Rosario, March 6th, 2014

Director, Editorial Office
Jin-Lei Wang
World Journal of Hepatology

Title: The challenge of liver disease in systemic lupus erythematosus: clues for diagnosis and hints for pathogenesis

Authors: Fernando Bessone, Natalia Poles, Marcelo G. Roma

Name of Journal: World Journal of Hepatology

ESPS Manuscript NO: 9055

Dear Jin-Lei Wang

Thank you for your letter of 20 of February, 2014, giving us the opportunity to revise our manuscript, according to the reviewers' suggestions. We would also like to thank the reviewers for their helpful comments.

We have introduced changes in the manuscript so that to fulfill all the reviewers' requirements. We hope these changes have addressed the suggestions, so that the manuscript is now acceptable for publication.

Below are the responses to the reviewer, where actions or comments relating to individual points raised by him/her are indicated.

We look forward to your response.

Yours sincerely,

Fernando Bessone

Gastroenterology and Hepatology Department.

University of Rosario School of Medicine,

Alvear 740, 1st floor

2000 Rosario, Argentina

E-mail: bessonefernando@gmail.com

Telephone: +54-341-425926

Fax: +54-341-4259265

RESPONSE TO REVIEWERS

REVIEWER #1

1. Please, change lupus by systemic lupus erythematosus in the title.

As suggested, the word "lupus" was replaced by "systemic lupus erythematosus" in the title.

2. *Although the review documented good studies on SLE, the authors must present the criteria used for their selection (English articles, citation, number of patients, impact factor of the journals?).*

When available, cohort studies were quoted only when written in English and published in peer reviewed journals. Studies in other languages were only exceptionally included. They were limited to topics where information was extremely scarce, and when the exception was worthy, after our critical evaluation of quality. Similarly, abstracts were quoted exceptionally, and only when coming from peer reviewed, highly prestigious meetings (e.g. AASLD, EASL). The number of abstracts was kept to a minimum, by searching the literature for related full papers until the very last moment before submission. We have stated these literature search criteria at the end of "Introduction" section.

3. Findings of altered unspecific laboratory tests as liver enzyme tests is the basis of this review. In addition, some studies as those investigating the involvement of anti-ribosomal P antibodies in SLE liver disease were performed using not well-standardized homemade immunoassays, and did not investigate the influence of environmental factors on the levels of these autoantibodies in patient population. Thus, studies that investigated liver histology of the SLE patients presenting abnormal results of liver laboratory tests are more clinically consistent.

In the original version of the manuscript, we had warned the readers that many studies quoted in there were based on liver function tests rather than on histological analysis. For example, at the end of the section entitled *"Prevalence of biochemical and histological hepatic alterations in patients with SLE"*, we had stated that "a high number of cases lack adequate histological documentation". Unfortunately, the state of the art of many of the topics we are covering in the review is still too poorly developed to allow for large-scale, well documented clinical studies to be available.

As for the limitation of some studies to properly support the involvement of anti-ribosomal P antibodies in SLE liver disease, we have discussed this issue more extensively in the new version of the manuscript. Taking into account the reviewer's comments, we have now warned the readers further about the limitations of the assays to detect anti-ribosomal P antibodies (see page 11, 2 last sentences of 2nd paragraph, new version). In addition, lack of consideration for putative environmental factors or appropriate stratification by ethnicity was also highlighted as putative limitations (see page 11, first sentence of 2nd paragraph, new version)..

4. *The association of SLE with chronic hepatitis C virus infection is anecdotal. NOSA production (e.g., ANA, SMA and aCL) in chronic hepatitis C has been associated with B cell dysfunction due to HCV lymphotropic property and the occurrence of antigen mimicry in chronic HCV infection. On the other hand, cryoglobulinemia is the main cause of glomerulonephritis and vasculitis in patients with chronic HCV infection. In addition, this infection can be easily excluded in SLE patients using routine anti-HCV serology and, HCV-RNA tests.*

We agree with the reviewer that association of SLE with chronic hepatitis C virus infection is a controversial issue, and that, perhaps, it can be anecdotal. We had somewhat reflected this debate in the original version of our manuscript by stating that "very little" association would

exist (see page 14, line 7 from the bottom, original version). Now, we have been more emphatic in the need for distinction between true SLE and HCV infection mimicking SLE, according to the reviewer`s comments.
For this purpose, we have now better balance the pros and cons of the existence of this association. On one hand, positive evidence are provided by cohort studies suggesting that the prevalence of HCV is higher among SLE patients than among the general population (see refs. # 48 and 50, original version), Now, we have made emphasis on the need for large-scale studies lacking potential bias, such as multiple admissions and blood transfusions, to confirm or ruled out this association (see page 15, last sentences of 2nd paragraph, new version). In addition, the factors that may make SLE easy to be confused with HCV infection have been now better described (similarities in NOSA production, cryoglobulinemia, etc.), including the mechanistic basis given by the reviewer (see page 15, 3rd paragraph, new version). In part, this had been already commented on in the original version of the manuscript, when overstimulation of lymphocytes B in HCV infection had been described (see last sentence of this item in the original version).

5. *Drug hepatotoxicity is a predictable finding in patients treated for different autoimmune and non-autoimmune diseases. Therefore, the authors need to compare the prevalence of drug hepatotoxic events in SLE patients with those reported in patients suffering from other autoimmune diseases treated with the same medications, mainly rheumatoid arthritis. On the other hand, the immune response of self-reactive lymphocytes for liver antigens and the genetic background of the patients need to be considered in these studies of drug hepatotoxicity in SLE subjects. Please include this limitation in their review.*

Unfortunately, to the best of our knowledge, there is not robust evidences in the literature establishing that autoimmune diseases other than SLE have an increased risk of hepatotoxicity, or randomized control trials comparing head to head drug safety in patients suffering rheumatoid arthritis (RA) *vs.* SLE. The true incidence rate of NSAID-induced hepatotoxicity in the general population carrying only an autoimmune disease is largely unknown. Most studies includes a mixture of patients including both rheumatologic and non-rheumatologic populations. Moreover, the existence of a pro-oxidant liver condition linked to a pro-inflammatory status (like reported in SLE) that becomes the liver more susceptible to hepatotoxic reactions remains still to be established.
As required by the reviewer, we have added a paragraph stating these limitations and the need for well controlled comparative studies in SLE *vs.* RA in the new version of the manuscript (see page 20, last sentence of the 2nd paragraph, new version).

REVIEWER #2

1. The paper needs to be proofread for some spelling and grammar errors.

We revised and corrected the manuscript throughout for spelling and grammar mistakes.

2. SLE was diagnosed in (2.8%) of AIH-PBC overlap patients and this should be cited (Efe C, Eur J Gastroenterol Hepatol. 2012 May;24(5):531-4.) Related this one, antidsDNA which is known to be strongly associated with SLE, were detected in 60% and 56% of patients with AIH-PBC(Muratori P, Am J Gastroenterol 2009; 104:1420–1425, Efe C,Am J Gastroenterol. 2010 Jan;105(1):226. Ds-DNA were also found to be associated with poor response in AIH(Czaja AJ,Hepatology. 1997 Sep;26(3):567-72.) The association between PBC and SLE is thought to be rare but 27 cases had also SLE among 1032 PBC patients(Gershwin ME, Hepatology 2005;

42:1194–1202.) similary, anti-dsDNA were detected in 22% of pure PBC patients (Agmon-Levin N,J Autoimmunity 2010;34:55–8.).

We thank the reviewer for these helpful references. All of them have been now quoted in the new version of the manuscript (see refs. 40, 43, 44, 48, 49, and 50, in the new version).

REVIEWER #3

1. In the section about Lupus hepatitis, the authors do not address the importance of discussion about the different methodologies available to detect what they called anti-P antibodies, more correct anti ribosomal P or anti-riboP antibodies. They talk about very insensitive methods like immunodiffusion and IIF. First of all, when we talk about the pattern of AAN by IIF is just a whole topic to discuss in detail, since the pattern of AAN and anti riboP antibodies is usually a fine speckled pattern in patients with positivity of ANA, so they cannot talked about immunodiffusion and AAN like if they were both methods to detect the specific presence of anti ribosomal P antibodies. Besides, it is important the authors include a paper published by Carmona Fernandes D in 2013 where he explored the Anti-ribosomal P protein IgG autoantibodies in patients with systemic lupus erythematosus: diagnostic performance and clinical profile. In this paper it is states the specificity, sensitivity, positive likelihood ratio, and negative likelihood ratio of anti-Rib-P for SLE diagnosis were 99.4%, 14.2%, 23.7%, and 0.86%, respectively. Caucasian ethnicity was associated with lower anti-Rib-P antibody levels. No relation was found between anti-Rib-P levels and clinical features and they tested these antibodiess using ELISA. Also it is important that they looked for papers where the anti ribosomal P antibodies are detected by the most reliable method such as immunoprecipitation for protein and for RNA and protein complexes to see which is the more accurate method to suggest to the readers as the best method published in relation to SLE and liver damage or different liver autoimmune conditions.

The expression “anti-P antibodies” was changed throughout by the more correct “anti-ribosomal P antibodies”.

Double Immunodiffusion (DID) and immunofluorescence (IIF) had been used by Jearn & Kim (ref. #29, original version) to check the results of Calich & Bonfa (ref. #28, original version) that anti-ribosomal P is present in autoimmune hepatitis. They found that, when using low sensitive but quantitative assays like DID and IIF instead of the highly sensitive but non-quantitative ELISA test used by Calich & Bonfa, no relationship among elevated anti-ribosomal P values and liver enzyme levels was founded; this cast doubts on the involvement of this autoantigen as a true etiologic factor of liver damage. We acknowledge that this topic was not properly discussed in the original version of the review, and that such a ¨fine” methodological discussion is beyond the scope of our review. Therefore, we prefer to remove this piece of text from the review, since it could be misleading for the readers.

In our original version, we had made the readers aware of the discrepancies among different studies to establish an association between anti-P antibodies and the occurrence of liver damage in SLE, and that this may be due to different features of the studied populations and lack of reliable methods to detect the specific presence of these autoantibodies (page 11, line 9, original version). We have now better described the limitations of the not well-standardized methods used in the literature to address this issue, and emphasized the need for larger cohort studies using reliable anti-Rib-P detection assays with better documented liver disease tests (see page 11, 2nd paragraph, new version).

By searching the literature, it becomes apparent that the better current immunoassays to assess anti-Rib-P levels that can be routinely used in large-scale studies are ELISA employing a mixture the ribosomal P antigens, such as P0, P1, and P2, three ribosomal phosphoproteins with the highest reactivity against anti-Rib-P. The study by Carmona Fernandes et al. mentioned by the reviewer is a good example of studies using this approach, and it has been quoted now

in the new version of the manuscript (see ref. 20 of the new version). By employing methods based on this strategy, both sensitivity and specificity were increased, so as to duplicate the frequency of detection of anti-Rib-P in SLE patients, a finding that is now commented on the new version of the manuscript (see page 9, first sentence of the 3rd paragraph). Unfortunately, to the best of our knowledge, immunoprecipitation for protein and for RNA and protein complexes has not been employed as yet to assess anti-Rib-P levels in clinical studies. We have now discussed further the whole methodological subject, so as to draw attention about the need for the use of more reliable methods to detect anti-Rib-P in future clinical studies. We thank the reviewer for having raised attention about this important methodological issue.

When they talked about CBP, it is important to include the well known AMA antibodies as the more important ones to differentiate the liver damage as CBP or other conditions.

Anti-mitochondrial antibodies (AMAs) had been mentioned in the original version of the manuscript, as a typical finding in patients with SLE-PBC overlap syndrome, when occurring together with anti-native DNA (see 2nd sentence of the item *"SLE-PBC overlap syndrome"*, page 13). We have now added thereafter a piece of text stating that occurrence of anti-native DNA and AMAs are pathognomonic signs of SLE and PBC, respectively (see page 13, line 7 from the bottom).