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Reviewer 1:

In the paper ?Cytochrome CYP3A4 modulators in Kidney Transplantation: 6 years results of two ketoconazole and calcineurin inhibitor based immunosuppressive regimens, one with an m-TOR inhibitor and the other with an anti-proliferative agent.“, the authors Gonz á lez and Valjalo present an interesting and important single-center analysis of their strategy of post-kidney-transplant immunosuppression. The work, although comparing to possible procedures, is a single-center, non-randomised study, as the authors state themselves, so there are some limitations to its relevance, however their observations are extremely interesting, and the paper should thus be published. However there are some major and minor points that have to be acknowledged.

Major points: There is no information about the number of patients the authors intended to include in their prospective study, and no information about an ethical committee vote.

As this experience was not a controlled clinical trial, we did not calculate the number of patients who could be invited to participate in the two cohorts and, of course, we did not estimate any sample numbers. The Comité de Ética Científica from the Hospital del Salvador, reviewed and approved this project.

Thus the work is rather organized like a retrospective study analyzing the results of a new standard operating procedure initiated in 2005. I believe that it should better not be called a ?prospective“ study.

This experience was considered “prospective” and not “retrospective”, because all data was collected as the patients came to their clinical visits and from the beginning, our aim was to perform a controlled observation of their clinical courses. We think that real retrospective experiences are those constructed merely collecting the registered information with no investigational intention from the beginning.

Further, the text does not contain any information about toxicity – neither immunosuppressant- oder ketokonazole-induced or unspecific toxicity; these data can only (in part) be seen in the tables. A summary of these findings should be given in the results section.

We added some new information about adverse effects.

Minor points p 1: instead of treatment consistent in a calcineurin inhibitor better write treatment consisting in a calcineurin inhibitor p. 1 instead of dose and were receiving better write dose and who were receiving p. 2 instead of cohort, comparative clinical trial better write cohort comparative clinical trial In the figures, there is no labeling of the Kaplan-Meyer curves explaining what the green or blue lines stand for.

We modified the text as was suggested by the reviewer and the figures were also corrected.

Reviewer 2:

It is an interesting manuscript evaluating the association of cyclosporine and ketoconazole in transplantation. This therapy reduces calcineurin inhibitors dosage causing thus fewer side effects and also saving money. Some suggestions were made to improve it for publication. In introduction I believe that a short information about the mechanism of interference when ketoconazole is used at the same time as CsA is missing.

We added the solicited information.

Few authors have already shown that for other clinical conditions than transplantation the proposed combination has no adverse effects and saves money. Please insert that on introduction. Please insert the adverse effects expected by the administration of ketoconazole. Please describe the dose of mycophenolate mofetyl used as IS.

The suggested sentence and clarifications were added.

In materials and methods it is described that "No induction therapy was allowed" but in Table 1 there is a description of 1 induction therapy which was not specified. Please clarify.

We added a clarification comment as suggested the reviewer.

I believe a graph with the correlation between cyclosporine trough levels and serum creatinine should be included. There is no indication of IS groups in Figures 4 and 5. There are two figures numbered as 7.

We constructed the solicited graphs and make the corrections in Figures and in their numbers

Regarding the Editor´s indications:

1. The original title was shortened to reach the suggested number of words: From: "Cytochrome CYP3A4 modulators in Kidney Transplantation: 6 years results of two ketoconazole and calcineurin inhibitor based immunosuppressive regimens, one with an m-

TOR inhibitor and the other with an anti-proliferative agent" to "Combining Cytochrome CYP3A4 Modulators and Cyclosporine or Everolimus in transplantation is Successful".

2. A running title was added: "Combining Ketoconazole and Immunosuppressive is Successful".
3. Authors´ complete names and affiliation were added as requested.
4. The authors´ contributions were clarified.
5. The Institutional Review Board that authorized the experience was indicated and the correspondent document will be available in the Journal´s web system.
6. The Informed Consent Form was also approved by the IRB, as can be seen in the correspondent document.
7. Biostatistics: One of the authors has a MBA degree and statistics is part of the degree study plan.
8. The conflict of interest statement was added to the manuscript.
9. A Data sharing statement was added as it is in the model sent as example by the Editor.
10. Correspondence address, phone and fax numbers were included.
11. Abstract, core tip and key words were added and it was the core tip audio record.
12. The long and short titles were added before the Introduction.
13. CYP3A4 definition was included as indicated.
14. A comment was added.
15. The references were modified as it was requested. We added all PMID data and also the doi.org addresses. References numbered 14, 16, 18, 20, 37 and 38 did not have a doi.org clue.
16. The figures were modified in order to allow you to access the originating data using Microsoft Excel. However, figures 6 and 7 are composed graphs drawn from two individual data series that we could not arrange as requested.

All modifications are in red.
Awaiting a favorable response

Fernando González MD, MBA.

Answering to Chief Editor

We can answer:

"As editor in chief I have several concerns in accepting this manuscript for publication. In my opinion the authors did not reply the reviewers in the right way. The manuscript does not not refer to a prospective study, but rather to an observational one".

We apologize because of our misunderstanding. In red, we add to the manuscript the observational nature of our experience (Abstract and Methods sections).

Several points are missing: a) the predetermined number of enrolled patients and the two groups are too much size different;

As can be read in the manuscript Methods section, our aim was to describe the clinical course of kidney transplant patients receiving a cytochrome P-450 modulator with standard immunosuppressive drugs (calcineurin inhibitors and everolimus). We, indeed, describe two study groups in order to fairly, but not strictly, compare them. Our experience was not a randomized clinical trial (in red in Methods section) and the inclusion and exclusion criteria to be recruited in the everolimus group precluded that both groups were statistically similar. For example, those patients recruited in everolimus group could not be suffering delayed graft function (DGF) (see in red in the Immunosuppressive sub section) because, when we start the study, there existed experimental evidence that another m-TOR inhibitor (sirolimus) could prolong DGF duration. For these reasons, both groups were numerically asymmetrical. Moreover, this issue is described in Renal function and Grafts survival sub section of the Results section (in red).

b) I do not see the Ethical committee statement: I should see the number and the date

The Ethical Committee statement was uploaded in WJT system. Anyway, I am sending with this letter a copy of that document (in Spanish, of course). It can be read that the Committee approved the project on May 17th, 2005 and also the Informed Consent form to be signed by all participants.

c) Nothing has been said on the potential ketokonazole toxicity. This is a relevant point as the drug is administered for a long time.

In the results section, paragraphs 3 and 4 and, also in Table 2, we describe the adverse events and complications observed in both groups. Ketoconazol usual doses in systemic fungal infections or Cushing syndrome are more than 200-400 mg/d (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/018533s040lbl.pdf). Nevertheless these doses can induce liver damage and the FDA recommended in 2013 to limit ketoconazole prescription because this adverse events risk (http://www.fda.gov/downloads/Drugs/DrugSafety/UCM362444.pdf). We have monitored liver function test for more than 10 years without observing any case of liver injury (see reference 51). Our experience with low ketoconazole dose (100 mg/d) has been very satisfactory as we describe in the manuscript and also in others (references 32 and 33).
Main adverse events after combining ketoconazole and calcineurin inhibitors result from over dosage of calcineurin inhibitors during the learning curve of co-prescription. This issue can be read in the fourth paragraph of the Results section (in red).

d) A cyclosporine pharmakokinetics study should have been done

We did those pharmacokinetic curves in the previous experience described in reference 51. This experience is commented in the Discussion section of the manuscript (in red).

e) Nothing is said about drops out from the study and the side effects of immunosuppression

We analyzed all patients transplanted in our unit who received ketoconazole and cyclosporine combined with everolimus or azathioprine/mycophenolate. The drop outs are described in the first paragraph of the Results section (in red).
Side effects observed are described in paragraphs 3 and 4 and, also in Table 2 of the Results section.

f) The AZA/MMF group is going to lose given the high number of DGF. This factor infers on all the results. Give please the results of the clean population (not DGF)

Our primary aim (in red in the Methods section) was: "To describe the pharmacological interaction between the CYP3A4 modulator ketoconazole and cyclosporine alone or in combination with everolimus in kidney transplanted patients". We did not intend to compare the two immunosuppressive schemes

neither in efficacy nor in adverse event profiles. In the second paragraph of the Results section (in red) we describe that both groups were not comparable.
Respectfully, if we clean those patients suffering DGF from the azathioprine/mycophenolate group, the results will obviously modify, but our primary aim will not. If you consider this cleaning process is important, we will perform it, but, it is certainly probable that long term graft and patient survival could improve compared with other clinical trials (see second and fourth paragraph in the Discussion section) in an unfair way that it is not our intention.

g) Going up with Everolimus dose is possible to reduce CsA administration (please look at paper: Everolimus With Very Low-Exposure Cyclosporine A in De Novo Kidney Transplantation: A Multicenter,Randomized, Controlled Trial, published on Transplantation

The paper that you cite (Transplantation. 2009 Nov 27; 88(10):1194-202. doi: 10.1097/TP.0b013e3181bb43ec) is in line with our results as it describes that patients well exposed to everolimus can maintain efficacy and safety even reducing their cyclosporine dosing and exposure. Nevertheless, our aim did not allude to this issue, but to the efficacy and safety of combining ketoconazole with usual drug blood levels of immunosuppressive agents in kidney transplantation. If you consider that this Transplantation paper merits a special comment, we will put it.

h) A warning should be stated on the use of CYP3A4 modulator, as with the new immunosuppressants we do not need of them.

A related warning and comment were added in red at the end of the Discussion section. A new reference (numbered 58) was also added in red.

I hope that this letter can contribute to clarify all doubts about our manuscript. But, if you need more clarifications or paper editions, please, do not hesitate to contact us. Our honest aim is to describe a long term clinical experience in kidney transplantation.
Sincerely yours

Fernando González F. MD, MBA