ANSWERING REVIEWR'S COMMENTS

Name of Journal: *World Journal of Transplantation*

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Manuscript Type: Observational Study

Title: Persistent Epstein-Barr Viral (EBV) Load in EBV Naïve Pediatric Heart Transplant Recipients: Risk of Late-Onset Post-transplant Lymphoproliferative Disease

Reviewer- 03309557

In this manuscript, the authors demonstrate an association of EBV loads an occurrence of late-onset PTLD. Although there are several weaknesses as the authors point out themselves (e.g. unknown EBV state of donor), the work provides valuable insight that may be of relevance for early diagnosis and treatment of PTLD. The research is methodological well performed, clearly written, and the data is honestly presented. Some points, however, should be addressed to improve the manuscript: Introduction: - EBV should be shortly introduced . This is missing completely. Methods: - 1st sentence: "All pediatric HT patients between 1995 and 2003…" does this relate to their birth year or transplantation date? - EBV measurement in blood: was there a DNAse treatment performed to distinguish between extracellular and intracellular EBV? Results/Discussion: - Although not significant, a smaller percentage of PTLD was developed by the group that was seronegative pre-HT. This is the opposite of what is expected since seropositive patients have (despite immunosuppression) some level of immunoprotection. Could the authors speculate about the reasons for this observation?

-Response: Changes in Introduction is done by introducing EBV as suggested. In methods, it is clarified that all patients transplanted (related to transplant dates) between 1995 and 2013. EMB measurement- whole blood PCR is done as per institutional protocol, no DNAse treatment done. A sentence is added to explain that late-onset PTLD is less likely to be associated with patients' EBV serostatus at the time of transplant. This is one of the unique finding of this paper, I agree with reviewer that it is in contrast to conventional wisdom and risk factors known. We believe the early onset PTLD is more likely in those who are seronegative before heart transplant

compared to late-onset PTLD. Late-onset PTLD are also more likely extra-nodal and heterogeneous.

Reviewer- 00503228

Methods: cyclosporine/tac levels, you didn't provided the data in the article and also what level you mean C0, C2 hours or what? - Because it is suggested that transplant patients who were EBV negative and subsequently develop EBV infection are at higher risk of PTLD, I recommend new categorization accordingly (in addition to the existing ones). - You may give pathological specificities of the PTLDs (poly, mono, …), CD20 positivity (5 received rituximab and 3 didn't, does it mean 5 were CD20 positive and 3 weren't or what?) and also staging and etc. - The results section is not well arranged. I recommend subsections to it. For example, before you talk about the number (percentage) of the PTLDs, you should not talk about PTLD associations. - I recommend time-dependent (survival) analyses for especially development of PTLD associations (including EBV load, EBV status change, and so forth). - Results "which means patients with younger age had high risk for high EBV during follow up. " The results section is not where you may talk about it - death "due to non-cardiac cause" Was the reason of death related to PTLD itself? Please specify what of them was the mortality? (The brain PTLD or who?) - P<0.05 is not accurate, you may give exact p values and preferably OR(95%CI) also - "a larger proportion of patients 72% (23/32) with persistently high EBV load had acute rejections versus 36% (41/113) patients with low or negative EBV load (p<0.05). " With this quotation, the past 2 sentences would become excessive and you may delete them - "Furthermore, there was an increase in frequency of total rejection episodes in patients with persistently high EBV load by 150% (48/32) vs. 72.5% (82/113) in patients with low or negative EBV load (p <0.05)." It is expectable due to reduction in IS. You may give this in the table instead, please only reserve major related findings in the Result's text .

-Response: According to our protocol for maintenance immunosuppression, we keep Tacrolimus trough level between 5 and10 and cyclosporine trough level between 150 and 250 in patients who did not have high EBV. For those who have high EBV, we keep their Tacrolimus trough level between 3-5 and Cyclosporine trough level between 50 and 75. This is added to manuscript. In Table-2: we have specified that those who have PTLD are polymorphic and those who are Hodgkin's or Large B-cell lymphoma are monomorphic. No changes are done. We added CD20 positivity to Table-2 as per reviewer's request. We have changed the result section as suggested by reviewer.

Reviewer- 01206087

Although this article is a retrospective study one, it has valuable information about the EBV and lymphoproliferative neoplasm. This article is worth to be published.

-Thank you very much for your expert review.