HAEMOSTASIS AND THROMBOSIS

Original article

Post-operative heparin reduces early venous thrombotic complications after orthotopic paediatric liver transplantation

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Background - Despite significant improvements in surgical techniques and medical care, thrombotic complications still represent the primary cause of early graft failure and re-transplantation following paediatric liver transplantation. There is still no standardized approach for thrombosis prevention.

Materials and methods - The study aimed to evaluate the effectiveness of early intravenous unfractionated heparin started 12 hours postoperatively at 10 UI/kg per hour and used a retrospective "before and after" design to compare the incidence of early thrombotic complications prior to (2002-2010) and after (2011-2016) the introduction of heparin in our institute.

Results - From 2002 to 2016, 479 paediatric patients received liver transplantation in our institution with an overall survival rate over one year of 0.91 (95% CI: 0.87-0.94). Of 365 eligible patients, 244 did not receive heparin while 121 did receive heparin. We reported a lower incidence of venous thrombosis (VT) in the group treated with heparin: 2.5% (3/121) *vs* 7.9% (19/244) (p=0.038). All clinical and laboratory variables considered potential risk factors for VT were studied. By multivariate stepwise Cox proportional hazards models, heparin prophylaxis resulted significantly associated to a reduction in VT (HR=0.29 [95% CI: 0.08-0.97], p=0.045), while age <1 year was found to be an independent risk factor for VT (HR=2.62 [95% CI: 1.11-6.21]; p=0.028).

Discussion - Early postoperative heparin could be considered a valid and safe strategy to prevent early VT after paediatric liver transplantation without a concomitant increase in bleeding. A future randomised control trial is mandatory in order to strengthen this conclusion.

Keywords: *heparin, thrombosis, paediatric liver transplant.*

INTRODUCTION

Paediatric liver transplantation (PLTx) is the treatment of choice for children suffering from end-stage liver disease[1-4], with a reported 5-year survival rate after PLTx >85%[5,6]. In our institute, 1-year patient and graft survival are currently estimated at 90% (95% CI: 86.7-93.3) and 80.7% (95% CI: 76.2-85.2), respectively[7,8].

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Over the last twenty years, different authors have provided insights into the donor and recipient factors affecting graft and patient survival[6-15]. A significant number of studies and research have demonstrated that, in paediatric patient cohorts, the postoperative period is characterised by a higher risk of hepatic vessel thrombosis, especially hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT)[5,8,14-20]. Indeed, thrombosis remains the primary cause of early graft failure and re-transplantation within the first 30 days following surgery, and it occurs despite prolongation of standard coagulation assays[21-25].

There are a number of recognised risk factors for the development of thrombotic complications in the paediatric population, such as age <30 months, weight ≥7 kg, period of PLTx (transplantation performed prior to 2010), previous abdominal surgery, and total ischaemia time ≥450 minutes[14-18]. Furthermore, there is evidence that, in children, post-liver transplant prothrombin time (PT) and activated partial thromboplastin time (aPTT) levels are rapidly normalised, while the anticoagulant proteins (antithrombin, protein C and protein S) show a delay in recovery resulting in a hypercoagulable status[22,25].

Prophylactic anticoagulation by heparin infusion is recommended to prevent thromboembolic complications in paediatric patients having a known higher thrombotic risk[26,27], but in paediatric liver transplantation this is not routine practice and, indeed, remains controversial[19,23]. Consequently, there is still no consensus regarding the use of anticoagulant therapies or how best to monitor for thrombosis in the early postoperative period in patients after PLTx. A recent systematic review of surgical and pharmacological prophylaxis and prevention of vascular thrombosis after PLTx reported there were no evidence-based practice strategies currently available[28]; in particular, no eligible studies evaluating pharmacological prevention strategies were found[29-32]. Furthermore, two recent surveys showed a wide variability in the type, timing and dose of anticoagulation therapy across the centres involved, concluding that the optimal antithrombotic strategy still needs to be defined[33,34].

In our institute, the practice of administering 5 mg/kg aspirin after PLTx was started at the beginning of the institutional PLTx programme following the experience of another centre[5]. In addition to aspirin, early administration of heparin after PLTx was started in January 2011. This strategy was directly derived from the guidelines for prevention of thrombotic complications in high-risk children and new-borns[27].

Therefore, a retrospective "before and after" study was performed with the aim of comparing the incidence of early thrombosis of the venous vasculature of the liver (venous thrombosis [VT]) and HAT in PLTx prior and after the introduction of the early heparin protocol.

MATERIALS AND METHODS

Patients

Data were prospectively collected from a transplant database at our institute.

A total of 479 consecutive patients (age <18 years) who underwent liver transplantation from deceased brain-dead donors at our centre between January 1st 2002 and December 31st 2016 were included in the study. Follow up data at September 2017 were included. According to the study protocol, exclusion criteria for statistical analysis were: combined organ transplantation, re-transplantation, and a critical event occurring within the first 24 hours (h) before heparin initiation, defined as vessel complication (stenosis and thrombosis) or death. The study was approved by the institutional ethical committee.

Surgical technique

Transplant types involved both full-size liver and split liver. Surgery was performed with conventional techniques; biliary reconstruction was a Roux-en-Y hepatic jejunostomy and, only occasionally, a duct-to-duct anastomosis. Three hepato-biliary surgeons performed all the PLTx considered in this series. ABO blood group identical or compatible grafts from deceased brain-dead donors were used for all patients. A total of three ABO incompatible grafts were implanted: one prior to 2011 and the remaining two from 2011. Organ procurement was performed according to the standard technique.

Blood transfusion policy

Tranexamic acid was administered intraoperatively to all patients. Transfusion therapy was based on weight (mL/kg), clinical blood losses, laboratory values, and haemodynamic monitoring. RBC transfusion was given with the aim of maintaining haematocrit between 0.25 and 0.30. Administration of other blood products such as fresh frozen plasma (FFP) and platelets (PLTs) was not solely guided by laboratory value (prolonged PT, or

prolonged r-value on thromboelastography, platelet count <50×10⁹/L), but also by the presence of blood loss which could not be controlled by standard surgical measures.

Thrombosis prophylaxis protocol and follow up

If 12 h-postoperative signs of active bleeding were excluded and laboratory test showed a trend of normalisation of hepatic function, a protocol involving intravenous unfractionated heparin infusion at 10 UI/kg/h was started (since January 1st 2011) and monitored to maintain aPTT ratio <3. This dose was chosen according to guidelines published in 2004 for prevention of thrombotic events in new-borns and children[27]. During the first week, graft vessels (both venous and arterial) were daily assessed by Doppler ultrasound examination in order to provide an early diagnosis of vascular or biliary complications. When clinically stabilised, patients were moved to the paediatric ward until discharged. Routinely, heparin prophylaxis was discontinued after 14 days, while it was prolonged in case of high thrombotic risk (known stenotic venous anastomosis, vessels size mismatch, previous venous thromboembolism [VTE], complicated surgery). Immune suppression was based on a double-drug regimen of tacrolimus and steroids for the entire period of the study. Any episode of clinical or laboratory suspicion of acute rejection was ruled out by a liver biopsy. Follow up was performed in the clinic through out-patient visits or hospitalisation when required.

Analysed variables, definitions and outcome

The analysed variables were divided into:

- *recipient's characteristics*: age, sex, weight, height, indication to transplantation (cholestatic cirrhosis, metabolic pathology, cancer and acute liver failure), paediatric end-stage liver disease (PELD) score (model end-stage liver disease [MELD] score was used if patients were ≥12 years), preoperative laboratory tests (creatinine, bilirubine, platelets, prothrombin time-international normalized ratio (PT-INR), fibrinogen, haemoglobin) congenital heart disease;
- *donor's characteristics*: age, gender, graft type (split or whole), macro-vesicular steatosis, arterial anomalies, norepinephrine (NA) requirement;
- *transplantation characteristics*: ischaemia time, blood product transfusion volume (mL/kg);
- *post-transplant characteristics*: days of mechanical ventilation and length of stay in Paediatric Intensive Care Unit (PICU).

Vessel thrombosis was classified as "venous" or "arterial", and each of these was further classified as "early" (occurring within 30 days post-transplant) or "late" (occurring after 30 days post-transplant). Diagnosis of VT was established by a minimum of suspicion on Doppler ultrasound, always confirmed by computed tomography scans, angiography, or surgical exploration. Primary outcome was defined as early hepatic graft VT or arterial thrombosis after PLTx (within 30 days). Major bleeding as complication after surgery was defined according to the International Society on Thrombosis and Haemostasis[35].

Statistical analysis

Calculation of sample size[36] was performed assuming that data will be evaluated in a 2-group time-to-event analysis, comparing the time it takes for the outcome (thrombosis) to occur between two groups (with/without heparin). For the purpose of this analysis, we considered α=0.05 and β=0.2 (1-β=0.80). As a result, the number of thrombosis events required to have an 80% chance of detecting a hazard ratio [HR] of 0.33[37] between two populations with 2:1 proportion is 28.

Data are summarised as frequencies and proportion for categorical variables and as mean ± standard deviation for discrete variables. Differences in frequency distributions between groups were compared by χ^2 test. Differences in means of continuous variables have been calculated with Student's t-test for independent samples. Kaplan-Meier curves and estimated VT/HAT cumulative incidence data were calculated from study inclusion until outcome occurrence, last follow up, patient's death or maximal length of follow up (30 days). Univariate analysis with log-rank test was used to screen for potential variables associated with outcome at 30 days. The variables chosen for the univariate analysis refer to donor and recipient characteristics or to surgery features that are considered to be related to the outcome of the study and they were selected on the bases of the literature review and the expected pathophysiologic mechanisms[7,8,38]. In the univariate and multivariate analysis, age is used as dichotomous variable: ≤1 year or >1 year. All variables with p≤0.10 in the univariate analyses were considered in multivariate regression analysis with backward stepwise selection, in order to identify independent risk factors for VT. Hazard ratios and correspondent 95% confidence intervals (CI) were estimated using a multivariable Cox

proportional hazard model. Statistical significance was set at <0.05 for each analysis. Data were tabulated and processed with SPSS software (Statistical Product and Service Solutions, version 21, SPSS Inc., Chicago, IL, USA).

RESULTS

From 2002 to 2016, 479 paediatric patients received liver transplantation at our centre. Ninety-three of them were excluded because of combined organ transplantation (17 procedures) and re-transplantation (76 procedures). Twenty-one patients with a critical vessel complication were excluded because the critical event occurred within the first 24 h, before heparin initiation: 8 VT, 8 hepatic arterial events (1 stenosis and 7 thrombosis), 5 deaths (3 of them intraoperative). Data of patient inclusion are reported in **Figure 1**.

A total of 365 patients were included in the analysis. Median age was 16.7 months (0-215 months) and approximately 37% of patients were aged <1 year. The most frequent indication for liver transplantation was cholestatic cirrhosis accounting for 73.6% of the total included procedures, followed by metabolic pathology (7.4%), cancer or acute liver failure (respectively 6.6%) and other causes (5.8%). The overall survival (OS) calculated over one year was 0.91 (95% CI: 0.88-0.94).

According to the use of postoperative anticoagulation, patients were divided into two groups: 244 patients (from 2002 to the end of 2010) who did not receive heparin and 121 patients (from 2011 to the end of 2016) who received heparin prophylaxis. For all patients, heparin prophylaxis was started on day 1 post surgery and continued at least until day 15.

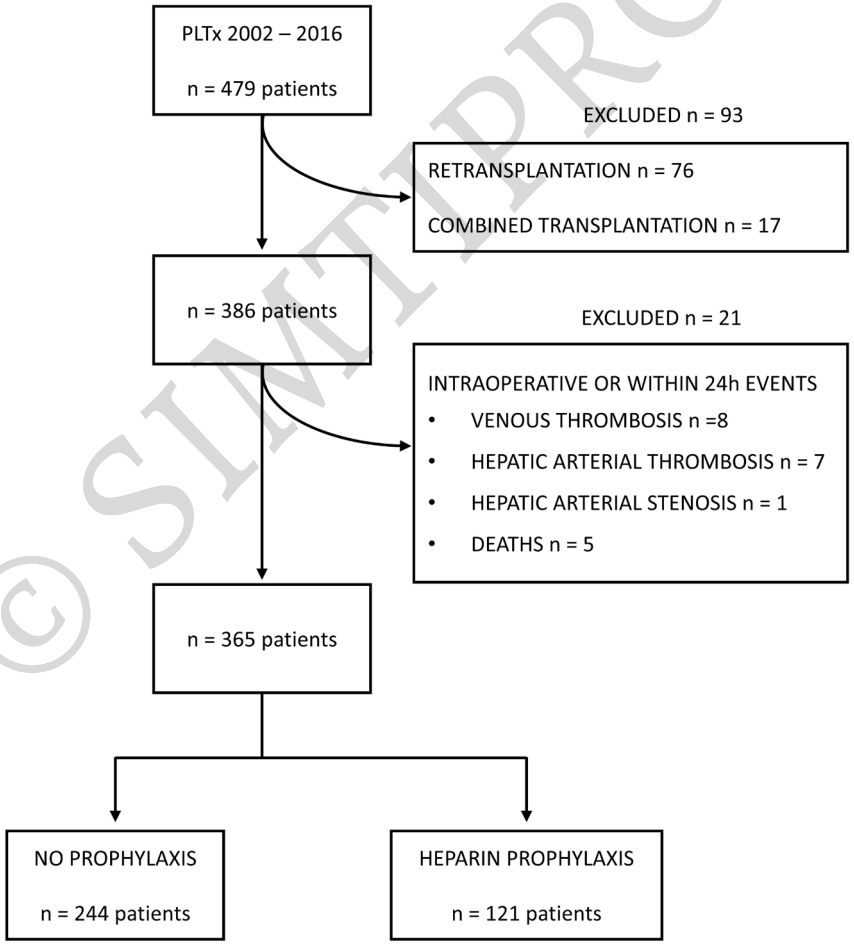

Figure 1 - Flow chart of patient inclusion in the analysis
PLTx: paediatric liver transplantation; n: number; h: hours.

The OS rate of each group at 1 year was 0.91 (95% CI: 0.87-0.94) from 2002 to 2010 and 0.89 (95% CI: 0.83-0.95) from 2011 to 2016. No statistically significant difference was found in OS between the two populations.

Descriptive data of the two groups are presented in **Table I**, and are divided into preoperative recipient variables, intraoperative variables, and donor variables. The total percentage of missing data was 0.0034%, whereas the maximum percentage of missing date per variable was 0.049%.

According to demography, anthropometric measurements (weight, height, body mass index) and preoperative data (creatinine, bilirubin, INR, platelets, fibrinogen), no significant differences were found between the two groups, except for recipient preoperative haemoglobin (Hb) level. Specifically, Hb levels were incidentally found to be significantly higher in the population treated with heparin: medium value 10.1 *vs* 9.5 gr/dL in the no-heparin prophylaxis group (p=0.005).

Among intraoperative variables, total ischaemia time and

Table I - *Patients' characteristics*

Characteristics	Patients with no heparin prophylaxis n=244	Patients with heparin prophylaxis n=121	p-value
Recipient variables			
Age (months)	40.7±49.4	44.2±56.1	0.554
Weight (kg)	14.44±13.76	15.74±14.58	0.405
Height (cm)	85.80±30.58	88.50±31.93	0.434
BMI (kg/m^2)	16.80±2.69	17.31±2.85	0.093
Preoperative creatinine (mg/dL)	0.35±0.22	0.45±0.64	0.104
Preoperative total bilirubin (mg/dL)	15.67±12.60	14.09±14.09	0.281
PELD/MELD	18.50±11.61	19.39±13.47	0.537
Preoperative platelets (10^3/µL)	169±133	153±115	0.240
Preoperative INR	1.9±1.0	1.8±0.8	0.246
Preoperative fibrinogen (mg/dL)	197±100	195±103	0.847
Preoperative Hb (mg/dL)	9.5±2.1	10.1±1.8	0.001
Gender (M/F)	128/116	61/60	0.713
Intraoperative variables			
Total ischaemia time (min)	429±157	373±91	0.000
RBC during surgery (mL/kg)	44±46	34±49	0.060
FFP during surgery (mL/kg)	36±40	20±32	0.000
PLT during surgery (mL/kg)	1±10	2±9	0.507
Postoperative variables			
Days of ventilation	3±6	3±7	0.650
Days in PICU	7±7	12±16	0.001
Donor variables			
Donor age (years)	25±16	30±17	0.019
Donor sodium level (mEq)	147.0±12.6	135.1±42.1	0.003
EqDoseNA (mcg/min)	2.23±2.07	0.88±1.70	0.000
Donor sex (M/F)	157/87	58/63	0.003
Graft (Split/whole)	206/38	103/18	0.862
Arterial anomalies (yes/no)	60/173	25/89	0.437

Results are expressed as mean±standard deviation (SD) or as frequencies. Equivalent dose of Norepinephrine was calculated as follows: EqDoseNE=norepinephrine (mcg/min) + dopamine (mcg/min)/2 + adrenaline (mcg/kg) + phenylephrine (mcg/kg)/2. BMI: body mass index; PELD: paediatric end-stage liver disease; MELD: model end-stage liver disease; INR: international normalised ratio; Hb: haemoglobin; M: male; F: female; RBC: red blood cell; FFP: fresh frozen plasma; PLT: platelets; PICU: Paediatric Intensive Care Unit; n: number.

amount of FFP transfused were significantly higher in the group without heparin prophylaxis (**Table I**).

Regarding donor variables, a higher distribution of male donors, sodium level and NA requirement in the no-heparin prophylaxis group were observed. Moreover, donors in the heparin group were older, with a median age of 30±17 *vs* 25±16 months (p=0.019). Furthermore, the post-operative variable PICU stay was significantly longer in the heparin prophylaxis group (12 *vs* 7 days; p=0.001) compared to other patients.

Venous thrombosis

Early VT occurred in 19 of 244 patients in the no-heparin prophylaxis group and in 3 of 121 in the group treated with heparin (χ^2=4.023, p=0.045). Cumulative incidence of VT by Kaplan-Meier analysis in the two groups is reported in **Figure 2**. Patients in the group treated with postoperative prophylaxis had a cumulative incidence of VT of 2.5% (95% CI: 0-5.2), while patients transplanted before the introduction of postoperative heparin had a cumulative incidence of VT of 7.9% (95% CI: 4.6-11.2) with an HR of 0.305 (95% CI: 0.09-1.03; p=0.056). Among the three VT events in patients treated with heparin, only one occurred during prophylaxis (at day 4), while the remaining two occurred 17- and 25-days post-transplant.

Univariate analysis for all the candidate variables considered to be potential risk factors for VT is presented in **Table II**. The analysis shows an association between early VT and heparin use, total ischaemia time, recipient age <1 year, and cholestatic disease as indication for liver transplantation (p<0.10). Evaluation of the prognostic value of these four variables was performed by a multivariate Cox proportional-hazard analysis with backward stepwise selection. As a result, in a multivariate model stratified for preoperative Hb value, heparin prophylaxis (HR=0.29 [95% CI: 0.08-0.97]; p=0.045) and recipient age <1 year (HR=2.62 [95% CI: 1.11-6.21]; p 0.028) were found to be independent predictive factors for early VT (**Table II**).

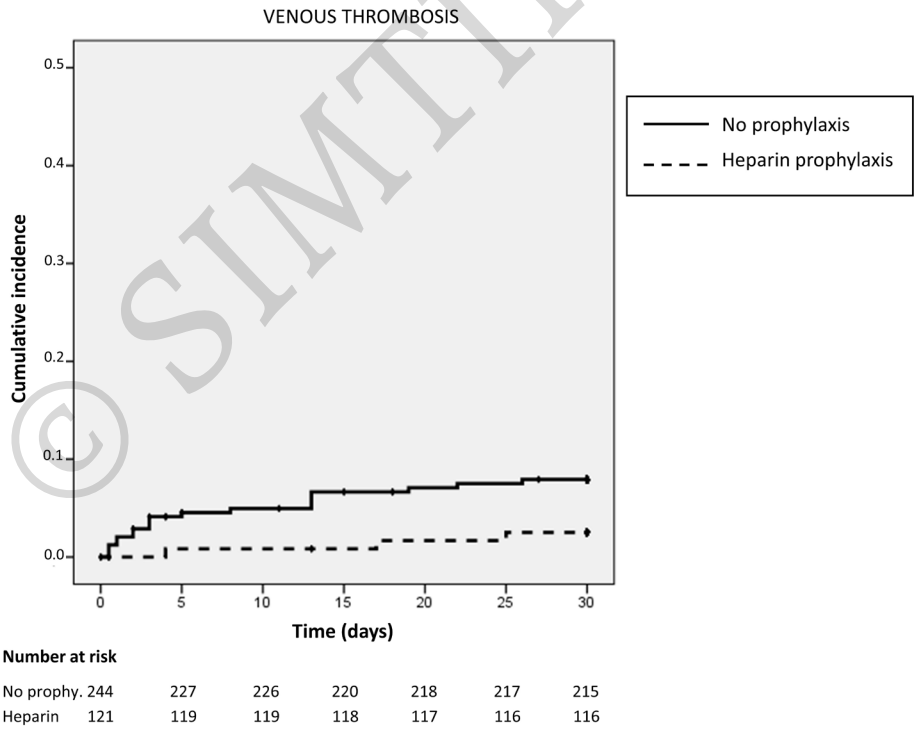

Figure 2 - Cumulative incidences of venous thrombotic events according to use of thromboprophylaxis by Kaplan-Meier analysis

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Table II - *Univariate and multivariate analysis of potential risk factors for venous thromboembolism at 30 days from liver transplant*

Baseline patient factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Recipient variables				
Age <1 year	2.45 (1.05-5.72)	**0.039**	2.62 (1.11-6.21)	**0.028**
Gender (male)	0.72 (0.31-1.69)	0.456		
Weight (kg)	0.97 (0.92-1.02)	0.205		
Transplant indication				
Cholestatic cirrhosis	3.6 (0.84-15.44)	**0.084**		
Metabolic pathology	0.57 (0.08-4.22)	0.580		
Cancer	0.68 (0.09-5.09)	0.711		
Acute liver failure	0.05 (0.00-94.8)	0.427		
PELD/MELD	1.01 (0.97-1.04)	0.782		
Preoperative creatinin (mg/dL)	0.88 (0.26-2.94)	0.832		
Preoperative total bilirubin (mg/dL)	1.01 (0.98-1.04)	0.645		
Preoperative platelets (10^3/μL)	1.00 (0.99-1.00)	0.274		
Preoperative PT-INR	1.23 (0.85-1.78)	0.280		
Preoperative fibrinogen (mg/dL)	0.99 (0.99-1.00)	0.418		
Preoperative haemoglobin (g/dL)	1.09 (0.89-1.33)	0.409		
Donor variables				
Age (months)	1.01 (0.99-1.04)	0.311		
Gender (male)	1.86 (0.73-4.76)	0.194		
Type of graft (full liver)	0.26 (0.04-1.93)	0.187		
Macro steatosis	0.99 (0.91-1.10)	0.977		
Arterial anomalies	0.75 (0.25-2.25)	0.610		
Sodium (mEq/L)	1.02 (0.98-1.07)	0.244		
EqDoseNA (mcg/min)	1.14 (0.96-1.37)	0.145		
Intraoperative variables				
Total ischaemia time (min)	1.00 (1.00-1.01)	**0.039**		
RBC during surgery (mL/kg)	1.00 (0.99-1.01)	0.806		
FFP during surgery (mL/kg)	1.00 (0.99-1.01)	0.525		
PLT during surgery (mL/kg)	0.98 (0.91-1.05)	0.596		
Heparin prophylaxis	0.31 (0.09-1.03)	**0.056**	0.29 (0.08-0.97)	**0.045**

Results of multivariate models are adjusted for quartile of haemoglobin. Statistically significant data in bold.
PELD: paediatric end-stage liver disease, PT-INR: prothrombin time-international normalized ratio; MELD: model end-stage liver disease, RBC: red blood cells, FFP: fresh frozen plasma, PLT: platelet, n: number.

Frequency of different types of early VT in each group and the relative outcomes are presented in **Table III**.

In both groups, VT occurred more frequently in the portal vein: 13 (68%) in the group without post-operative heparin and 2 (67%) in patients receiving heparin. The remaining events were localised in the supra-hepatic vein. No inferior vena cava thrombosis was registered in our study population.

In the no-heparin prophylaxis group, a total of 19 patients had a VT: 9 were treated with heparin infusion (therapeutic anticoagulant dose), obtaining reperfusion in 8 cases but one which required re-transplantation; 8 underwent surgical intervention ending in 4 reperfusions, 3 re-transplantations within 30 days and one patient's death; one patient underwent endovascular procedure using Fogarty balloon catheterization; one patient required prompt re-transplantation.

Table III - *Early venous thrombosis (VT) events according to groups, before and after introduction of heparin prophylaxis*

	Prior to heparin (2004-2010)		Heparin prophylaxis (2011-2016)	
Portal vein thrombosis	**n=13**		**n=2**	
Treatment	***n.***	***Final outcomes***	***n.***	***Final outcomes***
High-dose heparin	4	4 re-canalisations		
Surgical revision	7	1 death 2 re-transplantations 4 re-canalisations	2	1 re-transplantation 1 re-canalisation
Endovascular procedure	1	1 re-canalisation	-	
Re-transplantation	1		-	
Suprahepatic vein thrombosis	**n=6**		**n=1**	
Treatment	***n.***	***Final outcomes***	***n.***	***Final outcomes***
High-dose heparin	5	4 re-canalisations 1 re-transplantation at 2 months	-	
Surgical revision	1	1 re-transplantation	-	
Endovascular procedure	-		1	1 death at 2 months post-transplant

Type and frequency of VT, subsequent therapy or intervention, and final outcome are described for each group. n: number.

In the group of patients who received heparin prophylaxis, 2 underwent surgical intervention leading to reperfusion in one case and re-transplantation in the other case; one Fogarty procedure ended in the patient's death at 60 days (**Table III**).

No thrombotic events were observed for the three ABO incompatible patients and all of them are still alive.

Hepatic artery thrombosis

There was no difference in the proportion of early HAT in the no-heparin group (7/244 patients) and in the group with heparin prophylaxis (5/121 patients) (p=0.542).

Furthermore, in univariate analysis, the use of prophylactic heparin was not associated with occurrence of HAT (HR=1.446 [95% CI: 0.459-4.557]; p=0.534).

Major bleeding

According to the International Society on Thrombosis and Haemostasis definition[35], post-operative major bleeding complications occurred in 4.5% (11/244) of patients in the no-heparin group and in 7.4% (9/121) of patients in the group treated with heparin. There was no statistically significant difference in the proportions of events between groups (χ^2=1.274, p=0.259).

DISCUSSION

The incidence of hepatic vessel thrombosis after PLTx has decreased over the last decades; however, it remains one of the most frequent and serious complications that lead to a poor outcome after PLTx[5,8,14-25]. Furthermore, a valid prophylactic strategy has not yet been defined in paediatric antithrombotic recommendations[27,29,31]. In our study of early intravenous unfractionated heparin infusion started 12 h post operatively at 10 UI/kg/h, a lower incidence of early VT in the group treated with heparin prophylaxis compared to the no-heparin group was observed: 2.5 *vs* 7.9% of patients (p=0.038). No statistically significant difference was observed in incidence of HAT between the two groups: 2.9% in the no-heparin group and 4.1% in the heparin group (p=0.534).

To our knowledge, this is the first study involving a large sample of patients specifically designed to assess the feasibility and clinical impact of heparin prophylaxis in PLTx. In this regard, our group has recently carried out a systematic review[2] of different prophylactic strategies (either medical or surgical) for the prevention of thrombotic complications after PTLx without finding any evidence of efficacy; we found only a few retrospective studies and none concerning pharmacological prophylaxis that compared clinical outcomes according to the different treatments used.

Neither have strong expert-based recommendations published for North America in 2012 (intravenous dipyridamole and weight-adapted unfractionated heparin with transition to aspirin once the patient is being completely fed by mouth) served to standardise clinical

practice among centres[31]. More recently, results from two surveys on medical management of thrombotic risk after PLTx have been published describing a high degree of variability in anticoagulant and antiplatelet therapy between the centres taking part[33,34].

In this context, our study, although retrospective, has to be considered clinically relevant: it involves a large number of patients in a single study centre, with two comparable groups, carried out using the same group of surgeons/anaesthesiologists and perioperative protocol, including immunosuppression. It shows that postoperative heparin use results in a statistically significant reduction in incidence of VT without increasing the risk of bleeding. On the other hand, no impact was observed on artery vein thrombosis. These results suggest that our protocol with low-dose heparin could be a valid, simple and safe medical strategy for prevention of VT.

In terms of 1-year OS, no significant differences between the two groups were shown and these data may need a longer follow up. However, inferior graft survival was found in the group prior to heparin prophylaxis with 5 re-transplantations (2% of this group) *vs* one re-transplantation (0.8%) (**Table III**) and a related higher morbidity. In fact, **Table III** describes, from the clinical point of view, the consequences of the high rate of complications and required procedures to attempt re-canalisation following the thrombotic events.

The timing of heparin initiation and discontinuation requires some consideration. Sixteen cases of thrombosis occurred before heparin prophylaxis and for this reason they were excluded from the analysis. Earlier administration of heparin has certainly been considered a valid prophylactic option given a multifactorial aetiology of thrombosis in liver disease involving flow obstruction, chronic inflammation and a fragile haemostatic balance[23], which can be monitored by global coagulation assays like viscoelastic tests. For this reason, since 2017, in our centre, the G parameter of thromboelastography has been used to monitor hypercoagulability intraoperatively[39] and to decide on early heparin infusion.

Furthermore, we recommend continuing heparin prophylaxis for 14 days. Unfortunately, due to the retrospective nature of the study, data on the continuation of prophylaxis, which is decided according to the clinical judgment of increased thrombotic risk, are not available. Moreover, there are inconsistencies in the literature as to the duration of an anticoagulation protocol following paediatric liver transplantation[28,33]. According to the results of our study and the time required to restore liver function[25], it could be reasonable to prolong heparin prophylaxis; however, these indications should be evaluated in an *ad hoc* study.

Comparing the two study populations, most of the preoperative, intraoperative, and donor variables were similar. We observed an unexpected slightly higher preoperative Hb level in the population treated with heparin prophylaxis (10.1 *vs* 9.5). However, given the minimal difference involved, this can be considered not to have any clinical relevance.

Furthermore, we found total ischaemia time and amount of FFP transfused to be higher in the group without heparin prophylaxis as intraoperative variables, and a higher distribution of male donor, sodium level and NA requirement in this same group as donor variables. However, the statistical analysis was able to exclude any independent association between the outcome and these variables.

PICU stay was longer in the heparin group; this unusual result may reflect a more conservative policy of patient allocation between the ICU and the paediatric ward over recent years to promote prompt identification of life-threatening complications that mainly occur in the first month post PLTx[4,8]. Some may raise concerns about the unmonitored use and effectiveness of heparin and could suggest replacing antithrombin, protein C and protein S with FFP, as a delay in the recovery of anticoagulant proteins has been reported in PLTx, in contrast to adults[23,25]. These important physiopathologic observations are not supported by strong evidence in clinical studies; furthermore, FFP replacement post PLTx seems to be significantly associated with graft loss[38].

Interestingly, three patients with incompatible ABO transplantation were included in the study. The choice for this was supported by the fact that the literature did not show any increase in vascular thrombosis when compared with ABO compatible graft[40].

This study has potential limitations. Firstly, it was carried out retrospectively. Theoretically, a randomised clinical trial could be useful to confirm the results. Furthermore, the population receiving heparin prophylaxis is temporally subsequent to the no-heparin group, which implies the

surgical team may have experienced a "learning curve" that might have influenced the lower incidence of VT for the more recent patients. Nevertheless, the same surgical team performed the first PLTx in 1988 in Milan[41] and since 1997[3] has presented data regarding patient/graft survival and vessel complication that were already comparable to that of the present study. Moreover, since 1997, our centre has continued to maintain a high level of activity, performing more than 15 PLTx a year[3,18]. Another limitation of the study is the low number of thrombotic events that occurred compared to that expected (about 15% of the study population); this was probably due to the exclusion of some high-risk categories (combined-organ transplants or re-transplants) and of thrombosis occurring during transplantation. This low number of thrombotic events could compromise the statistical precision of the study and does not allow different confounding variables to be analysed simultaneously; for this reason, the multivariate analysis was conducted with the backward method with a maximum of two independent variables as final step.

CONCLUSION

Given the strong clinical impact of hepatic vessel thrombosis after PLTx[23], the issue regarding its prevention is crucial. Our results are 2-sided. On the one hand, they reaffirm that a standardised protocol for the ICU guarantees safety and high-quality care, and on the other identifies a viable strategy supported by good statistical evidence. There is a rationale for adopting a protocol involving early low-dose of heparin (10 UI/kg/h); this seems to safely reduce the incidence of early VT complications after PLTx, leading to lower morbidity and higher graft survival. However, a future randomised control trial is mandatory in order to strengthen this conclusion. Given the current absence of stronger evidence-based strategies[28], we suggest adopting this anti-thrombotic prophylactic strategy as standard of care after PLTx.

AUTHORSHIP CONTRIBUTIONS

CG and GC contributed equally to this manuscript as first Authors; NM and AF contributed equally to this manuscript as last Authors; CG is responsible for data collection and wrote the first draft of the manuscript; GC is responsible for statistical analysis and contributed to the writing of the manuscript; BA, FF, BD, CM, and AM are responsible for data collection; CM and BE critically reviewed the manuscript; NM designed the study, conceptualised the database for collecting data and wrote the first draft of the manuscript; FA conceptualised and designed the study, and critically reviewed the manuscript.

The Authors declare no conflicts of interest.

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