Clean version (Manuscript ID LT-16-389)

Title:

Successful donation after cardiac death liver transplants with prolonged warm ischemia time using normothermic regional perfusion

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Key words:

uncontrolled donors; machine perfusion; liver preservation; ischemic cholangiopathy; ischemic injury

Abbreviations:

ALT, alanine transaminase

CIT, cold ischemia time

DBD, donation after brain death

DCD, donation after cardiac death

ECMO, extracorporeal membrane oxygenation

EGD, early graft dysfunction

HCC, hepatocellular carcinoma

HMP, hypothermic machine perfusion

IC, ischemic cholangiopathy

MELD, Model for End-stage Liver Disease

NRP, normothermic regional perfusion

PNF, primary non-function

POD, postoperative day

WIT, warm ischemia time

Grants and financial support: Nothing to report

Conflict of interest: Nothing to report

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Abstract

The role of donation after cardiac death (DCD) in expanding the donor pool is mainly limited by the incidence of primary non-function (PNF) and ischemia-related complications. Even greater concern exists towards uncontrolled DCD, which represents the largest potential pool of DCD donors. We recently started the first Italian series of DCD liver transplantation, using normothermic regional perfusion (NRP) in 6 uncontrolled donors and in 1 controlled case to deal with the legally required no-touch period of 20 minutes. We examined our first 7 cases for the incidence of PNF, early graft dysfunction, and biliary complications. Acceptance of the graft was based upon the trend of serum transaminase and lactate during NRP, the macroscopic appearance, and the liver biopsy. Hypothermic machine perfusion (HMP) was associated in selected cases to improve cold storage. Most notably, no cases of PNF were observed. Median post-transplant transaminase peak was 1014 IU/L (range 393 – 3268). Patient and graft survival were both 100% after a mean follow up of 6.1 months (range 3 – 9). No cases of ischemic cholangiopathy occurred during the follow-up. The only one anastomotic stricture completely resolved with endoscopic stenting. In conclusion, DCD liver transplantation is feasible in Italy despite the protracted no-touch period. The use of NRP and HMP seems to earn good graft function and proves safe in these organs.

Introduction

As regards liver transplantation, the great potential of donation after cardiac death (DCD) in expanding the donor pool is limited by the ischemic injury during circulatory arrest, which makes these organs more prone to primary non-function (PNF) and ischemic cholangiopathy (IC) (1–3). Even greater concern exists towards uncontrolled DCD, which represents the largest potential pool of DCD donors (4). In Italy, death for cardiac arrest is legally declared on the absence of electrocardiographic activity for at least 20 min (5). This obliged no-touch period is much longer when compared to the worldwide practice of 5 min (6). This reason has long prevented the development of any DCD liver transplant program in Italy. In the last few years, normothermic regional perfusion (NRP) and hypothermic machine perfusion (HMP) have proved to limit ischemic injury and allow organ assessment before transplant (7,8). We therefore started in September 2015 the first Italian program of DCD liver transplantation, using NRP in 6 uncontrolled donors and in 1 controlled case (9). We associated HMP in selected cases. We present here the preliminary results of our activity in order to show the feasibility and safety of the procedure, despite the prolonged warm ischemia time (WIT).

Methods

Between September 2015 and March 2016, 9 DCD livers were proposed to our Center, and 7 transplants were performed. Donor and recipient data were collected prospectively. The Italian National Transplant Centre (Ministry of Health) validated the procedure.

Donor management and selection

The donors were managed in two hospitals different from our transplant center, according to local protocols for DCD kidney transplantation, which were adapted to the liver (5).

In the uncontrolled setting, the donors suffered a witnessed cardiac arrest outside the hospital and underwent high-quality cardiopulmonary resuscitation, with mechanical chest compression (AutoPulse®) and mechanical ventilation. Chest compression and ventilation continued with these modalities during transport. Upon the arrival at the emergency department, clinicians judged the cardiac arrest irreversible and not responsive to further treatment. Cardiopulmonary resuscitation was thus interrupted, and continuous ECG registration started (no-touch period). Death was declared following 20 min of absent electrocardiographic activity in continuous registration. Authorization to donation was obtained from the family during the no-touch period. After death declaration at the end of the no-touch period, two large-bore cannulae were placed in the femoral vessels, and a Fogarty balloon catheter was inflated in the supraceliac aorta to isolate the abdominal circulation. Anticoagulation started with full heparinization (3 mg/kg) and was maintained with boluses of 1.5 mg/kg. The NRP circuit relied upon cardiopulmonary bypass and extracorporeal membrane oxygenation (ECMO) technology.

Initial donor selection criteria were age < 65, witnessed cardiac arrest, no history of liver disease, absence of absolute infective and neoplastic contraindications for transplant.

In 3 cases, the patients were considered eligible for extracorporeal life support with ECMO during the resuscitation maneuvers. ECMO started with resuscitative intent after the arrival at the emergency department, but was subsequently judged futile for the onset of complications (thoracic aortic dissection and cardiac thrombosis) and irreversible asystole. ECMO support was thus withdrawn, continuous ECG recording started, and death was declared after 20 min of absent electrocardiographic activity. After death declaration ECMO was re-started and converted into NRP by the exclusion of the supraceliac circulation with a Fogarty balloon. The sequence of events for these and the other uncontrolled donors is summarized in **Figure 1**.

In the only one controlled DCD, the donor sustained stroke with consequent severe brain injury not fulfilling the brain death criteria and hemodynamic instability. Clinicians decided not to take further action in case of spontaneous circulatory arrest. Cardiac arrest occurred spontaneously, without withdrawal of life support measures. Vessel cannulation and NRP followed the same sequence described above.

In every case, total WIT was defined as time from cardiac arrest to the start of NRP. Total no-flow WIT was defined as total time of asystole (no-flow from cardiac arrest to the start of cardiopulmonary resuscitation + the 20-min no-touch period). Low-flow WIT was defined as the length of cardiopulmonary resuscitation.

Liver selection and procurement

Acceptance of each liver was based upon serum alanine transaminase (ALT) and lactate during NRP, the macroscopic aspect, and the liver biopsy. Arterial blood gas analyses including serum lactate were performed hourly during NRP until the retrieval. Liver function tests were obtained until the donor was moved form the intensive care unit, assuring at least 3 samples for each donor. The livers were considered for further evaluation if they did not show a massive elevation in ALT during NRP (>1000 IU/L) and presented a stable or downward trend in serum lactate. Macroscopic evaluation was based upon color, surface, margins, and consistency of the liver. Wedge liver biopsy was performed in all cases during the warm phase of procurement, immediately after incision and abdominal organ inspection. Frozen section results were available before in situ cooling. We accepted livers with macrovesicular steatosis ≤30% and fibrosis not greater than Ishak stage 1. Liver procurement followed the standard technique used for donation after brain death (DBD), including dissection of the hilar elements before in situ cooling. Cold Celsior® solution was flushed directly through the NRP circuit.

Machine perfusion

We used HMP to improve cold storage in case of expected long cold ischemia time (CIT). After organ delivery and back-table surgery, each graft was connected to the HMP (Liver Assist®) and perfused with recirculated cooled (10°C) and oxygenated (60 kPa) Belzer® solution, until the recipient hepatectomy was competed. Mean perfusion pressure was set on 4 mmHg for the portal vein and 25 mmHg for the hepatic artery. The flow was continuous in the portal vein and pulsatile in the hepatic artery (rate 60 bpm). HMP-treatment did not delay implantation.

Recipient selection, transplant procedure, and follow-up

The DCD livers were allocated among patients on waiting list at our center, according to the Italian center-based allocation system (10,11). We excluded as possible recipients patients requiring urgent transplant or retransplantation. Preference was given to patients in our list for liver transplant with multifocal hepatocellular carcinoma (HCC) in progression, within Milan criteria with or without down-staging procedures, but with compensated cirrhosis. Model for End-stage Liver Disease (MELD) score was calculated according to the laboratory tests, without extra-points for HCC. Each recipient was adequately informed about marginal organs and accepted this option at the inclusion in the waiting list. A specific written informed consent to receive a DCD organ was obtained from the recipient at the time the liver was proposed. Each graft was implanted after total caval-sparing hepatectomy without veno-venous bypass, using piggyback technique. The bile duct was reconstructed in all cases with a standard duct-to-duct anastomosis over a T-tube, to facilitate surveillance for biliary complications. The immunosuppressive protocol included induction by basiliximab, tapered steroids, and tacrolimus. Mean length of follow-up was 6.1 months (range 3 – 9). T-tube cholangiography was routinely performed on postoperative day 10 and 3 months post-transplant, before tube removal.

Outcome evaluation

Postoperative data were examined for the incidence of PNF, early graft dysfunction (EGD), and biliary complications. EGD was defined according to Olthoff et al. (12). IC was defined as non-anastomotic biliary strictures, irregularities, or dilatations, demonstrated by at least one adequate imaging, without the presence of concomitant hepatic artery thrombosis (2). Other complications such as infections, acute kidney failure requiring renal replacement therapy, biopsy-proven acute rejection, arterial thrombosis, and tumor recurrence were also considered.

Results

Main donor and recipient characteristics, and storage data are shown in **Table 1**.

Mean total no-flow WIT was 33.3 min. Donor ALT and lactate during NRP are illustrated in **Figure 2**. Two donors were discharged because of the macroscopic aspect (congested and irregularly perfused) and the liver biopsy. Of the 2 discharged donors, only one had higher ALT, while both showed a higher serum lactate trend.

Recipient ALT changes during the first postoperative week are shown in **Figure 3**. Median ALT peak was 1014 IU/L (range 393 – 3268). No cases of PNF were observed. Only one patient had grade 1 EGD, as defined by first-week aminotransferase peak > 2000 IU/L**.** Recipients' complications and outcome are summarized in **Table 2**. In one case hypertransaminasemia occurred after the first postoperative week due to biopsy-proven acute rejection, which was successfully treated with steroid boluses. The postoperative course was complicated in 2 cases by infective pneumonia. Patient and graft survival were both 100% at a mean follow-up of 6.1 months (range 3 – 9). No cases of IC were observed. One patient developed an anastomotic biliary stricture 45 days post-transplant, which resolved after stent placement via endoscopic retrograde cholangio-

pancreatography. At the third month post-transplant mean alkaline phosphatase was 111,3 IU/L (range 69-153), mean gamma-glutamyl transferase was 44,3 IU/L (range 17-79), and mean total bilirubin was 0,72 mg/dL (range 0,32-1,40).

Discussion

The percentage of DCD liver transplantations performed annually in the United States has plateaued around 6% since 2005, as a result of the decline in DCD liver utilization (3). Uncontrolled DCD, although representing the largest potential pool of DCD donors, still remains an almost entirely untapped resource (4,7). According to recent projections, any technology able to improve the outcome of DCD liver transplantation could more than double the expected amount of livers available over the next 10 years (13). Using NRP in type 2 DCD donors, the Barcelona group has recently obtained patient and graft survivals favorably comparable with both controlled DCD and DBD donors. (7).

Our results show that uncontrolled liver grafts with exceptionally prolonged WIT can be successfully transplanted using NRP. All the donors in our series were uncontrolled, except for one controlled case. In 3 donors ECMO was instituted during the resuscitation maneuvers and subsequently withdrawn for death declaration, configuring a hybrid category that anyway lies nearer to uncontrolled than controlled DCD. The main peculiarity of our series is the legally required no-touch period of at least 20 min, which may arise concerns about organ quality. The American Society of Transplant Surgeons guidelines for DCD liver transplantation advise against a WIT longer than 20-30 min (14). Along the same lines, a retrospective analysis of the Scientific Registry of Transplant Recipients data from all US liver-only DCD recipients has identified WIT ≥ 35 min as risk factor for graft failure (15). However, these recommendations have been extrapolated form series that did not include the systematic use of NRP, thus not considering its potential effects against warm ischemic injury. In our series no cases of PNF and only one case of grade 1 EGD

were observed. Furthermore, the peak and trend of ALT during the first post-transplant week, which are markers of ischemia-reperfusion injury, are surprisingly low considering the protracted WIT and the uncontrolled setting.

No cases of IC occurred in our series, and the only one anastomotic stricture resolved with endoscopic stenting. Although the follow-up is relatively short, it covers the period in which all major biliary complications were diagnosed in most series (1,2,16). In the Spanish experience with uncontrolled donors IC amounted to only 8%, and all cases fell within the first 3 months post-transplant (7). Oniscu et al. reported only 2 biliary complications and no cases of IC using NRP in 11 DCD liver transplants (17). Our results are consistent with these previous series, which suggest a possible beneficial effect of NRP upon ischemic-type biliary complications.

NRP provides essentially three main advantages. First, acting as a perfusion bridge between asystole and procurement, it provides a means of assessing liver function before transplantation. Secondly, numerous evidences exist that NRP enables organ repair, converting circulatory arrest into a period of ischemic preconditioning and preparing the liver graft for the subsequent cold storage period (18). Finally, NRP permits an unhurried donor operation, minimizing the risk of graft injury during the procurement (14).

Our donors presented higher serum transaminase during NRP than the superior limit accepted for transplantation in the Spanish series (7). This can reasonably be ascribed to the much longer WIT. We considered the trend in transaminase and lactate as indicator of ischemic injury and quality of the perfusion, but we did not adopt any strict cut-off to exclude the grafts a priori according only to the biochemical parameters. We conversely based final decision on the macroscopic appearance of the liver, in conjunction with the liver biopsy. No diffuse necrosis was observed in the livers accepted for transplant. Frozen section biopsy, although it may be not accurate in detecting early signs of ischemic

damage after circulatory arrest (19), assured that steatosis and fibrosis were acceptable for transplantation, not adding further risk to the procedure.

The Zurich group has recently reported that oxygenated HMP offers important benefits in preserving high-risk DCD livers and increasing graft survival in comparison to simple cold storage (8). The joint use of NRP and HMP combines the advantages of these two technologies and has not been reported in other clinical series so far. We adopted this combined approach to improve preservation in case of expected protracted CIT for logistic reasons. Uncontrolled donors require a very complex organization from early donor management to liver implantation, and timing of each step results even more difficult to define if donor management and transplant do not take place in the same hospital. NRP helps to buy time in donor management and evaluation, but it may be difficult to maintain good perfusion for long times. HMP resulted logistically essential in buying time for recipient transport, preparation and operation after the liver had been retrieved. We did not base graft selection upon HMP data, lacking sufficient evidence in literature as regards hypothermic perfusion.

In Italy, the livers are allocated regionally to the transplant centers in a rotation basis. Each center allocates the assigned livers amongst its own list, according to MELD-oriented criteria for cirrhosis and a shared policy for HCC prioritization (10,11). We decided to assign the DCD livers to less critic patients, preferring recipients with a clear indication for transplantation due to HCC progression, but with compensated cirrhosis. Given the exceptional nature of the procedure, we considered this matching the most equal and one with the highest margin of safety.

Our series includes a small number of transplants and lacks data on the long-term follow-up. Nevertheless it represents a fundamental step in Italy towards the exploitation of this previously unutilized donor source. Our results suggest that NRP might be of benefit, and HMP proves safe in uncontrolled DCD livers with prolonged WIT. Our approach could

reasonably extend the currently accepted WIT limits without an increase in ischemia-related complications, thus allowing DCD liver utilization despite non-ideal conditions.

Acknowledgements

The Authors thank Elisa Lodi and Marcella Gambino for the machine perfusion management.

Tables

Table 1: Donor and recipient characteristics in comparison with discharged livers.

Table 2: Recipient complications and outcome.

Figure Legends

Figure 1: Sequence of events in the donor management: uncontrolled donors (1) and uncontrolled donors with ECMO support prior to death declaration (2). Total no-flow WIT was defined as time of asystole (no-flow from cardiac arrest and start of resuscitation + the 20-min no-touch period). Low-flow WIT was defined as the length of cardiopulmonary resuscitation. In 3 donors ECMO was instituted during the resuscitation maneuvers and subsequently withdrawn for death declaration, configuring a hybrid category that anyway lies nearer to uncontrolled than controlled DCD. Dimension of squares and lines is arbitrary and not in proportion. *CPR = cardiopulmonary resuscitation, ER = emergency room*.

Figure 2: Donor alanine transaminase (A) and serum lactate (B) during normothermic regional perfusion. Final decision was based on the macroscopic appearance of the graft, in conjunction with the trend of transaminase and lactate, and the liver biopsy. Of the 2 discharged donors (dotted lines), only one had higher transaminase, while both showed a higher serum lactate trend.

Figure 3: Recipient alanine transaminase (ALT) during the first 7 postoperative days (PODs). Median ALT peak was 1014 IU/L (range 393 – 3268). Only one patient had grade 1 early graft dysfunction, as defined by aminotransferase peak > 2000 IU/L.

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Table 1

TRANSPLANTED LIVERS																				
	DONOR						LIVER BIOPSY				STORAGE			RECIPIENT						
Case	Type	Age (yr)	BMI	Tot. no-flow WIT (min)[1]	Tot. low-flow WIT (min)[2]	NRP (min)	Steatosis macro (%)	Steatosis micro (%)	Fibrosis (Ishak)	Necrosis (%)	Static CIT (min)	HMP (min)	Total CIT (min)	Age (yr)	BMI	Indication for transplant	Other liver disease	Lab MELD	CTP status	Follow-up (mo)
1	C	51	27.8	20	13	252	10	30	0	0	330	-	330	40	18.4	mHCC (M-in)	HBV	7	A	9
2	U*	57	26	44	84	360	<5	<10	0	0	350	-	350	54	27.7	mHCC (M-in)	HCV	7	A	8
3	U	40	26.2	38	107	350	<10	20	0	0	370	170	540	54	28.7	mHCC (M-in)	HBV	11	A	8
4	U	47	31.6	34	96	310	30	40	1	0	360	180	540	54	28.4	mHCC (M-in)	ETOH	22	C	7
5	U*	46	23.4	45	69	315	<5	20-30	0	0	210	230	440	58	35.9	mHCC (M-in)	NASH	9	A	5
6	U	61	25.2	23	75	305	15	30	1	0	240	150	390	56	21.3	mHCC (M-in)	HCV, HIV	8	A	3
7	U	31	26.8	29	104	335	<5	<5	0	0	305	-	305	62	27.4	mHCC (M-in)	HCV	10	B	3
Mean	-	47.6	26.7	33.3	78.3	318.1	-	-	-	-	309.3	182.5	413.6	54.0	26.8	-	-	10.6	-	6.1

DISCHARGED LIVERS										
	DONOR						LIVER BIOPSY			
Case	Type	Age (yr)	BMI	Tot. no-flow WIT (min)[1]	Tot. low-flow WIT (min)[2]	NRP (min)	Steatosis macro (%)	Steatosis micro (%)	Fibrosis (Ishak)	Necrosis (%)
I	U	58	32.7	33	117	390	35-40	40-45	2	10-20
II	U*	59	31.1	42	75	315	20-25	50-60	2	0

[1] Total time of asystole, including the 20-min no-touch period.

[2] Total length of cardiopulmonary resuscitation.

* Extracorporeal membrane oxygenation was instituted with resuscitative intent and stopped after the treatment had been deemed futile.

BMI = body mass index; CIT = cold ischemia time; C = controlled; CTP = Child-Turcotte-Pugh status; ETOH = alcoholic liver disease; HBV = hepatitis B virus; HCC (M-in) = multifocal hepatocellular carcinoma within Milan criteria; HCV = hepatitis C virus; HIV = human immunodeficiency virus; MELD = Model for End-stage Liver Disease; HMP = hypothermic machine perfusion; NASH = non alcoholic steatohepatitis; U = uncontrolled; WIT = warm ischemia time.

Table 2

Complication	Number (n=7)
Primary non-function	0/7
Early graft dysfunction	1/7
Biliary complications:	
- overall	1/7
- ischemic cholangiopathy	0/7
Arterial thrombosis	0/7
Acute rejection (biopsy proven)	1/7
Infection	2/7
Acute kidney failure	0/7
Tumor recurrence	0/7
Retransplantation	0/7

Survival[1]	
Patient	7/7
Graft	7/7

[1] Mean follow-up was 6.1 months.

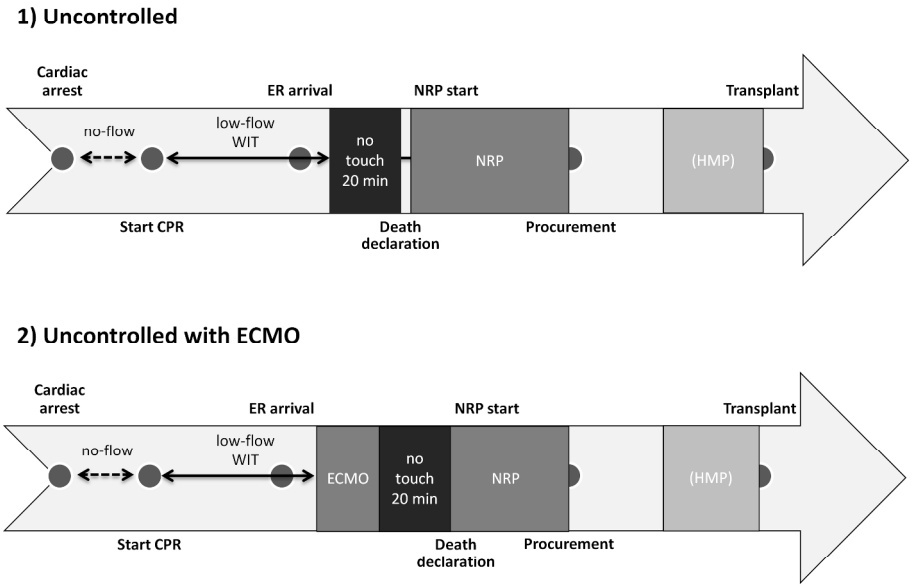

Figure 1: Sequence of events in the donor management: uncontrolled donors (1) and uncontrolled donors with ECMO support prior to death declaration (2). Total no-flow WIT was defined as time of asystole (no-flow from cardiac arrest and start of resuscitation + the 20-min no-touch period). Low-flow WIT was defined as the length of cardiopulmonary resuscitation. In 3 donors ECMO was instituted during the resuscitation maneuvers and subsequently withdrawn for death declaration, configuring a hybrid category that anyway lies nearer to uncontrolled than controlled DCD. Dimension of squares and lines is arbitrary and not in proportion. CPR = cardiopulmonary resuscitation, ER = emergency room.

Figure 1
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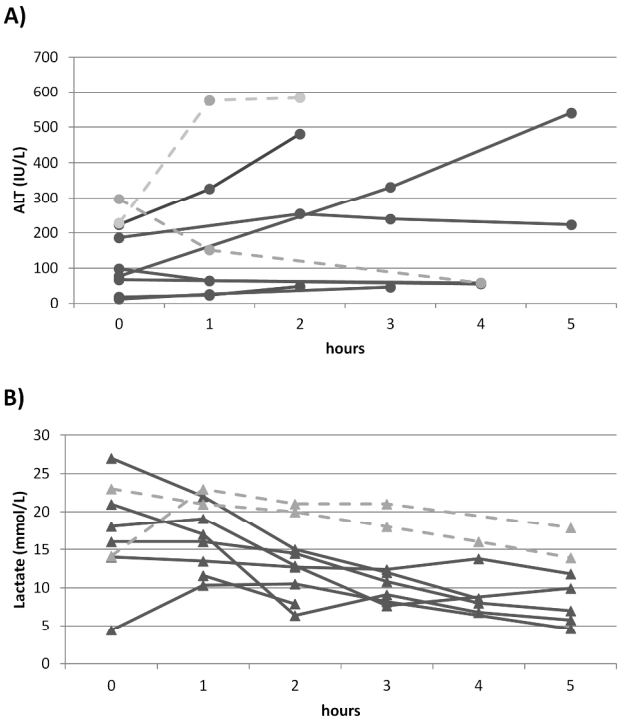

Figure 2: Donor alanine transaminase (A) and serum lactate (B) during normothermic regional perfusion. Final decision was based on the macroscopic appearance of the graft, in conjunction with the trend of transaminase and lactate, and the liver biopsy. Of the 2 discharged donors (dotted lines), only one had higher transaminase, while both showed a higher serum lactate trend.
Figure 2
254x190mm (300 x 300 DPI)

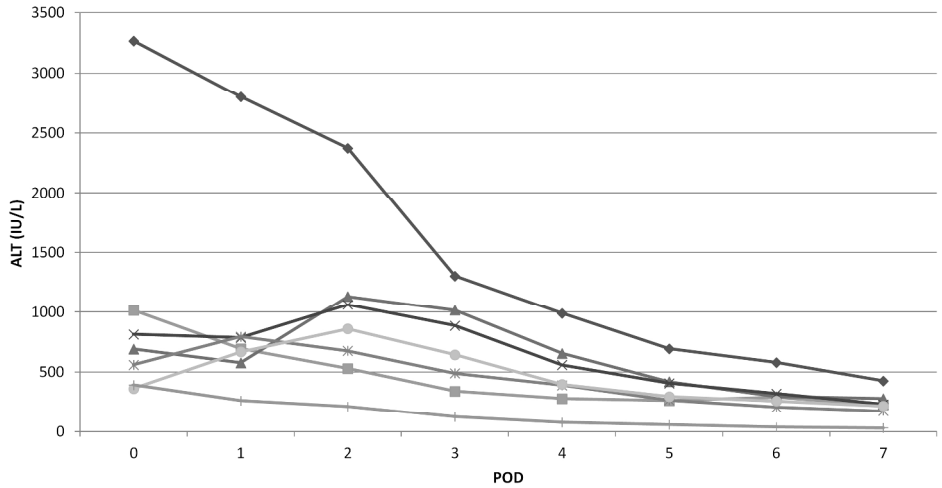

Figure 3: Recipient alanine transaminase (ALT) during the first 7 postoperative days (PODs). Median ALT peak was 1014 IU/L (range 393 – 3268). Only one patient had grade 1 early graft dysfunction, as defined by aminotransferase peak > 2000 IU/L.
Figure 3
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