Dear Editor and reviewer(s),

Thank you very much for revising our manuscript as we believe it has increased its quality significantly. Please find below our reply to your comments together with the summary of the main modifications (please note that for your ease, we have marked the changes in blue colour font within the manuscript text):

- *Methods: you should have controlled your case and control patients by age, transplant vintage, immunosuppression (tacrolimus, cyclosporine, monoclonal antibodies); most important factors. Fatal limitation!*

Thank you for this suggestion. Indeed, this is ideal but due to our small sample and the way of patient recruitment (consecutive patients from all available prospective patients needing a transplant biopsy) in a very heterogenous transplant population, matching to those characteristics was not possible.

- *Results: - Were all the rejection episodes the first one in the patients, and if the non-rejection patients had any rejection episodes in their history? - due to the limited sample size, I recommend you to give a table representing all 21 cases one by one with detailed data (including Fuggle's immune risk, HLA mismatches, immunohistochmy results, rejectionand so forth) - Why then the initial IS regimen were different between the groups?*

As commented in the discussion, our patient population and sample population was very heterogenous regarding many clinical characteristics including time post-transplantation: for some patients it was the first episode, but not for others. Focusing on first time-rejectors would be ideal but our small sample size does not allow any meaningful secondary analysis in such subgroup. Also in relationship to our patient heterogeneity, only in recent years we have standardised our immunosuppression protocols; in previous years the choice of immunosuppression depended greatly on the judgement of the treating nephrologist, who also modified it depending on side-effects and complications. Therefore, the reviewer(s)' suggestion of doing a summary table with the main patient characteristics is well-taken and we have added it (please see Tables 2a and 2b).

- *Results: - "a few ATCMR-KTx patients had higher infiltration by granzyme B+ and Foxp3+ cells" OK, what was its significance then? how it affected the outcome results?*

Our exploratory hypothesis was that perhaps the patients with higher degree of granzyme B+ cells could have worse outcomes, and the opposite for the patients with rich Foxp3+ infiltration. However, our correlation studies failed to show that, likely due to the limited power of our study, as commented in the discussion section. Larger studies would be necessary to test that as a primary hypothesis.

- *Results: "...This suggests that in ATCMR-KTx patients Foxp3+ Treg cells might be overwhelmed" this is not a thing that should be brought in the results, but the discussion section.*

Indeed, that sort of statement is more appropriate for the discussion section, we have deleted it. Thank you for pointing it out.

- *Results; - You say in either rejection and non-rejection groups, the number of type 17 helper T (Th17) cells are associated with worse outcome (serum creatinine and other outcome). It is expected because we know that Th17 cells can mediate glucocorticoid-resistant rejection. Please discuss this in the discussion, if this may*

mean that some of the non-rejection patients of yours detected by the current criteria, might have been diagnosed as rejection patients and treated with intensive immunosuppressives? Especially considering the observation of the ratio of Th17 cells over Foxp3+ Treg cells in the "non-rejection patients" were significantly positively correlated with the outcome, while you know Foxp3 cells have been suggested to be protective against rejections.

Thank you for this comment. We have added a couple of sentences to emphasise the role of Th17 cells in the discussion section. Indeed, Th17 cells (and CTL) were the centre of our hypothesis. It is interesting that Th17 cell infiltration is associated with worse outcomes irrespective of having confirmed rejection or not. The fact that others have showed similar results gives further validity to our observations. It is important to mention that the Banff classification is not fully accurate and rejection is a patchy process, so it is possible that some cases of rejection could have been misdiagnosed as non-rejection, but to our best knowledge and assessment the non-rejection patients indeed did not fulfil the criteria for acute rejection. We have added a statement that before the final statistical analysis, the non-rejection biopsies (classified according to the 2009 Banff classification – the one prevalent during the recruitment period- were confirmed to be within the non-rejection category using the Banff 2013 update. Alternatively, this observation could represent a different role of infiltrating Th17 cells. The immune system is always active and trying to tip over towards the rejection side if ‘given a chance eg reduction of immunosuppression’, so immune responses are detectable in patients with no rejection (perhaps also hold by different regulatory mechanisms). It is possible that patients with higher Th17 infiltration, irrespective of reaching the current thresholds for acute rejection or not, could be bound to worse outcomes due to the possibility that Th17 cells could be mediating smoldering inflammation or slow motion chronic rejection or the potential to transform into a rejection phenotype in the future if the circumstances allow eg minimisation of immunosuppression. This is a speculation, but the molecular microscope and a better classification of chronic T cell mediated rejection and i-IFTA could help us in the future to allocate a better meaning to this interesting observation.

Thank you very much once more for your useful comments. We hope that this new version is deemed of sufficient quality for publication.

Faithfully yours,
Dr F Salcido (on behalf of all the authors)