Thank you for taking the time to review our article. Your feedback was invaluable in helping us to improve the manuscript and we are grateful for your thoughtful suggestions. We have addressed your comments as follows.

Reviewer #1:

Comment 1: The groupings should include 45-70.

Reply 1: Thank you for addressing this important aspect. The main strength and significance of this study lie in the multivariate analysis of all 656 transplanted patients in the defined study time. Several multivariate models were constructed to investigate whether the donor's age has an impact on the observed outcome. This is elaborated in the Materials and Methods part of the paper: *"Several multivariate Cox proportional hazards models were developed using the model selection procedure laid out by Hosmer et al., in which the donor age was treated as a continuous variable [20]. Multivariate models were developed on all 656 patients transplanted in the defined period of the study. In the first Cox model, the donor age variable was kept during all steps of the model development regardless of statistical significance. Four other models were developed, without special treatment of the donor age variable: (1) using all variables; (2) without scores (MELD, BAR, and ET-DRI); (3) using all variables but without retransplanted patients; (4) without scores and retransplanted patients. This was done to gain an objective insight into the impact of donor age on patient survival."*

The division of donors into groups (donors ≤ 45 years vs. donors ≥ 75 years) was carried out as a supplement to the multivariate analysis and is by no means the main strength of this study. This comparative, univariate analysis was performed as an adjunct to the primary data analysis to better illustrate individual variables and exemplify the similarities of outcomes when using liver grafts of vastly different age groups, which according to the authors, were chosen arbitrarily. This is explained in the text in the introduction and discussion sections with statements: *"Additionally, we stratified liver recipients into two groups based on donor age (donors ≤ 45 years vs. donors ≥ 75 years) and conducted a comparative analysis."* and *"Moreover, the age limits of the younger and older group in univariate analysis were decided upon arbitrarily; however, we deliberately chose the difference between the two to be large enough to give more relevance to our results in addition to multivariate analysis where donor age was considered as a continuous variable."*

As a response to the reviewer's comments, the authors have incorporated changes into the manuscript, particularly in the abstract, the Materials and Methods section, and the presentation of results. These modifications aim to enhance clarity, ensuring that readers can better grasp the analysis's key points and minimize any potential sources of confusion. The authors believe these changes will contribute to the overall improvement of the manuscript.

Comment 2. For Table 5, do parameters for ALT, GGT, etc. meet the normal distribution?

Reply 2: Thank you for your valuable feedback regarding the clarity of the tables. We acknowledge your concern about the ambiguity of the numbers in parentheses and appreciate the opportunity to enhance the readability and

comprehension of our data presentation. In response to your comment, we have undertaken a thorough revision of the tables. To improve clarity, we have grouped the variables based on their nature and the type of data they represent. In Table 5 (now Table 7 in the revised manuscript), and in other tables, the legend indicates that data are presented as the mean +/- standard deviation with ranges (min-max) for continuous variables with a normal distribution. When the continuous variable does not follow a normal distribution, the data are presented as the median with interquartile ranges. Categorical variables are expressed as numbers and percentages. Accordingly, parameters like ALT, GGT, sodium, bilirubin, and CRP exhibited a normal distribution.

Comment 3. For Table 4, donor pre-procurement cardiac arrest, recipient age, indication for LT – hepatitis C virus, and indication for LT – retransplantation contribute to the outcomes of LT, how does the donor age have the same impact on LT?

Reply 3: Thank you for this insightful comment. In the Results section of the paper, it is explicitly mentioned and discussed that donor age did not emerge as a statistically significant variable in the conducted multivariate analyses. Tables 3 and 4 display the variables and coefficients of the two Cox multivariate models, but only significant variables are included in these tables. Donor age has not shown to be significant in the analysis and is not present in the final models or in the tables which are discussed in the Results section of the manuscript and the legend of the tables. In addressing the reviewer's feedback, the authors have refined the presentation of results to provide enhanced clarity on these specific aspects.

Comment 4. Histological findings of different groups should be better for this report.

Reply 4: Thank you for bringing out this important element. This study emphasizes pre-transplantation variables. The evaluation is centered on the baseline biopsy conducted before transplantation, specifically considering steatosis as a pivotal histological factor that can directly influence the decision-making process regarding the utilization of the graft. Grafts with incidental and unexpected liver lesions discovered during the explantation procedure, including newly identified primary tumors or extensive necrosis and granulomas, were not further considered for transplantation and were rejected. These cases are not incorporated into the scope of this study. It is essential to clarify that the study's focus does not extend to cases where a detailed analysis of the recipient's diseased liver is conducted post-transplantation. Such an analysis falls outside the parameters defined by this study.

Comment 5. Tables should be rearranged in order logically.

Reply 5: Thank you for your valuable suggestion regarding the arrangement of tables in our manuscript. We carefully reviewed the positioning and found that each table aligns logically with the corresponding sections of the manuscript, effectively supporting the study's progression from methodology to results. We believe that the current

arrangement facilitates a coherent and seamless flow of information. However, we are open to specific suggestions if there are particular areas of concern that you would like us to address.

In addition to addressing the reviewers' comments, the authors have undertaken grammatical polishing of the text, striving to enhance the overall quality of the manuscript.

Reviewer #2: The authors have conducted a retrospective cohort study aiming at evaluating the influence of donor age on LT outcomes. Some issues need to be addressed

Comment 1: Please clarify what “adult liver transplants” mean in your study- please include the age (>18y? >16y?).

Reply 1: We agree that the term “adult liver transplantation“ can be clarified in more detail. The term “adult liver transplantation“ refers to recipients aged 18 years and older. In response to the suggestion, the authors have modified the opening sentence of the Materials and Methods section to better explain the term as follows: *“In the period from April 2013 to December 2018, 656 adult liver transplants (≥18y) were performed at the University Hospital Merkur, Zagreb.”*

Comment 2: I would like to have a better explanation according to the allocation policy. It was not clear. It is not based on the MELD system? Why patients with hepatitis C are preferably transplanted with liver from younger donors… to avoid recurrence??

Reply 2: We appreciate the inquiry from the reviewer. As stated in the Materials and Methods chapter, the Allocation Policy section outlines that liver transplants are allocated according to the MELD system. Our center's policy is, whenever feasible, to allocate liver grafts from younger donors to patients with hepatitis C. This approach is based on our experience and various references provided below, where older donor age has been described as a negative prognostic factor for recipients with hepatitis C. In our ongoing efforts to achieve optimal matching within the allocation system, we closely monitor trends in the literature and adhere to Eurotransplant guidelines. In response to the reviewer's comment, the authors have introduced a change in the mentioned section of the manuscript that they believe enhances clarity for the reader.

References:

- Pratschke S, Bender A, Boesch F, Andrassy J, van Rosmalen M, Samuel U, Rogiers X, Meiser B, Küchenhoff H, Driesslein D, Werner J, Guba M, Angele MK; Eurotransplant Liver and Intestine Advisory Committee (ELIAC). Association between donor age and risk of graft failure after liver transplantation: an analysis of the Eurotransplant database. Transpl Int. 2019 Mar;32(3):270-279. doi: 10.1111/tri.13357. Epub 2018 Oct 31. PMID: 30260509.

- Goldaracena N, Barbas AS, Galante A, Sapisochin G, Al-Adra D, Selzner N, Galvin Z, Cattral MS, Greig PD, Lilly L, Bhat M, McGilvray ID, Ghanekar A, Levy G, Grant DR, Selzner M. Live donor liver transplantation with older donors: Increased long-term graft loss due to HCV recurrence. Clin Transplant. 2018 Aug;32(8):e13304. doi: 10.1111/ctr.13304. Epub 2018 Jul 16. PMID: 29947154.

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- Kamo N, Kaido T, Hammad A, Ogawa K, Fujimoto Y, Uemura T, Mori A, Hatano E, Okajima H, Uemoto S. Impact of elderly donors for liver transplantation: A single-center experience. Liver Transpl. 2015 May;21(5):591-8. doi: 10.1002/lt.24086. Epub 2015 Apr 15. PMID: 25641778.

Comment 3: Tables 1 and 2, as well as many other tables do not indicate what the numbers in parenthesis mean.

Reply 3: Thank you for your valuable feedback regarding the clarity of the tables. We acknowledge your concern about the ambiguity of the numbers in parentheses and appreciate the opportunity to enhance the readability and comprehension of our data presentation. In response to your comment, we have undertaken a thorough revision of the tables. To improve clarity, we have grouped the variables based on their nature and the type of data they represent. Specifically, we have segregated the variables into distinct categories:

1. **Percentage Variables**: We have grouped variables that are presented as percentages into a dedicated section. This arrangement will allow readers to quickly identify and understand these variables without confusion.
2. **Normally Distributed Variables**: Variables that follow a normal distribution are now presented together. For these, we have used the format of mean ± standard deviation (min-max), which is a standard approach for normally distributed data.
3. **Non-Normally Distributed Variables**: Similarly, variables that do not follow a normal distribution are grouped and presented using the median and interquartile range (IQR). This method is more appropriate for skewed data and provides a clearer picture of the distribution.

Furthermore, to address the specific issue of numbers in parentheses a clear and concise legend can be found at the bottom of the table. This legend explains the representation of data within the table, such as the use of parentheses for standard deviations and IQRs. This addition will guide readers through the table, ensuring a better understanding of the statistical measures employed. We believe that these modifications significantly enhance the table's

readability and effectively convey the statistical information in a comprehensible manner. We hope that these changes adequately address your concerns and contribute positively to the manuscript's overall quality.

Comment 4: Most of the figures are cut.

Reply 4: We appreciate your attention to detail and understand your concern about the figures appearing "cut" in the document. We would like to assure you that all figures were carefully checked and correctly inserted before submission. However, it seems that this issue might be due to variations in word processor versions or settings, which can sometimes lead to discrepancies in how documents are displayed on different systems. Furthermore, if the problem persists, we are more than willing to provide the figures in an alternative format, such as PDF or JPEG. These formats are generally more stable across different platforms and could be less susceptible to display variations. Please let us know if these steps help or if there's anything else we can do to assist in your review of our manuscript.

Comment 5: Please note that when you define your outcomes, in practice, overall graft survival is the same outcome as graft survival.

Reply 5: We thank the reviewer for stating this point about graft survival. Overall graft survival is essentially equivalent to graft survival, as highlighted by the reviewer. The authors' preference for this term is to offer a more detailed explanation of its significance, supported by relevant literature references, such as the studies conducted by Kantidakis G. et al. Survival prediction models since liver transplantation - comparisons between Cox models and machine learning techniques. BMC Med Res Methodol. 2020; 20: 277 and de Boer JD et al. Predictive Capacity of Risk Models in Liver Transplantation. Transplant Direct. 2019; 5: e457, which are referenced in the manuscript.

Comment 6: Please explain why the age cut points were selected as 45 and 75. On what basis? Why were the donors between 45 and 75 excluded from the analysis?

Reply 6: Thank you for addressing this important aspect. The main strength and significance of this study lie in the multivariate analysis of all 656 transplanted patients in the defined study time. Several multivariate models were constructed to investigate whether the donor's age has an impact on the observed outcome. This is elaborated in the Materials and Methods part of the paper: *"Several multivariate Cox proportional hazards models were developed using the model selection procedure laid out by Hosmer et al., in which the donor age was treated as a continuous variable [20]. Multivariate models were developed on all 656 patients transplanted in the defined period of the study. In the first Cox model, the donor age variable was kept during all steps of the model development regardless of statistical significance. Four other models were developed, without special treatment of the donor age variable: (1) using all variables; (2) without scores (MELD, BAR, and ET-DRI); (3) using all variables but without*

retransplanted patients; (4) without scores and retransplanted patients. This was done to gain an objective insight into the impact of donor age on patient survival."

The division of donors into groups (donors ≤ 45 years vs. donors ≥ 75 years) was carried out as a supplement to the multivariate analysis and is by no means the main strength of this study. This comparative, univariate analysis was performed as an adjunct to the primary data analysis to better illustrate individual variables and exemplify the similarities of outcomes when using liver grafts of vastly different age groups, which according to the authors, were chosen arbitrarily. This is explained in the text in the introduction and discussion sections with statements: *"Additionally, we stratified liver recipients into two groups based on donor age (donors ≤ 45 years vs. donors ≥ 75 years) and conducted a comparative analysis."* and *"Moreover, the age limits of the younger and older group in univariate analysis were decided upon arbitrarily; however, we deliberately chose the difference between the two to be large enough to give more relevance to our results in addition to multivariate analysis where donor age was considered as a continuous variable."*

As a response to the reviewer's comments, the authors have incorporated changes into the manuscript, particularly in the abstract, the Materials and Methods section, and the presentation of results. These modifications aim to enhance clarity, ensuring that readers can better grasp the analysis's key points and minimize any potential sources of confusion. The authors believe these changes will contribute to the overall improvement of the manuscript.

Comment 7: The outcome of the multivariate model was graft survival.

Reply 7: We confirm that the outcome of the multivariate analysis was overall graft survival as stated in the Material and Methods part of the manuscript: "*The primary outcome of this study, evaluated in multivariate analysis, is overall graft survival, the period between LT and graft failure or death, whichever occurs first."*

Comment 8: Please explain why the retransplants were excluded from group comparisons and then included in the multivariate model "Patients who underwent retransplantation were not compared between the two groups, but were included in the multivariate analysis" This doesn't make any sense to me.

Reply 8: As previously mentioned in reply 6, within the scope of this study, several multivariate models were developed to analyze data from 656 patients. In the multivariate analysis, two models were specifically created, excluding retransplanted patients due to the well-known negative impact of retransplantation on the observed outcome of the analysis. The rationale behind constructing multiple models is elucidated by aiming to obtain a more objective understanding of "donor age" as a variable in the observed dataset. Through multivariate analysis across various models, it has been demonstrated that donor age is not a significant variable in the observed dataset. However, in several models retransplantation emerged as a significant variable. This is another reason why the authors considered it reasonable to create models with excluded retransplanted patients, to observe whether "donor

age" as a variable behaves differently. In the comparative analysis of groups, a univariate analysis was conducted on two arbitrarily selected donor groups, with the aim of better illustrating individual variables in the dataset. The authors believe that by excluding retransplanted patients from this analysis, a more objective understanding of data influencing organ allocation can be obtained, considering the specific risks associated with retransplantations.

Comment 9: I couldn't find the donor age in any of the multivariate models. None of the tables 3 or 4. "When adding donor age to models (3) and (4) to observe behavior, the result was once more an insignificant, this time slightly positive coefficient for donor age, with negligible alterations to other coefficients." Where are these results?

Reply 9: Thank you for bringing out this important element. Tables 3 and 4 display the variables and coefficients of the two Cox multivariate models, but only significant variables are included in these tables. Donor age has not shown to be significant in the analysis and is therefore not present in the final models or in the tables which is discussed in the Results section of the manuscript and the legend of the tables. The authors have addressed the reviewer's comment by revising the manuscript, incorporating tables for models (3) and (4), and making additional adjustments in the Results section to enhance clarity for the readers.

Comment 10: How do you explain, in Table 1, that the median age of the donors was 60 if you only have selected donors under 45 or over 75? The median, in this case, does not reflect the actual sample.

Reply 10: Thank you for this comment. The median donor age in Table 1 reflects the sample of all 656 patients included in the multivariate analysis of the study. Comparative analysis of donor groups is presented in separate tables.

In addition to addressing the reviewers' comments, the authors have undertaken grammatical polishing of the text, striving to enhance the overall quality of the manuscript.