February 24, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8307-edited.doc).

**Title:** Hepatitis C Virus and diffuse large B-cell lymphoma: pathogenesis, behavior and treatment.

**Author:** Carlo Visco, Silvia Finotto

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 8307

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer.
Please see attached in the bottom all detailed responses to reviewers suggestions.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Carlo Visco, MD

Divisione di Ematologia,

Ospedale San Bortolo,

Via Rodolfi 37, 36100 Vicenza, Italy.

carlovisco@hotmail.com

# RESPONSES to 4 REVIEWERS

## FIRST REVIEWER

A. Epidemiology The association between HCV infection and DLBCL is particularly prominent in some geographical areas. What do you think are the possible causes?

RESPONSE: When large scale studies were performed in areas with low HCV prevalence, a link between virus and lymphoma was demonstrated. Instead, low number studies in areas with low prevalence did not reveal such association. This might be the explanation to this fact. We added a sentence in the end of the "epidemiology" paragraph: "Large scale studies including higher numbers of patients are warranted from regions with low HCV prevalence before driving conclusions on the association between virus and lymphomas in these areas."

B. Pathogenesis According to a large amount of important literatures, the author summarized an active pathogenetic role of HCV infection in the development of aggressive B-cell lymphomas. Do you think antiviral treatment is necessary in the patients with HCV-associated DLBCL?

RESPONSE: Differently from indolent B-cell lymphomas, antiviral treatment yet does not play a significant role in HCV-positive DLBCL. Since the treatment of DLBCL is usually based on rapidly active drugs due to the aggressive clinical behaviour of the disease, antiviral regimens have not been tested so far on large series, especially due to their slow effect on lymphoid proliferation, if any. Nevertheless, few anecdotal reports that show successful antiviral treatment in patients with DLBCL. To clarify our thoughts, as suggested by the reviewer, we added a sentence in the conclusions paragraph: "Unless contraindicated by the hepatologist due to older age or particular comorbidities, we believe that antiviral treatment should be strongly considered after the end of immuno-chemotherapy, when a remission of the lymphoma has been achieved.".

C. Clinical management and tolerance to treatment: For the HCV-associated DLBCL patients, CHOP associated with Rituximab is the standard of care. Some studies demonstrated that antiviral treatment yet does not play a significant role in HCV-positive DLBCL. What do you think of the necessity of antiviral treatment according to your clinic medical work studies? Do you have any good suggestions for clinician?

RESPONSE: See previous response. Experiences are few, but we believe that antiviral therapy should always be recommended when possible.

D. It is significant that care must be used to monitor for acute and chronic hepatotoxicity associated with therapy. When should discontinue therapy due to severe hepatic function and what is the next therapeutic schedule?

RESPONSE: The decision to stop treatment should be taken together with the hepatologist. Usually, a sudden transaminase increase of more than 3x should be enough to observe the patient postponing the cycle. If values worsen or do not recover a couple of weeks later, it is probably safer to discontinue treatment. No other less toxic therapeutic schedule compared to R-CHOP is available with comparable effectiveness. This sentence has been added in the end of the "tolerance" paragraph: "When sudden transaminase increase occurs, subsequent cycles should be postponed before deciding to definitively stop treatment.". A specific paragraph has also been added to specify how to behave in several conditions (see page 9 and 10).

E. According to your clinical researches, what are clinical characteristics for HCV co-infected HBV patients with DLBCL? Is antiviral therapy including anti-HBV or HCV necessary?

RESPONSE: HCV+HBV coinfected patients are an extremely high risk population and should be treated very cautiously. As stated in the abstract, tips, and article their probability of viral reactivation is higher than in HCV isolated infection. Lamivudine prophylaxis is required in uncomplicated cases, while others require active antiviral therapy before starting chemotherapy. This problem has now been discussed in the last paragraph "Summary and conclusions".

F. SUGGESTIONS FOR IMPROVEMENT Clinical presentation and Outcome Line 3, "…and elevated LDH compared to their HCV-negative counterparts" LDH should examine lactate dehydrogenase (LDH). Simultaneously, Line 15, LDH should examine.

RESPONSE: WE thank the reviewer and we amended such mistake.

## SECOND REVIEWER

This is an interesting and well revision addressing the interaction between HCV infection and aggressive malignant lymphoproliferative disorders. I have some comments that I believe will help to improve the paper prior acceptance. Comments:

A. Throughout the article (abstract; page 2 – introduction; page 7 – clinical management), the authors use the term "active hepatitis". The term "active hepatitis" is dubious because it may refer to the "Chronic Active Hepatitis" (an old term to define histological features associated

with chronic hepatitis, and no longer used) or merely the active replication of a hepatitis virus. Active hepatitis (chronic inflammation with or without fibrosis) is a common feature of chronic hepatitis C, so the authors must explain better what they meant.

RESPONSE: We thank the Reviewer for this useful hint. We modified the text accordingly substituting active hepatitis with "grade >2 transaminases elevation" or deleting the term when no necessary (as on page 7).

B. I believe that a section addressing future perspectives and conclusions would be helpful for the readers.

RESPONSE: We agree with this suggestion by the reviewer. Therefore, we have added a paragraph entitled "Summary and conclusions" has been added at the bottom of the manuscript.

C. Explanation of the abbreviations must be included in the table.

RESPONSE: abbreviations have been added to the table.

## THIRD REVIEWER

1) A comprehenssive review well written & well prepared but need slight modifications.
A -The term active hepatitis is no longer used as now all classification according to METAVIR OR Ishak scoring system. –

RESPONSE: We thank the reviewer for such suggestion and we agree the term "active hepatitis" should not be longer used. We used "grade >2 transaminase elevation" along the text to substitute that term, as appropriate. For the purpose of our study, which is based on hematological malignancy, transaminase level looks the most useful value to be monitored, and seems to fit with the previous meaning we gave to active hepatitis.

B- The guidelines used when chemotherapy induce hepatotoxicity must be mentioned.

RESPONSE: We added the reference to our criteria for establishing hepato-toxicity, which were the Common Terminology Criteria for Adverse Events (CTCAE), v4.0. See page 7.

C - Also more details about the use of antiviral ttt associated with chemotherapy better to be discussed

RESPONSE: Differently from indolent B-cell lymphomas, antiviral treatment yet does not play a significant role in HCV-positive DLBCL. Since the treatment of DLBCL is usually based on rapidly active drugs due to the aggressive clinical behaviour of the disease, antiviral regimens have not been tested so far on large series. As suggested by the reviewer, further discussion on this topic has been added in the last paragraph of summary and conclusions. We also added the suggested reference of two case reports dealing with two cases whose flare was treated with ribavirine concomitantly to chemotherapy.

D- What the condition of HCV &HBV coinfection associated with DLBCL ?

RESPONSE: As already stated in the response to Reviewer 2, HCV+HBV coinfected patients are an extremely high risk population and should be treated very cautiously. Their probability of viral reactivation is higher than in HCV isolated infection, as outlined in the abstract, tips and in the text. Further discussion on this topic has also been added in the last paragraph of the manuscript.

## FOURTH REVIEWER

My major concern is that the presentation given seems to fit more with a hematological than with a gastroenterological journal. In this regard,

A- the chapter "Clinical Management and tolerance to treatment" should be integrated (in the text or in a table) with all available precise data concerning liver toxicity (enzyme elevation, progression to liver failure, decompensation of pre-existing cirrhosis, liver-related death) from each of the few studies on HCV-positive patients with aggressive lymphoma undergoing treatment with chemotherapy with/without rituximab.

RESPONSE: We totally agree with the Reviewer that our view of the topic is purely hematological. We also verified the published paper and it seems that the argument has been only marginally followed by gastroenterologists so far. Our review is an “invited” one, and we are hematologists. This makes it difficult to answer these questions in detail, as only a liver specialist would probably do. We have added a sentence in the “Clinical management and tolerance to treatment” paragraph on page 7 to specify this point, and possibly drive the attention of hepatologists on this argument. Nevertheless, we make ourselves available for future collaborations with hepatologists to better discern these important points.

B- Moreover, in another chapter, Authors should suggest how to monitor these patients while on treatment (enzymes? RNA? what timing) and eventually if there has been any evidence, even if very preliminary or from short series, of possible "rescue" hepatoprotective treatment once liver toxicity has developed in these patients (see for example: Pellicelli AM, Zoli V. Role of ribavirin in hepatitis flare in HCV-infected patients with B cell non Hodgkin's lymphoma treated with rituximab-containing regimens. Dig Liver Dis 2011;43:501-2).

RESPONSE: We added a paragraph on page 9 with our suggestions on how to behave when treating these patients, with timing of samples. We also added in the previous paragraph, as correctly suggested, this letter regarding ribavirin treatment of hepatic flare, which seemed of great interest. Some hints on enzymes and RNA monitoring have also been added to the tips and to the last new paragraph.

C- The Figure on expected trends of HCV-RNA and transaminase levels in an "DLBCL ideal patient experiencing transient toxicity" seems speculative.

RESPONSE: We agree that this figure is speculative. For this reason we added a comment in the legend of the figure, stressing that this is an ideal behavior, speculative, based on subjective experience. We also added in the text (Page 8) that this represents a patient without known risk factors. We have no problems in removing it from the article, although we believe it could be useful to the clinicians in order to rapidly visualize what generally expected after treating these patients. Therefore, if the Editor agrees, we'd keep this figure with suggested modifications of the legend.