

COVER LETTER AND RESPONSE TO REVIEWERS

To: Editors of the World Journal of Clinical Cases

February 28th, 2021

Dear editor,

please find herewith our revised manuscript entitled „Liver injury associated with the use of selective androgen receptor modulators and post-cycle therapy, a case series” we are sending you back for consideration for publication in the World Journal of Clinical Journal.

We believe that the topic would be of interest to the readers of the World Journal of Clinical Cases and also attract the attention of a wider medical and regulator community.

Response to reviewers:

Reviewer #1:

Specific Comments to Authors: In this manuscript the authors present two cases of liver injury potentially associated with the use of ligandrol. The manuscript focuses on an important issue, the hepatotoxicity potential of muscle enhancing products, and it is important to publish this kind of case reports to increase the awareness of this type of liver injury.

1. However, the exact role of ligandrol in the reported cases is difficult to determine due to additional treatments taken by both of the patients and this should be more emphasized as not to mislead the reader.

We agree with the reviewers, that in our case reports it is impossible to differentiate the causative agent of DILI. We have corrected the title of our manuscript by omitting ligandrol from the title,

adding PCT to the title, and in the text we are also emphasizing an equal role of ligandrol, ostarine and PCT substances in causing the hepatotoxicity.

Suggestions for improvements to the manuscript that should be considered are outlined below:

1. I agree with the authors in that the two presented cases are drug-induced liver injury. However, to determine the causative agent is more difficult. Both cases took ligandrol followed by PCTs, which are not described in detail. In addition, the second patient is said to have taken a second SARM (ostarine). As all these products were initiated prior to the liver episodes it is very difficult to determine a single causative agent for the two presented cases. Hence, describing these cases as SARM toxicity is in my opinion not completely correct. The PCT products should also be considered, but their hepatotoxicity potential is of course dependent on the ingredients. In fact, the temporal sequence between drug intake and liver injury onset is more compatible with the PCT as "In both cases, patients did not develop symptoms or clinical signs while using ligandrol and those signs only appeared after stopping the drug and taking substances for so-called "post cycle therapy" (discussion page 8). I suggest that the author should discuss this issue in more details.

As mentioned above, we have edited the title, abstract, core-tip and the text accordingly. In the first patient, time delay might suggest PCT as a causative agent, but the patient did not recall the details of PCT substances. In the second patient, time delay of 4 days of PCT suggests ligandrol/ostarine, but content of the PCT was hepatotoxic as well. We agree therefore, that it is impossible to separate the effect of ligandrol, ostarine and PCT.

2. I assume that the authors is referring to this problem in their final sentence of the abstract "Among amateur athletes, other hepatotoxic substances are often involved". However, this sentence is not very specific and the message is not very clear. This sentence should be reworded so that it conveys a clearer message. This abstract conclusion sentence was entirely rephrased.

3. 2. Case presentation 1: Based on the biochemical data provided for this case, it does not fulfil the latest consensus biochemical criteria for clinically significant hepatotoxicity according to Aithal et al (Clin Pharmacol Ther 2011;89:806-815). This does not mean that the liver damage cannot be caused by a drug/dietary supplement. However, the authors should mention this fact, to demonstrate that they are aware of it. If the patient in fact had an ALT value above 3 xULN at any time point (not indicated in Table 1) this should be stated as if this is the case the patient did in fact fulfil the DILI criteria. We agree, that ALT (not being >3xULN) and ALP levels (not reaching 2,5xULN) did not meet the criteria for DILI or Common terminology for adverse events. We added this limitation in the extended limitations paragraph. Patients however did meet the Common terminology criteria for adverse events in hyperbilirubinemia.
4. In addition, determining liver injury pattern using R value should be based on the earliest identified liver chemistry elevations that qualify as DILI (Aithal et al, 2011). Furthermore, the use of the R value to determine the type of liver injury when ALT and ALP values are low is questionable. Both patients described had ALP values within the normal range on admission (Table 1). Hence, as these cases do not have elevated ALP they do not reflect cholestatic injury biochemically, i.e. the cases should automatically be considered as hepatocellular cases based on biochemical values. Histological findings can also determine type of liver injury and do not always coincide with R value determinations. This can be discussed or the authors may simply concentrate on one way of determining the type of liver injury, preferably histological findings as you provide more details here. R value calculated from the entry ALT and ALP showed a mixed type of injury (between 2 and). Histology actually confirmed the mixed-type of injury, but certainly the picture of cholestasis dominated the histological findings and cytolysis/hepatitis was less significant. We added to the limitations paragraph, that the mixed-type character of liver injury according to R value should be interpreted with caution due to low ALT and normal AST.
5. 3. Discussion: I find the discussion dealing with previously reported cases of ligandrol hepatotoxicity cases poor. The authors describe two cases from references 12 and 13,

however there is no comparing or contrasting with the cases described in the current manuscript. Such an analysis is clearly missing and should be included, rather than simply describing the external cases. We added to the described cases some new details and in the discussion section we tried to compare and discuss their findings with our case reports.

6. 4. In line with the previous comment, I think the wording "and review" should be omitted from the title. 5. "Review" was omitted from the title.
7. Possible mechanisms of hepatotoxicity, page 11. The authors state "Also, low HDL cholesterol levels in both reported cases likely indicate the shift of cholesterol transport towards bile acid synthesis." Is this related to the mechanism of hepatotoxicity or is it simply a consequence of ligandrol use independent of hepatotoxicity? While attempting to explain possible mechanisms of hepatotoxicity, low HDL cholesterol indicated a shift in transport towards bile acid synthesis in hepatocytes, which increased the concentration of toxic bile acids in the hepatocytes. The sentence was rephrased to be more clear.
8. Minor comments 1. The word "icterus" is used in the abstract, while "jaundice" is used in other parts of the manuscript. Please be consistent. I suggest to use jaundice throughout the manuscript. Jaundice is used instead of icterus.
9. 2. Introduction, page 5: "...the widespread use SARMs..." should be "the widespread use of SARMs" 3. corrected
10. Reference 14 is not correct. Lewis et al did not develop the RUCAM scale. Reference 14 is simply an article in which Lewis et al have applied this scale to cases to assess potential DILI. The RUCAM scale was first published by Danan and Benichou (J Clin Epidemiol 1993) as correctly stated by Lewis et al in reference 14. This was changed to the Danan and Benichou paper.
11. 4. Case presentation 1, page 6: "A transcutaneous liver biopsy has been performed..." is better said as "...was performed.." corrected

12. 5. Case presentation 2: The RUCAM category is said to be "likely". What does this mean?

What was the total score? The RUCAM scale classifies the likelihood of DILI into 5 categories: excluded, unlikely, possible, probable and highly probable. Likely is not a category in this scale. This was corrected to „possible“.

13. 6. Last sentence on page 12: What do the authors mean with "...prevent their ultimate disqualification...."? This sentence was rephrased to be more clear.

4 LANGUAGE QUALITY

Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs. This was verified.

We also corrected the manuscript according to the reviewers suggestions. Added FINAL DIAGNOSIS, TREATMENT, OUTCOME AND FOLLOW-UP. We also added PMID to all citations, where PMID could have been found. Uniform presentation of Figures and Table is provided. We also added 3 relevant citations. Original Figures files are available to upload.

Sincerely

Tomas Koller

First and corresponding author