ANSWERS TO REVIEWERS

February 5, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Manuscript_6589_revised.doc).

Title: HEPATITIS-C VIRUS ASSOCIATED GLOMERULOPATHIES

Authors: Abdullah Ozkok, Alaattin Yildiz

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6589

The manuscript has been improved according to the suggestions of reviewers:
1- Manuscript was reviewed by a native English speaker and language is improved.
2- References has been shortened.
3-A new part discussing the effectiveness of newer antivirals in the treatment of HCV-induced cryoglobulinemia and glomerulopathy has been added as requested by the reviewer.
4- Electron microscopic evidence of HCV-related kidney disease has been added.
5- Possible mechanisms of interferon-induced proteinuira were added.
6- Treatment of cryoglobulinemia-related GN has been extended as requested.

Revisions has been made according to the suggestions of the reviewers:

REVIEWER 1 (Reviewer no: 01714111):

1. Some misspellings should be corrected, language corrections should be done (last sent. in p. 8).

We thank the reviewer for this correction. We carefully reviewed the manuscript and made the appropriate corrections as requested.

2. Abbreviations should be explained properly if used.

All abbreviations were explained properly in the manuscript.

3.Some unnecessary information can be ommitted (p.9 renal transplantation...)

This part was also shortened as requested.

4. Too many references, not always necessary.

We have decreased the number of references as requested.

5. A few words should be said about the new drugs (LIKE SOFOSBUVIR) and their possible future in HCV+MCG.

We thank the reviewer for this valuable contribution. Any drug probably including sofosbuvir effective in sustained virologic response should also be effective for the treatment of HCV-induced cryoglobulinemia and glomerulonephritis. However when we vigorously searched the literature, we recognized that there is no data about sofosbuvir and its use in cryoglobulinemia or HCV-induced glomerulonephritis. We also searched the literature for other newer antiviral agents such as telaprevir and boceprevir as treatment of HCV-induced mixed cryoglobulinemia. We added the following parts and references to the related sections of the manuscript.

"In a recent study investigating the efficacy and safety of IFNα/ribavirin/protease inhibitor combination in HCV-induced-mixed cryoglobulinemia, telaprevir and boceprevir were found to be highly effective in HCV-MC (*). Moreover, all the patients with kidney involvement improved significantly. However telaprevir and boceprevir should be used carefully in patients with high risk of renal impairment because very recently, these drugs were found to be related to significant decrease in glomerular filtration rate (**)"

* Saadoun D, Resche Rigon M, Thibault V, Longuet M, Pol S, Blanc F, Pialoux G, Karras A, Bazin-Karra D, Cazorla C, Vittecoq D, Musset L, Decaux O, Ziza JM, Lambotte O, Cacoub P. Peg-IFNα/ribavirin/protease inhibitor combination in hepatitis C virus associated mixed cryoglobulinemia vasculitis: results at week 24. Ann Rheum Dis. 2013 Apr 20.

**Mauss S, Hueppe D, Alshuth U. Renal impairment is frequent in chronic hepatitis C patients under triple therapy with telaprevir or boceprevir. Hepatology. 2014 Jan;59(1):46-8.

REVIEWER 2 (Reviewer no: 00503530):

1. How is the clear characteristic of the electron microscopic evidence of HCV-related kidney disease?

We added the electron microscopic description of HCV-related kidney disease into the manuscript as requested.

" In renal biopsies, electron microscopic detection of viral like particles has been reported in a few studies performed on HCV-infected patients (*,**). In the study by Sabry et al. (**), viral like particles were found within the immune complexes. HCV-related virus-like particles were about 30–45 nm in diameter and located in electron-dense deposits in the paramesangial areas (**)."

* Bosman, C., Valli, M.B., Bertolini, L., Serafino, A., Bolderini, R., Marcellini, M., Carloni, G., 1998. Detection of virus like particles in liver biopsies from HCV-infected patients. Res. Virol. 149, 311 – 314.

** Sabry A, E-Agroudy A, Sheashaa H, El-Husseini A, Mohamed Taha N, Elbaz M, Sobh M. HCV associated glomerulopathy in Egyptian patients: clinicopathological analysis. Virology. 2005 Mar 30;334(1):10-6.

2. Proteinuria comes out by the beta interferon dosage in HCV, how is the connection?

We have added the possible mechanisms of interferon induced proteinuria as requested.

" IFN therapy has been used for the treatment of various diseases and causes proteinuria as an adverse effect in approximately 20% of the subjects (*). However, the mechanism of proteinuria induced by IFN is not fully known. One of the immunologic effects of IFN is the alteration of the balance of T helper cells type 1 (Th1) and T helper cells type 2. IFN-induced Th1-dominant immune response may be involved in the exacerbation of underlying glomerulonephritis. In a study by Kimmel et al (**), after

IFN therapy, immune complexes containing IFN were found in the circulation and renal tissue in a patient with MPGN. Foot process effacement may also be a factor in the pathogenesis of IFN-induced proteinuria(***)."

* Quesada JR, Talpaz M, Rios A, Kurzrock R, Gutterman JU: Clinical toxicity of interferons in cancer patients: A review. J Clin Oncol 4:234-243, 1986.

** Kimmel PL, Abraham AA, Phillips TM: Membranoproliferative glomerulonephritis in a patient treated with interferon-α for human immunodeficiency virus infection. Am J Kidney Dis 24:858-863, 1994.

*** Ohta S, Yokoyama H, Wada T, et al. Exacerbation of glomerulonephritis in subjects with chronic hepatitis C virus infection after interferon therapy. Am J Kidney Dis. 1999;33(6): 1040-1048.

3. How about the anti-viral drug of the interferon non-combination?

As we mentioned in our manuscript, combination therapies with IFN and ribavirin have been found successful in the treatment of HCV-associated glomerulopathies. Patients receiving combination therapy with IFN plus ribavirin achieved a higher SVR than those with IFN monotherapy. Even the most recent antiviral drugs such as telaprevir and boceprevir have been found effective only in combination with IFN and ribavirin. Several trials with direct acting antiviral (DAA) combinations have reported increased SVR and low resistance. In the near future, IFN-free short duration DAA combinations may be found effective in treatment of chronic HCV. Thus as far as we know, there is no data about the efficacy of antiviral drug monotherapy without IFN especially in the treatment of HCV-related glomerulopathies.

REVIEWER 3 (Reviewer no: 00053433):

MINOR ISSUES

1. Minor English polishing is needed, all through the manuscript.

We have improved the English language of the manuscript significantly.

2. Page 2, line 19. "…was also found".

We thank the reviewer for these corrections. Appropriate corrections were performed as requested.

3. Page 4, line 28 & Page 5, line 19. Please correct typo – "usually".

Appropriate corrections were performed as requested.

4. Page 8, lines 4 to 8. Sentences are confusing and need rephrasing.

We reconstructed the sentence and made it simple and deleted the second sentence. Accordingly:

"Incidence of DM following kidney transplantation was found to be higher with the use of donor-HCV-positive kidneys compared to the other types of post-transplant HCV positivity."

5. Page 8, lines 31 to 32. Sentence seems to be incomplete.

We are sorry for this mistake, we have erased this incomplete sentence.

6. Page 9, line 20 – " CTG". Abbreviations should be defined in the text when first mentioned.

We checked all the abbreviations throughout the manuscript and corrected these mistakes accordingly.

7. Page 10, line 16, section "TREATMENT OF HCV-ASSOCIATED GLOMERULOPATHIES". The title of this section is misleading, since the therapeutic options described are mainly applicable to HCV-related cryoglobulinemic renal disease.

We partly agree with the reviewer. We agree that most of the mentioned treatment modalities are applicable to cryoglobulinemia however the advices were obtained from KDIGO Clinical Practice Guideline for Glomerulonephritis. In this guideline, these recommendations were expressed under the heading of "9.2: Hepatitis C virus (HCV) infection–related Glomerulonephritis". Thus we changed the

title as **"TREATMENT OF HCV-ASSOCIATED GLOMERULOPATHIES AND CRYOGLOBULINEMIC RENAL DISEASE".**

8. Page 11, lines 5 to 7. As mentioned below, HCV genotyping is also important to define treatment duration.

We have mentioned the importance of HCV genotyping for determining the duration of the treatment as requested by the reviewer (please also see the answer to major issues - comment 4).

9. Page 11, lines 12 to 14. Sentence is truncated.

We have corrected the sentence appropriately.

"Combination therapy (ribavirin + IFN-α) has the most favorable outcome with higher SVR and lower proteinuria."

10. Page 13, line 26. The correct unit is microgram (/kg/week and /week, respectively).

We apologize for this mistake. We corrected the units as μg/ kg/week and μg/week.

11. There are too many redundant references, which could be easily left out without any harm to the value of the manuscript.

We have decreased the number of references as requested.

MAJOR ISSUES

1. Page , line 2. Although the figures can vary, most studies estimate that overt vasculitic manifestations are seen in only 2%-3% of patients (see Gorevich PD, Kassab HJ, Levo Y, et al. Am J Med.1980;69:287-308. Agnello V, De Rosa FG. J Hepatol 2004;40:341-352. Sène D, Limal N, Cacoub P. Metab Brain Dis 2004;19:357-381).

This knowledge has been added to the manuscript together with the references.

2. Page 9, section "RENAL TRANSPLANTATION IN THE COURSE OF HCV INFECTION". Regarding the impact of HCV infection on renal transplantation outcomes, the landmark study of Mathurin P et al. should be cited and briefly discussed (Hepatology 1999;29(1):257-63).

We thank the reviewer for this contribution. We have added this article and summarized the findings of this study.

"In the study by Mathurin et al, 834 renal transplant patients with positive serologic markers for hepatitis B virus and/or HCV were included. A total of 706 patients were HCV positive. At 10 years, among overall patients with HCV screening, presence of HCV antibodies was found to be an independent prognostic value in patient survival. The case-control study showed that anti-HCV positivity was independently associated with both patient and graft survivals."

3. Page 10, line 17 – "Three steps may be suggested…". I would recommend using the word "approach" instead of "steps", because it suggests an order of therapeutic strategies that is not possible in many cases of cryoglobulinemia with renal disease.

We have changed the term "steps" with the term "approach".

4. Page 10, lines 29 to 30 – "The recommended duration of therapy is 1 year." Although some experts advocate a one-year therapy, treatment duration generally is not fixed (1 year), but rather it depends on HCV genotype and on-therapy virological response, varying from 24 to 72 weeks.

We thank the reviewer for this contribution. We obtained the "1-year" recommendation from "Kidney Disease: Improving Global Outcomes. KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease 2008". We changed this statement

according to the request of the reviewer:

"The recommended duration of therapy according to some experts is 1 year (102). However treatment duration should be adjusted for HCV genotype and virological response to treatment and it may vary from 24 to 72 weeks (*)."

*Hadziyannis SJ, Sette Jr H, Morgan TR, et al. Peginterferon alpha 2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140:346–55.

5. Page 10, line 32 to Page 11, line 4. Please consider the comments below:

5.1. In order to provide readers with a global perspective on the treatment of cryoglobulinemia-related GN, the recommendations from three relevant publications should be briefly mentioned (Ghany M et al. Hepatology 2009;49(4):1335-74; Iannuzzella F et al. Am J Med 2010; 123;400-408; and Dammacco F, Sansonno D. N Engl J Med 2013;369(11):1035-45).

We thank the reviewer for this contribution. We have added a brief summary of information from the mentioned three publications into the relevant parts of the manuscript.

"The recommended therapeutic regimen for cryoglobulinemic vasculitis consists of a 48-week course of once-weekly Peg-IFN-α-2a (180 μg) or Peg-IFN-α-2b (1.5 μg/kg) combined with 1000-1200 mg of daily ribavirin for patients with genotypes 1 and 4, and a 24-week course of Peg-IFN- α combined with 800 mg/day of ribavirin for patients with genotypes 2 or 3(*). Taribavirin, a prodrug of ribavirin, does not significantly accumulate in erythrocytes, has shown an antiviral efficacy similar to that of ribavirin, with a lower occurrence of anemia (**).

*Iannuzzella F, Vaglio A, Garini G. Management of hepatitis C virus-related mixed cryoglobulinemia.

Am J Med. 2010 May;123(5):400-8. doi: 10.1016/j.amjmed.2009.09.038.

**Gish RG, Arora S, Rajender Reddy K, et al. Virological response and safety outcomes in therapy-naïve patients treated for chronic hepatitis C with taribavirin or ribavirin in combination with pegylated interferon alfa-2a: a randomized, phase 2 study. J Hepatol. 2007;47:51-59.

Corticosteroids may be used at high oral doses (eg, prednisone 0.5-1.5 mg/kg/day) or intravenous pulses (methylprednisolone 0.5-1.0 g/day for 3 days followed by oral prednisone) in the presence of severe mixed cryoglobulinemia manifestations. However, corticosteroids may also favor HCV replication and worsen the liver disease.

Mycophenolate mofetil (MMF) is more selective than cyclophosphamide in inhibiting lymphocyte proliferation and functions. MMF may be a less toxic alternative to cyclophosphamide for the induction of remission in mixed cryoglobulinemic vasculitis (*).

*Reed MJ, Alexander GJ, Thiru S, Smith KG. Hepatitis C-associated glomerulonephritis—a novel therapeutic approach. Nephrol Dial Transplant. 2001;16:869-871.

"Renal function should be evaluated in all patients before ribavirin is administered. Patients with renal impairment should be monitored for the development of hemolytic anemia, and the daily dose of ribavirin should be adapted to the glomerular filtration rate (*). If the serum creatinine level increases to more than 2.0 mg/dL, therapy with peginterferon alfa and ribavirin must be discontinued (**).

*Perico N, Cattaneo D, Bikbov B, Remuzzi G. Hepatitis C infection and chronic renal diseases. Clin J Am Soc Nephrol. 2009 Jan;4(1):207-20.

**Dammacco F, Sansonno D. Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. N Engl J

Med. 2013 Sep 12;369(11):1035-45. doi: 10.1056/NEJMra1208642."

5.2. It is essential to emphasize that there is no standardized classification of disease severity and that the recommendations for the management of HCV-related cryoglobulinemic glomerulonephritis are based on data from small uncontrolled studies, case series, and experts' opinions.

We have added a statement to our manuscript as suggested by the reviewer.

"Since there is no standardized classification of severity for HCV-related cryoglobulinemic glomerulonephritis, recommendations for the management of this disease are based on small uncontrolled studies, case reports, and experts' opinions."

5.3. It would be particularly important to clarify that in patients with severe/active disease (GN with progressive renal failure or nephrotic syndrome; or renal disease associated with cutaneous ulcers, intestinal ischemia, severe neuropathy, alveolar hemorrhage, etc.), antiviral treatment should be initiated only after the acute cryoglobulinemia flare has been controlled with immunosuppression and/or plasmapheresis. On the other hand, in mild-to-moderate kidney disease (stable renal function, non-nephrotic proteinuria, and mild or moderate histological lesions at renal biopsy), antiviral therapy including low-dose ribavirin could be tried before immunosuppression.

Actually we have already mentioned these issues in terms of kidney involvement in different parts of our manuscript. As requested by the reviewer, we have reconstructed this part of the manuscript and added several extra-renal manifestations that necessitates immunosuppressive treatment before antiviral treatment.

"In the very recent Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Glomerulonephritis, in patients with moderate proteinuria, stable renal functions and mild to moderate histological lesions at renal biopsy, anti-HCV therapy is advised to be started as pegylated interferon-α (PEG-IFN-α) plus ribavirin. However in patients with nephrotic-range proteinuria and/or progressive kidney injury and other extra-renal manifestations such as cutaneous ulcers, intestinal ischemia, severe neuropathy or alveolar hemorrhage, immunosuppressive therapy with cyclophosphamide (2 mg/kg/d for 2–4 months), rituximab (375 mg/m2 once a week for 4 weeks), steroid pulses (0.5–1 g/d for 3 days) and plasmapheresis (3 L of plasma thrice weekly for 2–3 weeks) should be administrated. Since IFN-α may exacerbate cryoglobulinemic vasculitis, it should be started only after the acute phase of the disease has been controlled with immunosuppressive agents."

Thank you again for evaluating our manuscript to be published in the *World Journal of Gastroenterology*.

Sincerely yours,

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