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The role of ex-situ hypothermic oxygenated machine perfusion and cold preservation time in extended criteria DCD and DBD

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Abbreviations:

AKI, acute kidney injury
CA, cardiac arrest
CD>3, complication grade > IIIa according to Clavien-Dindo
CPT, cold preservation time
DBD, brain death donor
DCD, donor after circulatory death
DHOPE, dual hypothermic oxygenated machine perfusion
EAD, early allograft dysfunction
ECD, extended criteria donor
fWIT, functional warm ischemia time
HOPE, hypothermic oxygenated machine perfusion
IRI, ischemia reperfusion injury
LT, liver transplant
MELD, model for end stage liver disease
MRCP, magnetic resonance cholangiography
POD, post-operative day
PNF, primary non function
PRS, post-reperfusion injury
SCS, static cold storage

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Abstract

Hypothermic-oxygenated machine perfusion (HOPE) has the potential to counterbalance the detrimental consequences of cold and warm ischemia time in both brain death donors (DBD) and donors after circulatory death (DCD). Herein, we investigated the protective effects of HOPE in extended criteria (ECD) DBD and over-extended warm ischemia time (WIT) DCD grafts.

The present retrospective case-series study included 50 livers subjected to end-ischemic HOPE or dual (D)HOPE in two liver transplant (LT) Centers from January 2018 to December 2019. All DCD donors were subjected to normothermic regional perfusion before organ procurement. Results are expressed as median (IQR).

In the study period, 21 grafts derived from over-extended WIT DCD donors (total WIT 54 (40-60) min and 75% classified as futile), while 29 from ECD DBD. Three biliary complications and one case of ischemia-type biliary lesions were diagnosed. The rate of early allograft dysfunction (EAD) was 20% and those patients had higher comprehensive-complication index. Through a changing point analysis, cold preservation time (CPT) >9h was associated with prolonged hospital stay (p=0.02), higher rate of EAD (p=0.009) and worst post-LT complications (p=0.02). Logistic regression analyses indicated a significant relationship between CPT and early allograft dysfunction. No differences were showed in terms of early post-LT results between LT performed with DCD and BDB. Overall, our data are fully comparable with benchmark criteria in LT.

In conclusion, the application of (D)HOPE allowed to obtain satisfactory and promising results using ECD-DBD and over-extended DCD grafts. Our findings indicate the need to reduce CPT also in the setting of DHOPE, particularly for grafts showing poor quality.

Introduction

The gap between the number of transplantable organs and the number of patients waiting for liver transplantation (LT) required an expansion of the "standard" pool of grafts [1]. As a consequence, organs that were initially considered unacceptable, including grafts derived from extended criteria donors (ECD) and from donors after circulatory death (DCD), are now used for transplantation purpose.

ECD grafts show sub-optimal post-LT outcome relative to "ideal" donor organs [2]. Indeed, ischemia/reperfusion injury (IRI) has a particularly detrimental impact [3] on ECD grafts and could cause early allograft dysfunction (EAD) [4].

DCD grafts represent a peculiar ECD category that, in addition to cold ischemia (CI), is subject to warm ischemic (WI) injury. As a result, DCD transplants have higher rates of EAD, ischemic-type biliary lesion (ITBL) and graft loss compared to transplantations from brain dead donors (DBD) [5–7]. Since the Italian law establishes that death declaration for circulatory arrest can only be made after 20 min of absent electrocardiographic activity (no-touch period), in the Italian setting DCD grafts are exposed to more prolonged WI time (WIT) if compared to other countries.

Cold preservation is necessary to allow organ preservation [8]. However, a prolonged static cold storage (SCS) could lead to graft damage with post-LT complications and possible graft loss, especially in ECD grafts [9]. Even unavoidable, SCS could be at least limited, thanks to transplant centers coordination, reduction of operative times and through the application of advanced techniques of organs preservation.

A further improvement in organ preservation involves the mitigation of organ damage to reduce postoperative risks. In DCD setting, normothermic regional perfusion (NRP) was reported as a beneficial strategy to restore cellular metabolism through the oxygenated blood in-situ reperfusion of kidneys, liver and pancreas after death until organ retrieval [10–12]. Indeed, when DCD grafts are exposed to prolonged WI effect as in the Italian setting, NRP is widely adopted to improve graft quality by reversing the detrimental impact of warm ischemic injury [13,11,14]. In addition, ex-situ hypothermic oxygenated machine perfusion (HOPE) was implemented as a strategy to improve the quality of both DCD and DBD grafts, by reducing IRI and improving the organ energetic status[15–20]. Isolated portal vein HOPE or "dual" (D) HOPE perfusion were successfully used to improve post-LT DCD results, while only preliminary data are published on DBD ECD.

This retrospective observational study describes a series of LT performed with over-extended WIT DCD and DBD ECD grafts preserved and treated with HOPE. The main focus was to evaluate the reconditioning and preservation potential of oxygenated hypothermic machine perfusions in these grafts of poor quality.

Material and Methods

Study design

A retrospective case-series study was performed including all consecutive LT performed at the liver transplant center, of the S. Orsola-Malpighi Hospital, University of Bologna (Bologna, Italy) and the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) from January 2018 to December 2019. We enrolled only recipient receiving an ECD graft that underwent ex-situ hypothermic machine perfusion (n=50). Inclusion criteria were: recipient age > 18 years and signed informed consent. In case a DCD graft was used, recipients were required to provide an informed consent at the time of the waiting list and once a potential DCD graft was available. The study was approved by the Institutional Ethical Committees.

Donor selection and liver procurement

Extended criteria donors are classified according to Vodkin and colleagues [21]. Graft derived from DBD ECD underwent ex-situ hypothermic machine perfusion when graft macro-steatosis was > 30%, hepatonecrosis markers were 4 times higher than normal value, expected SCS was > 10 hours.

Due to the peculiar Italian setting, DCD donation involves 20 min hands-off period and all DCD donors were subjected to mandatory in-situ regional perfusion.

In-situ perfusion was obtained through a NRP according to Fondevilla and colleagues [11]. Target blood flow was > 1.7 l/min and pre-membrane lung saturation > 70% with hematocrit > 25%. Anticoagulation was obtained with heparin. Additional heparin was administered before cross clamp if activated clotting time was < 250 sec. Pre-perfusion liver biopsies were collected in all DCD cases to assess chronic liver damage. Graft macro-steatosis > 30% and liver fibrosis METAVIR > 2 [22] were considered risk factors for graft failure and graft discard. As previously reported [23], DCD graft evaluation was multifactorial and based on donor characteristics (age, past medical history, peri-mortem events, and WIT), liver biopsy, and NRP parameters (blood flow, lactate trend, and transaminases).

Ex situ perfusion of both DCD and DBD livers was performed using Celsior solution (IGL, France). DCD grafts were perfused through the aorta and portal vein, while in DBD single aortic perfusion was performed, and the portal vein was perfused during back-table. All grafts were preserved in ice-box until the arrival at the transplant center. During the back table preparation the portal vein and/or the arterial cannula were placed to enable ex-situ perfusion.
Donor characteristics are summarized in Table 1.

Oxygenated hypothermic machine perfusion (HOPE)

After back-table preparation and cannulation, grafts were connected to ex-situ machine perfusion. Ex-situ perfusion is intended as an end-ischemic, back to base dynamic perfusion. Grafts were perfused in an operation room under medical supervision as described elsewhere [24–26] and until completion of recipient hepatectomy. Single HOPE and/or DHOPE were performed using Liver Assist (Organ Assist, Netherland) and Vitasmart (Bridge for Life Ltd, Columbia, SC, USA), respectively.

The perfusion system was primed with 3-4 L of Belzer MPS® UW Machine Perfusion Solution (Bridge for Life Ltd, Columbia, SC, USA). When DHOPE was applied the arterial and portal pressures were set at 25 mmHg with a pulsatile flow and at 3-4 mmHg with a continuous flow, respectively. Conversely, HOPE enable the single portal perfusion with a pressure of 3-4 mmHg with a continuous flow. The oxygen flow was titrated to obtain a pre-liver pO2 of 500-600 mmHg in the perfusion fluid. The target liver temperature was between 4 to 10°C. Graft temperature was measured with a custom-made probe applied to the liver surface, while the perfusate temperature was monitored using built-in probes. During hypothermic perfusion no electrolytes, glucose or drug administration was required. At the end of the machine perfusion, liver grafts were flushed with Ringer's lactate 1000 cc to reach a para-physiological electrolyte concentration.

Perfusate and tissue evaluation

Metabolite and biomarkers were normalized according to the perfusate volume. Perfusate glucose, transaminases, lactate were measured every hour. The release ratio was calculated according to the formula [27]: $(C_{time2} - C_{time1})/C_{time1}$ (C is the concentration of a metabolite or biomarker; time2 is a time point that follows time1). Liver biopsies were collected before donor aortic cross-clamp to assess steatosis, inflammation and necrosis: they were fixed and stained with H&E for standard

histopathological analysis and with periodic acid/Schiff (PAS) to detect changes in glycogen cell content.

Recipients

Liver grafts were allocated according to the National Transplant Center (CNT) guidelines. DCD grafts were allocated to recipients with laboratory MELD score <25. All LT recipients were evaluated in the pre-transplant setting and followed-up after LT according to each center's institutional policy. LT procedures were carried on using the piggy-back technique without veno-venous bypass. Termino-terminal caval anastomosis and temporary port-systemic shunt were used when needed. A T-tube was routinely placed for duct-to-duct biliary anastomosis and removed 3 months post-transplantation under cholangiography. Patients were followed-up at month 1 and 3, and then every 6 months to assess the graft function and the presence of anastomotic biliary or vascular complications. Ischemic-type biliary lesion (ITBL) was defined as any non-anastomotic stenosis associated with symptoms or signs and requiring an endoscopic or surgical procedure in the absence of vascular complications. Immunosuppressive regimen was based on steroids, tacrolimus and, according to patient's needs, mycophenolic acid, m-Tor inhibitor or basiliximab. Routine T-tube cholangiography or magnetic resonance cholangiography (MRCP) were performed before discharge, at 3,6, and12 months and then every 12 months or in case of clinical problems. Recipients characteristics (Table 1) and post-LT course were registered in a prospective database. Complications were categorized according to Clavien-Dindo [28] and continuously evaluated using the Comprehensive Complication Index (CCI). The post-LT data were compared with Muller and colleagues benchmark criteria [29] in liver transplantation to ass the risks connected to the use of (D)HOPE ECD grafts and to evaluate our overall results.

Definitions

In this section the most frequently used definition are summarized. According to DCD, refer to the terminology discussed during the *6th International Conference on Organ Donation after Circulatory Death* [30] and in the paper by Ghinolfi and colleagues [13].

In particular:

- NRP or extra-corporeal membrane oxygenation (ECMO): circulating *in situ* machine perfusion of organs using a device pumping blood applied at normothermic temperatures in the donor;
- agonal phase or agonic phase: time elapsed from withdrawal of life-saving therapy (WLST) to circulatory arrest (CA);
- no-flow period: time elapsed from the witnessed cardiac arrest (CA) to the start of cardiopulmonary resuscitation + the no-touch period in type II DCD and the time from CA to initiation of NRP in type III DCD;
- functional warm ischemia time (fWIT): the time from when the systolic blood pressure drops below 50 mmHg (irrespective of oxygen saturation) after WLST until start of NRP (fWIT=low-flow period + no-flow period);
- total warm ischemia time (totWIT): the time from the start of WLST to initiation of NRP (totWIT=agonal phase + no-flow period);
- Post-transplant ischemic-type biliary lesion (ITBL) was defined as any non-anastomotic stenosis associated with symptoms or signs and requiring an endoscopic or surgical procedure in the absence of vascular complications. Bile duct imaging was performed at 1-3-6 months and then every year;
- Cold preservation time: static cold storage + (D)HOPE duration;
- EAD was defined as the presence of one or more of the following postoperative laboratory analyses: bilirubin > 10mg/dL on day 7, international normalized ratio < 1.6 on day 7, and alanine or aspartate aminotransferases >2000 IU/L within the first 7 days [31];
- post-reperfusion syndrome (PRS) was defined as a decrease in the mean arterial pressure of more than 30% of the value observed in the anhepatic stage, for more than 1 minute during the first 5 minutes after reperfusion of the graft [32];
- L-GrAFT score was calculated according to Agopian and colleagues to estimate the individualized risk of 3-month graft failure following LT [33];
- Acute kidney injury (AKI) was defined according to KIDGO criteria [19];
- Time to return to normal values was the number of post-operative days (POD) required to reach transaminases values <50 UI/L, and total bilirubin <1.5 mg/dl, and INR<1.2.

Statistical analysis

Data were analyzed using SPSS software version 23.0 (Chicago, Ill, USA).

Continuous variables were reported as median (IQR), while categorical ones as number (percentage). The two groups were compared using Student's t-test for quantitative continuous variables, ANOVA for discrete continuous variables and Fischer's exact test for categorical ones. Logistic regression was applied to evaluate the impact of cold preservation time on post-LT results. For logistic regression a cut off value of 9 hours for CPT was used in order to identify the two groups. This value was interpreted as a change point in the time series of the parameters analyzed, as a CPT higher than 9 hours was found to be associated with post-LT complications. Specifically, such value is the minimum that can be used in order to identify statistically significant differences between groups. Differences were considered significant for $p<0.05$.

Results

Fifty grafts were evaluated, ex-situ perfused and transplanted in the study period. Twenty-two organs derived from DCD and 28 (56%) from ECD DBD underwent (D)HOPE. Among the DBD grafts, hypothermic perfusion was applied in 8 cases for organizational reasons (e.g. 3 cases change recipient), 5 cases due to CPT > 10 h, and 15 cases for reconditioning purposes (5 grafts showed steatosis > 40%, 3 livers with hepatocellular injury at procurement, 7 cases for donor age > 80 years) (Supplementary Table1). Twenty-one grafts were transplanted in Milan and underwent DHOPE, while 29 livers were used in Bologna and were subjected to HOPE perfusion. Median follow-up was 17 (10-26) months with 93% of the recipients reaching at least 6 months of follow-up.

Ex-situ perfusion and graft preservation

The DHOPE procedures lasted 169 (91-232) min. The resulting total CPT was 533 (427-607) min. During HOPE and DHOPE no adverse events were registered, except for 1 case of DHOPE in which an arterial pump malfunction during the graft connection was observed (high pressure of perfusion). This graft was promptly disconnected from the circuit, machine perfusion was reset, and, after re-connection of the liver to the system, ex situ perfusion was successfully completed. Right artery thrombosis (a right hepatic artery reconstruction was performed before DHOPE connection) was diagnosed in post-operative day (POD) 4 without any further graft complications. All grafts had stable vascular flow within 1 hour of perfusion. Portal flow ranged from 30 ml/min to 505 ml/min with a resulting portal vein resistance of 0.04 (0.02-0.04) mmHg/ml/min (Figure 1). DHOPE arterial flow ranged from 11 to 107 ml/min with a resistance of 0.38 (0.21-0.42)

mmHg/ml/min. During ex-situ hypothermic perfusion, lactate, glucose and potassium increased ($p<0.001$) (Figure 2).

Early post-LT outcome

Among the transplanted grafts, only 1 (2%) primary non function (PNF) was diagnosed 2 days after LT. Stage 2-3 AKI was observed in 21% (11/50) of the recipients and 12% (6/50) needed post-LT CVVH/CRRT (55% of the patients that developed post-LT AKI). PRS was diagnosed in 18% (9/50) cases and was directly related to AKI development ($p=0.006$; OR 2.33 CI95% 0.99-5.49).

AKI was also related to a higher post-LT transaminases peak ($p<0.001$) and longer time for bilirubin normalization ($p=0.004$). Interestingly, the decrease of portal vein resistances during DHOPE was lower in the AKI patients ($p=0.04$), and potassium release during DHOPE showed a trend towards lower values in AKI (potassium release ratio AKI 0.46 (0.24-0.57) vs nAKI 0.72 (0.42-0.83), $p=0.07$, Figure 3A).

EAD was diagnosed in 10/50 (20%) patients. L-Graft scores was -0.62 (-2.81-1.03) and 48% of the cases were classified as high risk (L-Graft 3, 4, and 5). Potassium release during DHOPE (potassium release ratio: EAD 0.05 (0.02-0.05) vs nEAD 0.08 (0.05-0.09), $p=0.04$, Figure 4A) and reduction of DHOPE portal resistances (EAD 0.15 (0.1-0.34) vs nEAD 0.28 (0.10-0.45), $p=0.04$) were related to EAD diagnosis.

One (2%) case of acute liver rejection was treated with steroids. Eleven (22%) recipients had a post-LT complication grade > IIIa (CD>3) according to Clavien-Dindo and 3 (6%) needed a relaparotomy due to hemoperitoneum. The patients that developed CD>3 had a higher post-LT transaminases peak (AST 871 (472-1270) IU/L vs 1398 (809-1987) IU/L, $p=0.001$; ALT 543 (369-716) IU/L vs 1205 (678-1732) IU/L, $p=0.008$), and a higher rate of AKI ($p<0.001$) and EAD ($p=0.01$). All these complications caused prolonged ICU ($p=0.001$) and hospital stay ($p<0.001$). Consistently, the patients that developed EAD had higher CCI during hospitalization ($p=0.001$), at 3 and 6 months ($p=0.004$ and $p=0.01$, respectively) (Figure 4B). Only one vascular complication was identified and consisted in the above-mentioned case of right hepatic artery thrombosis conservatively managed (the right liver was gradually revascularized by S4 hepatic artery branch).

Biliary complications, graft and patient survival

After a mean follow-up of 17 (10-26) months, 2 graft losses (2/50, 4%) were registered: one due to PNF (successfully re-transplanted) and the other case due to hepatic artery hypoperfusion. It led to graft failure and re-transplant (after 55 days), but a multiorgan failure was diagnosed secondary to acute pancreatitis and the patient suddenly died 2.4 months after the first LT. This is the only patient loss of the study series. The routine cholangiography and MRCP revealed in POD 191 a case of ITBL in a patient transplanted with 76 yr old DBD graft. In three (6%) patients anastomotic complications were diagnosed (1 leak in POD 11 and 2 anastomotic stenosis after 9 and 11 months post- LT) and successfully managed with endoscopic retrograde cholangiopancreatography. The T-tube removal caused choleperitoneum in 5 patients. They were treated in 2 cases with endoscopic retrograde cholangiopancreatography and in 3 cases with abdominal drainage.

HOPE and DHOPE characteristics are shown in Supplementary Table 2.

Effect of preservation time on early graft function

The relation of donor, preservation and recipient characteristics with EAD was investigated. A CPT>9h (Table 2) caused higher hepatocellular damage after reperfusion (AST peak: CPT ≤ 9h 509 (364-961) IU/l vs CPT>9h 994 (630-1747) IU/l, p=0.04; ALT peak: CPT ≤ 9h 486 (365-897) IU/l vs CPT>9h 709 (516-1723), p=0.03) (Figure 3B). CPT>9h was associated with EAD (p=0.009) and AKI (p=0.001). Consistently, CPT > 9h led to prolonged hospital stay (10 ± 4 days vs 14 ± 3 days, p=0.01) and increased rate of CD>3 (OR 5.09 CI 95% 1.15-20.79, p=0.02) with a trend toward statistically higher CCI at discharge (p=0.05). No differences in terms of graft steatosis (p=0.68) or total WIT (p=0.79) were found. After logistic regression, only the relation between CPT>9h and EAD remained statistically significant (OR 5.03 CI 95% 1.02-27.43) (Supplementary Table 3). Interestingly, once CPT>9h is associated to at least one additional ECD characteristic (e.g. type II DCD, macrovescicular steatosis > 30%, donor age > 80yr; n=13), the OR of developing EAD increase to 8.67 (CI 95% 1.55-48.49, p=0.007) and AKI to 7.34 (CI 95% 1.27-35.98, p=0.02).

Evaluation of benchmark criteria in our series

Early and medium-term post-LT results were compared with Muller and colleagues benchmark criteria (Supplementary Table 4). The rate of post-LT complications >III according to Clavine-Dindo was lower at all time points in our series. Consistently, the CCI score was lower in the

present study at all time-point except at discharge. In addition, the use of CVVH/RRT was more frequent in our series, as well as the duration of LT procedure was longer. However, graft and patient survival were not affected by the over-extended characteristics of our grafts.

Comparison between DBD ECD and DCD

Twenty-eight organs (56%) derived from DBD ECD donors, while 22 (44%) from DCD (Table 3). Three donors were type 2 and 19 type 3 according to Maastricht criteria. DCD and NRP timing are reported in Table 3. The donor age was lower in the DCD group (DCD 56 (48-59) yr vs DBD ECD 72 (58-77), p=0.01), while no differences in graft histology were found.

UK DCD score was 11 (8-13) and 76% of the DCD grafts were categorized as "futile transplanted". The total cold preservation time was 580 (468-666) min in ECD DBD, while 500 (421-561) in DCD (p=0.05). No statistically significant differences were found between DCD and ECD DBD grafts (Table 3) according to early post-LT course, biliary complications and graft survival. Only transaminases in the first 10 days showed a trend towards a significant higher value in DCD group (p=0.06). The 2 (9%) graft losses reported in the DCD group were due to PNF in one case (the graft was exposed to 290 min of fWIT) and liver hypoperfusion in the other.

Discussion

The study presents a series of ECD that underwent hypothermic oxygenated dynamic preservation. The low rate of graft loss and biliary complications are the most interesting results obtained in this study, in particular, due to the fact that most DCD grafts were classified as futile according to the UK DCD score. In addition, our analysis indicates that, even if promising results in terms of graft survival are showed, CPT length affects post-reperfusion damage and early post-LT outcome. This result emphasizes the importance to reduce CPT, even when a combination of innovative preservation strategies is applied.

Among the preservation technologies, HOPE and DHOPE were introduced in the clinical setting to counterbalance the detrimental effect of IRI after static cold storage [34]. A reduction of graft loss, biliary complications and ITBL is shown when DHOPE was applied to DCD [35,36]. Conversely, the effect on DBD grafts needs to be further investigated, especially if grafts affected by different ECD characteristics are considered [3,23,37]. Comparison of our post-LT results relative to other published series (both machine perfusion or standard preservation), indicates that our patients showed comparable or even better results in terms of graft failure, biliary

complications and early post-LT outcomes [38–42]. In our opinion, these data add further evidence supporting the reconditioning potential of HOPE because they are obtained using ECD DBD grafts whose characteristics are poorly described in machine perfusion series.

To confirm this idea, we evaluated our results according to the benchmark criteria for LT identified by Muller and colleagues [11]. These authors, based on the analysis of data collected in a multicenter study, identified index values that could serve as reference for other studies and help standardize worldwide results. Due to the high donor age and incidence of metabolic disease of the Italian donor population [43–45], a standard preservation group is hardly identified in our general LT series and we decided to use Muller's benchmark criteria (Supplementary Table 4) to evaluate our results. Despite the ECD characteristics of our grafts, the post-LT outcome parameters fit the benchmark criteria and they could be considered as optimal results. However, our higher rate of CVVH/RRT and the higher CCI at discharge can be seen as the downside of using overextended grafts. Accurate donor-recipient match, DCD graft selection and, especially, the use of machine perfusion could significantly counterbalance the additional risks on outcome.

Together with (D)HOPE, NRP could have played a central role in facing the detrimental impact of extremely prolonged WIT in DCD grafts. The combination of in-situ and ex-situ perfusion could improve the success rate of transplantation of "over-extended DCD" grafts that, according to UK-DCD score [46], would be futile transplanted in 76% of the cases.

Besides its reconditioning potential, preservation is one of the most relevant advantage provided by DHOPE [47]. Some authors already hypothesized the possible organizational impact of prolonged preservation of liver grafts [24,48]: to change a recipient or accept a graft discarded after in-situ perfusion by other centers, prevents the loss of a possible transplantable graft. Our results showed that a prolonged CPT could impact the rate and severity of post-LT complications with a prolonged hospital stay, regardless donor and graft characteristics. According to our analysis the cut off value associated to post-LT complications is estimated to be 9hr. Even though the graft loss was not affected by CPT and considering the nature of the study, the possible increase in post-LT complications, especially in low MELD patients, could strongly impact the transplant benefit [49]. For this reason, we would suggest avoiding a routine application of DHOPE to "safely" prolong CPT, while its use when a sudden organizational problem takes place, could save a useful graft. We are aware that during (D)HOPE phase of CPT reoxygenation take place, but no evidences are published on prolonged use of (D)HOPE. Therefore, in this situation, especially when an ECD graft is "rescue" perfused, LT procedure and early course must be

optimized to minimize post-LT complications. Interestingly, our study showed a relation between post-LT transaminases peak, complications and overall hospitalization. The heterogeneity of our donor population and the combination of different perfusion technologies could affect our results and additional prospective data are needed to identify proper duration of hypothermic perfusion.

Among the biological mechanisms involved in post-LT complications, IRI plays a central role, especially in ECD grafts [50]. HOPE and DHOPE can reduce IRI [51] by limiting IR-induced ATP depletion. In fact, oxygenation during reperfusion was shown to preserve mitochondrial function through improvement of complex I and II activity [52,53]. This leads to increased ATP production and reduced reactive oxygen species (ROS) formation during ex vivo perfusion. In this scenario, it is possible that a transient opening of mitoK_{ATP} channels [54] occurs, resulting in activation of downstream signaling pathways mediating regulation of organelle volume and function as well as in induction of gene programs involved in cell survival and tolerance to ischemia. As a result, (D)HOPE could elicit a number of pre-conditioning and ischemic protection mechanisms that allows a significant reduction of IRI on ECD grafts and enables satisfactory post-LT results with extended and over-extended criteria grafts, as in our series.

The retrospective multicenter nature of the study, the sample size and the heterogenicity of the population represents the main limitations. However, the centers were selected according to homogenous procedures and the cases were consecutive to reduce the effect of the selection bias. . Another limitation consists in the use of EAD and post-LT transaminases to evaluate early post-LT outcome, although their accuracy in predicting post-LT outcome in machine perfusion studies is still being debated.

In conclusion, our remarkable results in terms of survival and biliary complications with “futile” DCD and ECD-DBD may be related to the preservation strategies applied with HOPE and DHOPE.

The CPT remains a fundamental variable to be controlled for improving transplant outcome. Application of innovative preservation strategies would allow to reduce IRI and mitigate the post-LT reperfusion injury, ultimately leading to improved post-LT results.

References:

1. Trapianti CN. Sistema Informativo Trapianti 2010. *Disponibile sul sito: https://trapianti. sanita. it/statistiche*

2. Durand F, Renz JF, Alkofer B, Burra P, Clavien P-A, Porte RJ, Freeman RB, Belghiti J. Report of the Paris consensus meeting on expanded criteria donors in liver transplantation. *Liver transplantation: official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2008;14:1694–1707.

3. Czigany Z, Lurje I, Schmelzle M, et al. Ischemia-Reperfusion Injury in Marginal Liver Grafts and the Role of Hypothermic Machine Perfusion: Molecular Mechanisms and Clinical Implications. *Journal of clinical medicine research [Internet]* 2020;9. Available from: http://dx.doi.org/10.3390/jcm9030846

4. Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, Greenstein SM, Merion RM. Characteristics associated with liver graft failure: the concept of a donor risk index. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2006;6:783–790.

5. Blok JJ, Detry O, Putter H, et al. Longterm results of liver transplantation from donation after circulatory death. *Liver transplantation: official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2016;22:1107–1114.

6. Croome KP, Lee DD, Perry DK, Burns JM, Nguyen JH, Keaveny AP, Taner CB. Comparison of longterm outcomes and quality of life in recipients of donation after cardiac death liver grafts with a propensity-matched cohort. *Liver transplantation: official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2017;23:342–351.

7. Kalisvaart M, de Haan JE, Polak WG, Metselaar HJ, Wijnhoven BPL, IJzermans JNM, de Jonge J. Comparison of Postoperative Outcomes Between Donation After Circulatory Death and Donation After Brain Death Liver Transplantation Using the Comprehensive Complication Index. *Annals of surgery* 2017;266:772–778.

8. Chedid MF, Pinto MA, Juchem JFG, Grezzana-Filho TJM, Kruel CRP. Liver preservation prior to transplantation: Past, present, and future [Internet]. *World Journal of Gastrointestinal Surgery*2019;11:122–125. Available from: http://dx.doi.org/10.4240/wjgs.v11.i3.122

9. Lozanovski VJ, Döhler B, Weiss KH, Mehrabi A, Süsal C. The Differential Influence of Cold Ischemia Time on Outcome After Liver Transplantation for Different Indications—Who Is at Risk? A Collaborative Transplant Study Report. *Frontiers in immunology* 2020;11:892.

10. Fondevila C. Is extracorporeal support becoming the new standard for the preservation of DCD grafts? *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*2010;10:1341–1342.

11. Oniscu GC, Randle LV, Muiesan P, Butler AJ, Currie IS, Perera MTPR, Forsythe JL, Watson CJE. In situ normothermic regional perfusion for controlled donation after circulatory death--the United Kingdom experience. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2014;14:2846–2854.

12. Miñambres E, Suberviola B, Dominguez-Gil B, Rodrigo E, Ruiz-San Millan JC, Rodríguez-San Juan JC, Ballesteros MA. Improving the Outcomes of Organs Obtained From Controlled Donation After Circulatory Death Donors Using Abdominal Normothermic Regional Perfusion. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2017;17:2165–2172.

13. Ghinolfi D, Dondossola D, Rreka E, et al. Sequential Use of Normothermic Regional and Ex Situ Machine Perfusion in Donation After Circulatory Death Liver Transplant. *Liver transplantation: official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society [Internet]* 2020;Available from: http://dx.doi.org/10.1002/lt.25899

14. Wang L, Thompson E, Bates L, et al. Flavin Mononucleotide as a Biomarker of Organ Quality-A Pilot Study. *Transplantation direct* 2020;6:e600.

15. Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors [Internet]. *Transplant International*2015;28:665–676. Available from: http://dx.doi.org/10.1111/tri.12530

16. Moers C, Smits JM, Maathuis M-HJ, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *The New England journal of medicine* 2009;360:7–19.

17. Zhong Z, Lan J 'nan, Ye S, et al. Outcome Improvement for Hypothermic Machine Perfusion Versus Cold Storage for Kidneys From Cardiac Death Donors. *Artificial organs* 2017;41:647–653.

18. Wight JP, Chilcott JB, Holmes MW, Brewer N. Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. *Clinical transplantation* 2003;17:293–307.

19. De Deken J, Kocabayoglu P, Moers C. Hypothermic machine perfusion in kidney transplantation. *Current opinion in organ transplantation* 2016;21:294–300.

20. Ravaioli M, De Pace V, Comai G, et al. Preliminary experience of sequential use of normothermic and hypothermic oxygenated perfusion for donation after circulatory death kidney with warm ischemia time over the conventional criteria - a retrospective and observational study [Internet]. *Transplant International*2018;31:1233–1244. Available from: http://dx.doi.org/10.1111/tri.13311

21. Vodkin I, Kuo A. Extended Criteria Donors in Liver Transplantation. *Clinics in liver disease* 2017;21:289–301.

22. Ferri F, Molinaro A, Poli E, et al. Donor liver small droplet macrovesicular steatosis is associated with reduced graft survival after liver transplantation [Internet]. *Journal of Hepatology*2018;68:S389. Available from: http://dx.doi.org/10.1016/s0168-8278(18)31010-9

23. Ghinolfi D, Lai Q, Dondossola D, et al. Machine Perfusions in Liver Transplantation: The Evidence-Based Position Paper of the Italian Society of Organ and Tissue Transplantation. *Liver transplantation: official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2020;26:1298–1315.

24. Dondossola D, Lonati C, Zanella A, Maggioni M, Antonelli B, Reggiani P, Gatti S, Rossi G. Preliminary Experience With Hypothermic Oxygenated Machine Perfusion in an Italian Liver Transplant Center. *Transplantation proceedings* 2019;51:111–116.

25. Ravaioli M, Grande G, Di Gioia P, Cucchetti A, Cescon M, Ercolani G, Del Gaudio M, Morelli C, Pinna AD. Risk Avoidance and Liver Transplantation: A Single-center Experience

in a National Network. *Annals of surgery* 2016;264:778–786.

26. Ravaioli M, Neri F, Lazzarotto T, et al. Immunosuppression Modifications Based on an Immune Response Assay [Internet]. *Transplantation*2015;99:1625–1632. Available from: http://dx.doi.org/10.1097/tp.0000000000000650

27. Dondossola D, De Falco S, Kersik A, et al. Procurement and ex-situ perfusion of isolated slaughterhouse-derived livers as a model of donors after circulatory death. *ALTEX [Internet]* 2019;Available from: http://dx.doi.org/10.14573/altex.1909131

28. Clavien PA, Barkun J, de Oliveira ML, et al. The Clavien-Dindo classification of surgical complications: five-year experience. *Annals of surgery* 2009;250:187–196.

29. Muller X, Marcon F, Sapisochin G, et al. Defining Benchmarks in Liver Transplantation: A Multicenter Outcome Analysis Determining Best Achievable Results. *Annals of surgery* 2018;267:419–425.

30. Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ, Neuberger J, Ploeg R. New classification of donation after circulatory death donors definitions and terminology [Internet]. *Transplant International*2016;29:749–759. Available from: http://dx.doi.org/10.1111/tri.12776

31. Agopian VG, Harlander-Locke MP, Markovic D, et al. Evaluation of Early Allograft Function Using the Liver Graft Assessment Following Transplantation Risk Score Model [Internet]. *JAMA Surgery*2018;153:436. Available from: http://dx.doi.org/10.1001/jamasurg.2017.5040

32. Aggarwal S, Kang Y, Freeman JA, Fortunato FL, Pinsky MR. Postreperfusion syndrome: Hypotension after reperfusion of the transplanted liver [Internet]. *Journal of Critical Care*1993;8:154–160. Available from: http://dx.doi.org/10.1016/0883-9441(93)90021-c

33. Agopian VG, Markovic D, Klintmalm GB, et al. Multicenter validation of the liver graft assessment following transplantation (L-GrAFT) score for assessment of early allograft dysfunction. *Journal of hepatology [Internet]* 2020;Available from: http://dx.doi.org/10.1016/j.jhep.2020.09.015

34. Schlegel A, Muller X, Dutkowski P. Machine perfusion strategies in liver transplantation. *Hepatobiliary surgery and nutrition* 2019;8:490–501.

35. Ghinolfi D, Pezzati D, Rreka E, Biancofiore G, De Simone P. Transient Cold Storage Prior to Normothermic Liver Perfusion May Facilitate Adoption of a Novel Technology [Internet]. *Liver Transplantation*2020;26:164–165. Available from: http://dx.doi.org/10.1002/lt.25656

36. Ravaioli M, De Pace V, Angeletti A, et al. Hypothermic Oxygenated New Machine Perfusion System in Liver and Kidney Transplantation of Extended Criteria Donors:First Italian Clinical Trial [Internet]. *Scientific Reports*2020;10. Available from: http://dx.doi.org/10.1038/s41598-020-62979-9

37. Czigany Z, Schöning W, Ulmer TF, et al. Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD): a prospective multicentre randomised controlled trial (HOPE ECD-DBD). *BMJ open* 2017;7:e017558.

38. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2010;10:372–381.

39. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic Machine Preservation Facilitates Successful Transplantation of “Orphan” Extended Criteria Donor Livers [Internet]. *American Journal of Transplantation*2015;15:161–169. Available from: http://dx.doi.org/10.1111/ajt.12958

40. Schlegel A, Dutkowski P. Impact of Machine Perfusion on Biliary Complications after Liver Transplantation. *International journal of molecular sciences [Internet]* 2018;19. Available from: http://dx.doi.org/10.3390/ijms19113567

41. Muller X, Marcon F, Sapisochin G, et al. Defining Benchmarks in Liver Transplantation [Internet]. *Annals of Surgery*2018;267:419–425. Available from: http://dx.doi.org/10.1097/sla.0000000000002477

42. Patrono D, Surra A, Catalano G, et al. Hypothermic Oxygenated Machine Perfusion of Liver Grafts from Brain-Dead Donors. *Scientific reports* 2019;9:9337.

43. Zanierato M, Dondossola D, Palleschi A, Zanella A. Donation after circulatory death: possible strategies for in-situ organ preservation. *Minerva anestesiologica [Internet]* 2020;Available from: http://dx.doi.org/10.23736/S0375-9393.20.14262-7

44. Ghinolfi D, Lai Q, Pezzati D, De Simone P, Rreka E, Filipponi F. Use of Elderly Donors in Liver Transplantation: A Paired-match Analysis at a Single Center. *Annals of surgery* 2018;268:325–331.

45. Cescon M, Zanello M, Grazi GL, et al. Impact of very advanced donor age on hepatic artery thrombosis after liver transplantation. *Transplantation* 2011;92:439–445.

46. Schlegel A, Kalisvaart M, Scalera I, et al. The UK DCD Risk Score: A new proposal to define futility in donation-after-circulatory-death liver transplantation. *Journal of hepatology* 2018;68:456–464.

47. Clavien P-A, Dutkowski P. Advances in hypothermic perfusion [Internet]. *Liver Transplantation*2017;23:S52–S55. Available from: http://dx.doi.org/10.1002/lt.24844

48. De Carlis R, Lauterio A, Ferla F, Di Sandro S, Sguinzi R, De Carlis L. Hypothermic Machine Perfusion of Liver Grafts Can Safely Extend Cold Ischemia for Up to 20 Hours in Cases of Necessity. *Transplantation* 2017;101:e223–e224.

49. Jasseron C, Francoz C, Antoine C, et al. Impact of the new MELD-based allocation system on waiting list and post-transplant survival—a cohort analysis using the French national CRISTAL database [Internet]. *Transplant International*2019;32:1061–1073. Available from: http://dx.doi.org/10.1111/tri.13448

50. Barshes NR, Horwitz IB, Franzini L, Vierling JM, Goss JA. Waitlist mortality decreases with increased use of extended criteria donor liver grafts at adult liver transplant centers. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2007;7:1265–1270.

51. Kron P, Schlegel A, Mancina L, Clavien P-A, Dutkowski P. Hypothermic oxygenated

perfusion (HOPE) for fatty liver grafts in rats and humans. *Journal of hepatology [Internet]* 2017;Available from: http://dx.doi.org/10.1016/j.jhep.2017.08.028

52. Dutkowski P, Odermatt B, Heinrich T, Schönfeld S, Watzka M, Winkelbach V, Krysiak M, Junginger T. Hypothermic oscillating liver perfusion stimulates ATP synthesis prior to transplantation. *The Journal of surgical research* 1998;80:365–372.

53. Muller X, Schlegel A, Kron P, Eshmuminov D, Würdinger M, Meierhofer D, Clavien P-A, Dutkowski P. Novel Real-time Prediction of Liver Graft Function During Hypothermic Oxygenated Machine Perfusion Before Liver Transplantation. *Annals of surgery* 2019;270:783–790.

54. Paggio A, Checchetto V, Campo A, Menabò R, Di Marco G, Di Lisa F, Szabo I, Rizzuto R, De Stefani D. Identification of an ATP-sensitive potassium channel in mitochondria. *Nature* 2019;572:609–613.

	Overall population (n=50)
Donor characteristics	
Age, years	60 (50-74)
Male sex, n (%)	34 (68)
BMI, kg/m2	25 (23-26)
Already in ECMO, n (%)	7 (14)
Brain death donors, n (%)	28
CPT tot, min	533 (427-607)
ALT peak during NRP, UI/L	129 (76-798)
Biopsy	
Macrosteatosis, %	10 (5-20)
Microsteatosis, %	5 (0-10)
Fibrosis, Ishak	1 (0-1)
Machine perfusion characteristics	
Duration, min	169 (91-232)
Max Lactate, mmol/L	3 (2-4.3)
Flow portal Start, ml/min	200 (110-252)
Flow portal End, ml/min	250 (205-280)
Pressure portal Start, mmHg	5 (5-5)
Pressure portal End, mmHg	5 (4-5)
Flow arterial Start, ml/min	52 (35-63)
Flow arterial End, ml/min	69 (55-81)
Pressure arterial Start, mmHg	25 (24-25.5)
Pressure arterial End, mmHg	25 (24-25)
Recipient characteristics	
Age, years	58 (53-63)
Male sex, n (%)	42 (84)
BMI, kg/m2	25 (23-27)
MELD score	11 (9-15)

Na-MELD	13 (10-16)
Indication for LT	
Alchoholic cirrhosis, n (%)	10 (20)
Hepatitis B cirrhosis, n (%)	10 (20)
Hepatitis C cirrhosis, n (%)	14 (28)
Hepatitis B+D+C cirrhosis, n (%)	3 (6)
Non-alcoholic steatohepatitis, n (%)	6 (12)
Primary sclerosing cholangitis, n (%)	2 (4)
Metastasis, n (%)	2 (4)
Other, n (%)	3 (6)
Concurrent HCC, n (%)	37 (74)
Bilirubine at LT, mg/dL	2 (0.88-2.79)
Creatinine at LT, mg/dL	1 (0.69-0.99)
Portal vein thrombosis at LT, n (%)	6 (12)
T Tube use, n (%)	44 (88)

Table 1. Donor, machine perfusion and machine characteristics in the overall population. BMI, body mass index; ECMO, extracorporeal membrane oxygenation; DCD, donor after cardiocirculatory death; WIT, warm ischemia time; CPT, cold preservation time; ALT, aspartate alanine transferase; MELD, model for end stage liver disease.

	CPT≤ 9H (n=25)	CPT > 9H (n=25)	p
Donor characteristics			
Age, years	60 (52-73)	58 (50-75)	0.32
Male sex, n (%)	19 (76)	15 (60)	0.22
BMI, kg/m2	24 (22-26)	25 (24-27)	0.08
CPT tot, min	425 (390-480)	610 (565-690)	<0.001
ALT peak during NRP, UI/L	92 (61.7-122.5)	936.5 (140.2-2468.2)	0.10
Lactate peak during NRP, mmol/L	9.9 (8.1-12.8)	11.1 (7.2-11.7)	0.48
Biopsy			
Macrosteatosis, %	5 (0-5)	10 (5-15)	0.38
Microsteatosis, %	0 (0-7)	5 (4-11)	0.41
Fibrosis, Ishak	1 (1-1)	0 (0-1)	0.30
Macrovescicular steatosis >40%, n (%)	3 (12)	4 (16)	0.68
Functional WIT (controlled DCD), min	40 (33-52)	40 (35-48)	0.52
Low flow time (uncontrolled DCD), min*	85	110-87	/
No flow time (controlled DCD), min	28 (25-32)	32 (29-34)	0.18
No flow time (uncontrolled DCD), min	33	30-38	/
Total WIT, min	49 (39-65)	56 (53-74)	0.79
Normothermic regional perfusion time, min	240 (181-285)	250 (217-318)	0.33
Machine perfusion characteristics			
Duration, min	120 (75-168)	210 (170-290)	0.04
Lactate max, mmol/L	2.4 (1.3-3.7)	3.4 (2.5-4.6)	0.70
Flow portal Start, ml/min	200 (104-252)	185 (118-255)	0.87
Flow portal End, ml/min	250 (220-285)	250 (230-280)	0.43
Pressure portal Start, mmHg	5 (5-5)	5 (4-5)	0.56
Pressure portal End, mmHg	5 (5-5)	4 (4-5)	0.12
Flow arterial Start, ml/min	53 (44-56)	50 (35-68)	0.42
Flow arterial End, ml/min	69 (67-74)	68 (55-84)	0.80
Pressure arterial Start, mmHg	25 (24-25)	25 (24-26)	0.31

Pressure arterial End, mmHg	25 (25-25)	24 (24-25)	0.58
Recipient characteristics			
Age, years	58 (56-65)	58 (51-62)	0.25
Male sex, n (%)	23 (92)	20 (80)	0.22
BMI, kg/m2	25 (24-28)	25 (22-27)	0.08
MELD score	10 (8-12)	12 (9-16)	0.38
Na-MELD	11 (10-16)	13 (9-16)	0.80
Concurrent HCC, n (%)	20 (80)	17 (68)	0.33
Portal Thrombosis at LT, n (%)	4 (16)	2 (8)	0.38
T Tube use, n (%)	20 (80)	24 (96)	0.08
Post-LT hospitalization			
ICU stay, days	3 (2-5)	4 (2-6)	0.09
Overall hospitalization, days	13 (11-15)	16 (13-24)	0.02
Early allograft dysfunction, n (%)*	2 (8)	8 (32)	0.009
Primary non-fucntion, n (%)	1 (4)	0 (0)	0.73
Post-LT AST peak, UI/L	509 (364-961)	994 (630-1747)	0.04
Post-LT ALT peak, UI/L	486 (365-897)	709 (516-1723)	0.03
Post-LT INR peak	1.6 (1.4-1.8)	1.7 (1.5-1.9)	0.60
Complications >3a Clavien Dindo, n (%)	3 (12)	10 (40)	0.02
Comprehensive Complication Index			
Hospitalization	24.2 (15.1-33.4)	34.3 (27.5-41-0)	0.05
3 months	27.9 (15.8-44.7)	37.9 (31.3-44.5)	0.13
6 months	32.7 (16.6-48.7)	41.8 (34.9-48.7)	0.17
Anastomostic biliary complications*, n (%)	3 (12)^	1 (4)	0.72
Vascular complications, n (%)	1 (4)	1 (4)	0.84

Table 2. Donor, machine perfusion and recipient characteristics of grafts that suffered cold preservation time (CPT) over (CPT>9h) or below (CPT<9h) 9 hours. BMI, body mass index; DCD, donor after cardiocirculatory death (n=22); controlled DCD, Maastricht class III DCD (n=19); uncontrolled DCD, Maastricht class II DCD (n=3); WIT, warm ischemia time; ICU,

intensive care unit; ALT, aspartate alanine transferase; MELD, model for end stage liver disease; AST, aspartate transferase; INR, international normalized ratio; *, excluded T-tube removal complications; ^, ITBL case

	DBD ECD (n=28)	DCD (n=22)	p
Donor characteristics			
Age, years	72 (58-77)	56 (48-59)	0.01
Male sex, n (%)	15 (53)	19 (86)	0.01
BMI, kg/m2	25 (23-27)	24 (24-26)	0.45
Maastricht class III, n (%)	-	19 (86)	-
Functional WIT (controlled DCD), min	-	40 (33.5-51)	-
Low flow time (uncontrolled DCD), min*	-	85-87-110	-
No flow time (controlled DCD), min	-	30 (25-33)	-
No flow time (uncontrolled DCD), min	-	33-30-38	-
Total WIT, min	-	54 (40-66)	-
Normothermic regional perfusion time, min	-	240 (181-300)	-
Lactate end NRP, mmol/L	-	4.7 (3.8-6.2)	-
UK DCD Score	-	11 (8-13)	-
Cold preservation time, min	580 (468-666)	500 (421-561)	0.05
Biopsy			
Macrosteatosis, %	7.5 (5-20)	10 (0-12.5)	0.70
Microsteatosis, %	5 (0-15)	5 (0-10)	0.98
Fibrosis, Ishak	1 (0-1)	1 (0-1)	0.62
Macrovescicular steatosis >40%, n (%)	5 (18)	2 (9)	0.37
Machine perfusion characteristics			
Duration, min	162 (97-210)	180 (91-240)	0.72
Max Lactate, mmol/L	3 (1.6-4.6)	3 (2.3-4.1)	0.68
Flow portal Start, ml/min	115 (108-212)	220 (200-285)	0.002
Flow portal End, ml/min	240 (205-257)	255 (210-302)	0.77
Pressure portal Start, mmHg	5 (5-5)	5 (4-5)	0.90
Pressure portal End, mmHg	5 (4-5)	4 (4-5)	0.48
Flow arterial Start, ml/min	43 (30-71)	54 (40-61)	0.62

Flow arterial End, ml/min	67 (53-77)	70 (57-88)	0.21
Pressure arterial Start, mmHg	25 (24-26)	25 (24-25)	0.99
Pressure arterial End, mmHg	25 (24-25)	24 (24-25)	0.88
Recipient characteristics			
Age, years	57 (51-59)	60 (54-65)	0.24
Male sex, n (%)	23 (82)	20 (90)	0.22
BMI, kg/m2	25 (23-27)	25 (24-29)	0.18
MELD score	12 (9-16)	10 (9-12)	0.10
Na-MELD	14 (10-19)	11 (9-13)	0.02
Concurrent HCC, n (%)	21 (75)	16 (72)	0.10
Portal Thrombosis at LT, n (%)	2 (7)	4 (18)	0.38
T Tube use, n (%)	22 (78)	22 (100)	0.09
Hospitalization Data			
ICU stay, days	3 (2-5)	4 (2-5)	0.43
Overall hospital stay, days	10 (6-13)	11 (9-15)	0.66
Early allograft dysfunction, n (%)	5 (17)	5 (22)	0.67
CVVH/RRT, n (%)	2 (7%)	4 (18%)	0.22
Post-LT AST peak, UI/L	575 (367-1243)	969 (829-1306)	0.10
Post-LT ALT peak, UI/L	593 (258-1162)	692 (464-1077)	0.25
Post-LT INR peak	1.6 (1.5-1.7)	1.5 (1.3-1.8)	0.71
Post-LT bilirubine peak, mg/dL	4.4 (2.8-6.2)	3.5 (2.1-6.6)	0.32
Complications >3a Clavien Dindo, n (%)	8 (28)	5 (22)	0.64
Comprehensive Complication Index			
Hospitalization	32.2 (23.3-41.1)	30.6 (22.6-36.8)	0.76
3 months	33.1 (24.4-41.9)	36.5 (26.5-46.6)	0.59
6 months	39.0 (29.2-48.8)	39.3 (32.9-45.4)	0.96
Primary non function, n (%)	0 (0)	1 (4)	0.87
Re-transplant, n (%)	0 (0)	2 (11)	0.41
Biliary complications*, n (%)	3 (10)^	1 (4)	0.81
Vascular complications, n (%)	1 (3)	1 (4)	0.98

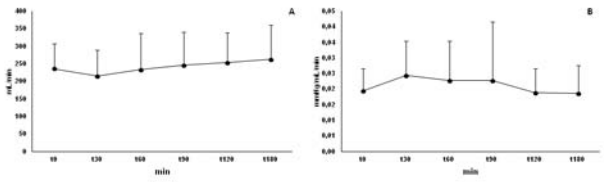
Table 3. Comparison between donor, machine perfusion and recipient characteristics of grafts procured from DCD e DBD; BMI, body mass index; DCD, donor after cardiocirculatory death; WIT, warm ischemia time; ALT, aspartate alanine transferase; MELD, model for end stage liver disease; AST, aspartate transferase; INR, international normalized ratio; *, excluded T-tube removal complications; ^, ITBL case

Figure 1. Portal flow (A) and resistances (B) during hypothermic oxygenated machine perfusion. Results are showed until t180 (15 (D) HOPE procedure lasted>180 min).

Figure 2. Main metabolites trend during hypothermic oxygenated machine perfusion (glucose (A), lactate (B) and potassium (C)). Results are showed until t180 (15 (D)HOPE procedure lasted>180 min).

Figure 3. Potassium release ratio in patients that developed acute kidney injury (AKI) or not (nAKI) (A). Post-LT transaminases peak (B) in patients that were transplanted with graft affected by prolonged cold preservation time. *, p=0.03 °, p=0.04

Figure 4. Potassium release ratio during hypothermic oxygenated machine perfusion (A) and comprehensive complication index (CCI) in patients that developed or not early allograft dysfunction (EAD) (B).



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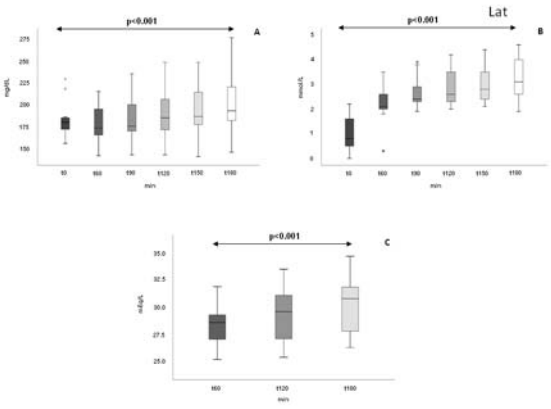

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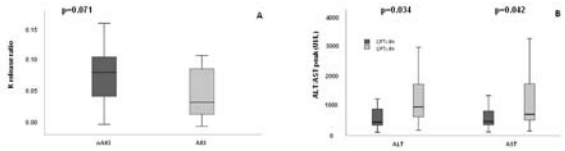

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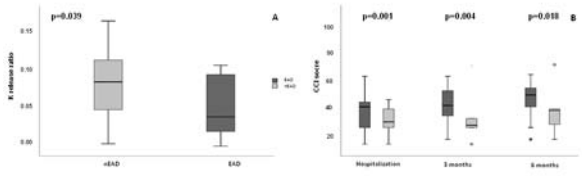

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