Dear Dr. Thevenot,

We are pleased to inform you that, after preview by the Editorial Office and peer review as well as CrossCheck and Google plagiarism detection, we believe that the academic quality, language quality, and ethics of your Manuscript NO.: 53825 basically meet the publishing requirements of the World Journal of Transplantation. As such, we have made the preliminary decision that it is acceptable for publication after your appropriate revision. Upon our receipt of your revised manuscript, we will send it for re-review. We will then make a final decision on whether to accept the manuscript or not based on the reviewers' comments, the quality of the revised manuscript, and the relevant documents.

In order for you to publish a high-quality academic article in the World Journal of Transplantation, lead the development of the discipline, and attract more readers, the first author and corresponding author are requested to follow the steps outlined below to revise your manuscript to meet the requirements for final acceptance and publication. If you have any questions in revising the manuscript, please feel free to contact the Editorial Office by E-mail. Please note that you have only two chances for revising the manuscript.

Step 1: Verify the accuracy of general information for your manuscript

Name of journal: World Journal of Transplantation

Manuscript NO.: 53825

Column: Retrospective Cohort Study

Title: Links between donor macrosteatosis and circulating IL-33 and activated complement immediately after liver transplantation as an early indicator of liver dysfunction

Authors: Kelley Nunez, Mohammad Hamed, David Bruce, Paul Thevenot and Ari Cohen

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Reviewer code: 01221666, and 03021264

First decision: 2020-03-26

Reviewer #1: 03021264

The article quantified plasma IL-33 and complement proteins, C3a and C5a in recipients immediately following reperfusion, found these test variables elevated in patients receiving macrosteatotic grafts. The authors suggested that MaS donor may lead to more injury post-transplant and circulating IL-33 and activated complement immediately after liver transplantation as an early indicator of liver dysfunction. This paper provides a new index for the prognosis of macrosteatotic donor, which is helpful for clinical work.

limitations:

1. there are fewer cases in Mas Group. Will the statistical results be affected?

> **Author's response:** Reviewer #1 brings up an excellent point and an inherent limitation in the study of transplant donor macrosteatosis (MaS). The stigma of donor MaS ≥ 30% often results in the organ being declined. In a prospective study, balancing replicates among MaS tiers is extremely challenging. Propensity score-based approaches are also tricky, as several complicated variables regarding clinical utilization of extended criteria donors are at play. At our center, the ≥ 30% MaS risk is managed by selecting ideal waitlist candidates, specifically with low MELD priority and minimal surgical complexity. The ideal recipient hypothesis is practice by several centers utilizing MaS grants. The approach yields subgroup demographics which can be quite different, specifically regarding recipient age, MELD at transplant, and even cold ischemia time (accepting secondary organ offers from outside our allocation region) can be difficult to match. Where appropriate, we have utilized Welch's test for unequal variance and non-parametric Mann-Whitney test.
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> **Author's changes in manuscript:** In order to more clearly define these underlying experimental factors, we have provided clarification within the discussion. "To avoid stacking risk factors, moderate MaS grafts were implanted

in lower risk recipients, reflected by younger age with similar CIT and MELD scores at transplant." [page 13, lines 320-321]

2. The cold ischemia time of Mas Group were longer, although the author thought that it was not enough to cause adverse effects, but it can not completely exclude the effect on liver function recovery, just as many opinions shows that MAS will not cause adverse effects also;

Author's response: Several studies have determined CIT >9 hours are associated with early allograft dysfunction (EAD) and graft failure [PMID: 29476693, 26952540]. The association between CIT and increased liver dysfunction is well investigated, however, there are limited studies that investigate both graft steatosis and cold ischemia time (CIT) with outcome. Westerkamp et al. demonstrated safe utilization of moderate macrosteatotic livers when CIT were <8 hours [PMID: 25545740]. Within our study, the median differences in CIT between the absent/mild and moderate macrosteatotic graft was 42 minutes with the longest CITs of 7 hours represented in both groups. This time frame is well below the previously published CITs associated with EAD and graft failure.

Reviewer #1 states an important contradiction observed in the literature involving macrosteatotic grafts and outcome. Studies demonstrating safe utilization of macrosteatotic grafts have focused on selection of low-risk recipients.

Author's changes in manuscript: To provide additional clarification and further justify our conclusions, we added the following lines within the discussion: "While the median CIT between the two groups differed by 42 minutes, we do not believe this impacted the greater risk of EAD observed within moderate MaS group." [page 13, lines 322-323] and "Additionally, Westerkamp et al.

demonstrated no difference in post-transplant outcomes between moderate MaS and nonsteatotic grafts with median CIT below 8 hours" [page 13, line 328-329]

3. The article is to evaluate the effect of MaS on liver dysfunction, and whether the survival rate can also be introduced?

Author's response: We thank Reviewer #1 for pointing this out and agree this data should be included in the manuscript.

Author's changes in manuscript: We have added the following paragraph to manuscript: "Survival outcomes within the cohort were as follows: six patients (6%) experienced graft failure at a median time of 139 days post-transplant with four of these patients having graft-related death (4%). None of the patients were transplanted with moderate MaS grafts experienced graft failure or death, despite 60% meeting criteria for EAD. While recipients with EAD had significantly longer length of stay ($P<0.01$) with mean of 22 days for those with EAD compared to 10.5 days for those without EAD." [page 14, lines 336-341]

4. Should the increased incidence of early allograft dysfunction (18% vs 60%) data in the Results of Abstract be reversed?

Author's response: We thanks Reviewer #1 for being this to our attention and agree that this sentence was confusing in the abstract.

Author's changes in manuscript: To provide clarification, we have modified the sentence in the abstract. [page 3, line 72-73].

5. The authors hope to take the circulating IL-33 and activated complex as an early indicator of liver dysfunction. Can you compare the level of IL-33 and activated

complex between the cases with liver dysfunction and those without liver dysfunction and get more meaningful results?

> **Author's response:** A thoughtful suggestion was brought up by Reviewer #1. We found no significant associations between levels of IL-33 and activated complement with early allograft. Given this limitation, ideally a larger cohort would give us more information.
>
> **Author's changes in manuscript:** None.

Re-review of manuscript 53825 by Reviewer #1: 03021264

1. This paper provides a new index for the prognosis of macrosteatotic liver transplantation. I believe that the academic quality, language quality, and ethics of the manuscript meet the publishing requirements of the World Journal of Transplantation after the appropriate revision.

> **Author's response:** Thank you, we appreciate your time in reviewing this manuscript.
>
> **Author's changes in manuscript:** None.

Reviewer #2: 01221666

The authors try to understand the relationship between IL-33 and complement in recipients immediately following liver reperfusion as a marker of liver dysfunction.

Comments

1. Liver dysfunction in this manuscript seem to be elevation of AST/ALT. Does this "dysfunction" cause any real disease, for example, graft failure, primary nonfunction, or cholestatsis?

Author's response: We apologize for the confusion. The OLTHOFF EAD index was used to measure early allograft dysfunction. This index utilizes post-operative peak levels of AST or ALT within the first 7 days, along with bilirubin and INR on day 7 to determine EAD post-transplantation. This index was validated within a large multicenter patient population combined with bilirubin and INR to measure liver injury instead of relying solely on AST/ALT levels. We have incorporated survival data to provide clarification. We have also included additional data that demonstrates patients with EAD had significantly longer length of stay at our center.

Author's changes in manuscript: We have included the following paragraph: "Survival outcomes within the cohort were as follows: six patients (6%) experienced graft failure at a median time of 139 days post-transplant with four of these patients having graft-related death (4%). None of the patients were transplanted with moderate MaS grafts experienced graft failure or death, despite 60% meeting criteria for EAD. While recipients with EAD had significantly longer length of stay ($P<0.01$) with mean of 22 days for those with EAD compared to 10.5 days for those without EAD." [page 14, lines 336-341]

2. The markers, IL-33 and complement, derived from recipients immediately after liver reperfusion were linked to early liver "dysfunction". The time period between sampling and outcome is very short (a few days to 1 week). The clinical significance seems limited. Does this have any impact on longer outcome or short-term survival?

Author's response: We apologize to Reviewer #2 for not clearly stating outcome and short-term survival. While early allograft dysfunction is determined after the first week post-transplantation, our study provides insight into recipients at risk of developing EAD immediately following transplantation. The patients that

developed EAD also have significantly longer length of stay, doubling those that do not experience EAD. We have added this information to the manuscript.

Our manuscript also provides potential therapeutic options to treat macrosteatotic grafts prior to transplantation in an effort to decrease adverse effects post-transplantation. As an example, there is a clinical trial underway (NCT03468140) targeting complement C5 through inhibition in macrosteatotic grafts.

Author's changes in manuscript: To better state the clinical significance of this work, we have provided additional data in terms of short-term impacts of EAD on length of stay and the long-term significance of therapeutic targets for decreasing liver injury post-transplant. We have added the following sentences:

"Survival outcomes within the cohort were as follows: six patients (6%) experienced graft failure at a median time of 139 days post-transplant with four of these patients having graft-related death (4%). None of the patients were transplanted with moderate MaS grafts experienced graft failure or death, despite 60% meeting criteria for EAD. While recipients with EAD had significantly longer length of stay ($P<0.01$) with mean of 22 days for those with EAD compared to 10.5 days for those without EAD." [page 14, lines 336-341]

"Currently, a clinical trial (NCT03468140) is underway using a C5 inhibitor, Eculizumab, in macrosteatotic grafts to decrease post-transplant liver dysfunction. Our results provide further justifications into clinical trials targeting complement in macrosteatotic grafts." [page 15, lines 362-365]

3. Can you explain more clear about the univariate analysis result in your study?

Author's response: We thank Reviewer #2 for pointing this out and apologize for the omission.

Author's changes in manuscript: We have added the following sentence to the methods section: "Univariate analysis was performed to determine differences of donor and recipient demographics and variables between absent/mild and moderate macrosteatotic grafts." [page 8, lines 198-200]

Re-review of manuscript 53825 by Reviewer #2: 01221666

1. I think the authors may need to provide KM curves and do the statistical test to compare the survival rates and confirm the results.

Author's response: The primary outcome measured in this manuscript is length of stay. Length of stay was longer in those patients with early allograft dysfunction. As requested, we have provided a KM curve of EAD and 1-year survival. As stated in the manuscripted [see prior comments], four patients died within 1-year with one having EAD. We did not add the KM curve to the manuscript because we feel this graph did not provide meaningful information.

Author's changes in manuscript: None.