DR. PAULO N MARTINS (Orcid ID : 0000-0001-9333-0233)

DR. DAVIDE GHINOLFI (Orcid ID : 0000-0001-7933-8941)

Article type : Hepatology Elsewhere

Corresponding author mail id: paulo.martins@umassmemorial.org

A Call for Randomization in Clinical Trials of Liver Machine Perfusion Preservation

Paulo N. Martins[1], Pierre-Allain Clavien[2], Rajiv Jalan[3], Davide Ghinolfi[4]

1. Division of Organ Transplantation, Department of Surgery, University of Massachusetts Memorial Hospital, University of Massachusetts, Worcester-MA, USA
2. Swiss HPB and Transplantation Center, Department of Surgery and Transplantation, University Hospital Zurich, Zurich, Switzerland.
3. Liver Failure Group, UCL Institute for Liver and Digestive Health, UCL Medical School, Royal Free Campus, London, UK.
4. Division of Hepatobiliary Surgery and Liver Transplantation. University of Pisa Medical School Hospital, Pisa, Tuscany, Italy.

Key words: clinical trials, liver machine preservation, normothermic machine perfusion, ex-situ preservation, liver transplantation, randomized controlled trials, study design, bias, study endpoints, biomarkers, limitations, outcomes

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/HEP.31686

Liver transplantation is a lifesaving operation for patients with advanced liver failure. Although mortality from liver disease continues to increase, the number of available grafts remain relatively unchanged, leading to the death of about 15-30% patients on the waiting list. Therefore, transplant teams have resorted to using marginal organs with often poorer outcomes. In order to improve the quality of 'marginal' organs and reduce the severity of liver injury following transplantation, extracorporeal techniques such as ex-situ normothermic machine perfusion (NMP) have been explored, but data whether these approaches improve outcome are still pending[1].

The authors of the VITTAL study address these questions suggesting that evaluating marginal livers using NMP could avoid discarding many organs on subjective assessment only[2]. This study had two primary objectives: 1) To establish the feasibility of NMP to assess graft function and increase the number of transplantable livers, 2) To achieve successful transplantation of these previously rejected livers evaluating 90-day patient survival. In their work, Mergental et al. assessed 31 livers discarded by all UK centers (n=7) using NMP and were able to transplant 22 (71%) of them with a 100% 1-year patient and 86.4% 1-year graft survival. In our opinion, the main message of the study is that NMP can support the decision-making process providing the surgeons with additional information on liver function. This study did not compare NMP preservation with standard cold preservation but its design and choice of the end-points would not be able to provide convincing evidence of NMP superiority to the standard static cold storage (SCS) in terms of preservation quality. The capacity of NMP to maintain grafts in a near-physiological state allows the evaluation of both liver injuries and synthetic capacities. However, an ideal biomarker, specifically able to predict clinically relevant outcomes, has not been identified yet[1]. Mergental's work represents a milestone in the pursuit of establishing reliable graft viability criteria and the adaptive trial design in this study lays the groundwork for a future randomized trial where NMP and SCS in a high-risk donor cohort are compared head-to-head. Unfortunately, the parameters chosen to assess graft quality are all focused on hepatocellular function/damage alone (no qualitative bile parameter was considered such as pH, LDH, glucose or bicarbonate) and, even if their adoption may reduce the risk of primary-non-function or severe graft dysfunction, they are unable to predict or prevent ischemic cholangiopathy. As a matter

of fact, six patients (27%) developed biliary complications at 6 months, four (18%) needed re-transplantation after a median follow up of 542 days, and four (18%) more patients had radiological findings compatible with ischemic-type biliary lesions. This highlights that hepatocellular viability is likely physiologically separate from biliary/cholangiocyte viability and should be included in future trials to check viability[3].

While such studies are important and welcome, it is necessary to point out the limitations: a) Lack of randomization, b) Lack of objective, universally reproducible inclusion and exclusion criteria (e.g. liver biopsy) and c) Inappropriate control group. We must disagree with the authors' statement: "…*the study design had to be non-randomized because to conduct a similar study using previously declined livers in a randomized way would be ethically unacceptable*". NMP can be considered standard of practice in LT, as its safety and feasibility have been already shown[4], however its clinical superiority to SCS is not available yet[5,6], and a large randomized trial did not show differences in relevant clinical outcomes like patient and graft survival, biliary complications, use of blood products, overall complication rate, hospital or ICU stay[3]. A randomized comparison to SCS would have been unethical only if NMP was undisputedly associated with significantly better transplant outcomes.

Achieving good results with perfused grafts that were previously declined by other centers does not necessarily mean that NMP was responsible for graft "rescue" or quality improvement. Several other single center experiences reported good or excellent outcomes when using discarded livers without using ex-situ machine perfusion (MP) (Table1). Indeed, the same center that performed the VITTAL trial recently reported good outcomes of 206 previously discarded grafts after SCS compared with a matched low-risk cohort of primarily accepted livers, showing that even without MP an appropriate recipient selection may grant comparable outcomes in terms of incidence of primary-non-function (2.4% vs 1.7%; p=0.5483), in-hospital mortality (6.3% vs 4.1%; p=0.2293), and 3-year graft (82.5% vs 84.1%; p=0.6872) and patient (85.4% vs 87.6%; p=0.8623) survival[7].

Randomization is crucial because data on organ utilization with MP are difficult to interpret since decision to use or not a graft is based on a subjective decision, as acknowledged by the authors. No definitive objective viability criteria are available and the decision of whether to transplant or discard a liver rather depends on the peculiar

practice of the respective transplant centers. Organ acceptance have been shown to be influenced by logistics, day of the week and time of the day, costs, center volume and performance, and regulatory boards. MacConmara et al., recently observed in a retrospective study of 228 livers preserved with MP that the number of discarded grafts in USA decreased from 13.3% to 3.5% when a MP strategy was used, with no difference in 1-year patient and graft survivals[8]. We have challenged this enthusiasm in view of the use of MP restricted to few high-volume centers, thus providing significant biases in their conclusion. For example, while moderate-severe macrosteatosis and advance donor age are absolute reasons to discard a graft in smaller centers, those grafts are routinely transplanted by several large and experienced programs[9].

In the VITTAL trial only 22 out of 126 discarded grafts (17.4%) which qualified for NMP, were eventually transplanted. This may suggest a strict selection of grafts with favorable characteristics for inclusion in the MP arm of the study. Most of the grafts considered for the VITTAL study were previously discarded based on a subjective evaluation of steatosis (64 grafts, 35%), but only 3 out of those eventually perfused had macrosteatosis>30%. Furthermore, no objective histological characteristic (e.g.: steatosis, necrosis, fibrosis) has been taken into consideration to define these organs as 'marginal'. A protocol biopsy of all discarded livers would have been extremely helpful in promoting a gradual expansion of acceptability criteria using objective reproducible parameters and a comparable control group.

Analyzing the 31 perfused organs in this study, there were two main reasons for considering them as high-risk grafts: 1) A Donor Risk Index (DRI) >2.0 (23/31, 74%) and 2) An expected prolonged cold ischemia time (CIT) (10/33, 32%). Prolonged CIT has been widely regarded as a risk for post-LT complications. It might be postulated that NMP has the potential to improve transplant logistics, delay LT and counterbalance longer CIT. However, to appraise this effect, timing of pre-SCS-NMP and duration of NMP should have been matched as well for a fair comparison. In general, matching patients on pre-specified objective and reproducible criteria is important to mitigate the impact of any bias.

The international Liver Transplantation Society (ILTS) through the Special Interest Group (SIG) "DCD, Preservation and Machine Perfusion" established a working group to

evaluate and discuss the relevant literature and establish consensus statements and suggestions on how design future clinical trials in liver perfusion during the "DCD, Liver Preservation, and Machine Perfusion" consensus conference held in Venice, Italy on January 31st, 2020. Recommendations privileged randomized trials using clinically relevant end-points such as 1-year graft/patient survival, ischemic cholangiopathy or other relevant complications (https://ilts.org/education/lectures/machine-perfusion-and-clinical-trials-session-special-considerations-and-pitfalls-in-clinical-trials-using-machine-perfusion/)[6].

Randomized studies to validate viability criteria are therefore, not only ethically acceptable, but necessary, as they can minimize bias commonly involved in liver graft selection and allocation. To design well-powered studies a large number of transplants and multi-center collaborations may be necessary. The main question which remains unsolved after this study is if and how MP will lead to superior outcomes of these higher risk "declined" grafts when compared to non-perfused organs to justify the increased costs and complexity in logistics and extra personnel requirement associated with MP. It is important to establish whether MP really improves graft quality and reliably predicts outcomes or if it is just another element subjectively used to increase surgeons' confidence to accept a higher risk liver graft. The burden of proof required to do "more" using a more complex approach should probably be higher than to do "less," particularly if doing "more" is likely to increase overall health care costs.

Table 1. All studies that have investigated the use of discarded livers by other centers (rescue allocation) without ex-vivo machine perfusion. The definition of rescue allocation and extended criteria grafts was not uniform. These livers have been declined by at least three other transplant centers. Despite the fact that machine perfusion was not used in any one of these studies with discarded livers, most of them showed similar graft and patient survivals to livers that were standardly allocated.
Abbreviations: DCD: donor after cardiac death, DBD: donor after brain death, PNF: primary non function.

REFERENCES:

1. Eshmuminov D, Becker D, Bautista Borrego L, et al. An integrated perfusion machine preserves injured human livers for 1 week. *Nat Biotechnol*. 2020;38(2):189-98.
2. Mergental H, Laing RW, Kirkham AJ, Perera MTPR, Boteon YL, ATTARD J, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. *Nat Commun*. 2020;11:2939.
3. Bruggenwirth I, de Meijer VE, Porte RJ, Martins PN. Viability criteria assessment during liver machine perfusion. Nature Biotech 2020. Oct 26. doi: 10.1038/s41587-020-0720-z
4. Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Ceresa CDL, Chiocchia V, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018;557:50-56.
5. Ghinolfi D, Lai Q, Dondossola D, DeCarlis R, Zanierato M, Patrono D, et al. Machine perfusions in liver transplantation: The evidence-based position paper of the Italian society of organ and tissue transplantation. *Liver Transpl*. 2020;10.1002/lt.25817.
6. Martins PN, Rizzari MD, Ghinolfi D, Jochmans I, Attia M, Jalan R, Friend P. Design, Analysis, and Pitfalls of Clinical Trials Using ex-situ Liver Machine Perfusion: The International Liver Transplantation Society (ILTS) Consensus Guidelines Transplantation. In press.
7. Marcon F, Schlegel A, Bartlett DC, Kalisvaart M, Bishop D, Mergental H, et al. Utilization of Declined Liver Grafts Yields Comparable Transplant Outcomes and Previous Decline Should Not Be a Deterrent to Graft Use. *Transplantation*. 2018;102:e211-e218.
8. MacConmara M, Hanish SI, Hwang CS, DeGregorio L, Desai DM, Faizpour CA, et al. Making Every Liver Count: Increased Transplant Yield of Donor Livers Through Normothermic Machine Perfusion. *AnnSurg*. 2020;10.1097.
9. McCormack L, Petrowsky H, Jochum W, Mullhaupt B, Weber M, Clavien PA. Use of severely steatotic grafts in liver transplantation: a matched case-control study. Ann Surg. 2007;246:940-6.

Author/Center	Publication	Number of patients Declined livers group vs standard allocation	Country/Rate of decline	Outcomes
Sotiropoulos (Essen University)	Transp Proc 2008,40:3196-7	45 (no control group)	Germany (single center) (Eurotransplant) Livers declined by at least 3 centers	-PNF 13.3%. -1-year graft survival 76%. -1-year patient survival 82%.
Schemmer (Heidelberg University)	Clin Transpl 2009;23:42-8	85 (vs 168 controls)	Germany (single center) (Eurotransplant) Livers declined by at least 3 centers	-PNF 3.5% vs 0% (p=n.s). -1-year graft survival 75% vs ~62% (p=0.14). -1-year patient survival 84% vs ~66% (p=0.14). -No differences in delayed graft function, PNF, and complications.
McComarck (German Hospital, Buenos Aires)	HPB 2010, 12:523-30	26 (vs 25 controls)	Argentina (single center) Livers declined by at least half (8) centers AND refused at least 30 times prior to our final acceptance.	-1 year graft survival 84% vs 80% (p=0.94). -1 year patient survival 84% vs 84% (0.74). -No differences in complications
Doenecke (Regensburg University)	Scand J Gastroenterol 2010; 45: 1516-7	38 (vs. 150 controls)	Germany (single center) (Eurotransplant) Livers declined by at least 3 centers	-No difference in 5-year graft (~70%) and patient (~80%) survivals. -No difference in complications.
Mossdorf (Aachen University)	Transpl Intern 2013; 26:886-92.	53 (vs 49 controls)	Germany (single center) (Eurotransplant) Livers declined by at least 3 centers	-1-year graft survival 80.7% vs 87.8%). -1-year patient survival (96.2% vs 87.9%). - Re-transplant rate 7.5% vs 2% (p=0.36).
Halazun (Columbia Presbyterian Hospital)	Annals of Surg 2017; 266:441-9	649 (vs. 1,401). Fair comparison is compromised because the analysis included other marginal grafts that were not declined by other groups. The comparison group also included living donor recipients.	USA (single center) Livers declined by all local centers (n=7), centers in other regions and offered nationally	-1 year graft survival 83% vs 87% (p=0.002) -1-year patient survival 86% vs 88% (p=0.007) These differences did not reach statistical significant in most recent era.
Giretti (University Hospital of Tours)	Transplantation 2018;102:775-82	33 (vs. 321 controls)	France Livers declined by at least 5 centers	-1-year graft survival 65% vs 83% (p=0.02). -1-year patient survival 81% vs 86% (p=0.85). - Survival benefit (81% vs. 44% waitlisted patients (p=0.004). -Increased hepatic artery thrombosis (15.2% vs 3.1%, p=0.001). -Retransplant rate (18.2% vs 4.7%, p=0.002).
Marcon (Birmingham University)	Transplantation 2018,102:e211-8	206 (vs 347) DBD and DCD	UK (single center) Livers declined by a median of 4 other UK centers	-3-year graft survival (82.5% vs 84.1%, p=n.s). -3-year patient survival (85.4% vs 87.6%, p=n.s). PNF (2.4% vs 1.7%, p= n.s).
Kitano (Paulo Brousse Hospital)	Clin Transp 2020; July 19:e14046	102 (vs 1,533 controls)	France (single center) Livers declined by at least 5 centers	-5-year graft survival 70.3% vs 67.5% (p=0.89). -5-year patient survival 74.6% vs 71.9% (p=0.9). -Delayed graft function 44.1% v. 32.4% (p=0.02). -No differences in complications
Azoulay (Paul Brousse Hospital)	World J Surg 2020; 44:912-24	64 (vs 185 controls)	France (single center) Livers declined by at least 5 centers	-5-year patient survival was 67% vs 67% (p=0.07). -5-year patient survival was 70% vs 76% (p=0.08). -No difference in delayed graft function, PNF, retransplantation rate, ICU stay, morbidity and mortality in 90 days.
Winter	J Hep Rep 2020; 2: 100118	336 (vs. 4,882 controls)	France (multicenter study) Livers declined by at least 5 centers	-1-year graft and patient survival not reported. -13% increased risk of death or graft loss (but in experienced centers there was no difference). -PNF 2.6% vs 2.2% (n.s.)