Rescue of Discarded Grafts for Liver Transplantation by Ex Vivo Subnormothermic and Normothermic Oxygenated Machine Perfusion: First Experience in Spain

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ABSTRACT

Background. Ex vivo machine perfusion (MP) has been reported as a possibly method to rescue discarded organs. The main aim of this study was to report an initial experience in Spain using MP for the rescue of severely marginal discarded liver grafts, and to, secondarily, define markers of viability to test the potential applicability of these devices for the real increase in the organ donor pool.

Methods. The study began in January 2016. Discarded grafts were included in a research protocol that consisted of standard retrieval followed by 10 hours of cold ischemia. Next, either normothermic (NMP) or controlled subnormothermic (subNMP) rewarming was chosen randomly. Continuous measurements of portal-arterial pressure and resistance were screened. Lactate, pH, and bicarbonate were measured every 30 minutes. The perfusion period was 6 hours, after which the graft was discarded and evaluated as potentially usable, but never implanted. Biopsies of the donor and at 2, 4, and 6 hours after ex vivo MP were obtained.

Results. A total of 4 grafts were included in the protocol. The first 2 grafts were perfused by NMP and grafts 3 and 4 by subNMP. The second and third grafts showed a clear trend toward optimal recovery and may have been used. Lactate dropped to levels below 2.5 mmol/L with stable arterial and portal pressure and resistance. Clear biliary output started during MP. Biopsies showed an improvement of liver architecture with reduced inflammation at the end of the perfusion.

Conclusion. This preliminary experience has demonstrated the potential of MP devices for the rescue of severely marginal liver grafts. Lactate and biliary output were useful for viability testing of the grafts. The utility of NMP or subNMP protocols requires further research.

LIVER transplantation (LT) is a feasible and safe option for several liver diseases. However, the balance between the number of recipients and potential donors remains unfavorable, leading to death on waiting list and restricted criteria for listing in order to optimize utilization of the grafts. Several alternatives have been proposed to increase the donor pool. Living donor liver transplantation (LDLT) is the main source of liver grafts in some parts of

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Table 1. Descriptive Analysis of the 4 Cases Included in Our Research

	Case 1	Case 2	Case 3	Case 4
Age	67	46	81	83
COD	Stroke	Polytrauma	Stroke	Stroke
BMI	34	35	36	34
AST	35	409	230	17
ALT	22	320	130	11
GGT	169	117	95	40
ITU stay	2	15	2	2
DRI	1.56	1.67	1.95	1.88
Lactate	1.4	1.2	1.7	2.3
Comorbidities	Dialysis + cardiac insufficiency	Dislipemia	AF + cardiac insufficiency	HT, DM
Steatosis	50%	25%	60%	50%

Abbreviations: AF, atrial fibrillation; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; COD, cause of death; DM, diabetes mellitus; DRI, donor risk index; GGT, Gamma-glutamyltransferase; HT, heart transplant; ITU, intensive care unit.

the world, but in Western countries cadaveric donation remains the main source of organs.

The profile of donors has changed in recent years. Nowadays, most donors are older and have severe comorbidities [1]. Fatty content has also been considered a contraindication to transplantation. However, mild to moderately fatty livers have been used extensively in recent years, with acceptable results [2–4]. These variables have led to defining the concept of marginal or extended criteria donors, which is now accepted among liver transplant teams. Unfortunately, some liver grafts are discarded due to excess risk and are considered unsuitable for transplantation [5,6].

The use of ex vivo machine perfusion (MP) devices has shown excellent results in the most recent decade [7,8]. The potential of these devices is enormous as they may reduce cold ischemia time, ischemia-reperfusion injury, and allow for real-time testing of the grafts. Results from the first randomized, controlled trials have been reported, showing excellent safety and improved posttransplant liver function testing [9]. Several techniques and protocols have been reported, including hypo-, normo-, and subnormothermic machine perfusion [10,11]. The main aim of our research is to report the first preliminary experience using MP devices for the rescue of discarded human liver grafts by normothermic (NMP) or subnormothermic (subNMP) ex vivo perfusion. In addition, we aimed to define markers of viability to test the potential applicability of these devices for the real increase of organ donor pool.

MATERIALS AND METHODS

(a) *Inclusion protocols and exclusion criteria.* All donors from our geographic area, starting on January 2016, were considered as potentially suitable for inclusion in our study. Relatives were consented for potential retrieval of the graft if previously discarded by all liver transplant teams in Spain. After this, the graft was included in the research. We considered suitable for a "potential rescue" all livers that could be potentially implanted after perfusion. This criterion excluded cirrhotic livers. Also, grafts discarded due to intraoperative findings of tumors in the donor were discarded, as these were not pathologic grafts, and thus the need for rescue under MP devices would be futile.

(b) *Procedures.* After inclusion of the graft in the study, standard procurement was performed with cold perfusion using University of Wisconsin (UW) or Celsior fluids with both portal and aortic perfusion. Thereafter, a 10-hour period of cold ischemia time (CIT) period was defined. After this, if the graft was allocated to the NMP group, a 33°-37°C perfusion was started by using a combination of blood transfusion with several additives, as reported previously [12]. After this, the NMP was maintained for 6 hours. If the graft was allocated to subNMP, then perfusion in the MP would start with machine perfusion with UW solution at 10°C, raised 5°C every 10 minutes until reaching 20°C. Next, after a 10-minute period of UW perfusion at 20°C, the system was poured out and a quick refill was performed using the previously cited combination of blood and additives. Another sequential increase of 5°C every 10 minutes was performed until reaching 33°-37°C, after which 6 hours of normothermic perfusion was applied. In all cases, a 30-minute warm ischemia time (WIT) was initiated by unplugging the MP device, simulating an actual transplant. All MP tests were oxygenated as reported previously. The Liver Assist (Organ Assist, The Netherlands) device was used in our research.

(c) *Measurements.* Liver biopsies were performed at 2, 4, and 6 hours during MP after WIT and before the recovery procedure. During the MP, continuous measurements of portal and arterial flows and pressures were screened. Blood gas tests were then performed, which included lactate, bicarbonate, and pH every 30 minutes during the perfusion. Bile output was measured qualitatively (inspection of clear or thick bile) every hour and quantitatively. A graft was considered as usable for potential transplant if adequate flows with no increased pressure were obtained and if lactate levels followed a clear trend toward a decrease during normothermic perfusion.

(d) *Ethics.* Informed consent was obtained from every case included in our protocol. The informed consent and all the research was previously approved by the ethics committee for Human Research of the University Hospital Reina Sofia, Cordoba, Spain, on March 26 (Acta 239, ref 2780; Protocol PI14/01559).

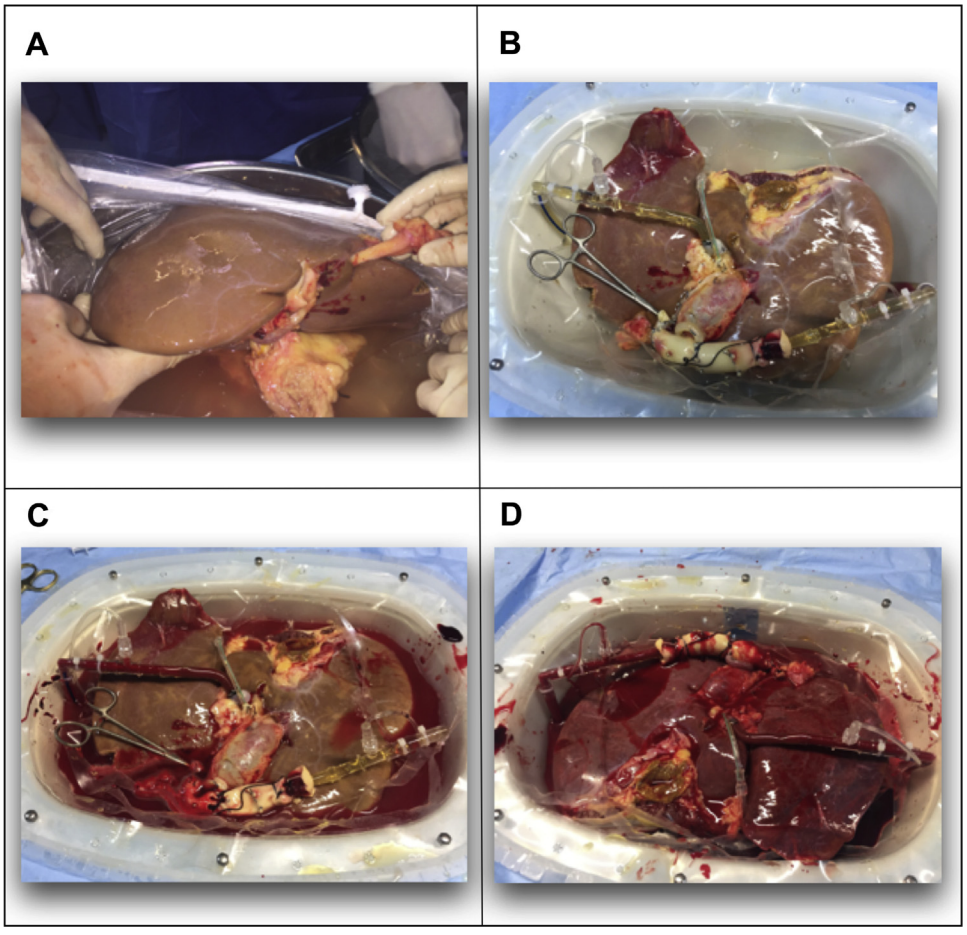

Fig 1. External view of the liver graft: during the procurement **(A)**; inside the machine perfusion device during cold perfusion **(B)**; at the exact moment in which cold perfusion switches to subnormothermic perfusion **(C)** where clear perfusion fluid is seen in the arterial cannula and blood through the portal vein cannula; and during normothermic perfusion **(D)**.

RESULTS

(a) *Baseline results.* A total of 4 cases were included in the research during the study period. Descriptive baseline data are presented in Table 1. All cases were discarded due to pathologic aspects of the graft with moderate to severe steatosis and comorbidities. The first 2 cases were included in the NMP protocol and the others were included in the subNMP protocol. Identifying external aspects of the graft was important to assess a proper perfusion with absence of ischemic or unperfused areas (Fig 1).

(b) *Recovery parameters.* During the 6-hour simulation period, grafts 1 and 4 had unfavorable parameters of recovery. Grafts 1 and 4 needed increased pressure rates to obtain portal flows below 600 mL/min. Graft 1 actually did not reach 400 mL/min during the first 2 hours after perfusion, showing important pressure rates. In this sense, lactate was permanently above 20 mmol/L and did not show any decrease during the perfusion. A similar situation occurred with graft 4, in which permanent high lactate levels were detected in the perfusate, with no decreased trends. A different situation was observed with grafts 2 and 3. The arterial and portal flows were stable and required no increase in pressure (Fig 2). Similarly, although lactate started at 20 mmol/L, it decreased during the NMP, and was stable around 2.5 mmol/L at the end of the simulation period. Biliary output was present and clear in these grafts, with a rate of 20 mL at the end of the perfusion period. Grafts 1 and 4 showed no bile output (graft 1) or it was thick hematic fluid (graft 4). Biopsies showed severe inflammation immediately after the perfusion with progressive recovery of the liver architecture, with important preservation of areas 1 and 2.

DISCUSSION

The use of discarded liver grafts may be a viable option in the Western countries in which the donor pool still consists of mostly cadaveric organs. In this context, donation after

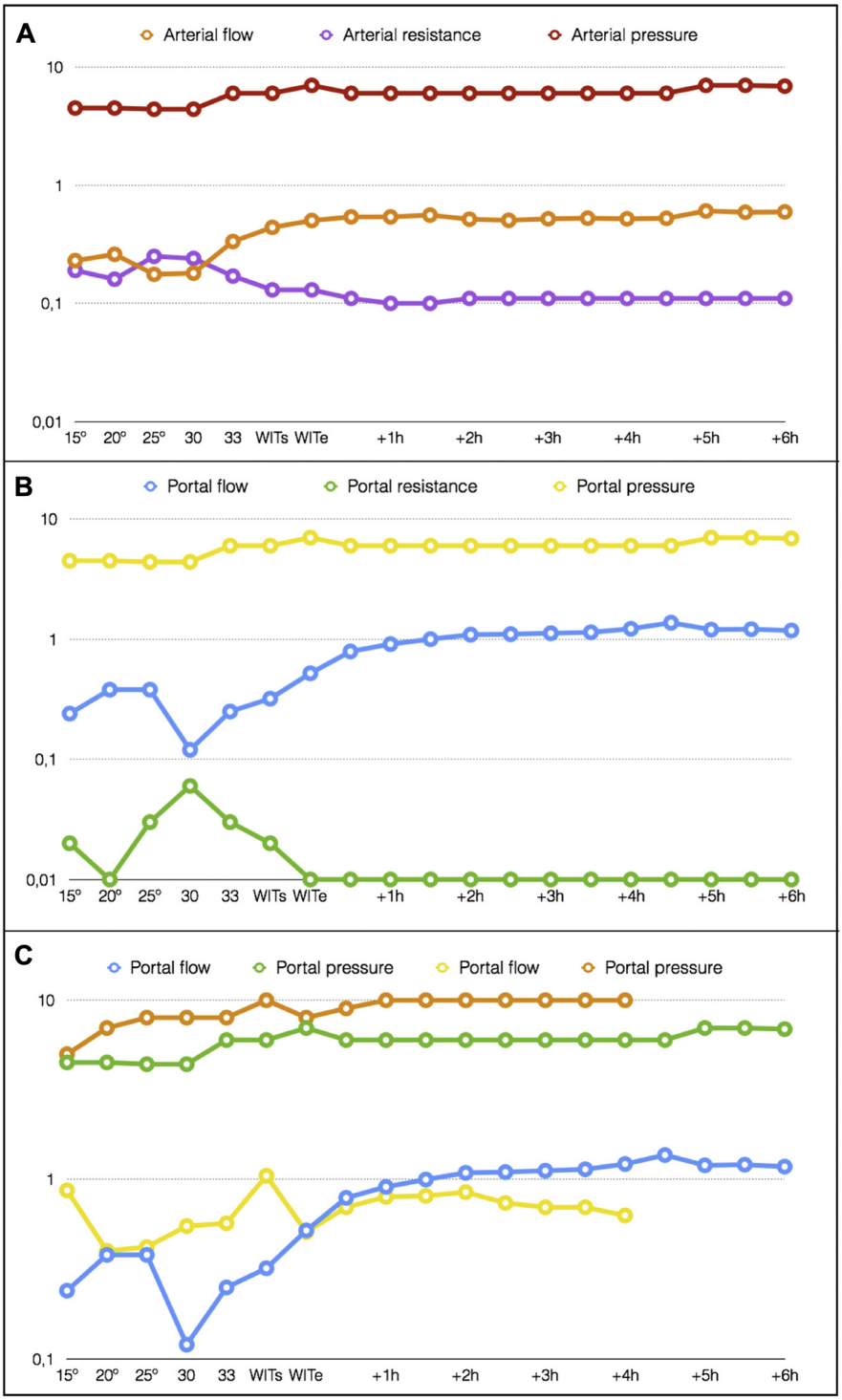

Fig 2. Measurements during machine perfusion. As observed, adequate flows should be obtained during the perfusion with decreased resistances and constant pressure in both arterial **(A)** and portal **(B)** measurements. Increased pressure to maintain flow (case with orange and yellow lines) reflects the graft's increasing resistance, contrary to optimal functioning (case with blue and green lines) in which adequate flow is observed with maintained stable pressure **(C)**.

cardiac death (DCD) donors have also become a source of organs but they may also become marginal grafts when extended use with increased criteria may be considered. For both brain-dead and DCD grafts, the use of MP devices may be useful and could potentially recover borderline or clearly marginal grafts. Recently, UK liver transplant teams have reported an initial experience using NMP on discarded grafts, with acceptable outcomes [13]. Similarly, a group from Zürich

recently reported the first international matched case analysis of static cold storage vs hypothermic oxygenated perfusion in DCDs, also with excellent results [14].

It is still unknown which protocol (hypo-, normo-, or subnormothermic) is better. This may well be multifactorial and it is likely the same protocol would not work for fatty, DCD, or aged grafts. In our preliminary experience, both NMP and subNMP worked adequately in the recovery of 2 grafts. It is important to note that our protocol was an extreme protocol in which severely marginal grafts underwent a prolonged CIT, followed by a strict long WIT period. In this situation, recovery of grafts was a clear challenge for every protocol. To date, no previous reports on human extremely marginal liver grafts has been reported to be effective when using subNMP. In our experience, subNMP, including a gradual increase of between 20°C and 35°C, has been useful. Previous protocols of subNMP excluded this range of temperature [10]. Although we hypothesize that controlled rewarming in this range of temperature may be useful in severely marginal grafts, further research is needed. It is also important to note that lactate trends and bile production during the perfusion seem to be the most effective markers to define whether a graft may work well when finally implanted. We can conclude that, based on our preliminary experience, MP devices may be a valuable tool for recovery of discarded grafts. In our opinion, MP devices that can work with perfusion ranges between 4° and 37° may offer an extra opportunity to work with increasing ranges of temperature and define optimal strategies for different marginal grafts.

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