Effect of Aprotinin on Liver Injury After Transplantation of Extended Criteria Donor Grafts in Humans: A Retrospective Propensity Score Matched Cohort Analysis

Anna Roehl (✉ aroehl@ukaachen.de)
  Department of Anesthesiology RWTH Aachen University, Faculty of Medicine, Aachen

Marc Hein
  Department of Anesthesiology RWTH Aachen University, Faculty of Medicine, Aachen

Anne Andert
  Department of General, Visceral and Transplantation Surgery, RWTH Aachen University, Faculty of Medicine, Aachen,

Rolf Rossaint
  Department of Anesthesiology RWTH Aachen University, Faculty of Medicine, Aachen

Karsten Junge
  Department of Anesthesiology RWTH Aachen University, Faculty of Medicine, Aachen

Ulf Neumann
  Department of General, Visceral and Transplantation Surgery, RWTH Aachen University, Faculty of Medicine, Aachen,

Felix Kork
  Department of Anesthesiology RWTH Aachen University, Faculty of Medicine, Aachen

---

Abstract

Background: The number of patients awaiting liver transplantation still widely exceeds the number of donated organs available. Patients receiving extended criteria donor (ECD) organs are especially prone to an aggravated ischemia reperfusion syndrome during liver transplantation leading to massive hemodynamic stress and possible impairment in organ function. Previous studies have demonstrated aprotinin to ameliorate reperfusion injury and early graft survival.

Methods: In this single center retrospective analysis of 84 propensity score matched patients out of 290 liver transplantation patients between 2010 and 2014 (OLT), we describe the association of aprotinin with postreperfusion syndrome (PRS), early allograft dysfunction (EAD: INR ⬚ 1,6, AST/ALT >2000 within 7-10 days) and recipient survival.

Results: The Incidence of PRS (52,4% vs 47,6%) and 30-day mortality did not differ (4.8 vs 0%; p=0.152) but patients treated with aprotinin suffered more often from EAD (64,3% vs 40,5%, p=0.029) compared to controls. Acceptable or poor (OR=3.3, p=0.035; OR=9.5, p=0.003) organ quality were independent predictors of EAD.

Conclusion: Our data does not support the notion that aprotinin prevents nor attenuates PRS, EAD or mortality.

Background

Increasing organ shortage for liver transplantation is a major challenge to transplant hepatology. To address this situation and to overcome the discrepancy between organ demand and supply, marginal donors or extended criteria donors (ECD) are more often accepted to increase the number of available donor organs.[1, 2]

There is no standard definition of extended criteria donors. Eurotransplant considers liver graft donors as extended criteria donors if one of the following criteria is fulfilled: Donor age >65 years, ICU stay with ventilation >7 days, body mass index >30, steatotic liver >40% serum sodium >165 mmol/L, SGPT >105 U/L, SGOT >90U/L or serum bilirubine >3mg/dL. In the Eurotranplant region, available donor organs are primarily offered to patients matching the ABO blood group with the highest Model of end stage liver disease (MELD) score nearest to the explantation site [3]. Organs form extended criteria donors on the other hand are offered to several centers closest to the explantation site. Remarkably, patients with a special urgency are exempted from the MELD-based allocation (e.g. acute liver failure, primary organ nonfunction). This leads to an increased acceptancy of organs from extended criteria donors being transplanted to increasingly sick patients[4],[5].

Moreover, these liver grafts from extended criteria donors are especially susceptible to ischemia-reperfusion injury.[6] During the liver transplantation itself, one of the most crucial time points for the recipient is the reperfusion of the liver graft, potentially resulting in post-reperfusion syndrome (PRS) [7].

PRS has been shown to be associated with poorer short- and long-term outcomes, in particular hyperfibrinolysis, early allograft dysfunction (EAD) and mortality.

Several measures improving ischemia-reperfusion of the liver transplantation graft and attenuating PRS have been subject to investigation and extensive discussion: changes in preservation solution, external organ perfusion as well as pharmacologic pre-conditioning by, among others, aprotinin[6]. Aprotinin is a reversible binding, competitive serine protease inhibitor widely used to reduce blood loss during heart as well as liver surgery [8-13]. One of the first descriptions of the applications of aprotinin in liver transplant patients has been described by Neuhaus and colleagues [11, 12, 14]. Aprotinin activates plasminogen and has been demonstrated to prevent microthrombosis, to improve microcirculation and consequently oxygen supply, and to ameliorate systemic inflammatory response [9, 15, 16]. Aprotinin reduces liver ischemia reperfusion injury in animal models[17, 18] and improves 1-month graft survival in liver transplant recipients.[9] The double-blinded European Multicenter Study on the Use of Aprotinin in Liver Transplantation (EMSALT) found that aprotinin reduced hyperfibrinolysis and consequently led to a 50% reduction of blood loss and 30% reduction of transfusion requirement.[10] However, aprotinin was temporarily suspended as preliminary results from the BART trial demonstrated higher mortality for patients receiving aprotinin [19].

At our center, aprotinin was an essential element in the perioperative management when transplanting liver grafts from extended criteria donors. We therefore conducted a single-center retrospective analysis to investigate the effect of aprotinin on intraoperative and postoperative outcomes of liver graft recipients compared to a cohort of propensity score matched patients who were not treated with aprotinin.

Methods

Patients

The local ethics committee (University Hospital Aachen, EK 291/13) approved the analysis and waived the requirement of informed consent. Our analyses was conducted with adherence to the *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE) guideline [20].

From January 2010 to June 2015 we performed 290 liver transplantations in our tertiary care university hospital. Records of patients who received a liver transplantation during the observation period from 2010 to 2014 were reviewed for the intraoperative treatment of aprotinin. Using organ quality, donor age and CIT, we identified a propensity score matched control group with the nearest neighbor method (SPSS 24.0, MatchIt package for R, IBM Corporation, Armonk, USA). Matching variables were selected a priori, as these were the criteria used to apply aprotinin at our center at that time.

Donor data

The covering letter from Eurotransplant provided the donor data: age, sex, body mass index, cold ischemic time (CIT) as well as sodium concentration, alanine transaminase (ALT), aspartate

transaminase (AST) and bilirubin (Bili).

Donor organ assessment

The transplanting surgeon assessed the liver graft macroscopically by inspection and palpations as described before [21], assessing liver texture, yellowness, absence of scratch marks and round edges[22] . The organs were classified to either good, acceptable or poor quality following the Eurotransplant criteria. Macro- and microvesicular fat content were determined by histology and described as affected hepatocytes in percentage [7] including microsteatosis (MIS; the cytoplasm of the hepatocyte contains multiple tiny lipid vesicles without nuclear dislocation) or macrovesicular steatosis (the cytoplasm of the hepatocyte contains a univacuole lipid vesicle with nuclear displacement) [23].

Liver transplantation management

Liver transplantation was performed using an extracorporeal venovenous/portalvenous bypass. Bypass, surgical and anesthesiologic management as well as peri- and postoperative immune suppression regimen have been described earlier in detail [7, 21]. The patients received a maximum of one liter of balanced electrolyte solution. Adjacent volume replacement was hold up by transfusion of fresh frozen plasma (FFP) in order to anticipate coagulation disorders. Transfusion triggers for red blood cell units (RBC) were dependent on the patient's comorbidities and transfusions were conducted at the discretion of the providing anesthesiologist. Our standard operation procedure (SOP) scheduled a thrombelastometry (TEM, Rotem®) after induction of anesthesia as well as 15-30 min and 45-60 min after reperfusion for early correction of coagulation disorders [24].

Aprotinin application

After allocation of the organ, the treating anesthesiologist and transplant surgeon decided jointly considering i) the visual assessment of the liver, ii) to the cold ischemic time (CIT) and iii) the donor age, whether the patient should receive aprotinin in order to attenuate PRS and early allograft dysfunction. Aprotinin infusion was started immediately after the surgical incision with a testing dose of 1ml (equivalent to 10 000 IE) to rule out any allergic reaction. After that, aprotinin was infused at a rate of $2x10^6$ IE/h, a rate of $4x10^6$ IE/h during the an-hepatic phase and reduced to $2x10^6$IE/h until the end of surgery.

Recipient data

Recipient data were abstracted from the patient's medical chart: recipient age, diagnosis leading to transplantation and the laboratory model of end stage liver disease score (labMeld: 10x (3.8*ln(bilirubin[mg/dl])+11,2*$\log_e$(INR)+9,6*ln(creatinine level [mg/dl])+6,4*(etiology: 0 if cholestatic or alcoholic, 1 otherwise) [3] were recorded at the evaluation procedure before patients were enlisted for transplantation. Clinical chemistry data (creatinine, AST, ALT, Bili, GGT, GLDH) were extracted from the electronical chart after admission closest to the beginning of the surgery (preoperatively), at ICU

admission immediately after surgery (postoperatively) and on day 1, 3, 7 and 14. The number of intraoperatively transfused units of red blood cell units (RBC; units), fresh frozen plasma (FFP), platelets, fibrinogen and 4 factor prothrombin complex concentrate (4F-PCC) were extracted from the paper-based anesthesia protocol. Bilirubin, INR, AST/ALT, acute rejection (clinical diagnosis), surgical revisions, re-transplantation, sepsis, need for renal replacement therapy (RRT), intensive care unit (ICU) length of stay (LOS). Early allograft dysfunction is defined as bilirubin ⎕ 10mg/dl on postoperative day (POD)7 and/or INR ⎕1,6 on POD 7 and/or AST or ALT >2000 IU/L within in the first 7 days, were abstracted from the patient's chart after the transplantation.

Postreperfusion syndrome [7]

Postreperfusion syndrome was defined as occurrence of one of the following criteria: (1) decrease in mean arterial pressure (MAP) of at least 30% at time of reperfusion, (2) administration of an intravenous bolus of norepinephrine >2 μg kg body weight (BW)-1, (3) increase of continuous norepinephrine (NE) infusion of ≥0.1 μg kg BW− 1 within 5 to 30 min after reperfusion, or (4) initiation of continuous vasopressin infusion after reperfusion. According to our department's SOP, PRS was treated as follows: (i) 0,5 mg atropine before reperfusion if heart rate < 80, (ii) NE boli and NE infusion to maintain MAP, (iii) epinephrine boli and infusion in case of significant bradycardia with hypotension and decrease of SVO2 during reperfusion, (iv) infusion of vasopressin if high doses of NA are necessary or NA therapy ineffective.

Statistics

For a detailed description of the propensity score matching please refer to the the patients section above. Differences between groups were analyzed using the t-test for continuous, Chi-Square test for categorical and variance analysis for repeated measurements for continuous variables over time. Kaplan-Meyers curves were generated to display the effect of trasylol on patient and graft survival. A logistic regression analysis was used to analyse the effect of trasylol, MELD, organ quality, donor BMI and donor AST on EAD. Parameter were included in multivariate (stepwise forward) approach if univariate analysis was significant (SPSS 24.0). Results were displayed as mean and standard deviation or absolute and relative number of cases. Figures were created using Prism 6.0 (GraphPad Software, San Diego, USA). A two-sided p-value ≤0.05 was considered statistically significant.

Results

Patients

A total of 290 patients received a liver transplantation graft during the five-year study period and 42 of these patients were treated with aprotinin. Whether the patient was treated with aprotinin to attenuate PRS and EAD had been a joint decision of the attending anesthesiologist and the transplant surgeon based on the following criteria: i) organ quality (good, acceptable, poor), ii) cold ischemia time and iii) age of the donor. In a propensity score analysis using these three criteria, 42 statistically similar patients

were identified who were not treated with aprotinin and were assigned to the control group. Matching reduced the relative multivariate imbalance L1 (0.634 vs. 0.782) and the χ2 balance test showed no significant imbalance ($\chi 2 = 1.08$, df = 4, p = 0.897) in the matched cohort (n = 84).

Liver Graft Recipient Characteristics

Demographic and clinical characteristics of the study population are described in detail in **Table 1**. Patients who were treated with aprotinin intraoperatively during a liver graft transplantation were of similar age and sex and had a similar BMI compared to controls. Patients treated with aprotinin did not differ regarding the reason for liver graft transplantation, preoperative clinical chemistry, amount of intraoperative transfusions and intraoperative complications. Notably, patients who were treated with aprotinin did tend to have a higher preoperative labMELD score (19.9±8.5 vs. 16.4±8.6; p = 0.061).

Liver Graft Donor Characteristics

Demographic and clinical characteristics of the liver graft donors for the matched study population of liver graft recipients are described in **Table 2**. Donors did not differ in age, sex and ICU length of stay for recipients treated with aprotinin compared to controls. Overall organ quality, graft fat content and cold and warm ischemia time did not differ as well. However, donors for recipients treated with aprotinin had a higher BMI (34.7±8.9 vs. 30.3±7.5, p = 0.018) and a higher ALT (85.7±114.2 vs. 43.6±38.6, p = 0.032).

Table 1: Demographic and clinical characteristics of 42 liver graft recipients were intraoperatively treated with aprotinin and 42 controls. The 42 statistically similar recipients who were not treated with aprotinin were identified by propensity score matching in a cohort of 290 single center liver graft recipients. Propensity scores were calculated using organ quality, cold ischemia time and donor age.

	Matched Cohort (n = 84)	Aprotinin (n = 42)	Controls (n = 42)	p
Age [years]	57.0 ± 6.9	57.0 ± 6.6	57.1 ± 7.2	0.937
Sex [female]				
BMI [kg/cm^2]	27.6 ± 5.5	27.0 ± 5.3	28.1 ± 5.7	0.361
labMELD score	18.1 ± 8.5	19.9 ± 8.1	16.4 ± 8.6	0.061
Reason for transplantation				
Alcoholic Cirrhosis [n]	32 (38.1%)	15 (35.7%)	17 (40.5%)	0.596
Hepatocellular Carcinoma [n]	23 (27.4%)	9 (21.4%)	14 (33.3%)	
Acute Liver Failure [n]	4 (4.8%)	3 (7.1%)	1 (2.4%)	
Primary Biliary Cirrhosis [n]	4 (4.8%)	2 (4.8%)	2 (4.8%)	
HBV or HCV Cirrhosis [n]	5 (6.0%)	3 (7.1%)	2 (4.8%)	
Graft Failure [n]	3 (3.6%)	3 (7.1%)	0 (0.0%)	
Nonalcoholic Steatohepatitis [n]	4 (4.8%)	2 (4.8%)	2 (4.8%)	
Other [n]	9 (10.7%)	5 (11.9%)	4 (9.5%)	
Preoperative Clinical Chemistry				
Creatinine [mg/dl]	1.6 ± 1.5	1.8 ± 1.5	1.4 ± 1.4	0.189
AST [U/L]	302.5 ± 1432.2	518.5 ± 1998.9	80.6 ± 100.7	0.188
ALT [U/L]	163.3 ± 721.9	269.5 ± 1007.9	54.2 ± 55.3	0.205
Bilirubin [mg/dL]	5.7 ± 7.5	7.0 ± 8.5	4.3 ± 6.3	0.113
GGT [U/L]	167.1 ± 234.5	160.8 ± 278.4	173.4 ± 184.2	0.817
GLDH [U/L]	132.9 ± 871.3	275.2 ± 1274.6	8.8 ± 10.6	0.249

BMI: body mass index; labMELD: laboratory model of end-stage liver disease; HBV: hepatitis B virus; HCV: hepatitis C virus; AST: aspartate transaminase: ALT: alanine transaminase; GGT: gamma glutamyl transpeptidase; GLDH: glutamate dehydrogenase; 4F-PCC: four-factor prothrombin complex concentrate.

Outcomes

Differences in outcome between liver graft recipients who were treated with aprotinin compared to recipients who were not treated with aprotinin are displayed in **Table 3**. During the intraoperative period, recipients did not differ regarding the incidence of postreperfusion syndrome (52.4% vs. 47.6%; p = 0.414)

and hyperfibrinolysis (7.1% vs. 9.5%; p = 1.0). Similarly, the number of transfusion units of packed red blood cells, fresh frozen plasma and platelets as well as the amount of fibrinogen and four-factor prothrombin complex concentrate did not differ between liver graft recipients who were treated with aprotinin compared to liver graft recipients who were not.

Table 2: Demographic and clinical characteristics of the liver graft donors and grafts data for 42 liver graft recipients who were intraoperatively treated with aprotinin and 42 controls. The 42 statistically similar recipients who were not treated with aprotinin were identified by propensity score matching in a cohort of 290 single center liver graft recipients. Propensity scores were calculated using organ quality, cold ischemia time and donor age.

	Matched Cohort (n = 84)	Aprotinin (n = 42)	Controls (n = 42)	p
Age [years]	60.3 ± 12.7	59.0 ± 10.8	61.6 ± 14.5	0.354
Sex [female]	41 (48.8%)	24 (57.1%)	17 (40.5%)	0.127
BMI [kg/cm^2]	32.5 ± 8.5	34.7 ± 8.9	30.3 ± 7.5	0.018
Clinical chemistry				
AST [U/L]	99.3 ± 188.1	127.9 ± 256.8	72.2 ± 75.9	0.869
ALT [U/L]	64.7 ± 87.3	85.7 ± 114.2	43.6 ± 38.6	0.032
Bilirubin [mmol/L]	1.0 ± 1.2	0.7 ± 0.9	1.1 ± 1.5	0.193
Sodium [mmol/L]	148.9 ± 7.5	149.4 ± 7.9	148.4 ± 7.2	0.182
ICU length of stay [days]	6.2 ± 8.8	6.1 ± 9.7	6.4 ± 8.0	0.587
Organ Quality[a]				
good	24 (28.6%)	12 (28.6%)	12 (28.6%)	0.960
acceptable	43 (51.2%)	22 (52.4%)	21 (50%)	
poor	17 (20.2%)	8 (19%)	9 (21.4%)	
Graft fat content				
Macrovesicular [%]	27.8 ± 24.2	32.1 ± 23.0	23.5 ± 25.1	0.319
Microvesicular [%]	46.8 ± 26.5	50.6 ± 22.9	42.7 ± 29.9	0.246
Time to transplantation				
Cold Ischemia Time [min]	517.1 ± 128.0	524.6 ± 137.5	509.5 ± 119.0	0.281
Warm Ischemia Time [min]	45.2 ± 8.5	46.2 ± 8.0	44.2 ± 9.1	0.593
Extended donor criteria				
Age > 65 years [n]	29 (34.5%)	12 (28.6%)	17 (40.5%)	0.340
BMI > 30 [n]	36 (42.9%)	24 (57.1%)	12 (28.6%)	0.008
ICU stay > 7 days [n]	18 (21.4%)	7 (16.7%)	11 (26.2%)	0.287
Elevated Transaminases[b] [n]	15 (17.9%)	10 (23.8%)	5 (11.9%)	0.154
CIT > 10 h	21 (25%)	12 (28.6%)	9 (21.4%)	0.450
Bilirubin > 3 mmol/L	6 (7.1%)	1 (2.4%)	5 (11.9%)	0.645
Steatosis > 40%	35 (41.7%)	20 (47.6%)	15 (35.7%)	0.268

Sodium > 165 mmol/L	5 (6.0%)	2 (4.8%)	3 (7.1%)	0.645
Number of Extented donor criteria				
0 [n]	8 (9.5%)	4 (9.5%)	4 (9.5%)	0.682
1 or 2 [n]	60 (71.4%)	28 (66.6%)	32 (76.2%)	
≥ 3 [n]	16 (19.0%)	10 (23.8%)	6 (14.3%)	

[a]organ quality definition as described by Kork et al.[7]; [b]ALT > 105 U/L or AST > 90 U/L per definition of the marginal donor criteria of Eurotransplant [25]

BMI: body mass index; AST: aspartate transaminase: ALT: alanine transaminase; ICU: intensive care unit; GGT: gamma glutamyl transpeptidase; GLDH: glutamate dehydrogenase; 4F-PCC: four-factor prothrombin complex concentrate.

Liver graft recipients who were treated with aprotinin had more postoperative complications than recipients who were not treated with aprotinin: They suffered more often from early allograft dysfunction (64.3% vs. 40.5%; p = 0.029), suffered more often from acute kidney injury (48.8% vs. 26.2%; p = 0.033) and required more often renal replacement therapy (24.4% vs. 7.1%; p = 0.015) than controls. Multivariable regression analysis confirmed this finding: Only intraoperative treatment with aprotinin was associated with a 4-fold (OR 4.12, 95%CI 1.21–14.00; p = 0.023), acceptable donor organ quality with a 5-fold (OR 4.95, 95%CI 1.26–19.5; p =0.022) and poor donor organ quality with a 12-fold (OR 11.77, 95%CI 2.00–69.5, p = 0.007) risk for developing early allograft dysfunction after adjusting for for recipient age, sex, BMI and labMELD, donor age, BMI and AST, cold ischemic time and organ quality (**Table 4**). An additional sensitivity analysis reconfirmed this: Matching of a cohort using a propensity score including age, sex, BMI, labMELD, donor age, donor BMI, donor AST, cold ischemia time and organ quality led to a cohort of 70 liver graft recipients, 35 of whom were treated with aprotinin and 35 of whom were not. In this alternate matched cohort, recipient and donor data did not differ in any of the above reported variables, but recipients still suffered more often from early allograft dysfunction when treated with aprotinin compared to controls (68.6% vs. 37.1%, p = 0.017; data not shown).

In accordance with the higher incidence of early allograft dysfunction, recipients who were treated with aprotinin had higher maximum AST (3193.6 ± 2273.5 vs. 1984.2 ± 1883.9; p = 0.010) and ALT (1611.3 ± 1152.4 vs. 975.2 ± 937.4; p = 0.007) after transplantation of the graft. A multivariable linear regression model confirmed this by demonstrating that having been treated intraoperatively with aprotinin was associated with a 1.324 U/L (95%CI 354–2.295) higher maximum AST after adjusting for recipient age, sex, BMI and labMELD, donor age, BMI and AST, cold ischemic time and organ quality (**Table 4**).

Liver graft recipients treated with aprotinin did not differ regarding mortality from recipients not treated with aprotinin: 30-day mortality, 1-year mortality, and overall mortality (p > 0.152; **Table 3**) as well as recipient survival time and graft survival time did not differ from controls (**Figure 1**).

Table 3: Intraoperative and postoperative complications and mortality of 42 liver graft recipients who were intraoperatively treated with aprotinin and 42 propensitiy score matched controls. The 42 statistically similar recipients who were not treated with aprotinin were identified by propensity score matching in a cohort of 290 single center liver graft recipients. Propensity scores were calculated using organ quality, cold ischemia time and donor age.

	Matched Cohort (n = 84)	Aprotinin (n = 42)	Controls (n = 42)	p
Intraoperative Complications				
Postreperfusion Syndrome [n]	42 (50%)	22 (52.4%)	20 (47.6%)	0.414
Hyperfibrinolysis [n]	7 (8.3%)	3 (7.1%)	4 (9.5%)	1.000
Intraoperative Transfusions				
Packed Red Blood Cells [U]	11.3 ± 23.3	13.7 ± 32.0	8.8 ± 6.9	0.374
Fresh Frozen Plasma [U]	16.2 ± 9.3	15.2 ± 9.0	17.1 ± 9.5	0.380
Platelets [U]	1.0 ± 1.2	1.2 ± 1.2	0.8 ± 1.1	0.191
Fibrinogen [g]	3.0 ± 3.2	3.6 ± 3.2	2.4 ± 3.0	0.094
4F-PCC [IU]	1090.5 ± 1581.9	1200.0 ± 1450.1	986.8 ± 1710.4	0.566
Postoperative Complications				
Early Allograft Dysfunction [n]	44 (52.4%)	27 (64.3%)	17 (40.5%)	0.029
Rejection Episodes [n]	18 (21.4%)	12 (14.3%)	6 (7.1%)	0.241
Acute Kidney Injury [n]	31 (37.3%)	20 (48.8%)	11 (26.2%)	0.033
Renal Replacement Therapy [n]	13 (15.7%)	10 (24.4%)	3 (7.1%)	0.015
Retransplantation				
30 Day Retransplantation [n]	5 (6.0%)	4 (9.5%)	1 (2.4%)	0.167
1 Year Retransplantation [n]	7 (8.3%)	4 (9.5%)	3 (7.1%)	0.693
Reasons for Retransplantation				
Arterial Thrombosis [n]	1 (2.4%)	1 (2.4%)	0	0.306
Primary Non-Function [n]	4 (4.8%)	3 (7.1%)	1 (2.4%)	
Ischemic Type Biliary Lesions [n]	2 (4.8%)	0 (0.0%)	2 (4.8%)	
Tumor [n]	1 (2.4%)	0 (0.0%)	1 (2.4%)	
Postoperative Clinical Chemistry				
Creatinine, peak [mg/dL]	2.7 ± 1.9	2.7 ± 1.6	2.6 ± 2.2	0.812
AST, peak [U/L]	2588.9 ± 2162.5	3193.6 ± 2273.5	1984.2 ± 1883.9	0.010

ALT, peak [U/L]	1293.3 ± 1092.0	1611.3 ± 1152.4	975.2 ± 937.4	0.007
Bilirubine, peak [mg/L]	6.9 ± 4.7	7.6 ± 5.1	6.2 ± 4.2	0.167
GGT, peak [U/L]	463.5 ± 430.3	502.9 ± 499.7	424.1 ± 349.1	0.405
GLDH, peak [U/L]	1100.9 ± 1210.4	1199.4 ± 894.8	1002.3 ± 1464.7	0.459
Mortality				
30 Day Mortality [n]	2 (2.4%)	2 (4.8%)	0 (0.0%)	0.152
1 Year Mortality [n]	9 (10.7%)	5 (11.9%)	4 (9.5%)	0.724
Overall Mortality [n]	13 (15.5%)	6 (14.3%)	7 (16.7%)	0.763

4F-PCC: four-factor prothrombin complex concentrate; AST: aspartate transaminase: ALT: alanine transaminase; ICU: intensive care unit; GGT: gamma glutamyl transpeptidase; GLDH: glutamate dehydrogenase;

Table 4: Intraoperative treatment with aprotinin is associated with early allograft dysfunction (EAD) and peak aspartate transaminase (AST) after liver graft transplantation. Two multivariable models in a cohort of 84 liver graft recipients (42 treated with aprotinin and 42 propensity score matched controls) describe this association. On the left, a binary logistic regression model for EAD and on the right a linear regression model for peak AST.

	Early Allograft Dysfunction			peak AST after Transplantation		
	OR	(95%CI)	p	beta	(95%CI)	p
Recipient data						
Age [years]	0.95	(0,86–1,04)	0.242	-33.4	(-107.8–41.0)	0.373
Sex [female]	0.64	(0,15–2,66)	0.534	-437.6	(-1632.9–757.7)	0.467
BMI [kg/cm^2]	1.02	(0,9–1,15)	0.768	-19.0	(-120.3–82.3)	0.709
labMELD score	0.98	(0,92–1,06)	0.671	-47.4	(-104.7–10.0)	0.104
Donor data						
Age [years]	0.98	(0,94–1,03)	0.478	-64.0	(-103.7–-24.3)	0.002
BMI [kg/cm^2]	0.96	(0,89–1,02)	0.181	-22.9	(-79.6–33.9)	0.423
AST [U/L]	1.00	(1,00–1,00)	0.833	-2.0	(-4.6–0.5)	0.115
Cold Ischemia Time [minutes]	1.00	(1,00–1,01)	0.994	1.4	(-2.5–5.2)	0.483
Organ Quality[a]						
acceptable	4.95	(1,26–19,46)	0.022	1770.0	(814.4–3031.2)	0.001
poor	11.78	(1,99–69,55)	0.007	1324.8	(387.6–3152.4)	0.013
Treatment with Aprotinin	4.12	(1,21–14,00)	0.023	-33.4	(354.0–2295.6)	0.008

[a]compared to good organ quality, definition as described Kork et al. [7]

BMI: body mass index; labMELD: laboratory model of end-stage liver disease; AST: aspartate transaminase.

Conclusions

In this single center retrospective analysis of 84 propensity score matched liver graft recipients of organs from extended criteria donors, we sought to determine the association of intraoperative treatment of aprotinin with hyperfibrinolysis, PRS, EAD and mortality. We found that patients receiving intraoperative aprotinin did not differ in incidence of intraoperative hyperfibrinolysis, PRS or mortality compared to controls. However, liver graft recipients suffered more often from EAD (64% vs. 41%) and had higher postoperative peak transaminases compared to controls. In fact, multivariable regression analyses determined intraoperative treatment with aprotinin to be independently associated with a 4-fold risk of

EAD and 1.300 U/L higher peak AST after liver graft transplantation in patients receiving organs from extended criteria donors.

All of the 84 liver graft recipients in this analysis treated at the same center with the same operative technique (intraoperative venovenous/portalvenous bypass) [21] and with an SOP guided intra-operative management [7]. Although this has led to a homogenous single center study sample, it also limits the external validity of the results. Limited external validity is a common problem when analyzing liver transplant patient data. In 2019 in Germany alone, there were 22 liver transplanting centers transplanting 1,571 liver grafts, each with its own characteristic treatment modalities. However, this limitation will only be overcome by an efficient multicenter registry that collects comprehensive perioperative datasets of adequate granularity. Furthermore, the retrospective design of our analyses could have impaired data quality.

In our sample, the incidence of EAD was 52%. While the incidence of EAD has been reported in the literature between 23% [26] and 39% [27], the notably high incidence in our sample is most likely due to the fact over 90% of the patients analyzed in this study received a liver graft from a donor that fulfilled at least one criterium for an extended donor; i.e. the high incidence of EAD may be attributable to the transplantation of marginal organs with a high proportion of moderate and poor organs containing above-average microvesicular and macrovesicular fat. Evidence describing risk factors for EAD is rather scarce but moreover limited by the multitude of treatment modalities as described above. Evidence suggests that mainly graft steatosis, recipient MELD score and CIT [7] but also donor BMI [26-28] are risk factors for EAD. Our sample may have been biased with regard to this fact, as donors for patients treated with aprotinin had a tendentiously higher BMI compared to controls. This may be due to fact that we considered only donor age, organ quality and CIT for matching, as those were the criteria for the clinical consensus decision to treat the graft recipient with aprotinin. Interestingly, it could be that – although considering only those three criteria – transplant surgeon and attending anesthesiologist inadvertently chose recipients of organs with higher fat content or poorer quality to be treated with aprotinin. However, a sensitivity analysis controlling especially for these potential confounders confirmed the independent association of aprotinin with the increased risk of EAD.

Aprotinin is a protease inhibitor derived from bovine or porcine lungs. As such, aprotinin inhibits human proteases as e.g. trypsin, kallikrein but moreover plasmin, thus decelerating hyperfibrinolysis. In 1989, aprotinin was first demonstated to reduce blood loss, transfusion requirements and duration of surgery in liver graft recipients [14]. A finding that was later confirmed in single and multicenter studies [29], [30], [31], [10]. We could not demonstrate any effect of aprotinin on hyperfibrinolysis in our sample. This may be attributed to the small sample size of the matched cohort in combination with the overall low hyperfibrinolysis incidence of 8.3% but also due to our standardized transfusion regime [7, 21]. Moreover, evidence questioning the usefulness of aprotinin cumulated since large multicenter trials had found an increased risk of major cardiac events, stroke and mortality in cardiac-surgery patients [32], [33]. This ultimately led to the withdrawal of aprotinin in many countries. A study by Schofield and colleagues

suggests that although the incidence of hyperfibrinolysis is higher in patients not receiving aprotinin, there has been no increase in transfusion requirements [34].

Renal impairment

Molenaar et al. described an improvement in EAD by aprotinin [9]. Improvement in EAD could not be detected in our patients applying aprotinin. Surgical variables as to cold ischemia times were not different between our studies. Differences in aprotinin concentration have to be considered for these divergent results. From studies in cardiac surgery aprotinin has been described to ameliorate the systemic inflammatory response due to cardiopulmonary bypass [35].

Aprotinin is a serine protease inhibitor. Of these proteases, interaction with plasmin and kallikrein are likely to be the most important for haemostasis and the reduction of inflammation. Additionally, aprotinin also inhibits matrix metalloproteinases (MMP). MMPs are zinc-binding proteolytic enzymes, who are responsible for the degradation of extracellular matrix proteins and basement membranes. The main injury target in the liver following cold storage as well for ischemia reperfusion injury are the liver sinusoidal endothelial cells (LSEC) [36]. An 8-fold increase of MMP-9-levels have been shown 30 min after reperfusion in human OLTs [15]. The inhibition of MMP especially MMP-9 in liver injury has a relevant impact on attenuation and repair of LSEC [36]. An intravenous infusion of aprotinin during the liver transplantation leads to an unselected inhibition of MMP's hereby inhibiting bone marrow progenitor cells which are needed to repair injured LSEC. Wang et al. described an even more pronounced effect when the MMP-2,9 inhibitor is directly injected to the donor organ in a steatotic rat liver I/R model [36]. This might be an interesting approach while using hypothermic machine perfusion for marginal liver grafts. In lung transplantation addition of aprotinin to organ preservation solution decreases reperfusion injury significantly [37].

Aprotinin is transiently stored in the tubular cells for 5-6 days [38]. This might help explain the significant difference of renal impairment in aprotinin treated patients within one week after the transplantation. The 30 day as well as the 1-year survival in the aprotinin treated patients did not differ. The influence of aprotinin on renal function in liver transplant patients has been described to a similar extend by Warnaar et al. [11]. The incidence on hyperfibrinolysis has been expected to be higher, especially in steatotic donor organs.

A bias in patients' selection for aprotinin treatment may not be ruled out due to the treating anesthesiologists experience in transplantation of EDC organs as well the clinical impression of the patient awaiting the liver transplantation. Histological quantification of hepatic steatosis is strongly observer dependent and not always reproducible at the transplant site [22, 28, 39].

Cold ischemic time (CIT) is an independent risk factor of early as well as delayed organ dysfunction [40]. PRS may result in a condition of severe hemodynamic compromise of the recipient as well leading to hyperfibrinolysis. Two major risk factors for PRS are steatosis of the graft liver as well as the cold ischemic time (CIT) [7, 40].

This may help to explain why the survival rate after liver transplantation has declined after implementation of the MELD system [2]. Especially due to the estimated decrease in transplantable organs due to diabetes, obesity and aging population graft utilization will decrease from 78% in 2010 to 44% in 2030 in the United States. This will additionally influence the outcome for patients on the waiting list [36, 41].

Regarding our results presented in this study we changed our standard protocol for liver transplantation in and do not further applicate aprotinin regularly. The idea of pharmacologic preconditioning by aprotinin of the OLT recipient in case of liver transplantation of ECD organs did not improve the short nor the long-term outcome in this study.

Abbreviations

ECD extended criteria donor

OLT orthotopic liver transplantation

PRS postreperfusion syndrome

EAD early allograft dysfunction

ICU intensive care unit

MELD model of end stage liver disease

ALT alanine transaminase

AST aspartate transaminase

GGT gamma glutamyl transpeptidase

GLDH glutamate dehydrogenase

HBV hepatitis B virus

HCV hepatitis C virus

Bili bilirubin

RBC red blood cell units

SOP standard operation procedure

TEM thrombelastometry

CIT cold ischemic time

FFP fresh frozen plasma

4F-PCC 4 factor prothrombin complex concentrate

RRT renal replacement therapy

LOS length of stay

POD postoperative day

MAP mean arterial pressure

NE norepinephrine

BW body weight

BMI body mass index

OR odds ratio

MMP matrix metalloproteinase

LSEC liver sinusoidal endothelial cells

Declarations

Autors' contributions

AR, AA, MH designed the research. AR, MH, FK, KJ, UN participated in the acquisition of the data. AR, FK, MH conducted the data analysis. AR drafted the manuscript. FK and MH participated in writing the manuscript, all authors interpreted the data and revised the manuscript critically for important intellectual content, approved the final version to be published and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

The local ethics committee (University Hospital Aachen, EK 291/13) approved the analysis and waived the requirement of informed consent.

Consent for publication:

All authors aggree for publication.

Competing interests:

The authors declare that they have no competing interests

References

1. Manns, M. P., Liver cirrhosis, transplantation and organ shortage. *Dtsch Arztebl Int* **2013,** 110, (6), 83-4.

2. Weismuller, T. J.; Negm, A.; Becker, T.; Barg-Hock, H.; Klempnauer, J.; Manns, M. P.; Strassburg, C. P., The introduction of MELD-based organ allocation impacts 3-month survival after liver transplantation by influencing pretransplant patient characteristics. *Transpl Int* **2009,** 22, (10), 970-8.

3. Kamath, P. S.; Wiesner, R. H.; Malinchoc, M.; Kremers, W.; Therneau, T. M.; Kosberg, C. L.; D'Amico, G.; Dickson, E. R.; Kim, W. R., A model to predict survival in patients with end-stage liver disease. *Hepatology* **2001,** 33, (2), 464-70.

4. Tacke, F.; Kroy, D. C.; Barreiros, A. P.; Neumann, U. P., Liver transplantation in Germany. *Liver Transpl* **2016,** 22, (8), 1136-42.

5. Schlitt, H. J.; Loss, M.; Scherer, M. N.; Becker, T.; Jauch, K. W.; Nashan, B.; Schmidt, H.; Settmacher, U.; Rogiers, X.; Neuhaus, P.; Strassburg, C., [Current developments in liver transplantation in Germany: MELD-based organ allocation and incentives for transplant centres]. *Z Gastroenterol* **2011,** 49, (1), 30-8.

6. Czigany, Z.; Lurje, I.; Schmelzle, M.; Schoning, W.; Ollinger, R.; Raschzok, N.; Sauer, I. M.; Tacke, F.; Strnad, P.; Trautwein, C.; Neumann, U. P.; Fronek, J.; Mehrabi, A.; Pratschke, J.; Schlegel, A.; Lurje, G., Ischemia-Reperfusion Injury in Marginal Liver Grafts and the Role of Hypothermic Machine Perfusion: Molecular Mechanisms and Clinical Implications. *J Clin Med* **2020,** 9, (3).

7. Kork, F.; Rimek, A.; Andert, A.; Becker, N. J.; Heidenhain, C.; Neumann, U. P.; Kroy, D.; Roehl, A. B.; Rossaint, R.; Hein, M., Visual quality assessment of the liver graft by the transplanting surgeon predicts postreperfusion syndrome after liver transplantation: a retrospective cohort study. *BMC Anesthesiol* **2018,** 18, (1), 29.

8. Lentschener, C.; Roche, K.; Ozier, Y., A review of aprotinin in orthotopic liver transplantation: can its harmful effects offset its beneficial effects? *Anesth Analg* **2005,** 100, (5), 1248-55.

9. Molenaar, I. Q.; Veldman, M.; Begliomini, B.; Groenland, H. N.; Januszkiewicz, A.; Lindgren, L.; Metselaar, H. J.; Terpstra, O. T.; Porte, R. J., Improved early graft survival in patients receiving aprotinin during orthotopic liver transplantation. *Transplant Proc* **2001,** 33, (1-2), 1345-6.

10. Porte, R. J.; Molenaar, I. Q.; Begliomini, B.; Groenland, T. H.; Januszkiewicz, A.; Lindgren, L.; Palareti, G.; Hermans, J.; Terpstra, O. T., Aprotinin and transfusion requirements in orthotopic liver transplantation: a multicentre randomised double-blind study. EMSALT Study Group. *Lancet* **2000,** 355, (9212), 1303-9.

11. Warnaar, N.; Mallett, S. V.; de Boer, M. T.; Rolando, N.; Burroughs, A. K.; Nijsten, M. W.; Slooff, M. J.; Rolles, K.; Porte, R. J., The impact of aprotinin on renal function after liver transplantation: an

analysis of 1,043 patients. *Am J Transplant* **2007,** 7, (10), 2378-87.

12. Warnaar, N.; Mallett, S. V.; Klinck, J. R.; de Boer, M. T.; Rolando, N.; Burroughs, A. K.; Jamieson, N. V.; Rolles, K.; Porte, R. J., Aprotinin and the risk of thrombotic complications after liver transplantation: a retrospective analysis of 1492 patients. *Liver Transpl* **2009,** 15, (7), 747-53.

13. Royston, D.; De Hert, S.; van der Linden, J.; Ouattara, A.; Zacharowski, K., A special article following the relicence of aprotinin injection in Europe. *Anaesth Crit Care Pain Med* **2017,** 36, (2), 97-102.

14. Neuhaus, P.; Bechstein, W. O.; Lefebre, B.; Blumhardt, G.; Slama, K., Effect of aprotinin on intraoperative bleeding and fibrinolysis in liver transplantation. *Lancet* **1989,** 2, (8668), 924-5.

15. Kuyvenhoven, J. P.; Molenaar, I. Q.; Verspaget, H. W.; Veldman, M. G.; Palareti, G.; Legnani, C.; Moolenburgh, S. E.; Terpstra, O. T.; Lamers, C. B.; van Hoek, B.; Porte, R. J., Plasma MMP-2 and MMP-9 and their inhibitors TIMP-1 and TIMP-2 during human orthotopic liver transplantation. The effect of aprotinin and the relation to ischemia/reperfusion injury. *Thromb Haemost* **2004,** 91, (3), 506-13.

16. Bittner, H. B.; Richter, M.; Kuntze, T.; Rahmel, A.; Dahlberg, P.; Hertz, M.; Mohr, F. W., Aprotinin decreases reperfusion injury and allograft dysfunction in clinical lung transplantation. *Eur J Cardiothorac Surg* **2006,** 29, (2), 210-5.

17. Lie, T. S.; Seger, R.; Hong, G. S.; Preissinger, H.; Ogawa, K., Protective effect of aprotinin on ischemic hepatocellular damage. *Transplantation* **1989,** 48, (3), 396-9.

18. Maksan, S. M.; Maksan, M. O.; Gebhard, M. M.; Herfarth, C.; Klar, E., Reduction of hepatic reperfusion injury by antithrombin III and aprotinin. *Transpl Int* **2000,** 13 Suppl 1, S562-4.

19. Tuffs, A., Bayer withdraws heart surgery drug. *BMJ* **2007,** 335, (7628), 1015.

20. von Elm, E.; Altman, D. G.; Egger, M.; Pocock, S. J.; Gotzsche, P. C.; Vandenbroucke, J. P.; Initiative, S., The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* **2007,** 370, (9596), 1453-7.

21. Mossdorf, A.; Ulmer, F.; Junge, K.; Heidenhain, C.; Hein, M.; Temizel, I.; Neumann, U. P.; Schoning, W.; Schmeding, M., Bypass during Liver Transplantation: Anachronism or Revival? Liver Transplantation Using a Combined Venovenous/Portal Venous Bypass-Experiences with 163 Liver Transplants in a Newly Established Liver Transplantation Program. *Gastroenterol Res Pract* **2015,** 2015, 967951.

22. Yersiz, H.; Lee, C.; Kaldas, F. M.; Hong, J. C.; Rana, A.; Schnickel, G. T.; Wertheim, J. A.; Zarrinpar, A.; Agopian, V. G.; Gornbein, J.; Naini, B. V.; Lassman, C. R.; Busuttil, R. W.; Petrowsky, H., Assessment of hepatic steatosis by transplant surgeon and expert pathologist: a prospective, double-blind evaluation of 201 donor livers. *Liver Transpl* **2013,** 19, (4), 437-49.

23. McCormack, L.; Dutkowski, P.; El-Badry, A. M.; Clavien, P. A., Liver transplantation using fatty livers: always feasible? *J Hepatol* **2011,** 54, (5), 1055-62.

24. Gorlinger, K., [Coagulation management during liver transplantation]. *Hamostaseologie* **2006,** 26, (3 Suppl 1), S64-76.

25. Eurotransplant, In *Annual Report* 2013; Vol. http://www.eurotransplant.org/wp-content/uploads/2019/12/AR2015.pdf.

26. Olthoff, K. M.; Kulik, L.; Samstein, B.; Kaminski, M.; Abecassis, M.; Emond, J.; Shaked, A.; Christie, J. D., Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* **2010,** 16, (8), 943-9.

27. Hoyer, D. P; Paul, A.; Gallinat, A.; Molmenti, E. P; Reinhardt, R.; Minor, T.; Saner, F. H.; Canbay, A.; Treckmann, J. W.; Sotiropoulos, G. C.; Mathe, Z., Donor information based prediction of early allograft dysfunction and outcome in liver transplantation. *Liver Int* **2015,** 35, (1), 156-63.

28. Andert, A.; Becker, N.; Ulmer, F.; Schoning, W.; Hein, M.; Rimek, A.; Neumann, U.; Schmeding, M., Liver Transplantation and Donor Body Mass Index >30: Use or Refuse? *Ann Transplant* **2016,** 21, 185-93.

29. Patrassi, G. M.; Viero, M.; Sartori, M. T.; De Silvestro, G.; Rossaro, L.; Burra, P.; Nolli, M. L.; Piccinni, P.; Bassi, N., Aprotinin efficacy on intraoperative bleeding and transfusion requirements in orthotopic liver transplantation. *Transfusion* **1994,** 34, (6), 507-11.

30. Marcel, R. J.; Stegall, W. C.; Suit, C. T.; Arnold, J. C.; Vera, R. L.; Ramsay, M. A.; O'Donnell, M. B.; Swygert, T. H.; Hein, H. A.; Whitten, C. W., Continuous small-dose aprotinin controls fibrinolysis during orthotopic liver transplantation. *Anesth Analg* **1996,** 82, (6), 1122-5.

31. Molenaar, I. Q.; Begliomini, B.; Martinelli, G.; Putter, H.; Terpstra, O. T.; Porte, R. J., Reduced need for vasopressors in patients receiving aprotinin during orthotopic liver transplantation. *Anesthesiology* **2001,** 94, (3), 433-8.

32. Mangano, D. T.; Tudor, I. C.; Dietzel, C.; Multicenter Study of Perioperative Ischemia Research, G.; Ischemia, R.; Education, F., The risk associated with aprotinin in cardiac surgery. *N Engl J Med* **2006,** 354, (4), 353-65.

33. Fergusson, D. A.; Hebert, P. C.; Mazer, C. D.; Fremes, S.; MacAdams, C.; Murkin, J. M.; Teoh, K.; Duke, P. C.; Arellano, R.; Blajchman, M. A.; Bussieres, J. S.; Cote, D.; Karski, J.; Martineau, R.; Robblee, J. A.; Rodger, M.; Wells, G.; Clinch, J.; Pretorius, R.; Investigators, B., A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. *N Engl J Med* **2008,** 358, (22), 2319-31.

34. Schofield, N.; Sugavanam, A.; Thompson, K.; Mallett, S. V., No increase in blood transfusions during liver transplantation since the withdrawal of aprotinin. *Liver Transpl* **2014,** 20, (5), 584-90.

35. Sodha, N. R.; Boodhwani, M.; Sellke, F. W., Is there still a role for aprotinin in cardiac surgery? *Drug Saf* **2007,** 30, (9), 731-40.

36. Wang, X.; Walkey, C. J.; Maretti-Mira, A. C.; Wang, L.; Johnson, D. L.; DeLeve, L. D., Susceptibility of rat steatotic liver to ischemia-reperfusion is treatable with liver-selective matrix metalloproteinase inhibition. *Hepatology* **2020**.

37. Roberts, R. F.; Nishanian, G. P.; Carey, J. N.; Darbinian, S. H.; Kim, J. D.; Sakamaki, Y.; Chang, J. Y.; Starnes, V. A.; Barr, M. L., Addition of aprotinin to organ preservation solutions decreases lung reperfusion injury. *Ann Thorac Surg* **1998,** 66, (1), 225-30.

38. Baran, D.; Tenstad, O.; Aukland, K., Aprotinin uptake in the proximal tubules in the rat kidney. II. Uptake site relative to glomerulus. *J Struct Biol* **2003,** 142, (3), 409-15.

39. El-Badry, A. M.; Breitenstein, S.; Jochum, W.; Washington, K.; Paradis, V.; Rubbia-Brandt, L.; Puhan, M. A.; Slankamenac, K.; Graf, R.; Clavien, P. A., Assessment of hepatic steatosis by expert pathologists:

the end of a gold standard. *Ann Surg* **2009,** 250, (5), 691-7.

40. Pezzati, D.; Ghinolfi, D.; De Simone, P.; Balzano, E.; Filipponi, F., Strategies to optimize the use of marginal donors in liver transplantation. *World J Hepatol* **2015,** 7, (26), 2636-47.

41. Orman, E. S.; Mayorga, M. E.; Wheeler, S. B.; Townsley, R. M.; Toro-Diaz, H. H.; Hayashi, P. H.; Barritt, A. S. t., Declining liver graft quality threatens the future of liver transplantation in the United States. *Liver Transpl* **2015,** 21, (8), 1040-50.
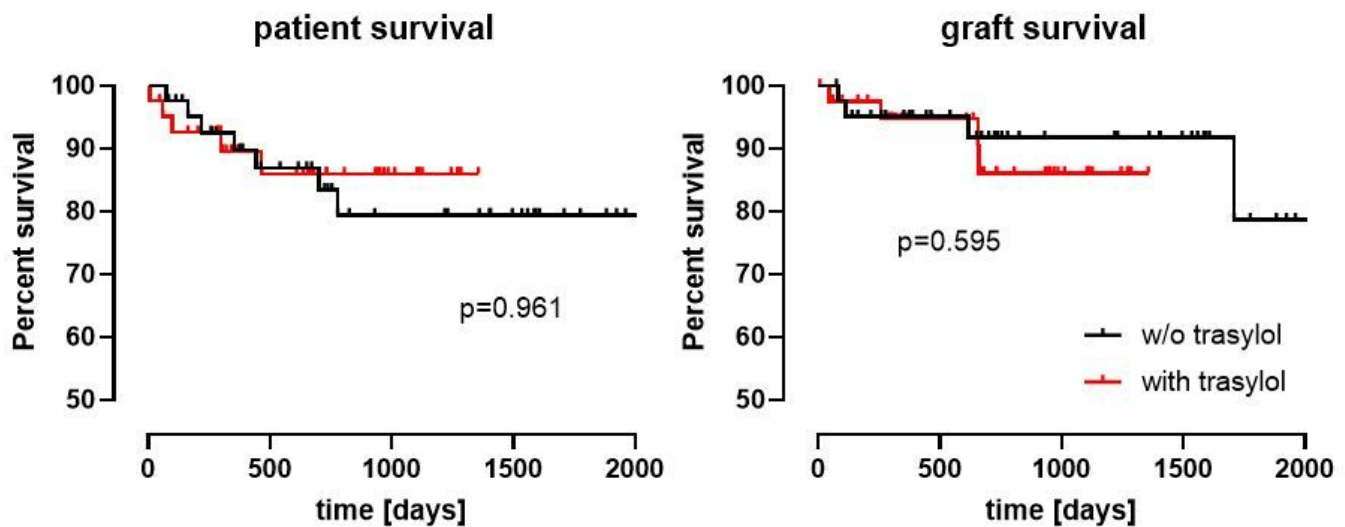
Figures

Figure 1

Patient survival (left) and graft survival (right) did not differ between 42 liver graft recipients receiving aprotinin and 42 matched controls.