 g fibrinogen preemptively in the fibrinogen group. Nine patients in the saline group (20.9%) required fibrinogen at graft reperfusion (compared with one patient [2.1%] in the fibrinogen group; p = 0.005). Blood was transfused to 52.9% (95% confidence interval [CI] 42.5–63.3%) in the fibrinogen group and 42.74% (95% CI 28.3–57.2%) in the saline group (p = 0.217). Relative risk for blood transfusion was 0.80 (95% CI 0.57–1.13). Thrombotic events occurred in one patient (2.1%) and five patients (11.4%) in the fibrinogen and saline groups, respectively. Seven patients (14.6%) in the fibrinogen group and nine (20.3%) in the saline group required reoperation. Preemptive administration of fibrinogen concentrate did not influence transfusion requirements.

Abbreviations: CRF, case record form; CI, confidence interval; EXTEM, assay for tissue factor activation; FFP, fresh frozen plasma; FIBTEM, assay for tissue factor activation and platelet inhibition; Hb, hemoglobin; Htc, hematocrit; ICU, intensive care unit; INR, international normalized ratio; IRB, institutional review board; LOS, length of stay; LT, liver transplantation; MA10, maximum amplitude at 10 min; MCF, maximum clot firmness; MELD, Model for End-Stage Liver Disease; NA, not applicable; pO_2, partial pressure of oxygen; PTT, partial thromboplastin time; RBC, red blood cell

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Introduction

Packed red blood cells (RBCs) are transfused in 20–86% of patients in liver transplantation (LT) (1). A Cochrane review on methods for attenuating blood loss and decreasing transfusion requirements in LT concluded that antifibrinolytic therapy and use of viscoelastic point-of-care testing may potentially reduce blood loss and transfusion requirements, although they pointed out the need for well-designed randomized trials (2). The reviewers, however, did not explore the role of fibrinogen. Hemostatic and coagulation disorders related to severe liver disease are known to cause massive bleeding beyond what can be expected from surgical trauma in LT. Fluid resuscitation after surgical bleeding may aggravate the problem because the concentrations of fibrinogen and major antifibrinolytic proteins decrease in proportion to hemodilution (3). In one LT series with low preoperative plasma fibrinogen (≤2 g/L), the transfusion of RBCs was significantly higher than in the cohort with fibrinogen values >2 g/L (4).

Although the altered hemostasis of end-stage liver disease is multifactorial, given that preoperative hemoglobin

plays a major role, we hypothesized that preemptive administration of concentrated fibrinogen in patients with a low preoperative plasma fibrinogen level would reduce requirements for blood product transfusion, assuming that other risk factors for transfusion were similar. We aimed to test this hypothesis in a randomized controlled trial.

Materials and Methods

Study design

This randomized, multicenter, hemoglobin-stratified, double-blind, placebo-controlled trial was conducted in five teaching hospitals in Spain after approval was obtained from the institutional review board (IRB) of the lead hospital (Hospital Universitari de Bellvitge, IRB approval number AC 123/10 and protocol number 1553-H-459), the other participating centers and the Spanish Ministry of Health and Science. The trial was registered in the European Clinical Trials Database (EudraCT, 2010-024584) and at ClinicalTrials.gov (NCT01539057).

Patients

Eligible participants were all adults aged 18–80 years who were scheduled for LT. Exclusion criteria were a history of allergic reaction to fibrinogen concentrate, known history of thromboembolic events in the last 30 days, known or suspected pregnancy, previous randomization in this trial, known presence of congenital bleeding disorder, and aspirin or warfarin therapy. Also excluded were the following indications for transplantation: familial polyneuropathy and living donors, because of variability in surgical technique; acute liver failure, biliary cirrhosis and sclerosing cholangitis, because of high rates of hypercoagulation; and non–heart-beating donors, because of higher blood requirements in comparison with heart-beating donors. Patients with a plasma fibrinogen concentration >2.9 g/L in the 24 h prior to LT were also excluded. We made this decision based on our previous study of a low-fibrinogen LT series (≤2 g/L) in which 87% required intraoperative blood products (4). The critical level of plasma fibrinogen (1 g/L) was reached after graft reperfusion in 39% of cases in that study, and the mean decrease of plasma fibrinogen at reperfusion was 0.9 g/L. Consequently, we hypothesized that preemptive administration of concentrated fibrinogen would be of value only in patients with a preoperative plasma fibrinogen level of ≤2.9 g/L.

The trial was explained to all patients, who were also given written information. Patients were enrolled if they gave their written informed consent. Recruitment took place at two hospitals in Barcelona (Hospital Universitari de Bellvitge and Hospital Clinic Universitari) and three in other parts of Spain (Hospital Universitario de Cruces in Bilbao; Hospital Universitario Virgen del Rocio in Seville; and Hospital Universitario Virgen de la Arrixaca in Murcia).

Anesthesia and surgical management

The protocols were monitored to ensure consistency and compliance across all research centers.

All patients were placed on a convective air blanket (WarmTouch; Mallincrod Medical, St. Louis, MO). Oxygen was given for 5 min before standard anesthesia management was started. Arterial and central venous cannulas were placed in all patients. Crystalloid fluid replacement (7 mL/kg per hour) was used to maintain blood volume, and colloids were used to improve hemodynamics at the discretion of the anesthesiologist. Sodium bicarbonate 1/6 M was given to maintain pH 7.3. Intravenous calcium was administered to keep the plasma calcium ion concentration within the ranges of reference stipulated by each hospital's laboratory. Normothermia was maintained. Vena cava preservation was attempted in all patients. If preservation was not feasible, venovenous bypass or a complete caval clamp was used and registered in the patient's electronic case record form (CRF). The liver allograft was preserved in University of Wisconsin solution. Prior to reperfusion of the graft, it was flushed with 1000 mL Hartmann's solution at 38°C to remove air and detritus from the wall of the graft's inferior vena cava. Next, the distal end of the donor's vena cava was closed with a vascular stapler. Vasoconstrictor drugs were administered to compensate for reperfusion syndrome. At the end of surgery, all patients remained mechanically ventilated and were transferred to a surgical intensive care unit.

Intraoperative and postoperative transfusion management

The protocols for blood transfusion were monitored to ensure consistency and compliance across all the research centers, according to the following transfusion criteria: (i) packed RBCs to maintain hemoglobin >80 g/L, (ii) platelet concentrates if a blood platelet count fell to <50 000/mm^3, (iii) fresh frozen plasma transfusion (2 U/30 min) only in cases in which there was continuous bleeding uncorrected by the aforementioned measures, and (iv) intravenous tranexamic acid boluses of 500 mg if fibrinolysis (>15% lysis at 60 min) was detected on thromboelastometry. Cell saver devices were not used in any case. Hemostatic surgical management followed standard protocol.

Randomization and masking

All trial data were anonymously collected and stored in the electronic CRF for each patient; each was assigned a unique study number and a unique randomization number. The randomization sequence was created using a computer-generated random list, which was then stratified according to whether the baseline hemoglobin concentration was <95 or ≥95 g/L and by center (1:1 ratio, in blocks of multiples of 2 U). Patient allocation took place just prior to surgery. Personnel involved with patient management or study design were uninvolved with randomization. No center could enroll >40% of the patients.

The drug and saline solutions were previously prepared by an authorized pharmacy and distributed to the central pharmacy department of each hospital in consecutively numbered, sealed boxes (kits) for distribution, according to the random number sequence supplied by the independent clinical research organization that also created the electronic CRF. The kits were then dispensed to the nurses for storage in an agreed-on location in the operating room. The sequence was concealed from the researchers, the caregivers and the statistician who analyzed the results.

Once a patient's plasma fibrinogen level was known, a kit containing the intervention drug (fibrinogen) or saline was assigned. An independent nurse who was not otherwise involved in patient management opened the box with the assigned solution and loaded it into a special injection set prepared to allow physicians and nurses to remain blinded. The anesthesia nurse then administered the solution to the patient before the induction of anesthesia.

Procedures

Patients randomized to two groups were managed as follows.

In the intervention group, the preemptive fibrinogen concentrate dose was automatically calculated by the CRF based on the dose requirements for patients with acquired fibrinogen deficiency (5). The dose of fibrinogen that would probably be required by a patient to reach the target was available to the nurse, who provided the appropriate number of kits containing 1 g/L of fibrinogen for each patient assigned to the intervention.

We administered 1 g fibrinogen expecting to obtain a mean plasma fibrinogen value increase of 0.29 g/L to reach the target value of 2.9 g/L.

In the saline group, the dose of fibrinogen required by the patient to reach the target plasma concentration was also calculated and registered on the CRF. The appropriate number of saline-containing kits could be assigned automatically to each patient.

After the assigned dose was administered, blood samples were extracted for analysis of fibrinogen levels and thromboelastometry; the investigators remained blinded during this process. Once surgery started, nonblinded plasma fibrinogen levels were determined at successive stages of LT. If the level fell to <1 g/L, fibrinogen concentrates could be administered in either group, following a therapeutic correction strategy.

In each center, a data quality-monitoring procedure was established to ensure that these level checks were done and that the results were recorded and reported in accordance with the study protocol and good clinical practice. Members of the IRB and the public health funding agency had access to patient data throughout the study. Assessments were done regularly at preset follow-up intervals as patients were included in the trial.

Outcomes

The primary end point was the percentage of patients requiring transfusion of packed RBCs during the LT procedure. The data were locally assessed and confirmed centrally by a data monitoring committee at the lead hospital.

The secondary outcomes were as follows: percentages of patients requiring fresh frozen plasma, fibrinogen concentrate and platelets during surgery and within 24 h within each group; number of units of packed RBCs, fresh frozen plasma and platelets transfused during and within 24 h of surgery; grams of fibrinogen administered during and within 24 h of surgery; number of patients and grams of tranexamic acid administered during surgery; mortality during the operative period until hospital discharge; liver graft survival; and thrombotic complications of any type and cause during the hospital stay or 30 days after surgery.

All adverse events related to fibrinogen administration or the surgical procedure were recorded on the CRF and communicated to the principal investigator (A.S.). To safeguard participants, the data monitoring committee also assessed all adverse events, and an annual safety report was sent to the Spanish Drug Agency and to the IRB that approved the protocol. The following specific adverse events were considered: massive hemorrhage, hepatic arterial thrombosis, portal vein thrombosis, retransplantation, any other systemic thrombosis (pulmonary embolism, myocardial ischemia, cerebral ischemia), and death.

Statistical analysis

Sample size: With a total of 132 patients (66 per group), the study had 80% statistical power to detect an absolute rate reduction of 30% in patients requiring transfusion, assuming an 80% rate of transfusion in the control arm, a two-sided α level of 0.0294 adjusted for the interim analysis (overall two-sided α, 5%), and a 10% dropout rate. The interim analysis (by an independent data monitoring committee) was preplanned to take place when data were available for 80 patients in case of early termination for rejection of the null hypothesis, using the Pocock group's sequential method, or for futility, using the conditional power cutoff point of 30%.

Statistical methods: The main end point was assessed by per-protocol comparison (of patients without protocol violations), followed by an intention-to-treat analysis (including all randomized patients regardless of protocol adherence), which was used to assess sensitivity of the main outcome to control for bias. Safety was assessed in data for all intervention-exposed patients. Inferential testing for the main efficacy variable was conducted using log-binomial regression models adjusted by baseline hemoglobin stratum. Other variables were analyzed using the Fisher exact test to compare categorical variables and the Mann–Whitney test for ordinal and continuous data. The Wilcoxon test was used for intragroup comparison. Before the database was locked, exploratory analyses were undertaken to assess the uniformity of treatment contrasts. All analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC), and the level of significance was established at 5% for a two-sided comparison.

Results

From August 8, 2012, to February 25, 2014, we assessed 128 patients for eligibility; 29 patients signed the informed consent but were excluded before randomization because they revoked their written consent, were excluded after written consent or were set aside on early termination of the study (Figure 1). Interim analysis was performed for the first 81 patients to evaluate both the efficacy end point and safety. The trial was halted at that point because efficacy differences between the treatment and saline groups were inconclusive and unlikely to be significant at the end of the study (conditional power <17%); however, because patients were being enrolled while the committee deliberated, 99 patients were finally randomized, with 51 to the fibrinogen group and 48 to the saline group (Figure 1). Three patients in the fibrinogen group and four patients in the saline group were lost when the CRFs were offline during data recording or malfunctioned and group assignment could not be processed or were unavailable online or malfunctioned and group assignment could not be processed. No patients were lost to follow-up in either group. The primary end point of a 30% reduction in packed RBCs and the secondary outcomes were assessed using data for the 92 patients who completed the study. Data for all 99 patients randomized were used to assess safety. They were also used in the intention-to-treat analysis of the primary end point. Patients were distributed similarly among the five centers. Participants' baseline characteristics were similar in the two groups (Table 1). Nearly 20% of the patients had hemoglobin concentrations <95 g/L. Donor and surgical data were also similar in the two groups (Table 2). Table 3 shows the fibrinogen levels and thromboelastometry determinations before and after the active or placebo intervention and at different stages of LT as well as blinded and unblinded doses of fibrinogen concentrate and total fibrinogen administered during and 24 h after surgery. We injected a median of 3.54 g of fibrinogen preemptively (blinded) in the fibrinogen group and a median of three saline kits in the placebo group. The total amount of fibrinogen (blinded and unblinded) administered was significantly higher in the intervention

group (Table 3), and the plasma fibrinogen level was also significantly higher. Differences in coagulation time and maximum clot firmness after preemptive injections were significant within the fibrinogen group and between groups (Table 3). Additional fibrinogen was required at reperfusion of the graft in nine patients (20.9%) in the saline group and in one patient (2.1%) in the fibrinogen group (p = 0.005).

Between-group differences in packed RBCs and other blood products transfused during and within 24 h of surgery were not significant at any LT stage or during the surgical procedure as a whole (Table 4). The percentages of patients requiring RBC transfusion during LT (primary end point) were 52.9% (95% confidence interval [CI] 42.5–63.3%) in the fibrinogen group and 42.74% (95% CI 28.3–57.2%) in the saline group (p = 0.217). The between-group difference in the primary end point was 10.2% (95% CI 6–26.3%); relative risk was 0.80 (95% CI 0.57–1.13). Intention-to-treat analysis for all 99 patients randomized during LT gave similar results: 49.5% (95% CI 38.1–60.9%) in the fibrinogen group and 38.1% (95% CI 24.9%–51.3%) in the saline group required RBC transfusion (p = 0.129).

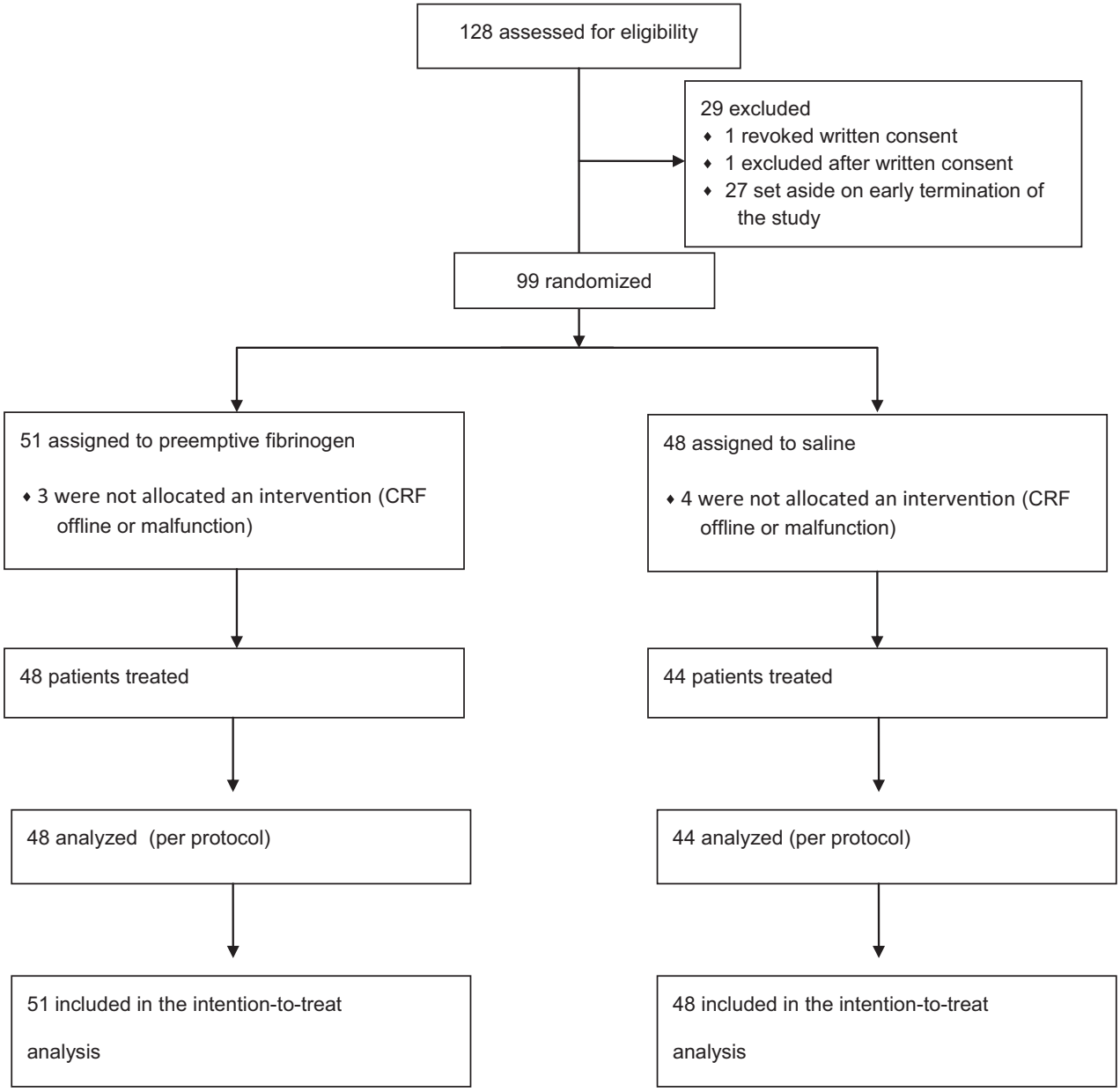

Figure 1: Trial profile. CRF, case record form.

Table 1: Patient characteristics

	Total (n = 92)	Fibrinogen (n = 48)	Saline (n = 44)	p-value
Hb ≥95 g/L	74 (80.4%)	38 (79.2%)	36 (81.8%)	0.798
Hb <95 g/L	18 (19.6%)	10 (20.8%)	8 (19.2%)	
Age (years)	55 (50–63)	54.5 (49–60)	57 (50–64)	0.271
Male	78.3%	81.3%	75%	0.614
Female	21.7%	18.8%	25%	
Weight (kg)	76.65 (64·5–90)	79.5 (66.8–90.85)	72.5 (64.5–89.5)	0.377
Height (cm)	168 (162–174.5)	170 (164.5–175)	166 (159.5–172)	0.042
BMI (kg/m^2)	26.81 (24.62–30·1)	26.84 (24.69–29.96)	26.75 (24.07–30)	0.978
Cirrhosis	63%	66.7%	59.1%	>0.999
Tumor	37%	33.3%	40.9%	0.520
Prior abdominal surgery	12%	12.5%	11.4%	>0.999
Diabetes	19.6%	18.8%	20.5%	>0.999
Abnormal arterial pO_2	15.2%	20.9%	9.1%	0.302
Portal thrombosis	9.8%	10.4%	9.1%	>0.999
Kidney dysfunction	13%	16.7%	9.1%	0.360
MELD score	16 (11–20)	16 (12–20.5)	16 (10–20)	0.456
Altered echocardiogram	41.3%	37.5%	45.5%	0.526
Pulmonary hypertension	22.8%	25.0%	20.5%	0.975
Hepatopulmonary syndrome	12.5%	13.6%	13%	>0.999
Hb (g/L)	115 (99.5–134.5)	113.5 (97–136)	115.5 (103.5–131.5)	0.643
Htc (%)	33.6 (28.4–39)	33.3 (27.4–38.7)	33.8 (29.7–39)	0.359
Platelet count (10^3/mm^3)	72.5 (51–98)	77.5 (50–107)	69 (62.5–93.5)	0.621
PTT	1.23 (1.09–1.41)	1.24 (1.13–1.48)	1.21 (1.05–1.36)	0.122
INR ratio	1.45 (1.21–1.91)	1.38 (1.21–1.93)	1.5 (1.21–1.88)	0.660
Fibrinogen (g/L)	1.9 (1.52–2.39)	1.84 (1.48–2.43)	2 (1.6–2.38)	0.629

Data are expressed as median (interquartile range) or percentage. Abnormal arterial pO_2: pressure of oxygen <80 mmHg in arterial blood sample. Kidney dysfunction: creatinine values >1.3 mg/dL. Altered echocardiogram: any pathologic findings in preoperative echocardiography exploration.

Hb, hemoglobin; Htc, hematocrit; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; pO_2, partial pressure of oxygen; PTT, partial thromboplastin time.

Table 2: Donor and surgical characteristics

	Fibrinogen (n = 48)	Saline (n = 44)	p-value
Donor characteristics			
Age (years)	60 (47–70)	59 (51–70)	0.532
Height (cm)	170 (160–175)	165 (160–176)	0.531
Sodium (mmol/L)	136 (131–139)	135 (133–138.4)	0.975
Cold ischemia (min)	350 (254–426)	327 (260–422.5)	0.614
Surgical technique: (%)			
Piggyback	48 (100)	43 (97.7)	0.481
Portocaval shunt	7 (15.2)	9 (20.5)	0.583
Vena cava clamp	0 (0)	2 (4.5)	0.220
Lowest mean blood pressure (mmHg)	60 (45–68)	56 (44–63)	0.220
Temperature before reperfusion (°C)	35.25 (34.6–35.9)	35.5 (35–36)	0.098
Temperature after reperfusion (°C)	34.85 (34–35.4)	35.2 (34.8–35.6)	0.131
Warm ischemia (min)	38 (25–50)	39 (30–55)	0.503
Reperfusion syndrome (%)	13 (27.1)	12 (27.3)	>0.999
Vasoconstrictor requirements (%)	33 (68.8)	32 (72.7)	0.814
Hemorrhagic complications (%)	1 (2.1)	6 (13.6%)	0.051
Thrombotic complications (%)	0 (0)	2 (4.5)	0.220

Data are expressed as number of patients (percentage of group) or median (interquartile range), as appropriate.

There were no between-group differences in the rates of adverse events related to drug administration or complications related to LT (Table 5). Hepatic artery, portal vein and other systemic thromboses were present in one patient (2.2%), none (0%) and none (0%), respectively, in the fibrinogen group and in three (6.8%), one (2.3%) and one (2.3%) in the saline group. Considering all thrombotic events in combination, we observed one event in the fibrinogen group (2.2%) and five (11.4%) in the saline group (p = 0.102). Reoperation was required

Table 3: Fibrinogen levels and thromboelastometry determinations before and after the preemptive intervention and total fibrinogen administered during and 24 h after surgery

	Fibrinogen (n = 48)	Saline (n = 44)	Between-group p-value[1]	Intragroup p-values[2]	
				Fibrinogen	Saline
Baseline					
Fibrinogen in plasma (g/L)	1.84 (1.48–2.43)	2 (1.6–2.38)	0.629		
EXTEM					
Coagulation time (s)	60 (54–71.5)	62 (54–77)	0.540		
MA10 (mm)	37 (31–41.5)	37 (31.5–42.5)	0.904		
MCF (mm)	45.5 (41–50)	46 (41–51)	0.979		
Lysis (%)	5 (3–9)	5 (1–8)	0.675		
FIBTEM					
MA10 (mm)	8 (6–10)	9 (7–11)	0.149		
MCF (mm)	9 (6–11)	9.5 (7–12)	0.203		
Kits administered (n)	4 (1.5–5)	3 (2–4)	0.663		
After preemptive fibrinogen					
Fibrinogen in plasma (g/L)	2.2 (1.86–2.41)	1.76 (1.3–2.2)	<0.001	0.002	<0.001
Difference basal-after preemptive					
Fibrinogen in plasma (g/L)[3]	0.32 (0.13–0.51)	−0.18 (−0.3 to −0.06)		0.0023	<0.001
EXTEM					
Coagulation time (s)	54.5 (48.5–61.5)	63.5 (50–79)	0.012	<0.001	0.647
MA10 (mm)	40 (35–46.5)	35.5 (32–43)	0.029	<0.001	0.714
MCF (mm)	49 (44.5–55)	46 (40–53)	0.040	<0.001	0.655
Lysis (%)	5 (2–8)	5 (1–8)	0.606	0.958	0.788
FIBTEM					
MA10 (mm)	11 (9–14)	8 (7–11)	<0.001	<0.001	0.247
MCF (mm)	11 (10–15)	9 (7–12)	0.013	<0.001	0.501
Anhepatic fibrinogen in plasma (g/L)	1.7 (1.23–2.04)	1.55 (1.19–1.99)	<0.001		
Reperfusion fibrinogen in plasma (g/L)	1.7 (1.4–2.11)	1.5 (1.8–1.85)	0.042		
End-surgery fibrinogen in plasma (g/L)	2.1 (1.66–2.55)	1.7 (1.22–1.95)	0.306		
24-h surgery fibrinogen in plasma (g/L)	2.88 (2.19–3.53)	3.06 (2.38–3.68)	0.430		
Fibrinogen administered (g)[3]					
Preemptive	3.54 (2.95–4.14)	0	NA		
Intraoperative	0.13 (0–0.28)	0.45 (0.11–0.8)	0.079		
Intra-24-h postoperative	0.63 (0.26–1)	2.58 (1.59–3.57)	0.004		
Total administered	4.14 (3.38–4.92)	2.58 (1.59–3.57)	0.013		
Fibrinogen administered (%)					
Dissection/anhepatic	4.2%	18.2%	0.042		
Reperfusion/end of surgery	2.1%	20.9%	0.005		
24-h postoperative	2.1%	9.5%	0.178		

Data are expressed as median (interquartile range) unless otherwise specified.
EXTEM, assay for tissue factor activation; FIBTEM, assay for tissue factor activation and platelet inhibition; MA10, maximum amplitude at 10 min; MCF, maximum clot firmness; NA, not applicable.
[1]Comparison between groups; Mann–Whitney test.
[2]Intragroup comparisons, baseline versus after preemptive fibrinogen; Wilcoxon test.
[3]Expressed as mean (95% confidence interval).

by seven patients (14.6%) in the fibrinogen group and nine (20.3%) in the saline group. One patient (2.1%) in the fibrinogen group and three (6.8%) in the saline group died in the hospital.

Discussion

Similar intraoperative and 24-h RBC transfusion or tranexamic acid requirements and similar requirements for fresh-frozen plasma and platelets were observed whether preemptive fibrinogen concentrate or saline was administered before LT. During surgery and within the next 24 h, packed RBC transfusion was needed by 68% of the total number of patients enrolled, consistent with the high risk of bleeding indicated by the median Model for End-Stage Liver Disease score of 16 in this study and the median hemoglobin concentration of 115 g/L.

Fibrinogen level and thromboelastometry measures were significantly higher after preemptive administration of fibrinogen, whereas lysis remained unaffected. More patients in the saline group required fibrinogen replacement at all stages of LT and afterward. Even so,

Table 4: Blood product and fluid therapy requirements

	Fibrinogen (n = 48)	Saline (n = 44)	p-value[1]	p-value[2]
During surgery				
RBCs[3,4] (units)	52.9%; 0 (0–1)	42.74%; 0 (0–0.5)	0.217	0.364
≥6 units RBCs	0%	4.60%		
≥10 units RBCs	0%	2.30%		
FFP[3] (units)	6.3%; 0 (0–0)	15.9%; 0 (0–0)	0.143	0.184
Platelets[3] (mL)	16.7%; 0 (0–325)	20.5%; 0 (0–300)	0.67	>0.999
Tranexamic	20.80%	27%	0.544	
Fluid therapy				
Crystalloids (mL)	——; 2459 (1650–3125)	——; 2350 (1625–4000)		0.53
Colloids (mL)	33.3%; 0 (0–500)	38.6%; 0 (0–1075)	0.281	0.665
Bicarbonate (mL)	45.8%; 0 (0–125)	61.4%; 112 (0–400)	0.005	0.148
During & 24 h after surgery				
RBC[3] (units)	68.8%; 2 (0–6)	68.2%; 3 (0–6)	0.727	1
≥6 units RBCs	29.20%	27.40%	>0.999	
≥10 units RBCs	2.10%	6.80%	0.356	
FFP[3] (units)	29.2%; 0 (0–1)	43.2%; 0 (0–2)	0.143	0.195
Platelets[3] (mL)	43.8%; 0 (0–387)	45.5%; 0 (0–300)	0.986	>0.999
Fibrinogen[3] (g)	22.9%; 0 (0–0)	47.7%; 0 (0–4)	0.003	0.018

Data are expressed as percentage of patients followed by median (interquartile range), if shown.
FFP, fresh frozen plasma; RBCs, red blood cells.
[1]The p-value refers to percentage comparison.
[2]The p-value refers to absolute numbers comparison.
[3]Adjusted percentages from the log-binomial model.
[4]Unadjusted percentages: 35.4% and 25% for the fibrinogen and saline groups, respectively (p = 0.391).

fibrinogen levels remained higher in the fibrinogen group during surgery. The lack of efficacy of preemptive fibrinogen cannot be explained by differences between groups regarding fluid therapy or surgical technique, by timing or magnitude of blood transfusion based on clinical assessment, or by the number of outliers in each group, given that >6 U of packed RBCs were needed by nearly a third of patients in both groups. A possible compensatory effect between fibrinogen and platelets could not be assessed in the present study because platelet counts and the need for replacement were similar in the two groups.

Only a single thrombotic complication and no retransplantations were seen in the fibrinogen group, whereas there were five and three, respectively, in the saline group. Consequently, fibrinogen administration was not linked to harm, consistent with a large surveillance program that found no increased risk of thromboembolic events with the use of fibrinogen concentrate (6).

Although we excluded patients with cholestasis who had thromboelastography values above the reference range (7), patient characteristics in our series were similar to those of previous observational studies in large series (4). It can be inferred that the patients included in this trial closely matched unselected patients scheduled for LT.

There are few published randomized controlled trials on the management of hemostasis and coagulation in surgery in general or LT in particular. A Cochrane review on methods to decrease blood loss and blood transfusion requirements for LT published in 2011, including 33 trials involving 1913 patients, concluded that antifibrinolytic therapy and use of thromboelastography guidance may potentially reduce blood loss and blood transfusion requirements, although the authors pointed out the need for well-designed randomized trials (2); however, the role of fibrinogen was not specifically explored by the Cochrane reviewers. We searched PubMed and Scopus (between 1990 and 2015) with no language restrictions, using the terms *fibrinogen, liver transplantation, coagulation,* and *blood product replacement*. Controlled trial and observational studies were selected on the basis of their relevance to clinical practice. Most articles presented expert opinion, which provides the grounds for the recent European guideline recommendation (8) to provide fibrinogen concentrate when a surgical patient's plasma level falls to 1.5–2 g/L. No randomized controlled trial had yet been done to ascertain the value of fibrinogen administration in LT when we registered this trial.

Although fibrinogen is the most abundant coagulation factor in plasma, large amounts are captured to form a stable thrombus (3). High fibrinogen levels (3 g/L) are considered adequate for hemostasis *in vitro* (9); however, the administration of fibrinogen concentrate above the critical level of 1 g/L was questioned in a recent systematic review when the authors concluded that only weak evidence supports the use of fibrinogen in surgical patients who are not currently bleeding (10). Although

Table 5: Adverse events and postoperative complications

	Fibrinogen (n = 48)	Saline (n = 44)	p-value
Any adverse event	33 (68.8)	33 (75)	0.644
Serious adverse event	11 (22.9)	21 (47.7)	0.016
Complications			
Infective	10 (20.8)	11 (25)	0.795
Neurological	2 (4.2)	3 (6.8)	0.67
Cardiac	2 (4.2)	5 (11.4)	0.252
Respiratory	6 (12.5)	12 (27.3)	0.114
Renal replacement therapy	1 (2.2)	5 (11.4)	0.104
Any thrombosis	1 (2.2)	5 (11.4)	0.102
Hepatic artery	1 (2.2)	3 (6.8)	0.346
Portal vein	0 (0)	1 (2.3)	0.466
Other systemic thrombosis	0 (0)	1 (2.3)	0.466
Retransplantation	0 (0)	3 (6.8)	0.116
Reoperation, for bleeding	3 (6.3)	4 (9)	0.707
Reoperation, other	4 (8.3)	2 (4.5)	0.687
Tracheotomy	0 (0)	2 (4.5)	0.22
Mechanical ventilation (h)	10.5 (6–17)	13 (7.5–27)	0.22
Surgical ICU (days)	4.5 (3–6.5)	6 (4–8)	0.108
Hospital LOS (days)	18 (13–20.5)	17 (13–36.5)	0.317
In-hospital mortality	1 (2.1)	3 (6.8)	0.346

Data are expressed as number of patients (percentage). An adverse event was defined as any untoward medical occurrence, which does not necessarily have to have a causal relationship with the study medication. A serious adverse event was defined as any untoward medical occurrence that results in death or requires inpatient hospitalization or prolongation of existing hospitalization. Complications: infections, any complication associated with fever and signs of local, organ or systemic inflammatory process; neurological, any deterioration of consciousness or sensory or motor deficit observed during hospitalization; cardiac, any episode of arrhythmia, thoracic pain or heart failure during hospitalization; respiratory, any episode of postoperative lung collapse requiring additional respiratory therapy.
ICU, intensive care unit; LOS, length of stay.

voices have been raised to warn against the indiscriminate use of fibrinogen supplementation (11), the lack of evidence contrasts with widespread use and the widely held opinion that fibrinogen improves clotting function and reduces blood loss (12,13).

Ex vivo addition of fibrinogen concentrate to samples taken during LT substantially improves structural properties of the fibrin clot (14). In the single trial evaluating the influence of fibrinogen administration on blood transfusion in LT, an observational study by Roullet and coworkers (15) found that a thromboelastometry-based algorithm led to increased fibrinogen transfusion; however, like us, they saw no decrease in blood transfusions. To our knowledge, ours is the only completed randomized trial to assess the efficacy of preemptive fibrinogen administration in LT. Although preemptive fibrinogen increased plasma fibrinogen levels in our study, giving a mean increase of 0.32 g/L (95% CI 0.13–0.51 g/L), the fibrinogen values achieved were lower than expected (mean 2.19 g/L, 95% CI 2.05–2.33 g/L). One possible explanation for that finding would be an effect of hemodilution, as seen in the saline group (in which there was a mean decrease in plasma fibrinogen of 0.18 g/L). A second explanation would be the rise in fibrinogen level related to the amount of concentrate transfused, which has been validated in cardiac surgery patients (16) but not in other surgical procedures; in this scenario, plasma fibrinogen elevations in cirrhotic patients may be influenced by variations in the volume distribution, and such variation might have happened in our patients.

Also potentially relevant is the fact that the response to additional fibrinogen in controlled studies has been somewhat less than in observational studies (10). Furthermore, the evaluation of plasma fibrinogen itself is problematic. Functional fibrinogen may be calculated automatically on the basis of clot firmness by eliminating the platelet contribution in viscoelastic tests, but in bleeding surgical patients (17) and in LT after graft reperfusion (18), the calculated value overestimates the value measured by the conventional Clauss method (17,18). The intensity of platelet inhibition also influences the accurate assessment of clot firmness, on which calculations will be based (19). Finally, with severely low fibrinogen levels, maximum clot firmness in ROTEM FIBTEM (Tem International GmbH, Munich, Germany) has been reported to present high variability (20). Despite these limitations, in one LT series, cutoff values that best predicted the transfusion threshold for platelets and fibrinogen were those of clot firmness ROTEM EXTEM at 10 min (35 mm) and clot firmness FIBTEM at 10 min (8 mm) (21). Nevertheless, clear thromboelastometry target values for each blood product have not been established in LT. We used a specific protocol to replace blood products that would be more appropriate for both participant groups; therefore, we did not use thromboelastometry to guide blood product replacement, and this could be a limitation.

We conclude that preemptive administration cannot be recommended on the basis of our data, although no harm was directly related to the strategy tested. Preemptive administration of fibrinogen concentrate increased plasma levels of fibrinogen to normal values and increased maximum clot firmness; however, these gains did not reduce the need for RBC transfusions in LT. Our study confirms the marked loss of plasma fibrinogen during LT and the need for intraoperative fibrinogen replacement.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Sabate A, Dalmau A, Koo M, Aparicio I, Costa M, Contreras L. Coagulopathy management in liver transplantation. Transplant Proc 2012; 44: 1523–1525.
2. Gurusamy KS, Pissanou T, Pikhart H, Vaughan J, Burroughs AK, Davidson BR. Methods to decrease blood loss and transfusion requirements for liver transplantation. Cochrane Database Syst Rev 2011; 12: CD009052.
3. Bolliger D, Gorlinger K, Tanaka KA. Pathophysiology and treatment of coagulopathy in massive hemorrhage and hemodilution. Anesthesiology 2010; 113: 1205–1219.
4. Costa M, Dalmau A, Sabate A, Koo M, Aparicio I, Contreras L. Low plasma fibrinogen levels and blood product transfusion in liver transplantation. Minerva Anestesiol 2014; 80: 568–573.
5. Danés AF, Cuenca LG, Bueno SR, Mendarte-Barrenechea L, Ronsano JB. Efficacy and tolerability of human fibrinogen concentrate administration to patients with acquired fibrinogen deficiency and active or in high-risk severe bleeding. Vox Sang 2008; 94: 221–226.
6. Dickneite G, Pragst I, Joch C, Bergman GE. Animal model and clinical evidence indicating low thrombogenic potential of fibrinogen concentrate (Haemocomplettan P). Blood Coagul Fibrinolysis 2009; 20: 535–540.
7. Krzanicki D, Sugavanam A, Mallett S. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. Liver Transpl 2013; 19: 852–861.
8. Kozek-Langenecker SA, Afshari A, Albaladejo P, et al. Management of severe perioperative bleeding: Guidelines from the European Society of Anaesthesiology. Eur J Anaesthesiol 2013; 30: 270–382.
9. Bolliger D, Szlam F, Molinaro RJ, Rahe-Meyer N, Levy JH, Tanaka KA. Finding the optimal concentration range for fibrinogen replacement after severe haemodilution: An *in vitro* model. Br J Anaesth 2009; 102: 793–799.
10. Lunde J, Stensballe J, Wikkelsø A, Johansen M, Afshari A. Fibrinogen concentrate for bleeding: A systematic review. Acta Anaesthesiol Scand 2014; 58: 1061–1074.
11. Wikkelso A, Lunde J, Johansen M, et al. Fibrinogen concentrate in bleeding patients. Cochrane Database Syst Rev 2013; 8: CD008864.
12. Rahe-Meyer N, Sorensen B. Fibrinogen concentrate for management of bleeding. J Thromb Haemost 2011; 9: 1–5.
13. Kozek-Langenecker S, Fries D, Spahn DR, Zacharowski K. Fibrinogen concentrate: Clinical reality and cautious Cochrane recommendation. Br J Anaesth 2014; 112: 784–787.
14. Groeneveld DJ, Adelmeijer J, Hugenholtz GC, Ariëns RA, Porte RJ, Lisman T. *Ex vivo* addition of fibrinogen concentrate improves fibrin network structure in plasma samples taken during liver transplantation. J Thromb Haemost 2015; 13: 2192–2201.
15. Roullet S, Freyburger G, Cruc M, et al. Management of bleeding and transfusion during liver transplantation before and after the introduction of a rotational thromboelastometry-based algorithm. Liver Transpl 2015; 21: 169–179.
16. Solomon C, Pichlmaier U, Schoechl H, et al. Recovery of fibrinogen after administration of fibrinogen concentrate to patients with severe bleeding after cardiopulmonary bypass surgery. Br J Anaesth 2010; 104: 555–562.
17. Ågren A, Wikman AT, Östlund A, Edgren G. TEG® functional fibrinogen analysis may overestimate fibrinogen levels. Anesth Analg 2014; 118: 933–935.
18. Yang L, Tanaka KA, Abuelkasem E, Planinsic RM, Sakai T. Clinical applicability of rapid thrombelastography and functional fibrinogen thrombelastography to adult liver transplantation. Liver Transpl 2014; 20: 1097–1105.
19. Schlimp CJ, Solomon C, Ranucci M, Hochleitner G, Redl H, Schöchl H. The effectiveness of different functional fibrinogen polymerization assays in eliminating platelet contribution to clot strength in thromboelastometry. Anesth Analg 2014; 118: 269–276.
20. Seo H, Choi JH, Moon YJ, Jeong SM. FibTem of thromboelastometry does not accurately represent fibrinogen concentration in patients with severe hypofibrinogenemia during liver transplantation. Ann Transplant 2015; 20: 342–350.
21. Blasi A, Beltran J, Pereira A, et al. An assessment of thromboelastometry to monitor blood coagulation and guide transfusion support in liver transplantation. Transfusion 2012; 52: 1989–1998.