Author’s response to reviews

Title: Uncontrolled donation after circulatory death: comparison of two kidney preservation protocols on graft outcomes.

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Author’s response to reviews:

Pr Alexandre Hertig
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Dear Professor Hertig,

Please find appended a revised version of the manuscript entitled “Uncontrolled donation after circulatory death: comparison of two kidney preservation protocols on graft outcomes” (one version with modifications made apparent and a second clean version) as well as a point-by-point reply to comments made. We hope that the manuscript is now acceptable for publication, but remain at your disposal to make further changes if required.

Thank you for your consideration

Yours sincerely,

Claire Delsuc, on behalf of the co-authors

REVIEWER REPORTS:

Sarah Osgood (Reviewer 1): This manuscript compares in-situ cold perfusion and normothermic recirculation perfusion in uncontrolled DCD kidneys in two centres. In the first era 2006 - 2010 in-situ cold perfusion (ISP) was used and from 2010 - 2013 (n = 32) normothermic recirculation (NR) (n = 32). After retrieval all kidneys were preserved by cold machine perfusion.
The primary end point was graft function at 1 year. mGFR and eGFR was statistically higher in the NR group at 1 and 2 years. Protocol biopsies 3 and 12 month were also compared. Fibrosis, borderline changes and the Banff 07 score were assessed. Borderline changes and fibrosis were less in the NR group but these did not reach statistical significance.

This is an important manuscript and the only study to compare in-situ cold perfusion with normothermic regional perfusion in uncontrolled DCD donors. The differing protocols, differences in cold ischaemic times and post-transplant care during the two eras is a significant limitation of the study. However, a randomised controlled trial comparing the techniques is unable to be performed due to ethical reasons in France and Spain. Therefore, this likely to be the only study of its kind.

The results of both techniques are excellent with an extremely low rates of primary non function. Although the delayed graft function rate is high, graft function at 1 and 2 years is good. Kidneys undergoing normothermic perfusion had improved graft function at 1 and 2 years compared to cold perfusion.

Reviewer 1, Comment 1

Based on the biopsy information the authors speculate that normothermic perfusion may reduce activation of the innate immune system. I would be cautious in making such a conclusion with limited number of biopsies and without baseline samples.

The discussion could be improved by simply discussing the results of normothermic perfusion compared to the previous in-situ cold perfusion technique rather than making direct comparisons and speculating about the mechanisms.

Baseline biopsies were not available. Therefore it is difficult to conclude any differences between the groups at 3 and 12 months. The change between 3 and 12 months would be more appropriate.

Authors: This comment highlights a limit of our study (the absence of baseline biopsies) and we thank Reviewer 1 for this point. The difference between the 2 groups concerning borderline change appears at M12 and not at M3, and we found a trend towards the same difference for interstitial fibrosis. Following this comment, we have removed from Table 3 the line “M3 and M12”. Concerning overall difference in borderline change between NR and ISP we also removed from the Discussion section (paragraph 5, line 312 of the original version; line 319 of the revised
version) the phrase concerning the activation of the innate system to avoid any form of speculation. In addition, we have revised the start of the fifth paragraph to highlight this limit, indicating that we did not analyse baseline biopsies, and that the difference found at M12 was not present at M3.

Reviewer 1, Comment 2

What do the authors mean in the discussion page 11 line 274 'duration of no flow or low flow'?

Authors: This part refers to the results in Table 1, no flow duration was similar in both groups as was low flow duration. No flow duration was 10 min in both groups and low flow duration was 118 min in the ISP group and 123 min in the NR group (p=0.38 and p=0.16). No flow duration is the length of time without cardiopulmonary resuscitation, and low flow the duration of cardiopulmonary resuscitation before preservation. We have made modifications to clarify this point in this part of the manuscript in the Discussion section (line 274 of the original version; line 278 of the revised version)

Reviewer 1, Comment 3

What temperature is ISP performed at?

Authors: As pointed out, preservation solution temperature is not detailed in the Methods section and therefore we have added this information to the Organ preservation protocol paragraph. ISP was performed using a refrigerated preservation solution at 4°C in the intraaortic catheter and hypothermia was achieved with peritoneal refrigeration using 4 L of isotonic saline at 4°C.

Sarah Drouin, M.D. (Reviewer 2): This article attempts to compare the outcomes of kidney graft realized from uncontrolled donner after circulatory death using 2 preservation methods: normothermic recirculation recently and cold in situ perfusion. The authors showed that renal graft function was better at one year in the normothermic perfusion group

This is a retrospective study comparing graft survival, graft filtration rate at short term and at one year.
The study is well done with clear objectives, results and conclusions. These results are interesting as very few studies are available on the topic.

Reviewer 2, Comment 1

Few data are however missing.

Regarding the donor, the cause of death should have been given. The distribution of Maastricht I and II category should have been given for each type of perfusion.

The global follow up should have mentioned in the text. Regarding the follow up and the fact that the inclusions end in 2013 it would have been interesting to have a longer follow up.

The mean day of restart of the diuresis should have been indicated as well as the time for diuresis > 1000ml.

The complications should have been detailed: thrombosis, ureteral leak,...

Authors: Concerning the cause of death, due to the retrospective design, the exact cause was unknown or recorded as sudden cardiac arrest. All patients were Maastricht I category, we have added this information to the Study population paragraph of the Results section.

The study was performed in 2015 and, in order to include the patients of 2013, we chose a follow-up of 2 years; and therefore all patients were followed 2 years. We have revised the Study design and Patients paragraph of the Methods section to clarify this (line 120 of the original version; line 120 of the revised version).

For early recipient graft outcome, we chose the duration of haemodialysis and time for diuresis > 1000ml, as reported in Table 2. Due to the retrospective design, the delay of diuresis restart was not always available and therefore not selected.

Concerning complications, 5 occurred in the ISP group: 1 arterial stenosis, 2 ureteral stenoses, 2 venous thromboses, and 6 in the NR group: 5 arterial stenoses and 1 haemorrhage. We have added these data to the Early graft outcome paragraph of the Results section.

Reviewer 2, Comment 2
Regarding the difference on cold ischemia time, it is indeed a confounder, even if the data were adjusted.

The authors should indicate how long the kidney remain on pulsatile perfusion machine and what were the resistance index immediately before the graft as it can also be a confounder.

Authors: The kidney remains on pulsatile perfusion machine throughout cold ischemia time; following this comment we have added this information to the paragraph Organ preservation protocol of the Methods section (line 157 of the original version; line 157 of the revised version). Concerning the resistance index, our team is currently conducting a retrospective study on these data, and therefore this was not reported in the present manuscript.

Reviewer 2, Comment 3

Recent references regarding normothermic perfusion should also be included as: Oniscu et al, Am J Transplant, 2014 or Demiselle and al, Transplant Int, 2016.

Authors: We thank Reviewer 2 for these suggestions. Indeed, Demiselle et al. add an argument in favour of NR concerning uncontrolled DCD. We have cited this reference in the Discussion section (reference 36). Oniscu et al. investigated the feasibility of NR in controlled DCD, and using NR they observed a lower rate of delayed graft function than that currently seen in the United Kingdom at the time of their study, and we have therefore added this reference to the Discussion section (reference 37).