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Impact of the duration of normothermic regional perfusion on the results of liver transplant from controlled circulatory death donors: a retrospective, multicentric study

Louise Barbier^{1,2}, Thomas Guillem³, Eric Savier⁴, Olivier Scatton⁴, Federica Dondero⁵, Anne-Gaëlle Si Larbi⁶, Petru Bucur^{1,2}, Laurent Sulpice⁷, Fabien Robin⁷, Claire Goumard⁴, Xavier Muller⁸, Jean-Christophe Venhard^{2,3,9}, Marc-Antoine Allard¹⁰, Gabriella Pittau¹⁰, Olivier Soubrane⁵, Emmanuelle Mercier¹¹, Marc Laffon³, Mickael Lesurtel⁸, Ephrem Salamé^{1,2}

Affiliations:

1: Department of Digestive Surgery and Liver Transplantation, University Hospital of Tours, University of Tours, Tours, France

2: FHU Suport, Tours, France

3: Anesthesiology and Surgical Critical Care Medicine Department, Tours University Hospital, University of Tours, Tours, France

4: Digestive Surgery and Liver Transplantation, Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, Sorbonne University, Paris, France; St Antoine Research Center (CRSA), Sorbonne University, INSERM, Paris, France

5: Hepatobiliary Surgery and Liver Transplantation, Beaujon hospital, Assistance Publique-Hôpitaux de Paris, Paris-Diderot University, Clichy, France

6: Medical and Surgical Intensive Care Unit, Foch Hospital, Suresnes, France

7: Department of Hepatobiliary and Digestive Surgery, Pontchaillou University Hospital, Rennes, France

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8: Department of Digestive Surgery and Liver Transplantation, Croix Rouse University Hospital, University of Lyon I, 69004 Lyon, France.

9: Coordination for organ and tissue donation and procurement, Tours University Hospital, University of Tours, Tours, France

10: AP-HP Hopital Paul Brousse, Department of HPB surgery and liver transplantation, Centre Hépatobiliaire, Paris-Saclay University, Villejuif, France.

11: INSERM CIC 1415, Intensive Care Medicine, University Hospital of Tours, University of Tours, Tours, France

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Corresponding author:

Louise BARBIER, Department of Digestive Surgery and Liver Transplantation, University Hospital of Tours, avenue de la République, Chambray-lès-Tours

Tel +(33)2-47-47-81-23, Fax +(33)2-47-47-59-10, louisebarbier@hotmail.fr

Barbier L, Guillem T, Savier E, Scatton O, Dondero F, Si Larbi AG, Bucur P, Sulpice L, Robin F, Goumard C, Muller X, Venhard JC, Allard MA, Pittau G, Soubrane O, Mercier E, Laffon M, Lesurtel M, Salamé E

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Clin Transpl.

Abstract

In France, the program of controlled donation after circulatory death (cDCD) was established with routine use of *in situ* normothermic regional perfusion (NRP). There is currently no consensus on its optimal duration. The purpose was to assess the impact of NRP duration on liver graft function and biliary outcomes. 156 liver recipients from NRP-cDCD donors from six French centers between 2015 and 2019 were included. Primary endpoint was graft function assessed by early allograft dysfunction (EAD, according to Olthoff's criteria) and MEAF (model for early allograft function) score. Overall, three (1.9%) patients had primary non-function, 30 (19.2%) patients experienced EAD, and MEAF score was 7.3 (± 1.7). Mean NRP duration was 179 (± 43) min. There was no impact of NRP duration on EAD (170 ± 44 min in patients with EAD vs. 181 ± 42 min in patients without, $p=0.286$). There was no significant association between NRP duration and MEAF score ($p=0.347$). NRP duration did neither impact on overall biliary complications nor on non-anastomotic biliary strictures (overall rates of 16.7% and 3.9%, respectively). In conclusion, duration of NRP in cDCD donors does not seem to impact liver graft function and biliary outcomes after liver transplantation. A 1 to 4-hours perfusion represents an optimal time window.

Keywords: liver transplantation, ischemia-reperfusion injury, delayed graft function, normothermic regional perfusion, controlled circulatory deceased donors

Corresponding author: Louise BARBIER, Department of Digestive Surgery and Liver Transplantation, University Hospital of Tours, avenue de la République, Chambray-lès-Tours

Introduction

With growing indications for liver transplantation (LT), the number of recipients has increased (+3.3% in France in the past 5 years ¹), while new leads are being developed to overcome organ shortage. Among those, donation after circulatory death (DCD) has been proposed in addition to donation after brain death ^{2,3}. In France, the program of donation after controlled circulatory death (cDCD, Maastricht category III) started in 2015. Based on the experience of donation after uncontrolled circulatory death, the French Steering Committee for donation after circulatory death made the choice to mandatorily use normothermic regional perfusion (NRP) after cardiac arrest ⁴ instead of rapid retrieval, in an attempt to decrease the higher rates of biliary complications first described with grafts from DCD donors ⁵.

The use of *in situ* abdominal NRP allows abdominal organs (liver and kidneys) to receive non-pulsatile oxygenated blood at 37°C provided by an extracorporeal membranous oxygenation device. Although no randomized trials have ever been conducted, the rates of ischemic cholangiopathy with NRP seem to be reduced to 0-2%. NRP could also help in reducing the damage associated to warm ischemia and ischemia-reperfusion injury. It is currently used in eight European countries ⁶. However, protocols are different in every country, including duration of warm ischemia before the setup of NRP, and the duration and pump flow of the NRP. Recently, the international liver transplantation society (ILTS) guidelines recommended NRP to last between 1 to 4 hours ⁶, mostly based on the actual clinical practice. Initially, preclinical models first demonstrated that 30 minutes of NRP should be sufficient to allow graft recovery ^{7,8}. Hence, the question remains whether extensive NRP could also improve the graft function and post-transplant outcomes.

The objective of this study was to assess the outcomes of NRP-cDCD liver grafts according to *in situ* NRP duration in terms of graft function and biliary complications.

Patients and Methods

Study design and Setting

This is a retrospective study including data from six French LT centers. All consecutive patients who received a liver graft from controlled DCD Maastricht category III between 2015 and 2019 were included. *In situ* NRP was used according to the French protocol, and no *ex situ* machine perfusion was used during the time of this study. Methodology followed the STROBE cohort guidelines⁹.

NRP-cDCD program in France (Figure 1)

We describe here the conditions of cDCD Maastricht category III as they were during the study period. Conditions have slightly changed with longer functional warm ischemia time (WIT) up to 45min, and donors age up to 70years and are available on the Biomedicine agency website (see¹⁰ for full protocol).

Eligibility criteria for donors and recipients are described in **Figure 1**. Canulation was performed either with percutaneous or direct groin incision *post mortem*, although guide wires may have been placed *ante mortem*. The agonal phase, defined by the time between withdrawal and circulatory arrest, should last 3 hours maximum. Heparin was administered at the time of support withdrawing. The “no-touch” period after cardiac arrest was 5 minutes. Functional donor WIT, defined by the time between mean arterial pressure below 45mmHg and the NRP set up, was 30 min maximum. Cold ischemia time (CIT), defined by the time between NRP cessation and graft reperfusion, was maintained below 8 hours. NRP pump flow was maintained between 2 and 3.5L/min, and NRP lasted between 1 and 4 hours. In case of concomitant lung procurement, two techniques were possible: (i) either first lung procurement while NRP was ongoing for more than one hour and then usual abdominal procurement, (ii) or concomitant procurement of lungs and abdominal organs with duration

of NRP of one hour. The latter technique was chosen by some teams in order not to experience the pump flow drop at the time of inferior vena cava clamping.

Data collection and Ethics

Data were collected both prospectively (regarding NRP procedure and donor) and retrospectively (regarding recipient and post-transplant outcomes) from local databases declared to the French data protection authority. Anonymized data on donors were obtained from the on-line secured Biomedicine Agency database (Cristal) approved by the CNIL. This observational study did not require any written consent in accordance with the bioethical French Law (Jardé's law). The study has been performed in accordance with the 2000 declaration of Helsinki and the declaration of Istanbul 2008.

Variables and outcomes

NRP duration was expressed in minutes (min) and used as a continuous variable. The UK-DCD Risk score was recorded according to the definition published by Schlegel et al.'s ¹¹. Function of NRP-cDCD liver grafts was quantitatively assessed with the model for early allograft function scoring (MEAF, as previously described by Pareja et al. ¹²); early allograft dysfunction (EAD) was defined according to Olthoff et al.'s as the presence of one or more of the following: bilirubin superior to 10mg/dL on day 7, international normalized ratio superior to 1.6 on day 7, and alanine (ALT) or aspartate aminotransferases (AST) peaks superior to 2000 IU/L within the first 7 days ¹³. Primary non-function was defined according to the United States Organ Procurement and Transplantation Network ¹⁴ and evidenced within 7 days of transplant by: AST superior to 3000 IU/L and at least 1 of the following: international normalized ratio superior to 2.5, arterial pH inferior to 7.30, and lactate superior to 4 mmol/L, leading either to retransplantation or to death within 7 days after LT without any identifiable cause of graft failure. Total biliary complications included biliary fistula,

anastomotic and non-anastomotic strictures. Acute kidney injury in the first week post transplant was defined by Kidney disease improving global outcomes (KDIGO)¹⁵.

Statistical analysis

Continuous data were presented as mean with standard deviation. Categorical data were summarized as numbers and percentages. Non-parametric Mann-Whitney tests were used to compare quantitative variables. A linear regression analysis was used to search for an association between two quantitative variables. Two-sided probability values less than 0.05 were considered statistically significant in all tests. Statistical analyses were performed using Prism v8.

Results

Donors and recipients characteristics (Table 1)

During the study period, 157 LT procedures were performed with NRP-cDCD donors in the six centers; only 156 procedures for which duration of NRP was available were included in the study. Mean follow-up was 2 (± 1) years. Donors had a mean age of 48 years. Recipients were mostly male patients with hepatocellular carcinoma and a low MELD score. Underlying hepatopathy was mostly alcoholic, followed by viral hepatitis and non-alcoholic steatohepatitis.

Procurement and LT procedures (Table 2)

Functional donor WIT was below 30 min according to protocol with a mean of 22 min. Mean NRP duration was 179 (± 43) min. Procurement procedures were performed using colloid solutions for abdominal organs (IGL-1® in 74% and SCOT 15® in 23% of the donors). Regarding LT procedure, CIT was maintained below 8 hours according to protocol with a mean duration of 5.8 hours.

Outcomes of LT in the whole cohort

Three (1.9%) patients had primary non-function, including one patient with ABO incompatibility, and 30 (19.2%) patients experienced EAD according to Olthoff's criteria. Among the latter, six patients had elevated bilirubinemia, whereas the others had only increased ALT and/or AST. MEAF score was 7.3 (± 1.7). Four patients developed an arterial thrombosis, including three (1.9%) patients in the early postoperative period. Regarding biliary complications, the overall rate was 16.7% (26 patients), including 6 (3.9%) patients with non-anastomotic biliary strictures. Of note, 67 (42.9%) patients had a magnetic resonance cholangiography during their follow-up. Overall, 6 (3.9%) patients underwent a

retransplantation: three patients for primary non-function, one for early hepatic artery thrombosis, and two for non-anastomotic biliary strictures.

Impact of NRP duration on post transplant outcomes

Mean duration of NRP was 179 (± 43) min. NRP duration was below 60min only in two patients, below 90min in six patients, whereas it lasted 240min and more in seven patients. There was no impact of NRP duration on EAD according to Olthoff's criteria (170 ± 44 min in patients with EAD vs. 181 ± 42 min in patients without, $p=0.286$). There was no significant association between NRP duration and ALT levels at post transplant day one ($p=0.837$), AST levels/peaks at post transplant day one ($p=0.626$), and MEAF score ($p=0.347$, data available for 147 patients, see **Figure 2**). Although the MEAF score was significantly lower when IGL-1[®] was used (6.7 ± 1.6 vs. 8.1 ± 1.4 with SCOT 15[®], $p < 0.0001$), it was not different according to NRP duration when either IGL-1[®] ($n=112$, $p=0.104$) or SCOT 15[®] ($n=34$, $p=0.303$) were used for static preservation.

There was no difference in NRP duration in terms of biliary outcomes ($p=0.600$ for overall biliary complications, and $p=0.196$ for non-anastomotic biliary strictures).

Fifteen patients experienced acute kidney injury in the first week after transplantation. There was no difference regarding NRP duration (178 ± 44 min in patients with KDIGO 1, 2 and 3 vs. 188 ± 31 min in patients without acute kidney injury, $p=0.209$).

Discussion

We did not identify in this study any impact of *in situ* NRP duration on liver graft function nor on biliary complications. These results confirm that NRP represents a “safe bridge” to liver procurement in cDCD donors, and that a 1 to 4-hour perfusion indeed seems to be an optimal time frame to preserve graft function.

The French experience of NRP-cDCD donors has been recently reported ^{16,17} and has showed similar results to those with brain-dead donors, especially in terms of graft function and cholangiopathy. In accordance with recent studies ¹⁸⁻²⁰, the choice of routine systematic *in situ* NRP instead of rapid retrieval remains an on-going question. Although demanding in terms of logistics and organization, NRP had largely contributed to these excellent results and to the implementation of the cDCD donors’ programme across France. Like in other countries ⁶, the duration of NRP was empirically set between 1 and 4 hours. This time frame allows coping with different logistical issues, including the access to the operating room during daytime. Of note, when there was a concomitant lung procurement, some teams chose to stop NRP just after lung procurement, in order to avoid the drop in pump flow after the vena cava clamping, and accounted for the donors with only 60 to 90min of NRP duration. A reduced NRP time (1 hour) could also speed up the retrieval process, reducing the strain on retrieval and anaesthetic teams, while reducing blood products requirements during NRP.

In the setting of *ex situ* liver graft preservation, the use of normothermic machine perfusion has been associated with less liver graft injury, hepatocellular enzyme release ²¹ and reduced ischemia-reperfusion injury ²². These results raised the question of a potential benefit of *in situ* NRP on ischemia-reperfusion injury, decreasing histological ischemia-reperfusion lesions on reperfusion biopsies or decreasing the rates of renal failure. In our study, we failed

to observe any relation between NRP duration and postoperative creatinin level in the liver transplant recipients during the first post-operative week. Unfortunately, there was no assessment in this retrospective study of inflammation and ischemia-reperfusion injury on reperfusion biopsies, such as centralized pathological assessment with measurement of hypoxia inducible factor (HIF)-1 α , vascular endothelial growth factor (VEGF) or reactive oxygen species, and plasma tumor necrosis factor (TNF)- α , interleukin (IL)-6 and IL-10.

One limitation of this study is that it is limited to the outcomes of the liver graft, and not of all organs, especially the kidneys. At an experimental level, this issue has been addressed in kidney transplantation from NRP-cDCD donors in a preclinical study by Kerforne et al. ²³, where the authors set up an abdominal NRP in male pigs after 30 minutes of potassium-induced cardiac arrest. The authors demonstrated that 4 hours might be the optimal duration of NRP for kidneys, enabling better function and decreased inflammatory markers, although renal histology was not affected.

Prospective studies in liver and kidney transplantation evaluating ischemia and reperfusion injury according to characteristics of *in situ* NRP are required to confirm our results and address these unresolved issues.

Authorship:

TG, XM, FR, CG, GP, ES, AGSL, EM collected data.

TG, PB, LB performed statistical analysis and wrote the original manuscript.

LB supervised and wrote the original manuscript.

ES, ML, MAA, PB, ES, OS, JCV, AGSL, EM edited the manuscript.

OS, FD, AGSL, JCV, OS, LS collected data and edited the manuscript.

MLe, MLa and ES supervised, designed research, and edited the manuscript.

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Table 1. Donors' and recipients' characteristics

Donors	Total (N=156)
Age (years)	48.3 (\pm 13.2)
Male gender	112 (71.8%)
Days in intensive care unit	13.8 (\pm 14.6)
Body mass index (kg/m ²)	24.8 (\pm 4.9)
Cause of death	
- Cerebrovascular accident	51 (32.7%)
- Traumatic brain injury	41 (26.3%)
- Anoxia	64 (41.0%)
Recipients	Total (N=156)
Age, years	57.6 (\pm 6.8)
Male	140 (89.7%)
MELD score	12.5 (\pm 5.3)
Etiologies	
Hepatocellular carcinoma	103 (66.0%)
Alcoholic cirrhosis	67 (42.9%)
Hepatitis B virus	16 (10.3%)
Hepatitis C virus	41 (26.3%)
Hepatitis B+C viruses	5 (3.2%)
NASH	21 (13.5%)
NASH and alcoholic cirrhosis	22 (14.1%)

Data are expressed as mean (\pm standard deviation) and absolute numbers (percentages);;

MELD: model for end-stage liver disease; NASH: non-alcoholic steatohepatitis

Table 2. Procurement and LT procedures

Procurement	Total (N=156)
Time from therapeutics withdrawal to NRP (min)	35 (\pm 19)
Functional donor WIT (min)	22 (\pm 5)
Time from circulatory arrest to NRP (min)	17 (\pm 5)
NRP duration (min)	179 (\pm 43)
IGL-1 as preservation solution	116 (74.4%)
SCOT 15 as preservation solution	36 (23.1%)
Other preservation solution	4 (2.5%)
UK DCD risk score	5.9 (\pm 3.0)
Liver transplantation	Total (N=156)
Cold ischemia time (h)	5.8 (\pm 1.5)
Duration of transplant procedure (h)	5.8 (\pm 1.6)

Data are expressed as mean (\pm standard deviation) and absolute numbers (percentages); IGL: institute George Lopez; NRP: normothermic regional perfusion; SCOT: *solution de conservation des organes et des tissus*; WIT: warm ischemia time

Figures

Figure 1. Liver graft procurement in the setting of NRP-cDCD donor in France at the time of the study

Freely adapted from the 6th version of the protocol from the Biomedicine agency

CIT: cold ischemia time, MAP: mean artery pressure, LT: liver transplantation, MELD: model for end-stage liver disease, NRP: normothermic regional perfusion, PVT: portal vein thrombosis, WIT: warm ischemia time

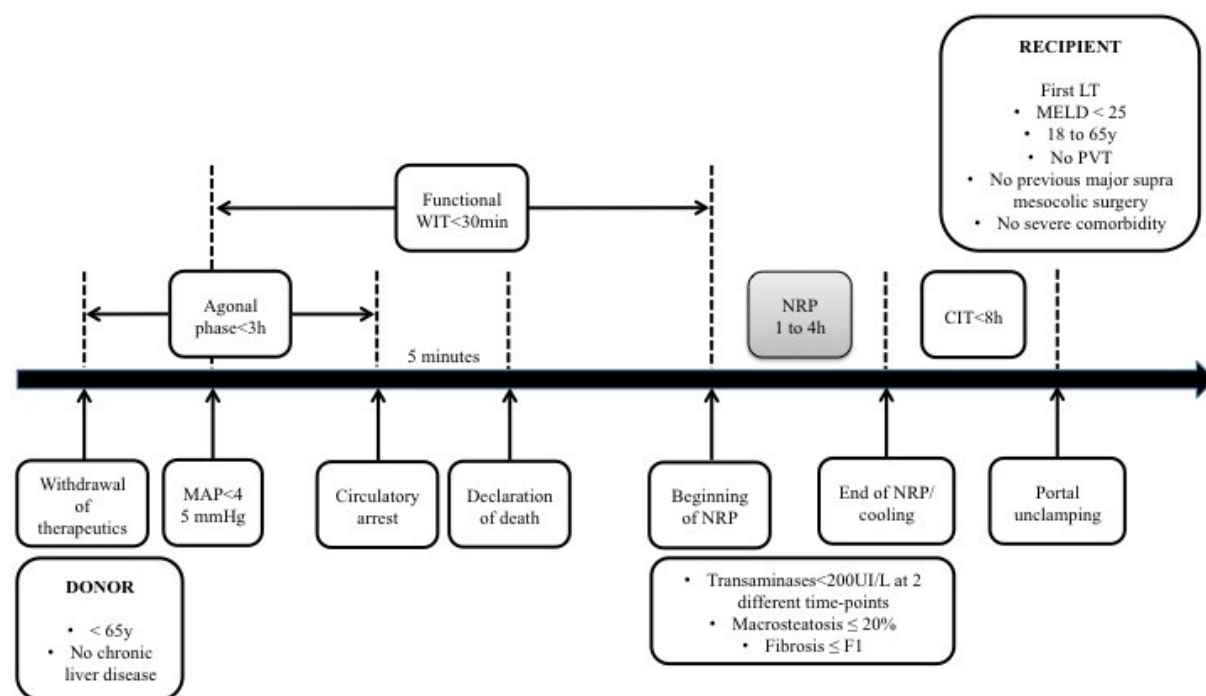


Figure 2. Distribution of MEAF according to NRP duration (linear regression)

NRP: normothermic regional perfusion

