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**Novel Graft Preservation Strategies in
Liver Transplantation**

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Abstract

Dynamic preservation strategies improve outcomes after liver transplantation and have now entered routine clinical practice. In this thesis, we follow a bench-to-bedside approach with the ambition to improve and expand their use with the main focus on hypothermic oxygenated perfusion (HOPE). First, we tested a novel preservation solution tailored for the combined use with HOPE and static cold storage in a preclinical model of marginal liver grafts. Preservation with this novel solution resulted in graft ischemia-reperfusion injury comparable to a combination of 2 different preservation solutions (UW +Belzer MPS). Second, using the same preclinical model we investigated the ideal preservation sequence with HOPE and could show that end-ischemic portal HOPE is the benchmark for cold perfusion. Finally, with the aim of expanding the indications of HOPE, we focused on partial liver grafts and high-risk donor-recipient matching. We conducted two phase I clinical trials which show a benefit of HOPE in terms of graft preservation for both indications. These results have allowed to set-up larger multicenter validation trials. In the second phase of the thesis, we focused on normothermic perfusion. We developed a preclinical porcine model for normothermic regional perfusion (NRP) to identify novel biomarkers for graft viability assessment prior to procurement. In parallel, we initiated a prospective multicenter clinical study in the French NRP cohort to transpose the identified biomarkers into clinical practice. Our work on cold and warm perfusion allowed us to envision the use of a combined cold and warm perfusion strategy in order to improve outcomes in high-risk donor recipient combinations. The presented work led to a significant increase in the use of machine perfusion at our center and allowed to develop and adopt novel strategies in dynamic preservation of liver grafts.

Key Words: Liver Transplantation, Machine Perfusion, Ischemia-Reperfusion Injury, Organ Donation, Organ Preservation, Partial Grafts

Résumé

La conservation dynamique des greffons hépatiques permet une amélioration des résultats après transplantation. Ce travail de thèse suit une approche translationnelle et a pour ambition de développer les connaissances fondamentales et applications cliniques des stratégies de conservation dynamique, en se concentrant sur la perfusion froide oxygénée (HOPE). Nous avons d'abord validé, dans un modèle porcin de greffons hépatiques marginaux, une nouvelle solution de conservation spécifiquement conçue pour l'utilisation combinée en conservation statique et pendant HOPE. Les résultats montrent que la conservation statique et dynamique avec cette solution induit des lésions d'ischémie-reperfusion comparables au standard constitué par 2 solutions différentes (UW+ Belzer MPS). Nous avons ensuite comparé dans le même modèle préclinique, différentes séquences de conservation avec HOPE. Les résultats identifient la modalité *end-ischemic single portal HOPE* comme le standard de la perfusion froide oxygénée. Troisièmement, nous avons réalisé deux études cliniques de phase I pour des nouvelles indications de HOPE qui sont les greffons partiels et les appariements donneurs-receveurs à haut risque. Les résultats ont montré un effet bénéfique de HOPE et ont permis d'initier des études prospectives multicentriques. Dans la deuxième phase de cette thèse, nous nous sommes intéressés à la perfusion normothermique oxygénée. Nous avons établi et standardisé un modèle porcin de circulation normothermique régional (CRN) afin de déterminer des biomarqueurs de viabilité du greffon hépatique avant la transplantation. En parallèle, afin de valider ces biomarqueurs, nous avons débuté une étude clinique multicentrique prospective sur la cohorte française de donneurs avec CRN dans le cadre du protocole Maastricht III. En conclusion, les travaux de cette thèse ont permis de développer l'utilisation clinique de la conservation dynamique en validant dans une approche translationnelle de nouvelles indications et modalités de perfusion en transplantation hépatique.

Mots Clés : Transplantation hépatique, Conservation d'organe, Greffons marginaux, Greffons partiels, Lésions d'ischémie-reperfusion

Résumé Substantiel

Stratégies de conservation des greffons en transplantation hépatique

Travaux de thèse réalisés par Muller Xavier au sein du Centre de Recherche en Cancérologie de Lyon, UMR Inserm 1052 - CNRS 5286 - UCBL – CLB, dirigé par Patrick Mehlen.

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Introduction

La pénurie de greffons hépatiques disponibles pour la transplantation justifie une utilisation croissante de greffons dits marginaux. Alors que la conservation froide statique reste aujourd’hui la référence pour les greffons hépatiques de bonne qualité, les résultats après transplantation de greffons marginaux sont améliorés avec l’utilisation de nouvelles stratégies de conservation dynamique. Le principe de ces stratégies est de perfuser activement les greffons avant la transplantation afin de suppléer les tissus en oxygène et de réduire les lésions induites par l’ischémie-reperfusion. Les deux modalités principales sont la perfusion hypothermique oxygénée et la perfusion normothermique oxygénée. Ces différentes stratégies ont été validées dans des essais randomisés de phase III en montrant une réduction du temps d’ischémie froide statique, des lésions d’ischémie-reperfusion et des complications après transplantation. Cette thèse s’inscrit donc dans le cadre de cet avènement rapide des stratégies de conservation dynamique en transplantation hépatique. Il persiste néanmoins des questions importantes sur les mécanismes physiopathologiques ainsi que sur l’optimisation des modalités et indications de ces stratégies de conservation des greffons hépatiques.

Objectif du projet de thèse

L’objectif de cette thèse est de développer les connaissances fondamentales, d’améliorer l’utilisation et de valider de nouvelles applications cliniques des stratégies de conservation des greffons hépatiques.

Matériels et Méthodes

L'approche de cette thèse est translationnelle avec comme ambition de développer et standardiser des modèles de conservation dynamique précliniques permettant une transposition rapide des hypothèses de recherche en pratique clinique. La structure de cette thèse va donc suivre la logique clinique en commençant par les mécanismes physiopathologiques de l'ischémie froide et chaude du greffon pour ensuite se focaliser sur la perfusion froide oxygénée (HOPE) et finalement s'intéresser à la perfusion normothermique oxygénée.

Le modèle préclinique choisi est celui de greffons hépatiques porcins soumis à une reperfusion *ex-situ* isolée pour simuler la transplantation et étudier les lésions d'ischémie-reperfusion précoces. Afin d'obtenir un modèle lésionnel proche de la clinique, les greffons porcins sont exposés à une phase d'ischémie chaude dans le donneur suivie d'une phase d'ischémie froide statique. Ce modèle permet ensuite de soumettre les foies porcins à différentes modalités de perfusion afin de réaliser des comparaisons directes et d'en élucider les mécanismes. Les comparaisons sont basées sur la caractérisation des lésions d'ischémie-reperfusion au niveau tissulaire et plasmatique. Cette caractérisation comporte une analyse fine des différents compartiments fonctionnels notamment hépatocytaires, cholangiocytaires, immunitaires et vasculaires. En parallèle des expériences animales, une des priorités de cette thèse est d'initier des études cliniques afin de valider les résultats obtenus dans le modèle préclinique.

Résultats

Après une revue des mécanismes lésionnels de l'ischémie froide et chaude sur le greffon hépatique ainsi que les effets protecteurs de la perfusion d'organe, nous nous sommes d'abord intéressés à une nouvelle solution de conservation IGL2 spécifiquement conçue pour une utilisation combinée en conservation statique et perfusion hypothermique oxygénée (HOPE). En comparant dans notre modèle préclinique, cette solution unique au standard actuel qui combine deux solutions différentes (UW + Belzer MPS), nous avons montré que la conservation avec IGL2 induit des lésions d'ischémie-reperfusion comparables à l'utilisation

de UW + Belzer MPS. La solution de conservation IGL2 permet donc une simplification logistique considérable dans le cadre de HOPE et les résultats obtenus vont nous permettre de débiter une étude de phase I afin d'étudier IGL2 en pratique clinique.

Nous avons ensuite, en collaboration avec deux doctorants en Master 2, étudié la meilleure modalité de HOPE dans la séquence de conservation. En comparant HOPE par la veine porte seule à l'abord combinée veine porte et artère hépatique nous n'avons pas mis en évidence de différences significatives en termes de lésion d'ischémie-reperfusion. En ce qui concerne le *timing* de HOPE dans la séquence de préservation, une perfusion continue remplaçant la phase de conservation statique n'a pas montré de bénéfice par rapport à l'utilisation en fin de conservation froide statique (*end-ischemic* HOPE). En revanche, une perfusion suivie de ≥ 2 h de conservation froide statique annule l'effet protecteur de HOPE sur les lésions d'ischémie-reperfusion hépatocytaires. Nous concluons donc que la modalité *end-ischemic single portal HOPE* est le standard de la perfusion froide oxygénée.

Troisièmement, nous avons réalisé deux études cliniques de phase I afin d'étudier la faisabilité et la sécurité de nouvelles indications de HOPE qui sont les greffons partiels et les appariements donneur-receveur à haut risque. Lors de l'étude monocentrique de phase I HOPE-SPLIT, l'application de HOPE pendant la bipartition *ex-situ* de greffons hépatiques a montré une réduction significative de l'ischémie froide statique, ce qui se traduit par des lésions d'ischémie-reperfusion diminuées sur les biopsies de reperfusion en comparaison à la bipartition standard pendant la conservation froide statique. L'utilisation de HOPE permet donc d'améliorer la conservation des greffons de bipartition et facilite la logistique de cette procédure. Ces résultats ont permis d'obtenir le financement d'un PHRC national comparant les résultats après transplantation de greffon HOPE-SPLIT au split standard chez les receveurs pédiatriques et adultes (étude de phase III). L'étude monocentrique de phase I HOPE-MATCH s'intéresse au bénéfice de l'utilisation de HOPE pour les greffons marginaux qui sont attribués à un receveur avec un MELD > 25 . Les résultats préliminaires, après les 10 premiers patients inclus, permettent de conclure à la faisabilité et à la sécurité de la procédure et montrent une réduction significative de l'ischémie froide statique et une diminution du taux de non-fonction primaire par rapport à un collectif historique de 27 appariements à haut risque sans HOPE. Dans la suite de cette thèse, nous allons initier une étude de phase II au niveau national afin de valider cette stratégie de conservation pour les appariements à haut risque en transplantation hépatique.

Dans la deuxième phase de cette thèse, nous nous sommes intéressés à la perfusion normothermique oxygénée en collaboration avec une troisième doctorante en Master 2. Nous avons établi et standardisé un modèle de circulation régionale normothermique (CRN) dans le même modèle porcin décrit précédemment. Ce modèle préclinique est basé sur le protocole français Maastricht III qui prévoit une CRN obligatoire chez les donneurs décédés par arrêt cardiocirculatoire. Il nous permettra d'identifier des biomarqueurs de viabilité du greffon hépatique lors de la CRN afin d'optimiser le processus de sélection et réduire le nombre de greffons non-prélevés. Simultanément, afin de valider ces biomarqueurs, nous avons initié une étude clinique multicentrique prospective sur la cohorte française de donneurs avec CRN dans le cadre du protocole Maastricht III (NCT05361044).

Conclusions et Perspectives

Les travaux de cette thèse ont permis premièrement d'optimiser l'utilisation des stratégies de conservation dynamique par la validation d'une solution de conservation unique IGL2 et de la modalité *end-ischemic portal HOPE* comme modalité de conservation dynamique froide optimale. Deuxièmement, l'application de HOPE à la conservation des greffons partiels et pour les appariements donneurs-receveurs à haut risque va permettre d'élargir les indications et faire bénéficier un plus grand nombre de patients des technologies de perfusion. Troisièmement, nos travaux sur la normothermie ont permis d'établir un modèle préclinique de CRN permettant d'identifier des marqueurs de viabilités des greffons afin de les valider dans une étude clinique. Enfin, ces expériences réalisées avec la perfusion froide et chaude lors de cette thèse aboutissent à la perspective d'un protocole de perfusion innovant combinant hypothermie et normothermie afin de bénéficier des avantages des deux modalités.

Table of Contents

1. Introduction	12
2. Ischemia-Reperfusion Injury of Liver Grafts	13
2.1. Donor Warm Ischemia	14
2.2. Static Cold Ischemia	15
2.3. Recipient Warm Ischemia	17
2.4. Combined Ischemic Injury	17
2.5. Graft Reperfusion	18
2.5.1. Sterile inflammation in the liver graft	19
2.5.2. Impact of IRI on graft microcirculation	19
3. What is the Current Gold Standard for Liver Graft Preservation?	20
4. How to Improve Liver Graft Preservation?	20
4.1. Dynamic Graft Preservation Strategies	22
4.2. Available Clinical Evidence	24
5. Experimental Ischemia-Reperfusion Injury Model	29
5.1. Procurement	30
5.2. Induced Ischemic Injury	31
5.3. Ex-situ Isolated Reperfusion	31
6. Hypothermic Oxygenated Perfusion	32
6.1. Reoxygenation in Hypothermic Conditions	32
6.2. Clinical Evidence	34
6.3. Universal Preservation Solution	34
6.4. Optimal HOPE Strategy	41
6.5. Viability Assessment during HOPE	46
6.6. Partial Grafts	48
6.6.1. Split Grafts	48

6.6.1.1.	<i>Technical Considerations</i>	49
6.6.1.2.	<i>The HOPE Split Pilot-Study</i>	50
6.6.1.3.	<i>The HOPE Split Randomized Controlled Trial</i>	53
6.6.2.	Graft Reduction.....	56
6.6.2.1.	<i>Anatomical Rational for H67 Graft Reduction</i>	56
6.6.2.2.	<i>Anthropometric Criteria for ex-situ H67 Reduction</i>	57
6.6.2.3.	<i>Ex-situ H67 Graft Reduction during HOPE</i>	58
6.7.	High-Risk Donor-Recipient Matching	59
6.7.1.	Study Protocol.....	60
6.7.2.	Preliminary Results	62
6.8.	Perspectives for HOPE	63
6.8.1.	Prolonged Perfusion.....	63
6.8.2.	Immunomodulation.....	64
6.8.3.	Graft Treatment.....	65
7.	Normothermic Oxygenated Perfusion	65
7.1.	Reoxygenation in Normothermic Conditions	66
7.2.	Normothermic Regional Perfusion	67
7.2.1.	Clinical Outcomes.....	67
7.2.2.	Technical Considerations.....	69
7.2.3.	Viability Assessment during NRP	71
7.2.4.	A Preclinical NRP Model	71
7.3.	Ex-situ Normothermic Perfusion	76
7.4.	Combination of Cold and Warm ex-situ Perfusion	77
7.4.1.	Controlled Oxygenated Rewarming.....	78
8.	Conclusion and Outlook	79
9.	Summary of Research Activity	82
10.	References	84
11.	Supplementary Material	89

1. Introduction

Transplantation of a liver graft is a fascinating technical and biological endeavour. Although the liver graft is procured from a deceased donor, it remains in a state of latent life during which biological processes are slowed down and allows it to regain full function after transplantation into recipient. In transplantation this crucial step is referred to as **graft preservation**. Optimizing liver graft preservation was already a major concern during the first liver transplantations in the 1960s. Indeed, Starzl and colleagues used a specifically designed device to allow ex-situ perfusion of the graft at hypothermic temperature and additional oxygen prior to transplantation.¹

Interestingly, the vision of keeping organs alive outside the human body is far older than the first successful transplantation. It was the French surgeon Alexis Carrel born in Lyon, who besides inventing techniques for vascular sutures and patches, developed a perfusion device that could keep organs alive outside of the organism.² Together with the aviator and engineer Charles Lindbergh whom he met at the Rockefeller Institute in New York, they built a perfusion set-up made out of glass and consisting of several chambers connected by tubes. Although Carrel and Lindbergh experimented only on animals, their first results were astonishing: *“Thyroids were amazingly well preserved with pulsating arteries after a period of up to 30 days. ... Cat hearts maintained their contractions for about 12 hours.”*^{3,4} Carrel’s perfusion chamber laid the conceptual foundations for today’s perfusion machines in the field of liver transplantation.

The need for such innovative preservation strategies is related to the shortage of available liver grafts in a context of increasing success of liver transplant as a lifesaving treatment of end-stage liver disease.⁵ Thus, the transplant community is forced to use so-called marginal liver grafts which have a higher susceptibility to preservation and reperfusion injury and expose the recipient to a higher risk of graft failure and post-transplant complications.^{6,7} It is in this context that the clinical use of dynamic preservation strategies has rapidly increased over the last decade. There is now accumulating evidence on the benefit of dynamic preservation strategies from 7 major randomized controlled trials and many centers now use dynamic preservation strategies in routine clinical practice.⁸ It is in this context of a rapid adoption of machine perfusion technology, that the presented thesis is set.

The structure of the thesis will follow a bench-to-bedside approach with the aim of testing a hypothesis in a preclinical model followed by a validation in a clinical study. As a prerequisite, we will first discuss the different physiopathological mechanisms involved in liver graft preservation with special emphasis on graft ischemia under its various forms. Given the large amount of available data and the very heterogenous practices of dynamic preservation across centers, we chose a pragmatic approach starting with the current gold standard in liver preservation: static cold storage (SCS). The next logical step was to focus on hypothermic oxygenated perfusion (HOPE) which shares some common ground with SCS and is the most easily applicable perfusion technology. To optimize the use of HOPE we focused first on a novel universal preservation solution and the best perfusion modality followed by application of HOPE for new indications such as partial grafts and high-risk recipient donor matching. In the second part of the thesis, we will focus on normothermic perfusion in search of novel viability markers. The final consideration and outlook of the thesis is the development and implementation of a combined cold and warm perfusion approach.

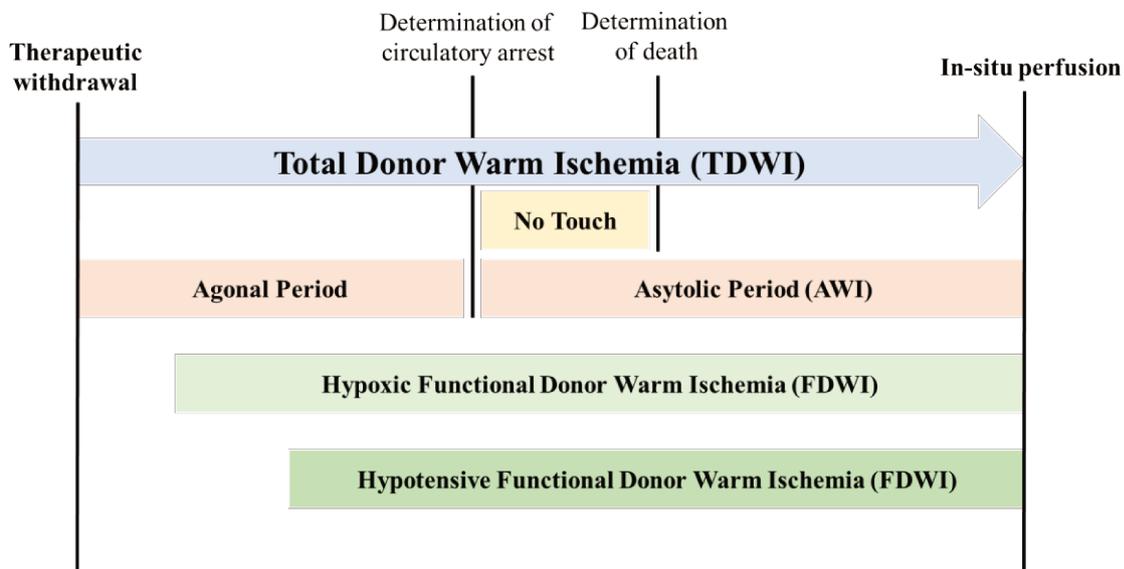
2. Ischemia-Reperfusion Injury of Liver Grafts

Liver transplantation is as a process that starts hours before the implantation of the graft into a recipient (Figure 1). The first step is to match a potential liver donor to a recipient, followed by procurement of the liver graft. (1) The graft will then be stored and transported in a preservation solution on ice (static cold storage, SCS) (2) until the recipient hepatectomy is completed and implantation starts (3). Besides donor and recipient factors, the liver graft undergoes ischemic damage during the first 2 steps of the transplant process, which will cause metabolic and structural alterations and induce ischemia-reperfusion injury (IRI) upon implantation of the graft into the recipient ⁹. Not only are the different ischemic phases important risk factors for post-transplant graft loss but they are also the main factors which may be modulated by dynamic graft preservation strategies. Therefore, we will provide a brief overview of the different ischemic injuries to the liver graft during the transplant process and describe their physiopathological mechanisms.

2.1. Donor Warm Ischemia

In order to increase the number of available grafts, many centers have now included donation after circulatory death (DCD) in their transplant program.¹⁰ The specificity of DCD is the active withdrawal of live sustaining therapies which results in a cardiac arrest of the donor. Associated with this process is a warm ischemia period while the graft is still in the donor. This period is referred to as donor warm ischemia (DWI)¹¹. Donor warm ischemia can be subdivided in different time periods which have different definitions and a specific impact on liver graft IRI as shown in the Figure below.

Figure : Donor warm ischemia in donation after circulatory death (adapted from Kalisvaart et al¹¹)



The first phase is total donor warm ischemia (TDWI) which ranges from therapeutic withdrawal until graft flushing or reperfusion using normothermic regional perfusion in the donor. This phase is constituted by an agonal and an asystolic period as shown in the Figure above. These two phases are purely chronological descriptions and do not necessarily correlate with ischemic damage to the graft. The asystolic period is commonly referred to as asystolic warm ischemia (AWI) and starts from the moment of cardiac arrest until in-situ (re)perfusion. This period varies depending on the different no-touch periods across countries. For example, in France the no-touch period is 5 min while for example in Italy the delay is 20 minutes. International recommendations suggest using functional donor warm ischemia

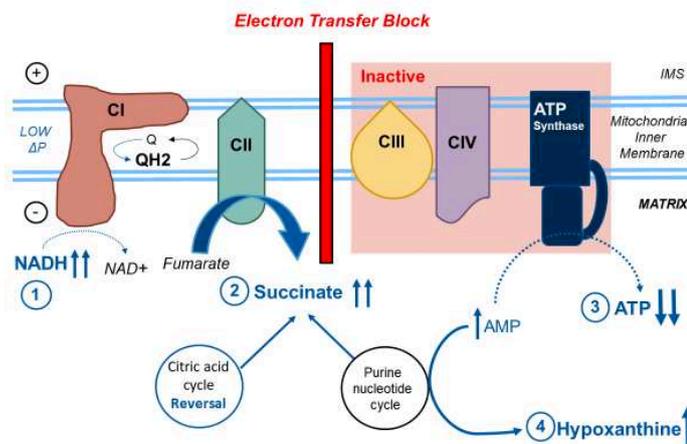
(FDWI) as reference, which is a second phase of DWI.^{11,12} This phase can be defined as hypoxic (pO₂) or hypotensive (MAP, systolic pressure) depending on the starting point which is chosen. In France for example, the *Agence de la Biomédecine* has defined the onset of hypotensive FDWI as mean arterial pressure (MAP) ≤ 45 mmHG. In contrast, the recent consensus guidelines by the International Society of Liver Transplantation set the threshold at a MAP < 60mmHg.¹¹

Based on the aforementioned definitions, we conclude that there is a clear lack of a widely accepted definition of DWI periods which makes direct comparisons among countries centers but also preservation techniques difficult. In addition, the DWI thresholds for accepting DCD liver grafts also vary across countries. In France for example, the FDWI threshold is 45min while the American guidelines recommend 30min. The impact of DWI on clinical outcomes has been extensively studied with conflicting results. A large retrospective study using the UK and the UNOS database has identified FDWI as a major determinant of graft loss as well as ischemic cholangiopathy when combined with other unfavourable donor and recipient characteristics.¹³ In contrast, a more recent study analysed 1114 DCD from the UNOS database found that the risk of graft loss increases with increasing duration of the hypoxic phase (oxygen saturation $\leq 80\%$) until 16 minutes.¹⁴ From 16 min to 50 min, there was no increased risk of graft failure. The authors conclude that one should be cautious on declining DCD grafts solely based on prolonged hypoxic periods. Further work is needed to clarify the impact of the different DWI phases on graft outcomes.

2.2. Static Cold Ischemia

During static cold storage (SCS), liver grafts are stored on ice in a specific preservation solution with the aim of slowing down the metabolic activity of the graft.¹⁵ This protective mechanism is however limited in time given that during SCS the graft is exposed to ischemic conditions which leads to a complete inhibition of oxidative phosphorylation and a switch to an anaerobic metabolism by glycolysis.¹⁶ Studies dating back to the 1980 have shown that with prolonged cold ischemia there is a continuous reduction in adenine nucleotides notably ATP which reduces graft survival after transplantation.^{17,18} Besides a continuous breakdown of ATP and a reduction of the ATP/ADP ratio there is also a complete consumption of the glycogen storage over the course of 8-10 hours of SCS.¹⁹ In addition, there is an inhibition of the enzyme bound membrane transporter Na/K ATPase which ultimately leads to cell death.²⁰

Figure: Metabolic changes at the mitochondrial level during static cold ischemia (adapted from Dutkowski et al²¹)



As shown in the figure above, the respiratory chain of the mitochondria suffers major metabolic impairment during cold ischemia. Several recent studies have shown that succinate accumulates following the reduction of electron carriers such as NADH in combination with an electron transfer block across the respiratory chain.^{9,19,22} This triggers the metabolic alterations described above, for example ATP breakdown.

The first major preservation solution for static cold storage and in-situ graft flushing was the University of Wisconsin (UW) solution developed by Belzer and colleagues.^{15,23} The UW preservation solution had four main characteristics to counteract some of the ischemia-induced metabolic disorders: (1) reduce hypothermia induced cell swelling; (2) prevent intracellular acidosis; (3) reduce formation of oxygen free radicals and (4) provide metabolites for energy production.²⁴ The main novel constituents of the UW solution were hydroxyl starch which replaced albumin as colloid agent, adenosine to stimulate ATP production and lactobionate and gluconate to prevent cell swelling induced by hypothermia.²³ In 1988, Belzer reported on outcomes after transplantation of 17 liver grafts which were static cold stored with UW for a mean of 12,7 hours.²⁵ Since these early days of organ preservation several novel solutions have been developed notably IGL-1 which has been proven to be a safe and effective preservation solution for the liver.²⁶ In comparison to UW, IGL-1 includes polyethylene glycol 35 000 Da (PEG35) as an oncotic agent and presents extracellular electrolyte composition (high sodium, low potassium) with a reduced viscosity.²⁷ PEG35 has been shown to offer a better protective effect of the endothelium and the cytoskeleton

integrity of the hepatocytes thus limiting the oedema and reducing the ischemia-reperfusion injury.²⁸ In addition, PEG35 has been shown to mitigate the deleterious effects of IRI on glycocalyx, a protective layer on the endothelium by counterbalancing the shear stress alterations via remodelling of the cells actin cytoskeleton and favouring the integrity of liver glycocalyx as a “cytoprotective barrier” .^{29,30}

2.3. Recipient Warm Ischemia

Besides cold ischemia, liver grafts also undergo warm ischemia during the implantation into a recipient which is referred to as recipient warm ischemia (RWI). This period ranges from from the start of the vascular anastomosis until reperfusion in the recipient. During RWI there is gradual rewarming of the graft under ischemic conditions. Interestingly, warm ischemia is far more deleterious than cold ischemia.^{11,19} This is due to the fact that the above-described injury mechanisms are significantly accelerated under normothermic conditions. Recent data showed that succinate accumulation after 6 min of warm ischemia was significantly higher compared to 4h of SCS. In line with this observation, the ATP/ADP ratio declined by 50% during the first five minutes of warm ischemia while five minutes of cold ischemia did not induce a significant reduction in the ATP/ADP ratio.¹⁹ As recently shown in a large cohort of over 600 LTs, prolonged RWI translates into significant higher early allograft dysfunction and post-LT acute kidney injury rates as well as poorer 3-year graft survival.³¹ The authors identified 44min as the cutoff above which there is a clear negative impact of RWI on post-LT outcomes.³¹

2.4. Combined Ischemic Injury

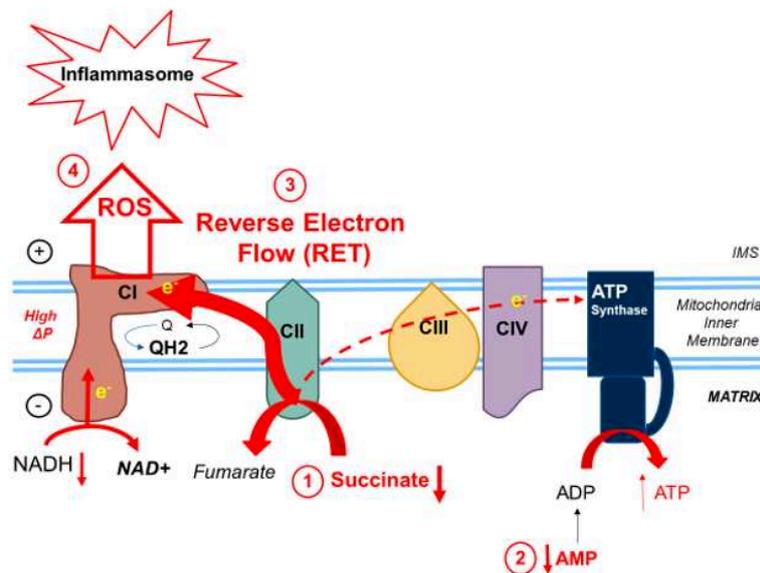
Ultimately, the most important prognostic factor is likely to be the combination of DWI, SCS and RWI. For example, a publication by the Birmingham group showed that if the combined duration of DWI and RWI is > 60min, there is a significant increase in severe post-transplant acute kidney injury in recipients receiving a DCD liver graft.³² In addition, the majority of available donor risk scores combine ischemic and non-ischemic risk factors such as donor and recipient characteristics. However, FDWI and SCS often remain the main prognostic factors.¹³ The data on ischemic damage highlights that novel preservation strategies should target all three ischemic phases in order optimize graft preservation. It should also be noted that the beneficial effect of one intervention on a specific ischemic

period may be canceled out by the following ischemic period, especially in the case of prolonged RWI.

2.5. Graft Reperfusion

The mechanisms underlying graft damage upon implantation have been extensively studied and one of the most cited mechanisms is depending on mitochondrial metabolism.^{9,33} There are 3 consecutive phases of IRI depending on the dominant source of mitochondrial reactive oxygen species namely the hyperacute, acute and chronic phase.²⁹ The hyperacute phase is triggered upon reperfusion of the ischemic liver graft with oxygen rich blood at normothermic temperatures (37°C) in the recipient. There is rapid oxygenation of the accumulated succinate during the first 5 minutes following reperfusion as shown under (1) in the Figure below.^{19,22}

Figure: Metabolic changes at the mitochondrial level during normothermic reoxygenation (adapted from Dutkowski et al²¹)



In combination with low adenine nucleotide levels and a reduced coenzyme Q pool following static cold ischemia, this triggers a reverse electron transfer (RET) across the mitochondrial respiratory chain (2+3).⁹ RET has been shown to cause release of reactive oxygen species (ROS) from complex I.²² In addition, results obtained in brain mitochondria and liver grafts showed release of the metabolite flavin mononucleotide (FMN) as a signature of ROS release from complex I.³⁴

2.5.1. Sterile inflammation in the liver graft

Reperfusion induced ROS release causes cell death resulting in release of danger associated molecular patterns (DAMPs) in the circulation which have the ability to activate various immune cells.³⁵ Examples of DAMPs include high mobility box 1 (HMGB-1), hyaluronic acid, mitochondrial DNA and uric acid.^{29,35} As a consequence, cellular damage is rapidly amplified during the acute phase of IRI and reaches a plateau within 1 hour after reperfusion. One prominent receptor participating in the amplification of the inflammatory signal is the toll like receptor 4 (TLR4) found in Kupfer cells. Following activation of TLR4, Kupfer cells release various cytokines to recruit other immune cells for example neutrophils and induce a sterile inflammation of the liver graft (chronic IRI phase).^{29,35} For example, following TLR4 activation, Kupfer cells release IL-1 β which activates sinusoidal endothelial cells by upregulation of endothelial ICAM-1 enabling neutrophil adhesion.

2.5.2. Impact of IRI on graft microcirculation

Sterile inflammation of the liver graft following IRI has a deleterious effect on the graft microvasculature.³⁶ ROS release leads to an imbalance between vasodilation and vasoconstrictive agents with a shift towards a vasoconstrictive state. In addition, leucocyte recruitment in the liver sinusoids leads to a mechanical obstruction by leucocyte plugging. Altogether, this results in perfusion deficits in the liver graft and rapid microcirculatory deterioration. A key event of IRI induced microvascular impairment is the degradation of the glycocalyx which is a protective layer of the endothelium.^{29,36}

We conclude from the pathophysiological mechanisms of IRI that ischemic damage to the graft conditions later occurrence of IRI which is an event occurring rapidly during the first 5 min of reperfusion with a subsequent inflammatory amplification. Key players in hepatic ischemia-reperfusion injury are complex I and II of the mitochondrial respiratory chain. Strategies to optimize graft preservation and mitigate IRI should target all three ischemic phases namely donor warm ischemia, static cold storage and recipient warm ischemia.

3. What is the Current Gold Standard for Liver Graft Preservation?

The initial efforts for better graft preservation focused on developing specific preservation solutions to reduce cellular energy decline and prevent cell death during SCS.¹⁵ It is important to note that SCS using modern preservation solutions achieves excellent post-transplant outcomes, especially in low-risk transplant scenarios.³⁷ In addition, SCS greatly facilitates procurement and graft transport. Accordingly, SCS is by far the most widely used preservation strategy in the world today.²¹ However, there are at least two scenarios where SCS may not provide an optimal preservation: marginal liver grafts and prolonged static cold storage.

Marginal grafts are defined as grafts procured from donors with multiple risk factors including advanced age, higher BMI or longer intensive care unit stay and show poorer results when preserved with SCS.^{7,38} One explanation is the altered metabolic state of these grafts at the time of procurement, which increases their susceptibility to IRI.^{39,40} The best example of the deleterious effect of prolonged SCS are partial grafts transplanted after ex-situ split procedures. Despite the fact that split grafts are procured from highly selected donors, the longer the SCS duration the poorer are the post-transplant graft survival rates.⁴¹

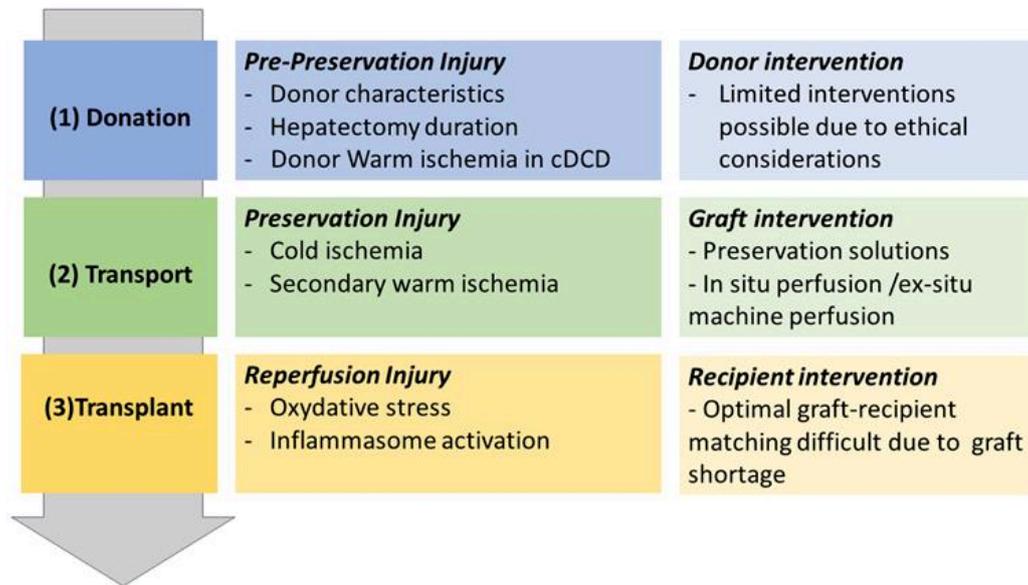
We conclude that SCS remains the gold standard of liver graft preservation due to its simple use and excellent outcomes in optimal donor-recipient matchings. However, marginal grafts and long static cold ischemia storage result in poorer outcomes and requires optimized preservation strategies.

4. How to Improve Liver Graft Preservation?

Interventions to reduce ischemia-reperfusion in liver grafts during preservation are limited. As shown in the Figure below, there are 3 main clinical phases of hepatic IRI where potential therapeutic interventions can be applied. First, interventions in the donor are difficult due to logistical and ethical constraints (1). Second, while interventions during the reperfusion phase are theoretically possible, the rapid occurrence of oxidative stress upon reperfusion is a major limiting factor (2). Finally, the optimal target seems to be the actual preservation phase

of the graft, which coincides with the transport of the graft from the donor to the transplant center (3).

Figure: The clinical phases of ischemia-reperfusion injury (adapted from Muller et al⁴²)



We conclude that interventions to reduce IRI should focus on the preservation phase of liver grafts.

4.1. Dynamic Graft Preservation Strategies

Personal Contribution: We performed a review on currently available dynamic preservation modalities and its clinical applications. Given the importance of national allocation rules and distribution of transplant center, the review focused on the specific French context.

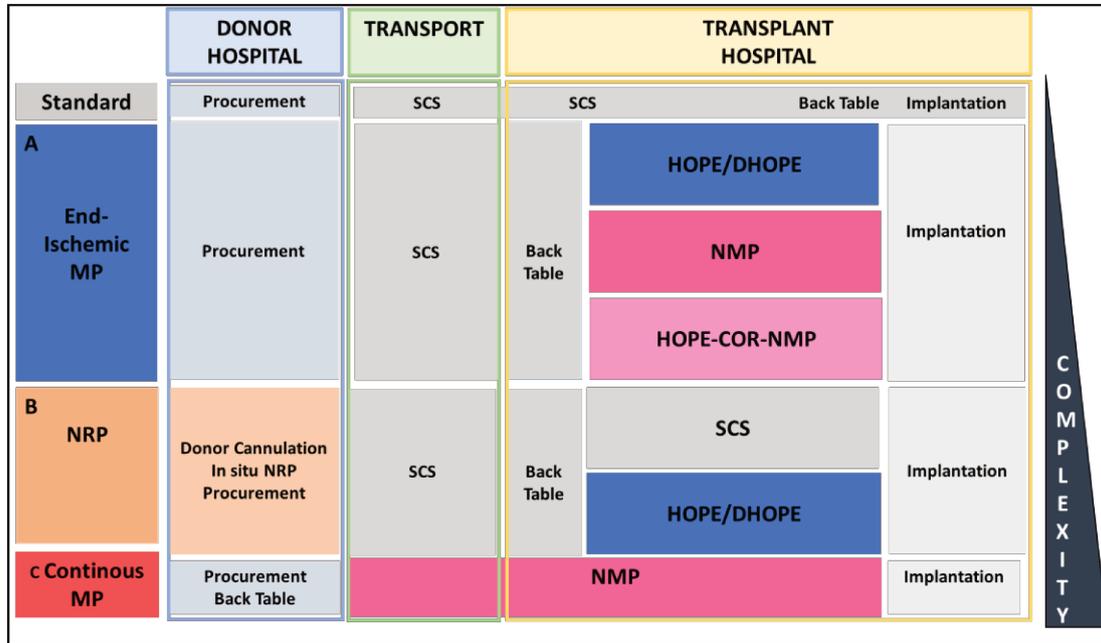
Muller, X., Rossignol, G., Mohkam, K., & Mabrut, J Y. Novel strategies in liver graft preservation - The French perspective. Journal of visceral surgery 2020, 159(5), 389–398. <https://doi.org/10.1016/j.jviscsurg.2022.06.006>

Muller X, Rossignol G, Mohkam K, Lesurtel M, Mabrut JY. Dynamic Liver Graft Preservation in Controlled Donation After Circulatory Death: What Is the Best Fit? Liver Transpl. 2022 Feb;28(2):330-331. doi: 10.1002/lt.26333. Epub 2021 Oct 25. PMID: 34628725.

Dynamic graft preservation is one of the main interventions during the preservation phase. The common ground of all available dynamic preservation strategies, is the use of a perfusion machine to active perfuse the graft with oxygen in order to reduce ischemic damage. There are three main perfusion modalities depending on the applied temperature: either at hypothermic temperatures (8-12°C), normothermic temperatures (37°C) or a combination of both with a gradual rewarming from 8-37°C. In addition, different perfusion solutions exist which are either acellular and supplemented with oxygen under hypothermic/subnormothermic conditions or red blood cell-based perfusion fluids under normothermic temperatures. Normothermic perfusion can be performed in-situ in the donor during procurement or ex-situ during graft transport. Another important factor is the timing of perfusion which may be initiated during procurement, during transport or at arrival at the transplant center.

Figure: Available clinical dynamic preservation strategies (adapted from Muller et al⁴²)

COR: controlled oxygenated rewarming; DHOPE: dual hypothermic oxygenated perfusion; HOPE: hypothermic oxygenated perfusion; MP: machine perfusion; NMP: normothermic machine perfusion; NRP: normothermic regional perfusion



The most commonly used clinical perfusion strategies are shown in the Figure above. Briefly, during ex-situ end-ischemic machine perfusion (A), the graft undergoes perfusion with either hypothermic oxygenated perfusion (HOPE), normothermic machine perfusion (NMP) or a combination of both, once it has been transported to the transplant center by standard SCS. A second strategy is in-situ normothermic regional perfusion (NRP) which consists in perfusing the liver graft with an extracorporeal membrane oxygenation device in the donor (B). Procurement is then performed during NRP followed by static cold storage for transport. A third strategy is continuous (during transport) perfusion of the liver graft with NMP (C). Of note, there is on single-center randomized trial on ischemia-free LT using normothermic perfusion.⁴³ In this strategy, procurement, transport and implantation are performed under continuous NMP. The logistic requirements and complexity of the perfusion procedure increases from strategy A to C. This has direct consequences on practical implementation and costs.

4.2. Available Clinical Evidence

To date there are 7 major randomized controlled trials available including 1062 transplanted patients comparing either cNMP (n=3) or HOPE (n=4) against SCS (Table 1).^{43–49} A total of four studies focus on DBD grafts while one study focuses only on controlled DCD grafts (cDCD). The remaining two studies included both DBD and cDCD grafts.^{44,46} Of note the French RCT HOPEExt comparing HOPE to SCS in extended criteria donor has finished inclusions and results are awaited by the end of 2023.⁵⁰ Beyond the clinical endpoints, this trial also includes an economical evaluation of HOPE which will be of major importance in order to identify reimbursement schemes and promote wider adoption of machine perfusion technology. A summary of the main 7 available RCTs can be found in the Table below.

Table: Published randomized controlled trials in the field of dynamic preservation

cDCD: controlled donation after circulatory death; *DBD*: donation after brain death; *EAD*: early allograft dysfunction;; *NAS*: non anastomotic stenosis, *NMP*: normothermic machine perfusion; *SCS*: static cold storage;

	Graft Types	Treatment	Device	Center	Patients	Primary Endpoint	Results	Conclusion
Nasralla et al, 2018 ⁴⁶	cDCD / DBD	Continuous NMP vs SCS	OrganOX DUAL Perfusion	7 centers (EU)	121 NMP 101 SCS	Peak serum AST within 7 days post LT	<i>NMP</i> 484 <i>AST</i> vs <i>SCS</i> 973 <i>AST</i>	Continuous NMP reduces post-LT peak AST
Markmann et al, 2022 ⁴⁴	cDCD / DBD	Continuous NMP vs SCS	Portable Organ Care System	20 centers (US)	151 NMP 142 SCS	EAD	<i>NMP</i> 18% vs <i>SCS</i> 31%	Continuous NMP reduces EAD rates
Guo et al, 2023 ⁴³	DBD	Total NMP vs SCS	Liver Assist© DUAL Perfusion	1 center (China)	32 tNMP 33 SCS	EAD	tNMP 6% vs SCS 24%	Total NMP reduces EAD rates
Van Rijn et al, 2021 ⁴⁹	cDCD	End- ischemic DHOPE vs SCS	Liver Assist© DUAL Perfusion	6 centers (Eu)	78 DHOPE 78 SCS	Symptomatic NAS at 6 months	<i>DHOPE</i> 6% vs <i>SCS</i> 18%	End- ischemic DHOPE reduces NAS at 6 months
Czigany et al, 2021 ⁴⁸	Extended criteria a DBD	End- ischemic HOPE vs SCS	Liver Assist© SINGLE perfusion	4 centers (EU)	23 HOPE 23 SCS	Peak Serum ALT within 7 days post LT	<i>HOPE</i> 418 <i>ALT</i> vs <i>SCS</i> 796 <i>ALT</i>	End- ischemic HOPE reduces post-LT peak ALT
Ravaioli et al, 2022 ⁴⁷	eDBD	End- ischemic HOPE vs SCS	VitaSmart SINGLE Perfusion	1 center (Italy)	55 HOPE 55 SCS	EAD	<i>HOPE</i> 13% <i>EAD</i> vs <i>SCS</i> 35% <i>EAD</i>	End- ischemic HOPE reduces EAD rates
Schlegel et al, 2023 ⁴⁵	DBD	End- ischemic HOPE vs SCS	Liver Assist© SINGLE perfusion	10 centers (EU)	85 HOPE 85 SCS	One CD≥III during 1y	<i>HOPE</i> 51.8% vs <i>SCS</i> 54.1%	No difference in CD≥III

Based on the results from the 7 RCTs detailed in the Table above, HOPE and cNMP allow for a reduction of allograft injury by reducing post-LT transaminase release and early allograft dysfunction rates according to the Olthoff criteria.^{8,51} In addition, HOPE has shown a reduction of symptomatic non-anastomotic strictures (NAS) in cDCD grafts procured by the super-rapid technique.⁴⁹ Beyond the primary endpoints of the trials, we we can make the following 3 observations (see Table below):

First, with the exception of the trial on ischemia-free transplantation (IFLT), all liver grafts in the perfusion groups undergo a period of SCS prior to cNMP or HOPE. However, duration of SCS differs greatly between perfusion and control groups within the different trials. For example, in the 2 RCTs using cNMP there is a significant reduction of SCS in the perfusion arm compared to the control arm (48% and 70% respectively).^{44,46} In the 4 RCTs using HOPE, there is a large variation in SCS reduction in the perfusion arm and we may distinguish two different subgroups: In the trials by Schlegel and Van Rijn et al, the reduction in SCS is < 20% while in the studies by Czigany and Ravaioli et al reduction in SCS is > 20% (25% and 39% respectively).^{45,47-49}

Second, there are important differences in duration of SCS when comparing the different perfusion groups across the trials. For example, in both cNMP trials SCS is kept < 180min while in the HOPE trials SCS ranges from 255min to 375min. This makes a direct comparison of the different trials difficult.

Third, the perfusion durations are also different across the presented trials. In the 4 HOPE trials, perfusion durations are significantly shorter (95.5-145min) compared to the 2 cNMP trials (276.6-547.5min). The main preservation characteristics of the different trials are summarized in the Table below.

Table: Preservation characteristics across the 7 major randomized controlled trials

	Perfusion group			Control group		% reduction in static cold storage
	Static cold storage (min)	Perfusion duration (min)	Total ex-vivo preservation (min)	Static cold storage (min)	Total ex-vivo preservation (min)	
<i>Guo et al,</i>	0	426	426	414	414	100
<i>Nasralla et al</i>	126	547.5	673.5	465	465	70
<i>Markmann et al</i>	175.4	276.6†	454.9†	338.8	338.8	48
<i>Ravaioli et al</i>	255	145	400	420	420	39
<i>Czigany et al</i>	375	145	520	502	502	25
<i>Schlegel et al</i>	373.0	95.5	474	427	427	13
<i>Van Rijn et al</i>	371	132	524	409	409	9

Based on these three observations, we can further identify 4 different perfusion strategies which are summarized in the Table below.

Table: Four different machine perfusion strategies validated by a phase III trial

	End-ischemic HOPE Treatment	End-ischemic HOPE Preservation	Continuous NMP	Total NMP Preservation
Impact on SCS	Minor reduction of SCS <20%	Moderate reduction of SCS 20%-50%	Major reduction ≥50%	Avoids SCS
Impact on total ex-situ preservation	Moderate Increase	No Increase	Major Increase	Major Increase
Technical Specificities		Start perfusion during back-table	Start at donor center Graft Transport during NMP	Donor already at recipient center No graft transport
Outcomes (Level I)	Reduction of NAS at 6 months in cDCD No effect in DBD	Reduction of EAD major complications and improved graft survival	Reduction of EAD	Reduction of EAD

1. **End-ischemic HOPE** treatment is applied after standard procurement and back-table preparation. This results in a **minor reduction of SCS** compared to the control arm (<20%). The trial by Van Rijn et al, which was conducted in cDCD livers after super-rapid procurement showed a significant reduction in occurrence of ischemic cholangiopathies in the first 6 months after LT in the HOPE arm.⁴⁹ In contrast, the trial by Schlegel et al which included only DBD grafts could not show a significant reduction in severe post-LT complications.⁴⁵
2. **End-ischemic HOPE preservation**, resulted in a **significant reduction of SCS** but without a significant increase in total ex-situ preservation time compared to the control group. For example, in the study by Ravaioli et al, HOPE was started during back-table preparation which allowed to reduce SCS by 39% compared to the control group.⁴⁷ The authors could show a significantly lower rate of EAD (13% vs. 35%, p = .007) in the HOPE group which was associated with improved 1-year graft survival.

3. **Continuous preservation with NMP** starts at the donor center with a portable perfusion device.^{44,46} This strategy resulted in a significant reduction of SCS in addition to extended ex-situ preservation compared to the control arm. In the trial by Nasralla et al, first-week post-LT peak AST was reduced by 49.4%.⁴⁶ In the more recent trial by Markmann et al, there was also a significant decrease in early allograft dysfunction in the NMP group compared to the control group.⁴⁴ However, NMP duration and total ex-situ preservation was significantly shorter in the Markmann trial compared to Nasralla et al. Of note, continuous preservation with NMP is however rarely used outside clinical trials today due to its logistical complexity. Most centers in Europe have adopted end-ischemic normothermic machine perfusion which has however never been evaluated in a randomized controlled trial.
4. **Total NMP preservation** in the case of ischemia-free LT is the most recently described strategy which aims at completely avoiding both SCS and RWI by using NMP from procurement to the end of implantation.⁴³ In the RCT published by Guo et al in 2023, a total 32 DBD grafts underwent IFLT and showed a significant reduction in early allograft dysfunction compared to SCS group (2 vs 24%).⁴³

It should be noted that we focused only on level I evidence from large RCTs and did not include the large set of retrospective studies. While the latter give important information on real world use of machine perfusion, focusing on the RCTs allows to present a more concise account of the current state of available evidence in the field of machine perfusion.

We conclude that there is robust level I evidence supporting the use of HOPE and continuous NMP in cDCD and DBD liver grafts. Differences in SCS duration, perfusion timing and preservation characteristics identify 4 different clinical applications of machine perfusion strategies.

5. Experimental Ischemia-Reperfusion Injury Model

Personal Contribution: We established, standardised and validated an isolated ex-situ porcine liver graft reperfusion model to study various applications of different dynamic preservation strategies.

Muller X, Rossignol G, Couillerot J, Breton S, Hervieu V, Lesurtel M, Lohlam K, Mabrut JY. A Single Preservation Solution for Static Cold Storage and Hypothermic Oxygenated Perfusion of Marginal Liver Grafts: A Preclinical Study. Transplantation 2023, DOI: 10.1097/TP.0000000000004714, in press

In order to allow for a comprehensive bench-to-bedside approach, with the aim of rapid translation of our research work into clinical practice, we opted for ex-situ isolated reperfusion model using porcine liver grafts. This model has been well described in the literature and has several advantages when it comes to perfusion⁵²⁻⁵⁴.

First, porcine livers have similar weight and morphological characteristics as human livers which allows to perform procurement with the same surgical technique, use the same amount of preservation fluid and the same perfusion devices as in clinical practice. This has obvious translational advantages.

Second, warm reperfusion with whole blood to simulate transplantation has been largely used in the literature to validate various preservation strategies in the past.⁵²⁻⁵⁵

Third, we wanted to study the hyperacute and acute phase of IRI. As described in paragraph 2.5, the early events of IRI trigger the hepatic inflammasome and the late consequences of IRI.²⁹ In order to perform analysis of the early phase of IRI in an animal model, it is important to limit the bias induced by the surgical stress to the animal. This is why we decided to not use an actual transplant model. Furthermore, repeated serial biopsies and plasma samples are needed and those are easier to perform in an ex-situ isolated reperfusion model compared to a transplant model.

We decided to limit the ex-situ reperfusion to 2h in all our experiments which is sufficient to study the hyperacute and acute phase of IRI. In order to study the chronic phase of IRI, perfusion needs to be prolonged over 6h which induces important bias due to perfusion circuit and isolated organ setting. For example, recent data has shown that neutrophile recruitment may be blunted in an isolated organ setting due to adhesion to the perfusion circuit and oxygenator.⁵⁶ In addition, there is evidence that ex-situ perfusion artificially increases IRI when perfusion is prolonged owing for example to haemolysis.⁵⁷

There are also clear limitations to the proposed model. First, large animal models are costly and thus the number of subjects per study groups are limited. Second, the model does not allow to draw conclusion on long-term outcomes of IRI such as non-anastomotic biliary strictures, graft rejection and primary non function rates.

In the following, we will briefly describe our experimental model. Approximately 15 pigs were needed to develop, standardize and validate the model. This important preliminary work was made possible with the help of Dr. Rossignol and two Master students (Mr. Couillerot and Mr. Breton) as well as the excellent collaboration of the vet team at the Ecole de Chirurgie de Lyon. Data from the 15 preliminary experiments are omitted from this report but they were crucial in refining the procurement, reperfusion and anaesthesiologic protocol.

5.1.Procurement

Three months year old fasted landrace pigs weighting approximately 35 kg were anesthetized and equipped with a central arterial line for invasive blood pressure monitoring. The procurement procedure followed standard practice from human retrievals. Of note, the common bile duct was cannulated with a paediatric feeding tube and the hepatic artery was dissected up to the celiac trunk. Once the dissection was completed, pigs were heparinized (150-200 UI / kg) and exsanguinated via the previously cannulated aorta and inferior vena cava. During exsanguination, a total of 1,5-2 liters of autologous blood were collected in a blood bag with 5000 IU of heparin (heparin LEO 5000 IU/ml, LEO Pharmaceutical Products, Denmark), supplemented with PIPERACILLINE/TAZOBACTAM KABI 4 g/500 mg and stored at room temperature and protected from ambient light.

After exsanguination, cardiac arrest was declared via the invasive blood pressure monitoring, followed by a period of 30 min AWI. Liver grafts were then flushed by gravity via the portal vein with 1 L of cold NaCl 0.9% (Baxter BV, Utrecht, the Netherlands) supplemented with 5000 IU of heparin, followed by 2 L of cold preservation solution via the portal vein and the aorta. This marked the start of static cold storage (SCS). Finally, the hepatectomy was completed leaving a large aortic patch for adequate cannulation. During backtable preparation, livers were flushed with another additional liter of cold preservation solution and aortic and portal cannula provided with the LiverAssist© device were placed. Liver grafts then underwent static cold storage (SCS) at 4°C in the respective preservation solution for 6 hours.

5.2. Induced Ischemic Injury

We exposed the liver grafts to a combination of 30min of AWI in the donor and 6h of static cold ischemia which corresponds to a high-risk combination in the clinical setting. The rationale of choosing a combined ischemic injury model including DWI and SCS was to amplify the potential benefits of machine perfusion strategies. In addition, as noted in the introductory part of the thesis, machine perfusion strategies in the clinical setting are foremostly used in marginal liver grafts with prolonged warm and cold ischemia times.

5.3. Ex-situ Isolated Reperfusion

All livers in the study underwent ex-situ isolated warm reperfusion at normothermic temperatures with autologous pig blood using the Liver Assist© device (XVIVO©, Sweden). This experimental set-up enables serial tissue and serum sampling. Portal perfusion was pressure controlled with a maximum pressure set at 10mmHg. Arterial perfusion was also pressure controlled with a target mean arterial pressure of 65 mmHg and a pulsatile flow. No bile salts, sodium bicarbonate or parenteral nutrition were added during reperfusion to avoid blunting of IRI.

Figure: Ex-situ isolated porcine liver graft reperfusion

A: Exsanguination by cannulation of the inferior vena cava and infrarenal aorta, B: machine perfusion devices for reperfusion, C: ex-situ HOPE, D: ex-situ warm reperfusion



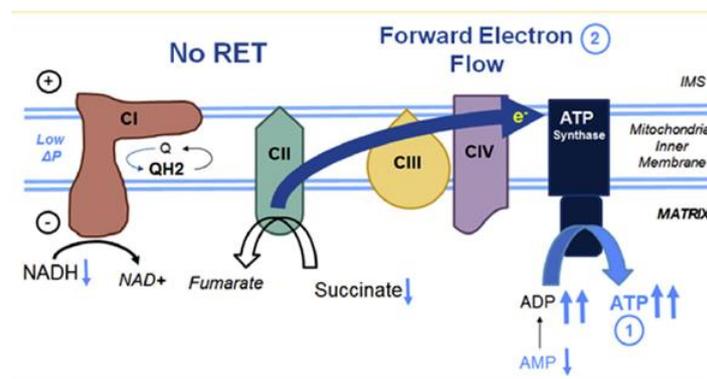
6. Hypothermic Oxygenated Perfusion

The first clinical report using HOPE dates back to the first human liver transplantation performed by Starzl and colleagues.¹ Interestingly, it was only in 2010 that Guarrera et al rehabilitated and refined the technology in clinical practice.⁵⁸ In parallel to the clinical implementation there was a large body of experimental work in small and large animal models to elucidate the protective mechanisms of HOPE which will be rapidly described below.

6.1.Reoxygenation in Hypothermic Conditions

The particularity of HOPE is the reoxygenation of ischemic liver grafts under cold temperatures (8-10°C). This has several advantages in comparison to warm reoxygenation as seen during implantation of the graft. The mitochondria have been identified as key player in the protective mechanism of HOPE.⁵⁹

Figure: Metabolic changes at the mitochondrial level during hypothermic reoxygenation (adapted from Dutkowski et al²¹)



In contrast to SCS, there is an active production of ATP during HOPE at the level of the respiratory chain (1).⁶⁰ The respiratory activity of the mitochondria is significantly slowed down while still allowing electron flow which reduces the risk of electron overload seen during ischemia.⁶¹ This results in a slow metabolization of ischemia induced metabolites such as succinate.⁶⁰ Thus, after HOPE the liver graft is in a more favourable energetic state than after SCS which is beneficial during reperfusion in the recipient.

In addition to the cold and oxygenated conditions, the active perfusion with a preservation fluid a low pressure allows wash-out of toxic residues accumulated during SCS with a protective effect on the graft's microcirculation.⁵³ A hypothesis is that the so called reflow paradox, which under warm conditions induces endothelial damage and leucocyte adhesion due to cytotoxic effects of ROS, is also dampened during HOPE.^{36,53}

Upon reperfusion, HOPE perfused livers have been shown to display reduced activation of the liver inflammasome by reduction of HMGB-1 release and subsequent activation of the TLR4 receptor in a porcine model of ex-situ reperfusion.⁵³ We could confirm the dampening of downstream effects of IRI after HOPE in our cohort of HOPE treated partial grafts. We found significantly less neutrophil infiltration on reperfusion biopsies in partial grafts preserved with HOPE in comparison to SCS.⁶²

Importantly, preclinical data has shown that the addition of oxygen is key.⁵⁹ Indeed, when perfusion is performed with a deoxygenated perfusate, the beneficial effect of HOPE on IRI is lost.⁵³ In addition, several studies have shown the importance of hyperoxygenation of the perfusate. Data from a pig model of DCD kidneys has shown that ATP resynthesis is only observed in the presence of oxygen levels around 100kPa.⁶³ This is significantly higher than the oxygen tension in non-actively oxygenated fluids. These observations were confirmed in an isolated rat liver reperfusion model which showed less transaminase release and a higher bile production in 100% oxygenated cold perfusion compared to perfusion with air only.⁶⁴ Interestingly, the protective effects of HOPE on the bile ducts have not been extensively studied to date and the mechanisms are still largely speculative.⁶⁵

We conclude that reoxygenation of ischemic tissue in cold conditions as seen with HOPE allows to upload cellular energy levels and reduces mitochondrial damage such as succinate accumulation.⁶⁰ This results in reduced ROS release during the hyperacute IRI phase and dampens downstream activation of the hepatic inflammasome.⁵³

6.2. Clinical Evidence

As described in paragraph 4.2, there are currently 4 RCTs on HOPE available of which 3 focus on DBD grafts and one on cDCD grafts.^{45,47-49} In addition to reducing early allograft dysfunction rates compared to SCS, a recent meta-analysis found that HOPE is also associated with a reduction of major post-LT complications, retransplant rates and improved graft survival compared to SCS in the 3 RCTs including DBD grafts.⁸ It is important to note that HOPE requires less logistics compared to other perfusion strategies and allows return to default SCS in case of device failure. The available level I evidence on HOPE clearly makes it the gold standard for dynamic preservation for both extended criteria DBD and cDCD grafts. However, many important questions remain and we have focused on the following 4 key aspects of HOPE during this thesis:

1. *Test a tailored perfusion solution*
2. *Define the optimal HOPE modality and timing*
3. *Refine viability assessment*
4. *Define novel indications*

6.3. Universal Preservation Solution

Personal Contribution: We tested a novel preservation solution designed for static and cold oxygenated perfusion in a preclinical model of isolated ex-situ liver graft reperfusion

Muller X, Rossignol G, Couillerot J, Breton S, Hervieu V, Lesurtel M, Mohlam K, Mabrut JY. A Single Preservation Solution for Static Cold Storage and Hypothermic Oxygenated Perfusion of Marginal Liver Grafts: A Preclinical Study. Transplantation 2023, DOI: 10.1097/TP.0000000000004714, in press

Interestingly, the first preservation solution developed by Belzer was designed for use with machine perfusion. After initially using human plasma as main oncotic constituent, it was replaced by sodium gluconate in combination with hydroxyethyl starch (HES).⁶⁶ The reason to use HES was its increased molecular weight anions which prevents quick denaturation and consequently reduces cell swelling in the cold. Several components of UW-MP are identical to UW for static preservation for example phosphate to prevent intracellular

acidosis and to stimulate ATP production, glutathione to scavenge reactive oxygen species (ROS) formation, adenosine or adenine and ribose as precursors for ATP synthesis besides glucose, buffers (HEPES) and Mannitol.²⁷ However, in contrast to standard UW, Belzer-MPS has an extracellular composition with high sodium and low potassium concentrations, which may be protective against microvascular injury.

Belzer MPS has two major drawbacks in the context of cold perfusion of marginal liver grafts. First, HES results in a high viscosity. Perfusion with a high viscosity solution may lead to an increase in shear stress under hypothermic conditions especially in marginal liver grafts with endothelial damage and presence of sinusoidal microthrombi.^{15,67} Designing a solution containing PEG35 may play a protective role on the glycocalyx during cold perfusion especially in steatotic and marginal grafts. Second, since Belzer MPS is not suited for SCS, a different preservation solution has to be used for SCS which results in mixing preservation solutions. For example, the sequential use of UW and Belzer MPS® exposes the graft to a solution with an intracellular-like electrolyte composition followed by a solution with an extracellular-like electrolyte composition. Hence, the need for repeated flushing during the preservation sequence.

In light of the biochemical and logistic shortcomings of combining 2 different preservation solutions, we studied a novel preservation solution suitable for both SCS and HOPE in a preclinical model of porcine liver grafts. The aim of this study was to test this novel single preservation solution (IGL2) in a preclinical model of hepatic IRI and compare it to the current gold standard of 2 different preservation solution for static and dynamic preservation.

Study design

We compared a novel preservation solution IGL2 against a combination of the standard solution for static cold storage (SCS), University of Wisconsin (UW, BEL-GEN™, IGL, France) solution and HOPE, Belzer Machine Perfusion Solution (BEL-GEN™, IGL, France) in an ex-situ isolated porcine liver reperfusion model.

The IGL2 preservation solution was designed for use in marginal grafts and tailored to the requirements of both static and dynamic cold preservation. In comparison to UW and Belzer MPS, IGL2 has a nearly 2x lower viscosity and the oncotic agent hydroxyl-ethyl starch (HES) has been replaced with the non-immunogenic agent polyethylene glycol 35. In comparison to the more recent preservation solution IGL1, PEG concentration is 5x higher in the present

solution. With the aim of optimizing cold perfusion, the vasoprotective and anti-oxydant agent zinc has been added under the form of ZnCl₂ to guarantee stability of the compound. Indeed, extracellular zinc has been shown to act at the level of vascular endothelial cells by a stimulating role in cell viability and proliferation via VEGFA as well as upregulation of co-factors involved in vasodilatation such as PTGIS and eNOS3.⁶⁸ The addition of Zn in the context of renal IRI has also been shown to reduce the activation of the IRI inflammasome and reduce autophagy and apoptosis.⁶⁹ In order to further improve vasodilatation which is important for vascular protection in the context of higher vascular resistance during cold, nitrite oxide (NaNO₂) was directly added to the solution in low concentration in order to avoid formation of reactive nitrogen species.

Table: Comparison of the characteristics of different preservation solutions

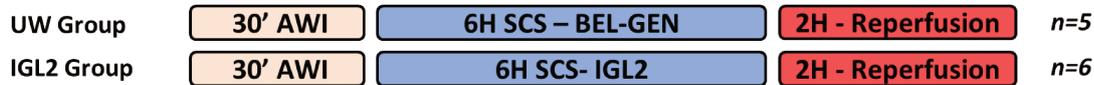
	Belzer MPS	UW	IGL2
Year	1970	1980	2020
Osmotic agent	Gluconate 90 mmol/l Glucose 10 mmol/l	Raffinose 30 mmol/l Lactobionate 100mmol/l	Mannitol 60 mmol/l Lactobionate 80mmol/l
Buffers	Phopshate HEPES	Phopshate Sulfate	Phosphate HEPES Histidine
Oncotic agent	HES 50 g/l	HES 50 g/l	PEG35 5g/l
Viscosity, cP	2.4	5.7	1.4
Anti-oxydants	Glutathion Allopurinol	Glutathion Allopurinol	Glutathion
Electrolytes	Na 100 mmol/l K+ 25 mmol/l = extracellular	Na 25 mmol/l K+ 120 mmol/l = intracellular	Na 125 mmol/l K+ 25 mmol/l = extracellular
Osmolality	300	320	360

Aim and Study groups

The aim of the study was to compare hepatic ischemia reperfusion injury (IRI) in marginal liver grafts preserved with IGL2 against the current gold standard UW for SCS and Belzer MPS for HOPE. For a detailed description of the experimental model refer to paragraph 5.1.

Figure: Study design and experimental groups

Can IGL2 be used for static cold storage (SCS) ?



Can IGL2 be used for static cold storage + HOPE ?



In a preliminary set of experiments, we compared IGL2 to UW (BEL-GEN™) for SCS only:

1. 30min of AWI ischemia followed by 6h of static cold storage in BEL-GEN™ solution followed by 2 h of ex-situ reperfusion (Static UW group, n=5)
2. 30min of AWI followed by 6h of static cold storage in IGL2 solution followed by 2 h of ex-situ reperfusion (Static IGL2 group, n=6)

In the main set of experiments, we then compared IGL2 to the combination of UW (BEL-GEN™) for SCS and Belzer MPS (PERF-GEN™) for HOPE:

3. 30min of AWI followed by 6h of static cold storage in BEL-GEN™ followed by 2 hours of ex-situ HOPE with PERF-GEN™ machine perfusion solution and 2 h of ex-situ reperfusion (MPS group, n=5)
4. 30min of AWI followed by 6h of static cold storage in IGL2 solution followed by 2 hours of ex-situ HOPE with IGL2 machine perfusion solution and 2 h of ex-situ reperfusion (IGL2 MPS group, n=6)

Endpoints

During 2h of warm reperfusion, IRI was assessed in 4 major functional compartments of the liver graft namely the hepatocyte, cholangiocyte, vascular and immunological compartments. Analysis was based on sequential perfusate, blood and bile samples as well as liver and bile tissue biopsies.

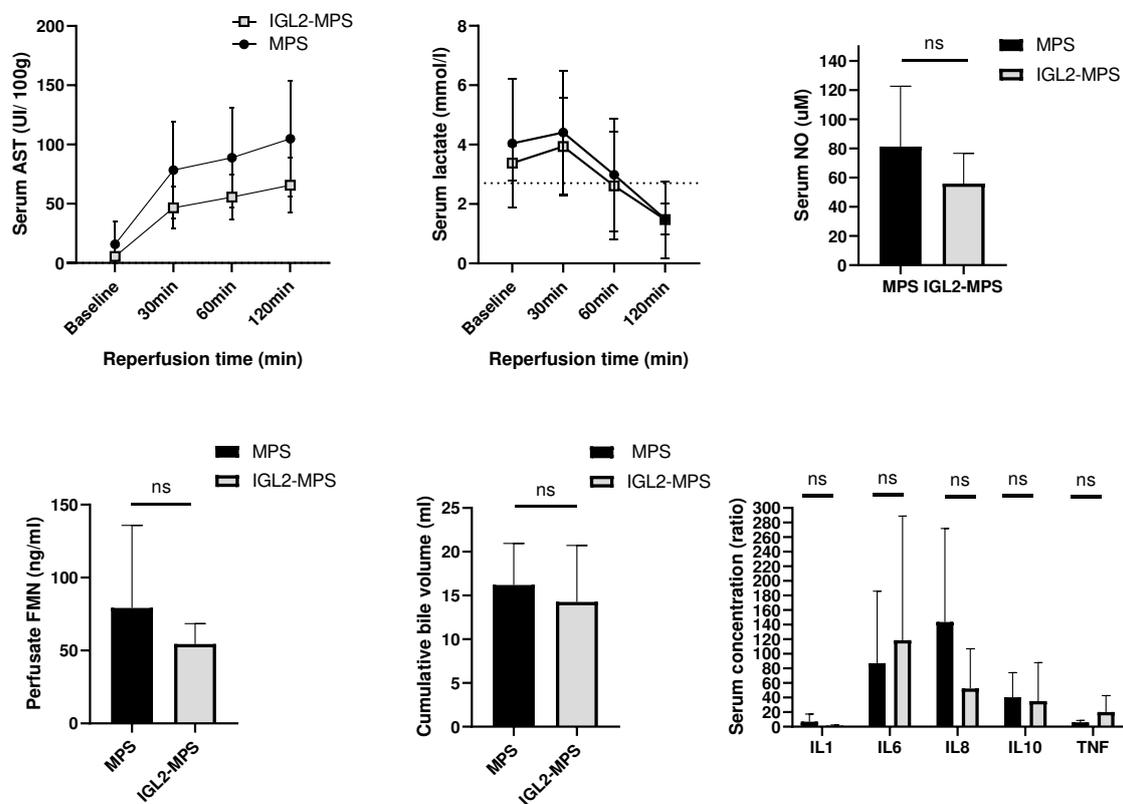
Table: Selected endpoints in the different function compartments of the liver graft

Target	Function	Detection Method	Sample	Timepoint
<i>Immunological Compartment</i>				
FMN	Marker of mitochondrial impairment	Spectrofluorimeter	Perfusate	During HOPE
NADH	Marker of Mitochondrial respiration	Spectrofluorimeter	Perfusate	During HOPE
Cytokines	Marker of hepatic inflammasome activation	Procartaplex 6 plex Assay	Serum	After Normothermic Reperfusion
<i>Hepatocyte Compartment</i>				
Architecture	Architectural integrity	Histology	Liver tissue	Before, during and after normothermic reperfusion, HOPE
AST	Cell death	Biochemistry	Serum	After normothermic reperfusion
Lactate	Metabolism	Biochemistry	Serum	After ischemia, after HOPE after normothermic reperfusion
<i>Cholangiocyte Compartment</i>				
Extra-hepatic bile duct epithelium	Arteriolenecrosis	Histology	Liver tissue	After ischemia, after HOPE after normothermic reperfusion
Bile quality	Cholangiocyte viability	Bile pH, base excess	Liver tissue	After normothermic reperfusion
<i>Vascular Compartment</i>				
TUNNEL	Vascular integrity, Endothelial Cells Apoptosis	Immunohistochemistry	Liver tissue	After normothermic reperfusion
Serum Thrombomodulin	Endothelial Integrity	ELISA	Serum	After normothermic reperfusion
von Willebrand Factor	Endothelial Integrity	ELISA	Serum	

Results and Discussion

In this preclinical study, we show that a novel single preservation solution (IGL2) allows safe preservation with SCS and HOPE of porcine liver grafts undergoing 30min of AWI. During warm reperfusion, hepatocyte, biliary vascular and immunological IRI were comparable to the current standard of combining UW for SCS and Belzer MPS for HOPE. A detailed description of the study results can be found in the final publication in supplementary material.

Figure: Comparison of early IRI between IGL2 MPS and UW + MPS



The main difference between Belzer MPS and IGL2 is the substitution of HES by polyethylene glycol 35kDa (PEG35) (Table 1). The oncotic agent PEG35 has been shown to confer a superior protective effect on mitochondrial metabolism and glycocalyx integrity during SCS of fatty livers compared to preservation solution without PEG35 in a rat model.⁷⁰ The use of a PEG35 based perfusion solution has also been shown to protect the liver glycocalyx during dynamic preservation with machine perfusion in a rat model of steatotic livers.^{67,71,72} Of note, IGL2 has a 5x higher concentration of PEG35 as other currently

available preservation solutions which may further improve mitochondrial and microcirculatory protection.^{73,74} To test this hypothesis, we performed a dynamic assessment of mitochondrial respiratory chain damage and activity during graft preservation by analysis of FMN and NADH in the HOPE perfusate as previously described.^{60,75} We could not show any significant differences in FMN release and NADH metabolism between the PEG35 based solution IGL2 and the HES based solution Belzer MPS which translated into similar hepatic inflammasome activation between both solutions.

A second main addition to the novel preservation solution are vasoactive metabolites such as NaNO₂ which have been shown to reduce IR when administered prior to reperfusion.⁷⁶ In addition, together with PEG35 these metabolites are hypothesized to upregulate eNOS and thus confer additional protection from IRI through a direct effect on the microcirculation.⁷⁷⁻⁷⁹ We found that IGL2-preserved liver grafts had a higher arterial flow during the first 30min of reperfusion albeit not reaching statistical significance. Direct quantification of total nitric oxide (NO) content, THBD and vWF levels in the blood after 2h of warm reperfusion were comparable among groups. Further explorations are warranted to identify a clear benefit of IGL2 on endothelial shear stress.

Although there were no statistically significant differences between IGL2 and the current gold standard of UW+Belzer MPS in terms of hepatic IRI, this study provides mandatory safety data to design a phase I first-in-human trial according to the IDEAL framework for surgical innovation.⁸⁰ Indeed, the use of a large animal model allowed to use the same volume of preservation solution and the same machine perfusion devices as in human clinical practice. The expected logistical benefit of using a single solution by avoiding repeated flushing with different solutions will likely present an economical benefit which needs to be confirmed in later stages of its clinical implementation. (Stage 3 and 4 of the IDEAL framework). We are currently working on setting up a first-in-human clinical trial to test IGL2 with HOPE in clinical practice.

We conclude that a novel single preservation solution allows for a safe preservation of marginal liver grafts with SCS and subsequent HOPE in preclinical model. Hepatic IRI was comparable with the current gold standard of combining 2 different preservation solutions for SCS and HOPE (UW + Belzer MPS).

6.4. Optimal HOPE Strategy

Personal Contribution: To establish the best preservation modality with HOPE, we used the previously described porcine model of hepatic ischemia-reperfusion injury and tested different preservation scenarios with HOPE.

Muller X, Rossignol G, Couillerot J, Breton S, Hervieu V, Lesurtel M, Mohlam K, Mabrut JY.: What is the optimal preservation strategy for marginal liver grafts using hypothermic oxygenated perfusion?

Manuscript in preparation

In the literature there are two main technical modalities to perform HOPE: either single portal HOPE or dual HOPE via the portal vein and the hepatic artery. The centers using D-HOPE claim that arterial perfusion allows a better oxygenation of the intrahepatic bile ducts hereby preventing the occurrence of ischemic cholangiopathy.⁴⁹ On the other hand, preclinical work has shown that the liver has well developed portal venous plexi around the intrahepatic bile ducts. This anatomical feature is the rationale of performing portal HOPE, as oxygenation through the portal vein at hypothermic temperature (8-10°C) is sufficient to reach and protect the bile ducts. In addition, there are still open questions about the best timing of HOPE in the preservation sequence. In the available RCTs, HOPE is always applied in an end-ischemic modality meaning after a period of SCS for transport of the graft from the donor to the recipient center. In contrast to this end-ischemic application of HOPE, data in kidney transplantation has shown that continuous HOPE during the entire organ transport achieves superior results.⁸¹ Furthermore, the perspective of creating centralised organ perfusion hubs, raises the question whether a second period of SCS after HOPE will cancel out the protective effect of the latter.

To answer these questions and establish the best preservation modality with HOPE, we used the previously described porcine model of hepatic ischemia-reperfusion injury (IRI) and tested different preservation scenarios with HOPE.

Aim and Study Groups

The study consisted of two consecutive experimental phases to determine the optimal preservation strategy using HOPE. The first phase of the study compared single portal HOPE (HOPE) with dual portal and arterial HOPE (D-HOPE) to determine the optimal perfusion modality. In the second phase of the study, the previously determined perfusion modality was

used to test different combinations of HOPE and SCS to determine the optimal timing of HOPE in the preservation sequence of liver grafts.

Phase 1: What is the optimal perfusion modality (HOPE vs. DHOPE)?

First, we investigated the optimal perfusion modality between single perfusion (HOPE) and dual perfusion (D-HOPE). In all groups, the livers were static cold stored (SCS) at 4°C in IGL2 preservation solution for 6 h followed by 2 h of HOPE. HOPE perfusion was performed through the portal vein only while for D-HOPE, perfusion was performed through the portal vein and the hepatic artery through an aortic patch. Pressure was set to 25 mmHg for the artery and 4 mmHg for the portal vein.

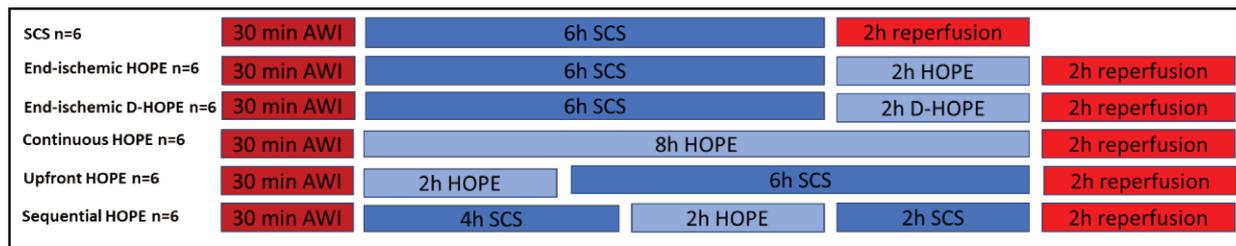
Phase 2: What is the optimal timing of HOPE?

After determining the optimal perfusion modality between HOPE and D-HOPE, we aimed at determining the best timing of HOPE in the preservation sequence. Liver grafts were randomly assigned to 5 different study groups. Each study group included 6 subjects.

1. *End-ischemic HOPE (HOPE-End): 6h of SCS followed by 2h of HOPE.*
2. *Upfront HOPE (HOPE-Front): 2h of HOPE followed by 6h of SCS*
3. *Continuous HOPE (HOPE-Cont): 8h of HOPE without SCS*
4. *Sequential HOPE (HOPE-Seq): 4h of SCS followed by 2h of HOPE and 2h of SCS*
5. *Control group (SCS): 6h of SCS*

All livers in each study groups underwent ex-situ isolated warm reperfusion for 2h to assess early IRI.

Figure: Study design and experimental groups



Endpoints

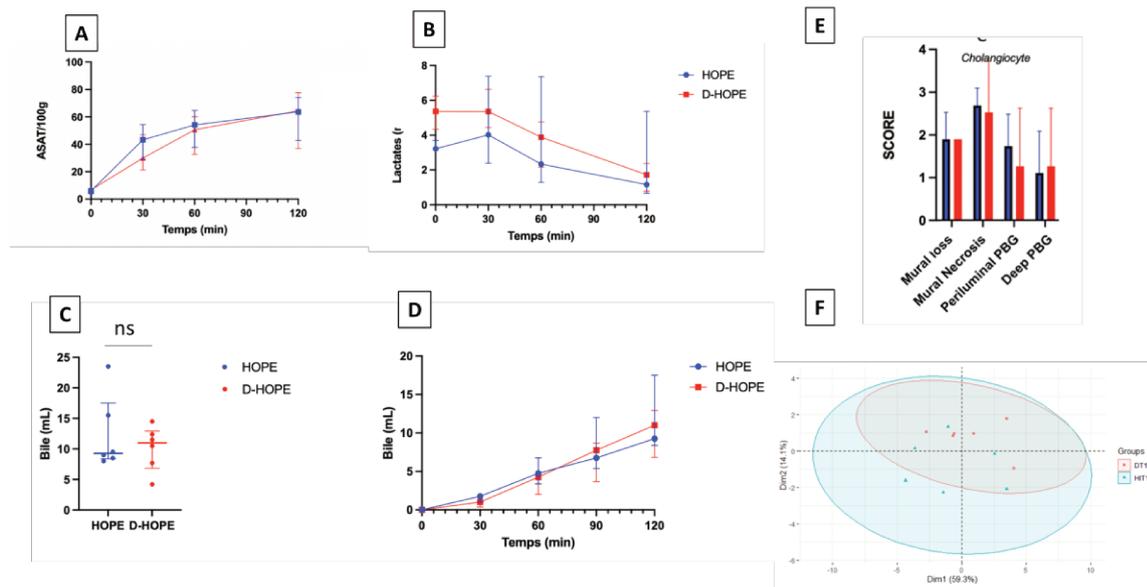
We assessed the same endpoints to quantify early IRI as for the IGL2 study mentioned in the previous paragraph 6.3. with peak AST being the primary endpoint. We would like to highlight that in order to address the hypothetical superiority on cholangiocyte viability of DHOPE we performed a detailed bile acid composition using liquid chromatography coupled with tandem mass spectrometry analysis (LC-MS).

Results

Phase 1: What is the optimal perfusion modality (HOPE vs DHOPE)?

We did not identify any significant differences in peak AST (63.7 IU/100g/L vs 64.5 IU/100g/L, $p=0.937$, Figure A) and lactate clearance (Figure B) between the HOPE and D-HOPE groups. Overall histological IRI were also not significantly different after 2h warm reperfusion among the 2 groups (Figure E). The median cumulative bile production in the HOPE group was 9.25 mL (IQ: 8.5-15.5) compared to 11 mL (IQ: 7.7-12.4) in the D-HOPE group ($p=0.82$). (Figure C+D). There were no differences in histological biliary IRI with comparable lesions at the level of the peribiliary glands. Bile acid phenotype was also not significantly different between HOPE and D-HOPE (Figure F).

Figure: Early IRI after warm reperfusion following HOPE and DHOPE



We conclude from phase 1 that there were no significant differences in hepatocyte and cholangiocyte IRI after 2h of warm reperfusion between HOPE and DHOPE. Thus, the HOPE modality was selected for the experimental set-up in phase 2.

Phase 2: What is the optimal timing of HOPE?

Mitochondrial injury

There were no significant differences between the different HOPE sequences in terms of FMN release after 30min and 60min of HOPE (Figure E).

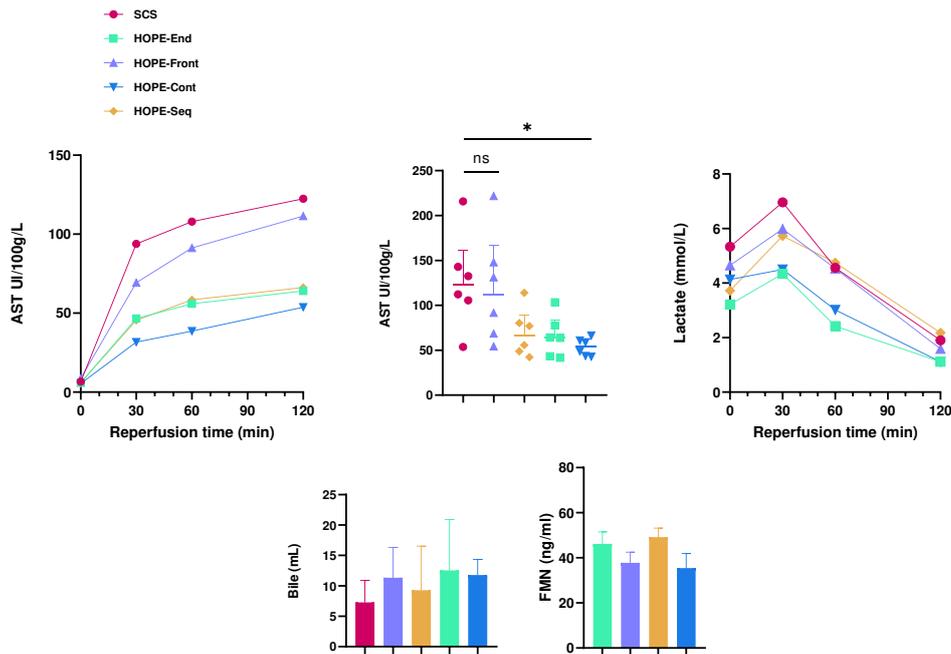
Hepatocyte compartment

During warm reperfusion, AST release increased in all groups. The HOPE-Front group showed similar peak AST than the SCS group (111 vs 122IU/L/100g p=0.9) while all other HOPE groups had significantly lower peak AST levels compared to SCS (Figure A+B). Direct comparison between HOPE groups showed that HOPE-front a 2x higher AST release compared to the others without reaching a statistically significant difference. Quantitative histologic analysis of apoptosis, ballooning, polynuclear infiltration and necrosis showed no significant difference between HOPE groups.

Cholangiocyte compartment

In the dynamic groups, HOPE-End group had the highest cumulative bile production with a median of 12.5 mL/2h which was 2x higher than the SCS group (6mL/2h) (Figure D). There were no significant differences across HOPE groups. Quantitative histologic analysis of the bile ducts showed a mean injury grade of 2 to the peribiliary glands in all groups

Figure: Comparisons of early IRI after different HOPE sequences



We conclude from phase 2 that HOPE-Front presented a higher AST release compared to other HOPE groups which was comparable to SCS group. No differences were found in the cholangiocyte compartment.

Overall Conclusion

We conclude from this preclinical study that HOPE and D-HOPE result in similar early IRI in liver grafts undergoing 30min of AWI and 6h of SCS. In addition, the HOPE-Front group had a higher peak AST compared to other HOPE groups, similar to that observed in the SCS group. This may point to loss of the HOPE effect if perfusion is followed by ≥ 2 h of SCS. Interestingly, we did not find any significant differences in hepatocyte or cholangiocyte injury in the end-ischemic HOPE and continuous HOPE group. There are several direct practical

applications of these results. First, HOPE in comparison to DHOPE seems sufficient to protect grafts from early IRI including bile duct damage which is in line with previous preclinical data.⁸² Another advantage of HOPE in comparison to DHOPE is that it avoids arterial cannulation. Second, the loss of the protective effect of HOPE after additional 2h of SCS questions the utility of centralized organ perfusion hubs and validates the current strategy of a center-based end-ischemic perfusion approach. However, in the context of very marginal grafts especially grafts with major macrosteatosis >30% a more consequent reduction of SCS may be needed. We will thus in the follow-up of this study develop a portable HOPE device to allow for continuous perfusion during transport hereby significantly reducing SCS. We conclude that the present preclinical study validates the current clinical strategy of using single portal HOPE in an end-ischemic application. Continuous HOPE should however be tested in the future especially for grafts with a high degree of macrosteatosis.

We conclude that single portal HOPE is the gold standard of cold perfusion which validates the currently used clinical strategy. Additional SCS after HOPE may cancelled out the protective effects of HOPE on early IRI.

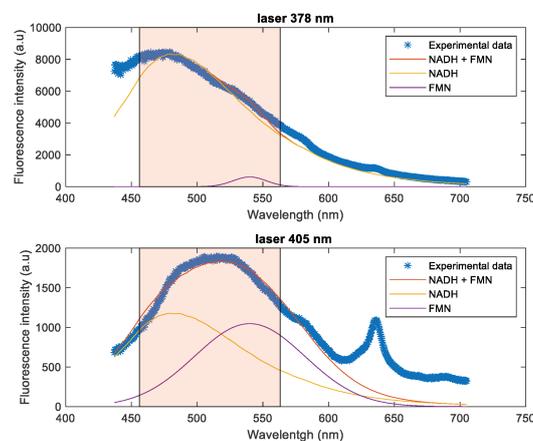
6.5. Viability Assessment during HOPE

Dynamic preservation does not only allow to prolong ex-situ preservation and protect liver grafts from IRI but additionally has to potential to allow real-time viability assessment.⁸³ There are several potential viability markers which have been studied during hypo- and normothermic machine perfusion. These biomarkers reflect the metabolic state and cellular damage of the liver graft during perfusion and include lactate, transaminases, pH as well as bile production and bile quality.^{83,84} More recently, studies have identified novel biomarkers based on metabolomics and glycomics which may provide superior clinical relevance by reflecting specific metabolic pathways in the liver. In 2018, we and others identified a promising biomarker Flavin Mononucleotide (FMN) in the perfusate of liver grafts undergoing HOPE.^{75,81,85} We have since confirmed that the accumulation of succinate during ischemia triggers FMN release upon normothermic reperfusion from complex I of the mitochondrial respiratory chain.⁶⁰ Second, we could show that this initial event leads to the activation of the hepatic inflammasome with subsequent liver graft damage. Third, these

findings in an animal model were translated into human cDCD liver transplantation where FMN release was shown to be a real-time biomarker for post-transplant liver graft function. The team from Zurich has recently developed an in-line measuring probe enabling to quantify FMN and NADH in the perfusate of liver grafts.⁸⁶

In the context of the current thesis, we have continued the investigation into real-time biomarkers including FMN during liver graft perfusion using our porcine model of ex-situ liver reperfusion. This was done in collaboration with a team of engineers from the *Institut Georges Lopez* in Lyon. Briefly, during the two aforementioned preclinical studies, liver grafts were continuously monitored using optical methods notably Laser Induced Fluorescence (LIF). This technology has been shown to enable monitoring of mitochondrial energy metabolism by detecting NADH/NAD⁺ and FMN which are both autofluorescent molecules with specific and different emission intensities.^{87,88} We obtained interesting preliminary results using LIF on porcine livers by measuring the evolution of NADH and FMN concentration in the tissue at the different steps liver preservation. As shown in the figure below, the established measuring model was able to extract the relative contribution of NADH and FMN in the 455-565 nm range during HOPE. Future steps will be to quantify FMN and NADH concentration during the whole protocol and correlate with conventional markers of liver graft IRI including transaminase release, lactate clearance and bile production. A major challenge remains the identification of autofluorescent molecules in context of a haemoglobin based perfusate.

Figure: Fluorescent measurement performed after 40 minutes of HOPE reperfusion with 2 excitation wavelengths (378 nm and 405 nm). FMN and NADH contribution in the fluorescent signal are extracted considering NADH experimental spectrum measurement with a phantom and a gaussian distribution for FMN spectrum



6.6. Partial Grafts

Personal Contributions: We developed and standardized the use of HOPE during ex-situ graft split and reduction procedures with the aim of reducing ischemic damage. The feasibility and safety of this novel application of HOPE were validated in a phase I clinical trial.

Muller X, Rossignol G, Mohkam K, Mabrut JY. Ex-situ graft reduction (H67) during hypothermic oxygenated perfusion to prevent large-for-size syndrome in liver transplantation-A technical report. Clin Transplant. 2023;37(6):e14995. doi:10.1111/ctr.14995

Rossignol G, Muller X, Hervieu V, Collardeau-Frachon S, Breton A, Boulanger N, Lesurtel M, Dubois R, Mohkam K, Mabrut JY. Liver transplantation of partial grafts after ex situ splitting during hypothermic oxygenated perfusion-The HOPE-Split pilot study. Liver Transpl. 2022 Oct;28(10):1576-1587. doi: 10.1002/lt.26507. Epub 2022 Jun 16. PMID: 35582790.

Rossignol G, Muller X, Mohkam K, Dubois R, Lesurtel M, Mabrut JY. Full left/full right liver graft ex situ split during hypothermic oxygenated perfusion. Pediatr Transplant. 2022 Aug;26(5):e14284. doi: 10.1111/ptr.14284. Epub 2022 Apr 18. PMID: 35437884

Mabrut JY, Lesurtel M, Muller X, Dubois R, Ducerf C, Rossignol G, Mohkam K. Ex Vivo Liver Splitting and Hypothermic Oxygenated Machine Perfusion: Technical Refinements of a Promising Preservation Strategy in Split Liver Transplantation. Transplantation. 2021 Aug 1;105(8):e89-e90. doi: 10.1097/TP.0000000000003775. PMID: 34291769.

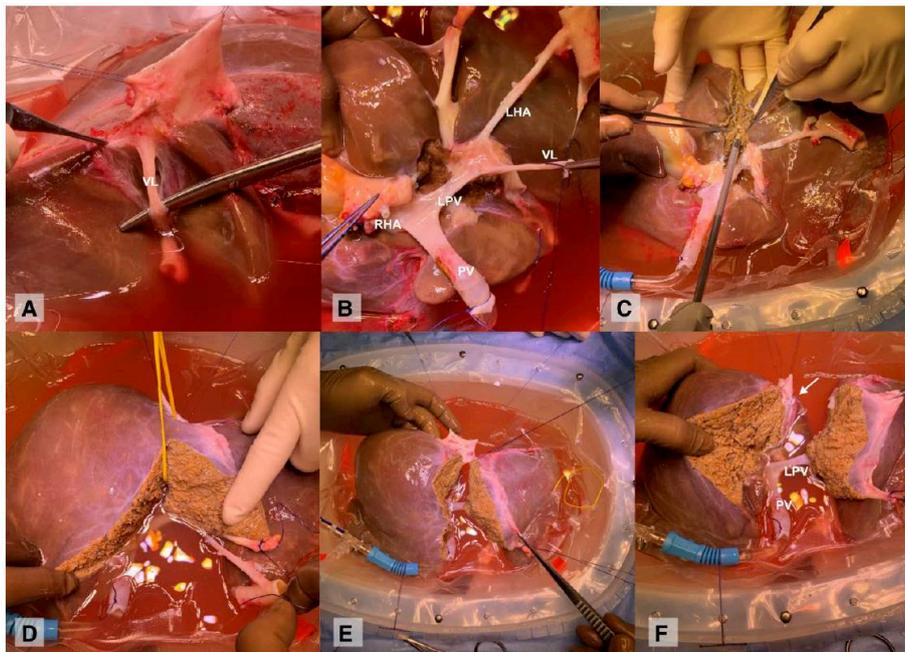
6.6.1. Split Grafts

Split-liver transplantation (SLT) offers rapid access to transplantation for paediatric recipients while also benefiting the liver graft pool for adult recipients. There are two major split procedures: in-situ and ex-situ. In situ liver graft splitting during procurement has achieved the best post-LT outcomes but is a technically and logistically very challenging procedure. In France for example, the majority of split procedures are performed ex-situ. The drawback of ex-situ splitting is the prolonged static cold storage time associated with the procedure which has been shown to negatively impact graft survival despite the use of optimal donors.⁴¹ In other words an ideal graft from a young donor is transformed into two marginal partial grafts owing to the ex-situ split procedure. The use of HOPE has shown to mitigate IRI and improved post-LT outcomes in whole liver grafts from extended criteria donor compared to SCS by reducing SCS and improving graft preservation.⁸ Thus, we envisioned to apply HOPE to the ex-situ split procedure in order to optimize outcomes in both paediatric and adult recipients SLT.

6.6.1.1. Technical Considerations

The first case report on ex situ liver splitting during end-ischemic HOPE was reported by Spada et al. in 2020 followed by a report by Thorne et al in 2021.^{89,90} Based on these preliminary reports we developed a standardized 2-step technique which allowed to perform a safe split procedure during ex-situ HOPE.⁹¹ The first step is performed during SCS and consists in the dissection of the vascular structures facilitated by the “star exposure” followed by a bench cholangiography to dissect the hilar plate (A+B). The second step is the parenchymal transection which is performed with simultaneous HOPE using a “split hanging manoeuvre” (C+D). This technique is applicable to full left/full right and left lateral/right extended split procedures.^{91,92}

Figure: The 2-step technique for ex-situ liver graft splitting during HOPE (adapted from Mabrut et al)⁹¹



In contrast to the first reports by Thorne et al and Spada et al, we made several important technical adaptations.^{91,92}

First, HOPE is performed through the portal vein only. This avoids cannulating the artery hereby reducing the risk of endothelial damage. In addition, portal division can be performed at the end of the procedure which allows to perfuse each partial graft until respective recipients hepatectomies are completed. This is specifically valuable in case of a

full left/full right graft, where reconstruction of SVIII or SV vein on the full right graft can be performed during HOPE while the full left graft is already implanted.

Second, the liver graft is placed in a supine position to allow bench cholangiography followed by a split-hanging manoeuvre to simulate the anterior approach of standard hepatectomies. The Split hanging manoeuvre also allows optimal perfusion by avoiding portal vein kinking and cannula compression.

Lastly, after performing several initial cases, we improved the technique and performed continuous HOPE during the entire split procedure, hereby further reducing SCS.

6.6.1.2. *The HOPE Split Pilot-Study*

After the aforementioned initial case reports, we initiated a prospective phase I study from 01/11/2020 to 31/12/2021 to test reproducibility and safety of HOPE-Split in an extended case series as recommended by the IDEAL guidelines for surgical innovation.⁸⁰

Study Design

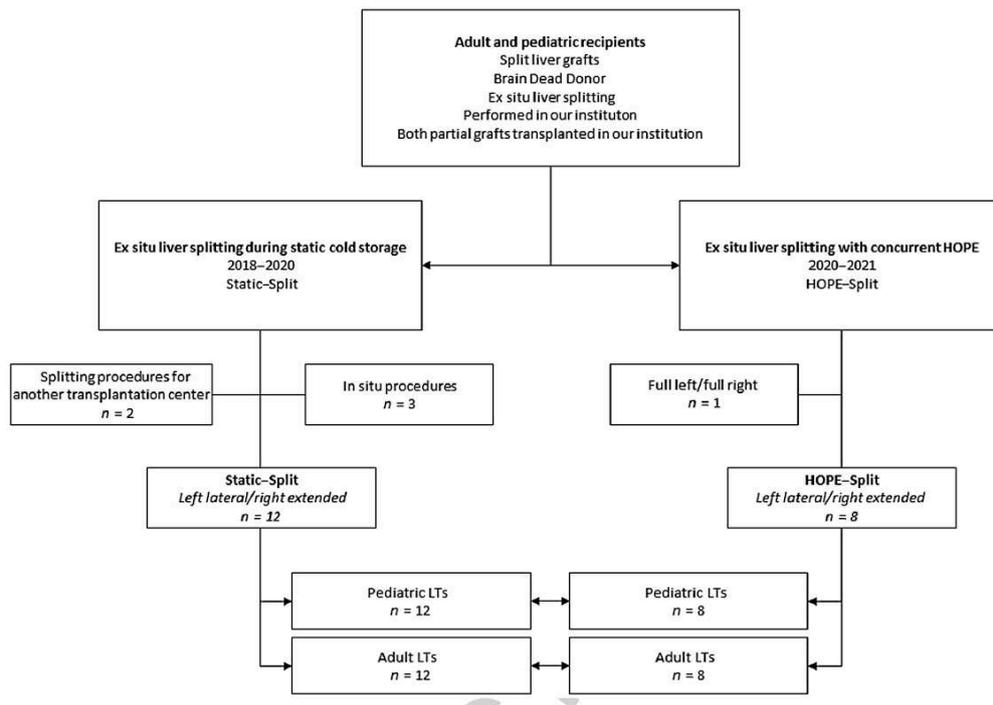
All consecutive HOPE splits in both adult and paediatric recipients were included in the study and compared to an historic cohort of pure ex-situ splits during SCS. All HOPE-Split procedures were performed using the Liver Assist© device (XVIVO©, Sweden) at 8-10°C with a portal vein pressure ranging from 3-5mmHg.

Endpoints

To test the safety of the novel procedure in contrast to the standard ex-situ Split during SCS, we used a composite safety endpoint defined as the mean number of liver graft-related adverse events (LGRAEs) per patient during the first 30 days after transplantation.⁴⁴

LGRAEs include primary nonfunction, biliary complications, hepatic vascular complications, and early relaparotomies.

Figure: Study Flowchart adapted from Rossignol et al⁶²



Results

We included a total of 8 HOPE–Split procedures resulting in 16 consecutive partial liver graft transplantations after HOPE–Split (8 adult recipients and 8 pediatric recipients) with a median follow-up of 7.5 months (IQR, 5.5–12.5). The comparator cohort consisted of 24 partial liver grafts (12 adult recipients and 12 pediatric recipients) transplanted after standard ex situ split during static cold storage (Static–Split group). All grafts included in the study were right extended/left lateral partial grafts.

Safety and Preservation

All 16 consecutive HOPE–Split partial grafts were transplanted with a 90-day graft and recipient survival of 100%. No technical problem with the perfusion device occurred during the split procedure. We recorded mean number of 0.31 ± 0.60 (a total of 5 events) LGRAEs in the HOPE–Split adult recipient group, which was not significantly different from the Static–Split group. (0.46 ± 0.83 ; 11 events; $p = 0.78$). In pediatric HOPE-Split recipients a mean number of 0.63 ± 0.74 (5 events) LGRAEs was observed compared to 0.75 ± 1.06 (9 events) in the Static–Split group ($p = 0.34$).

From a technical point-of-view, the duration of the split procedures in the HOPE-Split group was not significantly longer than in the Static-Split group (216 min [IQR, 170–240] vs. 180 min [IQR, 167–243]; $p = 0.45$).

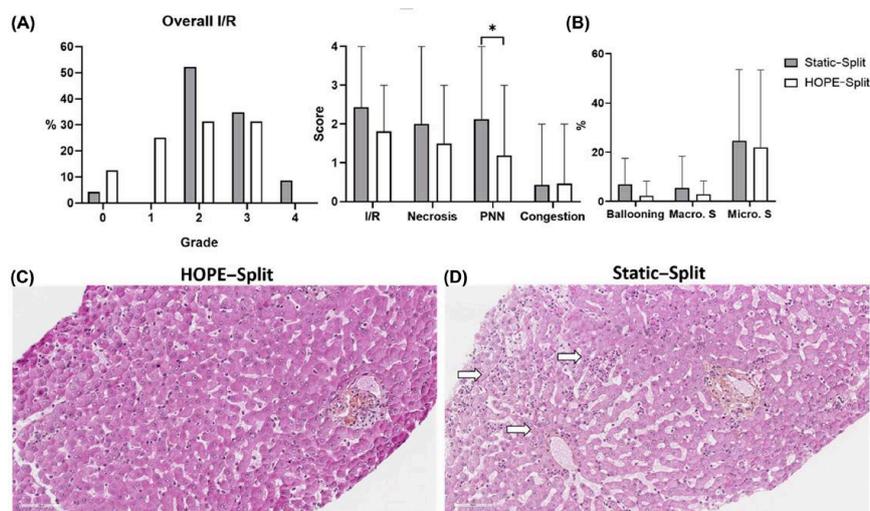
In terms of graft preservation, the median perfusion duration of the 16 partial grafts was 125 min (IQR, 95–165) which resulted in a significant reduction of static cold ischemia time (472 min [IQR, 410–516] vs. 544 min [IQR, 508–581]; $p = 0.001$) compared to Static-Split.

Ischemia Reperfusion Injury

The reperfusion biopsies of all HOPE-Split LTs ($n = 16$) were compared with the biopsies of 23 Static-Split LTs (one biopsy was not performed in the Static-Split group because of multiorgan failure). The majority of HOPE-Split grafts presented with grade 0–2 (none to mild) IRIs ($n = 11$, 68.8%) and no grade 4 IRI was observed. The Static-Split grafts showed a higher overall grade of IRI with two cases of grade 4 (severe) IRI. There was a significant reduction of lobular neutrophilic infiltrate upon reperfusion in the HOPE-Split group compared with the Static-Split group ($p = 0.04$).

Figure: Histological analysis of IRI in HOPE-Split compared to Static-Split (adapted from Rossignol et al)⁶²

A) Overall IRI grade in the HOPE-Split and Static-Split groups. (B) Detailed histological analysis of IRI in the HOPE-Split and Static-Split groups. Data are expressed as mean with range. $*p < 0.05$. (C and D) Representative histology of IRI on reperfusion biopsy. (C) Minimal IRI in the HOPE-Split group and (D) moderate IRI with neutrophilic infiltrate (arrow).



Discussion

This single-center prospective phase I study presents first feasibility and safety data for transplantation of partial liver grafts undergoing ex situ split during HOPE. In cohort of 16 successful SLTs after 8 HOPE–Split procedures, no early graft loss occurred and LGRAEs were comparable to the standard Static–Split procedure. HOPE–Split did not increase split procedure duration but resulted in reduced static cold storage duration leading to reduced IRI on reperfusion biopsies.

The primary safety endpoint 30-day LGRAEs was comparable between HOPE–Split and Static–Split groups, suggesting the safety of this standardized surgical technique. Importantly, the split procedure with concurrent HOPE was not significantly longer (216 min vs. 180 min; $p = 0.45$) compared to the standard Static–Split.

We would like to highlight that the encouraging results in terms of reduction of IRI on reperfusion biopsies highlight the potential of HOPE to improve preservation of Split grafts. All HOPE–Split grafts underwent perfusion for >1 h (158 min in adult recipients and 95 min in paediatric recipients), which has been shown to be sufficient to mitigate ischemia–reperfusion. In line with observations from whole liver grafts undergoing HOPE, the study shows a reduction in lobular neutrophilic infiltrate and the absence of severe IRI scores on reperfusion biopsies of HOPE–Split grafts. Importantly, the significant reduction of static cold ischemia in HOPE–Split compared to Static–Split may further improve preservation especially in more marginal grafts or difficult SLT scenarios requiring prolonged ex-situ preservation.

6.6.1.3. *The HOPE Split Randomized Controlled Trial*

According to the IDEAL recommendations for surgical innovation, the next step is to assess short and long-term clinical outcomes in a large-scale multicenter setting (stage 3).⁸⁰ We thus designed a national multicenter open-label randomized controlled trial comparing post-transplant of DBD grafts splitted during HOPE with the standard splitting during static cold storage. Besides the novelty of the procedure, the proposed study will be the first comparative clinical study on machine perfusion strategies in paediatric liver transplantation. HOPE Split may improve preservation and outcomes of SLT and allow to expand indications and selection criteria of split liver grafts. The RCT has been financed by the French authorities in the PHRC program and will start at the end of 2023 (PHRC 2021-0102).

Aim and endpoints

Primary Aim:

To investigate if the use of HOPE during ex-situ liver graft splitting reduces post-transplant liver-related morbidity in paediatric recipients in comparison to liver graft splitting during static cold storage.

Secondary Objectives:

To assess the impact of HOPE on the following features compared to static cold storage:

- Perioperative outcomes
- Perfusion and graft preservation characteristics
- Overall post-transplant 3 months and 1 year morbidity and specific post-transplant complications
- Overall post-transplant 3 months and 1 year graft and recipient mortality
- Comparison of post-LT outcomes after SLT in adult recipients

Primary Endpoint:

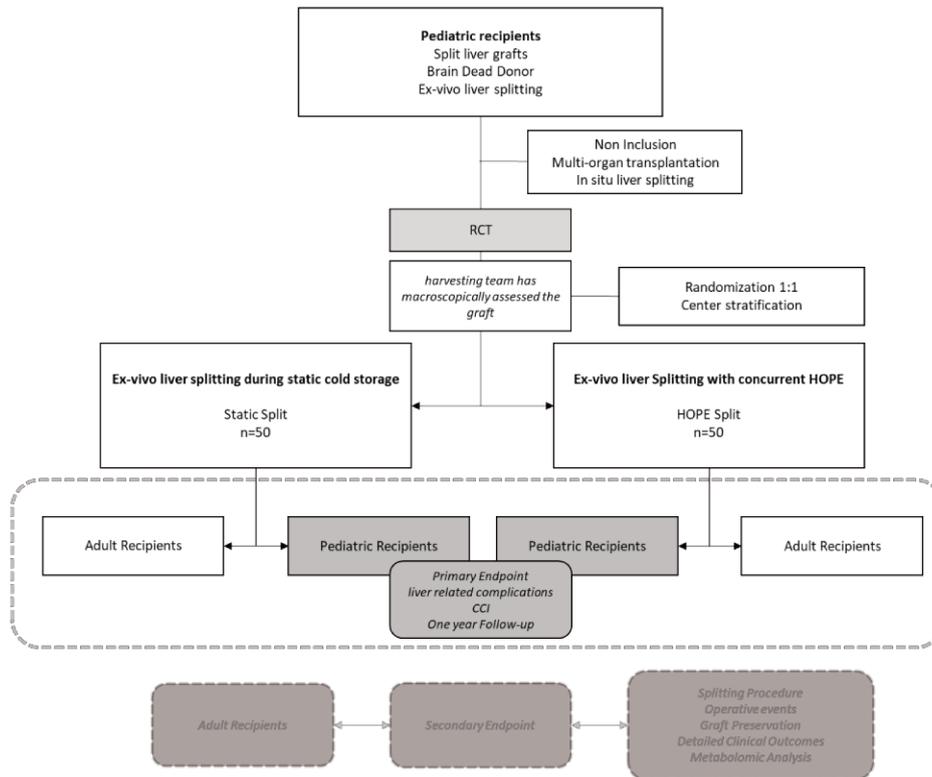
The majority of available RCTs on machine perfusion have either chosen transaminase release or early allograft dysfunction defined by the Olthof criteria as primary endpoint. The criticism of this endpoint is its lack of clinical relevance. Consequently, a more recent RCT by Schlegel et al focuses on recipient morbidity after transplantation.⁴⁵ However, the authors chose to only take into account the number of patients with at least one complication Grade Clavien-Dindo III or higher. In an ad-hoc analysis they could show that we should probably focus on the entire 12 months liver related morbidity in order to have a clinically meaningful endpoint. Thus, we did choose cumulative 12 months liver graft-related complications after transplantation as primary endpoint of the study. The primary outcome measure will be the Comprehensive Complication Index (CCI) which will be calculated as the total of all individual liver graft-related complications graded by the Clavien-Dindo score.

Liver graft-related complications include:

- Primary graft non function
- Post-transplant biliary complications
- Post-transplant arterial thrombosis
- Post-transplant portal vein thrombosis

- Early liver graft-related relaparotomies
- Post-operative bleeding
- Proven biopsy Acute rejection
- Post-transplant renal failure requiring dialysis

Figure: Study Flowchart



6.6.2. Graft Reduction

Transplantation of a “large” liver graft into a “small” recipient may lead to large-for size syndrome (LFS) which is commonly described as a morphological mismatch which leads to complications such as graft compression and subsequent graft ischemia with negative consequences on graft survival.^{93,94} However, in contrast to small-for size, perioperative hemodynamic data of the grafts are not considered in the definition of LFS.⁹⁵ However, we know from large retrospective studies that portal vein flow, hepatic artery flow and hepatic venous pressure gradient directly impact post-LT outcomes.⁹⁶ With this in mind, we reviewed our data on 257 consecutive liver transplantations with prospective hemodynamic measurements.⁹⁷ We could show that, low portal flow defined as <80ml/min per 100g of liver tissue, was encountered in 16.8% of all LT and was associated with a higher liver graft related morbidity (20.9% vs 7.5%, p=0.007) and a significant impaired 90-day graft and patient survival. This effect was independent of the classic LFS definition. Following these results, we propose the concept of large-for-flow which adds a hemodynamic component to the morphological evaluation. In addition to the importance of performing intra-operative flowmetric evaluation to optimize graft-recipient matching the large-for-flow paradigm raised our awareness of the possibility of performing graft reductions for both anthropometric but also hemodynamic purposes. We have thus analysed three such scenarios:

1. Very small adult recipients with a small abdominal cavity⁹⁸
2. Adolescent recipients⁹⁹
3. Small paediatric recipients matched to a paediatric donor¹⁰⁰

In these three different clinical scenarios, while SLT remains the priority, graft reduction is a promising strategy to optimize graft-recipient size match and prevent large-for-flow syndrome. In the following, we will focus on the adult scenario anthropometric size mismatch and discuss the relevance to perform an ex-situ H67 graft reduction during HOPE.

6.6.2.1. *Anatomical Rational for H67 Graft Reduction*

Anthropometric graft-recipient matching needs to be considered in a three-dimensional space as it depends on four main parameters: graft volume and morphology as well as volume and morphology of the recipient’s abdominal cavity. In a small abdominal cavity,

compression of a large liver graft is due to the anterior costal margin and the posterior costal margin and the lumbar spine and one should consider the posterior abdominal cavity space as shown in the Figure below. Consequently, strategies to reduce pressure such as open abdomen treatment or placing of a bridging mesh, may be insufficient. Therefore, some authors perform a marginal costotomy by combining an anterior costotomy and an additional lateral costotomy (ribs 8–10) to relieve pressure to the posterior liver segments.¹⁰¹

There are two other main arguments to perform a H67 graft reduction. First, volumetric studies have shown that segments VI and VII account for 26.8%–34.5% of the total liver volume.¹⁰² Consequently, liver reduction strategies should include these posterior segments to achieve a three-dimensional space gain. Second, a H67 resection allows the liver graft to rotate around the inferior vena cava (IVC) with a significant space gain in the anterior compartment and a reduction of the graft pressure on the IVC minimizing the risk of outflow obstruction described in LFS.⁹³

6.6.2.2. *Anthropometric Criteria for ex-situ H67 Reduction*

Besides the aforementioned anatomical and volumetric considerations, criteria for deciding to perform an ex-situ reduction can be identified in the preoperative setting.

The first key indicator is liver weight which is difficult to estimate prior to organ procurement. In our experience, while reduction is planned prior to organ procurement, the final decision is always made after actual morphological inspection and weighing of the graft during back table preparation.

Two important anthropometrical indicators are graft/recipient weight ratio (GRWR) and donor weight/recipient weight ratio (D/RW). The latter can now be assessed routinely before organ acceptance owing to the availability of abdominal CT scan for all donors.

Another important indicator when deciding to perform an ex-situ H67 reduction is the right anteroposterior distance (RAP) and graft weight/right anteroposterior distance (GW/RAP).⁹⁴ The RAP is commonly defined the longest right anteroposterior vertical distance between the anterior and posterior costal margin of the recipient. The accepted GW/RAP cut-off for occurrence of LFS in adult LT is >100 g/cm.⁹⁴

6.6.2.3. *Ex-situ H67 Graft Reduction during HOPE*

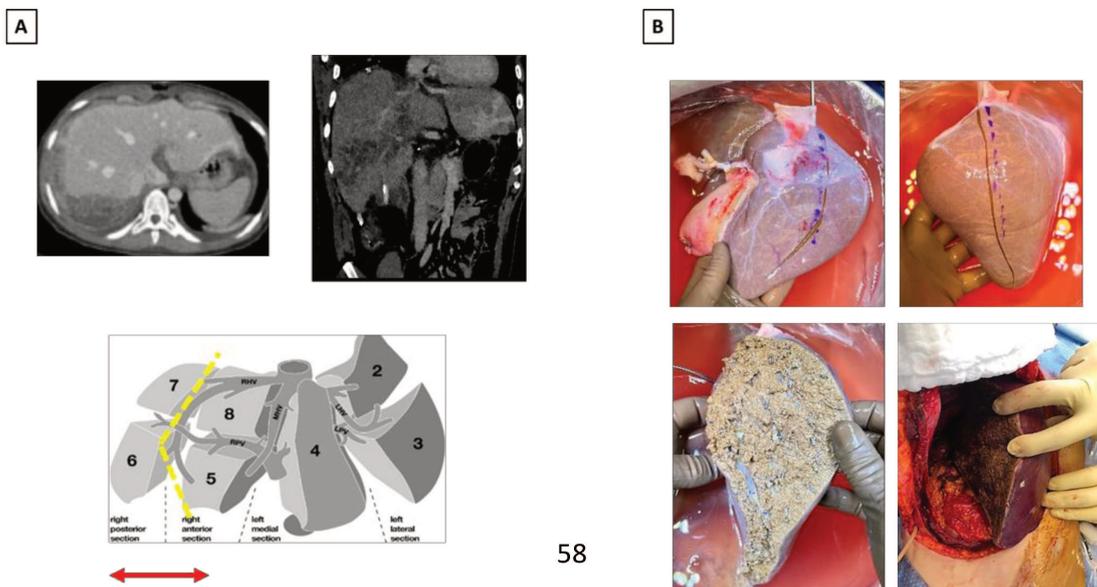
Ex-situ graft reduction during SCS exposes the graft to prolonged SCS which results in impaired outcomes as seen with ex-situ split grafts.⁴¹ We thus transposed our experience of HOPE-Split and applied this to ex-situ graft reduction.⁹⁸ We will briefly discuss several important technical considerations of this procedure.

The transection plane follows the axis of the right hepatic vein (RHV) while preserving the latter and only ligating the branches V6 and V7. The anatomical landmarks are the IVC cranially and the Rouvière sulcus caudally. Of note, the transection line is not straight but follows a curve in order to reduce the cranio-caudal and anterior-posterior length of the liver graft. During transection, we advocate intraparenchymal ligation of the right posterior Glissonian pedicle at the right border of the RHV. This allows to stay at a safe distance of the right anterior pedicle and the hilar plate. Of note, a common trunk forming the right posterior portal pedicle is observed in 73% of the cases which further facilitates safe ligation.

We perform the entire transection under HOPE similar to our experience with split grafts. There is a theoretical risk of small particles which are released during parenchymal transection to enter the perfusion system and cause subsequent vascular or sinusoidal obstruction. All the available machine perfusion devices have filters which allow to trap the majority of particles. The use of surgical gauze as an additional filter has been described in the literature.

Figure: Ex-situ H67 graft reduction during HOPE

A: Compression of segment 6 and 7 in an anthropometric mismatch B: Ex-situ H67 reduction



As general preliminary comment, while split procedures should be preferred to graft reduction given the possibility of transplanting two recipients, reduced grafts have the anatomical advantage of preserving the arterial, portal and biliary axis and avoiding hepatic vein reconstructions. Consequently, the implantation technique is more straightforward. One drawback of H67 reduction is the large transection plane which exposes the recipient to a higher risk of biliary leaks and bleeding compared to whole grafts. We, therefore, advocate the use of ultrasonic transection devices and perform a running suture on the transection plane with additional application of local hemostatic agents at the end of the procedure if necessary. It should be noted that hemostatic control of the posterior transection plane after reperfusion is facilitated by a natural compression of the latter due to the rotation of the liver graft around the IVC into the posterior abdominal cavity.

6.7.High-Risk Donor-Recipient Matching

Available studies on machine perfusion mainly focus on marginal grafts which are transplanted into highly selected recipients with low MELD scores in order to reduce the risks associated with an unfavourable matching. Indeed, when reviewing major trials in the field of machine perfusion by focusing on the Balance of Risk score (BAR), which quantifies the risk of donor-recipient matching, we can see conclude that the majority of trials focus on low-risk matching scenarios with a BAR score <10 (see Figure below).

Figure: Median Balance of Risk score across machine perfusion trials

Study	Bar score
Ravaioli et al, 2022	2-3
Schlegel et al al, 2023	7-8
Van Rijn et al, 2021	3
Lurje et al , 2020	5
Markmann et al, 2022	12
Nasralla et al, 2022	3
Cardini et al, 2022	4-8
Hann et al, 2022	12
Van Leuuwen, 2022	4
Liu et al, 2022	10

We know from large database studies that LT recipients with a higher BAR score present a significant increase in post-LT overall morbidity.¹⁰³ In a study by Schlegel et al, the comprehensive complication is 2 times higher in patients with a BAR score of 0-6 compared to 12-18 (26.3 vs 53.2).¹⁰⁴ In addition, a study published in 2020 by the *Agence de la Biomédecine* investigated optimal donor-recipient matches in over 8000 LT in the French program from 2009-2015.¹⁰⁵ The authors identify a subgroup of 207 LT recipients with a high MELD and receiving high-risk grafts. In these recipients, liver transplantation did not procure a survival benefit in contrast to remaining on the waiting list and wait for a graft with a lower risk. In contrast, low MELD patients did benefit from transplantation of a high-risk grafts. The hypothesis we established is that the subgroup of recipients with a high MELD receiving a high-risk graft may benefit from machine perfusion strategies. In accordance with the available data from the RCTs, we designed a single-arm prospective single-center phase I study (HOPE-MATCH) to investigate the impact of hypothermic oxygenated perfusion on outcomes in high-risk donor-recipient matching.

6.7.1. Study Protocol

The study study started in January 2023 and will finish in January 2024. All LT recipients with a MELD > 25 points and receiving a marginal graft according to the following criteria (based on the RCT HOPEExt):

4. *Age > 65 y*
5. *ICU stay > 7 days*
6. *BMI > 30*
7. *Macrosteatosis $\geq 30\%$*
8. *Natremia > 155mmol/L*
9. *AST max > 150 IU/L*
10. *ALT max > 170 IU/L*

All liver grafts will undergo end-ischemic HOPE upon arrival at the transplant center until the recipient hepatectomy is completed. Perfusion is performed as described in the RCT by *Schlegel et al* using single portal perfusion at 8-10°C with a perfusion pressure at 3mmHg and a target flow of 150-250 ml/min.⁴⁵

Endpoints

The primary endpoint will be cumulative liver-related morbidity at 1 year expressed by the Comprehensive Complication Index including primary-non-function rates, biliary complications, arterial complications and early relaparotomies (see Table below). Secondary outcomes will include 1-year recipient and graft survival rates, overall morbidity and number of recipients in the high-risk L-Graft7 category. HOPE-Match recipients will be matched in a 1-3 matching with a historical cohort based on the study inclusion criteria.

Table: Liver graft-related complications and associated treatments

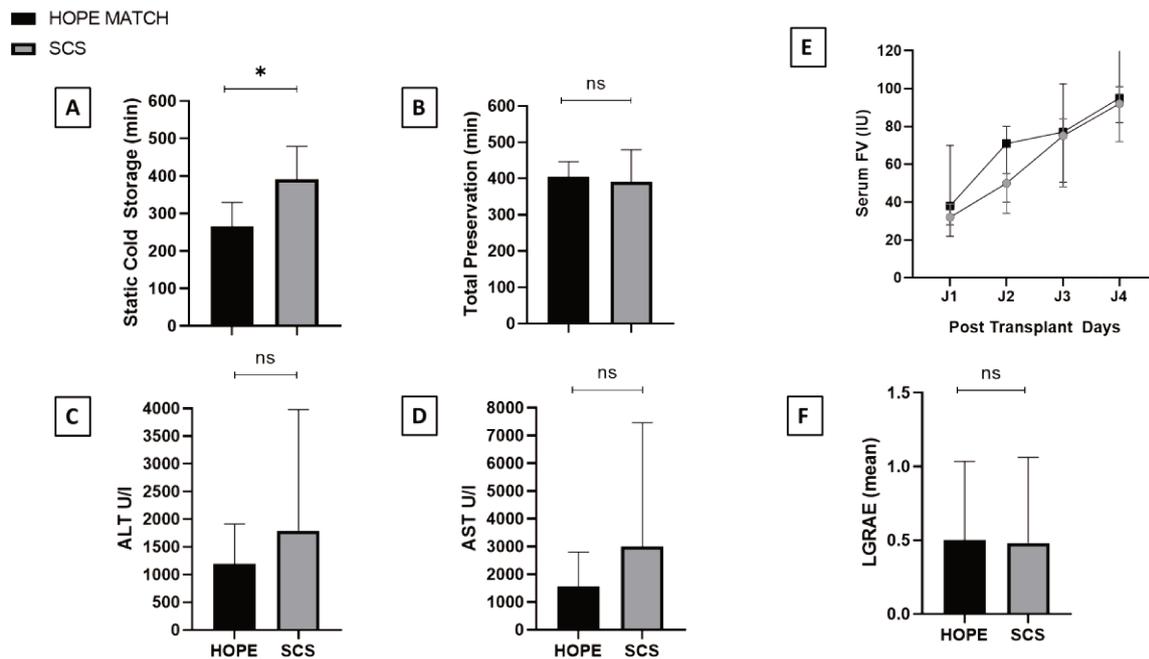
Liver Graft Related Complication	Definition	Potential treatment or evolution	Clavien Dindo
Primary Non-Function	<i>Graft loss within the first 7 Post-operative days (POD)</i> <i>And/or</i> <i>Patient's death within the first 7 POD</i>	Retransplantation Death	IVa V
Biliary complications	<i>Biliary leakage (anastomotic fistula, cut surface leak)</i>	Medical treatment	II
	<i>Symptomatic anastomotic stenosis or non-anastomotic stenosis</i>	Radiological Drainage Surgical Drainage Retransplantation ICU management (i.e. cholangitis, liver failure)	IIIa - IIIb IIIb IIIb Iva
Arterial complications	<i>Hepatic artery Thrombosis (HAT)</i>	Medical treatment Radiological intervention (i.e. thrombolysis) Surgical Intervention Retransplantation (i.e. early HAT (<1 week) or ischemic cholangiopathy)	II IIIa - IIIb IIIb

		ICU management (i.e. cholangitis and liver failure) Death	IVa V
Portal vein thrombosis	<i>Portal vein thrombosis demonstrated by Doppler ultrasonography or computed tomography angiography or surgical assessment</i>	Radiological intervention Surgical Intervention Retransplantation Liver failure requiring ICU management	IIIa - IIIb IIIb IIIb IVa
Early liver graft-related relaparotomies	<i>Early liver graft-related surgery during the first 90days post SLT in addition to aforementioned complications</i>	Surgical Management	IIIb
Post-operative bleeding	<i>Need for unattended transfusion after SLT and/or the need for specific radiological or surgical management</i>	Medical treatment Radiological Intervention Surgical Intervention ICU Management (i.e. Hemorrhagic shock) Death	II IIIa - IIIb IIIb IVa-IVb V

6.7.2. Preliminary Results

Since the start of the trial in February 2023, we have included 10 patients. We did not observe any device related adverse event. We have compared these 10 patients to a historical cohort of 27 patients with a MELD > 25 receiving a graft from a donor >65y after SCS. This preliminary analysis showed improved preservation with HOPE with a significant reduction (median 112 minutes) of static cold ischemia (Figure A). Total ex-situ preservation was not significantly different (Figure B). Early graft injury and function were comparable between the two groups and the mean number of liver graft related adverse events was also not significantly different. Interestingly, there was a higher rate of primary non-function in the SCS compared to the HOPE cohort (19% vs 0%).

Figure: Preliminary Outcomes from the HOPE-MATCH Study



6.8. Perspectives for HOPE

The presented results on new indications of HOPE pave the way for innovative applications of HOPE. We will focus on three perspectives for HOPE in LT for which there is already preliminary data available in the literature.

6.8.1. Prolonged Perfusion

Prolonging HOPE perfusion may have two major clinical impacts. First, it would allow to transform LT into a semi-elective procedure and avoid night-time LT. Second, it will allow to significantly reduce SCS in case of reallocation of the graft to another center, technical difficulties or other logistical challenges such as simultaneous LT in 2 different recipients. For example, in France in 2018, a total of 62 DBD grafts were discarded due to logistical problems. Currently, there exist no consensual cut-offs for minimal and maximal perfusion durations. A recent case report described a successful complex liver retransplantation with a graft undergoing HOPE for 11h and 20min.¹⁰⁶ A multi-center European registry study has shown benchmark graft and patient survival with prolonged end-ischemic HOPE for a median of 4h21min (range 4 to 8 h 35 min) and total ex-situ

preservation up to 21h.¹⁰⁷ The team from Groningen have initiated the DHOPE-PRO trial which will prospectively assess the feasibility and safety of perfusing liver grafts during the night for at least 4h to delay the transplant procedure until the regular opening hours.¹⁰⁸ They will include DBD and cDCD livers and outcomes will be compared to a prospective cohort of standard end-ischemic HOPE during recipient hepatectomy. Data from a preclinical porcine model even achieved 24 hours ex-situ preservation with HOPE.⁵⁴ In order to further improve LT logistics and allow transport of grafts over long distances without increasing SCS, a transportable device allowing HOPE is another promising perspective. Such a device is currently not available but several projects are ongoing.

6.8.2. Immunomodulation

As described in paragraph 6.1 HOPE dampens mitochondrial IRI and reduces the downstream activation of the hepatic inflammasome.^{53,60} Mitochondria have been shown to be the immunological powerhouse of the organism and there may thus be a potential effect of HOPE on the innate immune response of the graft after LT with a potential benefit on acute and chronic rejection.^{33,109} Available data from rodent models has shown that HOPE allows to downregulate post-LT immune response and reduce the need for immunosuppression after kidney and liver transplantation.^{110,111} A recent review of available data for rejection rates in trials using HOPE concluded to a significant reduction of acute cellular rejection compared to SCS ([OR 0.54 (95%CI 0.29–1), p=0.05].¹¹² Another interesting observation is the significant reduction of acute rejection in a RCT comparing HOPE with standard cold perfusion without oxygen in DCD kidney grafts.¹¹³ While these data are encouraging, none of the analysed trials have been specifically designed to investigate rejection rates and more robust studies are needed to confirm the role of HOPE in graft rejection.

In addition to rejection, liver transplantation for secondary liver tumours such as colorectal liver metastasis has increased and several countries, including France, have specific protocols to grant access to LT for these patients. In addition to refining oncological selection criteria there are two main challenges in the field of transplant oncology. First, the access to liver grafts in the absence of living donation is difficult which requires novel technical developments such as the RAPID concept.¹¹⁴ Second, post-LT disease-free and overall survival in these patients may be directly impacted by immunosuppression but also IRI to the graft which may increase susceptibility of tumour recurrence.¹¹⁵ Dynamic preservation and

especially HOPE may play an important role to tackle these two key challenges.¹¹⁶ In a retrospective single-center study, the authors observed lower hepatocellular carcinoma recurrence rates after transplantation of HOPE treated cDCD liver grafts compared to standard static cold storage.¹¹⁷ Again, while these data are encouraging they remain preliminary and need to be confirmed ideally by analysis of subgroups from the available RCTs.

6.8.3. Graft Treatment

Isolated ex-situ graft perfusion offers the perspective to deliver targeted treatments directly to the liver graft.¹¹⁸ While the biggest experience with graft treatment comes from the field of normothermic perfusion, there are several interesting preliminary experiences with HOPE. In a proof-of-concept study, Bonaccorsi-Riani et al showed that an adeno-associated virus (AAV) vector administered during HOPE was successfully engrafted and detectable by immunohistochemistry in the liver graft after transplantation in a rodent model.¹¹⁹ In a follow-up study, the authors administered small interfering RNA (siRNA) during HOPE to inhibit apoptosis and consequently hamper IRI in a rodent liver transplant model.¹²⁰ Using confocal microscopy, they could show presence of siRNA 24h after transplantation of grafts treated during HOPE. Administering such compounds during cold perfusion instead of perfusion at physiological temperatures may allow for a delayed activation or avoid denaturation of the compounds by DNases present in the blood based perfusates.

7. Normothermic Oxygenated Perfusion

Besides hypothermic oxygenated perfusion with acellular perfusion solution another widely used perfusion modality is normothermic perfusion.¹²¹ The particularity of normothermic perfusion compared to HOPE, is the reoxygenation of the graft under normothermic conditions, usually around 37°C. The rationale is to create a physiological environment for the liver graft in order to stimulate the different liver functions ranging from cellular respiration to bile production and immunological processes.

7.1. Reoxygenation in Normothermic Conditions

Normothermic reoxygenation of the liver graft after a period of SCS as seen with end-ischemic normothermic perfusion (NMP), induces a higher degree of mitochondrial injury compared to hypothermic reoxygenation as seen with HOPE.⁶⁰ This is due to a rapid oxidation of the accumulated succinate in a context of low adenine nucleotide levels with a longer delay to reconstitute the adenine nucleotide pool. This leads to an increase in damage associated molecular pattern release and hepatic inflammasome activation.¹²² A study by Friend et al in a porcine model of DCD liver grafts undergoing 60min of donor warm ischemia showed that a period of 4h of SCS prior to NMP induced higher hepatocellular and endothelial cell injury compared to continuous preservation with NMP without a period of SCS.⁵⁵ In line with this observation, continuous preservation with NMP has been shown to be associated with a reduction of pro-inflammatory cytokines as well as neutrophil infiltration, necrosis and apoptosis compared to SCS preserved liver grafts.¹²³ Recent clinical data by the Innsbruck group, which focuses on mitochondrial respiration in 50 liver grafts undergoing NMP after a median of 336min of SCS, shows aggravated necrosis and strong inflammatory response in a subgroup of discarded livers which were foremostly DCD graft.¹²⁴ We can conclude from this data that end-ischemic NMP should be used with caution especially in the context of prolonged SCS duration. Indeed the 2 published RCTs both have very short SCS times owing to the fact that they use a transportable machine.^{44,46} The only normothermic perfusion modality to avoid SCS prior to reoxygenation is Normothermic Regional Perfusion (NRP).

7.2. Normothermic Regional Perfusion

Personal Contributions: We have focused on investigating graft utilization rates during normothermic regional perfusion (NRP) and identified graft quality as a main factor. This has led to the development of a preclinical NRP model to identify biomarkers for graft quality and viability in parallel to the initiation of a prospective clinical trial.

Muller X, Rossignol G, Damotte S, Gregoire A, Matillon X, Morelon E, Badet L, Mohkam K, Lesurtel M, Mabrut JY. Graft utilization after normothermic regional perfusion in controlled donation after circulatory death—a single-center perspective from France. Transpl Int. 2021 Sep;34(9):1656-1666. doi: 10.1111/tri.13987. PMID: 34448267.

Muller X, Mohkam K, Mueller M, Schlegel A, Dondero F, Sepulveda A, Savier E, Scatton O, Bucur P, Salame E, Jeddou H, Sulpice L, Pittau G, Allard MA, Mabrut JY, Dutkowski P, Clavien PA, Lesurtel M. Hypothermic Oxygenated Perfusion Versus Normothermic Regional Perfusion in Liver Transplantation From Controlled Donation After Circulatory Death: First International Comparative Study. Ann Surg. 2020 Nov;272(5):751-758. doi: 10.1097/SLA.0000000000004268. PMID: 32833758.

*Liver Graft Viability Assessment During Normothermic Regional Perfusion: The MAASTR3BOLOMIC Study (NCT05361044), **Primary Investigator: Muller X***

7.2.1. Clinical Outcomes

Normothermic regional perfusion (NRP) consists in an in-situ normothermic reperfusion of the donor organs following cardiac arrest in the context of controlled or uncontrolled DCD. The basic principle is to restore blood supply to the abdominal organs using an ECMO-like device with the placement of an aortic occlusion balloon to avoid reperfusion of the brain. To date, there exists no randomized controlled trial comparing NRP to SCS or to another perfusion strategy and the available evidence is solely based on retrospective studies.¹²⁵ In addition, preclinical data from animal models or mechanistic approaches to NRP are scarce in the literature in contrast to HOPE and NMP. In a recent meta-analysis of 8 retrospective studies comparing NRP to SCS in cDCD grafts, the authors found low primary non-function rates ranging from 0-9% and low ischemic cholangiopathy rates ranging from 0-2% with a 75% lower risk in the NRP cohorts.¹²⁵ In France for example, a total of 282 cDCD liver grafts have been successfully transplanted from 2015 to 2020 with excellent results comparable to standard DBD LT.¹²⁶ We have performed the first direct comparison between 2 different dynamic perfusion techniques in cDCD in a study published in 2020.¹²⁷ In this collaborative work, we compared outcomes after cDCD LT with the French NRP protocol and the Swiss HOPE protocol after propensity score matching. In a cohort of

132 NRP and 93 HOPE cases we could show that the overall risk profile of cDCD donors in France was lower with a significant younger age (50 vs 61 years, $P < 0.001$), shorter FDWI (22 vs 31 minutes, $P < 0.001$) and a lower overall predicted risk for graft loss (UK-DCD-risk score 6 vs 9 points, $P < 0.001$). Both the NRP and HOPE cohort showed a $> 85\%$ 1-year tumour-censored graft survival which is comparable to benchmark values observed in standard DBD LT. Importantly, after propensity score matching, we found no differences in non-anastomotic biliary strictures, primary nonfunction and hepatic artery thrombosis between both cohorts. While this approach has inherent limitations given the different allocation rules, transplantation techniques etc., it allowed to highlight some important points:

11. NRP is an intervention on the donor whereas HOPE is applied to the graft
12. More technical adverse events related to perfusion were observed in the NRP cohort
13. Total ex-situ graft preservation was longer in the HOPE cohort
14. NRP allowed for a rapid viability assessment during procurement as well as perfusion of all abdominal organs
15. HOPE allowed for a liver specific viability assessment
16. Higher graft discard rates in the NRP cohort owing to a stricter selection protocol

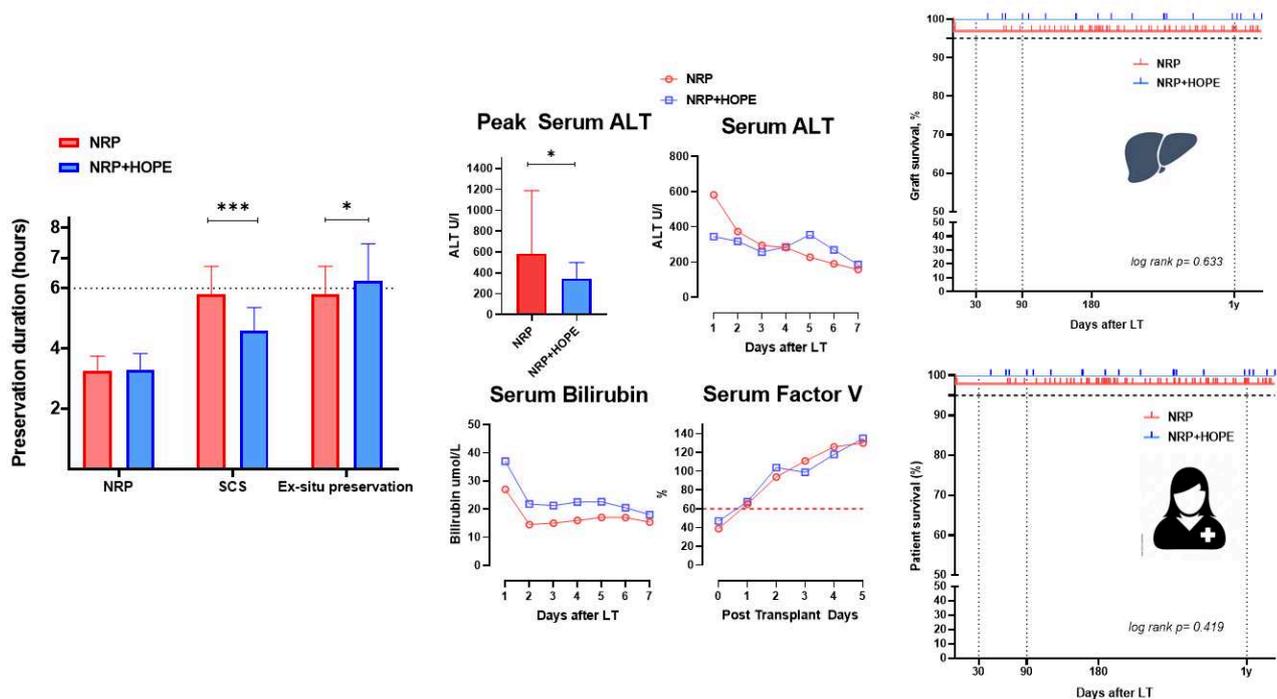
Since September 2021, the French NRP protocol applies extended donor and graft selection criteria for grafts undergoing additional HOPE after NRP hereby combining the benefits of both techniques. The following donor/graft criteria require additional ex-situ HOPE after NRP:

- Donor AST/ALT levels 4-8x normal value or ascending trend (three different measuring points)
- NRP AST/ALT levels 4-8x normal or ascending trend (three different measuring points)
- 20-30% macrosteatosis or F1 fibrosis on mandatory biopsy

We are currently completing a multicenter study including 8 French transplant centers comparing all cDCD LT with or without additional HOPE performed since the implementation of the novel selection criteria (manuscript in preparation). We included a total of 96 NRP LT and 20 NRP+HOPE LT. Donor age and warm ischemia times were not

significantly different. However, we could show a significant 71 minutes median reduction of SCS in the NRP +HOPE group with a significantly increased total ex-situ preservation duration. The NRP+HOPE group presented lower peak ALT (NRP group: 581 UI vs NRP+HOPE group: 345 UI, $p=0.0399$) after LT but similar graft function recovery. Overall, 1-year graft and patient survival was $>95\%$ for both groups. Importantly looking at our own center data, we were able to reduce the cDCD graft discard rates from 14% (14/99) prior to the implementation of the NRP+HOPE protocol to 2% (1/45). We conclude from this preliminary data that NRP+HOPE allows to reduce cDCD graft discard rates and achieves similar survival results with reduced hepatocyte injury and improved ex-situ preservation by a significant reduction of SCS.

Figure: Preliminary results comparing NRP to NRP +HOPE in the French cDCD program



7.2.2. Technical Considerations

Beyond the very promising clinical outcomes, the use of NRP requires specific technical experience to perform donor cannulation compared to the standard super-rapid procurement technique used for example in the Netherlands. Since the initiation of a NRP

program in France we have worked on 2 main technical features of NRP: donor cannulation and duration of NRP.

First, when analysing technical failures, it is important to note that the practical implementation of NRP is highly dependent on national ethical rules.¹²⁸ In this context, premortem cannulation in the donor to initiate NRP is permitted in Spain while in France only premortem placement of guidewires is allowed and cannulation must be performed postmortem. These technical differences which are based on ethical considerations have a direct impact on NRP outcomes. In a review of local data on NRP procurement from France, where donor cannulation is performed in the majority of the cases by an anesthesiologist at the donor center using the Seldinger technique over preplaced guidewires in the femoral vessels, we found that 25% of all discarded cDCD grafts were related to a cannulation failure.¹²⁹ This stands in contrast with a 6% cannulation failure rate reported in a study from the UK (34 cases) using exclusively surgical cannulation of the abdominal vessels.¹³⁰ Reports from Spain showed even lower cannulation failure rates with premortem cannulation, which reduces the time constraint and even allows to perform cannulation in an interventional radiology facility.¹³¹ Conclusively, we advocate a tailored cannulation approach depending on recipient criteria and the experience of the procurement team. For example, donors with peripheral vascular disease may present additional difficulty for percutaneous cannulation and a surgical approach in the operating room may have a higher success rate. Altogether, NRP is a technically challenging procedure and requires appropriate training and experience to overcome the learning curve.¹³² In the French setting, of the 30 centres in the French cDCD program, half performed ≤ 15 NRP procedures from 2015 to 2019. We did observe a trend towards a lower rate of NRP adverse events from 2018–2019 compared to 2015–2018 which highlights the importance of experience in the use of NRP.¹²⁹ A possibility to increase exposure to cannulation is to create regional NRP teams which travel to the donor hospital to perform cannulation. Feasibility of setting up a mobile team to allow hospitals without NRP experience to participate in cDCD programs have been reported.¹³³

Second, there is little clinical data available on optimal perfusion duration in the setting of NRP. The group from Poitiers tested different NRP durations ranging from 2-6h in a porcine model of NRP cDCD kidneys.¹³⁴ They found that the lactate/pyruvate ratio and LDH levels increased up to 2 hours and returned to baseline between 4 and 6 hours of perfusion. After transplantation of the porcine kidneys, there was an improved early renal function recovery after 4 h-NRP group compared to 2 h-NRP. In addition, NRP>4h increased

inflammatory damage to the grafts. The authors conclude that the ideal timeframe for NRP is 4h. We investigated the impact of NRP duration in a cohort of 156 cDCD procedures performed in French centers with a minimal perfusion duration of 1h and maximal of 4h. There was no significant impact of NRP duration on post-LT outcomes.¹³⁵

7.2.3. Viability Assessment during NRP

Normothermic regional perfusion offers a platform for quality and viability assessments of liver grafts prior to procurement. In the French cDCD program for example, there is mandatory assessment of transaminase levels over the course of perfusion which determines whether the graft is deemed transplantable or not. Recently, *Shurink et al.* reported their preliminary experience with a direct functional measurement of the liver graft during ex-situ normothermic perfusion using the LiMAX test.¹³⁶ This test directly measures cytochromal activity of the CYP1A2 enzyme and has been previously validated in functional assessment of future liver remnant in major liver surgery. This technology could be of interest in the context of NRP. The same authors also reported specific cholangiocyte viability assessment during NRP by analysis of bile samples. They assessed bile quality and defined viability criteria to predict occurrence of ischemic cholangiopathy in a cohort of 20 transplanted NRP cDCD grafts. They propose bile glucose <3.0 mmol/L, delta bile bicarbonate >5 mmol/L and delta pH bile >0.1 which are associated with a positive predictive value of 33% and a negative predictive value of 94% for ischemic cholangiopathy. A group from Italy went a step further and performed an assessment of various biomarkers for inflammation, regeneration and mitochondrial damage during NRP in 10 controlled DCD and 9 uncontrolled DCD grafts.¹³⁷ They identified several biomarkers which present significantly different profiles between the two groups for example α -GST and K-18 which are two liver specific proteins.

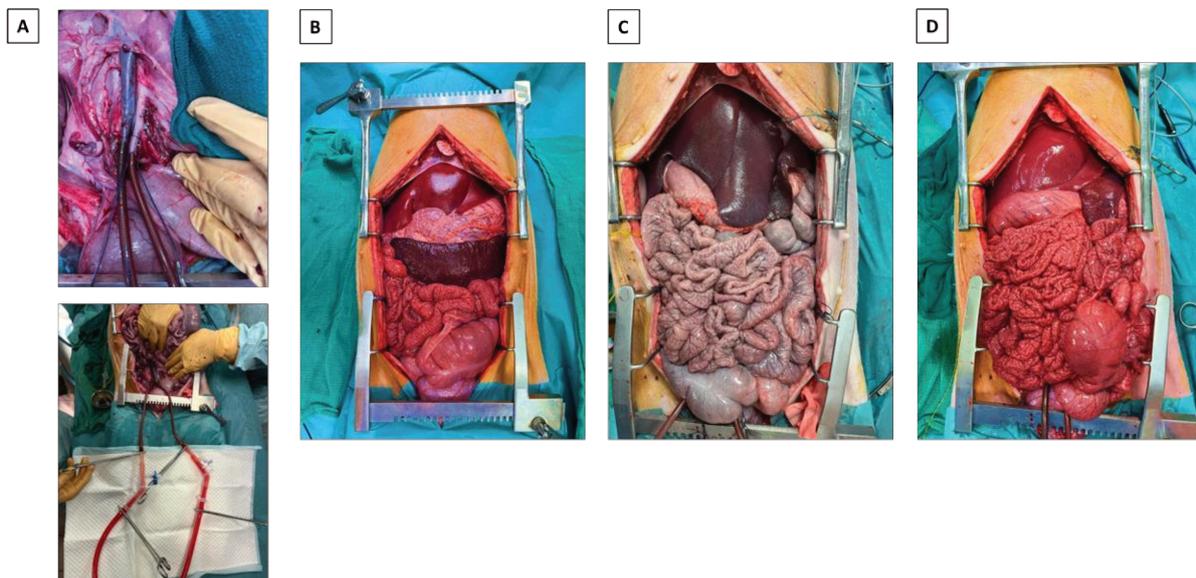
7.2.4. A Preclinical NRP Model

In order to further investigate the underlying physiopathological mechanism, detect novel viability markers and allow direct comparison with the other aforementioned preservation strategies, we established and standardized a preclinical model of porcine NRP in the context of a Master Thesis performed by Dr. Natacha Boulanger at our department (manuscript in preparation). We made substantial changes to the available models from the literature and adapted the protocol to our local requirements.¹³⁴ The final aim was to allow stable NRP

during 2h which is the median time of NRP in the French cDCD cohort.¹³⁵ We used the same animals and the same liver graft procurement procedure as for our other preclinical studies (see paragraph 6.3 and 6.4). This will allow direct comparisons of the different preservation strategies in our preclinical model. The perfusion circuit of the NRP device was primed with Ringer Lactate followed by addition of 1.5-1.7L of autologous pig blood.

Figure: Experimental Set-up of our porcine NRP model

A: Cannulation of the major abdominal vessels; B: Baseline view of the abdomen C: View of the abdomen during warm ischemia; D: view of the abdomen after initiation of NRP



Methods

The study was divided in 2 phases with a test phase including 4 animals and a consolidation phase including 5 animals. During the test phase the main pitfalls were important vasoplegia requiring >8L of fluid resuscitation which led to considerable blood dilution. As a consequence, there was important tissue oedema and continuous drop in NRP flows with subsequent suboptimal tissue oxygenation.

We made the following adjustment to correct the encountered pitfalls:

- 17. Invasive monitoring of mean arterial pressure by direct cannulation of the aorta to adjust flow of the NRP device to a target MAP of >50mmHg*
- 18. Priming of the NRP circuit with 250ml of Mannitol and 300ml of NaCl 0.9%*

19. *Continuous infusion of Ringer Lactate at 600 ml/h*

20. *Continuous infusion of norepinephrine at rate of 2mg/h*

21. *Continuous infusion of bicarbonate to potentialize the effect of the vasopressor at a rate of 125ml/h*

Preliminary Results

After implementation of these changes during the consolidation phase (n=5) we were able to perfuse for 2h with a stable flow around 1.3-1.5L, no visceral oedema and <1.5L of fluid resuscitation.

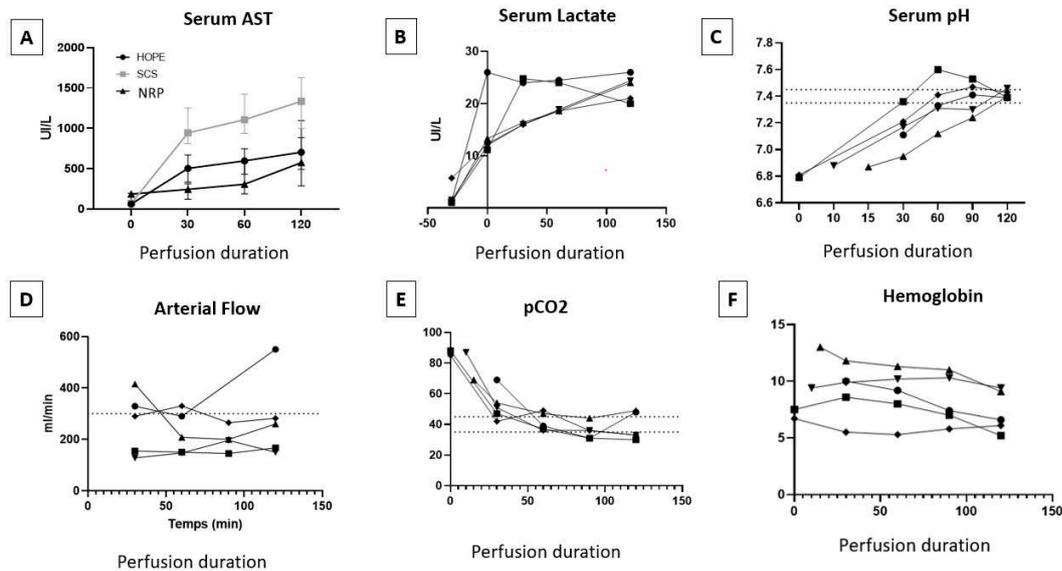
Hepatocyte Compartment

Interestingly, AST release during NRP was comparable to that observed in the group undergoing 6h of SCS and 2h of HOPE while it was inferior to the group undergoing only 6h of SCS (Figure A). Lactate clearance was not achieved but pH normalized at the end of NRP due to active addition of bicarbonate (Figure B+C). The high level of lactate may be due to the IRI to other abdominal organs notably the small bowel which is specifically fragile in pigs.

Vascular Compartment

Arterial flow remained stable with the new protocol allowing for an adequate tissue oxygenation with a rapid decrease of pCO₂ into the normal range (Figure D+E). In all but 1 experiment, hemoglobin levels remained stable and in a physiological range during the entire NRP duration (Figure F).

Figure: Early IRI during 120 min of NRP in a preclinical porcine model



Perspectives

After establishing a reproducible pig NRP model, we will in a next step apply 6h of static cold storage with ex-situ isolated reperfusion as performed for the other groups in the aforementioned preclinical preservation studies. This will allow to identify liver viability biomarkers by correlating the identified candidates during NRP with early IRI outcomes in the reperfusion model. This research has direct translational implications as it will serve to select and test biomarkers which will also be a target in the currently ongoing a prospective study on NRP in Liver Graft Viability Assessment During Normothermic Regional Perfusion (MAASTR3BOLOMIC Study) which will be described in detail in the next paragraph.

7.2.5. The MAASTR3BOLOMIC Study

Based on the aforementioned advances in graft quality assessment during NRP and the urgent need to reduce graft discard rates, we set-up an prospective non-randomised single-arm multicenter clinical trial including 60 cDCD liver grafts procured after undergoing NRP with the aim of identifying robust viability markers (NCT05361044)

The study has 2 main aims:

Aim 1: To characterise I/R injury in cDCD liver grafts during NRP by in-depth metabolomics to establish a robust biomarker for cDCD liver graft viability.

Aim 2: To develop a bio-clinical prediction model based on liver biomarkers in combination with donor and recipient data to predict graft function prior to transplantation.

The study period will be 24 months with a follow-up period of 1 year

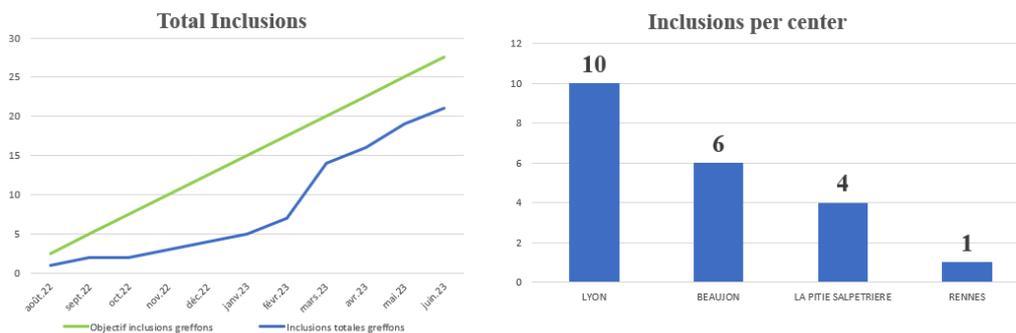
Table: Study samples types, collection time points, storage and analysis

	Type	Timepoint	Storage	Analysis
NRP Perfusate	Blood Serum	Pre-NRP, Every 30 min, during NRP	5x2ml aliquots stored at -80°C	Metabolomics, Biochemistry
Liver Samples	Liver Biopsy	After 120min of NRP + liver specific blood sample After cold storage After reperfusion in the recipient	Surgical biopsy divided into sample for formalin and snap frozen section at -80°C	Metabolomics, Histology
Bile Samples	Bile	End of NRP After reperfusion in the recipient	2ml aliquots Snap Frozen	Biochemistry
Recipient samples	Biochemistry Haematology Haemostasis	Standard of care at the transplant center	Standard of care at the transplant center	Standard of care at the transplant center

Primary objective: To identify and quantify I/R injury biomarkers markers for liver graft viability released during NRP and analyze their predictive value for major liver graft-related adverse events after transplantation.

Primary outcome measure: Metabolomics including FMN, hypoxanthine, succinate, xanthine and NADH, performed by nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography-mass spectrometry (LC-MS) in the NRP perfusate and liver graft tissue to be correlated with a composite endpoint of major liver graft-related adverse events including early allograft dysfunction as defined by Olthoff et al including primary non function, non-anastomotic strictures and early arterial complications within the first year after transplantation.⁵¹

The results of the present project will lay the foundation for a clinical trial on the use of extended criteria cDCD liver grafts with the aim to safely increase the cDCD donor pool. In addition, the identified biomarkers may also be validated for other organs procured during NRP such as hearts, lungs and kidneys. The study has been financed by the *Fondation de l'Avenir* and started in August 2022. To date, a total of 21 patients have been included and we are expecting to finish recruitment in 2024. The detailed study protocol can be found in supplementary material.



7.3.Ex-situ Normothermic Perfusion

Another form of reoxygenation at normothermic temperature is ex-situ normothermic perfusion (NMP). As stated under paragraph 4.2, there are 2 RCT's available on continuous NMP with a transportable device and initiation of perfusion at the donor center.^{44,46} A recent meta-analysis including 282 patients undergoing continuous NMP showed a reduction in early allograft dysfunction rates but no clear beneficial effects on biliary strictures and overall survival.⁸ Continuous NMP requires important logistics and expertise and currently the main focus is on optimizing perfusion protocols such as low oxygen tension to prevent vasoplegia and reperfusion syndrome in the recipient after reperfusion.^{138,139} In the recent RCT by Markmann et al, the safety endpoint of 90 days liver graft related adverse events was not different between NMP and SCS groups.⁴⁴ To reduce the risk of adverse events due to the complexity of the procedure, many teams have now opted to switch from continuous to end- ischemic NMP and recommend clear standard operating procedures, 24h emergency hotline and training of dedicated perfusion staff.¹⁴⁰ While switching to end-ischemic NMP may be interesting from a logistical point of view, this adds additional SCS which may increase the deleterious effects of reoxygenation at normothermic temperatures we have described in paragraph 7.1. , especially in marginal and steatotic grafts.

While the field of NMP is rapidly evolving, there are still only few transplant centers using this complex technology. In France for example, the prospective PENOFOR trial focusing on the use of continuous NMP with steatotic DBD grafts (>30% of macrosteatosis) initiated by three transplant centers from Paris (i.e, Paul Brousse, Pitié-Salpêtrière and Beaujon) was stopped after including 3 patients due to regulatory concerns and will restart recruitment after successful protocol amendments (NCT04154696). Following the first reports on elective LT using NMP, the team from Rennes have initiated a prospective trial using end-ischemic NMP to perfuse liver grafts during the night and allow the implantation to start in the morning (personal communication). In 2023, a prospective trial TRANSPERF will start in France and investigate the ability of end-ischemic NMP to increase utilization rates in marginal grafts by viability assessment.

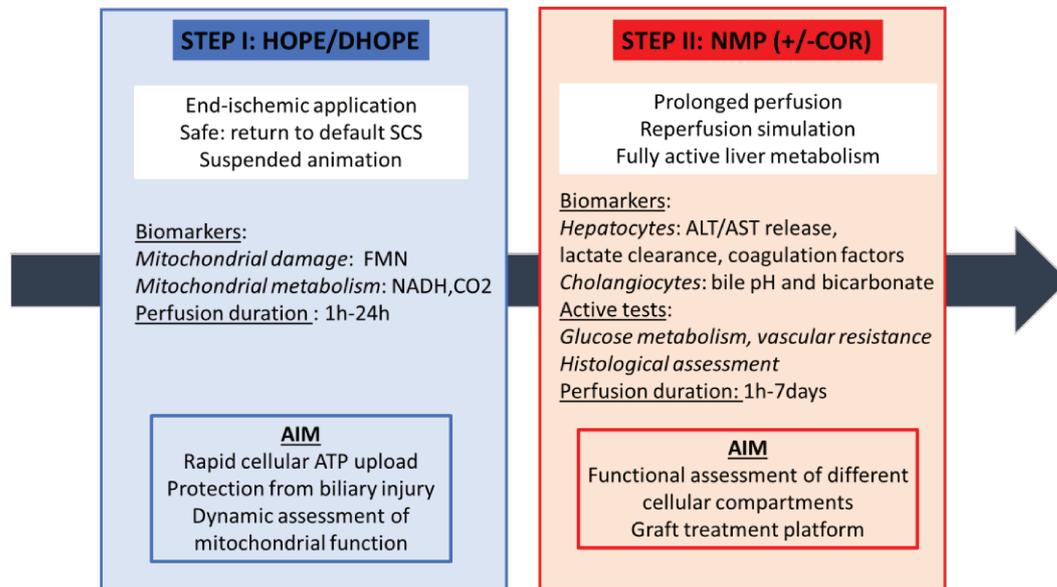
Following the data gathered during this thesis on HOPE, warm reperfusion and preservation injury, we have decided to focus on combining hypo- and normothermic ex-situ perfusion rather than isolated ex-situ NMP.

7.4. Combination of Cold and Warm ex-situ Perfusion

All the aforementioned dynamic preservation strategies have their advantages and disadvantages. Combining both cold and warm perfusion is thus very appealing since it allows to benefit from the advantages of both and compensate for the individual disadvantages. There are now several large single-center cohort studies available which present first encouraging outcomes with a 2-step approach combining HOPE and NMP for marginal liver grafts in order to combine the advantages of each technology.¹⁴¹ First, liver grafts undergo assessment of the metabolic state of the graft with HOPE/DHOPE while protecting from biliary injury.^{65,75} If the viability assessment is satisfactory, liver grafts can be immediately transplanted. On the contrary if there are signs of major metabolic impairment during HOPE, liver grafts will undergo additional NMP to simulate reperfusion and assess hepatocyte and cholangiocyte functions in metabolically fully active grafts.⁵⁷ Viability testing during NMP has been focusing on lactate clearance, glucose metabolism, bile quantity and quality and vascular resistance.^{57,121} NMP also offers the possibility to use graft treatments but to date, these applications are still in the preclinical phase.

Figure: Mechanistic rationale of combining cold and warm perfusion for liver graft preservation (adapted from Muller et al⁴²)

COR: controlled oxygenated rewarming; DHOPE: dual hypothermic oxygenated perfusion; HOPE: hypothermic oxygenated perfusion; NMP: normothermic machine perfusion;

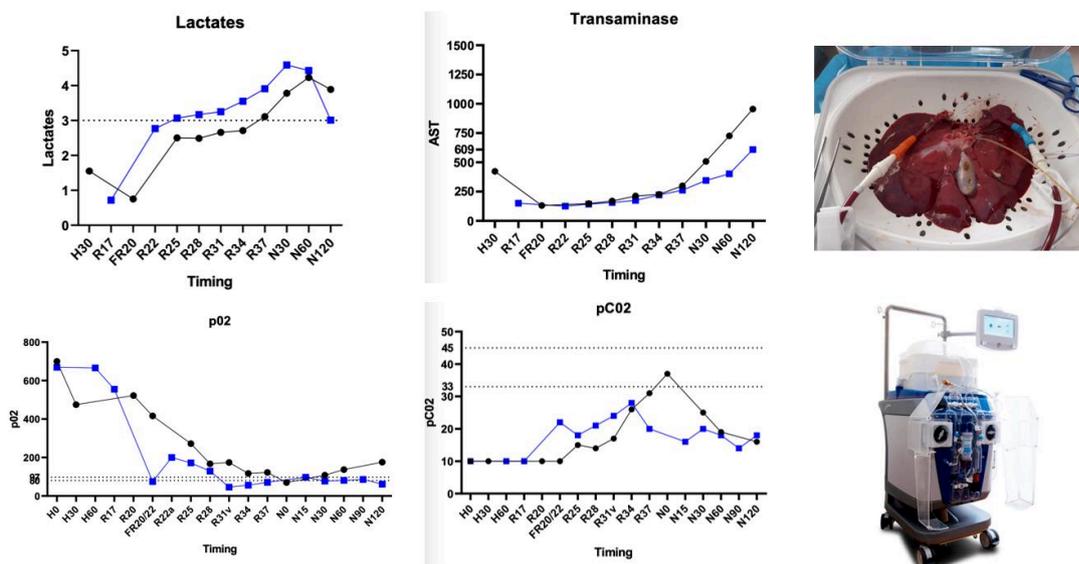


7.4.1. Controlled Oxygenated Rewarming

An even more sophisticated strategy is Controlled Oxygenated Rewarming (COR) which consists in rewarming the graft from 10 to 20°C using an acellular actively oxygenated preservation solution. The pathophysiological hypothesis of COR is to avoid the rapid rewarming of the graft during the switch from cold to warm perfusion in order to further protect the graft from mitochondrial damage and hamper IRI.¹⁴² A single center RCT published in 2022 included 40 extended criteria DBD liver grafts which were randomized to either end-ischemic COR from 10 to 20°C during 90 min or SCS prior to transplantation. The results show that there was a faster recovery of graft synthetic function (Factor V) in the COR group.¹⁴³ Another even more advanced perfusion modality is to perform COR prior to initiation of NMP. A single-center prospective study using HOPE-COR-NMP has shown promising results in marginal liver grafts, foremostly extended criteria DCD grafts with the possibility of performing viability assessment.¹⁴⁴ The clinical application of HOPE-COR-NMP requires however a sophisticated perfusion device which ideally allows to switch the perfusate from acellular to blood-based without breaching the sterility of the set-up. We have tested such a novel perfusion device using our preclinical porcine model to establish a HOPE-COR-NMP perfusion protocol applicable in our clinical practice. After 30 min of AWI and 6h

of SCS the procedure consisted in 1h of HOPE (8-10°C) followed by 1h of COR (10-37°C) and 2h of NMP at 37°C. At 20°C, we performed a switch from acellular perfusate to the autologous pig blood (15-20min) without disconnecting the liver. An example of 2 porcine grafts perfused with this protocol are shown in the Figure below. We conclude from the preliminary data that AST release after 120min of warm reperfusion in the HOPE-COR groups was in a similar range than observed in our HOPE model. Lactate clearance was also observed at the end of HOPE-COR-NMP. These very preliminary results encourage us to further refine the perfusion protocol with the aim of clinical implementation.

Figure: Preliminary results on liver injury and tissue oxygenation from a pig model using HOPE-COR-NMP



8. Conclusion and Outlook

Dynamic liver graft preservation was already in the minds of the pioneers of transplantation and has now reached the stage of routine clinical use. The context of graft shortage and novel indications have contributed to a rapid rise in the use of machine perfusion in the field of liver transplantation. The presented work does not aim at providing an exhaustive review of the literature and practices in dynamic preservation but rather reflects a personal tough process from static cold storage to HOPE and finally to normothermic perfusion. The final ambition of this work is to improve and extend the use of dynamic

preservation with a bench-to-bedside approach. To conclude, we would like to highlight five main conclusions of the presented work.

First, liver graft ischemia under its various forms remains a key factor in liver transplantation even in the era of machine perfusion. Indeed, with the exception of the complex protocol of ischemia-free LT. Indeed, perfused liver grafts are still exposed to a certain degree of ischemia. It is our understanding that in order to improve dynamic preservation strategies, more effort is needed to gather robust mechanistic data on the impact of the different ischemic phases on machine perfused liver grafts. Especially donor warm ischemia and recipient warm ischemia are areas where further research is needed.

Second, static cold storage still remains the gold standard of liver graft preservation and achieves excellent outcomes with relatively low costs and logistics. This is clearly reflected by the different machine perfusion RCTs where the static cold storage study groups achieve excellent graft and patient survival rates. While level I evidence on the benefit of machine perfusion is available, the clinical relevance of the primary endpoints is often questioned. Consequently, there is an urgent need to refine indications for machine perfusion by focusing on specific LT scenarios with a high expected ischemia-reperfusion injury. Perfusion of every single liver graft seems currently not justified. Thus, we have proposed several scenarios including partial grafts and high-risk donor-recipient matchings where we can expect a higher benefit of HOPE based on the pathophysiological mechanisms. The data gathered from the conducted phase I studies during this thesis will allow to set-up larger scale multicenter phase II and III trials. For example, the national RCT HOPE-SPLIT which includes adult and paediatric liver transplant centres and will start at the end of 2023.

Third, HOPE currently is the gold standard of liver graft preservation based on the available level I evidence as well as logistics and required expertise. It was thus the main aim of this thesis to improve and refine the use of HOPE. In a preclinical porcine model of ischemia-reperfusion injury, we tested a novel preservation solution IGL2 tailored to the use with cold oxygenated perfusion and could show that it achieves comparable results to the combination of 2 different preservation solutions (UW + Belzer MPS) for static and dynamic preservation. This preclinical data will allow to design and conduct a first-in-man clinical phase I study which will start at the beginning of 2024. Next, we aimed at defining the ideal perfusion modality and timing using HOPE in our preclinical model. We could show that end-ischemic portal HOPE achieves comparable IRI to DHOPE but also to continuous HOPE

without SCS. However, very marginal grafts especially steatotic grafts probably will benefit from a further reduction of static cold storage and we will test a portable HOPE device in the follow-up of the presented preclinical study.

Fourth, normothermic perfusion is a more complex and challenging procedure which requires considerably more expertise. This has direct impact on costs but also feasibility. More importantly, the pathophysiological mechanisms are different from HOPE and expose the graft to a higher degree of IRI in case of a prolonged SCS. Normothermic regional perfusion has the particularity of reperfusing the graft without prior SCS. While we could show the excellent clinical results of NRP, there are still many unanswered questions regarding mechanisms, discard rates and viability assessment. Consequently, we established and standardized a preclinical porcine model of NRP which will allow to conduct mechanistic studies and analyse potential viability biomarkers. In parallel we are currently conducting a multicenter prospective study in the French NRP DCD cohort to establish a biobank of liver tissue and perfusate to rapidly translate the findings from the preclinical model into clinical practice.

Finally, the data gathered during this thesis is in favour of combining both cold and warm perfusion with a period of controlled rewarming. Indeed, HOPE allows to protect the graft from ischemic biliary injury and the controlled rewarming will protect the mitochondria from the effect of warm reperfusion while NMP will allow to perform a more complete viability assessment. Based on the data from our preclinical model presented in the thesis, we will now work on the development of a clinical perfusion protocol for HOPE-COR-NMP. This technology is likely to have future application which are still in development such as for example graft treatment. We believe however that it is important for a transplant center to be able to have access to this technology and as a next step we will thus promote a national trial to introduce this novel perfusion modality in French LT centers.

9. Summary of Research Activity

Published papers in peer reviewed journals in chronological order (Original manuscripts in supplementary material)

1. **Muller X**, Mohkam K, Mueller M, Schlegel A, Dondero F, Sepulveda A, Savier E, Scatton O, Bucur P, Salame E, Jeddou H, Sulpice L, Pittau G, Allard MA, Mabrut JY, Dutkowski P, Clavien PA, Lesurtel M. Hypothermic Oxygenated Perfusion Versus Normothermic Regional Perfusion in Liver Transplantation From Controlled Donation After Circulatory Death: First International Comparative Study. *Ann Surg.* 2020 Nov;272(5):751-758. doi: 10.1097/SLA.0000000000004268.
2. **Muller X**, Tilmans G, Chenevas-Paule Q, Lebossé F, Antonini T, Poinot D, Rode A, Guichon C, Schmitt Z, Ducerf C, Mohkam K, Lesurtel M, Mabrut JY. Strategies for liver transplantation during the SARS-CoV-2 outbreak: Preliminary experience from a single center in France. *Am J Transplant.* 2020 Nov;20(11):2989-2996. doi: 10.1111/ajt.16082. Epub 2020 Jul 5
3. Mabrut JY, Lesurtel M, **Muller X**, Dubois R, Ducerf C, Rossignol G, Mohkam K. Ex Vivo Liver Splitting and Hypothermic Oxygenated Machine Perfusion: Technical Refinements of a Promising Preservation Strategy in Split Liver Transplantation. *Transplantation.* 2021 Aug 1;105(8):e89-e90. doi: 10.1097/TP.0000000000003775.
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5. **Muller X**, Rossignol G, Mohkam K, Lesurtel M, Mabrut JY. Dynamic Liver Graft Preservation in Controlled Donation After Circulatory Death: What Is the Best Fit? *Liver Transpl.* 2022 Feb;28(2):330-331. doi: 10.1002/lt.26333. Epub 2021 Oct 25.
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7. Rossignol G, **Muller X**, Mohkam K, Dubois R, Mabrut JY. Letter to the editor: Is there a place for machine perfusion strategies in pediatric liver transplantation? *Hepatology.* 2022 May;75(5):1341-1342. doi: 10.1002/hep.32343. Epub 2022 Jan 28.
8. Rossignol G, **Muller X**, Mohkam K, Dubois R, Lesurtel M, Mabrut JY. Full left/full right liver graft ex situ split during hypothermic oxygenated perfusion. *Pediatr Transplant.* 2022 Aug;26(5):e14284. doi: 10.1111/petr.14284. Epub 2022 Apr 18.
9. Rossignol G, **Muller X**, Hervieu V, Collardeau-Frachon S, Breton A, Boulanger N, Lesurtel M, Dubois R, Mohkam K, Mabrut JY. Liver transplantation of partial grafts after ex situ splitting during hypothermic oxygenated perfusion-The HOPE-Split pilot study. *Liver Transpl.* 2022 Oct;28(10):1576-1587. doi: 10.1002/lt.26507. Epub 2022 Jun 16.
10. Mohkam K, Nasralla D, Mergental H, **Muller X**, Butler A, Jassem W, Imber C, Monbaliu D, Perera MTPR, Laing RW, García-Valdecasas JC, Paul A, Dondero F, Cauchy F, Savier E, Scatton O, Robin F, Sulpice L, Bucur P, Salamé E, Pittau G, Allard MA, Pradat P, Rossignol G, Mabrut JY, Ploeg RJ, Friend PJ, Mirza DF, Lesurtel M; Consortium for Organ Preservation in Europe (COPE). In situ normothermic regional perfusion versus ex situ normothermic machine perfusion in liver transplantation from donation after circulatory death. *Liver Transpl.* 2022 Nov;28(11):1716-1725. doi: 10.1002/lt.26522. Epub 2022 Jun 29.
11. Schlegel A, Mueller M, **Muller X**, Eden J, Panconesi R, von Felten S, Steigmiller K, Sousa Da Silva RX, de Rougemont O, Mabrut JY, Lesurtel M, Cerisuelo MC, Heaton ND, Allard MA, Adam R, Monbaliu D, Jochmans I, Haring MPD, Porte RJ, Parente A, Muiesan P, Kron P, Attia M, Kollmann D,

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12. Rossignol G, **Muller X**, Dubois R, Mabrut JY, Mohkam K. Letter to the Editor: Reduced whole liver grafts from pediatric donors as an alternative for small recipients. *Liver Transpl.* 2023 Jun 1;29(6):E11-E12. doi: 10.1097/LVT.000000000000076. Epub 2023 Jan 30.
 13. Rossignol G, **Muller X**, Dubois R, Rode A, Mabrut JY, Mohkam K. Optimizing graft-recipient size matching in adolescent liver transplantation: Don't forget ex situ right posterior sectionectomy. *Pediatr Transplant.* 2023 Jun;27(4):e14510. doi: 10.1111/ptr.14510. Epub 2023 Mar 15. PMID: 36919397
 14. Rossignol G, **Muller X**, Couillerot J, Lebosse F, Delignette MC, Mohkam K, Mabrut JY. From large-for-size to large-for-flow: A paradigm shift in liver transplantation. *Liver Transpl.* 2023 Apr 12. doi: 10.1097/LVT.000000000000150. Epub ahead of print. PMID: 37039739
 15. **Muller X**, Rossignol G, Couillerot J, Breton S, Hervieu V, Lesurtel M, Mohlam K, Mabrut JY. A Single Preservation Solution for Static Cold Storage and Hypothermic Oxygenated Perfusion of Marginal Liver Grafts: A Preclinical Study. *Transplantation* 2023, DOI: 10.1097/TP.0000000000004714, in press

Randomized controlled trials

- **2022:** Design and co-investigator for Hypothermic Oxygenated machine Perfusion during ex-vivo Splitting in liver transplantation: A multicenter randomized controlled trial HOPE Split
- **2019-2022:** Design and local scientific coordinator for Hypothermic Oxygenated Perfusion for Extended Criteria Donors in Liver Transplantation (HOPEExt, NCT03929523)

Prospective Trials

- **2021:** Principal Investigator for Liver Graft Viability Assessment During Normothermic Regional Perfusion: The MAASTR3BOLOMIC Study (NCT05361044)
- **2023:** Principal Investigator for Hypothermic oxygenated perfusion to optimize outcomes in high-risk donor-recipient matching for liver transplantation- The HOPE-MATCH Study

Grants and Awards

- Industry Grant IGL 2020-2023 : 450.000 euros
- Bourse de la Fondation de l'avenir 2021 : 45.000 euros
- Prix Antonin Poncet 2021 : 20.000 euros
- Prix Pouyet 2021 : 4.000 euros

Oral Presentations (Presenting Author)

- European Hepato-Pancreato-Biliary Surgery Association Congress, Lyon, France, 2023
- Swiss College of Surgeons Annual Meeting, Basel, Switzerland, 2023
- Congrès de la Société Francophone de Transplantation, Lyon, France, 2022
- Congrès de la Société Francophone de Transplantation, Genève, Switzerland, 2021
- The European Society of Organ Transplantation Congress, Milano, Italy, 2021
- European Hepato-Pancreato-Biliary Surgery Association Congress, Bilbao, Spain, 2021
- Congrès Francophone de Chirurgie Digestive et Hépatobilio Pancréatique, Paris, France, 2020

Invited Speaker

- Journée de l'Institut d'Hépatologie de Lyon, France, 2023
- Congrès de la Société Francophone de Transplantation, Lyon, France, 2022
- XVIVO Perfusion Workshop, Groningen, Netherlands 2022
- 1st International Workshop on Machine Perfusion, Turin, Italy 2022

- Journée Francophone de Chirurgie Hépatobiliaire Pédiatrique et Transplantation, Lyon, France, 2022
- Journée de l'Institut d'Hépatologie de Lyon, France, 2021

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11. Supplementary Material

- **Original manuscripts of published papers in peer reviewed journals during the thesis in chronological order (p. 90-198)**
- **MAASTR3BOLOMIC Study Protocol (p.199-211)**

Hypothermic Oxygenated Perfusion Versus Normothermic Regional Perfusion in Liver Transplantation From Controlled Donation After Circulatory Death

First International Comparative Study

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Objective: To compare HOPE and NRP in liver transplantation from cDCD.

Summary of Background Data: Liver transplantation after cDCD is associated with higher rates of graft loss. Dynamic preservation strategies such as NRP and HOPE may offer safer use of cDCD grafts.

Methods: Retrospective comparative cohort study assessing outcomes after cDCD liver transplantation in 1 Swiss (HOPE) and 6 French (NRP) centers. The primary endpoint was 1-year tumor-death censored graft and patient survival.

Results: A total of 132 and 93 liver grafts were transplanted after NRP and HOPE, respectively. NRP grafts were procured from younger donors (50 vs 61 years, $P < 0.001$), with shorter functional donor warm ischemia (22 vs 31 minutes, $P < 0.001$) and a lower overall predicted risk for graft loss (UK-DCD-risk score 6 vs 9 points, $P < 0.001$). One-year tumor-death censored graft and patient survival was 93% versus 86% ($P = 0.125$) and 95% versus 93% ($P = 0.482$) after NRP and HOPE, respectively. No differences in non-anastomotic biliary strictures, primary nonfunction and hepatic artery thrombosis were observed in the total cohort and in 32 vs. 32 propensity score-matched recipients.

Conclusion: NRP and HOPE in cDCD achieved similar post-transplant recipient and graft survival rates exceeding 85% and comparable to the

benchmark values observed in standard DBD liver transplantation. Grafts in the HOPE cohort were procured from older donors and had longer warm ischemia times, and consequently achieved higher utilization rates. Therefore, randomized controlled trials with intention-to-treat analysis are needed to further compare both preservation strategies, especially for high-risk donor-recipient combinations.

Keywords: DCD, graft preservation, liver transplantation, machine perfusion, organ donation

(*Ann Surg* 2020;272:751–758)

Liver transplantation (LT) from controlled donation after cardiac death (cDCD) is a promising strategy to rescue patients with end-stage liver disease and reduce waiting-list dropout.¹ During the cDCD process, however, periods of hypoperfusion and circulatory arrest in the donor expose the liver graft to deleterious warm ischemic injury which may impair its functionality after transplantation.^{2–3} Consequently, recipients of cDCD grafts present an increased risk of graft loss mainly related to higher rates of primary non function or biliary complications, notably non-anastomotic strictures, with the need of re-transplantation.^{4–6}

To reduce the risk associated with cDCD grafts while respecting local ethical and regulatory constraints of the donation process around the world, 2 main dynamic preservation strategies have been implemented in clinical practice, that is, normothermic regional perfusion (NRP) and hypothermic oxygenated perfusion (HOPE).⁷

NRP in the donor before liver graft procurement followed by static cold storage may reduce the incidence of both graft loss and ischemic-type biliary complications after cDCD LT.^{8–9} It can additionally be beneficial for several types of organs from the same donor. Another preservation strategy is to apply static cold storage followed by end-ischemic HOPE of liver grafts after procurement.¹⁰ HOPE has been shown to allow transplantation of very high-risk cDCD donor-recipient combinations.¹¹ Despite these promising results a direct comparison is lacking. Thus, this study aims at providing the first international multicentric large-scale comparison of outcomes after NRP and HOPE in cDCD LT.

METHODS

Study Design

This is a retrospective study comparing outcomes after cDCD LT in one NRP cohort from 6 high-volume French centers and one HOPE cohort from the University Hospital of Zurich, Switzerland.

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X.M., K.M., P.D., and M.L. designed the study, acquired the data, performed the statistical analysis, interpreted the data, and wrote the manuscript. P.A.C. and J.Y.M. interpreted the data, critically reviewed the data and drafted a final version of the manuscript. M.M. acquired, analyzed, interpreted and verified the center data and critically reviewed the manuscript. A.S., F.D., A.S., E.S., O.S., P.B., E.S., H.J., L.S., G.P., M.A. acquired, analyzed, interpreted, and verified the individual center data and approved the final manuscript.

The authors declare no conflict of interest.

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The study period covered 4 years (2015–2019) for the French cohort and 6 years (2012–2019) for the Zurich cohort. Outcomes were assessed until January 1, 2020.

The cDCD program in France is a national program and procurement of liver grafts in the setting of cDCD is exclusively performed with NRP. All French centers included in this study follow the same cDCD procedure standardized by the national organ donation agency.¹² The French authorities chose a step-up approach in the selection of cDCD donors, for example limiting donor age to <61 years until May 2017 followed by <66 years until June 2018 and currently <71 years. Detailed criteria were: planned cold ischemia time (CIT) ≤8 hours, donor age ≤71 years, functional donor warm ischemia (fDWI) time <45 minutes and no-flow <25 minutes, liver transaminases (AST or ALT) increase less than 4 times the upper normal value during NRP, liver graft biopsy with frozen section showing macrovesicular steatosis <20%, and at least 60 minutes of NRP.¹³

The cDCD program in Switzerland has been established in 2012. In contrast to the French program selection of grafts was exclusively performed during HOPE, based on data from animal models and recent advances in assessment of mitochondrial metabolism (Supplementary material, <http://links.lww.com/SLA/C422>).^{14–16}

In-situ NRP

NRP is an extra-corporeal membrane oxygenation based perfusion system applied to the donor before the start of organ procurement. After cardiac arrest of the donor, a “no-touch” period of 5 minutes was mandatory before brain death could be declared. Femoral artery cannulas were then introduced over pre-placed guidewires to reconstitute blood flow at physiological temperatures to the potential donor organs (postmortem vessel cannulation). Of note, on treatment withdrawal heparin (300 UI/kg) was administered to the donor to prevent blood clotting. The minimal required duration of NRP for the procurement to proceed was 60 minutes. Once NRP was terminated, organs were cold flushed and a standard procurement followed by static cold storage was performed (Supplementary material, <http://links.lww.com/SLA/C422>).

Ex-vivo HOPE

In the setting of HOPE, super rapid en bloc multiorgan retrieval was performed. After documentation of circulatory arrest, brain death diagnosis was confirmed after a no-touch period of 10 minutes (lowered to 5 minutes since 2018). Importantly, no heparin was administered to the donor at treatment withdrawal. After brain death diagnosis, the iliac artery was cannulated unilaterally after laparotomy and the organs were cold flushed with Institute-George-Lopez-1 solution. Static cold storage was used to transport the graft to the transplant center.

After back-table preparation, HOPE was performed using the Liver Assist device (Organ Assist). Ex-vivo liver perfusion was performed through the portal vein only, with active oxygenation and low pressure and flow (3 mm Hg, 150–300 mL/min). The University of Wisconsin Machine Perfusion solution (Belzer MPS) was used as perfusion solution for all HOPE procedures (Supplementary material, <http://links.lww.com/SLA/C422>). After 30 minutes of perfusion, liver graft quality was assessed by fluorescence spectroscopic analyses of the perfusate, as previously described.¹⁶

Endpoints and Definitions

The primary endpoint was 1-year tumor-death censored graft and patient survival.

Secondary endpoints included early graft function indicators (International normalized ratio, factor V), level of liver transaminases (ALT, AST) and the composite score for early allograft dysfunction (EAD) by Olthoff et al.¹⁷

Functional donor warm ischemia (fDWI) time was defined as duration from mean arterial blood pressure below 50 mm Hg to cold aortic perfusion in the HOPE cohort and mean arterial blood pressure below 45 mm Hg to initiation of the perfusion in the NRP cohort. Asystolic warm ischemia started at the occurrence of cardiac arrest.

We defined 3 main phases of liver graft preservation: (1) duration of static cold storage which is common to HOPE and NRP, (2) duration of HOPE or NRP, and (3) total ex-vivo preservation which is defined as the time from aortic cross-clamp in the donor to graft reperfusion in the recipient.

Liver graft utilization rate was defined as the proportion of liver grafts which were transplanted after initiation of therapeutic withdrawal in donors eligible for liver graft donation.

Non-anastomotic biliary stricture (NAS) was defined as either multifocal, unifocal intrahepatic, or hilar strictures with or without the presence of concomitant hepatic artery thrombosis (HAT) or arterial complications. NAS was detected clinically and confirmed by magnetic resonance cholangiography.

Statistical Analysis

Categorical variables are expressed in quantities and percentages and continuous variables are expressed as median with interquartile range. Continuous variables were compared using the Mann-Whitney *U* test. Categorical variables were compared using the Chi-square test or the Fisher exact test. *P*-values < 0.05 were considered statistically significant. Survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests.

To adjust for covariate imbalances between the 2 groups, we performed a propensity score matching. The propensity score was calculated using a non-parsimonious binary logistic regression model, with treatment allocation as endpoint (NRP vs HOPE) and the 6 UK DCD Score variables (donor age, donor body mass index, recipient age, fDWI time, and CIT) as covariates.⁵ (Details in Supplementary Material, <http://links.lww.com/SLA/C422>). Approval by the French institutional local and national ethics committee and approval by the local Swiss ethics committee were obtained (KEK 2019-0100).

RESULTS

During the study period, 132 and 93 cDCD liver grafts were successfully procured and transplanted after NRP and HOPE, respectively. Due to more stringent donor selection criteria in the NRP cohort, the liver graft utilization rate was significantly lower compared to the HOPE cohort (63% vs 81%, *P* < 0.001, Fig. 1). Median post-LT follow-up was 20 [interquartile range (IQR) 9–25] and 28 (IQR 15–248) months for NRP and HOPE, respectively.

HOPE livers were procured from older donors (61 vs 50 years, *P* < 0.0001) with longer fDWI times (31 vs 22 minutes, *P* < 0.001) resulting in a higher donor risk index (DRI) (2.47 vs 2.01, *P* < 0.001) (Table 1). In the NRP cohort, procurement started after median 184 minutes of NRP in the donor, followed by static cold storage for 5.7 (IQR 4.7–6.6) hours before implantation. HOPE liver grafts first underwent 4 (IQR 3.1–5) hours of cold storage followed by a median 132 minutes HOPE perfusion and subsequent transplantation. Median total ex-vivo preservation was 5.7 and 6.4 hours after NRP and HOPE, respectively (*P* < 0.001).

Overall, recipients had low labMELD scores with 55% and 45% presenting hepatocellular carcinoma in the NRP and HOPE group, respectively (*P* = 0.96). Overall, donor-recipient combination presented a higher predicted risk of graft loss in the HOPE cohort (UK-DCD-risk score 6 vs 9 points, *P* < 0.001) (Table 2).

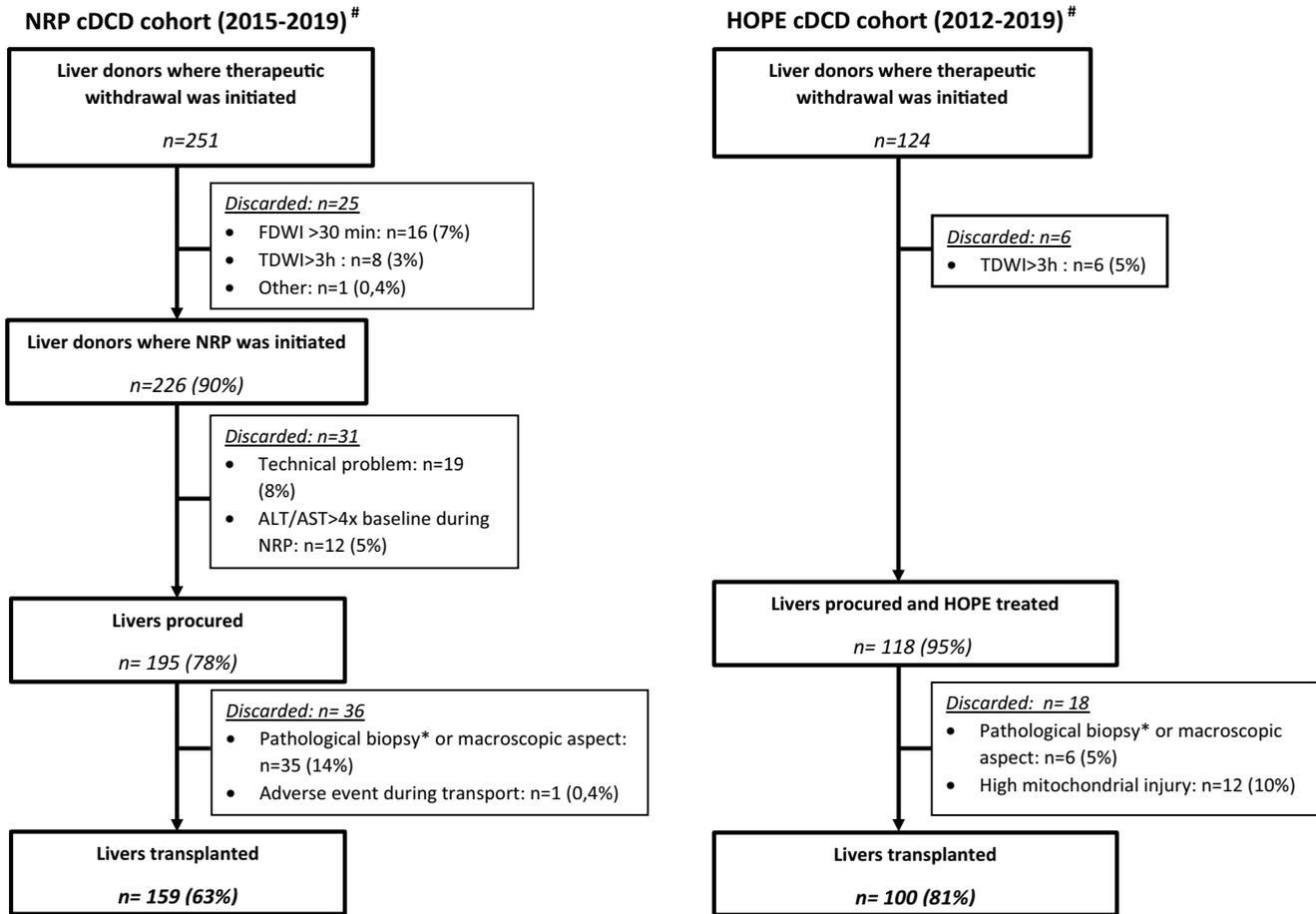


FIGURE 1. Liver graft utilization rates and reasons for graft discard. [#]To give a complete overview we included all the donors and liver grafts until January 1, 2020 in this figure. The total number is consequently greater than the actual study population for which the follow-up ended on the 01.01.2020. *In NRP pathological biopsy was defined as cirrhosis, fibrosis >F1, macrosteatosis >20%. In HOPE pathological biopsy was defined as cirrhosis, fibrosis >F1, macrosteatosis >60%. FDWI indicates functional donor warm ischemia; HOPE, hypothermic oxygenated perfusion; NRP, normothermic regional perfusion; TDWI, total donor warm ischemia.

Early Graft Function and Injury

Serum ALT and AST release peaked during the first 24 hours after transplantation with higher median peak values after HOPE (ALT 1197 vs 594; AST 1302 vs 489, $P < 0.001$; Supplementary Material Fig. S1, <http://links.lww.com/SLA/C422>). This translated into 68% of EAD in the HOPE cohort compared to 20% in the NRP cohort ($P < 0.001$). Synthetic liver function expressed by factor V and INR improved rapidly over the first 48 hours post-LT for both cohorts reaching a plateau after postoperative day 5. ALT/AST levels decreased to similar levels in both groups (Fig. 2).

Graft and Patient Survival

One-year tumor-death censored graft and patient survival was 93% versus 86% and 95% versus 93% after NRP and HOPE, respectively ($P = 0.12$; $P = 0.48$) (Fig. 3). No significant differences were observed for NAS (4.5% vs 8.6%, $P = 0.22$), primary non function (2.3% vs 4.3% $P = 0.39$) and HAT (3% vs 2.2%, $P = 0.69$) between NRP and HOPE, respectively (Table 3).

Risk Adjusted Donor-recipient Combinations

After propensity-score matching of donor and recipient risk factors for graft loss, a subgroup analysis was performed with 32

cases in each group. After matching, both cohorts presented with comparable fDWI time (27 minutes), donor age (58 vs 59 years) and cold ischemia time (HOPE 285 minutes vs NRP 309 minutes) resulting in a median UK-DCD risk score of 8 in both cohorts (IQR HOPE 6–11; IQR NRP 5–9) (Supplementary Material Fig. S2, <http://links.lww.com/SLA/C422>). Transaminase release remained higher in the HOPE cohort but 1-year tumor-death censored graft survival was still comparable (93% vs 87.3% for NRP and HOPE, $P = 1$). Importantly, rates of NAS, HAT, and primary non-function (PNF) remained similar in both cohorts. Serum creatinine levels at post-LT day 7 were higher after HOPE (111 vs 68 $\mu\text{mol/L}$, $P = 0.01$) but renal replacement rates were not significantly different (Table 3).

DISCUSSION

This is the first large-scale international multicentric study comparing 2 different preservation strategies, NRP and HOPE, in cDCD LT. Both, NRP and HOPE disclosed 1-year tumor-death censored graft survival rates >85% with similar rates of NAS, HAT, and PNF, comparable to the benchmark in DBD LT.¹⁸ However, the graft utilization rate was significantly higher in the HOPE group, despite longer donor warm ischemic times and higher donor age, compared to liver grafts transplanted after NRP. After propensity

TABLE 1. Donor, Graft, and Preservation Characteristics

	NRP n = 132	HOPE n = 93	P-value
Donor age, yr	50 (39–59)	61 (52–71)	<0.001
Donor BMI, kg/m ²	24 (22–27)	26.1 (24–27.8)	<0.001
Cause of death, n (%)			
Cerebrovascular accident	42 (31.8)	28 (30.1)	0.785
Hypoxic brain injury	57 (43.2)	48 (51.6)	0.212
Trauma	33 (25)	17 (18.2)	0.232
Donor ICU stay, days	9 (6–15.5)	4 (3–7)	<0.001
Donor AST (IU)	51 (31–79.5)	68 (40–111)	0.001
Donor ALT (IU)	42 (26.5–90.5)	61 (29–94)	0.276
Donor GGT (IU)	88 (41–191)	62 (27–155)	0.037
Total donor warm Ischemia, min	31 (26.5–36)	35 (30–39)	<0.001
Functional donor Warm ischemia, min	22 (19–26)	31 (26–35)	<0.001
Asystolic donor Warm ischemia, min	17 (15–20)	19 (17–21)	0.017
Procurement team, n (%)			
Local	81 (61.4)	65 (69.9)	0.187
Regional	51 (35.1)	28 (30.1)	0.187
NRP duration, min	184 (159–207)	—	—
Static cold storage, h	5.7 (4.7–6.6)	4 (3.1–5)	<0.001
HOPE duration, min	—	132 (105–165)	—
Total ex-vivo preservation, h	5.7 (4.7–6.6)	6.4 (5.6–7.4)	<0.001
Cold storage solution, n (%)			
IGL-1	96 (72.7)	93 (100)	<0.001
Scot 15	34 (25.7)	0 (0)	<0.001
Custodiol	2 (1.5)	0 (0)	<0.001

Categorical variables are expressed in quantities and percentages and continuous variables are expressed as median with interquartile range.

BMI indicates body mass index; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; IGL-1, Institute-George-Lopez-1; NRP, normothermic regional perfusion.

TABLE 2. Recipient Characteristics

	NRP n = 132	HOPE n = 93	P-value
Recipient age, yr	59.5 (54.5–63)	59 (54–63.6)	0.424
Recipient BMI, kg/m ²	27.1 (24–30)	27.5 (24.4–30)	0.907
Previous major surgery, n (%)	26 (19.7)	18 (19.4)	0.949
Indication for transplantaion, n (%) *			
Hepatitis B	12 (9.1)	8 (8.6)	0.899
Hepatitis C	29 (22)	31 (33.3)	0.580
Hepatitis C + B	4 (3)	2 (2.2)	0.687
Non-alcoholic-Steatohepatitis	16 (12.1)	11 (11.8)	0.947
Alcohol related liver disease	53 (40.2)	26 (28)	0.059
NASH + alcohol	19 (14.4)	5 (5.4)	0.031
Primary sclerosing cholangitis	0 (0)	1 (1.1)	0.232
Autoimmune Hepatitis	3 (2.3)	1 (1.1)	0.503
Hemochromatosis	7 (5.3)	0 (0)	0.024
Retransplantation	0 (0)	3 (3.2)	0.038
Other	1 (0.8)	0 (0)	0.403
Hepatocellular carcinoma, n (%)	81 (54.7)	67 (45.3)	0.960
Recipient pre-LT status, n (%)			
Home	125 (94.7)	83 (89.2)	0.128
In-hospital	6 (4.5)	9 (9.7)	0.129
Intubated	1 (0.8)	1 (1.1)	0.803
Pre-LT renal replacement therapy, n (%)	3 (2.6)	6 (6.5)	0.171
LabMELD score, points	12 (8–16)	12 (9–16)	0.833
DRI, points	2.01 (1.75–2.31)	2.47 (2.08–2.80)	<0.001
UK DCD-RISK score, points	6 (3–8)	9 (7–11)	<0.001
Low risk, n (%)	64 (48.5)	6 (6.5)	<0.001
High-risk, n (%)	56 (42.4)	45 (48.5)	0.376
Futile, n (%)	12 (9.1)	42 (45.2)	<0.001

Categorical variables are expressed in quantities and percentages and continuous variables are expressed as median with interquartile range.

*One patient may present a combination of indications.

BMI indicates body mass index; DRI, donor risk index; LT, liver transplantation.

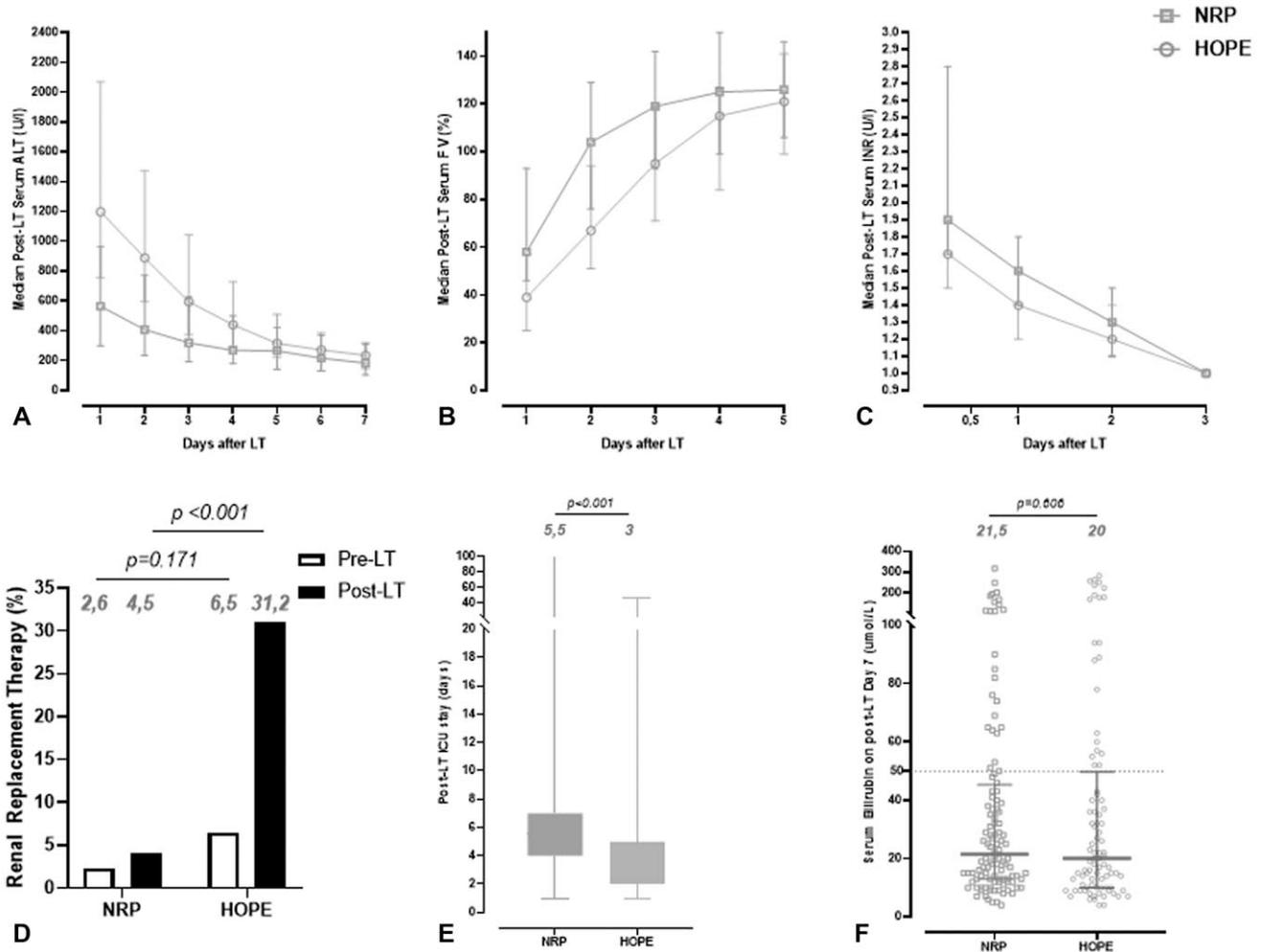


FIGURE 2. Early graft function and injury markers. A, Post-LT serum ALT release over the first 7 d. Early graft function expressed by Factor V (B) and INR (C). D, Pre- and Post-LT renal replacement therapy rates. E, ICU stay in days. F, Serum bilirubin on post-LT day 7. ICU indicates intensive care unit; LT, liver transplantation.

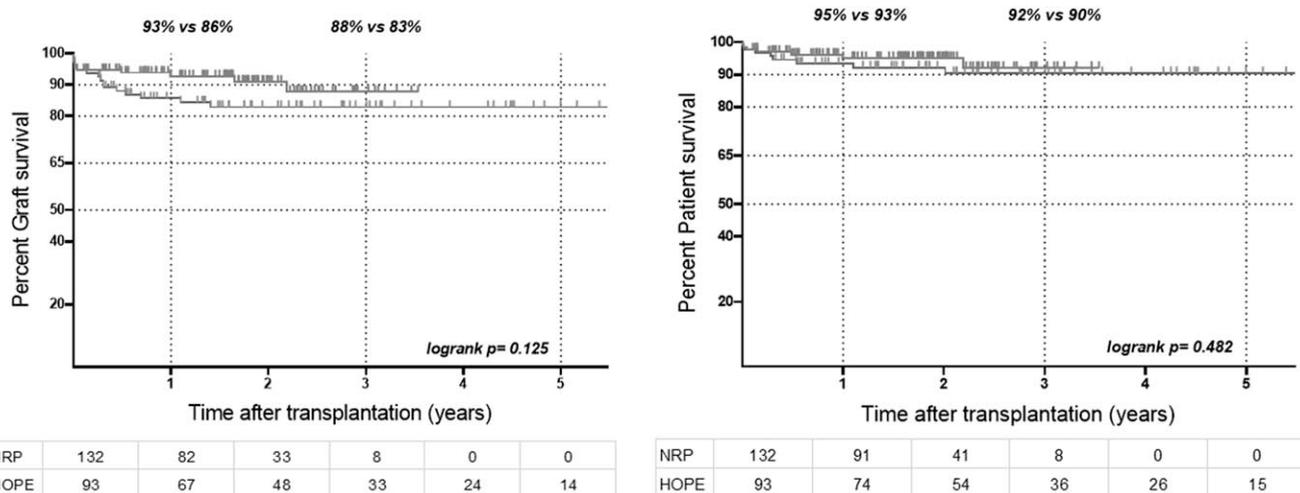


FIGURE 3. Tumor death censored graft and patient survival.

TABLE 3. Post-Transplant Outcomes in the Total and Propensity Score Matched Cohorts

	NRP n = 132	HOPE n = 93	<i>P</i> value	PSM NRP^X n = 32	PSM HOPE^X n = 32	<i>P</i> value
ICU stay, days	5.5 (4–7)	3 (2–5)	<0.001	6 (4–10)	3 (2–6)	0.010
Hospital stay, days	17 (13–24)	17 (13–23)	0.510	18 (13–32)	15 (10–25)	0.513
Serum Creatinine day 7	68 (58–90)	127 (76–233)	<0.001	68 (57–101)	111 (69–214)	0.012
Renal Replacement Therapy, n (%)	6 (4.5)	29 (31.2)	<0.001	4 (12.5)	11 (34.4)	0.092
Biliary Complication, n (%)	23 (17.4)	32 (34.4)	0.004	8 (25)	11 (34.4)	0.508
Anastomotic Strictures, n (%)	14 (10.6)	24 (25.8)	0.003	4 (12.5)	7 (21.9)	0.453
Non-Anastomotic Strictures, n (%)	6 (4.5)	8 (8.6)	0.215	2 (6.3)	4 (12.5)	0.688
Biliary Leak, n (%)	9 (6.8)	6 (6.5)	0.914	4 (12.5)	2 (6.3)	0.687
Overall Arterial Complications, n (%)	13 (9.8)	7 (7.5)	0.547	3 (9.4)	2 (6.3)	1
Thrombosis, n (%)	4 (3.0)	2 (2.2)	0.687	1 (3.1)	0 (0)	1
Stenosis, n (%)	8 (6.1)	5 (5.4)	0.829	2 (6.3)	2 (6.3)	1
Primary-Non-Function (PNF), n (%)	3 (2.3)	4 (4.3)	0.388	1 (3.1)	3 (9.4)	0.500
Overall Graft Loss, n (%)	18 (13.6)	24 (25.8)	0.021	5 (15.6)	8 (25)	0.727
Tumor-Death Censored Graft Loss, n (%)	10 (7.5)	14 (15.1)	0.075	4 (12.5)	5 (16.5)	1
<i>Cause of Graft Loss, n (%)</i>						
PNF	3 (3.8)	4 (4.3)		1 (3.1)	3 (9.4)	
HAT	0 (0)	2 (2)		0 (0)	0 (0)	
NAS	1 (0.8)	2 (2)		1 (3.1)	0 (0)	
Septic Shock	1 (0.8)	2 (2)		1 (3.1)	0 (0)	
Acute/Chronic Rejection	1 (0.8)	1 (1.1)		0 (0)	0 (0)	
Arterial Stenosis	0 (0)	1 (1.1)		0 (0)	1 (3.1)	
Tumor Recurrence	6 (4.5)	3 (3.2)		1 (3.1)	2 (6.3)	
Secondary Tumor	1 (0.8)	5 (5.5)		0 (0)	1 (3.1)	
Invasive Aspergillosis	0 (0)	1 (1.1)		0 (0)	0 (0)	
Budd Chiari	1 (0.8)	1 (1.1)		0 (0)	1 (3.1)	
Per-operative death	1 (0.8)	0 (0)		0 (0)	0 (0)	
Gas embolism	1 (0.8)	0 (0)		0 (0)	0 (0)	
Portal thrombosis	1 (0.8)	0 (0)		1 (0.8)	0 (0.0)	
Unknown	1 (0.8)	2 (2)		0 (0)	0 (0)	

Categorical variables are expressed in quantities and percentages and continuous variables are expressed as median with interquartile range.

X: Propensity score matched cohorts.

HAT indicates hepatic artery thrombosis; ICU, intensive care unit; NAS, non-anastomotic biliary stricture; PNF, primary non-function.

score adjustment of donor-recipient combinations (median UK-DCD score 8), both strategies achieved similar post-LT outcomes.

Although static cold storage is still the gold standard in LT of low-risk DBD grafts, extended criteria grafts including cDCD may benefit from dynamic preservation strategies.^{18–21} However, no direct comparison of such preservation strategies is currently available, making consensual conclusions impossible. Accordingly, this study aimed at providing a first comparison of NRP and HOPE in the setting of cDCD LT.

This is the largest series to date of NRP in cDCD LT. In 7 recently published studies including 8–95 patients, fDWI ranged from 10 to 28 minutes and donor age was <60 years (Supplementary Material, Fig. S5, <http://links.lww.com/SLA/C422>).^{8,20,22–26} Outcomes in terms of graft and patient survival, PNF and NAS reported in this study are similar to the 95 NRP cases published by Hessheimer et al.⁸ Of note, the Spanish series had a majority of pre-mortem cannulation with consequently shorter warm ischemic times.

In the direct comparison between HOPE and NRP, we observed a higher rate of EAD due to higher post-LT AST/ALT levels and a higher proportion of post-LT renal replacement therapy in the HOPE cohort. These differences did however not significantly impair early graft function nor reduce 1-year graft- and patient survival. The discrepancy between rates of EAD and post-LT liver function measured by INR and Factor V observed in the HOPE cohort warrants to review the current definition of EAD in the context of machine perfused cDCD grafts.^{19,27} The higher post-LT renal

replacement rate in the HOPE cohort may be partly explained by a 2× higher pre-LT renal replacement rate and the higher post-LT ALT/AST levels due to longer warm ischemia times.^{28,29} Of note, after adjusting for donor-recipient risk combinations in the propensity score analysis, rates of renal replacement therapy were not significantly different between the 2 groups.

The ethical and regulatory issues surrounding the therapy withdrawal in the donor lead to specific modalities of organ procurement in the setting of cDCD. In Switzerland for example, placing catheters or administering heparinization before death of the donor is prohibited. This led to differences in the application of HOPE or NRP in the context of cDCD.

First, HOPE is an intervention on the liver graft after transport to the transplant center with standard static cold storage (end-ischemic ex-vivo perfusion). In contrast, NRP is an intervention in the donor, performed by the procurement team, with the need of static cold storage for transport to the transplant center.

Second, NRP requires preliminary vessel cannulation in the donor which is an additional technical challenge to the cDCD process.³⁰ Indeed, during the study period, 8% of cDCD procurements were aborted as a result of a technical problem with the NRP set-up. Of note, this observation should be interpreted in the context of an ongoing learning curve with NRP in some of the French centers, given the recent implementation of the procedure (2015). In contrast, no technical adverse event occurred during the 93 HOPE procedures. Although technical problems potentially occur with every perfusion technique, HOPE has the advantage to keep liver grafts at 8–10°C. In

contrast to normothermic temperatures, this significantly reduces the risk of organ damage if perfusion problems occur.²

Third, the duration of ex-vivo liver graft preservation, from aortic cross clamp in the donor to graft reperfusion in the recipient, was longer in the HOPE cohort without significantly increasing the duration of static cold storage. Because static cold storage negatively impacts post-LT outcomes, the possibility of end-ischemic ex-vivo perfusion may provide an advantage for HOPE in the setting of marginal cDCD grafts, re-allocation or difficult recipient hepatectomy by reducing cold ischemia of the liver graft.³¹

Another notable difference is the assessment of liver graft quality during preservation to safely increase utilization rates without compromising post-LT outcomes.³² In the setting of NRP, some authors have suggested to use ALT/AST release and lactate clearance during perfusion as an indicator for graft quality.²⁰ Indeed, in this study, 5% of the cDCD liver grafts were discarded because AST/ALT levels rose to >4× baseline during NRP. Although transaminase release is a hepatocyte injury marker, it does not reflect bile duct viability or liver function and there's is no robust data from pre-clinical or human studies correlating AST/ALT to graft loss.^{20,33–34} Finally, a potential advantage of NRP for organ assessment may be the ability to perfuse several cDCD organs at once including for example kidney, pancreas, and even lungs.²² HOPE allows for a liver targeted metabolic assessment after the first 30 minutes of perfusion, based on a real-time measurement of perfusate levels of flavin mononucleotide (FMN), a fragment of complex I of the mitochondrial respiratory chain. FMN correlates with early graft function after transplantation in both DBD and cDCD.¹⁶ In the HOPE cohort reported here, real-time FMN detection led to a cDCD graft utilization rate of 81%, in contrast to 63% by NRP, with tumor-censored graft survival comparable to DBD LT.

This study has several limitations. Data was gathered retrospectively from centers which use different cold storage solutions, transplantation techniques for example piggy-back versus classic technique, immunosuppression protocols and treatment modalities of biliary and arterial complications. In addition, donor selection was very different in both groups and may lead to biases and the study did not aim to assess oncological outcomes in cDCD recipients with HCC. Finally, cost assessment could not be performed due to the multicentric design and important health-care differences between the 2 countries.

In conclusion, NRP and HOPE in cDCD LT achieved similar tumor-death censored graft and patient survival rates. Yet, fewer grafts in the HOPE cohort were discarded, despite being procured from older donors with longer warm ischemia times. Thus, in contrast to some recent statements on the supremacy of NRP in cDCD, the presented data advocates the need for a well-designed randomized controlled trial, with an intention to treat analysis of outcomes after HOPE and NRP, specifically in high risk recipient-graft combinations.^{35,36}

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Strategies for liver transplantation during the SARS-CoV-2 outbreak: Preliminary experience from a single center in France

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Liver transplantation (LT) during the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is challenging given the urgent need to reallocate resources to other areas of patient care. Available guidelines recommend reorganizing transplant care, but data on clinical experience in the context of SARS-CoV-2 pandemic are scarce. Thus, we report strategies and preliminary results in LT during the peak of the SARS-CoV-2 pandemic from a single center in France. Our strategy to reorganize the transplant program included 4 main steps: optimization of available resources, especially intensive care unit capacity; multidisciplinary risk stratification of LT candidates on the waiting list; implementation of a systematic SARS-CoV-2 screening strategy prior to transplantation; and definition of optimal recipient-donor matching. After implementation of these 4 steps, we performed 10 successful LTs during the peak of the pandemic with a short median intensive care unit stay (2.5 days), benchmark posttransplant morbidity, and no occurrence of SARS-CoV-2 infection during follow-up. From this preliminary experience we conclude that efforts in resource planning, optimal recipient selection, and organ allocation strategy are key to maintain a safe LT activity. Transplant centers should be ready to readapt their practices as the pandemic evolves.

KEYWORDS

cancer/malignancy/neoplasia, clinical decision-making, clinical research/practice, diagnostic techniques and imaging, infection and infectious agents – viral, liver transplantation/hepatology, organ procurement and allocation, risk assessment/risk stratification

Abbreviations: ACLF, acute-on-chronic liver failure; ALF, acute liver failure; BAR, balance of risk; DBD, donation after brain death; DRI, donor risk index; ELD, end-stage liver disease; HCC, hepatocellular carcinoma; ICU, intensive care unit; IQR, interquartile range; LT, liver transplantation; RT-PCR, reverse transcriptase polymerase chain reaction; SARS-Cov-2, severe acute respiratory syndrome coronavirus 2.

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Mickaël Lesurtel and Jean-Yves Mabrut share senior authorship.

1 | INTRODUCTION

Data on outcomes after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in liver transplantation (LT) recipients are scarce and the potential impact on LT activity remains uncertain. While reports on SARS-CoV-2 in long-term solid organ recipients including LT report a case-fatality rate as high as 27.8%, others suggest that LT recipients may be protected by immunosuppression-related mitigation of cytokine release.^{1,2} Besides unanswered questions on SARS-CoV-2 infections in transplant recipients, LT is particularly challenging during this pandemic, given the urgent need to reallocate healthcare resources, such as ventilators, intensive care unit (ICU) beds, and staff to treat SARS-CoV-2-infected patients.³ However, decreased LT activity has to be balanced against the potential negative impact for patients with end-stage liver disease or hepatocellular carcinoma.^{4,5} To face these challenges, guidelines suggest a phased reduction in LT activity based on available resources, ranging from performing only super-urgent transplantation to maintaining an active deceased donation-based program.^{6,7}

During the peak of the pandemic in France, a total of 7130 patients with SARS-CoV-2 infection required ICU treatment with a baseline availability of 5432 beds.⁸ In this context, we present the experience and preliminary outcomes from a LT program in one of the most exposed regions in France.⁸

2 | METHODS

This is a retrospective analysis of all consecutive adult patients undergoing LT at Croix Rousse University Hospital in Lyon, France during the first month after the beginning of the national SARS-CoV-2 Lock Down on March 16, 2019.

In accordance with the French National Organ Donor Agency's (Agence Nationale de Biomédecine) and the French Transplantation Society's recommendations to reduce LT activity, we reorganized our LT program based on available resources.^{7,9} This strategy included 4 major steps: (1) resource planning, (2) multidisciplinary risk stratification of LT candidates on the waiting list, (3) implementation of a systematic pre-LT SARS-CoV-2 screening strategy, and (4) definition of optimal recipient-donor matching to achieve benchmark outcomes.

2.1 | Resource planning

Our university hospital is one of the tertiary reference centers for SARS-CoV-2 infections, which forced us to substantially reorganize our LT program. Three separate surgical units were set up: 1 for all elective surgery in SARS-CoV-2-negative patients, 1 for SARS-CoV-2-positive patients, and a third SARS-CoV-2-negative unit for LT recipients. Of note, the transplant unit had only single rooms to avoid patient contact and post-LT visits by relatives were temporarily

suspended. Specific intrahospital SARS-CoV-2-free pathways especially for ultrasound and computed tomography (CT) scans were established.

The ICU capacity was a key consideration. Since this is the referral tertiary center for SARS-CoV-2 disease, the overall number of ICU beds in the center was increased by 67% during the early phase of the outbreak. The majority of the ICU beds were dedicated to SARS-CoV-2-infected patients and 17% of the ICU beds were dedicated to patients without SARS-CoV-2 infection including surgical patients and LT recipients. The ICU capacity available for LT including available beds, ventilators, and renal replacement therapy (RRT) were continuously reassessed during weekly multidisciplinary meetings.

Another important point was the implementation of strategies to mitigate in-hospital transmission of SARS-CoV-2 from healthcare personnel to LT recipients. Surgical face masks and scrubs were mandatory for all staff members upon entering the hospital compound and were worn during all clinical activity such as surgical rounds. All staff members were systematically screened with reverse transcriptase polymerase chain reaction (RT-PCR) if they presented symptoms compatible with SARS-CoV-2 and were put in quarantine until the RT-PCR results were available. Staff members with positive RT-PCR were quarantined for 2 weeks.

2.2 | Recipient risk stratification

Every week, a multidisciplinary team reviewed every LT candidate on the waiting list. We selected LT candidates with a MELD score >25 including acute liver failure (ALF) and/or with end-stage liver disease (ELD) with poor prognosis including refractory ascites, hepatopulmonary syndrome, or hepatocellular carcinoma (HCC). Except for ALF, we excluded LT candidates with expected high post-LT morbidity, long ICU stay, and continuous RRT requirements (eg retransplantations and multiorgan transplantations). Additionally, access to LT for patients admitted to the ICU with acute-on-chronic liver failure (ACLF) was discussed using risk stratification by the chronic liver failure consortium (CLIF-C) ACLF classification.¹⁰

2.3 | Recipient and donor SARS-CoV-2 screening

A systematic SARS-CoV-2 screening strategy was implemented for all recipients including (1) a questionnaire on prehospitalization symptoms and a clinical examination at hospital admission, (2) a nasopharyngeal swab for SARS-CoV-2 by RT-PCR IP2/4, and (3) a chest CT scan prior to LT. Chest CT images were interpreted according to the guidelines from the European Society of Radiology and the European Society of Thoracic Imaging. Of note, RT-PCR and chest CT scan were granted specific priority: results for RT-PCR were available within 4-6 hours after testing and LT recipients were prioritized for chest CT scan slots. LT was only performed if all 3 screening tests were negative. Following national recommendations, all donors

were screened by both nasopharyngeal swab and chest CT scan and donation only proceeded if all screening tests were negative.⁷

Post-LT SARS-CoV-2 screening was based on symptoms and no routine screening by chest CT scan or RT-PCR was implemented.

2.4 | Recipient-donor matching

The donation after brain death (DBD) program was maintained, while the donation after circulatory death and living donor program were stopped to preserve resources. To optimize available ICU resources, the organ allocation policy was based on ideal donor-recipient matching with low expected post-LT morbidity according to published LT outcome benchmarks, donor risk index (DRI), D-MELD, and balance of risk (BAR) score.¹¹⁻¹⁴ The DRI is a quantitative score including 7 donor characteristics predictive of post-LT graft failure.¹² Estimated post-LT 1-year graft survival decreases with an increasing DRI score. The D-MELD is the product of donor age and preoperative Model for End-Stage Liver Disease (MELD) score of the recipient.¹³ A score beyond the cutoff of 1600 score points is predictive of a longer post-LT length of hospital stay and poorer recipient survival.¹³ The BAR score combines 6 independent donor and recipient

characteristics associated with post-LT survival.¹⁴ The score balances 1 risk factor by optimal matching of the others, for example, high MELD with short cold ischemia and low donor age.

Standard post-LT immunosuppressive treatment included induction with basiliximab (20 mg after graft reperfusion and on post-LT day 4), corticosteroids during 7 days (perioperative bolus and withdrawal on post-LT day 7), mycophenolate mofetil and tacrolimus introduction on post-LT day 3. Target tacrolimus serum levels were 8-10 ng/mL during the first month post-LT.

3 | RESULTS

Our transplant center was situated in a high SARS-CoV-2 incidence zone, with 10-20 SARS-CoV-2-infected patients hospitalized per 100 000 inhabitants (Figure 1). Compared to the monthly average over the past 5 years, LT activity during the 30-day study period decreased by 29% in France (77 LT vs 108 LT), while LT activity increased by 42% at our center (10 LT vs 7 LT).⁸

In total, 39% (13 out of 33) of LT candidates on the waiting list were temporarily put on hold. These patients were either planned for a multiorgan transplant, had HCC controlled by bridging therapy,

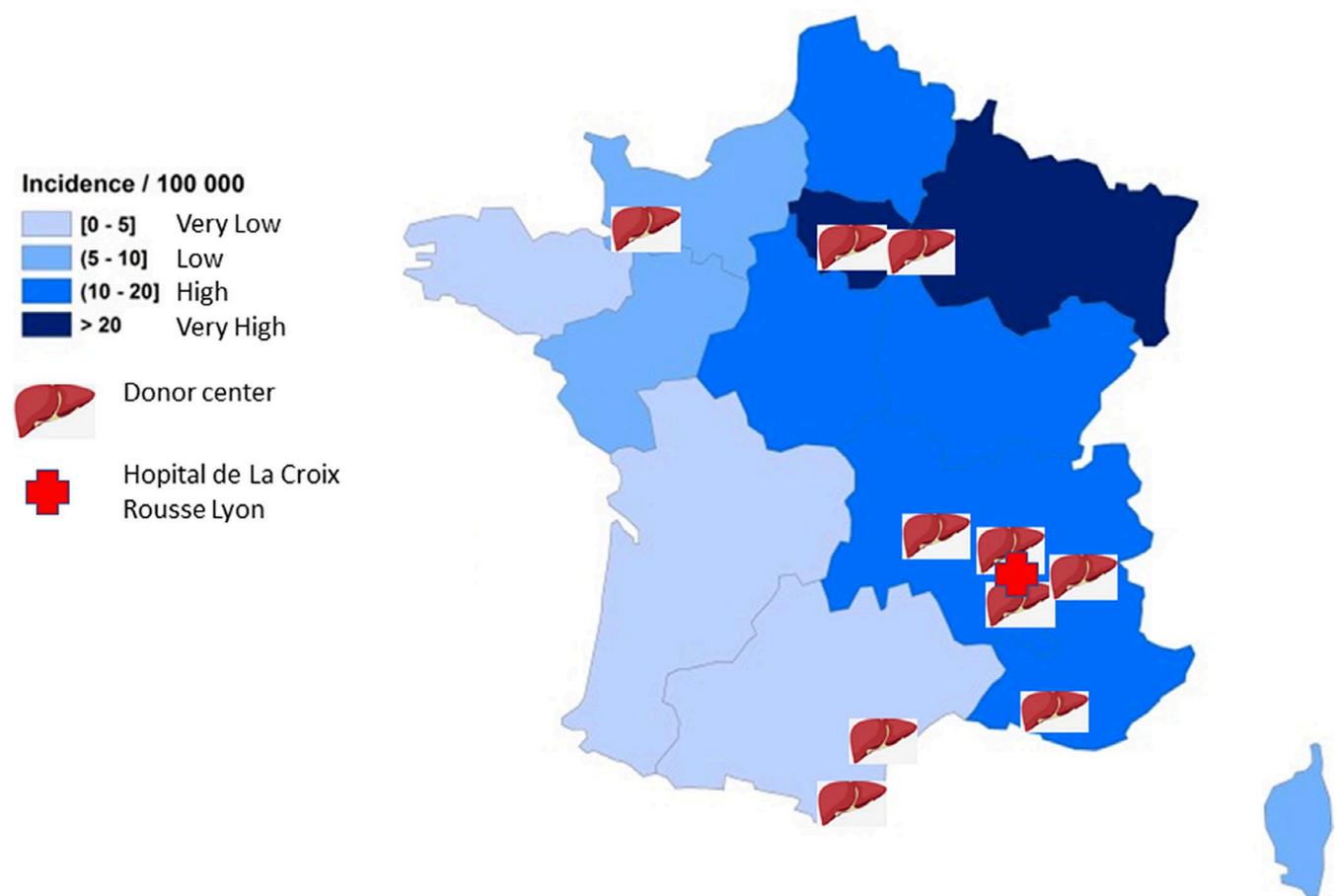


FIGURE 1 Geographical distribution of severe acute respiratory syndrome coronavirus 2 incidence based on number of hospitalizations per 100 000 inhabitants in France during the study period.⁸ The 10 donor centers included in the study as well as our transplant center are indicated on the map. (Figure adapted from reference 8) [Color figure can be viewed at wileyonlinelibrary.com]

or had severe cardiovascular or respiratory comorbidities. The median MELD on the waiting list was 14 (interquartile range [IQR] 10–20) and the median CLIF-C acute-on-chronic liver failure (ACLF) score was 7 (IQR 6–8).

A total of 10 successful DBD LT in adult recipients were performed during the study period. Recipients had a median age of 51 years (IQR 38–60 years) with a median MELD score of 19 (IQR 12–28). The majority had compensated ELD (70%) and were admitted from home. Three recipients were inpatients with a MELD score >25 points: 1 had ACLF grade 1 and 2 had ACLF grade 2. Overall, HCC was present in 40% of the recipients. All recipients were screened by RT-PCR prior to LT and 7 underwent additional chest CT scan. No selected recipient was diagnosed with SARS-CoV-2 infection during the pre-LT screening and all recipients underwent LT.

Overall, 7/10 (70%) liver donors were from centers in a region with a high or a very high SARS-CoV-2 incidence (>10 SARS-CoV-2-infected patients hospitalized per 100 000 inhabitants) (Figure 1). Liver donors had a median age of 33 years (IQR 25–59 years) with short cold ischemia times (median 7 hours, IQR 6–9 hours) resulting in a low median DRI of 1.37 (IQR 1.1–1.8). After recipient-donor matching, the median BAR score was 8 (IQR 2–11) (Table 1).

Median post-LT follow-up was 39 (IQR 35–45) days. Perioperative transfusion rates and posttransplant morbidity were within published benchmarks (Table 2) with a short median ICU stay of 2.5 (IQR 2–6) days. The median total hospital stay was 14 (IQR 13–21) days. The standard immunosuppressive protocol was followed for all patients. One liver graft recipient underwent liver biopsy for abnormal liver tests on post-LT day 30 and was diagnosed with an acute rejection classified BANFF 6, which was successfully treated by corticosteroids bolus.

During the 39 days of median post-LT follow-up, no case of SARS-CoV-2 was diagnosed in the 10 LT recipients.

4 | DISCUSSION

We report a single-center experience with LT during the peak of the SARS-CoV-2 outbreak in France. A careful assessment of available resources allowed the center to maintain an active LT program and to perform 10 successful DBD LT. The cornerstones of the implemented strategy were (1) flexible planning of ICU capacity including beds, equipment, and staff; (2) weekly multidisciplinary risk stratification of LT candidates; (3) systematic screening; and (4) optimal donor-recipient matching to reduce post-LT morbidity and ICU requirement. Results of this strategy show a low post-LT morbidity with short ICU stays. No SARS-CoV-2 infection during the post-LT follow-up was observed (Table 3).

The first question at the beginning of the SARS-CoV-2 pandemic was: Should LT activity be maintained? On the one hand, LT during the pandemic may have surpassed available capacities in ventilators, RRT, and ICU staff and thus jeopardized treatment options for SARS-CoV-2-infected patients.^{9,15} In addition, the risk of SARS-CoV-2-related morbidity and mortality in the context of

immunosuppression is being actively debated within the transplant community with only few data from single cases available.^{1,2,16–19} On the other hand, from a patient perspective, suspending LT may have a negative impact on patients with ELD or HCC without any other curative treatment option.^{4,5}

After balancing these considerations, we opted to maintain a LT activity by following center-specific decisional steps based on available guidelines (Table 3).

A first step was the reorganization of the LT program and a continuous evaluation of available resources. Despite the significant increase in ICU beds required for SARS-CoV-2-infected patients, we were able to maintain a SARS-CoV-2-free ICU dedicated to LT and surgical oncology (17% of total ICU beds). In addition, to further prioritize LT activity, major elective interventions (eg, major hepatectomy, esophagectomy) in frail patients with potential long ICU stays were reduced according to the national guidelines.^{20,21} The LT ward was reorganized into single rooms and medical staff wore face masks and scrubs and were tested and quarantined if they showed SARS-CoV-2 symptoms. While logistically challenging, setting up these SARS-CoV-2-free pathways to mitigate in-hospital transmission should be the first priority to allow safe LT activity for both recipients and medical staff.²¹

The second step was a case-by-case evaluation and risk stratification of every LT candidate on the waiting list, resulting in a 39% reduction of actively listed candidates. As reported in other LT centers, candidates listed for multiorgan transplants or retransplantations were temporarily put on hold due to an expected higher morbidity and to transitory shortage in blood products and RRT equipment.^{11,22} In contrast, LT candidates with MELD > 25 or ELD with poor prognosis but expected benchmark outcomes and short ICU stay were kept active on the waiting list. Of note, the median overall hospital stay was longer than the expected benchmark because of the mitigation strategies in place in France and reduced rehabilitation capacities.

The third step was the implementation of a screening strategy to avoid peri-LT SARS-CoV-2 infection. In contrast to some centers that only test symptomatic recipients, we opted for systematic testing in all recipients prior to LT.^{9,23} For the first 3 recipients, we used RT-PCR and quickly added chest CT scan to the systematic screening protocol, based on data showing good sensitivity of chest CT scan for detecting symptomatic and asymptomatic SARS-CoV-2-infected patients.²⁴ Chest CT slots were available 24/24 hours and in collaboration with our virology laboratory we were able to have pre-LT results from RT-PCR within less than 6 hours. Since recipients selected for LT were admitted to the hospital at least 6 hours before the transfer to the operating room, no significant delay due to pending test results occurred. Additionally, potential liver graft donors were screened by RT-PCR and chest CT during their ICU stay, and a negative SARS-CoV-2 status was mandatory to initiate the organ donation process.⁷ There were thus no delays due to SARS-CoV-2 diagnostics once the donation was initiated.

Finally, the organ allocation strategy played a major role. Our results showed optimal donor-recipient matches (median BAR

TABLE 1 Characteristics of 10 consecutive liver transplantations performed during the peak of the SARS-CoV-2 pandemic at a single center

	LT 1	LT 2	LT 3	LT 4	LT 5	LT 6	LT 7	LT 8	LT 9	LT 10
Donor and graft characteristics										
Donor age (y)	26	33	56	66	31	46	66	20	12	33
Donor center	National	Local	Regional	National	National	National	Local	National	National	National
SARS-CoV-2 incidence in donor center	Low	High	High	Very high	Very low	High	High	High	Very high	Very low
Donor ICU stay (d)	3	1	2	2	3	6	4	3	3	2
Type of grafts	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Right lobe/ex-situ split	Whole
Cold ischemia (h)	6	10	8	7	6	8	7	5	10	9
DRI (points)	1.19	0.936	1.53	1.89	1.089	1.898	1.719	1.047	1.719	1.213
Recipient characteristics										
Recipient age (y)	17	43	46	61	57	64	59	33	59	39
ELD cause	Auto-immune hepatitis	PSC	NASH/alcohol	Alcohol	NASH/alcohol	NASH/alcohol	Alcohol	Alcohol	HBV	HBV
HCC	No	No	No	No	No	No	Yes	No	Yes	Yes
Pre-LT status	Ward	Home	Home	Ward	ICU	Home	Home	Home	Home	Home
MELD (points)	32	10	18	38	27	18	21	19	7	12
CLIF-C ACLF (points)	10	6	7	11	9	7	7	7	6	6
ACLF grade	2	0	0	2	1	0	0	0	0	0
Pre-LT RT-PCR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pre-LT chest CT	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
SARS-CoV-2 positive	No	No	No	No	No	No	No	No	No	No
D-MELD (points)	832	330	1008	2508	837	828	1386	380	84	396
BAR score (points)	11	2	8	19	12	10	8	5	2	1

Abbreviations: ACLF, acute-on-chronic liver failure; BAR, balance of risk; CLIF-C, chronic liver failure consortium; D-MELD, product of donor age and Model for End-Stage Liver Disease; DRI, donor risk index; ELD, end-stage liver disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICU, intensive care unit; LT, liver transplant; NASH, nonalcoholic steatohepatitis; MELD, Model for End-Stage Liver Disease; PSC, primary sclerosing cholangitis; RT-PCR, reverse transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

	LT n = 10	Benchmark value at hospital discharge ¹¹
Peri-LT course		
Operation duration (h)	7.6 (5.4-9.4) ^a	≤6 h
Intraoperative blood transfusions (units)	1 (0-4)	≤3 units
Renal replacement therapy, n (%)	2 (20) ^a	≤8%
ICU stay (d)	2.5 (2-6)	≤4 d
Hospital stay (d)	14 (13-21) ^a	≤18 d
Morbidity and mortality at hospital discharge		
Any complication, n (%)	7 (70)	≤80%
Clavien Dindo grade II, n (%)	6 (60)	≤69%
≥Clavien Dindo grade IIIa, n (%)	3 (30)	≤42%
Biliary complications, n (%)	0 (0)	≤12%
CCI score	20.9 (0-34.3)	≤29.6
Graft loss, n (%)	0 (0)	≤4%
Mortality, n (%)	0 (0)	≤2%

Note: Continuous variables are presented as median and interquartile range.

Abbreviations: CCI, complication comprehensive index; ICU, intensive care unit; LT, liver transplant.

^aValues are out of the benchmark range.

TABLE 2 Posttransplant outcomes at hospital discharge compared to the available benchmark

TABLE 3 Center-specific decisional steps to maintain a liver transplant activity based on international guidelines

	International guidelines ^{6,9,14,19,25}	Practical implementation in our center
Resource planning	Evaluation and adaptation to available resources	Dedicated SARS-CoV-2-negative transplant unit and in-hospital pathways Weekly multidisciplinary meetings to adapt liver transplant activity to available ICU and operating room capacity
Liver transplant waiting list	Reduction of active patients on the waiting list	39% of recipients are temporarily put on hold
Recipient risk stratification	Prioritize urgent transplant indications (MELD > 25), ALF	Weekly multidisciplinary screening meetings ALF prioritized <u>High MELD</u> : CLIF-C ACLF Score assessment <u>Low MELD</u> : Recipients with HCC and expected benchmark outcomes
Recipient and donor screening before LT	Implement pre-LT SARS-CoV-2 screening	Recipient Questionnaire on prehospitalization symptoms and clinical examination Nasopharyngeal swap RT-PCR, results available within 4-6 h Chest CT scan prior to LT Donor Systematic donor screening by RT-PCR and chest CT scan
Recipient-donor matching	Reconsider organ allocation policies	Only DBD program maintained, DCD program suspended Optimization of donor-recipient matching to reduce post-LT morbidity and ICU stay (BAR, DRI)

Abbreviations: ACLF, acute-on-chronic liver failure; ALF, acute liver failure; BAR, balance of risk; DBD, donation after brain death; CLIF-C, chronic liver failure consortium; CT, computed tomography; DCD, donation after circulatory death; DRI, donor risk index; HCC, hepatocellular carcinoma; ICU, intensive care unit; LT, liver transplant; MELD, Model for End-Stage Liver Disease; RT-PCR, reverse transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

score 8) with liver grafts procured from very young donors presenting a low DRI. This allowed balancing the high pre-LT risk in

2 recipients with a MELD > 25 by the use of optimal grafts. One explanation of the availability of such grafts may be the selection

policy of the donor centers, focusing efforts on these young donors. Furthermore, similar to the transfer of SARS-CoV-2-infected patients from hospitals with insufficient ICU capacity to less affected hospitals across France during the study period, we also observed reallocation of liver grafts from regions with a high number of hospitalized SARS-CoV-2 patients to our center (Figure 1).²⁵ For example, recipient No. 8 (Table 1) received a liver graft declined by a center from a region with a high SARS-CoV-2 incidence because the initial recipient was screened SARS-CoV-2 positive. We conclude that all transplant centers should be ready to accept or decline liver grafts according to their local SARS-CoV-2 dynamics in order to guarantee optimal utilization of available grafts. In this context, centers may anticipate a back-up recipient in case of SARS-CoV-2-positive screening in the initial recipient. Furthermore, as a consequence of reallocation of liver grafts, cold ischemia time may be extended as in the case of recipient No. 8, where total static cold storage duration was 10 hours. This may increase the risk of allograft dysfunction or primary nonfunction, and centers may consider using ex-vivo machine perfusion strategies to recondition grafts with extensive ischemic damage.²⁶

This retrospective single-center report has inherent limitations. Regarding the small patient sample and short follow-up, more data are required to confirm our results. Additionally, the present report reflects a specific experience from a single center and thus may not be transferable to other centers, regions, or countries. However, given the unprecedented situation, this preliminary clinical experience helps in the process of moving forward: Continuous evaluation of both resources and outcomes may allow further extension of LT activity over the next weeks and to quickly respond in the event of a second SARS-CoV-2 peak.

In conclusion, we report the successful preliminary experience of a French LT program during the peak of the SARS-CoV-2 pandemic. Efforts in resource planning, optimal recipient selection, and organ allocation are key to maintain a safe LT activity. Transplant centers should be ready to readapt their practices as the pandemic evolves.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Ex Vivo Liver Splitting and Hypothermic Oxygenated Machine Perfusion: Technical Refinements of a Promising Preservation Strategy in Split Liver Transplantation

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Split liver transplantation (LT) has been developed to increase organ availability, but it results in prolonged cold ischemia and higher risks of postreperfusion bleeding and ischemia-reperfusion injury (IRI). Therefore, partial liver grafts are considered as marginal grafts. Dynamic preservation strategies have been proposed to minimize IRI and optimize post-LT outcomes, especially for extended criteria donor livers; however, all current trials assessing liver machine perfusion exclude partial grafts.¹ For instance, our team is currently conducting a multicenter randomized trial assessing end-ischemic hypothermic oxygenated perfusion (HOPE) before LT with extended criteria donor grafts (NCT03929523), with the exclusion of partial grafts.

We herein report an original 2-step technique of ex vivo left lateral/right extended graft splitting with concurrent HOPE: first, vessel dissection and hilar plate division are facilitated by the “star exposure” and performed during static cold storage; second, parenchymal transection is performed with simultaneous HOPE using a “split hanging maneuver” (Figure 1). After approval by the institutional ethics committee (CSE-HCL_21_202), we applied

this novel approach for 2 livers, allowing us to successfully transplant 2 adults (aged 56 and 45 y) and 2 pediatric recipients (aged 36 and 5 mo, weighting 15 and 5 kg, respectively). All 4 recipients showed satisfactory allograft function recovery. The 36-mo-old child had a history of previous LT for Alagille syndrome, which complicated with hepatic artery thrombosis and ischemic cholangiopathy. He was retransplanted using the present technique in a context of marked hemodynamic instability; despite 2 relaparotomies for postoperative hemorrhage, he recovered and normalized factor V on posttransplant day 6. The 3 other recipients normalized their factor V between posttransplant day 1 and 3, which is quite remarkable compared to our experience of ex vivo split LT without HOPE.

Ex vivo splitting with concurrent dynamic preservation represents a technical challenge, with very limited data available. Its feasibility has been suggested on discarded livers with normothermic perfusion,^{2,3} on swine using dual hypothermic perfusion,⁴ and during dual-HOPE.^{5,6} We herein report the 2 first cases of LT using concurrent liver splitting and HOPE through the portal vein only, which, according to us, represents the least demanding and most secure machine perfusion modality for ex vivo splitting. The graft is preserved in the cold during the entire procedure, which reduces the risk of warm ischemia due to technical problems or cannula kinking. In comparison with the report by Thorne et al,⁶ who perfused the graft in a supine position during both vascular dissection and parenchymal transection, our 2-step technique has the advantage of allowing bench top cholangiography, which is not currently possible after initiation of HOPE. Moreover, the split hanging maneuver in prone position allows performing parenchymal transection through an anterior approach, similarly to elective hepatectomies.

Of note, single perfusion through the portal vein facilitates the procedure and avoids arterial injury during graft handling, without compromising the protective effect of HOPE against IRI. This strategy allows to extend perfusion of both partial grafts through a single portal cannula, for example in case of a difficult recipient hepatectomy. Finally, although sequential perfusion of the 2 grafts after splitting may be easier, simultaneous splitting and perfusion enable the performance of parenchymal transection during HOPE, which hereby reduces graft rewarming caused by graft mobilization out of ice and heat exposure by the

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J.-Y.M. designed the research, performed the research, and wrote the article. M.L. and G.R. performed the research and participated in writing the article. X.M. and R.D. participated in research design and critically revised the article. C.D. performed the research and critically revised the article. K.M. participated in research design, performed the research, and participated in writing the article.

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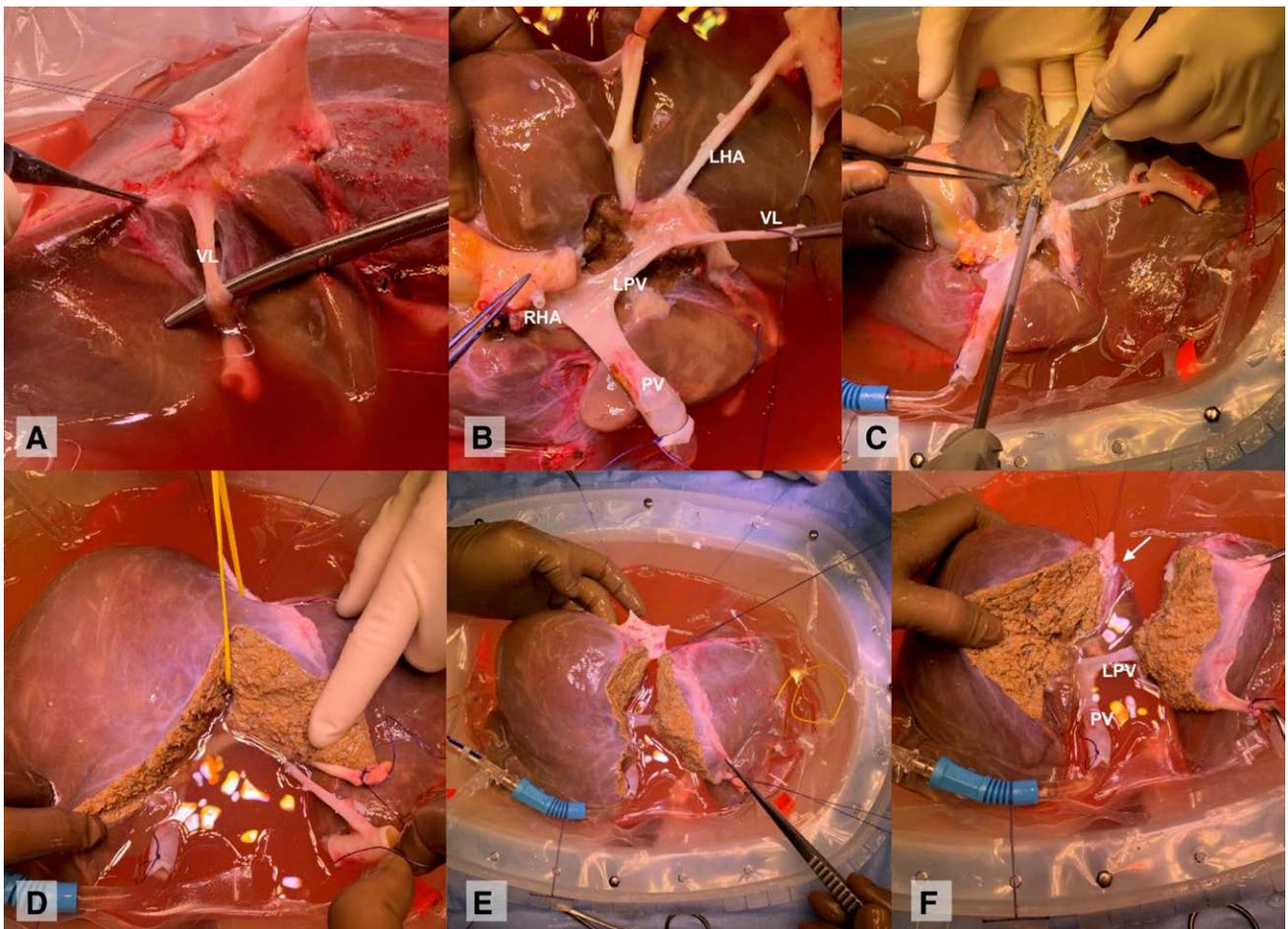


FIGURE 1. Ex vivo 2-step split technique with simultaneous transection and perfusion using hypothermic oxygenated perfusion (HOPE). The venous ligament (VL) is divided to allow identification and dissection of the left hepatic vein (A). The hepatoduodenal ligament is dissected until identification of the portal vein (PV) and hepatic artery division branches. Dissection of the left portal vein (LPV) is performed backward to the portal bifurcation, allowing division of S1 and S4 branches. The left hepatic artery (LHA) is in continuity with the full arterial axis to the left lateral graft. The right hepatic artery (RHA) is divided at its origin, leaving the left hepatic artery (LHA) in continuity with the full arterial axis to the left lateral graft. Sharp transection of the hilar plate is performed before parenchymal transection and guided by bench cholangiography to obtain a single bile duct for the left graft. This step is facilitated by the “star exposure,” which consists of the exposure of the following anatomical structures in a clockwise direction: the ligamentum teres, the LHA, the VL, the main PV, and the right Glissonean pedicle (including the RHA and the right PV [B]). Both grafts are then perfused with HOPE using the Liver Assist perfusion device (Organ Assist, The Netherlands) and a single portal perfusion via a 25F cannula under a 4 mm Hg pressure and a 150 mL/min portal flow at 10 °C. The vena cava is left open to allow free drainage into the organ reservoir. Parenchymal transection is then performed with simultaneous HOPE in 2 steps with an integrated bipolar/ultrasonic device (Thunderbeat, Olympus) and Prolene 5/0 stitches. A caudate approach is used to divide the left lateral graft from segment 1 and inferior part of S4 (C). After turning the graft upside down into the reservoir without interrupting portal perfusion, transection of S4 is carried out along the right side of the falciform ligament until the left hepatic vein, using an anterior approach. This step is facilitated by a “split hanging maneuver,” which allows raising the liver and offers 2 technical advantages: first, a better identification of the transection plane and, second, avoidance of an insufficient portal perfusion flow caused by graft compression (D). Finally, the left hepatic vein is divided, and the defect on the vena cava is closed (arrow) with a transverse running suture or a venous patch to avoid any stricture of the middle hepatic vein (E). The 2 grafts remain perfused by the main portal trunk as long as necessary, until hepatectomies in both recipients are carried out (F). Division of the LPV is performed as soon as 1 of the recipients is ready for graft implantation, possibly leaving the other graft perfused if necessary. Times from donor cold flush to start of back bench were 250 and 266 min, times from back bench to initiation of HOPE were 102 and 87 min, and times from initiation of HOPE to completion of parenchymal transection were 75 and 62 min, respectively. Total cold ischemia times (including HOPE) were 543 and 448 min for the 2 adult recipients and 640 and 520 min for the 2 pediatric recipients.

energy transection device. Further studies are needed to assess this promising dynamic preservation strategy, which may improve the outcome of split LT.

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ORIGINAL ARTICLE

Graft utilization after normothermic regional perfusion in controlled donation after circulatory death—a single-center perspective from France

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SUMMARY

Normothermic regional perfusion (NRP) in controlled donation after circulatory death (cDCD) is a promising procurement strategy. However, a detailed analysis of graft utilization rates is lacking. This retrospective study included all cDCD donors proposed to a single center for NRP procurement of at least one abdominal organ from 2015 to 2020. Utilization rates were defined as the proportion of transplanted grafts from proposed donors in which withdrawal of life sustaining therapies (WLST) was initiated. In total, 125 cDCD donors underwent WLST with transplantation of at least one graft from 109 (87%) donors. In a total of 14 (11%) procedures NRP failure led to graft discard. Utilization rates for kidney and liver grafts were 83% and 59%, respectively. In 44% of the discarded livers, the reason was poor graft quality based on functional donor warm ischemia >45 min, macroscopic aspect, high-transaminases release, or pathological biopsy. In this study, abdominal NRP in cDCD lead to transplantation of at least one graft in the majority of cases. While the utilization rate for kidneys was high, nearly half of the liver grafts were discarded. Cannulation training, novel graft viability markers, and *ex-vivo* liver graft perfusion may allow to increase graft utilization.

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Key words

donation after circulatory death, extended criteria donor, graft utilization, normothermic regional perfusion, organ donation

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Introduction

Normothermic regional perfusion (NRP) is a promising procurement strategy in controlled donation after circulating death (cDCD) [1]. During NRP, future grafts undergo in-situ perfusion at normothermic temperatures with an extra-corporeal membrane oxygenation (ECMO) perfusion [2]. Several cDCD programs worldwide have opted for the use of NRP as an alternative to super-rapid organ procurement [3,4]. France, Italy, and Norway have implemented a mandatory use of NRP for every cDCD procurement [1,5]. While studies report excellent outcomes after NRP in cDCD liver and kidney transplantation, this strategy requires additional logistics, donor cannulation training, and raises specific ethical questions [6-10]. In addition, a detailed analysis of utilization rates and reasons for graft discard including technical failures after NRP are currently lacking [3]. In the context of an increasing liver and kidney graft shortage, these data are urgently needed to optimize the use of available grafts, improve procurement and preservation strategies and further expand cDCD organ transplantation [11].

Materials and methods

Study design

This is a retrospective cohort study including all consecutive cDCD donors proposed for transplantation of at

least one abdominal organ to our center and for whom withdrawal of life sustaining therapies (WLST) was initiated. The study period covers 6 years (01.01.2015–31.12.2020).

Donor and recipient selection criteria

The French cDCD program started in 2015 with mandatory use of NRP for every procurement [5]. Strict donor and recipient selection criteria apply in order to select low-risk combination and achieve optimal post-transplant outcomes (Table 1). Of note, donor age limit was modified from <61 years until 2018 to <71 years in 2020. In addition, allocation of cDCD grafts was regional in contrast to DBD grafts, which are allocated at a national level to reduce static cold storage duration and facilitate coordination of the cDCD procedure. There were no cDCD lung or pancreas procurements performed in our study population.

Abdominal NRP and organ procurement

Abdominal NRP was applied to the donor after circulatory arrest, with the aim of reconstituting blood flow at physiological temperatures to the donor organs prior to procurement. Heparin (300 UI/kg) was routinely administered to the donor upon start of WLST. Once circulatory arrest occurred, a donor “no-touch” period of 5 min was mandatory before death could be

Table 1. Donor and recipient selection criteria for cDCD kidney and liver transplantation in France.

	Liver transplantation	Kidney transplantation
Donor criteria		
Age	<71 years	<71 years
Comorbidities	No chronic disease	No chronic disease
NRP	AST/ALT ≤ 4N	Normal renal function
Biopsy after NRP	AST/ALT ≤ 4N	No defined selection criteria
<i>Ex-vivo</i> hypothermic perfusion	Steatosis ≤ 20%, Fibrosis < F2	No routinely performed biopsies
TDWI	–	No defined selection criteria
FDWI	≤3 h	≤3 h
AWI	≤45 min	–
	≤30 min	If <66 years: ≤45 min If 66–71 years: ≤30 min
Recipient criteria		
Age	≥18 and ≤65 years	≥18 years
Hepatic/renal disease	Primary transplant, no PV thrombosis, MELD ≤25, no super-urgent transplantation	Primary transplant
Comorbidities	No major surgical history No ventilation, no inotrope UNOS 1	To consider but not mandatory: Vascular disease, surgical history

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AWI, asystolic warm ischemia time, FDWI, functional donor warm ischemia time; NRP, normothermic regional perfusion; TDWI, total donor warm ischemia.

declared. Once death was declared, arterial, and venous cannulas for NRP were inserted over preplaced guidewires into femoral vessels by an anesthesiologist (post-mortem vessel cannulation). The minimal required duration of NRP before procurement could proceed was 60 min with a maximum of 4 h (details in Appendix S1). Once NRP was terminated, organs were cold flushed and a standard organ procurement was performed. Liver grafts underwent static cold storage while kidney grafts underwent *ex-vivo* hypothermic machine perfusion until implantation (Appendix S1; Fig. S1).

Endpoints and definitions

Proposed donors included all donors who were screened by the national donor agency (Agence de la Biomédecine) and who were inside the aforementioned French cDCD selection criteria with consent by the donor and family (Table 1). Only once a donor was proposed for procurement, transplant teams were able to accept or decline the proposition.

The primary endpoint of the study was utilization rates for cDCD kidney and liver grafts defined as the proportion of transplanted organs procured from donors initially proposed for cDCD donation of the respective. Secondary endpoints included reasons and characteristics of discarded grafts, technical failures of NRP and graft and recipient survival after NRP cDCD kidney and liver transplantation at our center. We also investigated the impact of the time period on utilization rates and adverse events after NRP. For this purpose, we divided the cohort in two time periods, prior and after the change in donor age limit (2015–2018 vs. 2019–2020), and performed a subgroup analysis.

Functional Donor Warm Ischemia Time (FDWI) was defined as the duration from systolic blood pressure below 45 mmHg to initiation of NRP (Fig. S1). Asystolic Warm Ischemia (AWI) was defined as the period from occurrence of cardiac arrest until initiation of NRP (Fig. S1). Extended criteria for cDCD liver donors (EDCD) were based on the UK-DCD Risk score and included donor age >60, donor BMI >25 and FDWI >30 min [12]. A donor presenting at least 2 of these criteria was considered EDCD. Extended criteria for cDCD kidney donors were defined as a donor age >60 years or between 50 and 59 years with at least two of the three following criteria: cerebrovascular cause of death, renal insufficiency, and hypertension [13].

Data collection and ethical approval

All data for the present study were extracted from a prospective national database (CRISTAL) administered by the Agence de la biomédecine. Our center has signed a specific data sharing agreement to participate in this database. For the present study, nominative password protected access was limited to center-specific and anonymized data only. The study was conducted in accordance with French legislation and local ethics committee approval was obtained.

Statistical analysis

Categorical variables are expressed in quantities and percentages while continuous variables are expressed as median with interquartile range (IQR). Continuous variables were compared using the Mann–Whitney *U*-test. Categorical variables were compared using the chi-square test or the Fisher's exact test. Survival rates were calculated by Kaplan–Meier estimates. *P*-values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS software version 23 (Armonk, NY, USA) and GRAPHPAD PRISM 8.

Results

During the study period, a total of 125 cDCD donors were proposed for procurement of at least one kidney or liver graft (Fig. 1). Median donor age was 55 years and 68% had a cardiac arrest with a median of 30 min of no flow/low flow prior to ICU admission (Table 2). The delay from ICU admission to WLST was 9 days (IQR: 7–16 days). Overall, 20% of liver and 32% of kidney donors were EDCD donors (Table 3).

Donation process and normothermic regional perfusion

After WLST, cannulation was attempted in 118 donors (94%; Fig. 1). Successful initiation of NRP was followed by procurement and transplantation of at least one abdominal organ in 109 donors (87%). A median of two grafts per donor were transplanted. The median duration for postmortem vessel cannulation for NRP initiation was 12 min (IQR: 10–17 min). We registered 14 (11%) adverse events during NRP leading to discard of at least one potential graft (Table 4). The majority of adverse events were cannulation failures ($n = 7$, 54%). During the study period, the number of NRP cDCD procedures increased by 5× ($n = 7$ in 2016 to $n = 36$ in

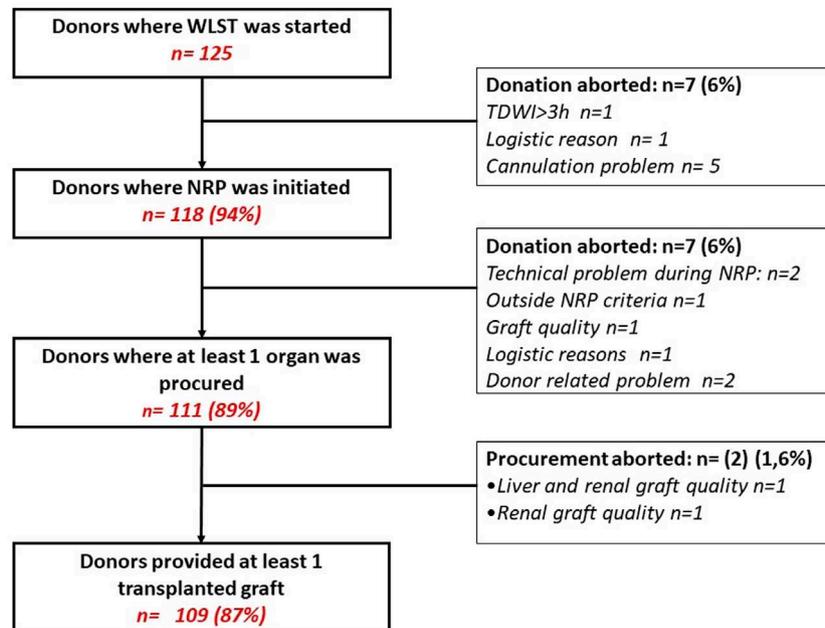


Figure 1 Study flow chart.

Table 2. Donor characteristics.

	All cDCD donors n = 125	Donor provided transplanted liver graft n = 58	Donor provided no transplanted liver graft n = 41	P	French cDCD Selection criteria
Donor characteristics					
Donor age, year	*	53 (45–61)	55 (47–61)	0.997	<71 years
Donor gender, male, n (%)	88 (70) [0]	41 (71)	25 (38)	0.313	
Donor BMI, kg/m ²	25 (22–29) [0]	25 (21–28)	25 (21–28)	0.428	
Cardiac arrest prior to ICU, n (%)	85 (68) [0]	44 (76)	21 (51)	0.011	
Duration of no flow/low flow, min	30 (20–39) [7]	30 (25–45)	26 (14–40)	0.180	
Peak donor serum lactate, mmol/l	2.1 (1.5–4.6) [2]	1.9 (1.5–4.1)	1.9 (1.3–2.6)	0.451	
Donor ICU stay					
Cause of ICU admission, n (%)					
Cerebrovascular accident	23 (18) [0]	6 (10)	12 (29)	0.016	
Hypoxic brain injury	78 (62) [0]	43 (74)	20 (49)	0.010	
Trauma	24 (19) [0]	9 (16)	9 (22)	0.414	
Donor ICU stay, days	9 (7–16) [0]	9 (7–16)	10 (7–21)	0.618	
Transfusions, n (%)	31 (25) [0]	15 (26)	9 (22)	0.655	
Donor serum Na, mmol/l	142 (138–145) [0]	141 (138–145)	142 (138–144)	0.806	
Donor serum Hb, g/l	8.7 (9–11) [1]	9.6 (9–10.9)	9.7 (9.1–10.6)	0.943	
Donor serum Hct, %	30 (28–34) [0]	30 (27–32)	30 (28–33)	0.428	
Donor serum AST, U/l	59 (26–85) [1]	54 (32–71)	56 (33–78)	0.432	
Donor serum ALT, U/l	51 (35–94) [1]	40 (29–60)	61 (36–83)	0.115	
Donor serum GGT, U/l	120 (60–253) [1]	92 (49–158)	120 (62–204)	0.145	
Donor serum creatinin, μmol/l	61 (43–76) [0]	63 (48–74)	58 (42–80)	0.621	
Donor creatinin clearance, ml/min	126 (96–168) [0]	126 (97–157)	115 (89–167)	0.707	

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; Hb, hemoglobin; Hct, hematocrit.

* Numbers between brackets indicate missing values.

Table 3. Procurement and NRP characteristics.

	All cDCD donors n = 125	Donor provided transplanted liver graft n = 58	Donor provided no transplanted liver graft n = 41	P	French cDCD Selection criteria
WLST characteristics					
Total donor warm ischemia, min	33 (28–43) [0]*	32 (28–38)	36 (28–52)	0.118	≤180 min
Functional donor warm ischemia, min	22 (18–29) [0]	21 (18–25)	27 (19–36)	0.01	≤45 min
Asystolic donor warm ischemia, min	17 (14–23) [0]	16 (15–20)	19 (14–25)	0.073	≤30 min for livers, ≤45 min for kidneys
Donor risk profile					
FDWI >30 min, n (%)	8 (6)	0 (0)	4 (10)	0.015	
Donor age >60 years, n (%)	34 (27)	16 (28)	11 (27)	0.934	
BMI >25 kg/cm ² , n (%)	62 (50)	25 (43)	22 (53)	0.300	
EDCD liver donors, n (%)	25 (20) [0]	30 (52)	26 (63)	0.248	
EDCD kidney donors, n (%)	40 (32) [0]	n.a.	n.a.	–	
NRP characteristics					
Donor vessel cannulation duration, min	12 (10–17) [0]	11 (10–15)	14 (9–20)	0.074	
NRP duration, min	204 (178–226) [0]	212 (195–229)	192 (142–229)	0.015	min 60 min and max 240 min
Mean arterial flow, l/min	2.6 (2.5–3) [2]	2.7 (2.5–3)	2.5 (2.5–3)	0.963	

EDCD, extended donation after circulatory death criteria; FDWI, function donor warm ischemia; NRP, normothermic regional perfusion; WLST, withdrawal of life sustaining therapies.

*Numbers between brackets indicate missing values.

2020) and the number of centers performing NRP increased from 1 to 11 (Fig. 2). Utilization rates for donors in 2015–2018 were 89% and in 2019–2020 were 86% ($P = 0.790$). Utilization rates for liver grafts in 2015–2018 were 57% and in 2019–2020 were 60% ($P = 0.831$). NRP adverse events decreased from 15% in 2015–2018 to 7% in 2019–2020 ($P = 0.263$; Fig. 2).

Graft specific utilization rates

Of the initially proposed 99 liver and 243 kidney grafts, a total of 58 liver and 201 kidney grafts were successfully transplanted resulting in graft utilization rates of 59% for livers and 83% for kidneys (Table 5). Overall, 1-year recipient and graft survival rates at our center were 93% respectively after liver transplantation and 97% and 96%, respectively, after kidney transplantation (Fig. 3). Donors in whom a liver graft was discarded had longer FDWI (21 min vs. 27 min, $P = 0.01$) but did not differ in age (55 years vs. 53 years, $P = 0.10$) nor EDCD criteria (63% vs. 52%, $P = 0.25$; Tables 2 and 3). In contrast, kidney donors in whom the kidney graft was rejected were more frequently EDCD donors (45% vs. 26%, $P = 0.022$) with a higher donor age (59 years vs. 53 years, $P = 0.026$) and higher rates of

arterial hypertension (48% vs. 26%, $P = 0.005$) and diabetes (21% vs. 6%, $P = 0.005$; Table S1). Nearly half of all liver grafts discards (44%) were due to poor graft quality based on either subjective evaluation ($n = 4/41$, 10%), transaminase increase during NRP $>4\times$ baseline ($n = 4/41$, 10%) or pathological liver biopsy (8/41, 20%; Table 5).

Discussion

This study presents graft specific utilization rates after cDCD procurement with the use of abdominal NRP at a single center from France. The study shows that the use of NRP in low-risk cDCD donors led to transplantation of a median of two abdominal grafts per donor with excellent post-transplant outcomes. However, while utilization rates for kidney grafts were 83%, liver grafts presented a significantly lower utilization rate of 59%. Technical failure of NRP leading to discard of at least one potential graft occurred in 11% of all cDCD procedures in the study.

The regulations, which apply to cDCD dependent on each country's legal and ethical framework and thus display important differences [3]. For instance, the *no-touch period* after cardiac arrest of the donor ranges

Table 4. Details of technical problems with NRP procedures resulting in discard of at least one proposed graft.

N	Adverse event during NRP	CVF in donor	Cause of death CVA	NRP initiation	NRP completion	Procurement of at least one organ	Kidney grafts	Liver graft
1	45 min for cannulation, prolonged AWI	No	No	Yes	Yes	Yes	Procured and transplanted	Liver not procured due to AWI >30 min
2	Arterial perforation during cannulation	No	No	No	No	No	Not procured	Not procured
3	Delayed occlusion balloon inflation, prolonged AWI	No	No	Yes	Yes	Yes	Procured and transplanted	Liver not procured due to AWI >30 min
4	Occlusion balloon failure	No	No	Yes	No	No	Not procured	Not procured
5	Low flow, addition of 1 l of saline	Yes	No	Yes	Yes	No	Not proposed	ALT/AST >4N, Liver not procured
6	Cannulation failure	No	No	No	No	No	Not procured	Not procured
7	Cannulation failure, venous perforation	No	No	No	No	No	Not procured	Not procured
8	Cannulation failure by percutaneous and surgical approach, anatomical variation	Yes	Yes	No	No	No	Not procured	Not procured
9	Occlusion balloon failure	Yes	No	Yes	No	No	Not procured	Not procured
10	Low flow, donor hemodynamic instability	Yes	No	Yes	Yes	Yes	Procured and transplanted	Liver not procured due to quality
11	Percutaneous cannulation impossible, surgical cannulation but prolonged AWI	No	No	Yes	Yes	Yes	Procured and transplanted	Liver not procured due to AWI >30
12	Aortic dissection during cannulation	Yes	No	Yes	Yes	Yes	Not procured	Procured and transplanted
13	Delayed occlusion balloon inflation	Yes	No	Yes	Yes	Yes	No decoloration of right kidney	Procured and transplanted
14	Arterial perforation during cannulation	Yes	Yes	No	No	No	Not procured	Not procured

AWI, asystolic warm ischemia times; CVA, cardiovascular accident; CVF, cardiovascular risk factors includes arterial hypertension, diabetes, history of smoking or peripheral vascular disease.

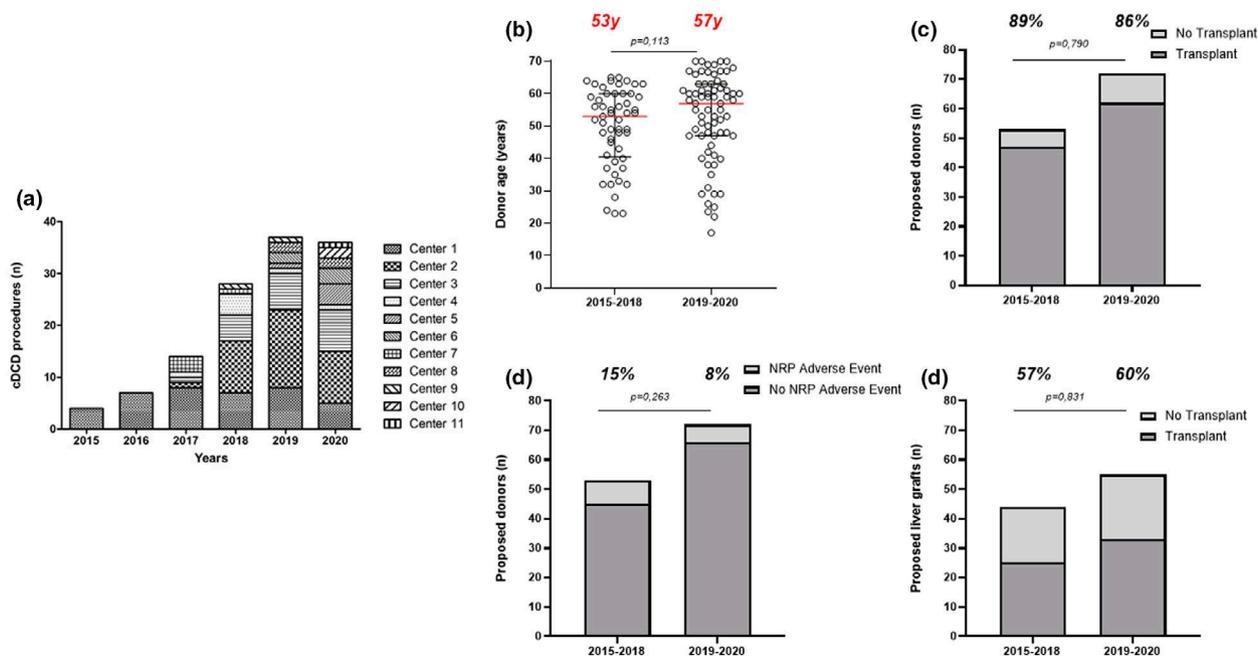


Figure 2 cDCD procedures and utilization rates according to volume and time period. (a) cDCD volume per center and year; (b) donor age, (c) donor utilization rate, (d) adverse events during NRP and (e) liver graft utilization rates according to the time period.

Table 5. Graft specific discard rates and reason for graft discard.

Reason for graft discard	Liver graft discarded 41/99 (41%)	Kidney graft discarded 42/243 (17%)
TDWI >3 h	n = 1	n = 2
FDWI >45 min	n = 2	–
Technical failure of NRP	n = 10	n = 16
Pathological biopsy*	n = 8	n = 4
AST/ALT >4 × N during NRP	n = 4	–
Graft quality concern†	n = 4	n = 2
Logistic reason‡	n = 4	n = 3
Donor history	n = 4	n = 6
Unexpected finding in donor	n = 2	n = 5
Adverse event during ex-vivo perfusion	–	n = 4
Unknown	n = 2	–

*Biopsy after NRP was mandatory for livers but not for kidneys. Pathological biopsy for livers was defined as cirrhosis, fibrosis >F1, macrosteatosis >20%; pathological biopsy for kidneys was based on the appreciation of the transplant team.

†Based on macroscopic aspect.

‡Adverse event or no-show of the recipient without an available backup recipient, no transfer of graft possible.

widely across countries from 5 min in France to 20 min in Italy. Furthermore, in Spain pre-mortem cannulation for NRP is permitted while in France only pre-mortem placement of guidewires is allowed and cannulation must be performed post-mortem [3]. These legal differences must be considered when analyzing donor selection and procurement techniques.

Since the beginning of the cDCD program in France, efforts were made to optimize post-transplant outcomes in order to promote acceptance of cDCD procedures among transplant professionals and other stakeholders. First, mandatory and strict donor and recipient selection criteria were established to achieve optimal post-transplant outcomes (Table 1). In liver transplantation

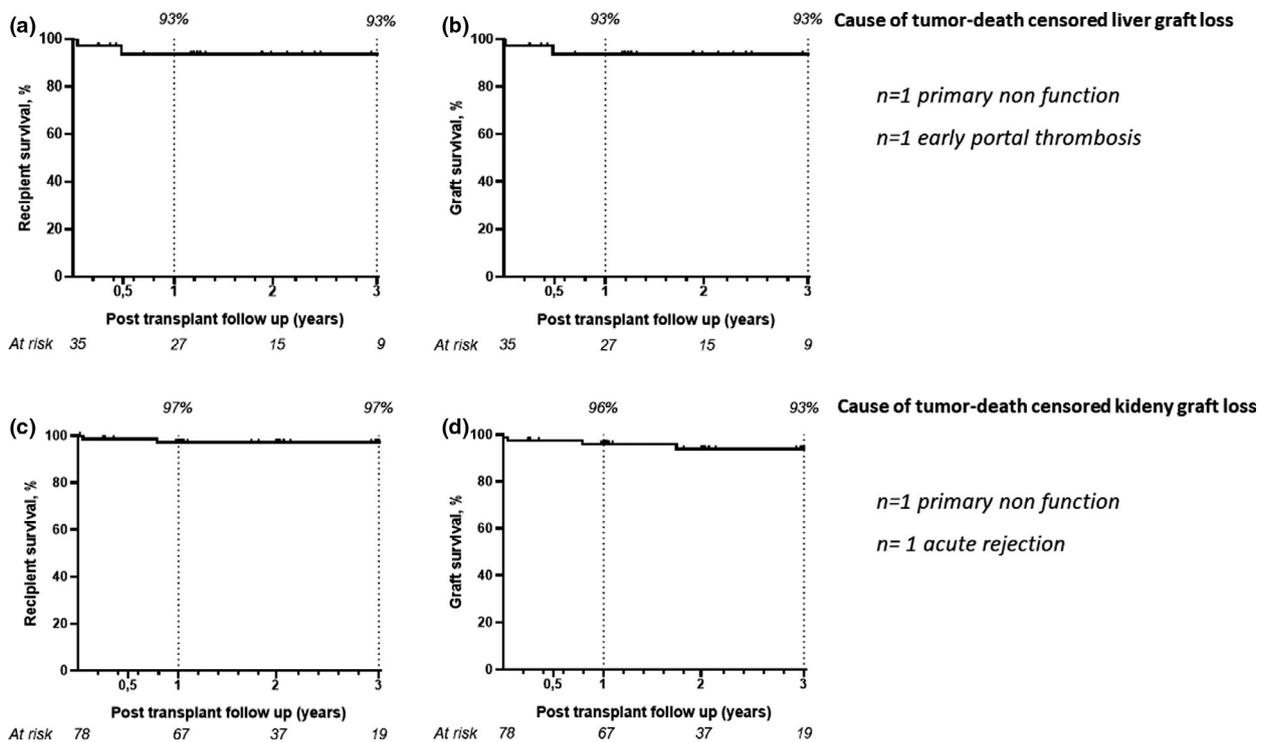


Figure 3 Recipient and graft survival after NRP cDCD kidney and liver transplantation at a single center. (a) Tumor-death censored recipient and (b) graft survival after liver transplantation; (c) overall recipient and (d) graft survival after kidney transplantation.

for example, these strict selection resulted in low-risk donor-recipient combinations with a median donor age of 50 years, FDWI of 22 min and a UK-DCD risk score of six points [14]. Other cDCD LT programs, for example, in Switzerland present significantly higher donor-recipient risk combinations with a median donor age of 61 years, FDWI of 31 min and UK-DCD risk score of nine points, resulting in higher liver graft utilization rates [15]. Second, allocation of cDCD grafts in France was regional instead of national as for DBD grafts, to shorten cold ischemia times and facilitate organization of cDCD procedures. It is in this context of optimal donor selection, that we present single-center data on cDCD kidney and liver graft utilization after abdominal NRP.

Currently, several countries use NRP for cDCD procurement but a detailed analysis of utilization rates is lacking [6,15,16]. A recent systematic review by van de Leemkolk *et al.* [16] identified 14 cohort studies on NRP in cDCD mostly focusing on either liver or kidney grafts in a single-center setting. Graft utilization rates were only reported in seven studies and definitions were very heterogeneous. For instance, Oniscu *et al.* defined utilization rates as grafts transplanted from donors who successfully completed NRP while Watson *et al.* [2,17] included all donors where NRP was initiated. Another

large study on NRP in cDCD kidney transplantation only included transplanted grafts in the analysis [7]. Reported graft utilization rates after NRP for cDCD kidneys range from 62.7% to 92.7% and for cDCD livers range from 61.4% to 62.5%, depending on the definition [16].

Given the heterogeneity in definitions of utilization rates in the literature, we propose a broader definition of utilization rates: The proportion of transplanted grafts procured from donors in whom the respective graft was proposed. This allows to identify adverse events at every step of the donation and propose pragmatic solutions to increase utilization rates, for example, by reducing cannulation failures (Fig. 1). Of note, in France donor screening is performed by the national donor agency and transplant centers are unable to interfere with the decision to propose donors. To prevent conflict of interest, transplant centers may accept or decline an organ offer once a proposition from the national agency is received. Conclusively, we opted to start the count from the moment a donor was proposed to our transplant center. Future randomized trials and cohort studies on marginal graft transplantation or novel preservation strategies should clearly state definitions and report utilization rates [1,18].

Regarding donor characteristics, an interesting finding was that significantly more cardiac arrests prior to ICU admission occurred in donors with successful graft utilization compared to those where grafts were discarded. The available literature and guidelines on cDCD donation only rarely report cardiac arrest prior to ICU admission and consequently its impact on graft quality and outcomes is not well studied [19]. A possible explanation for high utilization rates in this subgroup of donors is that donors with cardiac arrest have fewer comorbidities compared to donors with a cerebrovascular accident (CVA) in terms of age, smoking and arterial hypertension [20]. Indeed, in our study EDCD kidney donors had a 2× higher rate of CVA than non EDCD donors (data are not shown). This overall lower donor risk profile may increase utilization rates in this subgroup of donors.

We identified two major modifiable causes of graft discard in NRP cDCD: technical problems with NRP and poor quality after NRP, especially for liver grafts. First, technical issues with NRP were due to cannulation failure and balloon occlusion failure in the majority of the cases (Table 4). Donor cannulation in this study was performed by an anesthesiologist at the donor center using the Seldinger technique over preplaced guidewires in the femoral vessels. Of note in a few cases, a surgical approach of the femoral vessels or abdominal vessel was used in the case of failure of the Seldinger technique or as primary approach (data are not shown). In contrast to our data, Oniscu *et al.* [2] reported the preliminary UK experience (34 cases) with a 6% failure rate using surgical cannulation of the abdominal vessels in the operating room for all cases. Reports from Spain show even lower cannulation failure rates with pre-mortem cannulation, which reduces the time constraint and even allows to perform cannulation in an interventional radiology facility [6]. In France, cannulation by the Seldinger technique was chosen as the standard technique to allow end-of-life accompaniment of the donor and family in the intensive care unit. However, beyond those ethical considerations, our data raise the question of a tailored cannulation strategy based on specific donor criteria. For example, donors with peripheral vascular disease may present additional difficulty for percutaneous cannulation and a surgical approach in the operating room may have a higher success rate. In this study, we did however not observe a higher rate of cardiovascular risk factors or cerebrovascular cause of death in cases where femoral vessel cannulation failed (Table 4). Larger studies are needed to identify robust predictive criteria for difficult femoral

vessel cannulations, which may assist the choice of the cannulation strategy. We conclude from our data that percutaneous pre-mortem cannulation has several logistical advantages but remains a technically challenging procedure and requires appropriate training and experience to overcome the learning curve [7,21]. In this study, 5 centers performed all the NRP procedures during the first 3 years with six centers joining thereafter and thus having less experience (Fig. 2). Similarly, from the 30 centers in the French cDCD program, half performed ≤15 NRP procedures from 2015 to 2019 [22]. Given the very small number of cases performed by some centers included in the study (<4 cases), we were unable to identify a significant correlation between center volume and adverse events during NRP (data are not shown). A larger data set is needed to confirm the center volume hypothesis. However, we did observe a trend toward a lower rate of NRP adverse events from 2018–2019 compared to 2015–2018 (Fig. 2) which further supports the importance of experience in the use of NRP.

The issue of NRP experience raises the question if local teams at the donor hospital should perform cannulation for NRP or if a specialized mobile NRP team should be dispatched to each regional donor hospital to perform cannulation? In our study the majority of NRP procedures were performed by local teams, even in low volume centers. Feasibility of setting up a mobile team to allow hospitals without NRP experience to participate in cDCD programs have been reported [23]. However, this strategy presents additional logistical challenges, which may hamper broader acceptability of cDCD procurement.

A second major reason for graft discard, which was predominantly observed in liver grafts, was poor graft quality after initiation of NRP. Definitions for poor liver graft quality vary widely and assessment of graft viability is often based on visual assessment by the procurement surgeon or indirect markers such as transaminases [24]. A large series by Hessheimer *et al.* [6] including 152 cDCD liver grafts undergoing NRP reported a 21% discard rate due to poor macroscopic aspect on visual assessment. Interestingly in our cohort, other than FDWI included in the selection criteria, we did not find any significant differences in donor characteristics which differentiate transplantable from non-transplantable liver grafts. In this context, we see an urgent need for liver graft viability markers prior to transplantation. Such biomarkers may be identified by metabolomic analysis ideally focusing on mitochondrial metabolism [25–27]. A recent publication by Wang

et al. [28] suggests that a real-time assessment of a mitochondrial marker flavin mononucleotide during NRP is possible. A recent study from France on cDCD liver transplantation after NRP showed a 1-year graft survival of 68% for liver grafts with FDWI >30 min and prolonged cold storage >8 h [29]. Such marginal cDCD liver grafts may benefit from additional ex-vivo perfusion after NRP. Several Italian teams have proposed a sequential strategy with NRP followed by hypothermic oxygenated perfusion (HOPE) to rescue marginal cDCD liver and kidney grafts with FDWI up to 52.5 min for livers and 325 min for kidneys [30–32]. In the absence of validated biomarkers during NRP and following the positive results of two recent randomized trials on HOPE in cDCD liver and kidney transplantation, we suggest adding a period of HOPE after NRP in high-risk grafts to assess mitochondrial viability prior to implantation [12,24,33–36]. Of note in the French cDCD program, all kidney grafts already undergo mandatory hypothermic perfusion after NRP.

While this study is the largest to date to comprehensively report on utilization rates after abdominal NRP, it has certain limitations. The present study analysis discard rates from a single-center perspective which has the advantage of reflecting on a standardized practice. However, the reported discard rates need to be validated in a future study based on national data to capture all the discarded grafts in all the donor centers. Second, in order to perform a more precise statistical analysis of donor characteristic associated with graft discard and identify robust prediction factors, a larger cohort is needed.

In conclusion, the use of abdominal NRP in highly selected cDCD donors allowed transplantation of at least one graft in the majority of procedures resulting in excellent post-transplant outcomes. However, while utilization rates for kidneys were >80%, liver grafts had higher discard rates due to presumably poor quality after initiation of NRP. Dedicated training for NRP,

development of graft viability markers and the use of ex-vivo perfusion strategies for additional graft assessment may allow to further increase utilization rates.

Authorship

XM and GR: designed the study, acquired the data, performed the statistical analysis, interpreted the data and wrote the manuscript. JYM, KM and ML: interpreted the data, critically reviewed the data and drafted a final version of the manuscript. SD, AG, XMA, EM, and LB: analyzed, interpreted and verified the individual center data and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1: Donation Flow Chart for the French cDCD Procedure

Appendix 1: The French Normothermic Regional Perfusion Protocol

Table S1: Characteristics of Donors in which a Kidney Graft was Transplanted vs. Discarded.

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Dynamic Liver Graft Preservation in Controlled Donation After Circulatory Death: What Is the Best Fit?

TO THE EDITOR:

We read with great interest the study by Ruiz et al., and we would like to discuss 3 issues raised by their results.⁽¹⁾

First, as highlighted by the authors, their specific normothermic regional perfusion (NRP) protocol with premortem cannulation in an interventional radiology facility leads to less technical failures (1%) compared with postmortem cannulation (5.6%) but is not applicable in other countries for ethical reasons.⁽²⁾ The authors state that the use of super-rapid procurement in controlled donation after circulatory death (cDCD) would be unethical at their center, whereas a recent randomized controlled trial showed a reduction in symptomatic nonanastomotic biliary strictures after additional end-ischemic hypothermic oxygenated perfusion (HOPE).⁽³⁾ These examples illustrate the upcoming challenge for the transplant community to implement evidence on best practice in dynamic preservation within a specific ethical framework for organ donation.

Second, in this study, the functional donor warm ischemia (fDWI) threshold was <30 minutes with a median static cold storage <5 hours. In the initial French experience with NRP, 14 of 123 transplanted cDCD grafts had a fDWI >30 minutes (n = 9) or underwent static cold storage for >8 hours (n = 5). In this subgroup, a total of 3 early graft losses occurred (20%), with 1 primary nonfunction and 1 ischemic cholangiopathy.⁽⁴⁾ These results question the use of NRP in grafts with prolonged ischemia, and additional end-ischemic HOPE is now required in France in these grafts.

Third, the majority of discarded grafts (61%; n = 14/23) presented a visual quality concern or high transaminase release during NRP, similar to our experience (39%; n = 16/41).⁽²⁾ To further reduce discard rates, there is a consensus on the need to objectively assess graft function and injury beyond visual inspection, transaminase release, and lactate clearance. A promising candidate is flavin mononucleotide, a mitochondrial viability marker assessable in real time during NRP and/or ex situ HOPE and correlating with post-transplant outcomes.⁽⁵⁾

In conclusion, a direct comparison of different preservation strategies with validation of viability markers is needed to determine the best fit for cDCD grafts with prolonged ischemia.

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Xavier Muller designed the study and wrote the article. Guillaume Rossignol, Kayvan Mohkam, Mickaël Lesurtel, and Jean-Yves Mabrut critically reviewed the article and drafted a final version.

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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

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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. One-hundred and fifty-six 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 \pm 44 min in patients with EAD vs. 181 \pm 42 min in patients without, $P = .286$). There was no significant association between NRP duration and MEAF score ($P = .347$). 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-h perfusion represents an optimal time window.

KEYWORDS

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

1 | INTRODUCTION

With growing indications for liver transplantation (LT), the number of recipients has increased (+3.3% in France in the past 5 years¹), 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 arrest⁴ 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 and 4 h,⁶ mostly based on the actual clinical practice. Initially, preclinical models first demonstrated that 30 min 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.

2 | PATIENTS AND METHODS

2.1 | 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 guidelines.⁹

2.2 | 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 45 min, and donors age up to 70 years 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 h maximum. Heparin was administered at the time of support withdrawing. The “no-touch” period after cardiac arrest was 5 min. Functional donor WIT, defined by the time between mean arterial pressure below 45 mmHg 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 h. NRP pump flow was maintained between 2 and 3.5 L/min, and NRP lasted between 1 and 4 h. In case of concomitant lung procurement, two techniques were possible: (i) either first lung procurement while NRP was ongoing for more than 1 h and then usual abdominal procurement, and (ii) or concomitant procurement of lungs and abdominal organs with duration of NRP of 1 h. 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.

2.3 | 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.

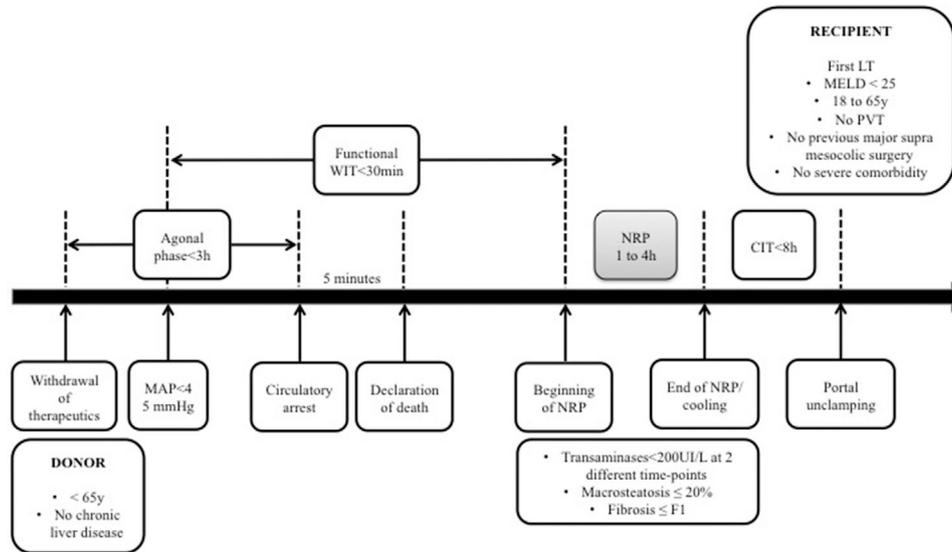


FIGURE 1 Liver graft procurement in the setting of NRP-cDCD donor in France at the time of the study. Freely adapted from the 6th version of the protocol from the Biomedicine agency. CIT: cold ischemia time, MAP: mean artery pressure, LT: liver transplantation, MELD: model for end-stage liver disease, NRP: normothermic regional perfusion, PVT: portal vein thrombosis, WIT: warm ischemia time

2.4 | 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 10 mg/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 Network¹⁴ 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

2.5 | 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 .05 were considered statistically significant in all tests. Statistical analyses were performed using Prism v8.

3 | RESULTS

3.1 | 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.

3.2 | 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 h according to protocol with a mean duration of 5.8 h.

3.3 | 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

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 gender	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).

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

postoperative period. Regarding biliary complications, the overall rate was 16.7% (26 patients), including six (3.9%) patients with non-anastomotic biliary strictures. Of note, 67 (42.9%) patients had a magnetic resonance cholangiography during their follow-up. Overall, six (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.

3.4 | Impact of NRP duration on post-transplant outcomes

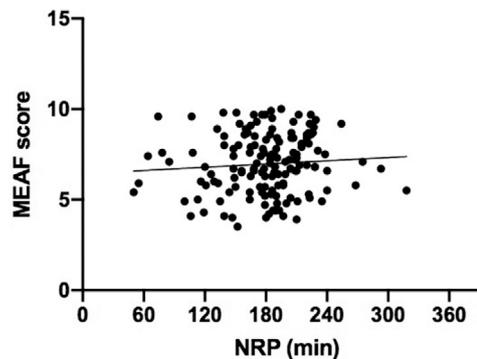
Mean duration of NRP was 179 (\pm 43) min. NRP duration was below 60 min only in two patients, below 90 min in six patients, whereas it lasted 240 min and more in seven patients. There was no impact of NRP duration on EAD according to Olthoff's criteria (170 \pm 44 min in patients with EAD vs. 181 \pm 42 min in patients without, $P = .286$). There was no significant association between NRP duration and ALT levels at post-transplant day one ($P = .837$), AST levels/peaks at post-transplant day one ($P = .626$), and MEAF score ($P = .347$, data available for 147 patients, see Figure 2). Although the MEAF score was significantly lower when IGL-1 was used (6.7 \pm 1.6 vs. 8.1 \pm 1.4 with SCOT 15, $P < .0001$), it was not different according to NRP duration when either

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).

Abbreviations: IGL, institute George Lopez; NRP, normothermic regional perfusion; SCOT, *solution de conservation des organes et des tissus*; WIT, warm ischemia time.

**FIGURE 2** Distribution of MEAF according to NRP duration (linear regression). NRP: normothermic regional perfusion

IGL-1 ($n = 112$, $P = .104$) or SCOT 15 ($n = 34$, $P = .303$) were used for static preservation.

There was no difference in NRP duration in terms of biliary outcomes ($P = .600$ for overall biliary complications, and $P = .196$ 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 \pm 44 min in patients with KDIGO 1, 2 and 3 vs. 188 \pm 31 min in patients without acute kidney injury, $P = .209$).

4 | 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-h 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' program across France. Like in other countries,⁶ the duration of NRP was empirically set between 1 and 4 h. 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 60–90 min of NRP duration. A reduced NRP time (1 h) could also speed up the retrieval process, reducing the strain on retrieval and anesthetic 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 min of potassium-induced cardiac arrest. The authors demonstrated that 4 h 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.

AUTHOR CONTRIBUTIONS

T.G., X.M., F.R., C.G., G.P., E.S., A.G.S.L., EM collected data. T.G., P.B., L.B. performed statistical analysis and wrote the original manuscript. L.B. supervised and wrote the original manuscript. E.S., M.L., M.A.A., P.B., E.S., O.S., J.C.V., A.G.S.L., E.M. edited the manuscript. O.S., F.D., A.G.S.L., J.C.V., O.S., L.S. collected data and edited the manuscript. M.Le, M.La and E.S. supervised, designed research, and edited the manuscript.

CONFLICT OF INTEREST

None of the authors declare a conflict of interest.

DATA AVAILABILITY STATEMENT

Data are not public but maybe available from the first author on reasonable request.

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Reply

With interest, we read the letter by Lee et al. and their study evaluating the value of serum thrombospondin-2 (TSP-2) as a predictive biomarker of advanced liver fibrosis ($F \geq 3$) (ALF) in diabetic patients with NAFLD.^[1] This large-scale cohort study demonstrates that serum TSP-2 level is capable of identifying those with ALF at high accuracy (area under the receiver operating characteristic curve [AUROC]: 0.80). They also show that serum TSP-2 level is independently associated with the presence of ALF. These findings are consistent with our study,^[2] further strengthening the high reliability of TSP-2 as a noninvasive biomarker of ALF in patients with NAFLD.

Meanwhile, as Lee et al. showed, there is a difference in the performance of TSP-2 in the diagnosis of ALF, which is comparable to Fibrosisi-4 index (FIB-4) and NAFLD fibrosis score (NFS) in our study, but significantly better than them in Lee et al.'s study. This may be attributed to the different clinical backgrounds of the patients in the two studies (diabetes mellitus complication rate was about 30% in our study and 100% in Lee et al.'s study), but it should be noted here that diagnostic accuracy of FIB-4 and NFS greatly differs between the two studies. Our data show relatively high accuracy of these tests (FIB-4 AUROC: 0.78; NFS AUROC: 0.81), which is consistent with the result of a recent meta-analysis of noninvasive tests for histologically defined ALF ($F \geq 3$) in patients with NAFLD (FIB-4 AUROC: 0.76 [$n = 5393$]; NFS AUROC: 0.73 [$n = 3248$]).^[3] In sharp contrast, their accuracy in Lee et al.'s study is surprisingly poor (FIB-4 AUROC: 0.60; NFS AUROC: 0.58).^[1] This result raises a concern of the accuracy of ALF diagnosis in Lee et al.'s study, which was done solely based on vibration controlled transient elastography value without histological evidence. Taken together, it is unclear whether discrepancy of diagnostic accuracy of FIB-4 and NFS for ALF between two studies is due to the suboptimal performance of these noninvasive tests in diabetic patients or to the difference of methodology used for ALF diagnosis. Therefore, it is desired for further careful comparison of the performance of TSP-2

and other noninvasive tests as a biomarker of ALF in diabetic patients with biopsy-proven NAFLD.

Nevertheless, these two studies complementary support the usefulness of serum TSP-2 in patients with NAFLD as a diagnostic biomarker of ALF and NASH as well as a predictive biomarker of future disease progression including fibrosis progression, shown by Lee et al., and occurrence of hepatic complications (liver cancer and decompensative events) shown by us.

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Letter to the editor: Is there a place for machine perfusion strategies in pediatric liver transplantation?

To the editor,
We would like to congratulate de Ville de Goyet et al. for their very interesting paper on the European experience with more than 16,000 pediatric liver

transplantations (LTs) over the last 50 years.^[1] Based on their results, we would like to highlight two potential applications of machine perfusion strategies in pediatric LT.

First, the study shows that there was a significant increase in the use of split liver grafts from deceased donors, representing one third of all pediatric LTs today. However, 10% of split liver grafts had prolonged cold ischemia time over 12 h (compared with only 5% for whole liver grafts), which was associated with a significant reduction in graft survival. Ex vivo machine perfusion is a promising strategy to reduce static cold ischemia and improve outcomes in split grafts. Our group has recently reported a standardized technique for liver graft splitting during HOPE, which is now prospectively used at our center and allows us to reduce cold ischemia time for split grafts.^[2]

Second, the study showed that over the last 50 years, only 86 whole donation after cardiac death (DCD) grafts were transplanted into pediatric recipients, with very encouraging 5-year graft survival of 93%. As for adult DCD LT, the use of machine perfusion may allow us to expand the use of DCD liver grafts for pediatric LT despite additional donor warm ischemia. For example, in a recent randomized controlled trial, HOPE has been shown to reduce ischemic cholangiopathies compared with static cold storage in DCD LT.^[3] Real-time viability assessment during machine perfusion could be an additional incentive to use DCD grafts for pediatric LT.^[4] Machine perfusion strategies may also allow us to split selected DCD livers by reducing cold ischemia time and mitigating the cumulative risk of cold and warm donor ischemia.

In conclusion, the study of de Ville de Goyet et al. identifies at least two promising applications for machine perfusion in pediatric LT. All of the stakeholders should set the necessary ethical and legal framework for future studies to investigate the feasibility and safety of machine perfusion in pediatric liver transplantation.

CONFLICT OF INTEREST

Nothing to report.

AUTHOR CONTRIBUTIONS

Manuscript draft: Guillaume Rossignol and Xavier Muller; *Manuscript review, revisions, and approval:* Jean-Yves Mabrut, Kayvan Mohkam, and Remi Dubois.

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Letter to the editor: Organ shortage and pediatric liver transplantation: David against Goliath...

To the editor,
Rossignol et al. reacted to our recent European Liver Transplant Registry (ELTR) analysis and questions whether our data support machine perfusion strategies in pediatric liver transplantation (PLT). The

authors argue that machine perfusion strategies may reduce total cold ischemia times. Rossignol et al. also interpret our data to extend indications for splitting non-heart-beating donor (NHBD) livers. Although we acknowledge that machine perfusion strategies are

Full left/full right liver graft ex situ split during hypothermic oxygenated perfusion

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Abstract

Background: Ex vivo split liver transplantation in pediatric recipients has shown inferior results compared with whole grafts. One factor among others contributing to split grafts being considered as marginal is the prolonged static cold storage time related to ex vivo liver splitting. End ischemic hypothermic oxygenated perfusion is a validated strategy to improve outcomes of marginal whole grafts and may thus also benefit split liver grafts.

Method: We present the first case of full left/full right split procedure performed during hypothermic oxygenated perfusion.

Results: We present a standardized surgical two-step approach where parenchymal transection was performed during end ischemic hypothermic oxygenated perfusion via the portal vein to shorten static cold storage duration. Both split grafts were successfully transplanted in a 4-year-old pediatric and a 38-year-old adult recipient. Despite high-risk procedure (retransplantation), extended donor criteria including a prolonged cardiac arrest and high donor risk index (2,25), both grafts showed early recovery of hepatic function and low serum transaminase release. At 6 months, both recipients were alive with a normal liver biology and a functioning graft.

Conclusion: Although challenging, full left/full right liver split procedure during end ischemic hypothermic oxygenated perfusion can be successfully performed and is a promising strategy to improve post-transplant outcomes.

KEYWORDS

machine perfusion, organ preservation, split liver transplantation

1 | INTRODUCTION

Split liver transplantation (SLT) has been developed to increase the number of available liver grafts for pediatric recipients. Liver graft splitting can be performed in situ during procurement but remains performed ex situ due to logistical challenges, resulting in prolonged

static cold storage (SCS) time.^{1,2} This negatively impacts postoperative outcomes^{2,3} and contributes to split liver grafts being considered as marginal grafts.⁴

End ischemic ex vivo hypothermic oxygenated machine perfusion (HOPE) has been shown to reduce ischemia/reperfusion injury, shorten static cold storage time and improve post-liver

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; DBD, donation after brain death; DRI, donor risk index; FL, full left; FR, full right; GGT, Gamma-glutamyl transferase; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; LHA, left hepatic artery; LT, liver transplantation; MHV, middle hepatic vein; POD, postoperative day; PV, portal vein; RHA, right hepatic artery; SCS, static cold storage; SFSS, small-for-size syndrome; SLT, split liver transplantation.

transplantation (LT) outcomes, especially in marginal grafts from extended criteria donor and donation after controlled circulatory death.⁵⁻⁷ Consequently, HOPE during liver graft splitting may improve graft quality and benefit both pediatric and adult recipients.⁸

In pediatric SLT, the left lateral graft is the gold standard. However, in some cases, a full left split liver graft is required to achieve an optimal graft/donor size matching. While left lateral segment splitting during HOPE has already been described⁹⁻¹¹ we present the first case of full left/full right (FL/FR) splitting during HOPE, a technically more challenging procedure, resulting in two successful liver transplantations in a pediatric and an adult recipient.

2 | CASE REPORT

The use of HOPE during ex vivo splitting procedure at our institution was approved by the local ethics committee (CSEHCL_21_202).

2.1 | Clinical history

2.1.1 | Pediatric recipient

The pediatric recipient was a 4-year-old girl weighting 15 kg and listed for a liver retransplantation. She underwent a first LT with a left lateral graft at the age of three for primary sclerosing cholangitis. She developed a hepatic artery thrombosis leading to a non-anastomotic biliary stricture with recurring biliary sepsis and secondary biliary cirrhosis. After multidisciplinary concertation, the patient was listed for a liver retransplantation.

2.1.2 | Adult recipient

The adult recipient was a 38-year-old women, weighting 62 kg, with a history of auto-immune liver cirrhosis complicated by liver insufficiency (MELD score 20) and portal hypertension.

2.1.3 | Donor characteristic

The donor was a brain-dead 12-year-old girl, weighting 40 kg. The cause of death was hypoxic brain injury with an initial cardiac arrest of 40 min (No Flow: 15 min; Low flow: 25 min).

Time from intensive care unit (ICU) admission to organ donation was 8 days. Serum transaminase levels after cardiac arrest were 10 times the upper limit of normal and returned to baseline during ICU stay. Of note, the prolonged duration of cardiac arrest and ICU stay resulted in a donor risk index (DRI) of 2,25 which were outside our center's split graft selection criteria. However, given the current organ shortage, the donor was deemed eligible for organ procurement regarding the young age and normalization of serum transaminase levels.

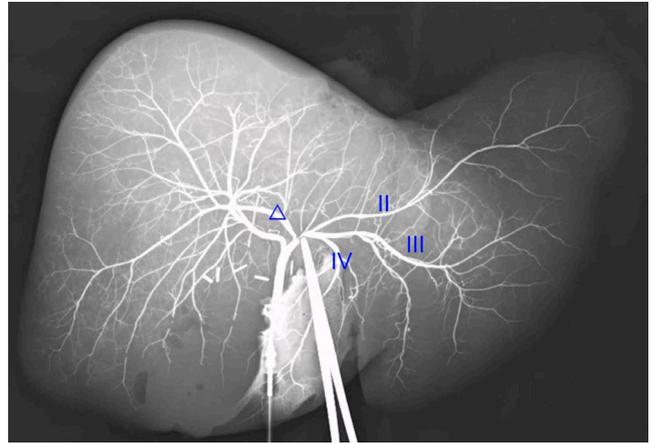


FIGURE 1 Bench cholangiography. Δ: Right posterior bile duct

2.2 | Split procedure

We used a two-step technique for liver splitting with concurrent HOPE as previously described for left lateral splitting.⁹ Surgical particularities of the FL/FR splitting were then applied to our previous knowledge of HOPE-splitting.

First, pedicular dissection was performed during static cold storage to identify the portal vein and the hepatic artery division. The portal vein (PV) was dissected but not divided in order to allow concurrent perfusion of both left and right split grafts with a single 25F portal cannula. The left hepatic artery (LHA) was divided at its origin leaving the right hepatic artery (RHA) with the common hepatic artery. The hilar plate was dissected after cholangiography which showed a branch of the right posterior hepatic duct draining into the left bile duct. In addition, the bile duct of segment IV drained into the segment III with a segment II closed to the convergence leading to a very short common left hepatic duct [Reichert III¹²]. The bile duct was divided to allow a single bile duct anastomosis in both recipients as presented on [Figure 1](#).

Secondly, parenchymal transection was performed during HOPE using the Liver Assist© perfusion device. Single perfusion was performed via a portal cannula at a constant pressure of 4 mmHg and a portal flow of 150 ml/min at 10°C. Transection was done using bipolar/ultrasonic device and facilitated by the "split hanging maneuver" as previously described.⁹ In detail, the caudate approach was used to draw the transection plane, leaving the gallbladder and segment I with the full right graft. A vessel loop was placed through the transection plane, underneath the PV division and between the distal part of the right hepatic vein and the common trunk of the hepatic veins. ([Figure 2-Panel A](#)) The liver was then turned upside down and suspended by the "split hanging maneuver" ([Figure 2-Panel B](#)). This enabled an optimal portal perfusion by avoiding graft compression and cannula kinking while exposing the transection plane. At the end of the split procedure, running sutures were performed on the transection surface for better hemostasis upon reperfusion in the recipient. The middle hepatic vein (MHV) remained with the FL graft to facilitate pediatric LT by avoiding any hepatic vein reconstruction.

FIGURE 2 Full left/full right liver splitting procedure during hypothermic oxygenated perfusion. Panel (A): Transection plan; Panel (B): Split hanging maneuver; Panel (C): Both grafts remained perfused through one single portal cannula; Panel (D-E): Segment VIII hepatic vein jump graft reconstruction

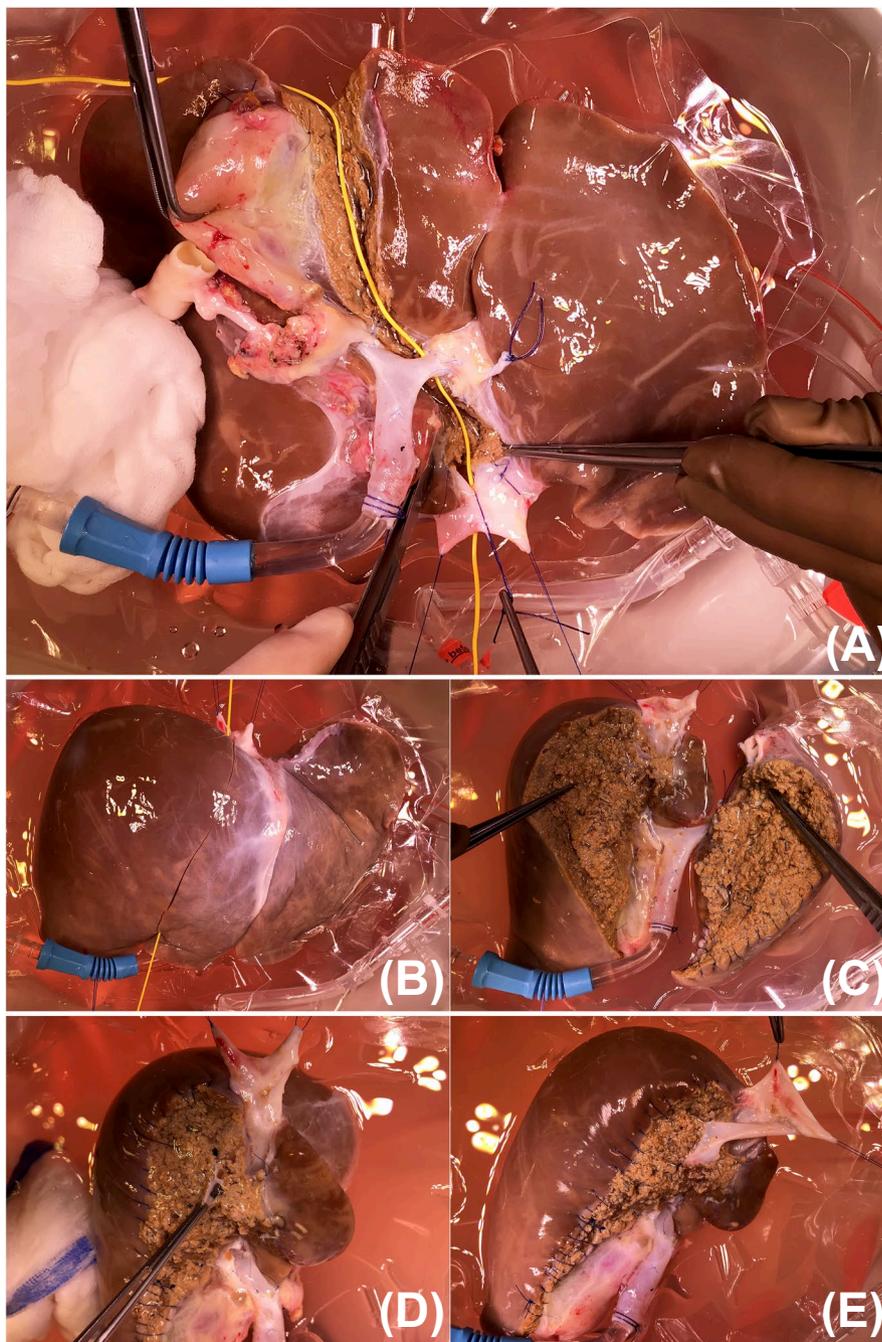
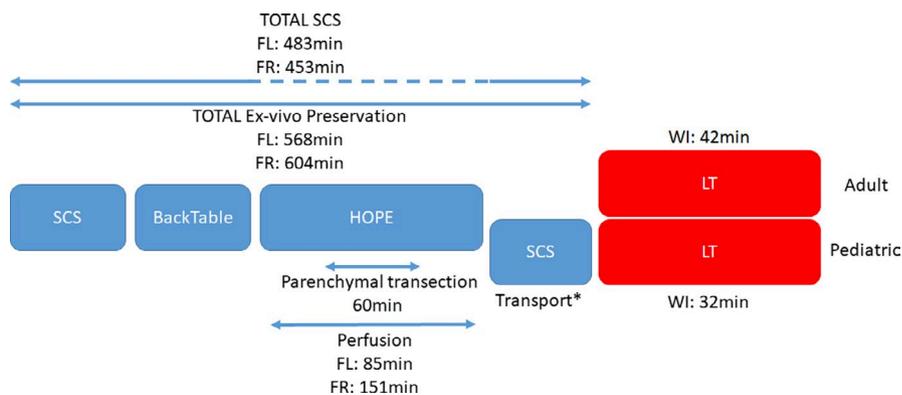


FIGURE 3 Liver transplantation procedure. (SCS: Static Cold Storage; FL: Full Left; FR: Full Right; LT: Liver Transplantation; WI: Warm Ischemia; *: transport to the pediatric transplant center)



Thus, in the FR graft a significant segment VIII hepatic vein was reconstructed during HOPE using a venous iliac jump graft from the same donor (Figure 2-Panel D-E).

Both grafts remained perfused until recipient hepatectomies were carried out (Figure 2-Panel C), resulting in total HOPE durations of 85 min for the FL graft and 151 min for the FR graft. Additional cold ischemia (30 min) was added after HOPE in the case of the FL due to transport to the pediatric transplant center. Total static cold storage time was 483 min and 453 min for the FL and FR liver graft, respectively (Figure 3). Full liver graft weight was 960 g and FL/FR liver grafts weighted 370 g and 590 g, respectively. The graft weight ratio for the pediatric and adult recipients were 2.4% and 0.95%, respectively.

2.3 | Outcomes

2.3.1 | Pediatric recipient

We observed no primary non-function and a normalization of the factor V at postoperative day (POD) 3. Transaminase peak was obtained at POD 2 (Figure 4). Reintervention was necessary at POD 10 for an infected intra-abdominal hematoma. During the post-LT course, increase of bilirubin and GGT at POD 90 revealed an anastomotic biliary stricture requiring percutaneous transhepatic

cholangiodrainage. After 6 months, hepatic function, transaminase levels, and bilirubin were normal.

2.3.2 | Adult recipient

We observed no primary non-function and a normalization of the factor V at POD 2. Transaminase peak was obtained at POD 1 (Figure 4). The adult recipient underwent a reintervention at POD 14 for an intra-abdominal collection and a percutaneous transhepatic cholangiodrainage for a biliary leak of the transection surface at POD 20. A small-for-size syndrome (SFSS) was encountered based on both clinical and biological parameters (Portal flow over 250 ml/min; ascites over 1000ml/day during the first week; prolonged hyperbilirubinemia during the first week) requiring splenic artery ligation during the first reintervention. After 6 months, hepatic function, transaminase levels, and bilirubin were normal.

3 | DISCUSSION

We herein report the first report of an ex situ FL/FR split procedure with concurrent HOPE in a high-risk donor resulting in two successful LT in a pediatric and an adult recipient. Using technical refinements including the "split hanging maneuver" after

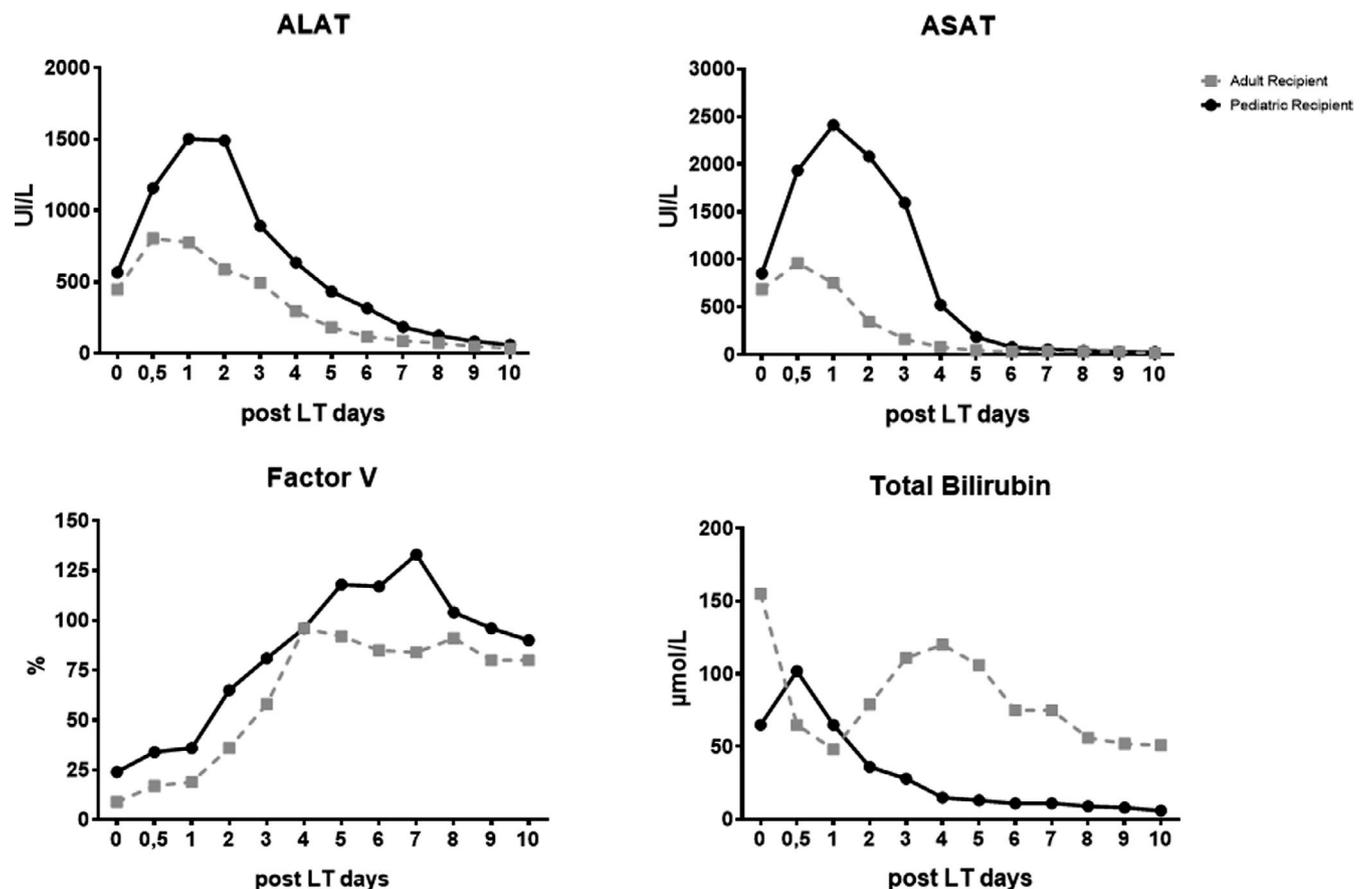


FIGURE 4 Serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), Factor V and Total Bilirubin, of full left pediatric recipient and full right adult recipient during the first postoperative days

mandatory bench cholangiography, HOPE was performed without any technical issue for 85min in the FL graft and 151min in the FR graft. Both recipients had well-functioning grafts at 6 months after transplantation.

HOPE is a validated strategy to reduce ischemia-reperfusion injury and improve post-LT outcomes of marginal grafts.^{5,7,13} Based on these findings, we propose to use HOPE to improve preservation of ex situ split grafts. Indeed, ex vivo liver graft splitting is a demanding surgical procedure, which remains often preferred to in situ splitting due to logistic advantages. However, ex situ splitting may lead to longer static cold storage time which negatively impact post-LT outcomes^{1,2} and rewarming of the graft during back table splitting with additional heat of the transection device. HOPE reduces static cold storage by adding a period of dynamic hypothermic oxygenation which can be prolonged until both recipients' hepatectomies are completed. It also prevents rewarming of the graft during ex vivo splitting by improving cooling of the graft. Of note, HOPE was performed selectively for each graft once the split procedure was completed resulting in a total perfusion time of more than 1 h for both grafts, which is known to be sufficient in order to decrease potential ischemia-reperfusion injury in DBD (donation after brain death) grafts.¹⁴

We would like to highlight several technical aspects of this case.

First, during the entire transection phase HOPE was performed at a constant perfusion flow (150–200 ml/min) at low portal pressure (3–4 mmHg) in order to prevent any liver damage in the setting of hypothermia (8–12°C).

Second, one challenging step during FL/FR splitting procedure is the division of the hilar plate without bile duct injury. When possible, this dissection should also leave one single bile duct to allow one single biliary anastomosis for both grafts. To ensure safe bile duct division, bench cholangiography should be considered as a mandatory step,¹⁵ especially during FL/FR split procedure. Anatomical variations which could compromise LT are encountered in 10% and a precise identification of case specific anatomy may avoid biliary injury.¹⁶ Cholangiography has to be done during back table as it is not possible during HOPE. In this case, bench cholangiography was helpful as it showed a right posterior sectorial bile duct draining into the left common bile duct. Hence, the surgical team was able to carefully preserve the right biliary drainage and performed sharp dissection of the bile duct to leave one common bile duct on both sides, thereby facilitating biliary anastomosis during SLT. During the postoperative course, the pediatric recipient presented with an anastomotic biliary stricture and the adult recipient with a biliary leakage of the transection plan. Both patients underwent percutaneous transhepatic cholangiodrainage. We did not identify any symptomatic non-anastomotic bile stricture during the 6 months after LT in both recipients.

Third, "Split hanging maneuver" facilitated parenchymal transection during HOPE. As described in this case, this maneuver allowed for a dorsal and caudate approach with the same anatomical landmarks as a standard hepatectomy. Furthermore, the liver remained steady and emerged in the cold perfusion solution during

transection. It allowed to decrease liver mobilization and graft rewarming and facilitated liver transection as FL/FR splitting has the largest cut surface plan.

Fourth, as described by Colledan et al,¹⁷ the MHV should remain with the FL graft in order to avoid any venous reconstruction thereby prioritizing the pediatric recipient. However, segment VIII or segment V hepatic veins often drain through the MHV thus requiring reconstruction of one or both veins of the FR graft with a jump graft. In this particular case, we performed a reconstruction of a significant segment VIII hepatic vein during HOPE without prolonging static cold storage.

Finally, at our center, we advocate the use of HOPE for SLT by single portal perfusion in contrast to dual portal and arterial perfusion (D-HOPE).¹⁰ Indeed, in case of D-HOPE, arterial cannulation may lead to arterial injury. It also requires the hepatic artery division to be performed at the end to allow a continuous dual perfusion of the liver which adds complexity to the procedure. Together with the absence of clear data on the benefit of D-HOPE over HOPE in DBD grafts we advocate single portal perfusion.^{18,19} However, despite a more challenging procedure in the setting of liver splitting, feasibility of D-HOPE has already been shown and remains a suitable option.¹¹

Another issue raised by this case report is the graft-recipient matching in SLT. Prolonged cardiac arrest and duration of ICU stay prior to organ donation were outside generally admitted split criteria²⁰ resulting in a DRI of 2,25 for the pediatric LT. However, given the age of the donor, the recipient status and current organ shortage, the liver graft was selected for a splitting procedure. In addition, the surgical procedure was challenging due to retransplantation scenario. Despite the high-risk donor-recipient matching and surgical difficulty, both factor V normalization and transaminase peak were observed before POD3. The adult LT was also challenging with poor size matching (<1%) and major portal hypertension in the recipient. Despite a SFSS requiring portal flow modulation by splenic artery ligation during the reintervention, biological parameters regarding hepatic function (transaminases release and factor V) normalized within 48h and no PNF nor vascular complication was encountered. We postulate that by reducing static cold storage and protecting from mitochondrial damage, HOPE may be beneficial in the setting of high-risk recipient-donor matching and difficult SLT scenarios.^{21,22} Further larger studies are needed to confirm this hypothesis.

We conclude that ex situ FL/FR liver graft splitting with concurrent HOPE is technically feasible and safe. We report the first case of an ex situ FL/FR performed during HOPE which facilitates parenchymal transection and reduces static cold storage resulting in two successful SLT in a pediatric and an adult recipient. High-risk SLT scenarios may benefit from machine perfusion and this strategy should be further explored in larger studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Guillaume Rossignol and Xavier Muller contributed equally as first authors, designed the study, acquired the data, performed the statistical analysis, interpreted the data, and wrote the manuscript. Kayvan Mohkam, Remi Dubois, Mickael Lesurtel, and Jean-Yves Mabrut designed the study, interpreted the data, critically reviewed the data, and drafted a final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. Restrictions may apply.

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ORIGINAL ARTICLE

Liver transplantation of partial grafts after ex situ splitting during hypothermic oxygenated perfusion—The HOPE–Split pilot study

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Abstract

Partial liver grafts from ex situ splitting are considered marginal due to prolonged static cold storage. The use of ex situ hypothermic oxygenated perfusion (HOPE) may offer a strategy to improve preservation of ex situ split grafts. In this single-center pilot study, we prospectively performed ex situ liver splitting during HOPE (HOPE–Split) for adult and pediatric partial grafts over a 1-year period (November 1, 2020 to December 1, 2021). The primary safety endpoint was based on the number of liver graft–related adverse events (LGRAEs) per recipient, including primary nonfunction, biliary complications, hepatic vascular complications, and early relaparotomies and was compared with consecutive single-center standard ex situ split transplantations (Static–Split) performed from 2018 to 2020. Secondary endpoints included preservation characteristics and early outcomes. Sixteen consecutive HOPE–Split liver transplantations (8 HOPE–Split procedures) were included and compared with 24 Static–Splits. All HOPE–Split grafts were successfully transplanted, and no graft loss nor recipient death was encountered during the median follow-up of 7.5 months (interquartile range, 5.5–12.5). Mean LGRAE per recipient was similar in both groups (0.31 ± 0.60 vs. 0.46 ± 0.83 ; $p = 0.78$) and split duration was not significantly increased for HOPE–Split (216 vs. 180 min; $p = 0.45$). HOPE–Split grafts underwent perfusion for a median of 125 min, which significantly shortened static cold storage (472 vs. 544 min; $p = 0.001$), whereas it prolonged total ex vivo preservation (595 vs. 544 min; $p = 0.007$) and reduced neutrophil infiltration on reperfusion biopsies

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BA, biliary atresia; BAR, Balance of Risk; BMI, body mass index; CCI, Comprehensive Complication Index; CI, cold ischemia time; COD, cause of death; DRI, Donor Risk Index; EAD, early allograft dysfunction; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOPE, hypothermic oxygenated perfusion; I/R, overall IRI score; ICU, intensive care unit; IDEAL, Innovation, Development, Exploration, Assessment, and Long-term study; IQR, interquartile range; IRI, ischemia/reperfusion injury; LGRAE, liver graft–related adverse event; LT, liver transplantation; macro. S, macrovesicular steatosis; micro. S, microvesicular steatosis; MELD, Model for End-Stage Liver Disease; NASH, nonalcohol-associated steatohepatitis; PELD, Pediatric End-Stage Liver Disease; PNF, primary nonfunction; PNN, neutrophilic infiltrate; POD, postoperative day; PSC, primary sclerosing cholangitis; RCT, randomized controlled trial; reTH, retransplantation; SLT, split-liver transplantation; UCLA, University of California, Los Angeles.

($p = 0.04$) compared with Static–Split. This clinical pilot study presents first feasibility and safety data for transplantation of partial liver grafts undergoing ex situ split during HOPE and suggests improved preservation compared with static ex situ splitting. These preliminary results will allow to set up large-scale trials on the use of machine perfusion in pediatric and split-liver transplantation.

INTRODUCTION

Split-liver transplantation (SLT) has been developed to increase the number of available grafts and offer a rapid access to transplantation for pediatric recipients. Since the first experience with SLT, outcomes have continuously improved, reaching a 5-year graft survival rate of 80% over the last decade.^[1] In situ liver graft splitting during procurement has achieved the best posttransplantation outcomes but consists in a technically and logistically challenging procedure.^[2] Consequently, ex situ liver splitting remains frequently performed.^[3] As a result of technical aspects inherent to the ex situ split, these partial grafts have prolonged static cold storage time, which negatively impacts graft survival despite the use of optimal donors.^[1,4–6] A recently validated strategy to optimize preservation of marginal whole-liver grafts is the use of end-ischemic hypothermic oxygenated perfusion (HOPE).^[7–9] This ex situ machine perfusion strategy has been shown to mitigate ischemia/reperfusion injury (IRI) of liver grafts by reducing static cold storage and uploading cellular energy levels with a positive impact on posttransplantation outcomes.^[10] Given these encouraging data from whole grafts, the use of HOPE may also benefit partial grafts from ex situ splits and improve outcomes in both pediatric and adult recipients.^[7,11] The first case report on ex situ liver splitting during end-ischemic HOPE was reported by Spada et al.^[12] and to date only single-case reports focusing on technical issues have been published.^[13,14] Consequently, we designed a clinical pilot study (IDEAL stage 2a; Innovation, Development, Exploration, Assessment, and Long-term study) to assess feasibility and safety as well as reporting preservation characteristics and early outcomes of adult and pediatric LT with partial grafts after ex situ splitting with concurrent HOPE.

MATERIALS AND METHODS

Study design

This is a prospective pilot study at a single institution covering 1 year (November 1, 2020 to December 1, 2021). During the study period, all consecutive liver graft split procedures were performed with concomitant HOPE by the same surgical team. The aim of the pilot study was

to show the feasibility and safety of HOPE–Split in a clinical setting and report early outcomes of this new procedure as recommended by the IDEAL guidelines (Stage 2a).^[15,16] To assess safety and identify relevant hypothesis for future large-scale trials, we added a comparator cohort, which included all consecutive pediatric and adult recipients transplanted with liver grafts split ex situ during static cold storage (the Static–Split group) at our center from January 1, 2018 to November 1, 2020 (Figure 1). The study protocol was approved by the local ethics committee (CSEHCL_21_202).

Study population

In France, partial liver grafts are allocated with prioritization to a pediatric recipient based on national allocation rules. By contrast, the contralateral partial liver graft obtained after splitting and used for an adult recipient is allocated to the regional center performing the ex situ splitting procedure, giving the opportunity to choose the recipient based on morphological characteristics and local priority. We did not modify our donor selection criteria and donor/recipient matching for HOPE–Split grafts and included all consecutive grafts performed during the study period in the HOPE–Split group. Whole grafts were considered for splitting based on donor age, body mass index, intensive care unit stay, no-flow time, and donor biology. The Donor Risk Index^[17] and Balance of Risk (BAR) score^[18] were used to stratify pretransplantation donor–recipient matching. Partial grafts from living donations, in situ liver splitting, full left/full right splits, and split and/or transplantation procedures performed by another surgical team were excluded. Of note, no in situ split was performed at our center during the study period and all split procedures were performed with concomitant HOPE during the study period.

HOPE–Split procedure

The procedure for liver graft splitting with concurrent HOPE has been previously standardized and reported.^[13] All HOPE–Split procedures included in the study were performed using the following steps. After procurement at the donor hospital, the liver graft was transported to the transplantation center in IGL-1 (Institut Georges Lopez,

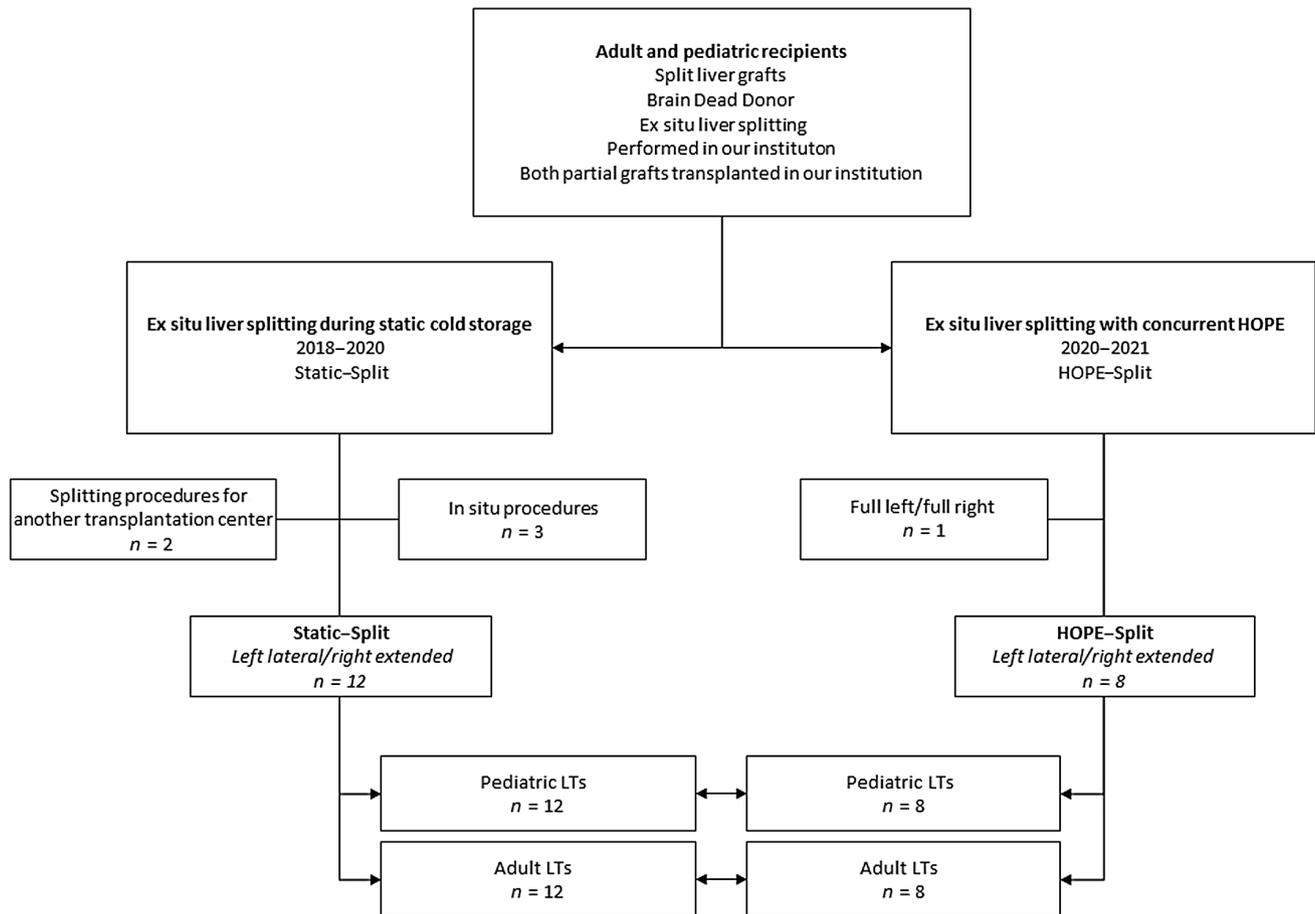


FIGURE 1 The HOPE-Split study flowchart

Lissieu, France) preservation solution. The first step of the HOPE-Split procedure was performed during static cold storage on the back table at the transplantation center. It included a pedicular dissection aimed at identifying the portal vein and the hepatic artery division. The portal vein was not divided to allow perfusion of both partial grafts with a single portal cannula. The right hepatic artery was divided at its origin for the right extended graft. The left hepatic artery and the common hepatic artery remained with the left lateral graft. A cholangiography was performed during back table for all cases to evaluate graft-specific biliary anatomy. The second step of the split procedure, namely, parenchymal transection, was performed during HOPE. HOPE was performed as previously reported through a 25F portal canula at 8–10°C using the Liver Assist Machine perfusion device (XVIVO, Groningen, The Netherlands) with Machine Perfusion Solution (Belzer MPS, Bridge to life, Northbrook, IL). Of note, both partial grafts were perfused through a single portal cannula at a perfusion pressure of 3–5 mm Hg to achieve a portal flow of 150–300 ml/min. Parenchymal transection was first performed with a caudate approach to determine the transection plan. Then, parenchymal transection was completed with an anterior approach facilitated by a “split hanging maneuver.” The left hepatic

vein was divided at the end of the parenchymal transection. Of note, the portal vein was only divided as soon as one recipient hepatectomy was carried out. This allowed to prolong perfusion of the remaining partial graft using the same portal cannula without interrupting perfusion (Figure 2).

Endpoints

The primary safety endpoint was defined as the mean number of liver graft-related adverse events (LGRAEs) per patient during the first 30 days after transplantation, similar to a recent randomized controlled trial (RCT) on machine perfusion of whole grafts.^[19] LGRAEs included primary nonfunction, biliary complications, hepatic vascular complications, and early relaparotomies. A second safety endpoint, focusing on technical feasibility, was the mean duration of the Split procedure.

Secondary endpoints included evaluation of preservation characteristics and early post-transplant outcomes. Static cold storage duration was defined as the time from in situ cold flush in the donor to HOPE. Total ex vivo preservation time was defined as the time from in situ cold flush in the donor to the start of implantation,

TABLE 1 Donor characteristics

Characteristics	Static-Split (<i>n</i> = 12)	HOPE-Split (<i>n</i> = 8)	<i>p</i> value
Sex			
Male	5 (42)	5 (63)	0.36
Female	7 (58)	3 (37)	
Age, years	20 (17–28)	21 (19–27)	0.65
BMI, kg/m ²	22 (20.8–23.1)	21.3 (17.8–24.2)	0.86
COD			
Trauma	5 (42)	4 (50)	0.74
Hypoxic brain injury	5 (42)	0 (0)	0.03
Cerebrovascular	2 (16)	4 (50)	0.11
Cardiac arrest	4 (33)	1 (12)	0.29
Vasopressive drugs	7 (58)	6 (75)	0.44
ICU stay, days	3 (2–4)	2 (2–4)	0.46
Na, mmol/L	147 (140–148)	146 (142–149)	0.86
AST peak, UI/L	51 (61–120)	57 (39–82)	0.19
ALT peak, UI/L	26 (20–51)	28 (20–43)	0.46
Lactate peak, mmol/L	3.4 (2.6–4.7)	3.6 [1.8–4.9)	0.86
DRI	2.08 [1.97–2.26]	2.04 [1.88–2.23]	0.86

Note: Data are expressed as *n* (%) or median (IQR).

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; COD, cause of death; DRI, Donor Risk Index; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; IQR, interquartile range.

including the HOPE and split procedures. Split duration was defined as the time from the beginning of back-table preparation during static cold storage to the end of parenchymal transection and cut-surface hemostasis.

Early graft function was assessed and graded according to the Olthoff criteria (early allograft dysfunction [EAD])^[20] and the L-GrAFT7^[21] score. Overall and 90-day graft and patient survival were assessed as well as morbidity using the Comprehensive Complication Index (CCI).^[22] Major posttransplantation complications with Clavien–Dindo grade ≥3 during the first 90 days were reported separately. A separate outcome analysis for pediatric and adult SLT was performed.

Histological analysis

Evaluation of IRI was based on reperfusion biopsies, which are routinely performed at our center during LT after graft reperfusion in the recipient. Biopsy samples were formalin fixed, paraffin embedded, and stained with (hematoxylin-eosin stained), Perls (to assess hemosiderin deposits), and Sirius Red (to assess fibrosis). A blinded reading by two independent and experienced pathologists was performed for all reperfusion biopsies. IRIs were assessed according to a standardized classification adapted from the UCLA (University of California, Los Angeles) classification^[23] and Suzuki classification.^[24] In detail, the presence of hepatocyte necrosis, neutrophilic infiltrate, and sinusoidal congestion were semiquantitatively scored, while hepatocyte ballooning, and micro and

macrosteatosis were quantitatively scored (Table S1). Based on this analysis of individual liver compartments, the overall IRI was ranked as grade 0 for absence of IRI, grade 1 for minimal IRI, grade 2 for mild IRI, grade 3 for moderate IRI, and grade 4 for severe IRI.

Statistical analysis

Categorical variables were expressed in quantities and percentages while continuous variables were expressed as median with interquartile range (IQR) except for LGRAE (mean ± standard deviation). Continuous variables were compared using the Mann–Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test.

Any *p* value <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows (version 26.0; IBM Corp., Armonk, NY) and GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, CA).

RESULTS

A total of 8 HOPE–Split procedures resulting in 16 consecutive partial liver graft transplantations after HOPE–Split (8 adult recipients and 8 pediatric recipients) were performed during the study period with a median follow-up of 7.5 months (IQR, 5.5–12.5). The comparator cohort consisted of 24 partial liver grafts

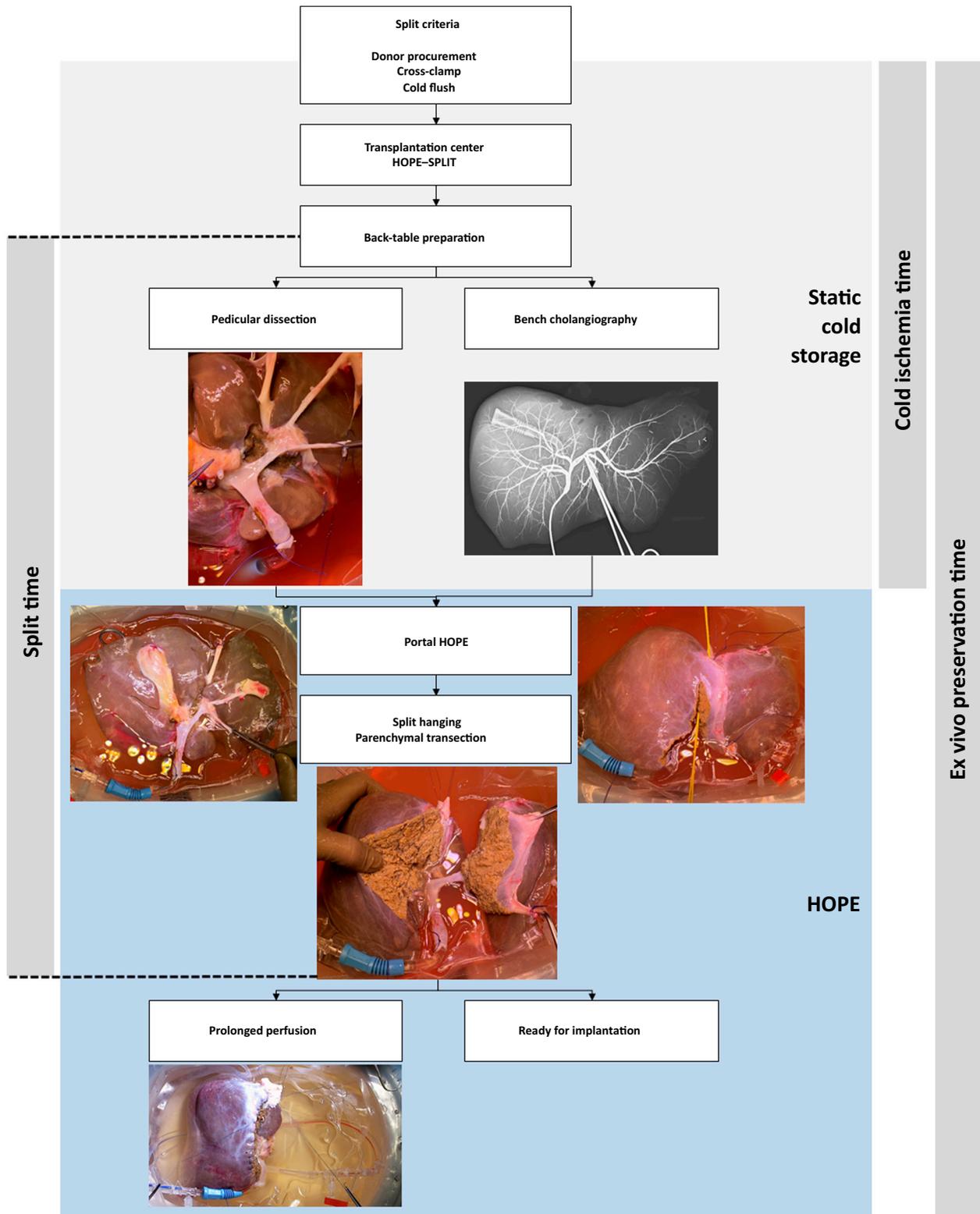


FIGURE 2 Detailed HOPE-Split procedure

(12 adult recipients and 12 pediatric recipients) transplanted after standard ex situ split during static cold storage (Static-Split group). All grafts included in the study were right extended/left lateral partial grafts.

Given the strict donor selection criteria, the median donor age was 20 years (IQR, 18–27) with a median donor intensive care unit stay of 3 days (IQR, 2–4) in the HOPE-Split group (Table 1).

TABLE 2 Recipient characteristics and surgical data

Group	Adult			Pediatric		
	Static-Split (n = 12)	HOPE-Split (n = 8)	p value	Static-Split (n = 12)	HOPE-Split (n = 8)	p value
Sex						
Male	9 (75)	4 (50)	0.25	4 (33)	2 (25)	0.69
Female	3 (25)	4 (50)		8 (67)	6 (75)	
Age ^a	47.5 (26–63.5)	56 (43–60)	0.52	24.8 (11.6–39.5)	42.1 (23.6–65)	0.27
Weight (kg)	60.3 (54.5–72)	63 (57.5–73)	0.57	11.0 (9.0–13.8)	15.5 (11–17.8)	0.18
Etiology ^b						
Adult						
Alcohol	3 (25)	1 (12)	0.49			
HBV	1 (8)	4 (50)	0.04			
HCV	3 (25)	0 (0)	0.13			
HCC	7 (58)	4 (50)	0.71			
NASH	1 (8)	1 (12)	0.76			
Pediatric						
BA				5 (42)	4 (50)	0.85
PSC				1 (8)	1 (12)	0.76
Metabolic				2 (17)	0 (0)	0.22
Ischemic cholangiopathy				0 (0)	2 (25)	0.27
Acute liver failure	0 (0)	0 (0)	–	3 (25)	1 (12)	0.49
Others	3 (25)	1 (12)	0.49	4 (33)	1 (12)	0.2
MELD/PELD	9 (8–13)	12 (8–18)	0.31	24 (16–39)	17 (13–21)	0.21
Major previous surgery	4 (33)	3 (37)	0.85	4 (33)	5 (62)	0.19
reTH	0 (0)	0 (0)	–	0 (0)	2 (25)	0.27
BAR	2 (1–4)	6 (4–7)	0.03	6 (5–15)	6 (4–10)	0.34
Split (min)	180 (173–244)	216 (170–240)	0.62	180 (162–228)	216 (170–240)	0.62
HOPE (min)		158 (124–165)	–		95 (86–126)	–
Ex vivo preservation (min)	543 (452–597)	604 (554–644)	0.04	544 (515–575)	591 (563–633)	0.047
Static cold storage (min)	543 (452–597)	432 (397–509)	0.08	544 (515–575)	490 (466–516)	0.005

Note: Data are expressed as n (%) or median (interquartile range).

Abbreviations: BA, biliary atresia; BAR, Balance of Risk; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOPE, hypothermic oxygenated perfusion; MELD, Model for End-Stage Liver Disease; NASH, nonalcohol-associated steatohepatitis; PELD, Pediatric End-Stage Liver Disease; PSC, primary sclerosing cholangitis; reTH, retransplantation.

^aAge is expressed as years for adult recipients and months for pediatric recipients.

^bThe recipients may have more than one underlying liver disease.

The adult recipients (Table 2) in the HOPE-Split group had a median age of 56 years (IQR, 43–60 years), and the median Model for End-Stage Liver Disease (MELD) score was 12 points (IQR, 8–18). The main transplantation indication was hepatocellular carcinoma (n = 4, 50%). The median BAR score was 6 (IQR, 4–7) in the HOPE-Split group.

The pediatric recipients (Table 2) in the HOPE-Split group had a median age of 42.1 months (IQR, 23.6–65 months), a median weight of 15.5 kg (IQR, 11–17.8), and a median Pediatric End-Stage Liver Disease (PELD) score of 17 points (IQR, 13–21 points). Biliary atresia was the main indication for LT (50%). Among the eight HOPE-Split pediatric LTs, two (25%) were

retransplantations due to ischemic cholangiopathy. A majority of pediatric HOPE-Split recipients had already undergone major abdominal surgery prior to transplantation (62%).

Safety endpoints

All partial grafts obtained after HOPE-Split were successfully transplanted with a 90-day graft and recipient survival of 100% (Table 3). No technical problem with the perfusion device occurred during the split procedure. A mean number of 0.31 ± 0.60 (a total of 5 events) LGRAEs were observed in the HOPE-Split

TABLE 3 Postoperative outcomes

Recipient Group	Adult			Pediatric		
	Static-Split (n = 12)	HOPE-Split (n = 8)	p value	Static-Split (n = 12)	HOPE-Split (n = 8)	p value
7 days						
PNF	0 (0)	0 (0)	–	1 (8)	0 (0)	0.40
EAD	7 (58)	2 (25)	0.14	8 (67)	4 (50)	0.45
L-GrAFT ^a	–3.8 (–4.2 to –3.4) 2.2 (1.5–3.3)	–3.6 (–4.1 to –3.3) 2.5 (1.7–3.5)	0.85	–2.7 (–3.8 to –1.2) 5.9 (1.4–10.6)	–2.5 (–3.6 to –12.2) 7.5 (2.7–10.1)	0.91
AST peak (UI/L)	1077 (665–3345)	971 (609–2777)	0.73	2554 (1148–4923)	1707 (1171–3038)	0.49
ALT peak (UI/L)	924 (700–2594)	1042 (699–1878)	0.68	1784 (564–2572)	969 (593–1299)	0.49
Early laparotomy	0 (0)	0 (0)	–	3 (25)	4 (50)	0.25
30 days						
LGRAE	0.17±0.39 (2 events)	0 (0 event)	0.57	0.75±1.06 (9 events)	0.63±0.74 (5 events)	0.34
90 days						
Overall complication rate (≥grade 3)	4 (42)	2 (25)	0.44	8 (67)	5 (63)	0.85
≥Grade 3 biliary complications	2 (16)	1 (12)	0.79	3 (25)	2 (25)	0.99
Anastomotic stricture	0 (0)	0 (0)	–	2 (16)	2 (25)	–
Nonanastomotic stricture	0 (0)	0 (0)	–	1 (8)	0 (0)	–
Anastomotic leakage	1 (8)	0 (0)	–	0 (0)	0 (0)	–
Cut-surface collection	1 (8)	1 (12.5)	–	0 (0)	0 (0)	–
≥Grade 3 artery complications	3 (25)	1 (12.5)	0.49	1 (8)	0 (0)	0.40
Hepatic artery stenosis	3 (25)	1 (12.5)	–	0 (0)	0 (0)	–
Hepatic artery thrombosis	0 (0)	0 (0)	–	1 (8)	0 (0)	–
Acute kidney injury requiring dialysis	0 (0)	0 (0)	–	2 (16)	1 (12)	0.78
Graft rejection ^b	0 (0)	0 (0)	–	1 (8)	1 (12)	0.76
CCI	34.5 (25.5–45.7)	20.9 (20.9–31.5)	0.43	69.2 (38.2–80.2)	53.2 (36.2–78.4)	0.67
ICU stay	3 (3–5)	5 (3–9)	0.21	12 (9–25)	6 (5–15)	0.13
Hospital stay	21 (12–30)	16 (14–27)	0.73	34 (24–67)	32 (23–84)	0.97
Graft survival	12 (100)	8 (100)	–	11 (92)	8 (100)	0.22
Patient survival	12 (100)	8 (100)	–	11 (92)	8 (100)	0.22

Note: Data are expressed as n (%) or median (interquartile range), except for LGRAE (mean±standard deviation).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, Comprehensive Complication Index; EAD, early allograft dysfunction; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; LGRAE, liver graft-related adverse event; PNF, primary nonfunction.

^aThe L-GrAFT score was presented (negative value) as well as the risk of graft loss (percentage) based on the L-GrAFT score.

^bProven biopsy graft rejection with the need to change immunosuppressive treatment.

group, which was not significantly different from the Static-Split group (0.46±0.83; 11 events; $p = 0.78$). When focusing on pediatric recipients a mean number of 0.63±0.74 (5 events) LGRAEs was observed in the HOPE-Split compared with 0.75±1.06 (9 events) in the Static-Split group ($p = 0.34$). The duration of the split procedures in the HOPE-Split group was not significantly longer than in the Static-Split group (216 min [IQR, 170–240] vs. 180 min [IQR, 167–243]; $p = 0.45$; [Figure 3](#)).

Graft preservation

The median duration of perfusion of the 16 partial grafts was 125 min (IQR, 95–165) with a median perfusion flow of 210 ml/min (14.3 ml/min/100 g). HOPE allowed to significantly reduce static cold storage duration (472 min [IQR, 410–516] vs. 544 min [IQR, 508–581]; $p = 0.001$), while total ex vivo preservation time was significantly increased (595 min [IQR, 562–639 min] vs. 544 min [IQR, 508–581 min]; $p = 0.007$; [Figure 3](#)).

The reperfusion biopsies of all HOPE–Split LTs ($n = 16$) were compared with the biopsies of 23 Static–Split LTs (one biopsy was not performed in the Static–Split group because of multiorgan failure; Figure 4). The majority of HOPE–Split grafts presented with grade 0–2 (none to mild) IRIs ($n = 11$, 68.8%) and no grade 4 IRI was observed. The Static–Split grafts showed a higher overall grade of IRI with two cases of grade 4 (severe) IRI (Figure 4A,B). There was a significant

reduction of lobular neutrophilic infiltrate upon reperfusion in the HOPE–Split group compared with the Static–Split group ($p = 0.04$).

HOPE–Split short-term outcomes

Early posttransplantation outcomes were comparable with Static–Split when stratified for adult and pediatric recipients. Serum transaminase peak, bilirubin levels, and factor V normalization are presented in Figure 5.

In detail, adult recipients in the HOPE–Split group had an EAD rate of 25% ($n = 2$) and a median CCI at 90 days of 20.9 points (IQR, 20.9–31.5; Table 3). We observed one arterial and one biliary complication (Clavien–Dindo \geq grade 3) in the HOPE–Split group. The arterial complication was a hepatic artery stenosis requiring a radiological stenting at postoperative day (POD) 80. The biliary complication was a cut-surface collection requiring a radiological drainage for sepsis at POD 36. Detailed arterial and biliary complications are presented in Table S2.

In pediatric recipients, the rate of EAD was 50% ($n = 4$) with a median CCI at 90 days of 53.3 points (IQR, 36.2–80.2). Two biliary complications (Clavien–Dindo \geq 3) occurred in the HOPE–Split group, which were two anastomotic strictures requiring percutaneous

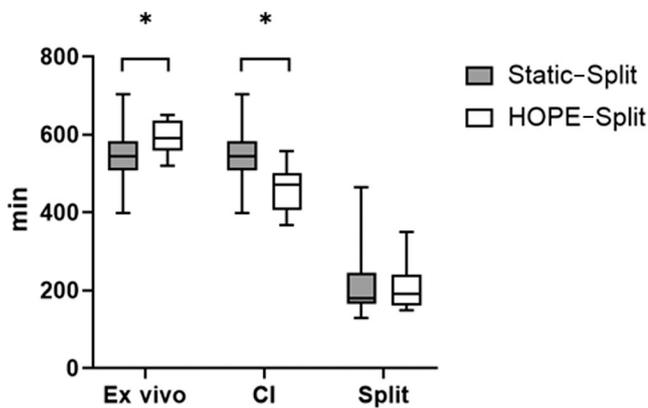


FIGURE 3 Detailed preservation time of HOPE–Split and Static–Split grafts. Data are presented as box plot with overall range. Ex vivo corresponds to total ex vivo preservation time; split indicates split duration; $*p < 0.05$

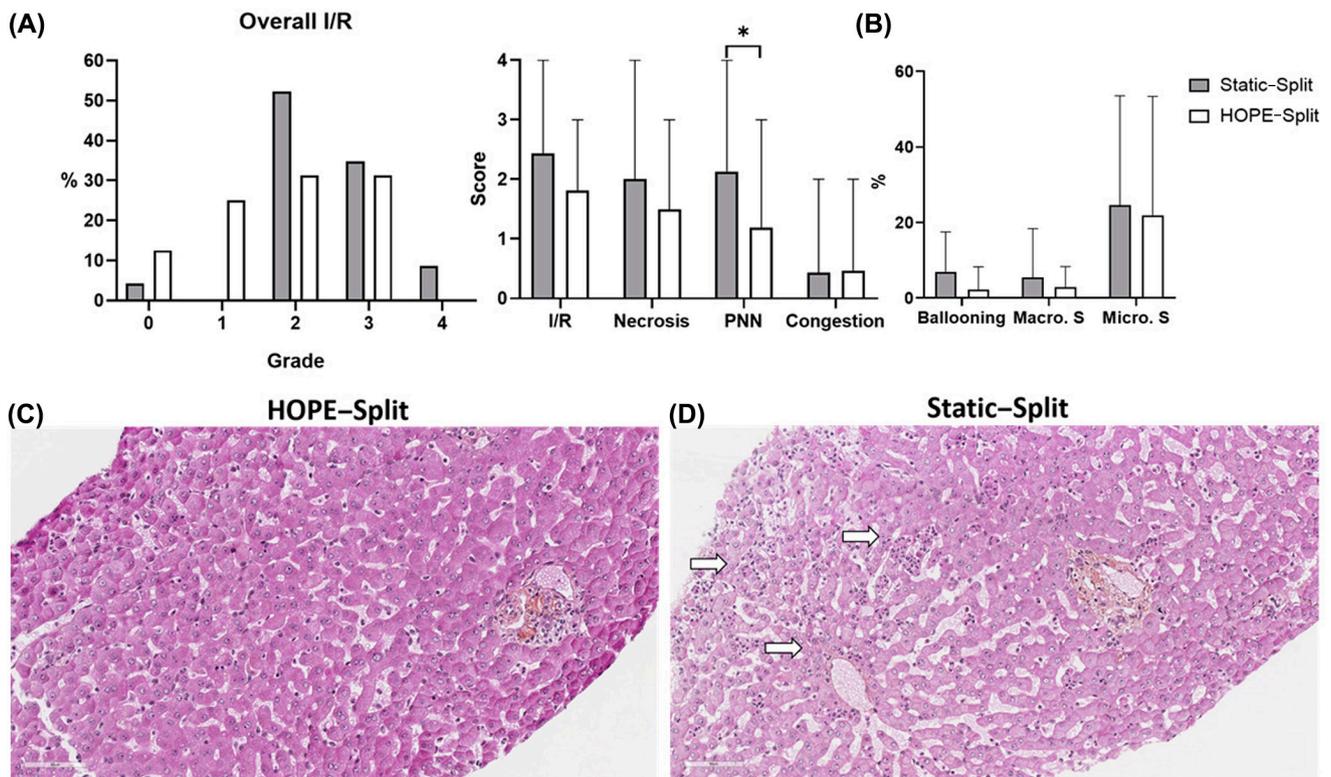


FIGURE 4 Histological analysis of IRI. (A) Overall IRI grade in the HOPE–Split and Static–Split groups. (B) Detailed histological analysis of IRI in the HOPE–Split and Static–Split groups. Data are expressed as mean with range. $*p < 0.05$. (C and D) Representative histology of IRI on reperfusion biopsy. (C) Minimal IRI in the HOPE–Split group and (D) moderate IRI with neutrophilic infiltrate (arrow)

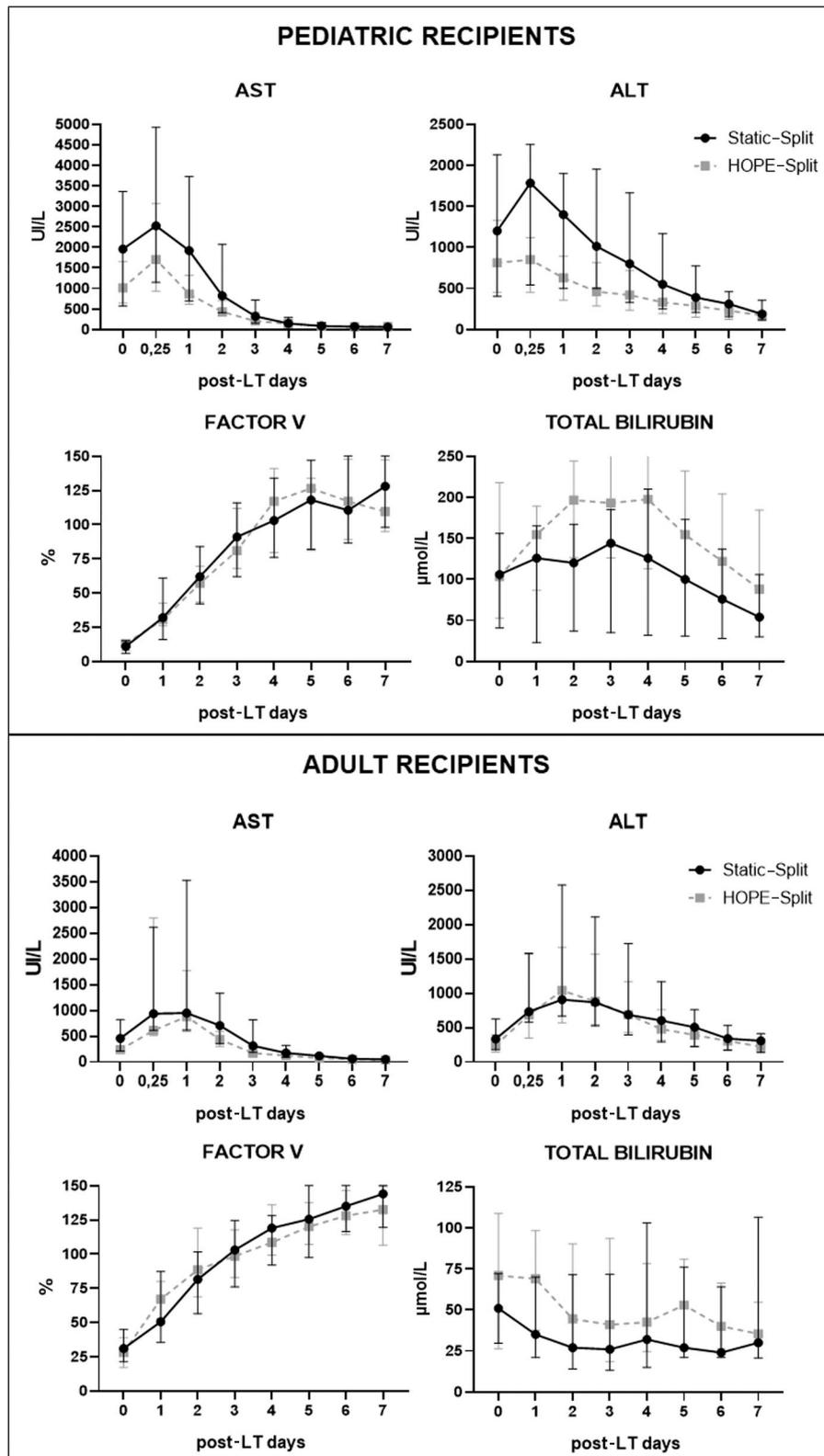


FIGURE 5 Serum ALT, AST, factor V, and total bilirubin of pediatric and adult recipients during the first PODs in the HOPE-Split and Static-Split groups. Data are expressed as median and IQR

transhepatic cholangiodrainage during the first month. No arterial complication occurred. Detailed arterial and biliary complications are presented in Table S2.

No graft loss or recipient death was encountered in the HOPE-Split group during the median follow-up of 7.5 months (IQR, 5.5–12.5; Table S2).

DISCUSSION

This single-center prospective pilot study presents first feasibility and safety data for transplantation of partial liver grafts undergoing ex situ split during HOPE. In a total of 16 successful SLTs after 8 HOPE–Split procedures, no early graft loss occurred and LGRAEs were comparable to the standard Static–Split procedure. HOPE–Split did not increase split procedure duration but resulted in reduced static cold storage duration leading to reduced IRI on reperfusion biopsies.

Outcomes after ex situ liver graft split have improved over the last decades.^[1] Because of the technical particularity of ex situ split, partial grafts undergo prolonged static cold storage. Indeed, in a recent European registry study, up to 10% of all SLT had a static cold storage time >12 h. This was associated with a significant reduction in graft and recipient survival.^[1,6] Consequently, improving graft preservation for ex situ split grafts is a key challenge. By adding oxygen to an active perfusion at cold temperatures, a short period of end-ischemic HOPE uploads cellular energy levels and protects the liver graft's mitochondrial metabolism.^[10,25] This leads to a mitigation of IRI upon implantation of the graft and translates into clinical benefits compared with static cold storage, as shown by recent randomized clinical trials.^[7,8] However, data on the use of HOPE in split LT are limited to small case series in either experimental^[26,27] or clinical^[12–14] settings. Previous reports from Spada et al.^[12] and Thorne et al.^[14] presented technical aspects of the procedure in single cases. Following these technical reports, we designed a clinical pilot study (stage 2a) as recommended by the IDEAL guidelines on surgical innovation to evaluate feasibility and safety in a series of consecutive single-center cases.

The primary safety endpoint was a composite indicator designed to capture 30-day LGRAEs as recently used in a recent RCT comparing machine perfusion with static cold storage.^[19] HOPE–Split was comparable to the Static–Split group, suggesting the safety of this standardized surgical technique. Furthermore, in terms of early relaparotomies and biliary complications, especially in pediatric recipients, the HOPE–Split group did not display significantly more adverse events. This supports the fact that ex situ liver splitting during concurrent HOPE did not differ from a standard split procedure for cut-surface hemostasis or bile duct division. In addition, the split procedure with concurrent HOPE did not last significantly longer (216 min vs. 180 min; $p = 0.45$) despite the logistical challenge imposed by machine perfusion.

Besides safety, we would like to discuss three interesting observations of our study. First, the use of HOPE may improve preservation of split grafts by significantly reducing static cold storage time and increasing total ex vivo preservation time. Preservation with HOPE has already been shown to be beneficial, especially in the

setting of marginal whole grafts by mitigating mitochondrial damage^[10] and reducing sterile inflammation upon reperfusion.^[27–29] In line with these observations, we were able to show a reduction in lobular neutrophilic infiltrate and the absence of severe IRI scores on reperfusion biopsies of HOPE–Split grafts compared with the Static–Split grafts. Of note, all HOPE–Split grafts underwent perfusion for >1 h (158 min in adult recipients and 95 min in pediatric recipients), which has been shown to be sufficient to mitigate IRI.^[30] A reduction of static cold storage may be of particular interest for complex SLT scenarios, such as difficult recipient hepatectomies in pediatric retransplantations. We encountered two such cases in our study in which HOPE allowed to optimize preservation of the pediatric split graft by prolonging perfusion until the recipient hepatectomy was completed.^[31,32]

Second, we would like to highlight several important technical points of the proposed standardized HOPE–Split procedure.^[13] In contrast to the first report by Spada et al.^[12] on using dual-HOPE through both the hepatic artery and the portal vein, we advocate single portal perfusion as performed in several ongoing randomized clinical trials.^[33,34] Single portal perfusion remains easier to perform from a technical point of view, and there is currently no clinical nor experimental data available showing the additional benefit of a dual perfusion compared with a single perfusion.^[35] In addition, single portal perfusion allows to remove one of the partial grafts from the perfusion device without interrupting the perfusion of the contralateral partial graft. In case of D-HOPE, arterial perfusion limits the possibilities of continuous selective perfusion due to the necessity to cannulate a vessel with a large diameter, ideally an aortic conduit. As stated by Spada et al.^[12] in their inaugural report, given the small caliber of the divided branches of the hepatic artery, cannulation might expose to arterial injury.

Third, our preliminary data on early outcomes after HOPE–Split showed an overall 90-day morbidity in adult recipients that reached benchmark values for whole-graft LT.^[36] In the subgroup of pediatric recipients, overall morbidity was comparable between HOPE–Split and Static–Split groups, although ~25% of the recipients underwent secondary LT in the HOPE–Split cohort and 62% had a previous major surgery prior to LT. Finally, we did not encounter any graft loss or recipient death during the follow-up.

A major limitation of the study is the small sample size and the short follow-up. In line with the IDEAL framework on surgical innovation, this study is a mandatory step to assess feasibility and safety of the HOPE–Split procedure in the routine clinical setting. Indeed, following stage 1, which consists in a proof-of-concept focusing on the description of a new technic,^[12,14] stage 2a aims at developing and refining the technique and

reporting short-term outcomes from a single-center prospective cohort. The data acquired from the stage 2a study will help design future large-scale trials, which will need to confirm the presented outcome data.^[15,16] Another limitation is that HOPE–Split was evaluated in well-selected donors and recipients. The next step would be to evaluate this procedure in higher-risk SLT scenarios for which HOPE may present a superior clinical benefit as shown for marginal whole grafts.^[37] Besides optimizing preservation, HOPE has the potential to assess viability and quality of the split grafts prior to transplantation, which may allow to extend donor and graft selection criteria for SLT.^[38]

In conclusion, this is the first study to evaluate HOPE in both pediatric and adult SLT in the routine clinical setting. HOPE–Split appeared as a feasible and safe procedure to improve preservation of partial grafts without jeopardizing early postoperative outcomes. The results of this pilot study provide the basis to set up large-scale trials on the use of machine perfusion strategies in pediatric and split LT.^[39]

AUTHOR CONTRIBUTIONS

Guillaume Rossignol and Xavier Muller contributed equally as first authors, designed the study, acquired the data, performed the statistical analysis, interpreted the data, and wrote the manuscript. Valérie Hervieu and Sophie Collardeau-Frachon performed the histological analysis and critically reviewed the data. Antoine Breton and Natacha Boulanger acquired the data. Jean-Yves Mabrut, Kayvan Mohkam, Mickaël Lesurtel, and Rémi Dubois designed the study, interpreted the data, critically reviewed the data, and drafted a final version of the manuscript.

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CONFLICT OF INTEREST

The authors of this manuscript have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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ORIGINAL ARTICLE

In situ normothermic regional perfusion versus ex situ normothermic machine perfusion in liver transplantation from donation after circulatory death

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Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; cDCD, controlled donation after circulatory death; CI, confidence interval; COPE, Consortium for Organ Preservation in Europe; DBD, donation after brain death; DCD, donation after circulatory death; DWI, donor warm ischemia; HAT, hepatic artery thrombosis; HCC, hepatocellular carcinoma; HOPE, hypothermic oxygenated machine perfusion; HR, hazard ratio; INR, international normalized ratio; IQR, interquartile range; ITU, intensive therapy unit; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; MRCP, magnetic resonance cholangiopancreatography; NAS, nonanastomotic biliary strictures; NMP, ex vivo normothermic machine perfusion; NRP, in situ normothermic regional perfusion; PSM, propensity score matching; SCS, static cold storage; SMD, standardized mean difference (absolute value); UK-DCD, UK-Donation After Circulatory Death.

SEE EDITORIAL ON PAGE 1701

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Abstract

In situ normothermic regional perfusion (NRP) and ex situ normothermic machine perfusion (NMP) aim to improve the outcomes of liver transplantation (LT) using controlled donation after circulatory death (cDCD). NRP and NMP have not yet been compared directly. In this international observational study, outcomes of LT performed between 2015 and 2019 for organs procured from cDCD donors subjected to NRP or NMP commenced at the donor center were compared using propensity score matching (PSM). Of the 224 cDCD donations in the NRP cohort that proceeded to asystole, 193 livers were procured, resulting in 157 transplants. In the NMP cohort, perfusion was commenced in all 40 cases and resulted in 34 transplants (use rates: 70% vs. 85% [$p = 0.052$], respectively). After PSM, 34 NMP liver recipients were matched with 68 NRP liver recipients. The two cohorts were similar for donor functional warm ischemia time (21 min after NRP vs. 20 min after NMP; $p = 0.17$), UK–Donation After Circulatory Death risk score (5 vs. 5 points; $p = 0.38$), and laboratory Model for End-Stage Liver Disease scores (12 vs. 12 points; $p = 0.83$). The incidence of nonanastomotic biliary strictures (1.5% vs. 2.9%; $p > 0.99$), early allograft dysfunction (20.6% vs. 8.8%; $p = 0.13$), and 30-day graft loss (4.4% vs. 8.8%; $p = 0.40$) were similar, although peak post-transplant aspartate aminotransferase levels were higher in the NRP cohort (872 vs. 344 IU/L; $p < 0.001$). NRP livers were more frequently allocated to recipients suffering from hepatocellular carcinoma (HCC; 60.3% vs. 20.6%; $p < 0.001$). HCC-censored 2-year graft and patient survival rates were 91.5% versus 88.2% ($p = 0.52$) and 97.9% versus 94.1% ($p = 0.25$) after NRP and NMP, respectively. Both perfusion techniques achieved similar outcomes and appeared to match benchmarks expected for donation after brain death livers. This study may inform the design of a definitive trial.

INTRODUCTION

Liver transplantation (LT) from controlled donation after circulatory death (cDCD) donors has been developed during the past two decades in an attempt to address the organ shortage but at the cost of inferior outcomes.^[1] Several studies have reported higher rates of early graft loss and increased rates of biliary complications in cDCD LT recipients compared with those receiving a donation after brain death (DBD) allograft.^[1–3] These inferior outcomes are attributed to allograft injury caused by prolonged donor warm ischemia (DWI) occurring after the withdrawal of life-sustaining therapies and before organ retrieval. Various dynamic preservation strategies have been developed to ameliorate this damage, including in situ normothermic regional perfusion (NRP) and ex situ normothermic machine perfusion (NMP).^[4,5]

The NRP approach consists of cannulating donor iliac vessels and commencing perfusion of the abdominal compartment organs shortly after donor circulatory

arrest. This strategy has been adopted by countries including France and Spain.^[4,6] Large retrospective studies from these programs suggested that NRP was associated with superior posttransplant outcomes in cDCD allografts compared with super-rapid recovery with static cold storage (SCS), achieving results similar to DBD livers.^[4,7]

In contrast, NMP involves the ex situ perfusion of livers with oxygenated blood and medications at body temperature to preserve the liver in a physiological, functioning state. This preservation strategy can be commenced at the donor hospital or at the recipient center after a period of SCS. NMP commenced at the donor hospital was compared with SCS in a randomized controlled trial conducted by the Consortium for Organ Preservation in Europe (COPE). The study demonstrated lower posttransplant peak aspartate aminotransferase (AST) levels and lower rates of early allograft dysfunction in the NMP group, with these benefits being greatest in the cDCD subgroup.^[8]

NRP and NMP have not yet been compared directly; this study is the first to compare the results of LT using these two different strategies in the context of LT from cDCD.

PATIENTS AND METHODS

Study population

The study enrolled 264 donation after circulatory death (DCD) donors, which comprised 224 NRP procedures performed according to French national guidelines between February 2015 and December 2019 and 40 NMP procedures performed between August 2014 and March 2016 in the COPE trial. The NRP procedures were performed in six French centers, whereas the NMP procedures were performed in six European centers from the United Kingdom, Germany, Spain, and Belgium. Graft acceptance criteria in the NRP French program were donor age ranging from 18 to 75 years, no circulatory arrest before organ procurement, and cold ischemia time less than 9 h. Although graft acceptance criteria in the NMP cohort were broader in theory, all grafts that were considered in the present NMP cohort conformed to the same criteria. The details of particular protocols were published elsewhere.^[5–8] In short, the NRP procedure was commenced in the intensive care unit using an oxygenated normothermic in situ perfusion through arterial and venous femoral canula. The perfusion was limited to abdominal organs only by using an endo-aortic balloon clamp positioned in the supraceliac aorta. Following a 1–4 h duration of NRP, the graft was procured in a standard fashion and transported to the recipient site using SCS. The NMP procedure was also commenced at the donor hospital following a short period of SCS during preparation of the liver for perfusion. NMP perfusate comprised packed red blood cells and gelofusine, with infusions of heparin, insulin, sodium taurocholate, and prostacyclin. NMP was maintained during transportation to the recipient center, where the graft was flushed with standard preservation fluid before being implanted.

Endpoints and definitions

Study endpoints included liver use rate; 30-day and 12- and 24-month patient and graft survival rates; incidence of clinically manifest biliary complications; early allograft dysfunction; and peak transaminase levels. Clinically manifest biliary strictures were divided into the following three categories: anastomotic strictures, nonanastomotic biliary strictures (NAS) that were unrelated to any hepatic artery complications, and ischemic biliary lesions related to hepatic artery thrombosis (HAT).

The use rate was calculated as the number of allografts that were transplanted divided by the total number of cDCD donors that proceeded to asystole within the time frame that allows procurement. Early allograft dysfunction was assessed using Olthoff's criteria.^[9]

Because of differences in terms of recipient selection—for example, the French program prioritizing recipients with hepatocellular carcinoma (HCC) for DCD graft allocation—patient and graft survival rates were calculated both with and without censoring for death from HCC recurrence. Clinically manifest biliary complications were defined as any biliary problem diagnosed on magnetic resonance cholangiopancreatography (MRCP) resulting in the need for invasive specific treatment and/or resulting in graft loss.

Variables related to graft ischemia were defined as follows: total DWI was defined as the time between therapeutic withdrawal in the donor and start of cold aortic perfusion in the NMP group or initiation of the perfusion in the NRP group. In the NMP group, functional DWI was defined as the time from systolic pressure below 50 mm Hg until start of aortic cold perfusion, whereas in the NRP group, it was defined as the time from mean arterial blood pressure below 45 mm Hg until initiation of NRP. Asystolic DWI started from the onset of cardiac arrest. Total ex vivo preservation time was defined as the time from donor aortic cross-clamp until graft reperfusion in the recipient. Cold ischemia time was defined as the duration of graft SCS. Donors were graded using Feng's donor risk index^[10] and the UK-DCD risk score.^[11]

Statistical analysis

Categorical variables were presented as number with percentages and compared using chi-square or Fisher's exact test, as appropriate. Continuous variables were presented as median values with interquartile ranges (IQRs) and compared using the Mann–Whitney U test. The survival rates were estimated using the Kaplan–Meier curve and compared with a log-rank test.

Because of differences in terms of organ allocation and recipient selection between the COPE trial and the French cDCD LT program, a few differences could be expected between the two groups. To overcome potential baseline covariate imbalances, we performed propensity score matching (PSM). The propensity score was calculated using a multivariable logistic regression model with group allocation as the outcome and included all variables involved in the UK-DCD risk score, a robust tool that predicts cDCD-LT outcome (namely, donor age, donor body mass index, functional DWI, recipient age, laboratory Model for End-Stage Liver Disease [MELD] score), with the exception of cold ischemia time (which is considered as an inherent benefit of the NMP strategy) and retransplantation (because

only primary LT cases were included in both treatment groups). Matching was performed with a 1:2 ratio using the nearest neighbor method, without replacement and with a caliper of 0.20. After PSM, balance assessment was performed using the absolute value of the standardized mean difference (SMD), and values less than 0.10 were considered well balanced. Outcome variables were only reported for the propensity score-matched cohorts.

All calculations were performed with SPSS software Version 23.0 (IBM Corp., Armonk, NY) and R software Version 3.1.3 (R Foundation for Statistical Computing). All tests were two-tailed, and statistical significance was established for $p < 0.05$.

RESULTS

Graft use rates

Of the 249 cDCD donors in the NRP cohort that were considered for procurement, 224 proceeded to asystole. Of these, 31 livers were not procured because of technical failure or dramatic postperfusion peak AST, whereas 193 livers were eventually procured, resulting in 157 transplants and 36 discarded livers, mainly as a result of steatosis or poor macroscopic aspect (Figure 1). In the NMP cohort, of the 63 cDCD donations that were considered for procurement, 40 proceeded

to asystole. Normothermic perfusion was commenced in all 40 cases and resulted in 34 transplants. The corresponding organ use rates were 70% and 85% ($p = 0.052$) in the NRP and NMP cohort, respectively (Figure 1).

Donor and recipient characteristics

Before PSM, the donor demographics of both study groups were comparable, including age, weight, body mass index, cause of death, functional DWI, and donor risk index. The donor intensive therapy unit (ITU) stay (9 vs. 4 days; $p < 0.001$) was longer in the NRP group. Because of differences in the donor cannulation and organ procurement technique between the two groups, the total DWI (31 [IQR, 27–37] vs. 25 [IQR, 22–29] min; $p < 0.001$) and asystolic DWI (18 [IQR, 15–20] vs. 12 [IQR, 10–13] min; $p < 0.001$) were longer in the NRP group. As expected, cold ischemia time (346 vs. 138 min; $p < 0.001$) was longer in the NRP group. The total dynamic perfusion time (184 vs. 525 min; $p < 0.001$) and total ex situ preservation times (516 vs. 651 min; $p < 0.001$) were longer in the NMP group. Details are provided in Table 1.

The NRP recipients were predominantly males (91% vs. 53%; $p < 0.001$) and older, although the difference was not statistically significant (59 vs. 56 years; $p = 0.052$). The main underlying liver disease in the

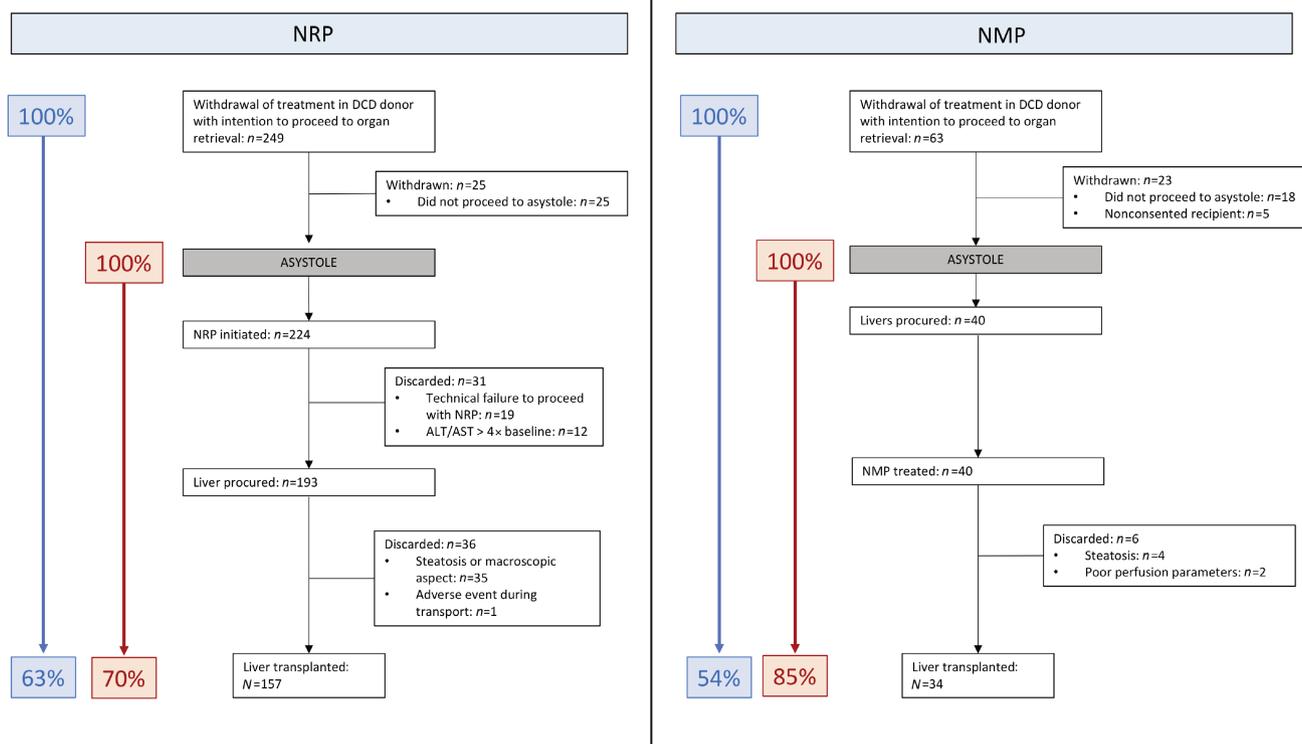


FIGURE 1 Study flowchart depicting graft use rates in both cohorts

TABLE 1 Donor, graft, and recipient characteristics

Variables	Before PSM			After PSM		
	NMP	NRP	<i>p</i> value	NMP	NRP	<i>p</i> value
<i>n</i>	34	157		34	68	
Donor age, year	48 (33–62)	50 (39–59)	0.970	48 (33–62)	49 (37–60)	0.673
Donor sex, male/female	20/14	113/44	0.680	20/14	48/20	0.235
Donor body mass index, kg/m ²	25 (23–29)	25 (22–29)	0.109	25 (23–29)	25 (23–29)	0.845
Donor intensive care unit stay, days	4 (2–5)	9 (6–15)	<0.001	4 (2–5)	9 (6–15)	<0.001
Cause of donor death			0.183			0.056
Trauma	3 (8.8)	41 (26.1)		3 (8.8)	23 (33.8)	
Anoxia	15 (44.1)	65 (41.4)		15 (44.1)	27 (39.7)	
Cerebrovascular accident	11 (32.4)	51 (32.5)		11 (32.4)	18 (26.5)	
Donor risk index	2.13 (1.90–2.42)	2.01 (1.69–2.35)	0.148	2.13 (1.90–2.42)	1.98 (1.68–2.43)	0.234
Functional DWI time, min	20 (17–25)	22 (19–26)	0.179	20 (17–25)	21 (18–25)	0.603
Cold ischemia time, min	138 (118–144)	346 (291–395)	<0.001	138 (118–144)	346 (285–397)	<0.001
Recipient age, year	56 (45–61)	59 (54–63)	0.052	56 (45–61)	57 (50–62)	0.949
Recipient sex, male/female	18/16	141/16	<0.001	18/16	58/10	<0.001
Recipient body mass index, kg/m ²	26 (22–33)	27 (24–30)	0.609	26 (22–33)	27 (24–30)	0.771
Main transplant indication			<0.001			<0.001
HCC	7 (20.6)	103 (65.6)		7 (20.6)	41 (60.3)	
End-stage liver disease	27 (79.4)	54 (34.4)		27 (79.4)	27 (39.7)	
Alcohol-associated liver disease	12 (35.3)	68 (43.3)		12 (35.3)	29 (42.6)	
Nonalcoholic fatty liver disease	2 (5.9)	21 (13.3)		2 (5.9)	10 (14.7)	
Viral hepatitis	1 (33.3)	54 (34.4)		1 (2.9)	25 (36.8)	
Primary sclerosing cholangitis	4 (11.7)	0 (0)		4 (11.8)	0 (0)	
Other	8 (23.5)	14 (8.9)		8	8	
Pretransplant renal replacement therapy	0 (0.0)	3 (1.9)	1.000	0 (0.0)	0 (0.0)	1.000
Laboratory MELD score	12 (9–13)	11 (8–15)	0.759	12 (9–13)	12 (9–13)	0.831
UK-DCD score	5 (3–6)	6 (3–8)	0.238	5 (3–6)	5 (3–7)	0.383
UK-DCD risk group			0.882			0.742
Low risk	18 (52.9)	77 (49.0)		18 (52.9)	34 (50.0)	
High risk	14 (41.2)	68 (43.3)		14 (41.2)	31 (45.6)	
Futile	2 (5.9)	14 (8.9)		2 (5.9)	5 (7.4)	

Note: Data are provided as *n*, *n* (%), or median (IQR).

Abbreviations: DWI, donor warm ischemia; HCC, hepatocellular carcinoma; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; NMP, ex vivo normothermic machine perfusion; NRP, in situ normothermic regional perfusion; PSM, propensity score matching; UK-DCD, UK–Donation After Circulatory Death.

NRP group was alcohol-associated liver disease while a majority of patients receiving LT for HCC (65.6% vs. 20.6%; $p < 0.001$), respectively. The laboratory MELD score, recipient body mass index, and UK-DCD score distribution were similar in both groups. The study median follow-up was 22 months (IQR, 14–32) in the

NRP group versus 24 months (IQR, 23–24) in the NMP group ($p = 0.75$), and each study patient still alive completed at least 12 months of follow-up.

After PSM, the two groups became well balanced for all variables included in the UK-DCD risk score except for cold ischemia time (Tables 1 and 2).

TABLE 2 Balance assessment for UK-DCD variables^a and score before and after 1:2 PSM

Variables	Before PSM			After PSM		
	NMP	NRP	SMD	NMP	NRP	SMD
<i>n</i>	34	157		34	68	
Donor age, year	48 (33–62)	50 (39–59)	0.040	48 (33–62)	49 (37–60)	0.077
Donor body mass index, kg/m ²	23 (25–29)	25 (22–29)	0.313	23 (25–29)	25 (23–29)	0.007
Functional DWI, min	20 (17–25)	22 (19–26)	0.272	20 (17–25)	21 (18–25)	0.091
Recipient age	56 (45–61)	59 (54–63)	0.559	56 (45–61)	57 (50–62)	0.019
Retransplant	0	0	–	0	0	–
Laboratory MELD score	12 (9–13)	11 (8–15)	0.051	12 (9–13)	12 (9–13)	0.011
UK-DCD score	5 (3–6)	6 (3–8)	0.210	5 (3–6)	5 (3–7)	0.087
UK-DCD risk group						
Low risk	18 (52.9)	77 (49.0)	0.078	18 (52.9)	34 (50.0)	0.058
High risk	14 (41.2)	68 (43.3)	0.042	14 (41.2)	31 (45.6)	0.089
Futile	2 (5.9)	12 (7.6)	0.065	2 (5.9)	5 (7.4)	0.059

Note: Data are provided as *n*, *n* (%), or median (IQR).

Abbreviations: DWI, donor warm ischemia; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; NMP, ex vivo normothermic machine perfusion; NRP, in situ normothermic regional perfusion; PSM, propensity score matching; SMD, standardized mean difference (absolute value); UK-DCD, UK–Donation After Circulatory Death.

^aAll UK-DCD score variables were included except cold ischemia time because the latter is expected to be decreased in the NMP group as part of its inherent benefits.

TABLE 3 Posttransplant outcomes

Variables	NMP	NRP	<i>p</i> value
<i>n</i>	34	68	
ITU stay, days	3 (2–5)	5 (4–7)	<0.001
Hospital stay, days	14 (8–17)	16 (13–20)	0.018
30-day graft loss	3 (8.8)	3 (4.4)	0.398
30-day patient death	2 (5.9)	0 (0.0)	0.109
Early allograft dysfunction	3 (8.8)	14 (20.6)	0.133
AST peak 7 days, IU/L	344 (216–701)	872 (538–1281)	<0.001
ALT peak 7 days, IU/L	311 (186–590)	725 (400–1304)	0.001
INR level Day 7	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.566
Total bilirubin Day 7, μmol/L	29 (16–57)	20 (12–43)	0.217
Serum creatinine Day 7, μmol/L	73 (51–97)	66 (55–83)	0.826
HAT	1 (2.9)	2 (2.9)	<i>p</i> > 0.99

Note: Data are provided as *n*, *n* (%), or median (IQR).

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; HAT, hepatic artery thrombosis; INR, international normalized ratio; ITU, intensive therapy unit; NMP, ex vivo normothermic machine perfusion; NRP, in situ normothermic regional perfusion.

Early postoperative outcomes

There was no difference between the groups in 30-day graft (97% [*n* = 65] after NRP vs. 91% [*n* = 31] after NMP; *p* = 0.39) and patient (100% [*n* = 68] after NRP vs. 94% [*n* = 32] after NMP; *p* = 0.11) survival rates. In the NRP group, the three cases of 30-day graft loss were attributed to primary nonfunction (*n* = 1), complete hepatic vein thrombosis (*n* = 1), and hyperacute rejection related to ABO incompatibility (*n* = 1), whereas in the

NMP group, the three cases were attributed to inferior vena cava thrombosis at reperfusion (*n* = 1), multiple organ failure caused by HAT (*n* = 1), and nonthrombotic graft infarction (*n* = 1). The NRP grafts showed higher peak AST and alanine transaminase (ALT) values within 7 days after LT, but there was no difference between the groups in early allograft dysfunction (Table 3). Recipients in both groups experienced similar rates of HAT (2.9% [*n* = 2] after NRP vs. 2.9% [*n* = 1] after NMP; *p* > 0.99).

Biliary complications

A total of eight (11.8%) versus seven (20.6%) recipients developed a clinically manifest biliary complication in the NRP and NMP groups, respectively ($p = 0.249$). Overall, 44 (64.7) and 25 (73.5%) patients underwent MRCP protocol ($p = 0.369$), showing that the majority of clinically manifest biliary complications consisted of anastomotic strictures (8.8% [$n = 6$] after NRP vs. 17.6% [$n = 6$] after NMP; $p = 0.208$). The incidence of symptomatic NAS was similar and low in both groups (1.5% [$n = 1$] after NRP vs. 2.9% [$n = 1$] after NMP; $p = 1.000$), leading to a single graft loss in each group (1.5% vs. 2.9%; $p = 1.000$) within the median 23-month follow-up period. Details regarding biliary complications are provided in [Table 4](#).

Survival rates

The 1- and 2-year graft survival rates were 93.9% versus 88.2% and 89.4% versus 88.2% (hazard ratio [HR], 0.67; 95% confidence interval [CI], 0.20–2.23; $p = 0.516$) for the NRP and NMP groups, respectively ([Table 5](#)). The 1- and 2-year patient survival rates were 98.5% versus 94.1% and 96.4% versus 90.9% (HR, 0.38 [95% CI, 0.06–2.30]; $p = 0.275$) for the NRP and NMP groups, respectively. Because of the high incidence of the HCC in the NRP group, we also calculated survival rates with censoring of recipients with HCC recurrence and found similar graft and patient survival rates ([Table 5](#), [Figures 2](#) and [3](#)).

DISCUSSION

This is the first study comparing NRP and NMP strategies in cDCD LT. The results show patient and

graft outcomes that are comparable with benchmarks established for DBD and much lower rates of NAS than contemporary cDCD series preserved by SCS.^[2,11–13] Our findings suggest that the NMP procedure allows longer total preservation times and may achieve higher organ use rates. The NRP intervention achieved similar posttransplant results and also potentially improves the quality of other donated abdominal organs.^[14]

As a result of the historically poorer outcomes of cDCD livers, the selection criteria are usually more restrictive, resulting in lower organ use and higher discard rates compared with DBD donors. An important objective of machine perfusion is to recover graft quality and increase use rates. In the randomized COPE trial, the organ discard rate was 50% lower for the NMP compared with the SCS group and yet, despite the increased use of suboptimal cDCD livers, the results were superior to the SCS group.^[8] This suggests that NMP allows increased organ use without compromising outcomes. Conversely, studies comparing the use of NRP with the standard cDCD super-rapid recovery have not yet demonstrated improvement in terms of cDCD graft use rates. In the study by Hessheimer et al., the liver use rates from withdrawal of life-sustaining therapies were 34% and 38% after NRP and super-rapid recovery, respectively.^[4] These findings seem to be confirmed by our results, which suggest that the use rate might be higher after super-rapid recovery followed by NMP commenced at the donor center than after NRP.

Although open-abdomen cannulation is preferred in some countries, as it appears to be technically less demanding and results in very low rates of technical failure,^[15] other countries advocate the use of percutaneous femoral performed in the ITU. Because open-abdominal aortic cannulation for cDCD donation is not allowed in France,^[16] the NRP cannulas needs

TABLE 4 Biliary complications

Variables	NMP	NRP	<i>p</i> value
<i>n</i>	34	68	
Clinically manifest biliary strictures ^a			
Any type	7 (20.6)	8 (11.8)	0.249
NAS ^b	1 (2.9)	1 (1.5)	1.000
HAT-related ischemic-type biliary lesions ^c	0 (0.0)	1 (1.5)	1.000
Anastomotic stricture	6 (17.6)	6 (8.8)	0.208
MRCP performed	25 (73.5)	44 (64.7)	0.369
Total biliary strictures (clinical and/or on imaging)			
Anastomotic stricture	13 (38.2)	7 (10.3)	<0.001
Nonanastomotic stricture	3 (8.8)	2 (2.9)	0.330

Abbreviations: HAT, hepatic artery thrombosis; MRCP, magnetic resonance cholangiopancreatography; NAS, nonanastomotic biliary strictures; NMP, ex situ normothermic machine perfusion; NRP, in situ normothermic regional machine perfusion.

^aRefers to biliary stricture requiring a specific treatment or resulting to graft loss and/or patient death.

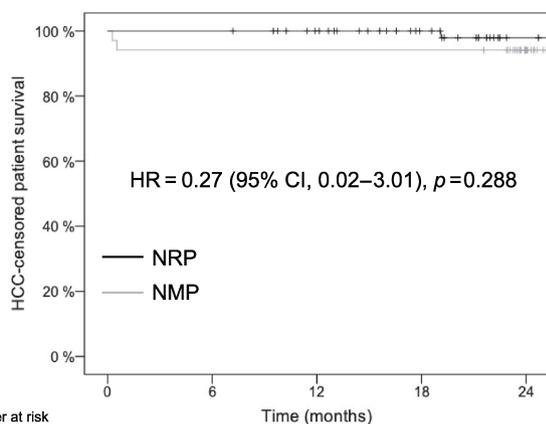
^bRefers to NAS with patent hepatic artery.

^cRefers to NAS related to HAT.

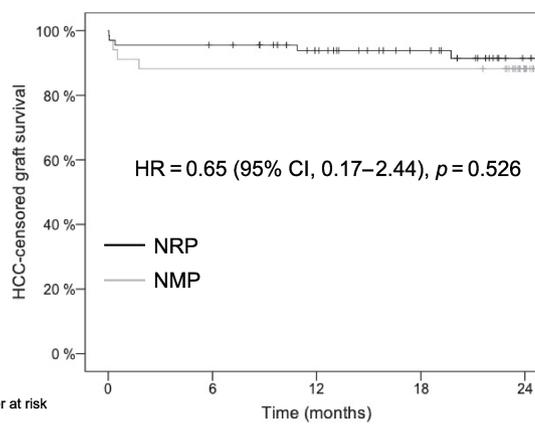
TABLE 5 Survival rates

Variables	NMP, %	NRP, %	p value
Graft survival rates			0.516
1 year	88.2	93.9	
2 years	88.2	89.4	
Tumor-censored graft survival rates			0.523
1 year	88.2	94.1	
2 years	88.2	91.5	
Patient survival rates			0.275
1 year	94.1	98.5	
2 years	90.9	96.4	
Tumor-censored patient survival rates			0.255
1 year	94.1	100	
2 years	94.1	97.9	

Abbreviations: NMP, ex situ normothermic machine perfusion; NRP, in situ normothermic regional machine perfusion.



Number at risk	0	6	12	18	24
NRP	68	68	62	50	36
NMP	34	30	30	30	30



Number at risk	0	6	12	18	24
NRP	68	64	53	42	28
NMP	34	32	32	32	31

FIGURE 2 HCC death-censored patient survival rates**FIGURE 3** HCC death-censored graft survival rates

to be inserted percutaneously by ITU doctors, which represents a demanding procedure associated with a steep learning curve.^[7] Technical failure to commence NRP was encountered in 19 of 224 donors in the NRP group, contributing to the reported use rates. The rates of technical success may be improved when cannulation is performed by skilled and well-trained operators^[17] or by using premortem cannulation, the latter of which is not allowed in several countries, including France.

Posttransplant AST or ALT are surrogate markers of hepatocyte injury used in definitions for early allograft dysfunction^[9,18,19] and also may predict the development of NAS in cDCD grafts.^[20] In comparison with SCS, both NMP and NRP are associated with a decreased AST peak after the LT of cDCD grafts. In the COPE trial, the peak AST was the primary endpoint that

was found to be significantly lower in both the cDCD and DBD subgroups.^[8] In the first comparative study of NRP versus ex situ hypothermic oxygenated machine perfusion (HOPE) LT from cDCD donors, the peak AST in the NRP group was significantly lower than in the HOPE group.^[21] This might suggest that commencing perfusion early in the cDCD liver preservation pathway is more efficient in preventing the graft damage compared with perfusion initiated at the recipient center. However, such an explanation must be taken with caution because of the hazardous interpretation of transaminase level variations after machine perfusion, the retrospective nature of these comparisons, and other differences between the studies. The present study also suggests that clinicians feel more confident in extending the preservation time during NMP than with NRP. Indeed, NRP protocol applied herein required not

to exceed a 4-h perfusion time while there is no data suggesting any deleterious effects of prolonged NRP (>4 h).

The biliary tract represents tissue that is most vulnerable to prolonged ischemia/reperfusion injury. The majority of studies assessing the outcome of cDCD versus DBD LT have reported increased rates of biliary complications in cDCD livers^[2] resulting either in decreased graft and patient survival rates^[22] or in more stringent selection criteria resulting in lower use rates.^[23] One of the main objectives of the use of machine perfusion in cDCD LT is to decrease the rate of NAS.^[24] The COPE trial was not powered to demonstrate any difference between SCS and NMP in terms of biliary complications; although it showed lower rates of radiologically diagnosed NAS in the NMP arm (11% vs. 26%), this difference did not achieve statistical significance, and the majority of these cases were clinically asymptomatic.^[8] In the Spanish cDCD transplant experience comparing super-rapid recovery versus NRP, the rates of both NAS and overall biliary complications were significantly reduced in the NRP group.^[4] All of these findings suggest that dynamic liver preservation strategies decrease the incidence of NAS in cDCD LT. In the present study, the rate of clinically manifest NAS was similar among the two groups (1.5% in the NRP group vs. 2.9% in the NMP group). This is considerably lower compared with the cDCD preserved by cold storage only,^[24] which suggests that both perfusion technologies are clinically efficacious in reducing biliary complications in cDCD LT.

DCD livers represent a valuable source of allografts that has helped to address the shortage of organs in several countries albeit at the cost of the inferior outcomes. In addition to the NAS that may lead to graft loss, the cDCD livers have also been associated with increased rates of posttransplant acute kidney failure and inferior patient survival rates.^[2,22,24,25] In the present study, recipients in both groups achieved a 90-day graft loss of less than 10% (4.4% after NRP, 8.8% after NMP), and 2-year graft and patient survival rates higher than 85%, thus achieving outcomes similar to those expected after LT from DBD nonmarginal grafts.^[12]

There are clear limitations to this study in relation to its retrospective nature and nonrandomized design. The two compared perfusion strategies were performed in different trials and in the context of different organ allocation policies, graft selection criteria, and health care systems. Use rates are also affected by waitlist pressure, which may vary across countries and centers. Despite some differences in recipient and donor baseline characteristics, the two groups were similar with respect to variables that could have an influence on the analyzed outcomes, and we performed an inverse probability treatment weighting adjustment, which allowed us to obtain two cohorts that were comparable for the most relevant variables, such as donor

age, donor risk index, UK-DCD score, and recipient laboratory MELD score. The main difference between the groups was a higher proportion of recipients who received transplants for HCC in the NRP group; we avoided the risk of a tumor-related survival bias by presenting both overall and tumor-censored graft and patient survival rates. The other shortcoming is the relatively small size of the NMP cohort.

In conclusion, the present study is the first to compare two different machine perfusion strategies applied to cDCD livers at a donor hospital. The results suggest that both interventions, NRP and continuous NMP, improve outcomes to a level expected for DBD livers in terms of 1- and 2-year graft and patient survival rates. On the other hand, we have produced no good evidence that either technology is superior and therefore propose that this state of equipoise might be the basis for a formal prospective, randomized, controlled clinical trial to compare not only the effectiveness of these two approaches to perfusion of DCD livers but also the logistic and cost elements.

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CONFLICT OF INTEREST

Darius F. Mirza owns stock in OrganOx. M. Tamara P. R. Perera is on the speakers' bureau for OrganOx. Xavier Muller received grants from Intitut Georges Lopez. Peter J. Friend is the cofounder of, owns stock in, is employed by, consults for, advises, received grants from, and is an inventor on a licensed patent for OrganOx.

ETHICS APPROVAL

The COPE study approval was obtained from the London–Dulwich National Research Ethics Committee and the Medicines and Healthcare Regulatory Agency in the United Kingdom and research ethics committees and medical device regulatory bodies in Belgium, Spain, and Germany. The NRP program was approved by the French institutional local and national ethics committee. The presented study was approved by the COPE steering committee and by the Hospices Civils de Lyon ethics committee (approval number: CSE-HCL_21_360).

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A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation

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Background & Aims: Machine perfusion is a novel method intended to optimize livers before transplantation. However, its effect on morbidity within a 1-year period after transplantation has remained unclear.

Methods: In this multicenter controlled trial, we randomly assigned livers donated after brain death (DBD) for liver transplantation (LT). Livers were either conventionally cold stored (control group), or cold stored and subsequently treated by 1-2 h hypothermic oxygenated perfusion (HOPE) before implantation (HOPE group). The primary endpoint was the occurrence of at least one post-transplant complication per patient, graded by the Clavien score of \geq III, within 1-year after LT. The comprehensive complication index (CCI), laboratory parameters, as well as duration of hospital and intensive care unit stay, graft survival, patient survival, and biliary complications served as secondary endpoints.

Results: Between April 2015 and August 2019, we randomized 177 livers, resulting in 170 liver transplantations (85 in the HOPE group and 85 in the control group). The number of patients with at least one Clavien \geq III complication was 46/85 (54.1%) in the control group and 44/85 (51.8%) in the HOPE group (odds ratio 0.91; 95% CI 0.50-1.66; $p = 0.76$). Secondary endpoints were also not significantly different between groups. A *post hoc* analysis revealed that liver-related Clavien \geq IIIb complications occurred less frequently in the HOPE group compared to the control group (risk ratio 0.26; 95% CI 0.07-0.77; $p = 0.027$). Likewise, graft failure due to liver-related complications did not occur in the HOPE group, but occurred in 7% (6 of 85) of the control group (log-rank test, $p = 0.004$, Gray test, $p = 0.015$).

Conclusions: HOPE after cold storage of DBD livers resulted in similar proportions of patients with at least one Clavien \geq III complication compared to controls. Exploratory findings suggest that HOPE decreases the risk of severe liver graft-related events.

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Introduction

Dynamic preservation strategies are an innovative approach for treatment and assessment of livers before transplantation. This is based on a number of experimental studies demonstrating that liver metabolism can be measured and controlled *ex situ* by either continuous normothermic,^{1,2} or short-term hypothermic oxygenated liver perfusion (HOPE),³ with multiple protective downstream effects, including less Kupffer and endothelial cell activation and subsequently a reduced immune response.^{4–6} Such promising results were translated from several preclinical studies and have demonstrated the prevention of liver ischemia

reperfusion injury.^{7–11} Despite this success, the effect of machine liver perfusion on clinically relevant endpoints, either normothermic or hypothermic, has remained unclear. This is important as liver transplantation (LT) is associated with exceptionally high morbidity even in benchmark cases,¹² despite excellent survival rates, and an observation period of at least one year is mandatory to capture all relevant complications.^{10,13} Most published trials or case series underestimate this fact, with primary endpoints consisting of laboratory values of questionable relevance, recorded only within the first week after LT.^{10,14,15} In contrast, there is an urgent need to investigate whether machine perfusion techniques also impact on clinically and patient relevant

Keywords: Liver transplantation; Randomised controlled trial; Hypothermic oxygenated machine perfusion; Cumulative complications; Liver-related complications.

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HOPE of grafts before liver transplantation

endpoints, the most convincing being complications, and the severity of complications after LT, to justify the additional efforts and costs. The first evidence in this context was presented only very recently with a study on HOPE-treated donation after circulatory death (DCD) livers,⁴ which showed less symptomatic cholangiopathies within 6 months after LT. Further recent randomized trials on donation after brain death (DBD) livers, treated by HOPE, showed less liver graft injury as a primary endpoint, and reported complications during a follow-up of 3 and 6 month.^{9,10} However, the effect of HOPE on cumulative morbidity within 1 year after LT remains unknown.

Patients and methods

Trial design

The HOPE (hypothermic oxygenated perfusion for human liver grafts) trial is an investigator-initiated multicenter randomized-controlled trial (RCT), which included 10 European transplant centers (Birmingham, Gent, Groningen, Leeds, Leuven, London, Lyon, Paris, Vienna, and Zurich) from six countries (Fig. 1).

Allocated livers were randomly assigned in a 1:1 ratio to be preserved either by conventional cold storage (control group), or by cold storage plus subsequent 1-2 h HOPE. Randomization

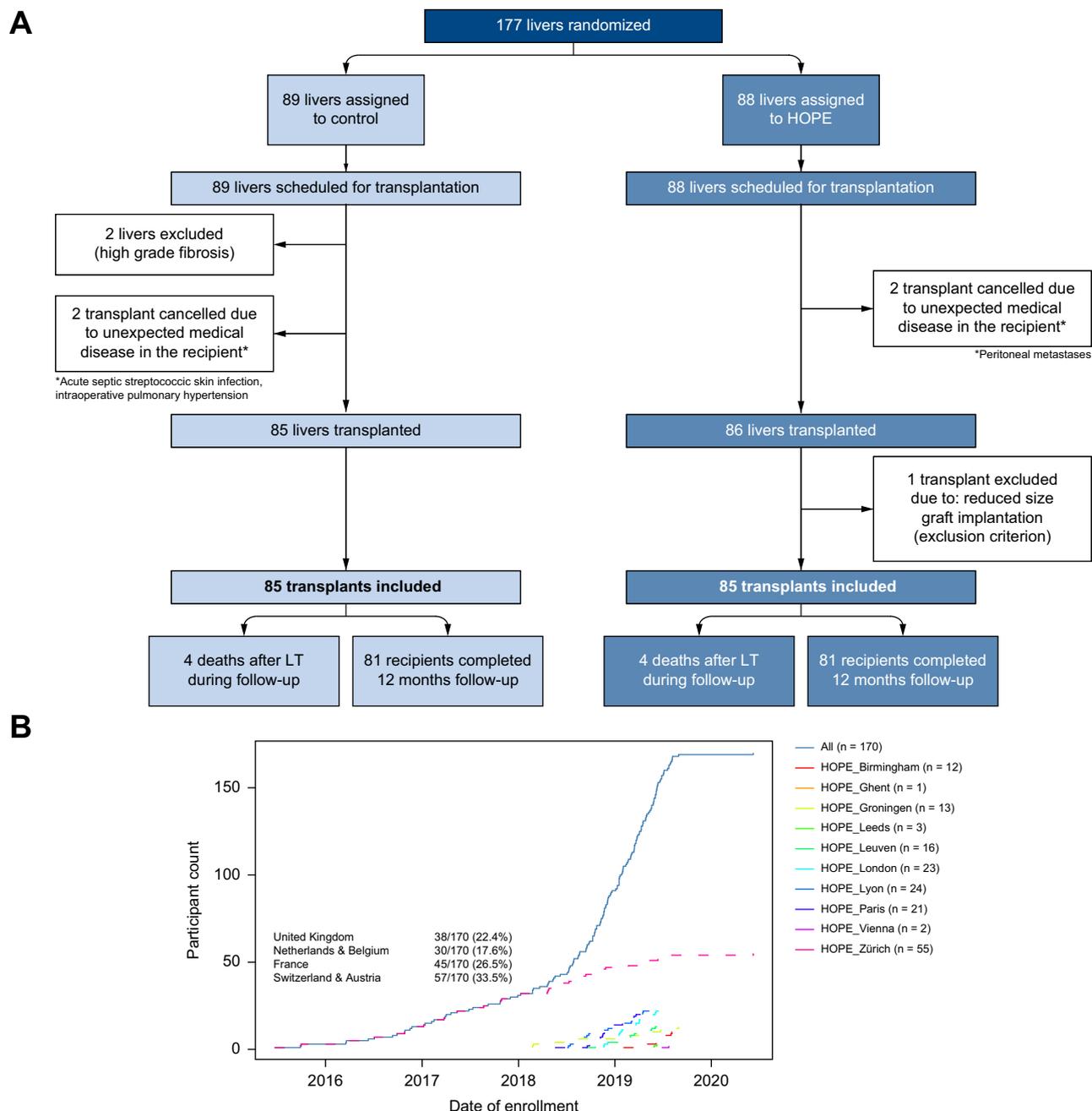


Fig. 1. CONSORT diagram and overall/center-specific recruitment. (A) CONSORT diagram for donor livers enrolled in the trial. 177 livers were randomized and 170 livers were transplanted according to protocol, e.g. 85 in the control group and 85 in the HOPE group (dropout rates 4/89, 4.5%; 3/88, 3.4%). (B) Overall and center-specific annual recruitment of cases. HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

was performed after the donor liver was accepted for transplantation. Center-stratified block randomization with a fixed block size of eight was used to generate a randomization list per center. The block size was not communicated to the investigators. A centralized web-based tool (Randomizer Software, Institute for Medical Informatics, Statistics and Documentation of the Medical University of Graz, www.randomizer.at) was used for randomization at the first center (Zurich), and randomization was performed by an independent person using Microsoft Excel for the other centers. Randomization lists were then stored in the electronic case report files (secuTrial[®]) to ensure allocation concealment.

The trial did not interfere with organ allocation or acceptance; patients, organ procurement teams, and the treating physicians were blinded to the trial group assignments, while surgeons were unblinded due to the perfusion procedure itself. The trial protocol and the amendments have been approved by the ethical committees and the national authorities, and are enclosed in the supplement. Data capturing was performed by electronic case report files using the secuTrial[®] platform. The trial was entirely funded by the Swiss National Science Foundation (33IC30_166909, 32003B_153012), including the perfusate, the perfusion machine disposables, and the monitoring. The funding party played no role in study design, performance, analysis, or the decision to publish. The participating centers provided the perfusion device (Liver Assist[®], Organ Assist, now XVIVO), and the training for machine perfusion for each center was supervised by the study PI.

Trial patients

All patients ≥ 18 years of age, who were listed for liver only transplantation with a whole DBD graft were eligible for inclusion in the trial. Exclusion criteria were all partial or combined liver transplants, living donor or DCD liver transplantation, cold ischemia times of more than 15 h, and an acute or unexpected medical contraindication for LT. All included patients provided written informed consent.

Perfusion procedure

All study centers used the Liver Assist[®] device for machine liver perfusion, with a pressure controlled oxygenated hypothermic liver perfusion through the portal vein only (Fig. S1), targeting a flow rate between 150–300 ml/min at a pressure of 3 mmHg, and a perfusate temperature between 8 and 12 °C. The perfusate consisted of 3 L re-circulating Belzer MPS[®] (Bridge to Life Ltd.) with active oxygenation (70–110 kPa). The minimum perfusion duration was defined as 1 h, while perfusion was generally continued until the recipient hepatectomy was completed.

Endpoint measures

The primary endpoint was the occurrence of one or more major post-transplant complication, defined as a Clavien score of $\geq III$, per patient (binary) within 1 year after LT.¹⁶ The Clavien score ranges from I (for any deviation from the normal postoperative course without pharmacological treatment or surgical, endoscopic, or radiologic interventions) to V (for death).

Secondary endpoints were the comprehensive complication index (CCI, from 0 for no complication to 100 for death),¹⁸ laboratory measurements (aspartate aminotransferase [AST],

alanine aminotransferase [ALT], bilirubin, alkaline phosphatase, gamma glutamyltransferase, international normalized ratio, and Factor V), biliary complications, duration of intensive care unit (ICU) and hospital stay, as well as recipient and graft survival at 1 year after LT. Laboratory measurements were taken at 6 h, 12 h, day 1–7, discharge and 3, 6, 9 and 12 months after LT. Measurements taken on day 1–7 were summarized as area under the curve (AUC), using natural cubic spline interpolation. Measurements taken 3, 6, 9 and 12 months after LT were considered as longitudinal data.

Outcomes analyzed post hoc

As many patients developed more than one major complication within the 12-month period after transplantation, we decided *post hoc* to also consider the number of complications per patient. The occurrence and grading of complications were assessed by the local investigators and controlled by the monitors. The final Clavien score was controlled by two independent clinicians, who were blinded to the preservation method (J.E., R.P.). All complications were additionally classified into three groups:

- Recipient-related complications: opportunistic infections, myocardial infarction, lung embolism, lung infections, hypertension, gastric ulcer, colitis, ileus, diabetes, diarrhea, pyelonephritis, seizures, cerebral ischemia, cerebral bleeding, mesenteric ischemia, ascites (without the need to drain), incarcerated umbilical or inguinal hernias (with the need for surgical repair), accidental traumas, recurrence of hepatocellular carcinoma, secondary cancer.
- Liver graft-related complications: primary non-function, biliary necrosis, biliary strictures (anastomotic and non-anastomotic), bile leaks, hepatic artery thrombosis, hepatic artery stenosis, hepatic artery aneurysms, portal vein thrombosis, hepatic vein thrombosis, acute biopsy proven liver rejection, cholangitis, cholangiosepsis, hepatic encephalopathy, elevated liver enzymes (three-fold over normal values), cholestasis, ascites (with the need for drainage).
- Transplant procedure-related complications: post-transplant hematoma in the first week (with the need for lavage), intermittent kidney failure (with the need for renal replacement therapy), wound infections (with the need for wound opening), elective incisional hernias (transplant incision).

In addition, graft survival was analyzed separately for recipient-related and liver graft-related graft loss.

Monitoring and safety

Monitoring and safety were organized and supervised by the GSO (Gesellschaft für Studienmanagement und Onkologie mbH) Hamburg with regular reporting of all serious adverse events to the national authorities. Adverse events were defined according to EN ISO 14155 as any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) whether or not related to the investigational medical device. Serious adverse events were defined as adverse events that:

- led to death, injury or permanent impairment to a body structure or a body function;
- led to a serious deterioration in health of the patient, that either resulted in: a life-threatening illness or injury, a permanent impairment of a body structure

HOPE of grafts before liver transplantation

or a body function, in-patient hospitalization or prolongation of existing hospitalization, or in the requirement for medical or surgical intervention to prevent life-threatening illness.

Statistical analysis

The trial was powered to detect a clinically relevant difference in the incidence of major complications, e.g. an absolute risk difference of 25% in the incidence of at least one Clavien \geq III complication by the treatment of liver grafts with HOPE. This calculation was based on initial data from the first clinical series on hypothermic liver perfusion,¹⁷ which showed a significantly decreased hospital stay by machine liver perfusion (10.9 vs. 15.3 days, $p = 0.006$, 29% less), and reduced early graft dysfunction (5 vs. 25%). It was assumed that the proportion of patients with at least one \geq grade III complication within 1 year will decrease from 60% in the control group to 35% in the HOPE group. For the sample size calculation, to achieve a power of 90%, a significance level of 0.05 was considered for a two-sided z-test with pooled variance.¹⁸ This resulted in a sample size of 82 per arm, 164 in total. The sample size was then increased to 85 per arm, 170 in total, to account for expected dropouts after transplantation.

Primary and secondary endpoint analyses were pre-specified in the protocol (supplementary information) and in the statistical analysis plan (Version 03, December 2020, supplementary information), which was finalized before the database was locked. The primary endpoint was analyzed by a generalized linear model (GLM) with binomial error and logit link and treatment as an explanatory variable to estimate an odds ratio (OR) with 95% CIs for the effect of HOPE vs. control. Two pre-specified sensitivity analyses were performed: We fitted a generalized linear mixed-effects model (GLMM), with a random intercept for center, for which the randomization was stratified (sensitivity analysis 1). We then added covariates expected to be associated with the primary outcome, i.e. 'recipient lab MELD score', 'cold storage time', 'age of recipient', 'age of donor' and 'previous transplantation', as fixed explanatory variables (sensitivity analysis 2). Due to some missing data in these co-variables, we used multiple imputation with 50 imputations. The imputation model contained the covariates mentioned above, the randomized treatment, the primary outcome and the CCI as well as donor and recipient sex, donor height and weight and treatment before liver transplantation. The results were pooled according to Rubin's rules.

The secondary outcome CCI was compared between groups by a linear regression model with treatment as an explanatory variable. The same two sensitivity analyses were performed as described for the primary outcome but using a linear mixed-effects model (LMM) instead of a GLMM. AUCs of laboratory values (AST and ALT) were log-transformed to better meet the normality assumption and were analyzed using LMMs. Binary secondary outcomes were analyzed by GLM with binomial error and logit link. Length of hospital stay and length of ICU stay were analyzed by cause-specific Cox proportional hazards models on time to discharge alive, accounting for death during hospital or ICU stay as a competing risk.

Number of major complications (per patient) was analyzed *post hoc* using a GLM with log link and quasi-Poisson error. Further, time to graft failure was analyzed *post hoc*, once

overall and once separated for liver-related graft failure and participant-related graft failure. Cause-specific Cox proportional hazards models were used for the two subtypes of graft failure (competing risks).

It should be noted that no adjustments were made for type I error rate inflation due to the analysis of multiple outcomes.

All statistical methods and results, including deviations from the original statistical analysis plan are reported in detail in the statistical report (Version 1.5, November 16th, 2022, supplementary information).

Results

Patients

Between April 2015 and August 2019, we randomized 177 livers, accepted for transplantation into eligible recipients. After randomization, six transplants were cancelled before any trial procedure, including four assignments to the control group, and 2 assignments to the perfusion group. The reasons for cancellations were high-grade fibrosis in two liver grafts, an unexpected severe pulmonary hypertension after intubation in one recipient, an acute streptococcal skin infection discovered at recipient hospital entry, and unexpected peritoneal metastasis after recipient laparotomy ($n = 2$). In one further case, the perfused liver was reduced to a left lobe before transplantation, to compensate for a severe size mismatch, resulting in transplantation of a partial graft. This patient was therefore excluded from the trial, according to the protocol criteria. These early dropouts, e.g., at the day of randomization, were compensated for by additional recruitment (Fig. 1).

Overall, 170 liver transplants were performed within the trial, and 85 patients in each study arm were included in the analysis (Fig. 1). All patients completed the 1-year follow-up, with the exception of deaths during this time ($n = 8$).

The baseline characteristics of the donors and preservation factors are shown in Table 1; baseline characteristics of recipients are shown in Table 2. Despite randomization, there were some imbalances between groups. For example, there were less cerebral hemorrhages and more other causes of death in the HOPE group. Liver weight was 126 g lower, and cold storage 54 min shorter in the HOPE group (Table 1). In addition, more female recipients (14.1%), less cases with Child-Pugh B/C cirrhosis, and less conservative treatment before LT were recorded in the HOPE group (Table 2).

Machine perfusion parameters were within the range defined in the protocol, e.g. median perfusion time 96 min, median perfusion flow 200 ml/min, median perfusion pressure 3 mmHg, median perfusate temperature 10 °C, and median oxygenation 100 kPa (Fig. S2).

Primary endpoint: number of patients with Clavien \geq III complications

A total of 1,190 complications were documented for all study patients during 1 year after LT with no patients lost to follow-up. The proportion of patients with at least one Clavien \geq IIIa complication did not significantly differ between groups – 54.1% (46/85) in the control group and 51.8% (44/85) in the HOPE group. This resulted in an unadjusted OR of 0.91 (95% CI 0.50–1.66, $p = 0.76$). The absolute risk difference was estimated as -2.35% (95% CI 16.96%–12.40%). In our sensitivity

Table 1. Characteristics of liver donors and liver graft preservation.

Variable	Overall	Control	HOPE	Missing (%)
N	170	85	85	
Before randomization				
Donor age, years	60.5 (47.0–72.0)	62.0 (44.0–71.0)	59.0 (48.0–72.0)	0
Donor sex, female	82 (48.5)	42 (50.0)	40 (47.1)	0.6
Donor height, m – mean (SD)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	1.2
Donor weight, kg – mean (SD)	76.3 (15.8)	77.8 (16.9)	74.9 (14.5)	1.2
Donor cause of death				6.5
Cerebral hemorrhage	73 (45.9)	39 (48.8)	34 (43.0)	
Cerebral trauma	37 (23.3)	18 (22.5)	19 (24.1)	
Anoxia	23 (14.5)	12 (15.0)	11 (13.9)	
Cerebral disease	1 (0.6)	0	1 (1.3)	
Suicide	4 (2.5)	3 (3.8)	1 (1.3)	
Other	21 (13.2)	8 (10.0)	13 (16.5)	
After randomization				
Preservation solution				0.6
Histidin-Tryptophan-Ketoglutarat (HTK)	4 (2.4)	1 (1.2)	3 (3.6)	
University of Wisconsin (UW)	53 (31.4)	27 (31.8)	26 (31.0)	
Institute George Lopez (IGL)-1	112 (66.3)	57 (67.1)	55 (65.5)	
Duration of cold storage, min	393.0 (320.0–482.0)	427.0 (356.0–487.0)	373.0 (299.2–471.8)	7.6
Duration of HOPE, min	95.5 (73.0–137.0)	–	95.5 (73.0–137.0)	57.6
Duration of total preservation time, min	451.0 (371.0–552.5)	427.0 (356.0–487.0)	474.0 (403.5–588.0)	13.5
Liver weight, g – mean (SD)	1517.0 (591.8)	1,583.0 (759.0)	1,457.3 (378.6)	17.1
AST HOPE perfusate, U/L		–	117.6 (60.0– 266.9)	76.1
ALT HOPE perfusate, U/L		–	177.1 (75.0–467.0)	76.1

Continuous variables are presented as median (IQR) and categorical variables as n (%), unless otherwise stated.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HOPE, hypothermic oxygenated perfusion.

Table 2. Characteristics of liver transplant recipients.

Variable	Overall	Control	HOPE	Missing (%)
n	170	85	85	
Recipient age, years	59.0 (50.2–64.0)	57.0 (49.0–64.0)	60.0 (51.0–64.0)	0
Recipient sex, female	48 (28.2)	18 (21.2)	30 (35.3)	0
Underlying disease				0
Acute liver failure	1 (0.6)	1 (1.2)	0 (0)	
Cirrhosis Child-Pugh A	49 (28.8)	23 (27.1)	26 (30.6)	
Cirrhosis Child-Pugh B,C	93 (54.7)	50 (58.8)	43 (50.6)	
Other	27 (15.9)	11 (12.9)	16 (18.8)	
Laboratory MELD	20.0 (11.0–27.0)	19.0 (12.0–26.0)	20.0 (11.0–28.0)	0
Treatment before liver transplant				0
TACE, RFA	41 (24.1)	21 (24.7)	20 (23.5)	
TIPS	10 (5.9)	5 (5.9)	5 (5.9)	
Conservative	38 (22.4)	23 (27.1)	15 (17.6)	
No treatment	61 (35.9)	34 (40.0)	27 (31.8)	
Other	20 (11.8)	2 (2.4)	18 (21.2)	
Previous liver transplant	7 (4.1)	2 (2.4)	5 (5.9)	0
Transplant center				0
Birmingham	12 (7.1)	7 (8.2)	5 (5.9)	
Ghent	1 (0.6)	0 (0)	1 (1.2)	
Groningen	13 (7.6)	7 (8.2)	6 (7.1)	
Leeds	3 (1.8)	2 (2.4)	1 (1.2)	
Leuven	16 (9.4)	8 (9.4)	8 (9.4)	
London	23 (13.5)	11 (12.9)	12 (14.1)	
Lyon	24 (14.1)	12 (14.1)	12 (14.1)	
Paris	21 (12.4)	11 (12.9)	10 (11.8)	
Vienna	2 (1.2)	1 (1.2)	1 (1.2)	
Zürich	55 (32.4)	26 (30.6)	29 (34.1)	

Continuous variables are presented as median (IQR) and categorical variables as n (%).

HOPE, hypothermic oxygenated perfusion; MELD, model for end-stage liver disease; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TIPS, transjugular intrahepatic portosystemic shunt.

analyses using GLMMs with random intercept for center, the ORs were estimated as 0.874 (95% CI 0.46–1.67, $p = 0.68$) and 0.91 (95% CI 0.47–1.78, $p = 0.787$), when adjusted for MELD, donor age, recipient age, duration of cold storage, and previous liver transplantation.

Secondary endpoints

The overall CCI of all complications was not significantly different between study groups, e.g. the median 12-month CCI was 49.5 (IQR 29.6–64.5) in the control group and 49.4 (IQR 33.2–63.9) in the HOPE group (Table 3). Laboratory

HOPE of grafts before liver transplantation

Table 3. Pre-specified secondary endpoints (recipient outcome within 12 months after LT) and additional outcome parameters.

Variable	Overall	Control	HOPE	p value	Effect size (95% CI)	Missing (%)
N	170	85	85			
CCI 12, months	49.4 (29.6–64.4)	49.5 (29.6–64.5)	49.4 (33.2–63.9)	0.89 [‡]	MD 0.685–7.202 to 8.338 [‡]	0
Peak AST, U/L	825 (430–1,705)	896 (409–2,478)	803 (435–1,303)			0
AST AUC, U/L – day 1-7	1,147 (687–2,171)	1,147 (683–2,752)	1,149 (693–1,856)	0.25*	MD -0.157 (-0.42 to 0.11)*	1.8
Peak ALT, U/L	654 (365–1,188)	695 (379–1,575)	636 (341–1,055)			0
ALT AUC, U/L – day 1-7	2,022 (1,242–3,750)	1,978 (1,232–4,128)	2,048 (1,252–3,475)	0.49*	MD -0.089 (-0.34 to 0.16)*	0
INR AUC, day 1-7	7.1 (6.6–7.8)	7.1 (6.5–8.1)	7.1 (6.6–7.8)			0
Bilirubin AUC, μmol/L – day 1-7	199 (103–438)	202 (95–542)	200 (119–381)			11.2
GGT AUC, U/L – day 1-7	1,653 (806–2,615)	1,774 (761–2,621)	1,531 (918–2,610)			11.8
AP AUC, U/L – day 1-7	846 (622–1,320)	874 (637–1,255)	803 (619–1,323)			0.6
Hospital stay, days	15 (13.0–25.0)	15 (13.0,25.0)	17 (12.0–24.5)	0.79 [#]	HR 0.958 (0.70 to 1.30) [#]	1.8
ICU stay, days	3.0 (2.0–5.0)	3.0 (2.0–6.0)	3.0 (2.0–5.0)	0.75 [#]	HR 1.051 (0.77 to 1.43) [#]	0
Any biliary complication	34 (20.0)	19 (22.4)	15 (17.6)	0.44 [§]	OR 0.744 (0.35 to 1.58) [§]	0
Overall graft loss in 1 year	11 (6.5)	7 (8.2)	4 (4.7)	0.36 [§]	OR 0.550 (0.140 to 1.896) [§]	0
Recipient death in 1 year	8 (4.7)	4 (4.7)	4 (4.8)	1.00 [§]	OR 1.000 (0.229 to 4.359) [§]	0
Additional outcome parameters after LT						
Duration of transplantation, min	380 (295–477)	384 (302–464)	371 (284–480)			3.5
Anastomotic biliary complications	32 (19.0)	18 (21.2)	14 (16.5)			0
Non-anastomotic biliary complications (NAS)	4 (2.4)	3 (3.5)	1 (1.2) ^{##}			0
Early allograft dysfunction**	53 (31.2)	39 (45.9)	14 (16.5)			0
Hepatic artery thrombosis	2 (1.2)	0	2 (2.4)			0
Hepatic artery stenosis	3 (1.8)	2 (2.4)	1 (1.2)			0
Liver-related graft loss due to:	6 (3.5)	6 (7.1)	0	0.004		0
Primary non function	3 (1.8)	3 (3.5)	0	0.015 [†]		0
NAS	3 (1.8)	3 (3.5)	0			0
Recipient-related graft loss	5 (2.9)	1 (1.2)	4 (4.7)	0.223 ^{††}	HR 3.90 (0.44 to 34.90) ^{††}	0
Primary tumor recurrence	1 (0.6)	0	1 (1.2)			0
Secondary tumor growth	3 (1.8)	1 (1.2)	2 (2.4)			0
Opportunistic infection	1 (0.6)	0	1 (1.2)			0
Retransplantation	3 (1.8)	3 (3.5)	0			0
CCI 3 month	41.8 (23.0–52.6)	42.4 (22.6–52.7)	41.8 (24.2–52.6)			0
CCI 6 month	46.0 (27.3–58.8)	42.4 (22.6–52.7)	46.8 (29.8–60.1)			0
CCI 9 month	48.3 (29.6–63.2)	48.2 (29.6–59.9)	48.9 (29.8–63.7)			0

Continuous variables are presented as median (IQR) and categorical variables as n (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, comprehensive complication index; GGT, gamma glutamyltransferase; HOPE, hypothermic oxygenated perfusion; HR, hazard ratio; INR, international normalized ratio; LT, liver transplantation; MD, mean difference; NAS, non-anastomotic strictures; OR, odds ratio.

[†]Linear model, with similar MD estimated in sensitivity analyses by simple and covariate-adjusted linear mixed-effects model.

*Linear mixed-effects model with a random intercept per center. AUCs were log-transformed to better meet the normality assumption.

^{††}Cause-specific Cox proportional hazards models on time to discharge alive from hospital or ICU (accounting for death during hospital or ICU stay as competing risk).

[§]Generalized linear model with log link function.

[†]Due to zero events in the HOPE arm, the HR could not be estimated. The p-value was calculated by the log rank test and the Gray test for comparing the two cumulative incidence function curves.

^{††}Cox proportional hazards model.

**Oloff criteria.

^{##}This graft was not lost, conservative treatment of biliary complication.

values (AST, ALT, international normalized ratio, gamma-glutamyltransferase, bilirubin, alkaline phosphatase) during the first week after liver transplant, assessed by AUC, and peak AST and peak ALT, were not significantly different between study groups (Table 3). Of note, the AUC for factor V was not calculated due to lack of data (missing values 46.5%). The further course of laboratory values at 3, 6, 9, and 12 months is shown in Fig. S3, together with those within the first week. Longitudinal analysis of laboratory measurements at 3-12 months using GLMM did not reveal significant differences between groups (supplementary information). ICU and hospital length of stay were also similar in both groups (Table 3). One-year overall graft survival was 95.3% (81/85) in the HOPE group with three tumor-related graft losses and one graft loss due to candida pneumonia, and 91.8% (78/85) in the control group with six liver graft-related and one tumor-related graft loss (OR 0.550; 95% CI 0.140–1.896, $p = 0.36$, Table 3).

Post hoc analysis: quantity of complications per patient

We recorded 574 complications in the control group and 616 complications in the HOPE group. The vast majority of complications were minor and graded as Clavien I-II in both arms (433/574, 75.4% and 494/616, 80.2%, Fig. 2). Likewise, the number of Clavien IIIa complications, treated under local anesthesia, was not significantly different, with 72/574 (12.5%) in the control group, and 81/616 (13.1%) in the HOPE group (Fig. 2), which underlines a comparable number of minor (anastomotic) biliary complications in both groups within benchmark values (Table 3B).¹²

In contrast, severe complications (Clavien-Grade ≥IIIb), e.g. operative re-exposures, single- and multi-organ failures, or death, occurred less frequently in the HOPE group (41 of 616 complications, 6.6%) than the control group (69 of 574 complications, 12.0%) (rate ratio 0.59, 95% CI 0.31–1.11), corresponding to a 41% reduction (Fig. 2). This was caused by a

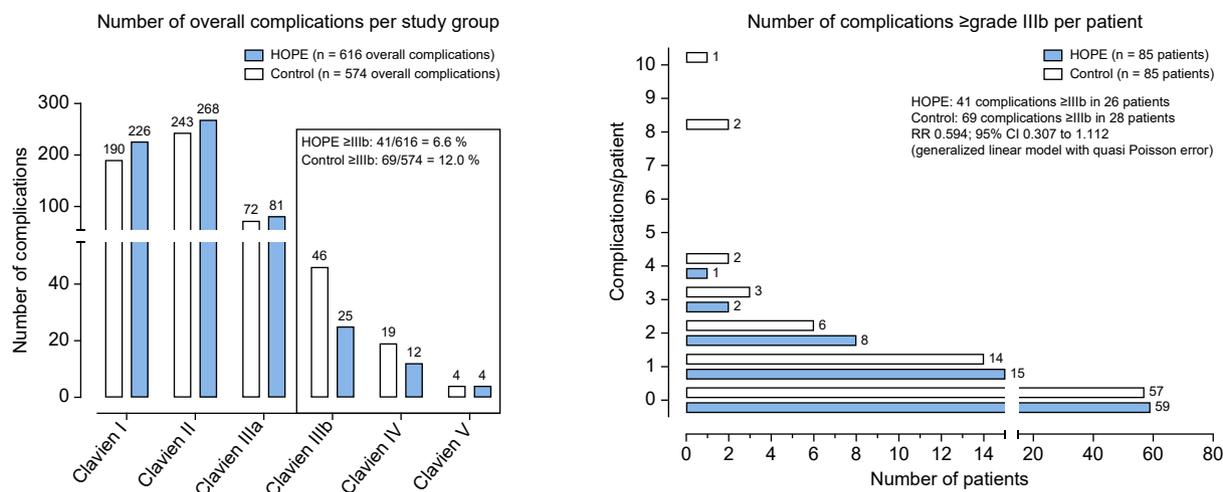


Fig. 2. Number of complications within 1 year after LT (1,190 recorded complications in 170 included patients). Number of complications per Clavien score by randomized treatment and study group (left panel). Number of Clavien >IIIb complications per randomized patient and study group (right panel). HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

74% lower number of liver graft-related Clavien ≥IIIb complications per patient in the perfusion group compared to the control group (11 complications in 7 patients vs. 42 complications in 17 patients (rate ratio 0.26; 95% CI 0.07–0.77; $p = 0.027$; Figs 3 and 4). Accordingly, the CCI for patients with liver graft-related complications ($n = 83$) within 1-year follow-up was lower in the perfusion group (median 30.6; IQR 20.9–37.1), compared to the control group (median 43.6; IQR 29.6–58.6, Table S1). Consistently, liver-related graft failure did not occur in the HOPE group, while six liver grafts were lost in the control group due to severe liver-related complications, e.g. primary non function or cholangiopathy (log-rank test, $p = 0.004$, Gray test $p = 0.015$, Table 3B and Fig. 5).

Safety and serious adverse events

The number of reported serious adverse events was comparable in the two study groups (Table S2). There was also no

relevant clinical difference between the two groups in the severity of these events. Four device malfunctions occurred in 88 machine liver perfusions (4.5%), which resulted in insufficient perfusion flow through the portal vein in three cases, and in excessive perfusion (>400 ml/min) despite low portal pressure in one case. In one of these cases, an unexpected peritoneal metastasis in the recipient was confirmed through histology, with consecutive cancelled transplantation. This case was therefore excluded from the analysis. The other three device malfunctions were included.

Discussion

Despite benchmark analysis and multiple reports on outcome after LT, quantifying morbidity in a liver transplant population remains a major challenge. We present the first randomized machine liver perfusion trial on cumulative recipient morbidity within a one-year period after transplantation, which is

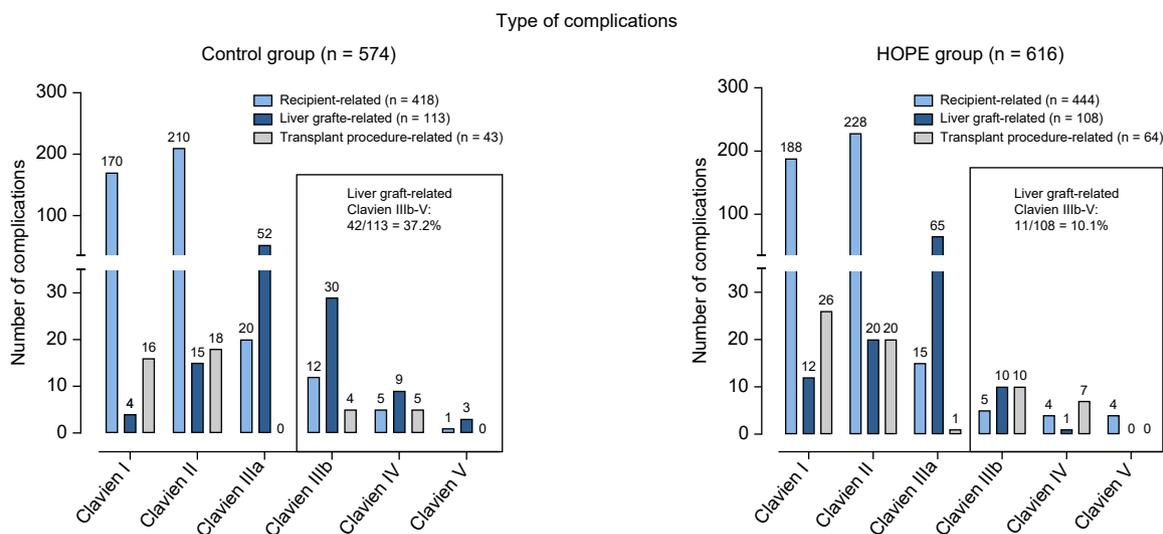


Fig. 3. Specification of complications within 1 year after LT. Number of complications per Clavien grading level by relatedness to recipient, liver graft, or transplant procedure for patients in the control arm (left panel) and in the HOPE arm (right panel). HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

HOPE of grafts before liver transplantation

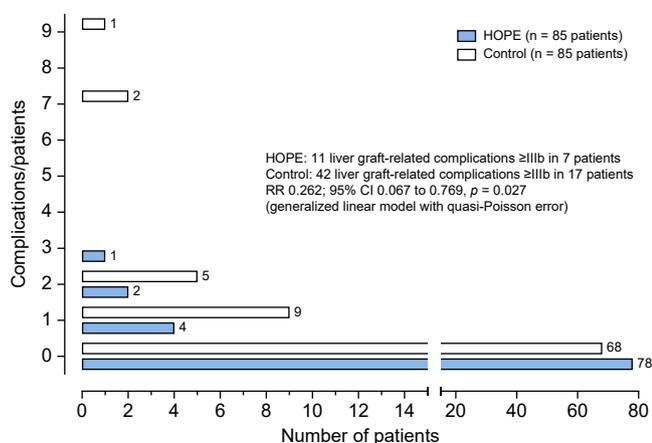


Fig. 4. Number of liver graft-related ≥IIIb complications per patient. Number of liver graft-related ≥IIIb complications per randomized patient and study group with comparison by a generalized linear model with quasi-Poisson error. HOPE, hypothermic oxygenated perfusion; RR, risk ratio.

mandatory for a reliable assessment of complications.¹² The trial shows that HOPE after cold storage of DBD livers did not significantly affect the number of patients with at least one grade ≥III complication within 1 year after LT. There was also no significant difference in all pre-specified secondary endpoints, which focus on laboratory values, initial ICU and hospital stay, and survival.

However, many patients developed more than one major complication within 1-year follow-up, which is ignored by the binary primary endpoint and is likewise not captured by average laboratory values, post-transplant ICU stay, or graft survival, with a subsequent potential underestimation of graft

treatment effects.¹⁹ Instead, the extent of post-transplant morbidity was only recognized by the frequency and the severity of complications, with a 74% lower number of liver-related Clavien ≥IIIb complications in the HOPE arm, compared to the control group. Yet, these results were found in a *post hoc* analysis, and are therefore of an exploratory nature. Further studies will be needed to confirm this potentially clinically important effect of HOPE on the most expensive complications after surgery (Clavien ≥IIIb).²⁰ A similar effect was recently shown for hypothermic oxygenated kidney perfusion,²¹ which may serve as a strong argument for reimbursement of this technology by healthcare providers.

Machine liver perfusion has attracted wide attention within the transplantation community over the last 5 years, but is still rarely applied by most transplant surgeons.²² This probably relies on the perception that this strategy is time consuming and costly, despite current research disclosing several advantages compared to conventional cold storage, including mitochondrial energy restoration or assessment of liver quality before implantation.^{23–26} Another reason for such restrictive use of machine liver perfusion is the lack of available convincing RCTs demonstrating clinically relevant benefits as primary endpoints, as opposed to data on recipient laboratory values or early allograft dysfunction within the first week after LT.^{10,14,15}

As a first step in this direction, a recently published RCT assessing the impact of D-HOPE (dual-HOPE, e.g. perfusion of both, the hepatic artery and the portal vein) on DCD livers, showed a decrease in symptomatic cholangiopathies.¹⁶ However, while intrahepatic cholangiopathies are a frequent and feared complication in DCD liver transplants, DBD liver recipients are rarely affected by this type of injury.^{6,27} Our results

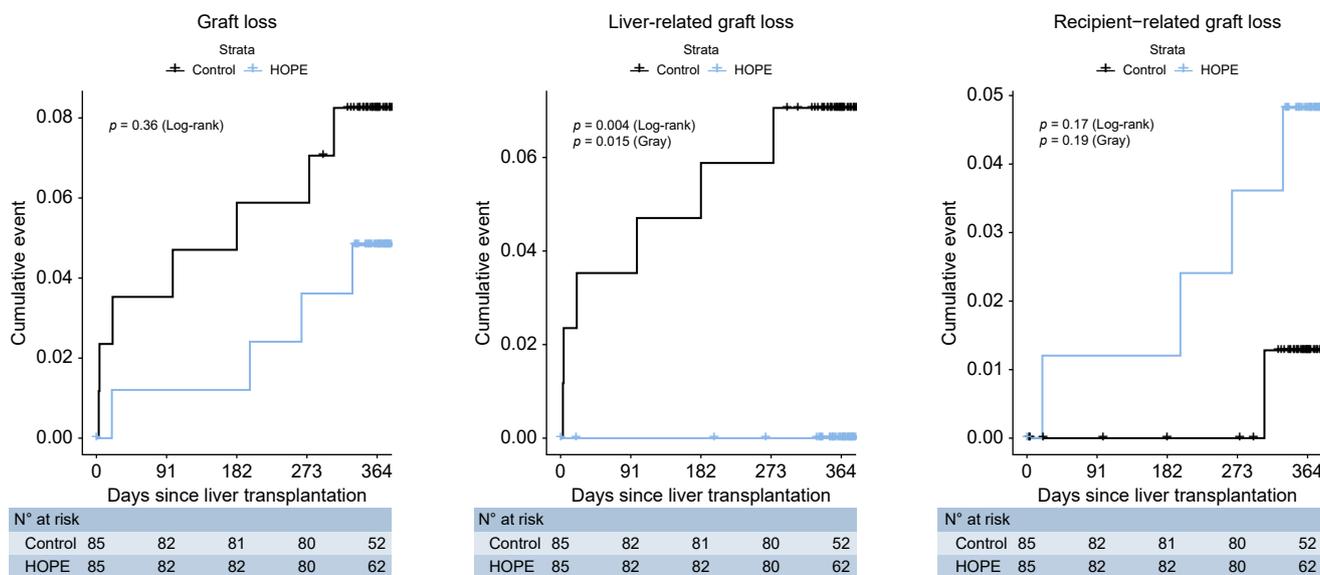


Fig. 5. Graft loss within 1 year after LT. 1-KM curves for graft loss (all types, left) and the two subtypes liver-related (middle) and recipient-related graft loss by treatment (right), which are treated as two competing risks by the Gray test. Liver related graft failure did not occur in the HOPE group, while six liver grafts were lost in the control group due to liver related complications, e.g. primary non-function (n = 3) or intrahepatic cholangiopathy (n = 3). The resulting HRs for HOPE vs. Control, estimated by (cause specific) Cox proportional hazards models, and the log-rank test for the 1-KM curve as well as the Gray test for the cumulative incidence function (CIF) curves (testing the null-hypothesis of no difference between curves) are as follows: • Graft loss: HR=0.57 (95% CI 0.17–1.94), log-rank test p value: 0.36; • Liver-related graft loss: HR = 0.00 (95% CI 0.00–inf), log-rank test p value 0.004, Gray test p value: 0.015 (due to zero events in the HOPE arm, the HR cannot be estimated); • Patient-related graft loss: HR = 3.90 (95% CI 0.44–34.90), log-rank test p value 0.17, Gray test p value: 0.19. HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

indicate that HOPE treatment may be also effective in preventing additional major complications in patients receiving DBD livers, the most common grafts in the West. This result is consistent with another published RCT on HOPE-treated extended criteria donor livers, which reported less Clavien \geq III complications with HOPE treatment.¹⁰ However, this trial focused on first week peak serum ALT levels as a primary endpoint, with only 23 patients included in each study arm, and a 3-month follow-up for complications. In addition, only the highest-graded complication per patient was counted, which limits interpretation regarding overall morbidity. In contrast, we present here a meticulous assessment of numerous complications per patient up to 1 year after LT.

The mechanism of HOPE has been investigated in several experimental studies and has been shown to be dependent on sufficient perfusate oxygenation under hypothermic conditions in livers, kidneys and in hearts.^{8,28,29} Oxygenated cold perfusion triggers a mitochondrial metabolic conversion with sufficient reduction of accumulated citric acid metabolites and electron donors, e.g., succinate and NADH, during perfusion, while avoiding reverse electron transfer to mitochondrial complex-I.²⁵ HOPE-treated livers are therefore up-loaded with ATP, without major oxidative stress, and simultaneously present low lactate and low succinate levels, and a well-preserved complex I-IV function, which enables immediate graft function after implantation.^{8,25,28-30} Based on this, the benefit of HOPE should increase with increasing graft injury.³

Despite these well described biochemical effects, end-ischemic HOPE liver treatment has failed to prevent anastomotic biliary complications, e.g. IIIa complications, even when applied additionally through the hepatic artery (D-HOPE).⁴ Accordingly, the extrahepatic bile duct epithelium, e.g. the common bile duct, appears more difficult to protect, compared to intrahepatic cholangiocytes and hepatocytes, and further research is needed, for example to investigate the effect of changes in perfusate compositions.

This study has limitations. First, the restrictions of using a binary primary endpoint are well known, and should have been anticipated when designing the study, which was in 2011 (first registration in clinicalTrials.gov.). At that time, very limited data on morbidity after LT was available and there was only scarce information on the effect of machine liver perfusion. Second, despite randomization, we noted imbalances between groups in terms of liver weight, cold storage time, sex distribution, underlying disease of recipient, and donor cause of death.

Third, the analysis is based on a modified intention to treat population, given only 170/177 recipients were actually

transplanted. Due to the small number of exclusions (7/177, 3.9%) and similar number of exclusions in both groups, a relationship with the intervention is unlikely and the consequences in terms of selection bias should be minor. Fourth, composite endpoints, such as the CCI, need to be adjusted in terms of complications caused by liver graft injury and those caused by the inherently high recipient morbidity in a liver transplant population. This should be carefully considered in future trial designs on LT. Lastly, given the high number of secondary endpoints and times of analysis, as well as the *post hoc* analyses, it is likely that some false findings could have occurred, given that no adjustment for multiplicity was performed.

One strength of our trial is the low and almost equal discard rate in both study arms. This is caused by late randomization, e.g. after arrival of procured livers in the transplant centers, in contrast to published normothermic perfusion trials, reporting high discard rates in the control group.^{14,15} Secondly, our trial also shows a difference in graft survival by machine liver perfusion technique, when looking at liver-related graft failure. This is important, as the study design was not selective, with no exclusion of sick recipients, marginal liver grafts, or retransplants, documented by a cumulative 12-month CCI clearly above the benchmark value in both groups.¹² Third, the trial illustrates that frequently used endpoints in previous studies, including CCI, serious adverse event counts, length of hospital stay, or liver function parameters, are insufficient for the assessment of liver-related morbidity after LT. The trial is furthermore representative of the real world, owing to the participation of 10 well-established European liver transplant centers from six countries, with a homogeneous case distribution per country and region. Finally, the applied machine liver perfusion technique appears safe with no graft loss due to pump malfunctions.

In summary, we demonstrate that the HOPE approach has no effect on the number of patients with one or more post-transplant Clavien \geq III complication. We believe however that morbidity after LT can only be captured by quantifying and specifying complications per patient. HOPE may be beneficial in this respect, by reducing the number of severe liver-related complications per patient. As it is a simple and quick perfusion technique, it can be applied easily after organ transport during recipient hepatectomy. This appears fundamental since concurring perfusion technologies need either perfusion at donor sites or continuous perfusion during organ transport, which are much costlier and more laborious. We conclude therefore that the *post hoc* findings of this trial should be further validated in future studies.

Affiliations

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HOPE of grafts before liver transplantation

Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; CCI, comprehensive complication index; DBD, donated after brain death; DCD, donation after circulatory death; GLM, generalized linear model; GLMM, generalized linear mixed-effects model; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; LMM, linear mixed-effects model; LT, liver transplantation; OR, odds ratio; RCT, randomized-controlled trial.

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Conflict of interest

This is an investigator-initiated trial (IICT) with no financial involvement of any perfusion or industrial companies.

Authors' contributions

AS: Conceptualization, Methodology, Investigation, Resources, Writing original draft, Writing review & editing, Supervision; MM: Validation, writing review & editing, Supervision; XM: Resources, Investigation, Supervision; JE: Validation, Writing review & editing, Supervision; RP: Validation, Writing review & editing; SvF: Software, Formal analysis; KS: Software, Formal analysis; RXSDS: Validation, Writing review & editing; OdR: Validation, Writing review & editing; JYM, ML, MCC, NDH, MAA, RA, DM, IJ, MPDH, RJP, AP, PM, PK, MA, DK, GB, XR: Resources, Investigation; KP: Validation, Project administration, Data Curation, Supervision; ALK, SA: Validation, Project administration, Data Curation, Supervision; BM: Resources, Conceptualization, writing & editing; PAC: Conceptualization, Methodology, Investigation, Resources, Writing review & editing; PD: Conceptualization, Methodology, Investigation, Resources, Writing Original draft, Writing review & editing, Visualization, Supervision, Funding Acquisition.

Data availability statement

The data used to support the findings of this study are included and available within the article.

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Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2022.12.030>.

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Author names in bold designate shared co-first authorship

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LETTER TO THE EDITOR

Letter to the Editor: Reduced whole liver grafts from pediatric donors as an alternative for small recipients

To the editor,
 We read with great interest the study from Wang et al^[1] on reducing the donor weight limit for split liver grafts in pediatric liver transplantation (LT). The authors described 22 split liver transplantations from donors weighing <25 kg with similar postoperative outcomes compared to other graft types.

The authors were able to perform a split procedure and 2 transplantations with the same team, which is a

logistical challenge. In addition, appropriate morphologic matching for 2 recipients remains challenging, especially for the right partial graft, and its reallocation may prolong cold ischemia time, which decreases graft survival.^[2]

In this context, we would like to discuss *ex-vivo* reduced whole liver graft (rWLG) as an alternative for split liver transplantations in small infants with pediatric donors. We report 2 LTs in small recipients (6.8–7.5 kg)

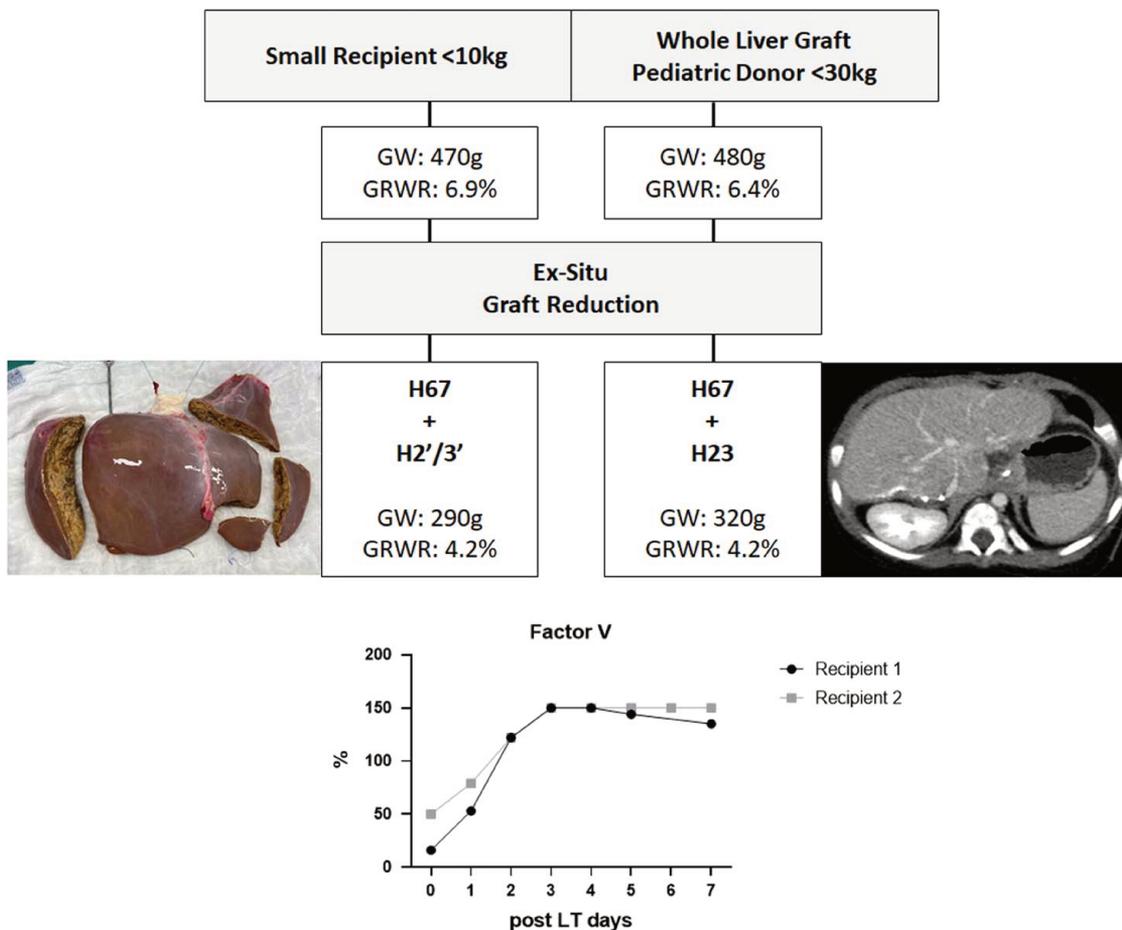


FIGURE 1 *Ex-vivo* whole liver graft reduction. H67: Right posterior sectionectomy; H2'/3': Non anatomical resection of S2 and S3; H23: Left lateral sectionectomy;

Abbreviations: GW, graft weight; GRWR, graft recipient weight ratio; LT, liver transplantation; rWLG, reduced whole liver graft.

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listed for acute liver failure with a liver graft from a pediatric donor (20–26 kg). The first graft was a rWLG following a right posterior sectionectomy (H67) and non anatomical resection of S2-S3 (H2'/3')^[3] the second one combined a H67 and a left lateral sectionectomy (H23) (Figure 1). Liver volume reduction ranged from 30% to 40%, allowing to reach a GRWR of 4%. Normalization of factor V was observed on day 2 without primary nonfunction or vascular complications.

In line with Wang and colleagues, an association of small pediatric donors and recipients is rare (5% of LT) but remains a surgical challenge. As discussed by Stoltz et al,^[4] expanding the limit of splittable grafts optimizes graft resources and decreases waiting time. Nevertheless, we would like to highlight 3 main arguments for rWLG. First, rWLG avoids the logistical challenge associated with 2 simultaneous LTs. Second, contrary to left partial grafts, rWLG offers optimal anthropomorphic matching by decreasing both cranio-caudal length and antero-posterior graft thickness. Third, it combines the advantage of whole graft LT by allowing caval replacement, duct-to-duct bile anastomosis and by preserving the entire portal and artery axis for vascular reconstruction.

AUTHOR CONTRIBUTIONS

Guillaume Rossignol and Xavier Muller drafted the manuscript. Jean-Yves Mabrut, Kayvan Mohkam, and Remi Dubois critically reviewed, revised, and approved the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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CASE REPORT

WILEY

Optimizing graft-recipient size matching in adolescent liver transplantation: Don't forget ex situ right posterior sectionectomy

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Abstract

Background: Graft-recipient size matching is a major challenge in pediatric liver transplantation, especially for adolescent recipients. Indeed, adolescents have the lowest transplantation rate among pediatric recipients, despite prioritization policies and the use of split grafts. In case of an important graft-recipient size mismatch, ex situ graft reduction with right posterior sectionectomy (RPS) may optimize the available donor pool to benefit adolescent recipients.

Methods: We present three cases of liver graft reduction with ex situ RPS for adolescent recipients. The surgical strategy was guided by GRWR (graft/recipient weight ratio), GW/RAP (right anteroposterior distance ratio), and CT-scan volumetric and anthropometric evaluation.

Results: Recipients were 12, 13, and 14-year-old and weighed 32, 47, and 35 kg, respectively. All liver grafts were procured from brain-dead donors with a donor/recipient weight ratio >1.5. RPS was performed ex situ, removing 20% of the total liver volume leading to a decrease of the GRWR <4% and the GW/RAP <100g/cm in each case. All three reduced grafts were successfully transplanted with a static cold storage time ranging from 390 to 510min without the need for delayed abdominal closure. We did not observe any primary non-function, vascular complication, or delayed graft function with a median follow-up of 6 months. One biliary anastomotic stenosis occurred which required surgical treatment.

Conclusion: Ex situ liver graft reduction with RPS allowed for successful transplantation in case of anthropometric graft-recipient size mismatch in adolescent liver transplant candidates. Although the use of split grafts remains the gold standard, RPS should be acknowledged as a way to optimize the donor pool, especially for adolescent recipients.

KEYWORDS

ex situ graft reduction, graft/recipient matching, pediatric liver transplantation, right posterior sectionectomy

Abbreviations: DBD, Donation after Brain Death; EAD, Early Allograft Dysfunction; GRWR, Graft/Recipient Weight Ratio; GW, Graft Weight; HOPE, Hypothermic Oxygenated Perfusion; LFS, Large-For-Size; LT, Liver Transplantation; RAP, Right Antero-Posterior Distance; RPS, Right Posterior Sectionectomy; SFS, Small-For-Size.

Guillaume Rossignol and Xavier Muller contributed equally as first authors.

1 | INTRODUCTION

Optimal graft-recipient size matching has been a major challenge in pediatric liver transplantation. In this context, split liver transplantation was first introduced as graft reduction strategy and later also performed to allow transplantation of two pediatric recipients.^{1,2} However, organ offers are still less favorable for adolescent recipients with a frequent mismatch between the donors' weight and the recipients' morphology. Finding the right balance between a small split graft leading to small-for-size syndrome (SFS) or a whole liver graft leading to large-for-size syndrome (LFS) is very challenging in adolescent recipients. This is reflected by the fact that adolescent recipients account for over 30% of the pediatric patients on the waiting list but less than 20% of all pediatric liver transplantations (LT), according to the ELTR registry³ and the OPTN/SRTR report.⁴ In other words, adolescent recipients have the lowest liver transplantation rate.⁵ Hence, there is a need to either adapt prioritization policies or use surgical strategies to optimize graft-recipient matching in order to increase access to transplantation for these recipients.

In adult LT, ex situ right posterior sectionectomy (RPS) has been described as a way to decrease LFS.⁶ It significantly reduces the GRWR (Graft Recipient Weight ratio) but also the GW/RAP (Graft Weight/Right Antero Posterior Distance ratio) ratio, hereby avoiding anterior and posterior graft compression in a small abdominal cavity. This surgical strategy may also benefit adolescent recipients and allow to transplant liver grafts initially declined for morphological reasons.

In this report, we present three cases of LT after ex situ liver graft reduction with RPS in adolescent recipients. We focused on key technical considerations transposed from adult LT, the matching process, and the morphological evaluation of both the donor and the recipient.

2 | CASE REPORT

2.1 | Donors and recipients

The first recipient (R1) was a 14-year-old boy, weighing 35 kg and presenting auto-immune hepatitis with portal hypertension. The second recipient (R2) was a 12-year-old girl, weighing 32 kg and presenting an alpha-1 antitrypsin deficiency with severe portal hypertension and ascites. The third recipient (R3) was a 13-year-old boy, weighing 47 kg and presenting cirrhosis of unknown origin with portal hypertension.

Liver grafts were procured from donation after brain death donors, weighing 58 kg (R1), 65 kg (R2), and 83 kg (R3). Donors were 13 (R1), 17 (R2), and 26-year-old (R3). The cause of death was traumatic in each case, without any cardiac arrest prior to organ donation. The respective donor weight/recipient weight ratios (D/RW) were 1.6, 2, and 1.7.

2.2 | Graft-recipient matching

Estimating the liver graft weight prior to organ procurement is challenging and relies on complex formulas which are often imprecise.^{7,8} Consequently, RPS was always planned prior to organ acceptance but not performed until morphological inspection and weighing of the graft during back table preparation at our center. GRWR and D/RW were assessed before and after graft ex situ reduction.⁹ Anthropometrical data including the right anteroposterior distance (RAP) and GW/RAP were used to guide surgical strategy. The published GW/RAP cut-off for occurrence of LFS in adult LT is >100g/cm.¹⁰ RAP was defined as the longest right anteroposterior vertical distance between anterior and posterior parts of the recipient's ribs. Of note, split liver transplantation allowing to transplant two recipients is always the priority at our center. Therefore, liver graft reduction was only performed when there was no suitable second recipient available or in case of unfavorable graft-size matching.

As described in Figure 1, GRWR was above the admitted 4% limit for each recipient.

The GW/RAP was above 100g/cm for R1 and R3, thereby suggesting the need for graft reduction prior to back-table preparation.

Volumetric assessment of the graft was available for R3 and allowed to refine the surgical strategy as shown in Figure 2. In this case, left lateral and full left partial grafts were ruled out for volumetric reasons with a theoretical risk of SFS. The discrepancy between donor and recipient anthropometrical data (length and thickness of the graft) suggested RPS rather than a right extended partial graft to avoid graft compression.

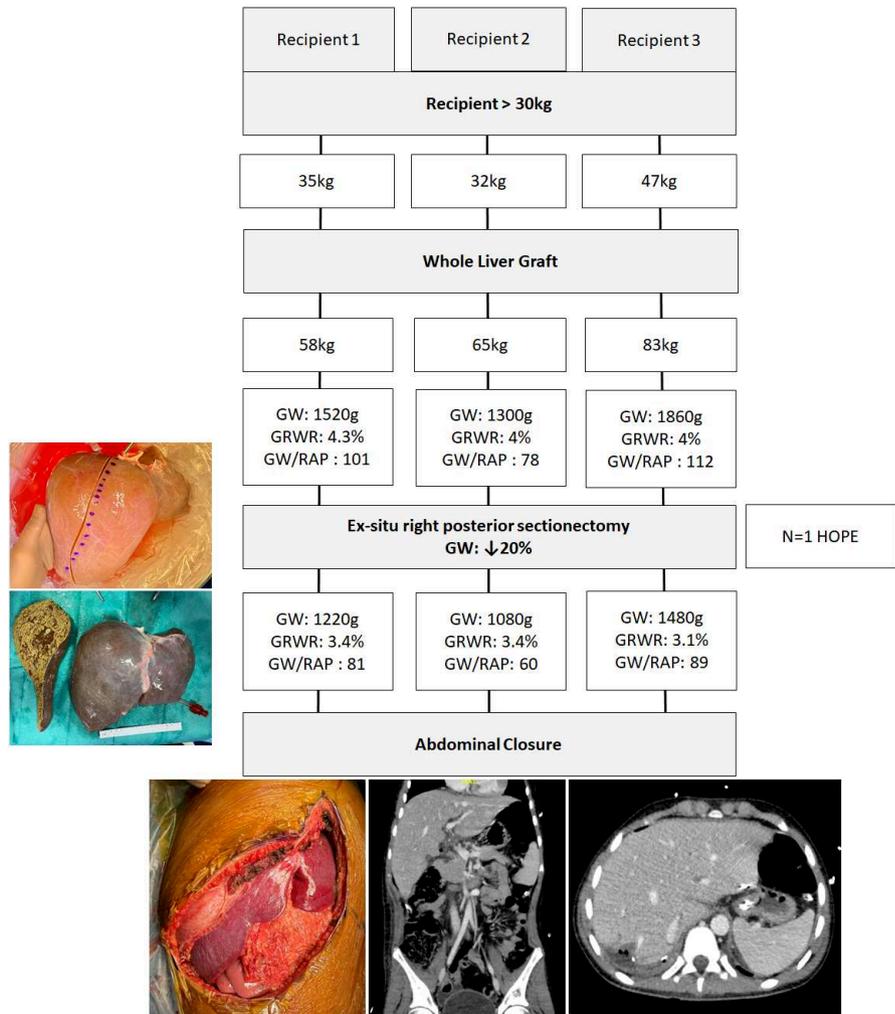
R2 presented with a GRWR >4% and a GW/RAP <100g/cm. The surgical strategy was based on the available CT-scan evaluation, including the cranio-caudate length and the anteroposterior thickness of the graft in comparison to the recipient's abdominal cavity. After confirmation of the actual graft anthropomorphology and the subsequent mismatch with the recipient's cavity, the decision to perform RPS despite a favorable GW/RAP was taken.

2.3 | Ex situ Right Posterior Sectionectomy

In contrast to left lateral sectionectomy, right posterior sectionectomy allows a reduction of at least 25% of the total liver volume¹¹ which translates into a significantly decreased GRWR. More importantly, RPS allows a three-dimensional volume gain in both cranio-caudal length and antero-posterior thickness, thereby optimizing graft-size matching.

From a technical point of view, the transection plane follows the right hepatic vein which remains with the graft, the right side of the inferior vena cava, and the Rouviere sulcus.⁶ The liver graft remains in a prone position and the parenchymal transection is performed from both cranial and caudate approaches with the same anatomical landmarks as in liver resection. This transection plane allows for intraparenchymal control of the venous branches

FIGURE 1 Donor-recipient matching strategy. (GW: graft weight; GRWR: graft/recipient weight ratio; RAP: right anteroposterior distance, HOPE: hypothermic oxygenated perfusion; RPS: right posterior sectionectomy).



of S6 and S7 and the posterior glissonian pedicle in the Rouviere sulcus. As stated by Zhang et al.,¹² this approach preserves both outflow and inflow integrity of S5 and S8. Of note, Minami et al.¹¹ studied variations of the portal vein and the right hepatic vein anatomy. The existence of an inferior right hepatic vein or the lack of a common left portal vein trunk is less frequent but must be acknowledged on donor CT-scan as these variations modify the anatomical landmarks during transection. Finally, we advocate to perform running sutures on the large transection plane to allow for mechanical hemostasis and biliostasis. An advantage of the RPS is that the liver graft will rotate into the posterior abdominal cavity after implantation allowing for an additional mechanical compression of the transection plane.

The total duration of RPS was 80, 90, and 130 min respectively. The liver volume reduction after RPS was 20% for R1, 17% for R2, and 21% for R3 (Figure 1). Consequently, the final GRWR ranged from 3.1 to 3.4% and the GW/RAP was below 100g/cm for each recipient.

In one case (R3), RPS was performed during hypothermic oxygenated perfusion (HOPE). The total perfusion time was 180 min, with a static cold storage time of 390 min and a total preservation

time of 570 min. In contrast, the non-perfused RPS grafts presented with a longer static cold storage time of 410 min (R1) and 510 min (R2).

2.4 | Liver transplantation and post-operative outcomes

Liver transplantation was performed with caval replacement and duct-to-duct bile anastomosis in each case. Primary abdominal closure was possible in each case and we did not observe any LFS (Figure 1). Post-LT transaminase peaks were 520 (R1), 1063 (R2), and 894 UI/l (R3) and no early allograft dysfunction occurred. Liver function expressed by factor V was in the normal range at post-operative day 2 for each recipient. We did not encounter any primary non-function or early vascular complication.

Two recipients had an uneventful post-operative course without any surgical complications. One recipient (R3) presented with an anastomotic biliary stricture requiring a surgical reintervention. No graft loss or recipient death was observed after a median follow-up of 6 months.

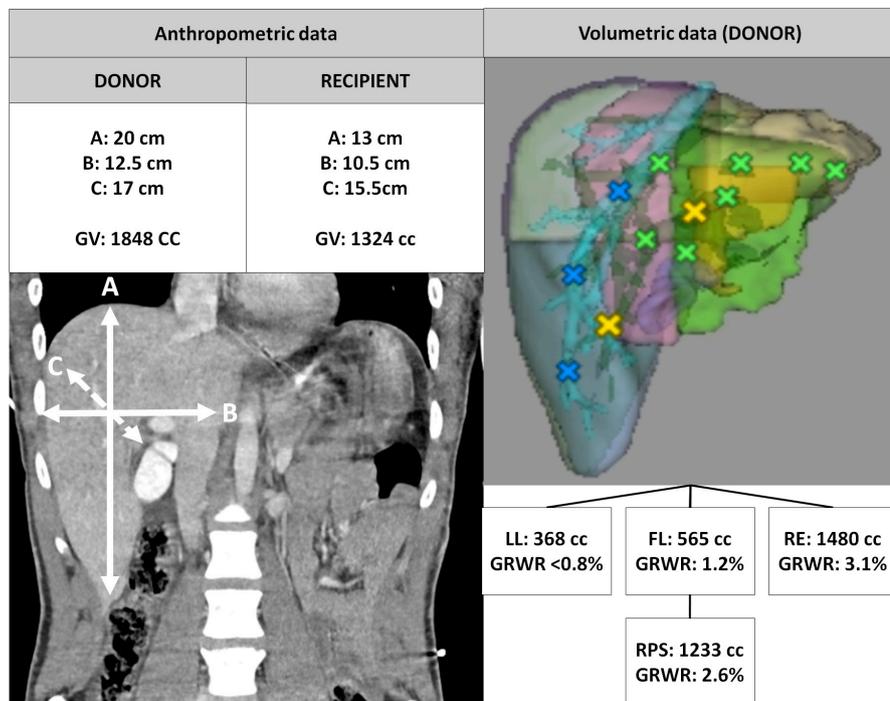


FIGURE 2 Radiological evaluation of case R3. (GV: graft volume; LL: left lateral graft; FL: full left graft; RE: right extended graft; RPS: right posterior sectionectomy).

2.5 | When to perform right posterior sectionectomy?

In order to propose a decision-making algorithm for indications of RPS (Figure 4), we reviewed a total of 20 LT in adolescent recipients during the same period (2018–2022). The recipients were 14-year-old [13.5–16] with a median weight of 45 kg [37–49].

The majority of the recipients ($n = 18$, 90%) underwent whole liver graft transplantation. The median D/RW was 1.4 [1.05–1.6], the median GRWR was 2.4% [2.07–2.7], and <3.5% in every recipient. The GW/RAP was <100g/cm in every recipient.

Two recipients received a full left partial graft with a D/RW >2, a GW/RAP >100g/cm, and an initial GRWR >4%.

3 | DISCUSSION

We herein described the first three cases of successful graft reduction by ex situ right posterior sectionectomy in pediatric liver transplantation as a way to improve graft-size matching for adolescent recipients. The surgical strategy was based on anthropomorphological data and RPS was able to decrease both the GRWR and the GW/RAP translating into a more favorable graft-recipient size matching. We did not observe any LFS, SFS, or delayed graft function. Following the reported data, we proposed the use of RPS in case of graft-recipient mismatch for adolescent LT candidates according to D/RW, GRWR, and GW/RAP evaluation (Figure 4). These are preliminary data based on a single-center experience requiring further confirmation in larger multi-center studies.

Liver donors allowing for optimal anthropometrical graft-recipient matching in adolescent transplant candidates are rare. In

addition, pediatric donors have declined over the last decade accounting for only 3% of all DBD donors in our country.¹³ This resulted in a low transplant rate for recipients older than 11 years.⁴ The use of split liver grafts is also challenging for adolescent recipients as smaller grafts could lead to SFS whereas whole grafts could cause LFS and potentially lead to a higher risk of graft loss.^{14,15} In adult LT, RPS has been described as a valid strategy to optimize graft-size matching but application in pediatric LT is lacking.⁶

From a technical point of view, RPS has three main advantages. It combines (a) an anthropo-morphological matching of the liver graft to the recipient cavity, (b) a functional matching by leaving enough parenchyma to avoid SFS or hypoperfusion, and allows (c) to perform LT using the same implantation technique as whole liver grafts.

First, the right posterior sector accounts for approximately 25%¹¹ of the liver volume. In our experience RPS was able to reduce the total liver weight by 20%, thus decreasing GRWR from >4% to 3%. Of note, with RPS, no delayed abdominal closure was needed in our case series. By definition, split liver grafts display the highest reduction in absolute liver volume, but this parameter does not take into account the shape of the liver and its positioning in the recipient's cavity. Despite a GRWR <4%, left or right-sided partial grafts fit according to their respective hepatic vein axis and primary abdominal closure may lead to graft compression in some recipients. In contrast, RPS enables the graft to rotate around the axis of the vena cava into the posterior abdominal cavity which greatly reduces anterior compression.

Second, portal flow has been described as a major determinant of graft loss. Both SFS and LFS conditions are consequences of inadequate perfusion of the liver due to either too little or too much portal flow normalized over graft weight.¹⁴ In case of graft reduction, the remnant liver volume allows for adequate perfusion of the graft.

As described in Figure 3, we indeed did not encounter any prolonged ascites and hyperbilirubinemia (SFS) or major transaminase release and EAD (LFS-hypoperfusion).

Third, partial liver grafts such as right extended or full left grafts are another option for adolescent recipients. Besides morphological consideration this allows two recipients to benefit from a single graft which in the context of organ shortage⁵ must remain a

priority. However, performing two procedures by the same team is a logistical challenge¹⁶ and requires having two suitable recipients on the waiting list. In addition, partial liver grafts presented with longer static cold storage,³ a higher rate of biliary complications¹⁷ and require more challenging implantation techniques. In contrast, RPS allows for caval replacement thereby facilitating recipient hepatectomy and providing optimal outflow. Furthermore, duct-to-duct

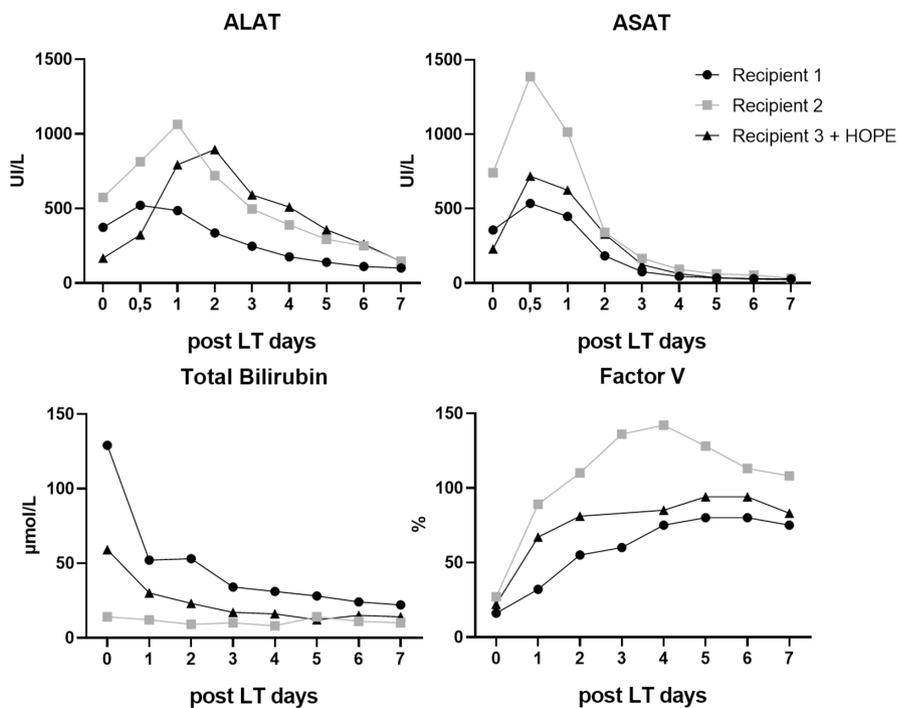


FIGURE 3 Post-transplant serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), factor V, and total bilirubin. (LT: liver transplantation; HOPE: hypothermic oxygenated perfusion).

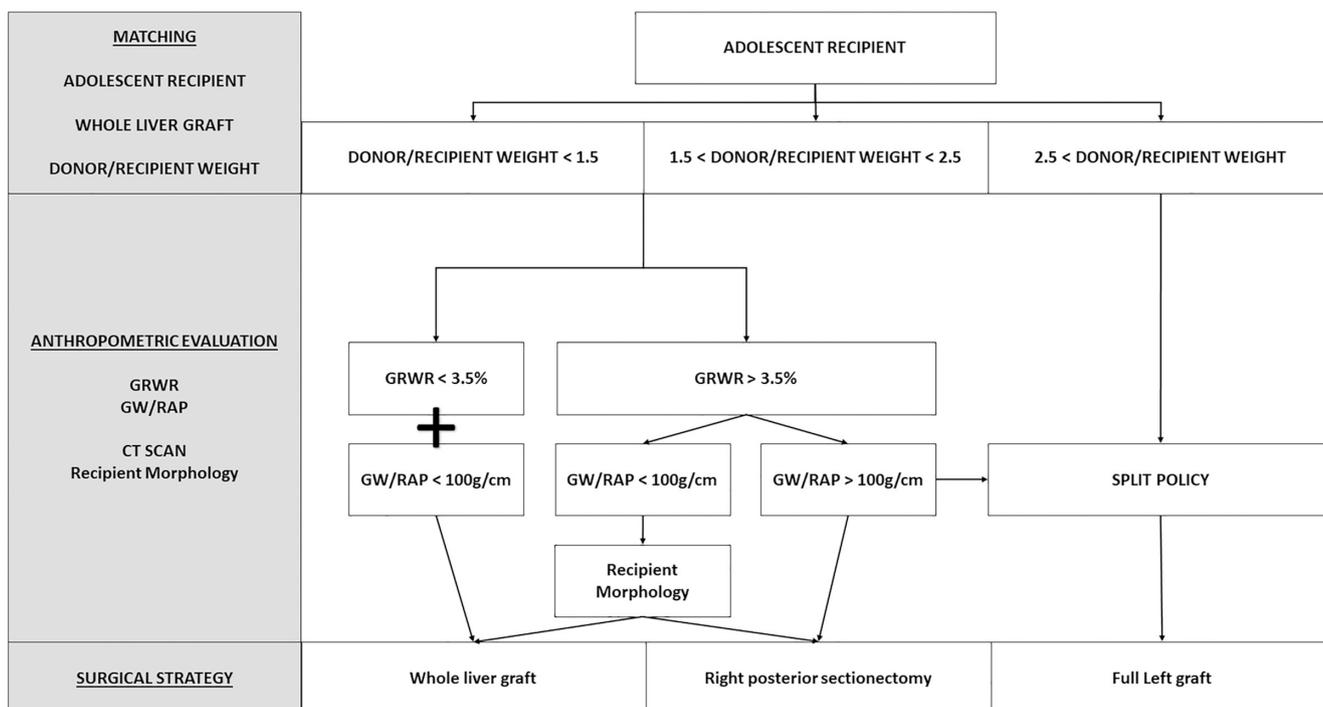


FIGURE 4 Clinical decision algorithm. (GRWR: graft/recipient weight ratio; GW: graft weight; RAP: right anteroposterior distance).

bile anastomosis was performed in all three described RPS cases and the entire portal vein and hepatic artery axis remained with the RPS grafts which facilitated vascular anastomosis.

In addition to the aforementioned benefits of RPS, the reported cases also highlight the need for anthropo-morphological evaluation prior to graft reduction to guide surgical strategy. As stated by Zhou et al.,¹⁸ the specific shape of the liver graft should be evaluated during the back table as it is insufficiently described by the GRWR or RAP. However, GW/RAP ratio, used in adult LT to estimate the risk for LFS¹⁹ was above 100g/cm in two recipients. This suggests its utility in pediatric LT for adolescent recipients as an indicator for graft reduction (Figure 2–4). Of note, in one of the reported cases, we had access to the donor CT scan with volumetric assessment of the donor liver which allowed for better planning of the subsequent graft reduction.²⁰ We thus advocate the use of D/RW, GRWR, and GW/RAP to guide surgical strategy as described in Figure 4. In our experience, all adolescent recipients with D/RW <1.5 had a GRWR <3.5% and a GW/RAP <100 which allowed for whole LT. On the other hand, partial liver graft should be considered when D/RW >2.5. For recipients with a 1.5 < D/RW < 2.5, we support the need for RPS when GRWR >3.5% and GW/RAP >100g/cm.

Finally, RPS is associated with prolonged cold ischemia when performed ex situ which impacts post-operative outcomes.²¹ In-situ RPS after graft implantation has been described as an alternative²² but remains seldom performed for logistic reasons. In this context, we performed RPS during HOPE in one of the reported cases which allowed to reduce static cold storage and may offer a promising strategy to optimize outcomes.^{23,24}

In conclusion, adolescent transplant candidates have limited access to size-matched liver grafts resulting in a low transplantation rate. In this scenario, intention to split policy must remain mandatory but ex situ graft reduction with right posterior sectionectomy should be acknowledged as a way to improve graft-recipient matching and increase access to LT for this specific population. Indicators such as GRWR or GW/RAP are important for decision-making and should be combined with anthropo-morphological evaluation on CT scan to guide surgical strategy.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. No specific financial support was granted for this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

From large-for-size to large-for-flow: A paradigm shift in liver transplantation

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Abstract

Liver graft-recipient matching remains challenging, and both morphologic and hemodynamic characteristics have been shown to be relevant indicators of post-transplant outcomes. However, no combined analysis is available to date. To study the impact of both morphologic and hemodynamic characteristics of liver grafts on transplantation outcomes, we retrospectively evaluated all consecutive 257 liver transplantations with prospective hemodynamic measurements from 2017 to 2020 in a single-center perspective. First, a morphologic analysis compared recipients with or without large-for-size (LFS), defined by a graft/recipient weight ratio > 2.5% and excluding extreme LFS. Second, a hemodynamic analysis compared recipients with or without low portal flow (LPF; <80 mL/min per 100 g of liver tissue). Third, an outcome analysis combining LPF and LFS was performed, focusing on liver graft-related morbidity (LGRM), graft and patient survival. LGRM was a composite endpoint, including primary nonfunction, high-risk L-Graft7 category, and portal vein thrombosis. Morphologic analysis showed that LFS (n = 33; 12.9%) was not associated with an increased LGRM (12.1% vs 9.4%; $p = 0.61$) or impaired graft and patient survival. However, the hemodynamic analysis showed that LPF (n = 43; 16.8%) was associated with a higher LGRM (20.9% vs 7.5%, $p = 0.007$) and a significantly impaired 90-day graft and patient survival. Multivariable analysis identified LPF but not LFS as an independent risk factor for LGRM (OR: 2.8%; CI: 1.088–7.413; and $p = 0.03$), 90-day (HR: 4%; CI: 1.411–11.551; and $p = 0.01$), and 1-year patient survival. LPF is a significant predictor of post-liver transplantation morbidity, independent of LFS when defined as a morphologic metric alone.

Abbreviations: AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CIT, cold ischemia time; CVS, coronary vein shunt; EAD, early allograft dysfunction; GRWR, graft-recipient weight ratio; ICU, intensive care unit; IQR, interquartile range; LFF, large-for-flow; LFS, large-for-size; LGRM, liver graft-related morbidity; LPF, low portal flow; LT, liver transplantation; MELD, model for end-stage liver disease; PF, portal flow; PNF, primary nonfunction; PSS, porto-systemic shunt; SFS, small-for-size; SRS, splenorenal shunt.

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Consequently, we propose the novel concept of large-for-flow, which may guide graft selection and improve perioperative management of LPF.

INTRODUCTION

Liver graft-recipient matching is not only based on the underlying liver disease of the recipient and donor characteristics but also on the morphologic evaluation of the graft. Indeed, in the case of an oversized graft for the recipient's abdomen, large-for-size (LFS) syndrome may occur, which increases the risk of vascular complications and impairs graft and recipient survival.^[1–3] The definition of LFS is solely based on a morphologic evaluation without considering hemodynamic liver graft characteristics, in contrast, for example, to the small-for-size (SFS) syndrome.^[4] However, intraoperative hemodynamic evaluation of portal vein flow, hepatic artery flow, and hepatic venous pressure gradient (HVPG) has been shown to provide valuable information on post-liver transplantation (LT) outcomes.^[5] For example, low portal vein flow (LPF) is a major risk factor for vascular complications and impaired graft survival.^[6–9] In addition, intraoperative measurement of portal flow allows to select LT recipients, which may benefit from portal flow modulation.^[10] Consequently, this study assesses for the first time the impact of both morphologic and hemodynamic characteristics of liver grafts on post-LT outcomes.

METHODS

Study design and participants

This study included all consecutive LT performed at our institution from 2017 to 2020 (donation after brain death) with a prospective intraoperative hemodynamic assessment, including portal vein flow, hepatic artery flow, and HVPG. In addition, a morphologic assessment, including the liver graft weight and the liver graft recipient weight ratio (GRWR), was performed for all included LT. Exclusion criteria were retransplantations, partial liver grafts, patients without hemodynamic evaluation, or missing data. Of note, one extreme LFS based on the definition by Allard et al^[11] was excluded from the study as this is a very specific condition outside the standard LFS.

First, the entire study cohort was divided according to morphologic characteristics based on the GRWR, where LFS was defined as a GRWR > 2.5%,^[12] and outcomes were assessed (Figure 1). The use of the GRWR to define LFS in our cohort was validated in a

preliminary comparison with the body surface area index^[3] and the donor-standardized total liver volume to recipient index^[13] (Supplemental Figure S1, <http://links.lww.com/LVT/A410>). Second, the entire study cohort was divided based on the perioperative hemodynamic findings in terms of LPF. As for the GRWR, a preliminary internal validation was performed in our cohort to select a cutoff value for LPF with high specificity (90%) in predicting the primary endpoint (Supplemental Figure S2, <http://links.lww.com/LVT/A411>). Similar to previous studies, the cutoff for LPF in our cohort was 80 mL/min/100 g^[7] (Figure 1).

Third, an outcome analysis combining morphologic and hemodynamic characteristics was performed.

The study met the requirements of the ethical guidelines of both the Declaration of Helsinki and Istanbul and written consent was given in writing by all included patients. The study was approved by the Institutional ethic and scientific committee (CSE_HCL 22_692).

Transplant procedure

The standard LT procedure at our center is the piggyback technique with a temporary porta-caval anastomosis. Portal and caval pressure and portal and arterial flows were measured after arterial and portal reperfusion and before bile duct anastomosis. Based on our previous published experience,^[14] significant portosystemic shunts (PSS) were ligated after primary hemodynamic assessment to increase the portal flow above 100 mL/min/100 g to a maximum of 250 mL/min/100 g. Of note, portal and arterial flows were assessed with time-to-transit flow meters (Transonic Systems, Inc.). A 10–12 mm probe was used for the portal vein flow, and a 6 mm probe was used for the hepatic artery flow. In all recipients, HVPG was an estimate of the pressure gradient between the portal vein and the inferior vena cava pressure, which was measured with a catheter.

Endpoints

The primary endpoint of the study was a composite endpoint for 90-day liver graft-related morbidity (LGRM). Patients with LGRM presented at least one of the following complications: primary nonfunction (PNF), high-risk L-Graft7 category, and portal thrombosis^[15] during the first 90 days after LT. This composite



FIGURE 1 Study flow chart. GRWR, graft-recipient weight ratio.

endpoint was defined to specifically capture expected liver-related morbidity in the settings of LFS or LPF.^[2,6,16] PNF was defined as patient death or the need for retransplantation within the first 7 postoperative days, excluding acute vascular complications, acute rejection, or acute cardiac failure.^[17] L-Graft7 was calculated for each included patient and divided into high and low-risk categories. High-risk included patients with L-graft7 < -1.5, as described in published data.^[18,19] Secondary endpoints included early allograft dysfunction (EAD),^[20] non-LGRM, including post-LT dialysis, surgical complications, 90-day and 1-year grafts, and recipient survival.

Statistical analyses

Categorical variables are expressed in quantities and percentages, whereas continuous variables are expressed as median with interquartile range (IQR). Continuous variables were compared using the Mann-Whitney test. Categorical variables were compared using the chi-square test or Fisher exact test. Kaplan-Meier curves were generated to show a univariate analysis of survival and analyzed using the log-rank test. Spearman correlation was used to test the correlation between GRWR and portal flow. Univariable analysis was performed, including relevant factors for

graft loss, related to recipient's and donor's baseline characteristics, liver grafts, and operative data. Variables with p values <0.10 in the univariable analysis were used in the multivariable analysis. Multivariable analysis was performed using binary logistic regression for LGRM and Cox regression for survival analysis. p values <0.05 were considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 26.0.; IBM Corp) and GraphPad Prism (Version 8.0.0 for Windows, GraphPad Software, www.graphpad.com).

RESULTS

A total of 289 LT were performed during the study period, of which 257 LTs with a prospective hemodynamic and morphologic assessment were included (Figure 1).

Morphologic analysis

Thirty-three recipients (12.8%) presented with an LFS and were compared with 224 (87.2%) recipients without LFS. The median donor body mass index and graft weight were significantly higher in the LFS group, 1780

g (IQR: 1580–1880) versus 1330 g (IQR: 1140–1565) ($p < 0.001$). In contrast, recipients in the LFS group had a significantly lower body mass index and were significantly younger without any significant difference regarding the model for end-stage liver disease. Detailed data on the donor's and recipient's characteristics are presented in Supplemental Table S1 (<http://links.lww.com/LVT/A412>).

Portal vein flow was similar in both groups [1600 mL/min (IQR: 1200–2300) vs 1800 mL/min (IQR: 1200–2300); $p = 0.57$]. However, portal vein flow normalized over liver weight was significantly lower in the LFS group [89 mL/min/100 g (IQR: 68–130) vs 129 mL/min/100 g (IQR: 94–186); $p < 0.001$]. In addition, there was a correlation between portal vein flow and GRWR (R spearman -0.312 , $p < 0.001$, Supplemental Figure S3, <http://links.lww.com/LVT/A413>). This resulted in a significantly higher rate of LPF in the LFS group (33.3% vs 14.3%; $p = 0.006$). Hepatic artery flow was similar in both groups with physiological HVPG in the LFS group.

The LFS group presented with a comparable rate of LGRM (12.1% vs 9.4%; $p = 0.61$) and an increased rate of EAD (39.4% vs 22.3%; $p = 0.03$). Postoperative outcomes were similar between both groups regarding, surgical complications, serum transaminase release,

TABLE 1 Postoperative outcomes

Outcomes	Morphologic comparison LFS versus no LFS			Hemodynamic comparison LPF versus no LPF		
	No LFS	LFS	p	No LPF	LPF	p
L-Graft 7	-3.34 (-3.74; -2.62)	-2.98 (-3.59; -2.29)	0.21	-3.38 (-3.76; -2.73)	-2.92 (-3.53; -2.03)	0.005
High-risk L-Graft	17 (7.6)	3 (9.1)	0.76	13 (6.1)	7 (16.3)	0.02
AST peak, UI/L	882 (515–1812)	909 (568–2666)	0.31	740 (386–1255)	1317 (771–3131)	< 0.001
ALT Peak, UI/L	774 (404–1345)	982 (500–2270)	0.27	835 (500–1545)	1648 (799–4908)	< 0.001
EAD	50 (22.3)	13 (39.4)	0.03	46 (21.5)	17 (39.5)	0.01
PNF	8 (3.5)	1 (3)	0.87	4 (1.6)	5 (11.6)	0.001
LGRM	21 (9.4)	4 (12.1)	0.61	16 (7.5)	9 (20.9)	0.007
Surgical complications ^a	66 (29.5)	11 (33.3)	0.65	57 (26.6)	20 (46.5)	0.009
Biliary complications ^b	18 (8)	2 (6.1)	0.69	16 (7.5)	4 (9.3)	0.69
Arterial complications ^b	14 (6.2)	3 (9.1)	0.54	16 (7.4)	1 (2.3)	0.26
AKI dialysis	29 (12.9)	8 (24.2)	0.08	24 (11.2)	13 (30.2)	0.009
Graft survival 3 mo	205 (91.5)	31 (93.9)	0.64 ^c	200 (93.5)	36 (83.7)	0.03 ^c
Graft survival 1 y	198 (88.4)	29 (81.9)	0.93 ^c	192 (89.7)	35 (81.4)	0.12 ^c
Patient survival 3 mo	208 (92.9)	32 (97)	0.38 ^c	204 (95.3)	36 (83.7)	0.004 ^c
Patient survival 1 y	200 (89.3)	30 (90.9)	0.78 ^c	195 (91.1)	35 (81.4)	0.06 ^c

Note: values are expressed as n (%) or median (interquartile range).

^aOverall surgical complications at 3 months.

^bCalvien Dindo \geq III biliary and arterial complications at 3 months.

^cLog-rank test.

Abbreviations: AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EAD, early allograft dysfunction; LGRM, liver graft-related morbidity; LFS, large-for-size; LPF, low portal flow; PNF, primary nonfunction.

graft, and patient survival at 90 days and one year (Table 1; Figure 2; Supplemental Figure S4, <http://links.lww.com/LVT/A415>).

(88.4% vs 95.8%; $p = 0.03$ and 83.7% vs 95.3%; $p = 0.004$ respectively) (Figure 2).

Hemodynamic analysis

Forty-three recipients (16.7%) presented with LPF and were compared with 214 (83.3%) recipients without LPF. The median donor body mass index was similar with a significantly higher median graft weight in the LPF group [1510 (IQR: 1280–1750) vs 1350g (IQR: 1140–1620); $p = 0.01$]. Baseline recipient characteristics were comparable between both groups with the exception of a higher rate of fulminant hepatitis in the LPF group (14% vs 4.2%; $p = 0.01$). Of note, there was no difference in the rate of native PVT between the two groups. No difference was observed regarding hepatic artery flow or HPVG between both groups.

There was a higher median GRWR in the LPF group [1.9% (IQR: 1.6–2.5) versus 1.7% (IQR: 1.4–2.2); $p = 0.008$], resulting in a higher rate of LFS (25.6% versus 10.3%; $p = 0.006$).

The LPF group presented a significantly higher rate of LGRM (20.9% vs 7.5%, $p = 0.007$) and PNF (11.6% vs 1.6%; $p = 0.001$). In addition, non-LGRM, including post-LT dialysis and surgical complications, was also higher in the LPF group. This translated into a reduced 90-day graft and recipient survival in the LPF group

Combined morphologic and hemodynamic analysis

Thirty-two recipients (12.4%) presented with LPF alone, 22 (8.6%) with LFS alone, 11 (4.3%) with both LPF + LFS, and 192 (74.7%) without LFS and LPF (Figure 1). Characteristics of the subgroups are listed in Table 2.

Recipients with LFS alone had a similar rate of LGRM compared with recipients without LPF and LFS (9.1% vs 7.3%, $p = 0.51$). Recipients with LFS + LPF and LPF alone had, however, a higher rate of LGRM compared with recipients without LPF or LFS (18.6% and 21.9% vs 7.3% respectively $p = 0.03$). In addition, LPF + LFS and, to a lesser extent, LPF recipients presented with a significantly higher post-LT aspartate aminotransferase and alanine aminotransferase peak compared with LFS alone (Supplemental Figure S5, <http://links.lww.com/LVT/A416>). The 90-day recipient survival was reduced in LPF recipients compared with recipients with LFS alone (81.3% vs 100%, $p = 0.03$) or without LPF and LFS (81.3% vs 94.8%, $p = 0.005$) (Figure 3).

A total of 13 shunt ligations were performed, mainly for splenorenal shunts ($n = 8$, 61.5%) with a median significant portal flow increase of 37 mL/100 g (26–48)

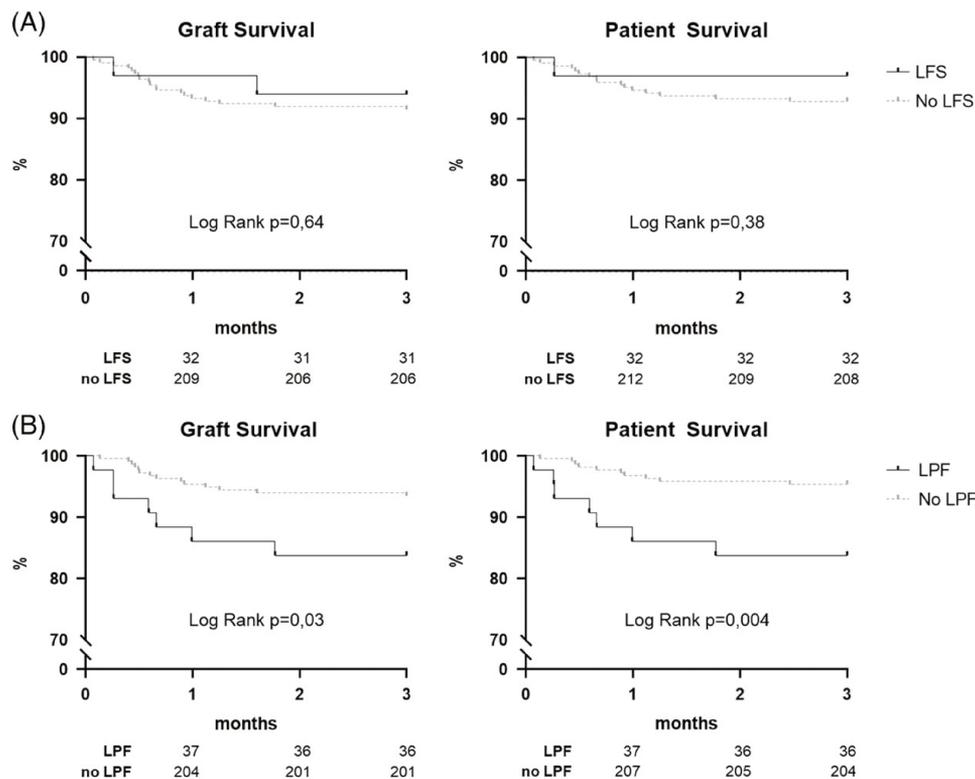


FIGURE 2 Patient and graft survival at 3 months. (A) Morphologic analysis—LFS versus no LFS. (B) Hemodynamic analysis—LPF versus no LPF.

TABLE 2 Overall and subgroup clinical characteristics of recipients and donors

	Total 257	No LPF–No LFS 74.7% (192)	LFS–No LPF 8.6% (22)	LPF–No LFS 12.4% (32)
Donor				
Age (y)	56 (43–70)	58 (44–71)	47 (30–59)	53.5 (44–66)
Sex (M)	151 (58.8)	104 (54.4)	15 (68.2)	24 (75)
BMI (kg/m ²)	24.7 (21.8–28.2)	24.3 (21.6–27.8)	25.1 (23.4–32.9)	24.9 (23.0–28.4)
ICU stay (d)	3 (2–4)	3 (2–4)	3 (2–5)	3 (2–5.5)
Graft weight (g)	1370 (1180–1640)	1310 (1120–1550)	1790 (1540–1880)	1430 (1240–1600)
CIT (min)	375 (313–450)	386.5 (313–463)	328.5 (287–393)	351 (312.5–425)
Recipient				
Age (y)	59 (51–65)	60 (53–65.5)	56 (37–61)	58 (51.5–63.5)
Sex (M)	181 (70.4)	5136 (70.8)	16 (72.7)	24 (74)
BMI (kg/m ²)	25.8 (22.2–30.1)	26.4 (23.3–30.4)	20.3 (18.0–22.6)	27.7 (24.3–32.0)
MELD	16 (10–28)	15.6 (10.0–25.5)	26.0 (13.5–33.0)	15 (9–28.2)
HCC	103 (40.1)	83 (43.2)	5 (22.7)	11 (34.4)
Alcohol	107 (41.6)	86 (44.8)	10 (45.5)	7 (21.9)
HCV	25 (9.7)	18 (9.4)	4 (18.2)	3 (9.4)
HBV	19 (7.4)	13 (6.8)	2 (9.1)	2 (6.3)
NASH	49 (19.1)	40 (20.8)	2 (9.1)	7 (21.9)
Fulminant	15 (5.8)	6 (3.1)	3 (13.6)	6 (18.8)
Ascite (L)	0 (0–2.6)	0.2 (0–2.8)	1.3 (0–3.5)	0 (0–1.1)
Portal flow (mL/min)	1780 (1200–2300)	1970 (1425–2500)	2075 (1600–2500)	880 (690–1005)
Portal flow (mL/min/100 g)	124.1 (89.4–173.4)	140.5 (106.5–193.9)	100.5 (89.9–161.1)	63.3 (53.3–73.8)
Arterial flow (mL/min/100 g)	18.6 (11.9–26.1)	18.1 (12.0–25.8)	22.6 (11.0–26.7)	19.8 (12.5–26.3)
Portal pressure (mm Hg)	12 (10–15)	12.5 (10–15)	13 (9.5–17)	10.5 (7–12.5)
HPVG (mm Hg)	3 (1–5)	3 (1–5)	1 (0–4)	3 (1–5)
GRWR (%)	1.8 (1.5–2.2)	1.7 (1.4–2.0)	3.0 (2.8–3.3)	1.8 (1.5–2.0)
PVT	20 (7.8)	14 (7.3)	3 (13.6)	3 (9.4)

Note: values are expressed as n (%) or median (interquartile range).

Abbreviations: BMI, body mass index; CIT, cold ischemia time; GRWR, graft recipient weight ratio; HPVG, Hepatic venous pressure gradient; ICU, intensive care unit; LFS, large end-stage liver disease.

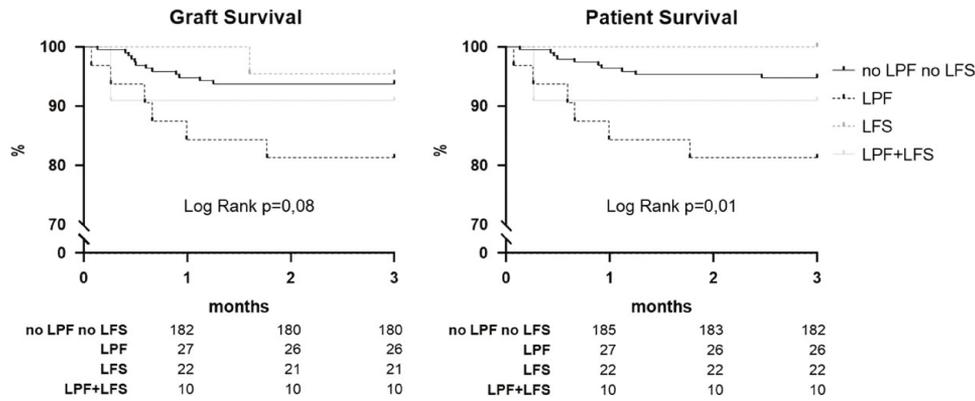


FIGURE 3 Subgroup analysis of patient and graft survival at 3 months and 1 year.

($p < 0.001$) [500 mL (400–800)]. Seven patients presented with LPF, and shunt ligation was able to increase portal flow to a normal range in 3 of them (42.8%). We performed reno-portal or mesenterico-portal anastomosis in 5 patients (no LPF $n = 2$ vs LPF $n = 3$; $p = 0.03$) with jump grafts from the cadaveric iliac vein in 3 of them. Of note, no shunt ligation was performed in both the LPF + LPS and LFS-No LPF groups due to the lack of significant PSS. Detailed data are presented in Figure 4.

Risk factors of graft-related morbidity and patient/graft loss

LPF was associated with LGRM on univariable analysis and was an independent risk factor for LGRM on multivariable analysis [OR: 2.840

(1.088–7.413); $p = 0.03$] (Table 3). LFS was not associated with LGRM.

LPF was associated with 90-day graft and patient survival on univariable analysis and was an independent risk factor of 90-day patient survival on multivariable analysis (HR: 4%; CI: 1.411–11.551; and $p = 0.01$). LFS was not associated with either graft or patient survival (Table 3; Supplemental Table S3, <http://links.lww.com/LVT/A412>).

The main causes of 90-day recipient death were PNF 2.3% (6), cardiopulmonary failure 1.9% (5), and hemorrhage 1.5% (4), with overall 3.9% (10) multiorgan failure.

Given the impact of fulminant hepatitis on patient and graft survival in our study, we performed a subgroup analysis excluding those recipients (Supplemental Table S4, <http://links.lww.com/LVT/A412>). In this subgroup analysis, LPF remained an independent risk

Recipient	Shunt	GRWR	Pre-PF	Post-PF	Increase (x)	Pre-LPF	Post-LPF	LGRM	Graft loss	Patient death
1	SRS	1,4%	26,7	63,3	2,4	Yes	Yes	1	1	1
2	SRS	1,7%	45,1	63,9	1,4	Yes	Yes	1	1	1
3	CVS	1,9%	54,9	79,3	1,4	Yes	Yes	0	0	0
4	SRS	1,6%	64,2	137,6	2,1	Yes	No	1	0	0
5	CVS	1,6%	77,7	113,0	1,5	Yes	No	0	0	0
11	SRS	2,4%	47,4	73,7	1,6	Yes	Yes	1	0	0
13	SRS	2,1%	73,3	133,3	1,8	Yes	No	1	0	0

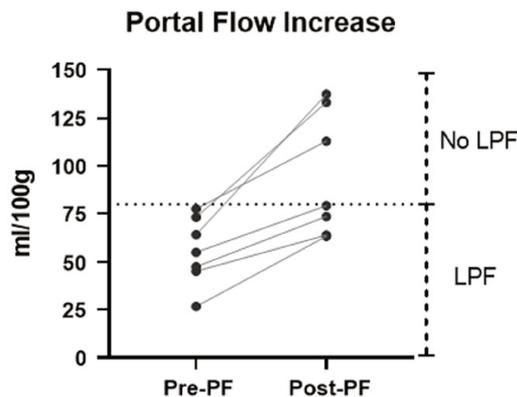


FIGURE 4 Detailed data on portal flow improvement during shunt ligation and postoperative outcomes.

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TABLE 3 Univariable and multivariable analysis of potential prognostic factors affecting LGRM and patient survival

Variables	LGRM						Patient survival 3 mo			
	Univariable			Multivariable			Multivariable			
	OR (CI 95%)	p	p	OR (CI 95%)	p	p	HR (CI 95%)	p	p	
LFS	1.333 (0.427–4.16)	0.62	—	—	—	—	0.420 (0.056–3.168)	0.40	—	—
LPF	3.276 (1.340–8.009)	0.01	2.840 (1.088–7.413)	0.03	3.746 (1.426–9.843)	0.007	4.037 (1.411–11.551)	0.007	—	0.01
Recipient age	0.975 (0.944–1.006)	0.11	0.991 (0.955–1.028)	0.62	0.988 (0.952–1.026)	0.53	—	—	—	—
Donor age	1.015 (0.992–1.038)	0.21	—	—	1.025 (0.996–1.054)	0.09	1.03 (0.997–1.063)	0.09	—	0.07
CIT (per hour)	1.05 (0.833–1.325)	0.67	—	—	1.088 (0.836–1.416)	0.53	—	—	—	—
Fulminant	7.825 (2.517–24.325)	< 0.001	3.079 (0.747–12.680)	0.12	5.577 (1.817–17.117)	0.003	1.562 (0.382–6.416)	0.003	—	0.56
MELD	1.053 (1.016–1.092)	0.004	1.034 (0.991–1.079)	0.12	1.068 (1.024–1.113)	0.002	1.059 (1.013–1.108)	0.002	—	0.01

Abbreviations: CIT, cold ischemia time; LFS, large-for-size; LGRM, liver graft-related morbidity; LPF, low portal flow; MELD, model for end-stage liver disease.

factor for LGRM [OR: 3.245 (1.113–9.372); $p = 0.03$], and 90-day patient and graft survival [HR: 3.4%, (1.049–1.073), and $p = 0.04$].

DISCUSSION

This study investigated, for the first time, the impact of both morphologic and hemodynamic characteristics of liver grafts on post-LT outcomes. The results showed that LPF was a significant predictor of post-LT morbidity and mortality, in contrast to LFS based on morphologic assessment using the GRWR. Following these results, we propose the concept of large-for-flow (LFF), which adds a hemodynamic component to the morphologic evaluation. This new concept emphasizes the need for flowmetric evaluation and may guide clinical decisions toward the optimization of graft-recipient matching and perioperative management in LT.

Morphologic assessment of liver grafts is a key metric for graft-recipient matching. According to previous studies, LFS is associated with graft survival,^[3] increased graft parenchymal injury and EAD^[13,21], or respiratory complications.^[12] In our study, LFS alone, solely based on morphologic analysis (GRWR), presented with a higher rate of EAD (39.4%, $p = 0.03$) but was not an independent risk factor for increased morbidity, graft loss, or recipient death. Of note, we excluded extreme LFS^[11] as this is a rare condition (one recipient in our cohort) where there is an anthropometric mismatch leading to graft compression. This condition illustrates that graft-size matching based on GRWR or BSA remains an important step to avoid this particular situation.

In addition to the morphologic assessment, several studies have shown the importance of perioperative hemodynamic analysis, specifically portal vein flow measurement^[10] with an association between LPF and PNF, EAD, and recipient survival.^[6–9] The data from our study confirmed LPF as an independent risk factor for LGRM and patient survival with a higher rate of PNF, EAD, and high-risk L-Graft7 category. The pathophysiological mechanisms of LPF are related to liver parenchymal injury as shown by the significantly higher serum transaminase release compared with grafts with normal portal vein flow (Supplemental Figure S5, <http://links.lww.com/LVT/A416>). As expected, we did not observe any compensatory increase in arterial flow in the presence of LPF. This lack of hepatic arterial buffer response leads to insufficient overall perfusion of the graft.^[22,23] As suggested by Matsushima et al,^[6] this situation is the exact opposite of SFS, in which portal hyperperfusion^[24] decreases the arterial flow through the hepatic artery buffer response^[22,23] and is associated with an increased risk of arterial thrombosis, biliary complications, and graft loss. Interestingly, in the LPF group, the portal vein pressure and the HVPG remained

in a physiological range, and we did not observe any arterial buffer effect, as described in the SFS syndrome.^[25]

When combining the morphologic analysis of LFS based on GRWR with the hemodynamic component of LPF, we made several interesting observations. First, we could not show any significant difference between overall portal vein flow in recipients with and without LFS. However, when portal vein flow was normalized over liver graft weight, the median portal vein flow was significantly lower in recipients with LFS (89 vs 129 mL/100 g/min; $p < 0.001$). In other words, portal vein perfusion of the graft was directly correlated to GRWR (R spearman -0.312 , $p < 0.001$; **Figure 3**), and consequently, LPF occurred more often in LFS grafts (33.3% vs 14.3%; $p = 0.006$). Second, LFS grafts with an adequate portal vein flow were not associated with an increased risk of LGRM or graft and patient loss in our study (**Table 3**). These results support the fact that hemodynamic assessment of the liver graft might refine morphologic evaluation alone. As in the case of SFS syndrome, which was recently redefined as small-for-flow,^[4] we propose the novel concept of LFF over LFS. The particularity of LFF is that it combines morphologic characteristics of the graft, such as GRWR, and hemodynamic criteria, including portal vein flow, and impacts both post-LT morbidity and recipient survival. Consequently, we propose a

pragmatic decision-making algorithm, including LFF with the goal of achieving optimal portal perfusion of the graft through morphologic graft-recipient matching, recipient characteristics, and hemodynamic analysis (**Figure 5**).

Given the fact that the actual portal flow can only be measured during LT, several pre-LT indicators should be assessed to optimize graft-recipient matching to reduce the risk of LFF. First, preprocurement imaging of the donor enables a direct measurement of graft size, which allows us to optimize matching with the anthropometric data of the recipient.^[26] Second, pre-LT CT-scan of the recipient allows for identifying surrogates of LPF, such as PVT,^[27,28] portal vein caliber,^[29] and PSS. Besides, pre-LT evaluation by CT-scan^[30] or 4D-MRI^[31] is a promising perspective to anticipate the surgical strategy for portal flow modulation by providing a precise anatomic and hemodynamic mapping of PSS. Second, in the case of high GRWR and potential LPF, recipients with portal hypertension, ascites, or PSS should be preferentially selected as they present the possibility of portal inflow modulation.^[14,32] Finally, perioperative assessment of portal flow and portal pressure should be mandatory in this scenario^[10] and may be assessed before implantation of the graft in case of temporary portocaval anastomosis. This may guide surgical strategies, such as PSS ligation to increase portal flow.^[32]

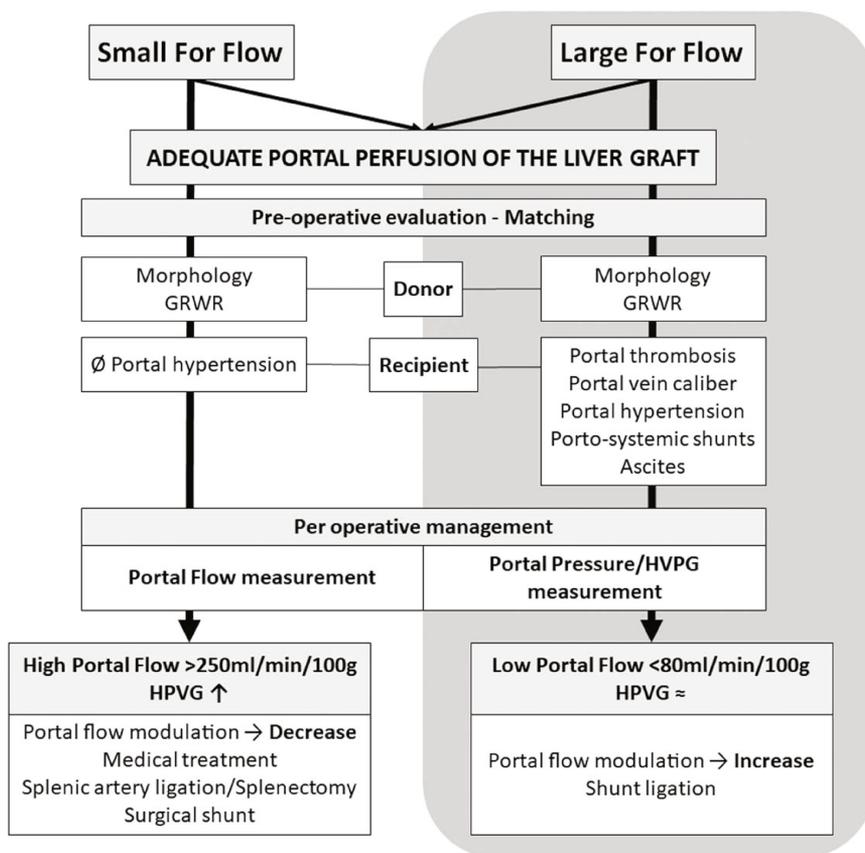


FIGURE 5 Clinical decision algorithm based on the morphologic and hemodynamic evaluation.

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For instance, shunt ligation was able to increase portal flow above the normal range in 3/7 patients in our experience. In recipients with LPF but without major PSS, for example, in the case of fulminant hepatitis (Supplemental Figure S6, <http://links.lww.com/LVT/A417>), graft reduction may offer a surgical alternative to adapt graft size to portal inflow and avoid LFF.^[33] However, graft reduction for hemodynamic purposes is a complex procedure and needs to be validated in future studies. Of note, given the impact of fulminant hepatitis on patient and graft survival in our study, we performed a subgroup analysis excluding those recipients (Supplemental Table S4, <http://links.lww.com/LVT/A412>). LPF was an independent risk factor for LGRM, patient survival, and 3-month graft survival (HR: 3.4%, (1.049–11.073), and $p=0.04$) for end-stage liver disease patients sustaining the LFF concept.

Our work has several limitations inherent to its single-center design with a limited sample size.

In addition, there are several definitions and cutoffs for LFS and LPF available in the literature.^[2,6,8] Those used in the present study were selected based on a preliminary internal validation to test their ability to predict LGRM (Supplemental Table S1, <http://links.lww.com/LVT/A412>; Supplemental Table S2, <http://links.lww.com/LVT/A412>). To the best of our knowledge, this study is, however, the first to combine morphological and hemodynamic characteristics of liver grafts to evaluate their respective impact on post-LT outcomes. LFF is also a concept which needed to be acknowledged to better anticipate recipient selection in some particular situations and point out the usefulness of hemodynamic assessment.

CONCLUSION

LPF increased LGRM and impacted patient survival, whereas LFS defined as a morphological metric alone did not impair the postoperative course. These results underlined the value of portal flow assessment in the setting of larger grafts. Combining hemodynamic and morphological characteristics defined the new concept of LFF, which might be anticipated based on the donor's and recipient's characteristics. This new approach may guide graft selection and will now need to be validated in large-scale studies.

AUTHOR CONTRIBUTIONS

Guillaume Rossignol and Xavier Muller: designed the study, acquired the data, performed the statistical analysis, interpreted the data, and wrote the manuscript. Joris Couillerot: acquired the data. Jean-Yves Mabrut, Kayvan Mohkam, Fanny Lebosse, and Marie-Charlotte Delignette: designed the study, interpreted the data, critically reviewed the data, and drafted a final version of the manuscript.

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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A Single Preservation Solution for Static Cold Storage and Hypothermic Oxygenated Perfusion of Marginal Liver Grafts: A Preclinical Study

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Background. Hypothermic oxygenated perfusion (HOPE) improves outcomes of marginal liver grafts. However, to date, no preservation solution exists for both static cold storage (SCS) and HOPE. **Methods.** After 30 min of asystolic warm ischemia, porcine livers underwent 6 h of SCS followed by 2 h of HOPE. Liver grafts were either preserved with a single preservation solution (IGL2) designed for SCS and HOPE (IGL2-Machine Perfusion Solution [MPS] group, n=6) or with the gold-standard University of Wisconsin designed for SCS and Belzer MPS designed for HOPE (MPS group, n=5). All liver grafts underwent warm reperfusion with whole autologous blood for 2 h, and surrogate markers of hepatic ischemia–reperfusion injury (IRI) were assessed in the hepatocyte, cholangiocyte, vascular, and immunological compartments. **Results.** After 2 h of warm reperfusion, livers in the IGL2-MPS group showed no significant differences in transaminase release (aspartate aminotransferase: 65.58 versus 104.9 UI/L/100 g liver; $P=0.178$), lactate clearance, and histological IRI compared with livers in the MPS group. There were no significant differences in biliary acid composition, bile production, and histological biliary IRI. Mitochondrial and endothelial damage was also not significantly different and resulted in similar hepatic inflammasome activation. **Conclusions.** This preclinical study shows that a novel IGL2 allows for the safe preservation of marginal liver grafts with SCS and HOPE. Hepatic IRI was comparable with the current gold standard of combining 2 different preservation solutions (University of Wisconsin+Belzer MPS). These data pave the way for a phase I first-in-human study and it is a first step toward tailored preservation solutions for machine perfusion of liver grafts.

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INTRODUCTION

With the success of liver transplantation (LT) as a lifesaving treatment, the increasing graft shortage has forced the transplant community to accept the so-called marginal grafts that are more susceptible to ischemia–reperfusion injury (IRI) and associated with a higher risk of graft failure after LT. To improve posttransplant outcomes of these marginal liver grafts, optimizing organ preservation is key.¹ Although preservation with static cold storage (SCS) has

shown excellent results in benchmark transplant scenarios, the currently used preservation solutions have been developed more than a decade ago and have thus not been tailored to the requirements of preserving marginal grafts.^{2,3} More importantly, although the use of hypothermic oxygenated perfusion (HOPE) has been shown to improve outcomes in both extended criteria DCD and donation after brain death LT, there is no preservation solution available to date specifically designed for the use during

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X.M. and G.R. designed the study, performed the experiments, acquired the data, performed the statistical analysis, interpreted the data, and wrote the article. A.B., J.C., and V.H. performed the experiments and acquired and analyzed the data. J.-Y.M., K.M., and M.L. interpreted the data, critically reviewed the data, and drafted a final version of the article.

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HOPE.³⁻⁶ With this in mind, a novel preservation solution was designed for SCS and cold perfusion of marginal liver grafts. This study aims to test this novel single preservation solution (IGL2) in a preclinical model of hepatic IRI and compare it with the current gold standard of 2 different preservation solutions for static and dynamic preservation.

MATERIALS AND METHODS

Ethical Statement

The presented research complies with all relevant ethical regulations, and the experimental protocol was approved by the Institutional Animal Care and Use Committee (project number DR2020-27).

Study Design

We compared a novel preservation solution IGL2 against a combination of the standard solution for SCS, University of Wisconsin (UW) solution, and for HOPE, Belzer Machine Perfusion Solution (Belzer MPS). Notably, we used the generic solutions for UW (BEL-GEN, IGL, France) and Belzer MPS (PERF-GEN, IGL, France), which have the same composition as their original counterparts. The IGL2 preservation solution was designed for use in marginal grafts and tailored to the requirements of both static and dynamic cold preservation. In comparison with UW and Belzer MPS, IGL2 has a nearly 2× lower viscosity and the oncotic agent hydroxyethyl starch (HES) has been replaced with the nonimmunogenic agent polyethylene glycol 35. Compared with the more recent preservation solution IGL1, polyethylene glycol (PEG) concentration is 5× higher in the present solution. To optimize cold perfusion, the vasoprotective and antioxidant agent zinc has been added under the form of ZnCl₂ to guarantee the stability of the compound. Indeed, extracellular zinc has been shown to act at the level of vascular endothelial cells by a stimulating role in cell viability and proliferation via vascular endothelial growth factor A as well as upregulation of cofactors involved in vasodilatation such as PTGIS and eNOS.⁷ The addition of Zn in the context of renal IRI has also been shown to reduce the activation of the IRI inflammasome and reduce autophagy and apoptosis.⁸ To further

improve vasodilatation, which is important for vascular protection in the context of higher vascular resistance in the cold, sodium nitrite was directly added to the solution in low concentration to avoid the formation of reactive nitrogen species.⁹ A detailed description of the composition of IGL2 and a comparison with UW and Belzer MPS can be found in Table 1 and Supplemental Material (SDC, <http://links.lww.com/TP/C812>).

Porcine Marginal Liver Graft Model

We used a previously validated model of porcine marginal liver grafts with 30 min of donor asystolic warm ischemia (AWI) followed by 6 h of SCS.¹⁰ After dissection of the liver graft, pigs were heparinized (150–200 IU/kg) and exsanguinated via the previously cannulated infrahepatic aorta and inferior vena cava. During exsanguination, a total of 1.5 to 2 L of autologous blood was collected in a blood bag with 5000 IU of heparin (heparin LEO 5000 IU/mL; LEO Pharmaceutical Products, Denmark), supplemented with PIPERACILLIN/TAZOBACTAM KABI 4 g/500 mg and stored at room temperature protected from ambient light until reperfusion. After exsanguination, cardiac arrest was declared via invasive blood pressure monitoring, followed by 30 min of AWI. Liver grafts were then flushed by gravity via the portal vein with 1 L of cold heparinized NaCl 0.9% (Baxter BV, Utrecht, the Netherlands) followed by 2 L of a cold preservation solution via the portal vein and the aorta. This marked the start of SCS. A detailed description of the experimental model can be found in Supplemental Material (SDC, <http://links.lww.com/TP/C812>).

Ex Situ HOPE

HOPE was performed ex situ after SCS and before warm reperfusion using the Liver Assist device (XVIVO, Sweden) as previously reported.¹⁰ Briefly, livers were perfused with either 3 L of PERF-GEN or the experimental solution IGL2. Notably, livers were not flushed out of the static preservation solution before connection to the perfusion device. The perfusate was oxygenated with 100% O₂ and perfusion was performed through the portal vein at 8 to 10 °C. The perfusion pressure was limited to 3 mmHg

TABLE 1.
Comparison of the main components of UW, Belzer MPS, and IGL2

	Belzer MPS	UW	IGL2
Year	1970	1980	2020
Osmotic agent	Gluconate 90 mmol/L Glucose 10 mmol/L	Raffinose 30 mmol/L Lactobionate 100 mmol/L	Mannitol 60 mmol/L Lactobionate 80 mmol/L
Buffers	Phosphate HEPES buffer	Phosphate Sulfate	Phosphate HEPES Histidine
Oncotic agent	HES 50 g/L	HES 50 g/L	PEG35 5 g/L
Viscosity, cP	2.4	5.7	1.4
Antioxidants	Glutathion Allopurinol	Glutathion Allopurinol	Glutathion
Electrolytes	Na 100 mmol/L K ⁺ 25 mmol/L = extracellular	Na 25 mmol/L K ⁺ 120 mmol/L = intracellular	Na 125 mmol/L K ⁺ 25 mmol/L = extracellular
Osmolality	300	320	360

HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HES, hydroxyethyl starch; MPS, Machine Perfusion Solution; PEG35, polyethylene glycol 35 kDa; UW, University of Wisconsin.

in the portal vein to reach a target portal flow ranging from 150 to 300 mL/min.

Ex Situ Warm Reperfusion

Warm reperfusion was performed ex situ using the Liver Assist device (XVIVO, Sweden) offering the possibility of serial tissue and serum sampling. Before connection of the liver, the perfusion system was primed with the autologous whole pig blood from the same donor as the graft and supplemented with calcium gluconate 10% (B. Braun Medical, Melsungen, Germany). The perfusate pH was adjusted to a physiological range using sodium bicarbonate 4.2% (B. Braun Medical, Melsungen, Germany). The temperature was set to 37 °C, and the blood was oxygenated with a carbogen mixture of 95% O₂ and 5% CO₂ at 1 L/min. After SCS or HOPE, liver grafts were flushed by gravity with 1 L of cold Ringer Lactate. The liver grafts were then connected to the perfusion circuit with the portal vein first followed by the arterial connection for reperfusion at 37 °C for 2 h. Notably, no gradual rewarming was performed. Portal perfusion was pressure controlled with a maximum pressure set at 10 mmHg. Arterial perfusion was also pressure controlled with a target mean arterial pressure of 65 mmHg and a pulsatile flow. No bile salts, sodium bicarbonate, or parenteral nutrition were added during reperfusion to avoid blunting of IRI.

Aim and Study groups

The aim of the study was to compare hepatic IRI in marginal liver grafts preserved with IGL2 against the current gold-standard UW for SCS and Belzer MPS for HOPE.

In a preliminary set of experiments, we compared IGL2 with UW (BEL-GEN) for SCS only (Figure 1):

1. 30 min of AWI ischemia followed by 6 h of SCS in the BEL-GEN solution followed by 2 h of ex situ reperfusion (static UW group, n = 5).
2. 30 min of AWI followed by 6 h of SCS in the IGL2 solution followed by 2 h of ex situ reperfusion (static IGL2 group, n = 6).

In the main set of experiments, we then compared IGL2 with the combination of UW (BEL-GEN) for SCS and Belzer MPS (PERF-GEN) for HOPE (Figure 1):

3. 30 min of AWI followed by 6 h of SCS in the BEL-GEN solution followed by 2 h of ex situ HOPE with PERF-GEN MPS and 2 h of ex situ reperfusion (MPS group, n = 5)
4. 30 min of AWI followed by 6 h of SCS in the IGL2 solution followed by 2 h of ex situ HOPE with IGL2 MPS and 2 h of ex situ reperfusion (IGL2 MPS group, n = 6)

Endpoints

During 2 h of warm reperfusion, IRI was assessed in 4 major functional compartments of the liver graft, namely the hepatocyte, cholangiocyte, vascular, and immunological compartments.¹¹ Analysis was based on sequential perfusate, blood, and bile samples as well as liver and bile tissue biopsies. Blood samples underwent centrifugation at 1500 rpm for 10 min, and the obtained plasma was snap-frozen at -80 °C together with bile and liver biopsies until further analysis.

Hepatocyte Compartment

Hepatocyte injury and function were assessed during reperfusion by serum aspartate aminotransferase (AST) levels, serum pH and glucose concentrations determined, and serum lactate clearance. AST serum levels were normalized for liver graft weight. Notably, as previously reported by others, there is no release of alanine aminotransferase in pig livers.¹² We could confirm this observation in our preliminary experiments, and we have thus excluded alanine aminotransferase measurements from the final analysis (Supplemental Data, SDC, <http://links.lww.com/TP/C812>).

Liver biopsies were formalin fixed, paraffin embedded, and stained with HES and Caspase-3 (Cell Signaling 9661). An histological IRI classification recently published by our group and adapted from the University of California, Los Angeles classification and Suzuki classification was performed in a blinded manner by an expert pathologist and applied to all liver biopsies.¹³⁻¹⁵ In detail, the presence of hepatocyte necrosis, neutrophilic infiltrate, and congestion were semiquantitatively assessed. Hepatocyte ballooning and apoptosis were quantitatively assessed (Table S1, SDC, <http://links.lww.com/TP/C812>).

Cholangiocyte Compartment

Total quantitative bile production was assessed after 2 h of warm reperfusion. Qualitative bile assessment

Can IGL2 be used for static cold storage (SCS) ?

UW Group	30' AWI	6H SCS – BEL-GEN	2H - Reperfusion	n=5
IGL2 Group	30' AWI	6H SCS- IGL2	2H - Reperfusion	n=6

Can IGL2 be used for static cold storage + HOPE ?

MPS Group	30' AWI	6H SCS - BEL-GEN	2H HOPE PERF-GEN	2H - Reperfusion	n=5
IGL2 MPS Group	30' AWI	6H SCS- IGL2	2H HOPE IGL2	2H - Reperfusion	n=6

FIGURE 1. Experimental set-up and study groups. AWI, asystolic warm ischemia; HOPE, hypothermic oxygenated perfusion; MPS, Machine Perfusion Solution; SCS, static cold storage; UW, University of Wisconsin.

was performed by analyzing bile acid composition using liquid chromatography coupled with tandem mass spectrometry analysis. Bile acids, bile acid ratios, and hydrophobicity index were evaluated.¹⁶ A detailed protocol of the analysis and a list of analyzed bile acids and bile acid ratios can be found in **Supplemental Data and Table S2** (SDC, <http://links.lww.com/TP/C812>). In addition to bile samples, intrahepatic and perihilar bile ducts were identified using CK7 staining (Dako M7018) to perform a bile duct-specific IRI injury score adapted from op den Dries et al¹⁷ in a blinded manner by an expert pathologist.

Vascular Compartment

Portal flow was constantly recorded during HOPE at a fixed pressure of 3 mmHg. During warm reperfusion, portal and arterial flow rates were also constantly recorded at a fixed portal pressure of 10 mmHg and a mean arterial pressure of 65 mmHg. The production of endothelial nitric oxide (NO) was assessed at the end of 2 h warm reperfusion by using a Nitrate/Nitrite Colorimetric Assay Kit (Sigma, 23479-1KT-F) as previously reported.¹⁸ Serum thrombomodulin (Pig Thrombomodulin [THBD] ELISA Kit, ABBEXA, abx155005) and von Willebrand factor (Pig von Willebrand factor [vWF] ELISA Kit, ABBEXA, abx255580) were both assessed as markers of endothelial IRI as previously reported.^{10,19}

Immunological Compartment

Ratios of proinflammatory and anti-inflammatory cytokines were analyzed in the serum during 2 h of warm reperfusion using a ProcartaPlex 6-Plex Assay (Life Technologies, ID: MXU64FK) and the Bio-Plex MAGPIX multiplex reader (Bio-Rad). To investigate the impact of the MPS on mitochondrial damage, flavin mononucleotide (FMN) was quantified using a spectrofluorometer (SAFAS Flx-Xeniusafter) during HOPE as previously described.²⁰⁻²² Similarly, the NADH ratio in the perfusate was determined after 5, 10, and 30 min of HOPE as a marker of mitochondrial respiration.²³

Statistical Analysis

The sample size was estimated on the basis of a non-inferiority of peak AST release during ex situ warm reperfusion between MPS and IGL2-MPS groups (**Table S3**, SDC, <http://links.lww.com/TP/C812>). Data are presented as mean \pm SD. Comparisons between 2 groups were made using the Mann-Whitney *U* test. A 2-sided *P* value of <0.05 was considered to be significant. Statistical analyses were performed with IBM SPSS Statistics for Windows (version 26.0; IBM Corp., Armonk, NY) and GraphPad Prism (version 8.0.0 for Windows; GraphPad Software, San Diego, CA).

RESULTS

Preliminary Experiments

In a set of preliminary experiments, we compared IGL2 with UW in the setting of SCS alone for 6 h followed by 2 h of warm reperfusion (Figure 1). We did not observe any significant differences in peak transaminase, lactate clearance, bile production, or early IRI scoring on histological

assessment (Figure S1, SDC, <http://links.lww.com/TP/C812>).

Dynamic Preservation With IGL2

Hepatocyte Injury and Function

During warm reperfusion, serum AST increased in both groups with a mean peak value of 65.58 UI/L/100 g liver for the IGL2-MPS and 104.9 UI/L/100 g for the MPS group ($P = 0.178$; Figure 2A and B). Lactate levels in the perfusate increased during the first 30 min after reperfusion in both groups with a peak serum lactate of 3.9 and 4.4 mmol/L in the IGL2-MPS and MPS group, respectively ($P = 1$; Figure 2C). Except for 1 liver in the IGL2-MPS group, all livers cleared lactate below the published viability threshold of <2.8 mmol/L²⁴ (1.49 mmol/L for MPS versus 1.47 mmol/L for IGL2-MPS $P = 0.126$; Figure 2D). Overall histological mean hepatocyte injury score was 2.8 and 4.6 for IGL2-MPS and MPS groups, respectively ($P = 0.353$; Figure 2E), and there were no significant differences in hepatic congestion ($P = 0.434$).

Cholangiocyte Injury and Function

Cumulative bile production was not significantly different between the 2 groups (MPS 16.2 mL/2 h versus IGL2-MPS 14.3 mL/2 h, $P = 0.701$; Figure 3A). A total of 16 bile acids were analyzed, including both primary and secondary bile acids (**Table S1**, SDC, <http://links.lww.com/TP/C812>). Univariable analysis of single bile acid and bile acid ratios did not show a significant difference between both groups (Figure 3B). Histological analysis of bile ducts showed a mean injury grade to the peribiliary glands <2 for both groups resulting in a cumulative mean injury score of 7.8 and 6 points for IGL2-MPS and MPS, respectively ($P = 0.370$; Figure 3C and 3D).

Hemodynamics and Endothelial Injury

During HOPE, the portal vein flow was stable and in a similar range for both groups (MPS 140–220 mL/h versus IGL2-MPS 160–280 mL/min) with a fixed portal vein pressure of 3 mm Hg (Figure 4A). During warm reperfusion, the mean arterial pressure was kept constant at 65 mm Hg) and we observed a more rapid increase in mean arterial flow during the first 30 min in the IGL2 MPS group (241 versus 182 mL/min, $P = 0.178$; Figure 4B). Serum NO content at the end of reperfusion was higher in the MPS group without reaching a statistically significant difference (81.34 versus 55.92 μ M, $P = 0.315$; Figure 4D). Levels of serum THBD and vWF increased during reperfusion in both groups and were not significantly different after 120 min of reperfusion ($P = 0.647$ and $P = 0.281$; Figure 4E and F).

Mitochondrial Injury and Inflammasome Activation

FMN increased in both groups during preservation and the mean perfusate concentration after 30 min of HOPE was 79.2 ng/mL in the MPS group and 54.5 ng/mL IGL2-MPS group ($P = 0.662$; Figure 5A). The NADH ratio in the HOPE perfusate was not significantly different between the 2 groups at the different time points (5 min: $P = 0.789$; 10 min: $P = 0.579$; 30 min $P = 0.196$; Figure 5B). The glucose in the perfusate increased in both groups during the

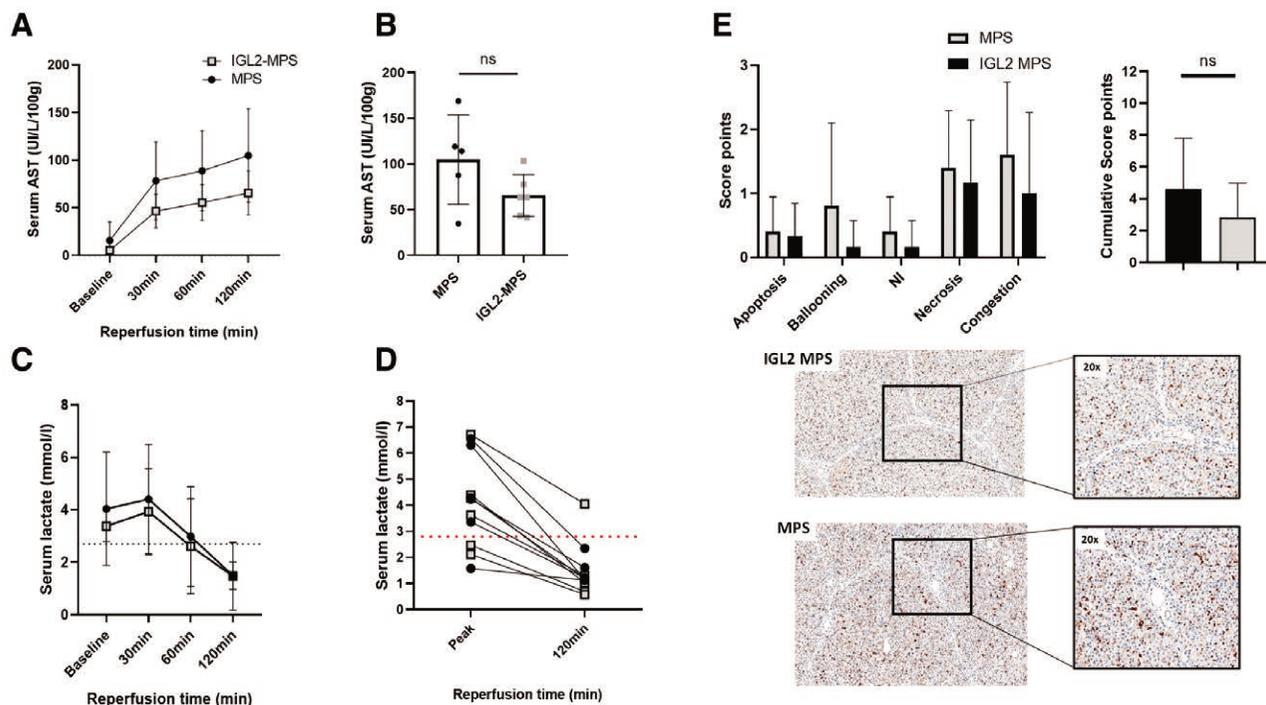


FIGURE 2. Hepatocyte injury and function. A, AST release during reperfusion normalized for liver weight. B, Peak AST during reperfusion for liver weight. C, Lactate clearance kinetics during warm reperfusion. D, Delta lactate during warm reperfusion. The red dotted line indicates the viability cut-off determined by Watson et al.²⁴ E, Histological analysis of different components of hepatocyte IRI and cumulative total injury score. CASPASE-3 staining for quantitative assessment of apoptosis. All data are represented as mean and SD. AST, aspartate aminotransferase; IRI, ischemia–reperfusion injury; MPS, Machine Perfusion Solution; NI, neutrophilic infiltrate.

first 30 of warm reperfusion minutes (4.9 mmol/L MPS versus 4.9 mmol/L IGL2-MPS, $P = 0.762$; Figure 5C) with no significant differences in both groups at the end of perfusion (3 mmol/L MPS versus 3.5 mmol/L IGL2-MPS, $P = 0.429$). Notably, no additional glucose was added to the perfusate during reperfusion. We observed an increase in proinflammatory cytokines IL-6 and IL-8 after 2 h of warm reperfusion in both groups without significant differences (Figure 5D).

DISCUSSION

This preclinical study shows that a novel IGL2 allows safe preservation with SCS and HOPE of porcine liver grafts undergoing 30 min of AWI. During warm reperfusion, hepatocyte, biliary vascular, and immunological IRI were comparable with the current standard of combining UW for SCS and Belzer MPS for HOPE.

The development of organ preservation solutions more than 3 decades ago allowed for prolonged SCS of liver grafts before transplantation. However, LT has rapidly changed during the past decade owing to the ever-increasing liver graft shortage. This has forced the transplant community to use grafts from extended criteria donors in addition to longer warm and cold ischemia times. In this context, HOPE of liver grafts before transplantation has been shown to reduce IRI and improve post-LT outcomes compared with SCS in both extended criteria donation after brain death and DCD grafts.^{4,5,25} To date, most published studies on HOPE in LT use Belzer MPS and an additional preservation solution for SCS.³ Belzer MPS has 2 major drawbacks in the context

of cold perfusion of marginal liver grafts. First, this solution has been designed for kidney perfusion and contains HES as the main oncotic agent resulting in a high viscosity (Table 1). Perfusion with a high-viscosity solution may increase shear stress under hypothermic conditions, especially in marginal liver grafts with endothelial damage and sinusoidal microthrombi.^{3,26} Second, because Belzer MPS is not suited for SCS, there is a need to use a different preservation solution for SCS, leading to a mix of different preservation solutions. Indeed, the sequential use of UW and Belzer MPS exposes the graft to a solution with an intracellular-like electrolyte composition followed by a solution with an extracellular-like electrolyte composition. Hence, the need for repeated flushing during the preservation sequence. Although UW is still the most widely used preservation solution for SCS, several centers are currently using IGL1 or Celsior instead of UW.^{27,28} These more recent preservation solutions have a lower viscosity, which may allow subsequent HOPE with Belzer MPS without the need for graft flushing before perfusion. Given the aforementioned physiopathological and logistical issues with Belzer MPS in the context of HOPE, this study aimed to test a novel IGL2 in a preclinical model of hepatic IRI and compared it with the current gold standard of using 2 different preservation solutions for static and dynamic preservation.

After validation of IGL2 as a preservation solution for SCS, we assessed the specific features of IGL2 in regard to cold oxygenated perfusion. The main difference between Belzer MPS and IGL2 is the substitution of HES for polyethylene glycol 35 kDa (PEG35; Table 1). The oncotic

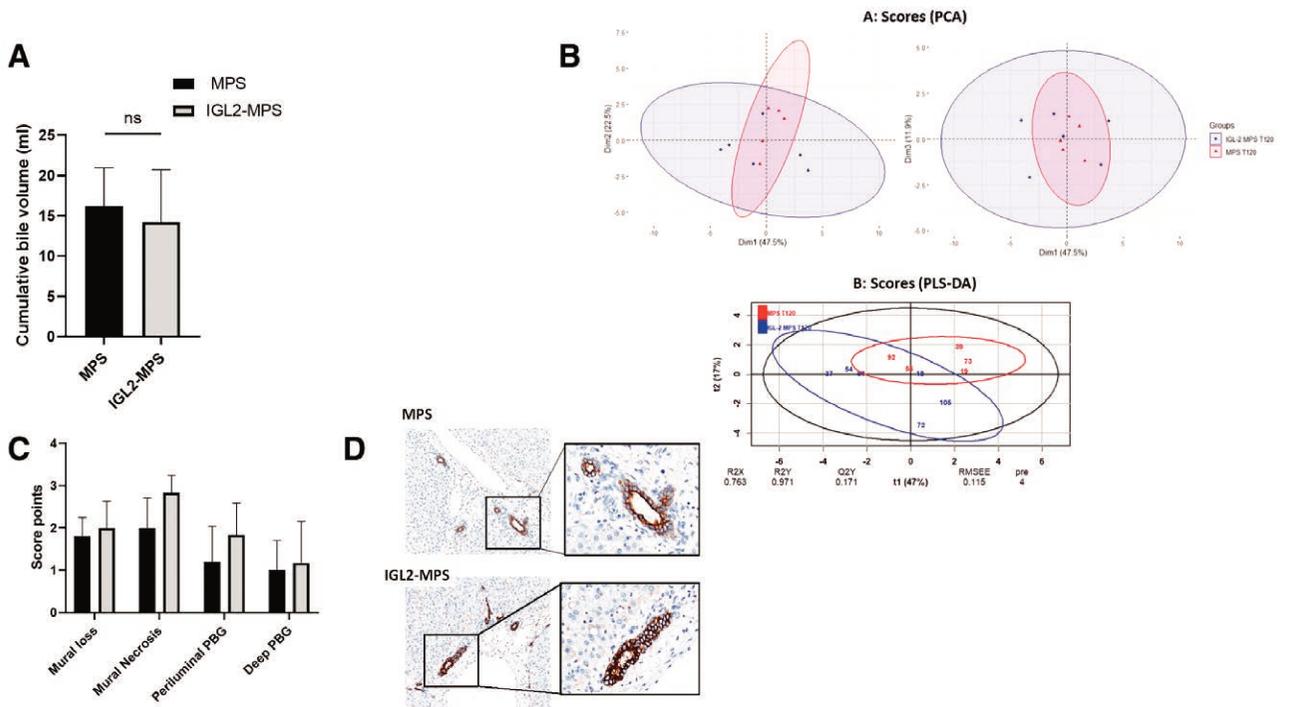


FIGURE 3. Cholangiocyte injury and function. A, Cumulative bile production after 120 min of warm reperfusion. B, PCA and PLS-DA analysis of BA in bile sample at the end of reperfusion in IGL2-MPS and MPS groups. Panel A exhibits PCA analysis without good discrimination between both groups. The 3 components explained 47.5%, 22.5%, and 11.9% of the variance. Panel B exhibits PLS-DA analysis without good discrimination between both groups (mean and SD. BA, bile acid; IRI, ischemia-reperfusion injury; MPS, Machine Perfusion Solution; PBG, peribiliary gland; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis.

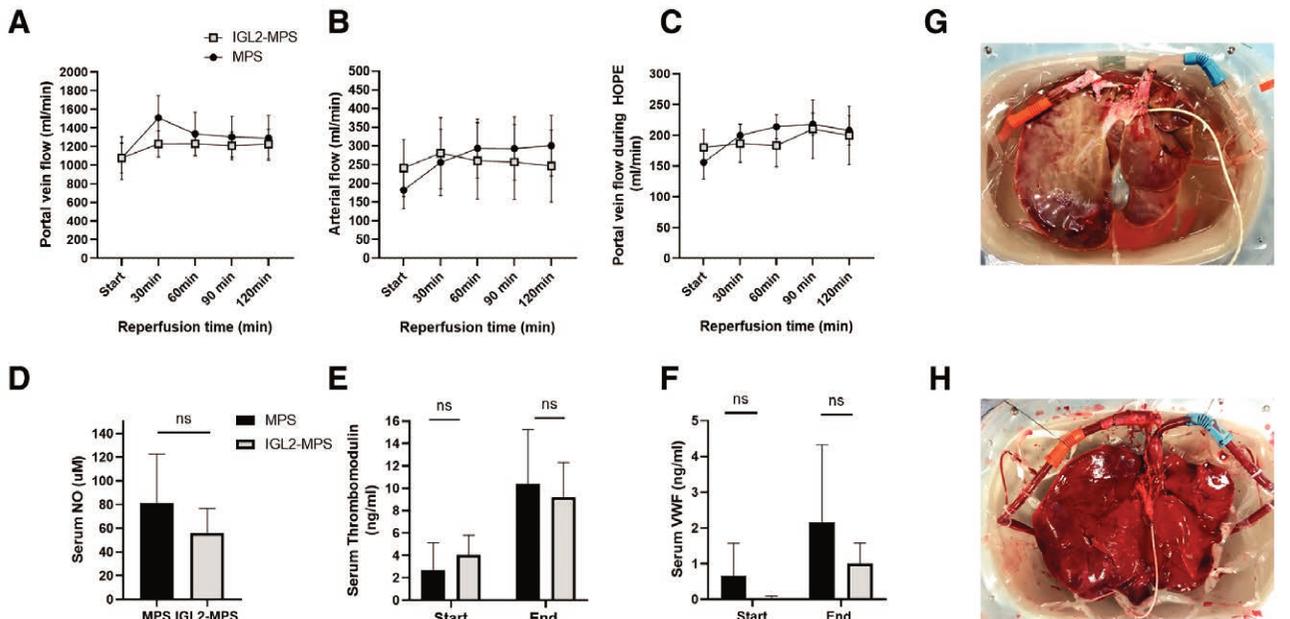


FIGURE 4. Hemodynamics and endothelial injury. A, Portal vein flow during warm reperfusion. Portal vein pressure was constant at 10 mmHg. B, Arterial flow during warm reperfusion. Arterial mean pressure was constant at 65 mmHg. C, Portal flow during HOPE. Portal vein pressure was kept constant at 3 mmHg. D, Serum NO after 2 h of warm reperfusion. E, Serum thrombomodulin at the start and at the end of warm reperfusion. F, Serum vWF at the start and end of warm reperfusion. G, Porcine liver graft during ex situ single portal HOPE using the Liver Assist device. H, Porcine liver graft during ex situ warm reperfusion using the Liver Assist device. HOPE, hypothermic oxygenated perfusion; MPS, Machine Perfusion Solution; NO, nitric oxide; vWF, von Willebrand factor.

agent PEG35 has been shown to confer a superior protective effect on mitochondrial metabolism and glycolyx integrity during SCS of fatty livers compared with a

preservation solution without PEG35 in a rat model.²⁹ The use of a PEG35-based perfusion solution has also been shown to protect the liver glycolyx during dynamic

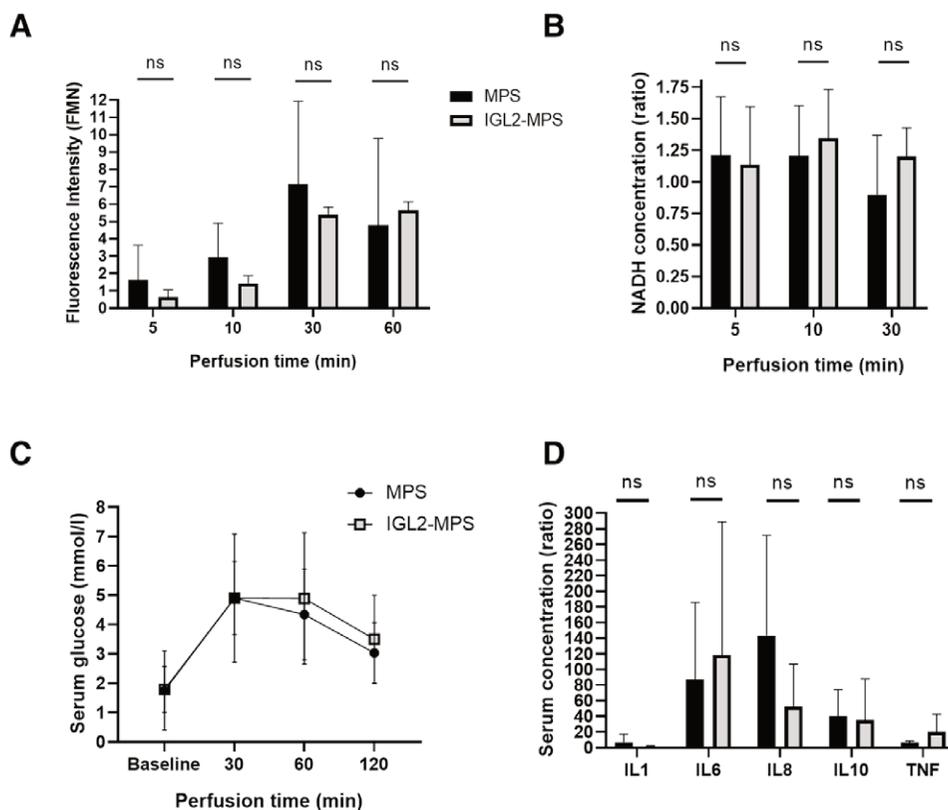


FIGURE 5. Mitochondrial injury and metabolic stress. A, FMN release measured spectroscopically at 5, 10, 30, and 60 min of HOPE. B, NADH ratio measured spectroscopically at 5, 10, and 30 min of HOPE. C, Glucose serum levels during warm reperfusion. D, Cytokine serum level ratios during warm reperfusion. FMN, flavin mononucleotide; HOPE, hypothermic oxygenated perfusion; MPS, Machine Perfusion Solution; ns, nonsignificant.

preservation with MPS in a rat model of steatotic livers.^{26,30,31} Notably, IGL2 has a 5× higher concentration of PEG35 than other currently available preservation solutions, which may further improve mitochondrial and microcirculatory protection.^{32,33} To test this hypothesis, we performed a dynamic assessment of mitochondrial respiratory chain damage and activity during graft preservation by analysis of FMN and NADH in the HOPE perfusate as previously described.^{20,21} We could not show any significant differences in FMN release and NADH metabolism between the PEG35-based solution IGL2 and the HES-based solution Belzer MPS, which translated into similar hepatic inflammasome activation between both solutions.

A second main addition to the novel preservation solution are vasoactive metabolites such as sodium nitrite, which have been shown to reduce IR when administered before reperfusion.³⁴ Additionally, together with PEG35, these metabolites are hypothesized to upregulate eNOS and thus confer additional protection from IRI through a direct effect on the microcirculation.³⁵⁻³⁷ We found that IGL2-preserved liver grafts had a higher arterial flow during the first 30 min of reperfusion, albeit not reaching statistical significance. Direct quantification of total NO content, THBD, and vWF levels in the blood after 2 h of warm reperfusion were comparable among groups. Further exploration is warranted to identify a clear benefit of IGL2 on endothelial shear stress.³⁸⁻⁴⁰

Although there were no statistically significant differences between IGL2 and the current gold standard of UW+Belzer MPS in terms of hepatic IRI, this study

provides mandatory safety data to design a phase I first-in-human trial according to the Idea, Development, Exploration, Assessment, Long-term study framework for surgical innovation.⁴¹ Indeed, using a large animal model allowed the use of the same volume of preservation solution and identical machine perfusion devices as in clinical practice. The expected logistical benefit of using a single solution by avoiding repeated flushing of the graft will likely present an economic benefit that must be confirmed in the later stages of its clinical implementation (stage 3 and 4 of the Idea, Development, Exploration, Assessment, Long-term study framework).

The main limitation of this study is the limited number of study subjects, which is inherent to a large animal model. A second limitation is the reperfusion time of 2 h, which does not allow us to draw any conclusions on the chronic phase of IRI and the long-term outcomes, such as nonanastomotic biliary strictures, graft rejection, and primary nonfunction rates. However, recent data showed that events occurring during the hyperacute and acute phase of IRI are key triggers for downstream activation of the hepatic inflammasome, directly impacting adverse events after LT.^{14,42,43} Nevertheless, the data provided on early IRI by this study must be verified in the clinical setting by focusing on the chronic IRI phase. Third, the absence of actual transplantation in our model may also be debated. However, in contrast to transplantation of pig livers, which is a complex procedure with high intersubject variability, isolated ex situ reperfusion is a long-standing and still commonly used model for studying liver graft preservation

with the advantage of allowing multiple tissue and blood samples in an isolated organ.^{10,44,45}

In conclusion, this preclinical study in a porcine model of IRI shows that a novel IGL2 allows for a safe preservation of marginal liver grafts with SCS and subsequent HOPE. Hepatic IRI was comparable with the current gold standard of combining 2 different preservation solutions for SCS and HOPE (UW + Belzer MPS). These data pave the way for a phase I first-in-human study and are a first step toward preservation solutions tailored for machine perfusion of liver grafts.

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Appel à Projet Fondation de l'Avenir 2021

Analyse métabolomique du greffon hépatique lors de la perfusion régionale normothermique chez le donneur décédé par arrêt cardiaque en transplantation hépatique- Etude *MAAS3BOLOMIC*

Madame, Monsieur,

Veillez trouver ci joint, en annexe de notre demande de financement pour l'Appel à Projet de la Fondation de l'Avenir 2021, le protocole détaillé de notre projet clinique MAAS3BOLOMIC, précisant son contexte réglementaire et les travaux en cours de notre équipe sur cette thématique.

En espérant que ce projet saura retenir votre attention.

Respectueusement

Xavier MULLER.

Contexte Reglementaire :

Il s'agit d'une étude humaine multicentrique, prospective, non interventionnelle.
Il s'agit donc d'une étude en RIPH (Recherche impliquant la personne humaine) n°3.
Le protocole de l'étude sera donc soumis à un CPP, enregistré auprès de la CNIL et référencé sur clinicaltrials.gov.

Projet d'Equipe :

L'équipe portant le projet a déjà réalisé la première étude comparant l'utilisation de la perfusion régionale normothermique dans les donneurs décédés par arrêt cardiaque à l'utilisation de la machine de perfusion Hypothermique Oxygénée (HOPE). Un PHRC national multicentrique, piloté par notre équipe est actuellement en cours sur l'utilisation et l'intérêt de la machine de perfusion HOPE pour les foies issus de donneurs à critères élargis.

Deux études, l'une animale et l'autre clinique fruit de la collaboration avec Zurich ont permis de mettre en évidence le rôle de la mitochondrie et du métabolisme cellulaire dans ces mécanismes d'ischémie-reperfusion, faisant de ces derniers des cibles de choix dans l'évaluation du greffon hépatique.

Notre équipe vient de décrire récemment l'utilisation de HOPE lors de la réalisation de la bipartition du foie en greffe adulte et pédiatrique.

Une étude préclinique est actuellement en cours sur un modèle porcin de donneurs décédés par arrêt cardiaque associée à l'utilisation de la perfusion Hypothermique Oxygénée et de la Perfusion Regionale Normothermique afin d'évaluer les mécanismes des lésions d'ischémie-reperfusion sur le plan métabolomique au sein du Centre de Recherche en Cancérologie de Lyon (UMR INSERM 1052 CNRS 5286).

Une collaboration scientifique a été mise en place pour ce projet avec l'Institut de Sciences Analytiques de Lyon pour la réalisation des études métabolomiques à l'aide de la spectrométrie de masse ou par résonance magnétique nucléaire.

Tout ceci dessine ainsi le rationnel scientifique du projet actuel.

1. Objectives and background:

1.1. Background and rational of the study

With the increasing shortage of available donor organs, liver transplantation from donation after circulatory death (DCD) has been established in many countries to offer a therapeutic strategy for patients with end-stage liver disease beyond standard donation after brain death (DBD)¹. In France, a controlled DCD (cDCD) program based on the use of normothermic regional perfusion (NRP) during organ procurement has been successfully implemented since 2015. This program has since shown excellent post-transplant outcomes with one-year patient and graft survival rates >90%.^{2,3} However, owing to very strict donor and graft selection criteria nearly one third of all potential liver grafts were not transplanted because of presumed poor quality.^{2,3} Furthermore, survival in cDCD liver transplantation which did not adhere to the current selection criteria was significantly lower (68% vs 94%) compared to the highly selected population.⁴ Thus, to further expand selection criteria and reduce discard rates without compromising outcomes, there is an urgent need for novel objective methods to assess graft quality prior to transplantation.

Graft quality assessment prior to transplantation has been a key challenge in liver transplantation for decades and still today the decision to accept or decline a graft before transplantation relies for the most part on “gut feeling” of the procurement or transplant team.⁵ Currently, selection criteria for cDCD in France are based on donor data, liver biopsy results and hepatocyte injury markers AST and ALT.^{2,3,4} While donor data are only indirect indicators of graft quality, liver graft biopsy are highly pathologist-dependant and do not provide a dynamic assessment. Hepatocyte injury markers during normothermic perfusion have been shown to only have limited value in predicting post-transplant graft function.⁶ In addition, several recent studies have suggested a superior predictive value of specific biomarkers in the liver through metabolomics.^{7,8} There is growing evidence that dynamic graft preservation such as NRP may even allow a direct analysis of key injury metabolites within solid organ grafts.^{9,10}

During cDCD procurement, liver grafts are exposed to donor warm ischemia which in combination with static cold storage causes ischemia-reperfusion (I/R) injury of the graft during implantation in the recipient.¹¹ I/R is directly correlated to detrimental post-transplant complications such as primary non function and ischemic cholangiopathy or even recipient death. Studies in animal models have revealed that mitochondria play a major role in IR injury and several mitochondrial signature metabolites for example succinate have been identified in various solid organ grafts such as livers, hearts and kidneys.¹² Based on these results, our team and others have recently identified the release of a small auto fluorescent molecular compound of the mitochondrial respiratory chain, Flavin Mononucleotide (FMN), during the early phase of hepatic I/R injury.^{13,14} Furthermore, FMN has been shown to serve as a surrogate marker for impaired cellular energy production of the liver graft prior to transplantation and enable accurate prediction of post-transplant liver graft viability.¹⁵ Given the natural fluorescence of FMN, a real time quantification method has been established allowing to rapidly assess viability of human liver grafts during the procurement process.^{13,14} Of note, a team from the United Kingdom has validated this real-time FMN quantification during NRP of cDCD liver grafts.¹⁴ Interestingly, since mitochondrial injury is a universal signature of graft injury during the transplant process, FMN has the potential to serve as viability marker in other solid organs such as hearts, lungs and kidneys.¹⁴

1.2. Objectives

Based on the recent findings on mitochondrial driven I/R injury of liver grafts in cDCD liver transplantation, the present study addresses two key objectives in organ preservation:

Aim 1: To characterise I/R injury in cDCD liver grafts during NRP by in-depth metabolomics to establish a robust biomarker for cDCD liver graft viability.

Aim 2: To develop a bio-clinical prediction model based on liver biomarkers in combination with donor and recipient data to predict graft function prior to transplantation.

To the best of our knowledge the proposed research project is innovative as it is the first investigating I/R injury during NRP in cDCD by metabolomics with the aim to establish robust biomarkers for graft viability. The data gathered in this study will provide a better understanding of I/R injury during NRP procurement in cDCD and allow to optimize donor selection and transplant outcomes.

1.3. Previous studies and preliminary results

The investigators of this research project are all part of a large-volume transplant center with active clinical and scientific interest in the field of cDCD organ donation, dynamic perfusion strategies and liver transplantation.^{2,16,17} The investigators have recently published the first direct comparison between NRP and ex vivo machine perfusion in cDCD liver transplantation in the highest-ranked surgical journal.² The results from this study have provided the background and rationale for the main aims of the proposed research project.

In addition, the investigators are currently supervising the first national multicenter randomized controlled trial *HOPExt* comparing different preservation strategies in liver transplantation (NCT03929523). The trial is currently recruiting and the lessons learned from setting up this trial and organizing national sample collection will contribute to the success of the present research project.

In parallel to the clinical activity, the investigators have recently participated in a basic research identifying and mitochondria induced I/R injury in cDCD liver grafts.^{13,15} First, the authors confirmed, using mass spectrometry analysis in a rodent model of cDCD liver grafts, the accumulation of succinate during ischemia which subsequently triggered FMN release upon normothermic reperfusion from complex I of the mitochondrial respiratory chain.¹³ Second, they showed that this initial event led to the activation of the hepatic inflammasome by ROS signalling with subsequent liver graft damage. Third, these findings in an animal model were translated into human cDCD liver transplantation where FMN release was shown to be an easily detectable predictor of post-transplant liver graft function.^{13,15}

In parallel to the present research project, the investigators have established a pig model of cDCD donation with NRP and ex-vivo reperfusion to further investigate mitochondrial mechanism of I/R injury in a preclinical model.

1.4. Significance and novelty of the planned research

The present research project will address key objectives in organ preservation today by transposing novel findings from basic research on I/R mechanism into clinical practice. Based on the preliminary results from previous studies of our team, we expect a high clinical significance as this may have a direct clinical application to allow safe transplantation of cDCD liver grafts and help to further develop the French cDCD program.

The results of the present project will lay the foundation for a clinical trial on the use of extended criteria cDCD liver grafts with the aim to safely increase the cDCD donor pool. In addition, the prediction model may be validated for other organs procured during NRP such as hearts, lungs and kidneys.

2. Project Description and Expected Results

2.1. Methods

2.1.1. Study design overview

The study is an open label prospective non-randomised single-arm multicenter clinical trial including 60 cDCD liver grafts procured and transplanted after undergoing NRP. The study period will be 24 months with a follow-up period of 1 year. The primary endpoint is to perform in depth metabolomics during NRP of perfusate and liver graft tissue including detection of flavin mononucleotide (FMN) during NRP to assess viability of cDCD liver grafts prior to transplantation.

2.1.2. Inclusion of cDCD donors

All cDCD donors where the liver is allocated to one of the four transplant study centers and NRP has been successfully started will be included in the study.

2.1.3. NRP, procurement and liver transplantation process

All livers will be retrieved with the standardised technique. No changes in the NRP or procurement technique will be required, that is to say NRP will be performed for at least for 60min and for maximum 4h. Following procurement at the donor hospital liver will be placed in ice-cold preservation solution on the back table and transported to the transport center (according to local protocol). There will be no delays in graft allocation, procurement, transport or implantation due to the study protocol.

2.1.4. Sampling during NRP

A detailed description of study samples and collection time points can be found in Table 1. The investigators will provide the study centers with a detailed standard operating procedure (SOP) on sampling types and timepoints. All study samples will be collected by the procurement team and transported back to the study center together with the organ graft.

2.1.5. Recipient follow-up

Based on the CRISTAL database (Agence de la biomédecine), the post-transplant follow-up will cover 12 months to capture all relevant complications notably non-anastomotic biliary strictures and 1-year survival.¹⁸ No additional study visits are required besides the routinely planned recipient follow-up visits in the respective centers. No study specific samples or analysis are required from the recipients and only routinely performed blood analysis will be analyzed in the study.

Table 1: Study samples types, collection time points, storage and analysis

	Type	Timepoint	Storage	Analysis
NRP Perfusate	Blood Serum	Pre-NRP, Every 30 min, during NRP	5x2ml aliquots stored at -80°C	Metabolomics, Biochemistry
Liver Samples	Liver Biopsy	After 120min of NRP + liver specific blood sample After cold storage After reperfusion in the recipient	Surgical biopsy divided into sample for formalin and snap frozen section at -80°C	Metabolomics, Histology
Bile Samples	Bile	End of NRP After reperfusion in the recipient	2ml aliquots Snap Frozen	Biochemistry
Recipient samples	Biochemistry Haematology Haemostasis	Standard of care at the transplant center	Standard of care at the transplant center	Standard of care at the transplant center

2.2. Objectives and outcome measures

2.2.1. Primary

Primary objective: Identify and quantify I/R injury biomarkers markers for liver graft viability released during NRP and analyze their predictive value for major liver graft-related adverse events after transplantation.

Primary outcome measure: Metabolomics including FMN, hypoxanthine, succinate, xanthine and NADH, performed by nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography-mass spectrometry (LC-MS) in the NRP perfusate and liver graft tissue to be correlated with a composite endpoint of major liver graft-related adverse events including early allograft dysfunction as defined by Olthoff et al¹⁹ including primary non function, non-anastomotic strictures and early arterial complications within the first year after transplantation.

2.2.2. Secondary

Secondary objective (1): Establish a risk prediction model to enable prediction of post-transplantation liver graft viability during NRP.

Secondary outcome measure (1): Combine detection of metabolomics data with donor data from the CRISTAL registry to establish a robust bio-clinical prediction model of liver graft viability prior to transplantation.

Secondary objective (2): Analysis of downstream graft inflammasome and metabolic changes occurring within the liver graft during NRP, after cold storage and implantation.

Secondary outcomes measures (2): metabolomics of liver tissue and NRP perfusate with focus on FMN and relevant purine metabolites (hypoxanthine, succinate, xanthine, NADH), Immunohistochemical analysis of liver tissue samples.

Secondary objective (3): Comparison of the predictive accuracy of the biomarker-based risk prediction model with available survival and early allograft dysfunction scores based on post-transplant indicators.

Secondary outcome measures (3): donor risk index score (DRI), L-Graft score, MEAF, tumour-censored graft and patient survival.^{20,21,22}

Secondary objective (4): Correlation of liver graft inflammation status and recipient morbidity after transplantation

Secondary outcome measures (4): Complications graded according to the Clavien-Dindo classification, requirement of renal replacement therapy; incidence of biliary complications, length of intensive therapy unit stay, length of hospital stay, recipient and graft survival.

2.2.3. Analytical methods

2.2.3.1. Donor and recipient data

Donor and procurement data will be collected from the CRISTAL database established by the Agence de la Biomedicine.

Recipient's blood samples will be analysed following the routine post-liver transplant guidelines of the respective center. No additional analysis will be required.

2.2.3.2. Metabolomics with Nuclear Magnetic Resonance spectroscopy (NMR) and liquid chromatography-mass spectrometry (LC-MS)

All metabolomic analysis will be performed by the "Institut de Sciences Analytiques" (ISA), UMR 5280, Lyon, France as part of a collaborative work. The ISA has a dedicated facility and team for Biological Analysis by Nuclear Magnetic Resonance spectroscopy (NMR) and Mass Spectrometry specialized in detection of biomarkers for disease in patients such as Alzheimer disease and more recently SARS-CoV-2. Consequently, the ISA disposes adequate facility to store, process and analyse human tissue and blood samples. The use of advanced Mass Spectrometry technology will allow to gain precise insights into the mechanisms of I/R injury. Our department has already an ongoing collaboration in the context of a research project on a cDCD model in pigs.

2.2.3.3. Detailed analysis plan:

Donor perfusate will undergo immediate centrifugation on site and plasma supernatant will be stored at -80°C in a transportable liquid nitrogen tank. Liver biopsy will be immediately snap-frozen.

First, an untargeted metabolomic profiling of the liver will be performed, mainly by NMR on both perfusate and snap frozen liver tissues. This high-sensitive method will allow a wide exploration of numerous metabolic pathways in the donor during NRP.

Second, targeted metabolomics of purine metabolites, known as surrogate markers of I/R injury in liver grafts, including nicotinate adenine dinucleotide (NAD), succinic acid, inosine monophosphate (IMP), adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), hypoxanthine, xanthine, fumaric acid, lactate, uric acid, α -hydroxybutyrate and flavin mononucleotide (FMN) will be performed by targeted LC-MS/MS on both perfusate and snap frozen liver tissues.

The obtained metabolomic fingerprint of cDCD liver grafts will be combined with donor and procurement data from the CRISTAL registry and correlated with post-transplant outcomes

2.2.3.4. Fluorescent spectrometry for detection of FMN and NADH

NRP perfusate samples will be immediately centrifuged and the supernatants are stored at -80°C in liquid nitrogen. After thawing, 200 µL of each sample in triplicates will be analyzed by the fluorescence. Spectrometry (Infinite 200 PRO, Switzerland) using the same settings detailed previously by our groups and others.^{13,14} For FMN measurement, a monochrome light with excitation wavelength of 450 nm and emission wavelength of 525 nm will be used. For NADH, an excitation wavelength of 360nm and an emission wavelength of 525nm will be used. The fluorescence readings in artificial unit (AU) will be correlated with the clinical outcome data. Fluoremetric analysis will be performed in "Institut de Sciences Analytiques" (ISA), UMR 5280, Lyon, France as part of a collaborative work.

2.2.3.5. Histology and Immunohistochemistry

Liver biopsy will be stored in formalin on site.

The following staining procedures targeting the hepatic inflammasome will be performed on formalin embedded liver tissue:

Haematoxylin-Eosin (H&E), Toll-Like-Receptor-4 staining, CD 68 staining, ICAM (Santa Cruz) and 8-OHdG. An independent liver histopathologist will perform all the histopathological assessments. Both will be blinded to the graft type and the primary and secondary outcome measures. Histologic and Immunohistochemistry stainings will be performed at the by the Department of Histology and Pathology, Hospices Civils de Lyon, Lyon, France.

2.3. Statistical justification and outcome analysis

2.3.1. Sample size justification

In a recent study published by our group analyzing outcomes after transplantation of 132 cDCD liver in France, the composite event of Early allograft dysfunction (EAD), non-anastomotic stenosis (NAS) and hepatic artery complications (HAC) was observed in 30% of the cases.²

The validation of the biomarker in a study of ex-vivo perfused liver by hypothermic oxygenated perfusion included 54 cDCD livers and was able to perform a reliable sensitivity and specificity analysis.¹⁶ In order to obtain a sufficient number of the composite event EAD/NAS/HAC to perform a robust sensitivity and sensibility analysis of the biomarker, we will include four liver transplant centers with high volume of cDCD liver transplantations (La Pitié, Strasbourg, Rennes and Lyon) to include 60 cDCD liver transplants during the 2 year study period.

2.3.2. Analysis of outcome measures

2.3.2.1. Primary analysis

To perform a sensitivity and sensibility analysis of a biomarker, a composite post-transplant endpoint will be used. The composite endpoint will include three major post-transplant complications specifically linked to the ischemic damage in cDCD liver transplantation: (a) Early allograft dysfunction and primary non-function, (b) non-anastomotic biliary strictures and (c) early arterial complications.

- (a) Early allograft dysfunction (EAD) describes initial poor liver graft function after transplantation and represents the clinical phenotype of severe ischemia-reperfusion injury due to a variety of recipient, donor, and perioperative factors. We will use the most widely validated definition by Olthoff et al¹⁹ : the presence of 1 or more of 3 variables, including (1) a peak serum aspartate aminotransferase (AST)

or alanine aminotransferase (ALT) level higher than 2000 U/L within the first 7 postoperative days (PODs), (2) a serum bilirubin level of 10 mg/dL or higher on POD 7, and (3) an international normalized ratio (INR) of 1.6 or higher on POD 7. Primary non-function (PNF) is defined as graft with poor initial post-transplant function requiring re-transplantation or leading to death within 7 days after transplantation without any identifiable cause of graft failure.

- (b) Non-anastomotic biliary strictures (NAS) will be defined as either multifocal, unifocal intrahepatic, or hilar strictures without the presence of concomitant hepatic artery thrombosis (HAT) or arterial complications and with clinical symptoms. NAS will be confirmed by magnetic resonance cholangiography.
- (c) Early arterial complications will be defined as arterial thrombosis, false aneurysm or stenosis occurring within the first 30 days after transplantation.

Sensitivity and specificity of the predictive value for the composite endpoint of biomarkers will be tested using receiver-operating characteristic curve (ROC) analysis. Cut-offs for highest specificity and sensitivity will be determined by the Youden index. Tests will be considered statistically significant at a 2-sided P value of <0.05.

2.3.2.2. Metabolomic analysis

Principal Component Analysis (PCA) followed by orthogonal projection to latent structures (O-PLS) method will be performed to evaluate whether metabolic profile differentiated groups of patients. The models quality and the models predictive ability will be evaluated.

2.3.2.3. Secondary analysis

The secondary analysis will be mainly descriptive. Categorical variables will be expressed in quantities and percentages and continuous variables are expressed as median with interquartile range. Continuous variables will be compared using the Mann-Whitney U test. Categorical variables will be compared using the Chi-square test or the Fisher exact test. P-values < 0.05 will be considered statistically significant. Survival rates will be estimated by Kaplan-Meier methods, with comparisons between groups performed using log-rank tests. Correlations between fluorimetric perfusate analysis and liver graft function will be calculated using Pearson correlation coefficient (R).

Comparison of the predictive value of the biomarkers and available risk scores will be tested using ROC curve analysis.

2.4. Ethical and regulatory approval

2.4.1. Competent authorities

The present study protocol will be submitted to the competent ethics committee. The notification of the favourable opinion from the competent ethics committee will be obtained prior to the initiation of the project.

If necessary, a specific ethic approval will be submitted including a consent form for the family of the donor in regard to the use of donor tissue for scientific purpose.

The principal investigator ensures that the start of the study only occurs after the favourable opinion of the respective ethical committees.

2.4.2. Substantial modifications

In the event that a substantial modification is made to the protocol by the investigator, the investigator must obtain a favourable opinion from the competent ethical committee.

2.4.3. Declaration of conformity

The investigator will ensure that the study is conducted:

- in conformity with the protocol,
- in conformity with both the French and international good clinical practices currently in force,
- in conformity with the current French and international legal and regulatory provisions

3. Conduction of the trial

3.1. Study group

A total of four French liver transplant centers with significant experience with cDCD liver transplantation and organ donation research will participate in the study (La Pitié Salpêtrière, Lyon, Rennes and Strasbourg, Rennes and Lyon). All cDCD liver grafts allocated to one of these centers and successfully undergoing NRP will be included in the study.

The included study centers take all part in the ongoing randomized trial HOPEXt and have thus a large experience with prospective data and sample collection in the setting of a study involving organ donation and liver transplantation. In addition, there exists an ongoing scientific collaboration between these centers in the context of the HOPEXt study, both at the level of the investigators but also at the level of the respective clinical and biological research centers.

Graft Inclusion criteria

- All cDCD liver grafts allocated to one of the study centers undergoing NRP and where procurement of any abdominal organ is initiated
- Procurement performed by one of the four transplant teams
- Consent from the donor family in CRISTAL

Graft Exclusion criteria

- cDCD procurement which are aborted due to prolonger total warm ischemia time
- cDCD procurement where NRP fails or is not initiated due to technical reasons
- Graft reallocation after procurement to a transplant team not included in the study
- No consent by the donor family in CRISTAL

Of note, there will be no donor laparotomy performed if the liver has been rejected by every French transplant center and no other abdominal organ is procured.

Recipient inclusion/exclusion criteria

No specific recipient selection criteria are required for the study. Recipient selection will be performed by the transplant center as for standard of care.

3.2. Study visits and follow-up

The cDCD donation process and liver transplantation course will not be altered in the study protocol and the process will follow standard procedures and recommendations from the ABM and the respective transplant centers.

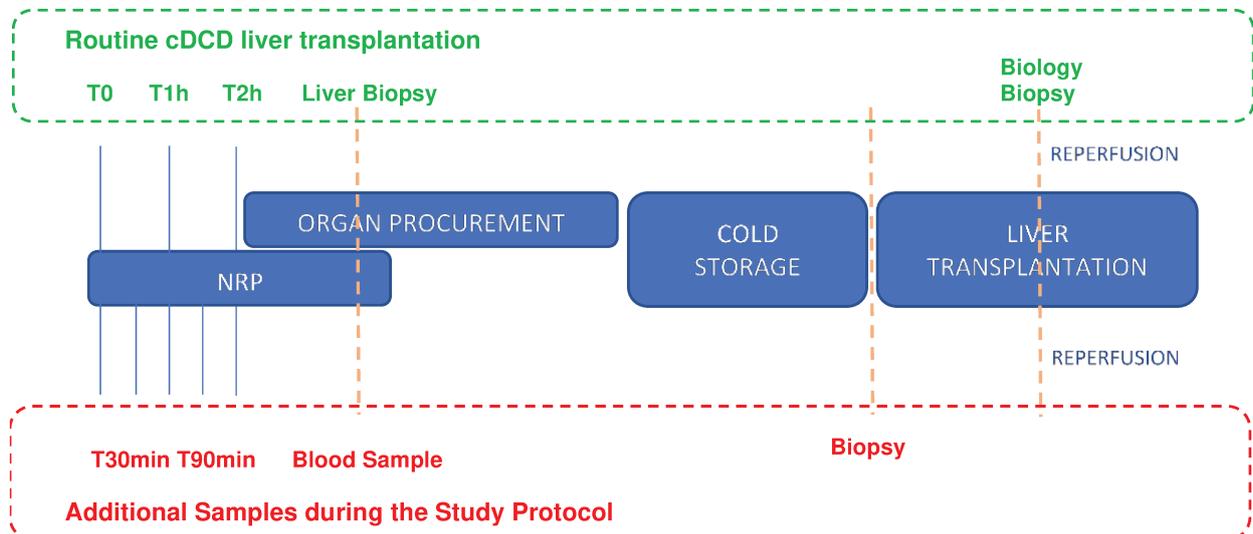
3.2.1. Procurement and Liver transplantation

The NRP perfusate will undergo point-of-care biochemical testing and samples will be centrifuged and plasma stored at -80°C. Donor biopsies will be performed by the procurement team and snap frozen on site. All donor samples will be transported back to the transplant center by the procurement team. A centrifuge and a specific transportable liquid nitrogen tank

will be provided to the procurement team as well as a short instruction on how to perform optimal sampling.

As shown in the study flowchart below, only few additional analyses will be performed (in red).

Figure 1. Study flowchart



3.2.2. Recipient Follow-up

The recipient follow-up after liver transplantation will be performed at the transplant center as standard of care. No additional recipient blood or tissue samples are required. In accordance with recent findings, follow-up will cover 12 months after transplantation to identify all relevant complications and mortality notably biliary complications.¹⁸

Study visits for the recipient overlap with the standard of care for transplanted patients:

- Visit 0 : Day of transplant
- Visit 1-7 : Post LT day 1-7
- Visit 8 : Post LT day 30 (+/-5)
- Visit 9: Post LT day 90 (+/-10)
- Visit 10: Post Lt day 120 (+/-10)
- Visit 11 : 12 months post-LT (+/-30)

On each visit the routine blood tests performed including FBC, urea, electrolytes, liver function tests, AST, GGT, eGFR, INR, FV will be included as outcome measure. Additionally, the routinely performed MRCP in the context of cDCD donation in France will also be included as outcome measure.

3.3. Adverse events reporting and analysis

Since there is no therapeutic intervention performed during the study, no adverse events related to the study are expected.

3.4. Storage of samples

Portable centrifuge devices will be provided to each of the procurement teams to proceed with centrifugation of the blood samples during procurement.

To allow storage of tissue and plasma samples in liquid nitrogen, the respective procurement teams will be equipped with a specially transport secured and transportable liquid nitrogen tank (LN2 Container made of Aluminium Type Voyageur 2). A similar tank is currently used for sample storage and transport in the HOPExt randomized trial.

NRP perfusate collected during procurement will undergo centrifugation on site with a portable centrifuge (IKA mini G) stored frozen in 0.5–1.0 mL aliquots at – 80°C and stored in the respective biological resource centers.

Liver tissue specimens will be collected in liquid nitrogen and stored at – 80°C.

The formalin fixed paraffin embedded segments will be stored, processed and analysed by the Institute of Pathology, Hospices Civils de Lyon, Lyon, France.

All donor samples will be initially stored in the respective biological resource centers.

All samples will be collected in accordance with national regulations and requirements including standard operating procedures for logistics and infrastructure. All tissue and blood samples will be destroyed at the end of the analysis.

Recipient blood samples taken as part of their standard of care will be processed and stored according to transplant center procedures. Results will be prospectively included in a secured online database by the center specific study coordinator.

3.5. Data handling, quality assurance, record keeping and retention

In each of the four study centers, a study coordinator will be designated who will supervise the logistics and data managing.

In accordance with provisions concerning the confidentiality of data to which persons responsible for the quality control of a study involving human individuals have access (article L.1121-3 of the public health code), and in accordance with the provisions regarding the confidentiality of information relating, in particular, to the trial, the persons who participate, and the results obtained (article R.5121-13 of the public health code), the persons having direct access to the data will take all necessary precautions to ensure the confidentiality of the information related to the trials, to the persons participating and, in particular, with regards to their identity as well as the results obtained.

These persons, as with the investigators themselves, are subject to professional confidentiality (in accordance with the conditions defined by articles 226-13 and 226-14 of the penal code).

During the research involving human individuals or at its end, the data collected on the persons participating and sent to the sponsor by the investigators (or any other specialists) will be made anonymous.

Under no circumstances should the names or the addresses of persons concerned appear. Only the first letter of the subjects' surname and the first letter of their first name shall be recorded, accompanied by a coded number specific to the study indicating the inclusion order of the subject.

Patient code: N°: Center number – inclusion order - 2 letters (surname and first name)

3.5.1. Case report form

The case report form will only include the data necessary for an analysis for a scientific publication. Other patient data necessary for their follow-up outside of this study will be collated in their medical file.

All information required by the protocol should be recorded in the case report form. Data must be collected as it is obtained and explicitly recorded in these case report forms. All missing data must be encoded.

This electronic case report form will be put in place in each center through an internet portal for recording the data. A help document for using this tool will be provided to the investigators. The completion of the case report form by the investigator through the internet allows the study coordination center to rapidly see the data at a distance. The investigator is responsible for the accuracy, quality, and pertinence of all the data entered.

3.5.2. Data management

Donor data will be retrieved from the CRISTAL database from the ABM. The recipient code (see the paragraph "data confidentiality") will be the only information which will link the data to the patient.

3.5.3. Statistical analysis

Statistical analyses will be conducted under the supervision of the biostatistician of the Clinical Research Center of the Croix-Rousse University Hospital. All analyses will be performed with IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp. Armonk, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

3.5.4. Rules relating to scientific publication

Scientific communications and reports related to this study will be carried out under the responsibility of the study's principal investigator with the agreement of the associated investigators. The co-authors of the report and the publications will be the investigators and doctors involved, in proportion to their contribution to the study, as well as the biostatistician and the associated researchers.

The publication rules will follow international recommendations (N Engl J Med, 1997; 336:309-315).

The study will be registered on the freely accessible clinical trials register (clinicaltrials.gov) before the inclusion of the 1st patient.

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