

Response to reviewers
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Dear editor and reviewers of World Journal of Transplantation,

Thank you for your feedback to our invited narrative review entitled 'Machine perfusion in abdominal organ transplantation: current use in the Netherlands'. Herewith, we would like to submit a revised version of our manuscript, which will be edited by dr. M.I. Bellini.

We address your feedback one by one in the addendum in a point-by-point response. In the manuscript, word change mode has been used to indicate new and deleted text.

On behalf of all authors, I am looking forward to hearing from you.

Yours sincerely,

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You supposed to give an overall perfect description of the safety and feasibility of the procedures in the world in general, and in specific in the Netherlands. The conclusions made for the abdominal regional perfusion of the transplant organs seem to be not based on meta-analyses and just based on the overall reports of the individual studies. For the ex-vivo practice, the meta analyses reviewed for the overall safety of the procedures are outdated; you better to make renewed meta-analyses yourself, including all the studies published up today.

Thank you for your suggestions. Indeed, those results are not based on a meta-analysis, as this is not available in the literature. We believe, from a methodological point, it is not possible to pool these studies because the differences between protocols and donor types used are too large. Also, indications for regional perfusion may vary largely. For example, results from regional perfusion in DCD Maastricht type II should not be pooled with studies investigating the use of NRP in Maastricht type III. The results in Table 2 and 3 show large differences for the outcomes between studies. This emphasizes the heterogeneity between studies.

For the second point regarding the meta-analysis: as this was an invited review, we were asked to write a narrative review concerning the use of machine perfusion methods with as focus the Netherlands. After we submitted this first version of the narrative review, a systematic review and meta-analysis was published describing outcomes of abdominal organ transplantation after machine perfusion (MI Bellini, Machine Perfusion for Abdominal Organ Preservation: A Systematic Review of Kidney and Liver Human Grafts. J Clin Med. 2019 Aug 15;8(8). pii: E1221. doi: 10.3390/jcm8081221). Therefore, we do not see an additional value of making our own meta-analysis.

And also it also should be emphasized in the text that patient and graft survival in the ex-vivo model offers superiority (versus abdominal regional perfusion which represented no end-point benefit).

We do believe we cannot make this comparison, as none of the studies actually made this comparison within 1 study. Of course, we can compare the outcomes, but because this has been shown in different studies with different populations, there is too much bias (confounding by indication and selection bias). For the other point, we did mention that kidney grafts transplanted after NRP had a lower incidence of DGF and PNF according to some of the studies (page 11). Graft survival was indeed not significantly improved. For liver grafts transplanted after NRP, there was a lower incidence of biliary complications, ischemic cholangiopathy, and a graft survival comparable to DBD grafts (page 12). However, superiority of one treatment over the other cannot be answered with the current data.

In the second part of your study, which is to report the situation in the Netherlands, you should be much more specific. The perfect approach is to find all the centers conducting researches on the issue, list them by registry number and center one by one, and give their methodology, the sample size, and results or preliminary data reports, if available.

Thank you for your suggestions. We added two tables to the review (Table 4 and 5), describing the studies that are currently carried out and their results, if they are already available. We hope this made it much more clear. As shown in the tables, many studies did not publish their results yet. Therefore, we cannot describe those results in our review.

Also how much do you think the new approach would enlarge the graft availability or person year/graft-year survival rates in the Netherlands. And it would be nice to extend your research to other important issues like for example cost-effectiveness, availability in different centers, expertises needed and so on.

This is indeed an interesting point. Only one study from the Netherlands has looked into this (van Leeuwen OB et al. Transplantation of High-risk Donor Livers After Ex Situ Resuscitation and Assessment Using Combined Hypo- and Normothermic Machine Perfusion: A Prospective Clinical Trial. Ann Surg. 2019 Nov;270(5):906-914. doi: 10.1097/SLA.0000000000003540.). They showed that the use of the DHOPE-COR-NMP protocol increased the amount of transplantable livers with 20%. HMP is not used for discarded organs, but may be cost-effective in terms of improving long-term graft survival. However, since we started this program in 2016, we do not have long-term follow-up results yet. This is also the case for the NRP project. For NMP: we have not been able yet to transplant kidneys that were initially discarded. So indeed, cost-effectiveness is useful, but in this stage it's hard to say whether machine perfusion is cost-effective. We need a longer follow-up to draw conclusions regarding cost-effectiveness.

Reviewer 2:

I think we will need more clarification with detailed machine perfusion procedure and regional perfusion regarding cannulas size, pump flow, use of anti coagulation, which additives to be given in the perfusates, how to assess viability of recovering organs, how to clamp thoracic aorta.

Thank you for your suggestions. We added a few paragraphs into the current manuscript to address this point:

Page 12/13: assessing viability of the liver during NRP, parameters during NRP and additives in NRP perfusate:

"Different protocols exist in the literature for pump parameters during NRP. In the Dutch NRP project, a pump flow of 2-3 liters per minute is pursued with a temperature starting at 33 degrees Celsius which is slowly increased to 37 degrees Celsius. For oxygen, a mix between air and oxygen is used with the aim to reach a PaO₂ of 110-150 mmHg. Loss of volume is supplemented by adding red blood cells concentrate, albumin and ringer's lactate. The circuit is primed with heparin to prevent the blood from clotting. Bicarbonate is added in case of acidosis to keep the pH within a physiologic range. For the liver, the following issues are considered to determine suitability for transplantation:

- *Aspartate aminotransferase (ALAT) less than 4 times the upper limit at the end of NRP.*
- *ALAT reaches its plateau phase between first and second hour*
- *Lactate below 5 at the end of NRP*
- *Glucose doubles at the end of NRP in comparison to the start of NRP.*
- *Glucose is above 10 at the end of NRP.*

After two years, results of this project will be analyzed to see whether this technique should be implemented nationwide in the Netherlands."

Page 14: perfusate used during NMP and HMP:

"As normothermia leads to metabolic activity, an oxygenated perfusate is essential. Therefore, a blood-based perfusate is often used, containing washed and leukocyte-depleted red blood cells. Another option is to use an acellular perfusion solution containing a hemoglobin-based oxygen carrier. No studies have investigated which of the

two is preferred. In practice, the blood-based perfusate is more popular, probably because this option is less expensive. For normothermic machine perfusion, additional substances are added to provide the best circumstances for active repair. The composition and number of additives in the perfusate differs. In general, antibiotics, vitamins, prostaglandins, heparin to prevent thrombosis and bicarbonate are added. Currently, there is no evidence favoring one perfusate solution over another. For HMP, KPS-1 is used as the standard solution for clinical machine perfusion, without additional substances."

Page 17: viability assessment during machine perfusion:

"Viability assessment

One of the benefits of machine perfusion is the possibility of viability assessment. However, rules concerning viability assessment are not set in stone. It still remains highly difficult, as often no highly predictive cut-offs of liver or kidney markers have been identified which could lead to either acceptance or rejection of the donor organ. Especially for HMP, viability assessment is largely unexplored.

Kidney

For NMP, Hosgood et al. developed a quality assessment score based on macroscopic perfusion, renal blood flow and urine output during NMP[8]. The total amount of urine produced during NMP has proven to be significantly less in kidneys deemed unsuitable for transplantation[8]. It is unknown whether parameters during perfusion, such as flow and intrarenal resistance, may predict post-transplant outcomes.

Liver

For HOPE, fluometric analysis of released mitochondrial flavoproteins showed to have a high predictive value of liver graft function after transplantation with an area under the curve (AUC) of 0.926 for 90-day graft loss[51]. During NMP, liver viability can be assessed using a combination of transaminase release, glucose metabolism, lactate clearance, and maintenance of acid-base balance[52]. Evaluation of bile pH may predict post-transplant biliary complications, such as ischemic cholangiopathy[52]. No correlation has been found for hepatic artery/portal vein resistance and hepatocellular damage[52]. Also, there was no difference in hepatic artery/portal vein resistance between non-transplanted livers and transplanted livers and transplanted and non-transplanted livers[46]. Liver enzymes, lactate and bile production has shown not to be sufficient for prediction of liver graft failure in the recipient[52]. The following criteria have been described as being associated with successful transplantation of a normothermically perfused liver[52]:

- Maximum bile pH > 7.5*
- Bile glucose concentration ≤ 3 mmol/L or ≥ 10 mmol less than perfusate glucose*
- Able to maintain perfusate pH > 7.2 without >30 mmol bicarbonate supplementation*
- Falling glucose beyond 2 hours or perfusate glucose under 10 mmol/L which, on challenge with 2.5 g glucose, does subsequently fall*
- Peak lactate fall ≥ 4.4 mmol/L/kg/h*
- ALAT <6000 iU/L at 2 hours."*

By how much did these procedures improve the wait-listed patients in Netherlands organ transplant program?

Interesting point, which was also mentioned by the first reviewer. Only one study from the Netherlands has looked into this (van Leeuwen OB et al. Transplantation of High-risk Donor Livers After Ex Situ Resuscitation and Assessment Using Combined Hypo- and

Normothermic Machine Perfusion: A Prospective Clinical Trial. Ann Surg. 2019 Nov;270(5):906-914. doi: 10.1097/SLA.0000000000003540.). They showed that the use of the DHOPE-COR-NMP protocol increased the amount of transplantable livers with 20%. HMP is not used for discarded organs and NMP is still in its infancy. So far, we have not been able yet to transplant kidneys that were discarded before by improving them with NMP.