

June 8th, 2020

Dr. Sami Akbulut & Dr. Vassilios Papalois
Editors-in-Chief
World Journal of Transplantation

Subject: Revised manuscript "Exploring the Safety and Efficacy of Adding Ketoconazole to Tacrolimus in Pediatric Renal Transplant Immunosuppression"

Dear Dr. Akbulut & Dr. Papalois-

We would like to extend our appreciation in the review of our manuscript. We found comments to be most helpful in clarifying and improving the manuscript. Please find the attached response letter addressing concerns of reviewers. We look forward to any additional comments.

Sincerely,
Brooke Ramay, Pharm D.
Researcher and Professor
Universidad del Valle de Guatemala
Guatemala City, Guatemala

We would like to confirm the following:

Name of journal: World Journal of Transplantation

Manuscript NO.: 56747

Column: Retrospective Cohort Study

Title: Exploring the Safety and Efficacy of Adding Ketoconazole to Tacrolimus in Pediatric Renal Transplant Immunosuppression

>> The title has been slightly modified to emphasize the exploratory nature of the manuscript.

(4) Special requirements for tables: Tables must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, *etc.*). Please verify that the tables are referred to in the text by their respective Roman numerals and that the numbering order is correct, and format the tables. Please verify that there are no missing or multiple spaces in the text and tables, *e.g.* before or after parentheses, between words, or before or after symbols like +, ×, ±, <, >, ≥, and ≤. Please verify that the special words or letters in the text and tables are correct, *e.g.* *P* (uppercase), *n* (lowercase), *via*, *vs* (lowercase, no punctuation), *in vivo*, *in vitro*, and *et al* (no punctuation) are italicized.

>> We have made minor edits in the table text to meet this requirement, thank you. Please find the changes in the attached manuscript in track-changes

(5) Special requirements for references: Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout. The author should provide the first page of the paper without PMID and DOI numbers. NOTE: The PMID is required, and NOT the PMCID; the PMID number can be found at <https://pubmed.ncbi.nlm.nih.gov>. (Please begin with PMID:) The DOI number can be found at <http://www.crossref.org/SimpleTextQuery/>. (Please begin with DOI: 10.**).

Please verify that the references are cited by Arabic numerals in square brackets and superscripted in the text, and that the numbering order is correct. There should be no space between the bracket and the preceding word or the following punctuation. When references in the text and tables are cited with author name(s), it is necessary to manually verify that the name(s) is consistent with the first author's surname in the corresponding reference list.

>> We have updated the DOI numbers and the citations in the text. Please see responses to the editors comments.

(6) Special requirements for article highlights: If your manuscript is an original study (basic study or clinical study), meta-analysis, systemic review, the "article highlights" section should be provided.

Detailed writing requirements for “article highlights” can be found in the Guidelines and Requirements for Manuscript Revision.

>> Thank you for inviting authors to add in the article highlights section. It has been added at the end of the article, beginning on line 619.

(7) Ethical documents: Please double check the accuracy of all ethical documents and verify the completeness of the documents according to the type of manuscript.

>> Confirmed, all documents are complete and accurate per documentation send in original submission documents.

(8) Approved grant application form(s) or funding agency copy of any approval document(s): If your manuscript has supportive foundations, the approved grant application form(s) or funding agency copy of any approval document(s) must be provided.

>> No supportive funds were received for this project

Reviewer #1:

Scientific Quality: Grade E (Do not publish)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Rejection

Specific Comments to Authors: Although this MS may be somewhat interesting, it remains only anecdotal clinical experience. 1. Follow-up duration in each patient remains too short to draw any conclusion regarding the efficacy and safety of authors' protocol. 2. Sample size is too small to draw any conclusion.

>>Regarding comments 1 and two, Text added to the limitation section (line 595):

“We recognize the limitations of this study and are those typical of retrospective chart reviews carried out with few patients during short periods of time.”

3. Some tables or figures which describe authors' protocol clearly are needed.

>>We have modified the table and the table description for clarification of study protocol and findings (beginning on line 345).

The title of the MS is strange.

>>Please note the correction in the manuscript title: “Exploring the Safety and Efficacy of Adding Ketoconazole to Tacrolimus in Pediatric Renal Transplant Immunosuppression”

We believe that the change in the title will also clarify the exploratory nature of the manuscript

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The study is interesting and showed that the addition of a small dose of ketoconazole minimizes the required dose of tacrolimus with safety. The latter is particularly important in low-income countries or low-income populations. The originality of the study is that it evaluated a pediatric population. Currently, there are no signs for improvement of the financial situation in many developing countries or in for attenuation of vast economic inequalities in certain other countries. Thus, the findings of the study could be characterized as significant. Comments In the statistical analysis, it may be more proper to use the paired t-test.

>> We have included a paired t-test to compare differences before and after ketoconazole addition. The following text has been added to the manuscript in various sections.

Abstract, line 89

Results, line 338

Article highlights, line 663

"There was a non-significant difference between the mean tacrolimus doses six months and two months prior to ketoconazole: -0.10 ± 0.04 (95%CI $0.007, -0.029$), $p = 0.23$. However, the difference between the mean tacrolimus doses six months prior to ketoconazole initiation and six months after ketoconazole addition was significant: 0.06 ± 0.05 (95%CI $-0.034, -0.086$) $p < 0.001$."

We have also re-worded sections of the results to clarify findings, beginning on line 324:

"The average recorded tacrolimus weight based doses at six, four and two months prior to ketoconazole initiation were 0.13 mg/kg/day ; 0.12 mg/kg/day ; and 0.11 mg/kg/day respectively. The average recorded tacrolimus weight based doses at two, four and six months post ketoconazole initiation were 0.09 mg/kg/day ; 0.07 mg/kg/day ; and 0.06 mg/kg/day respectively.

The mean tacrolimus blood levels at six, four and two months prior ketoconazole initiation were: $7.4 \text{ ng/dl} \pm 2.6 \text{ ng/dl}$; $7.4 \text{ ng/dl} \pm 2.5 \text{ ng/dl}$; $7.4 \text{ ng/dl} \pm 2.6 \text{ ng/dl}$. The mean tacrolimus blood levels recorded at two, four and six month visits post ketoconazole initiation were: $8.8 \text{ ng/dl} \pm 4.9$; $6.9 \text{ ng/dl} \pm 3.6$; and $6.8 \text{ ng/dl} \pm 3.2$ respectively. (Table 1)."

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: I have reviewed the article 56747. It reports on a topic that is of interest considering the patient population (ESRD children-renal transplant recipients) and the setting

(LMIC); and the strategy in question. The article is well written and clear. still, some minor revisions are needed (in my opinion).

1. Still some English polishing is needed (and there are a few typos scattered across the manuscript).

>>Thank you for pointing this out, the manuscript has been edited for English, the manuscript with track changes is included with several grammatical edits throughout.

2. I understand that it is accustomed to provide "statistical tests" - but here, this is really NOT needed (i.e., pre-post t-test). It would be more informative to provide pre-ketoconazole values, post-ketoconazole values (as they already are reported) - and DIFFERENCES (with 95%CI) between post- and pre-values. P-values really are not relevant here - a more important information is the AMOUNT of CHANGE (numerically provided).

>>This has been taken into account, as indicated above, please see responses to reviewer 2

3. A few things need to be additionally clarified: a): tacrolimus levels: when you say "median concentration"..what do you mean? Median trough concentration? or...?...The same with "minimum". In the methods section you should describe timing of blood sampling for tacrolimus concentration measurements. and clearly specify what do the reported numbers represent.

>>Thank you for this comment, we realize this was confusing and have made simplifications in the text as follows. Please see responses to reviewer 2

4. Table 1(or maybe one additional table) would be much more informative if it could include: a) liver transaminase levels (by time-point) b) information about time-span between pre-ketoconazole visits and time-span after inclusion of ketoconazole...so that one can have an idea about the time elapsed between the reported values.

>>Thank you for bringing this concern to our attention. First, we have changed the table to indicate the number of months prior to and after addition of ketoconazole to clarify the timespan. In regards to liver transaminase levels, transaminases are explored and noted in patient charts when patients manifest clinical effects of hepatic inflammation. No patients in this cohort experienced clinical signs hepatic inflammation, thus no values were documented in patient charts. We have added the following text in methods to clarify this point (line 297):

"Number of graft rejection episodes before and after ketoconazole were reported, additionally, the number of cases where transaminases were two times the normal limit compared to laboratory reference values during ketoconazole combination were monitored and used as indication of toxicity."

We have also modified the text in line 288 as follows:

"Ketoconazole hepatotoxicity was defined as an increase in liver enzymes greater than twice the normal value compared to the reference laboratory"

And in the results, line 372

"None of the patient charts documented a variation in serum transaminase levels during the visits pertaining to the use of ketoconazole-tacrolimus combination."

5. Finally - are there any other children who were NOT switched to ketoconazole?...The whole point that you would like to make would be much stronger if you could included a cohort (it does not matter whether it is small) of children who at that time ("in parallel" to the 25 reported children)...were kept constantly WITHOUT ketoconazole (and .e.g, provide...data on doses, concentrations, liver transaminases from control checks taken during an approximately similar time-period as for the reported cohort).

>>The patients who received ketoconazole were patients with much higher dose-requirements, we suspect that this is a result of genetic polymorphisms contributing to faster rates in tacrolimus clearance. Patients with higher dose requirements are suspected to have different metabolic profiles and requirements and were considered to be heterogeneous (not comparable) to patients who did not receive ketoconazole. Unfortunately, we were unable to measure cytochrome polymorphisms due to constraints in funding. We used the pre-post-study design in order to compare patients' profile before and after to explore safety and efficacy specifically in this group.

We have made a change in the title as well as in the text to emphasize that this is not a cohort comparing intervention and control, but rather we are exploring the effects in addition of ketoconazole as follows:

Title: Exploring the Safety and Efficacy of Adding Ketoconazole to Tacrolimus in Pediatric Renal Transplant Immunosuppression

Line 211 "We explore the safety, efficacy and the associated cost reduction of this combination from a retrospective cohort of children with kidney transplant in FUNDANIER."

Step 6: Editorial Office's comments

The author must revise the manuscript according to the Editorial Office's comments and suggestions, which listed below:

(1) *Science Editor*: 1 Scientific quality: The manuscript describes a retrospective cohort study of the safety and efficacy of adding ketoconazole to tacrolimus in pedantic renal transplant immunosuppression. The topic is within the scope of the WJT. (1) Classification: Grade C, Grade C, and Grade E; (2) Summary of the Peer-Review Report: The authors reported on a topic that was of interest considering the patient population (ESRD children-renal transplant recipients) and the setting (LMIC), which was well-written and clear. However, some tables or figures which describe authors' protocol clearly are needed. The questions raised by the reviewers should be answered; and (3) Format: There is 1 table. A total of 33 references

are cited, including 4 references published in the last 3 years. There is 1 self-citation. 2 Language evaluation: Classification: Grade B, Grade C, and Grade B. One author is a native English speaker. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and the Institutional Review Board Approval Form. Written informed consent was waived. The STROBE Statement is not qualified. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The topic has not previously been published in the WJT. 5 Issues raised:

(1) The "Author Contributions" section is missing. Please provide the author contributions;

>>This has been added beginning on line 16, thank you

(2) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout; and

>> We have updated the DOI numbers and the citations in the text, however, for some references (grey literature) there are no DOIs.

(3) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

>>This has been added beginning on line 621, thank you