Peer Review File

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Reviewer A: Why did the authors not retrieve serum samples from the donor before procurement to get a baseline value of the endothelial glycocalyx? Donor procurement influences and impacts ischemia-reperfusion injury.

Reply: We would like to thank the reviewer for raising this point. While obtaining baseline serum samples from donors prior to organ procurement might indeed provide valuable insights into endothelial glycocalyx status and degradation and elucidate potential associations with organ viability and postoperative outcome, this specific aim was not within the scope of our current study, as we aimed for identification of a biomarker during machine perfusion. Most importantly, we aimed to evaluate a biomarker which predicts postoperative outcome independently of donor characteristics. In addition, we aimed to explore Syndecan-1 as a mean for identification of organ damage during ischemia and machine perfusion. The association of donor characteristics with Syndecan-1 was not evaluated by us thus far. We evaluated this in more detail as a part of Supplemental Table 2. Interestingly, Syndecan-1 at 60 minutes seems to be directly associated with length of cold ischemic time. Accordingly, we suspect that Syndecan-1 might reflect the accumulation of damage during the period of cold storage. This information was added to the Results (see page 8, line 14-15) and Discussion section (see page 10, line 13-14).

Please provide information on donor organ procurement (aortic and portal flush?) and donors' demographic and clinical characteristics.

Reply: Thank you for pointing this matter out. We now provided additional information on donor demographics and clinical characteristics in Table 1. Further, we included more information on our institutional standard operating procedure to the Methods section of the manuscript (see page 5 & 6). Briefly, liver procurement at our center is carried out after aortic cold perfusion with cold HTK solution, portal flush is not used.

Why did the authors perform transplantation with cava replacement without a bypass? This is rather unusual. Please comment on this.

Reply: We thank the reviewer for this question. At our institution, we routinely perform liver transplantation via cava replacement, veno venous bypass is not routinely used. A recent analysis of techniques for liver transplantation conducted at 52 centers observed no preference on either cava clamping or cava sparing methods (Czigany et al. , J Gastrointestinal Surg. 2019). In fact, most included centers seem to adapt an institutional standard with 42% of centers using vena cava replacement and only 14% of centers using routine bypass. This is in line with our center standard of performing caval replacement without bypass.

Information of EAD patients is crucial: were these patients retransplanted?
Reply: Thank you for raising this issue, we added the necessary information to the Results section

(see page 7, line 16-17). Of the 14 patients who developed EAD, two required retransplantation.

Reviewer B: Summary: The authors present an interesting manuscript on the role of Syndecan-1 as a marker for glycocalyx degradation and subsequent early allograft dysfunction. With an increasing need for viable liver grafts for liver transplantation methods to predict organ viability are essential to increase the number of available organs. Hypothermic oxygenated machine perfusion of livers prior to transplantation has shown to improve outcome after liver transplantation. In their manuscript the authors address the need for metric assessment of organ quality. The manuscript is well written and only suffers from minor issues. Particularly the figures have been prepared with attention to detail. We are grateful for the thoughtful review and gracious feedback offered by the reviewer.

Minor issues: • While the authors present frequencies of EAD in patient groups defined by their Syndecan-1 cutoff, including a table with sensitivity, specificity, as well as positive and negative predictive potential for EAD may offer the reader more comprehensive information. Can the authors include such a table? To not go over the allowed number of tables and figures table 3 could easily be moved to the supplement.

Reply: Thank you for your valuable input. We have now included a table with sensitivity, specificity, and predictive values for EAD as new Table 3, and moved the former Table 3 to the supplementary material, as suggested by the reviewer. Of note, we observed a specifically good negative predictive value for the explored Syndecan-1 cut-off at 60 minutes. We included this information in the Results (see Page 8, line 26-28) and Discussion section of the manuscript. • There are minor grammatical errors, e.g., share stress instead of shear stress. Can the authors perform a thorough grammatical check? Reply: We need to thank the reviewer for this suggestion. In order to address this mater a thorough grammatical check was carried out on the manuscript and a series of linguistic errors were adapted by a native speaker.

Reviewer C: Very well written article with proper structure and good demonstration of hypothesis. Is there a potential selection bias - median MELD is 18 (max. 22) - what about Sdc-1 performance in high-MELD patients?

Reply: We would like to thank the reviewer for the kind comments. The cohort was collected prospectively and consists of consecutive patients. Of note, 22.5% of patients in this cohort underwent liver transplantation for HCC with lower MELD score when compared to cirrhotic patients. Accordingly, the median MELD score in this cohort is fairly low. We now clarified this in the Results section of the manuscript (page 7 lines 4-6). Additionally we would like to argue that Sdc-1 aims to evaluate organ viability and that recipient MELD is a variable which cannot be influenced at time of viability assessment as the organ is allocated according to the waiting list. Regarding the performance of Sdc-1 in high-MELD patients, our study did not specifically stratify by MELD score due to the limited sample size within certain MELD categories. However, investigating the performance of Sdc-1 in high-MELD patients could be an important direction for future research to elucidate its prognostic value across a broader spectrum of liver transplant recipients.