Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP) *CONICET-GCBA*

CONICET Buenos Aires Ciudad
I M I P P

ROUND 1

Lian-Sheng Ma,

Science Editor, Company Editor-in-Chief, Editorial Office
World Journal of Gastroenterology

Please find attached the revised manuscript entitled **"Viral hepatitis update: progress and perspectives"** (ID 63182) to be considered for publication as a review in the World Journal of Gastroenterology. Here after I include the point-by-point response to reviewers, and I submit a version of the manuscript with an active change track as supplementary material (only to be seen by the reviewer and the editor).

Sincerely yours,
Pamela Valva, PhD.
Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP- CONICET-GCBA) Laboratorio de Biología Molecular, División Patología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. Gallo 1330, CABA, Buenos Aires, C1425EFD, Argentina.

Response to reviewers

All the authors and I would like to acknowledge the editorial board and the reviewers the suggestions and corrections performed to our manuscript, as well as the interest in our work. We have made a point-by-point list with all the changes performed to the manuscript.

1) Very minor English language polishing is needed, some sentences have minor grammar errors e.g. page 5 "Despite that HAV was discovered more than 4 decades ago" e.g. page 13 "One of the most appealing strategies implies targeting HBV cccDNA and its transcriptional activity" page 21 "However, it should be bear in mind that besides treatment is expected to eliminate the risk" page 22 "it is expected a risk reduction since the viral clearance lower morbidity and mortality rates"

Response:

English language polishing listed above were corrected. Moreover, the English of the revised version of the manuscript was again reviewed to verify that the language of the manuscript reached grade A.

2) The sections on each type of hepatitis should have subheadings to break them up, for ease of reading (e.g. molecular basis, epidemiology, treatment, vaccination)

Response:

We agree with the reviewer's suggestion and, consequently, we added subheadings to each section.

3) Given how wide-ranging and discursive this article is, I think an additional table/box would be helpful that concisely summarizes the key recent advancements for each hepatitis, the strategies currently under investigations, and the future research needs.

Response:

In accordance with the reviewer's comment, we have added different boxes summarizing the most relevant aspects addressed in the manuscript.

4) In the abstract the sentence "Viral acute hepatitis can be resolved without intervention or it can sometimes turn into a chronic infection" should be clarified that this pertains only to hepatitis B, C, D and E, not hepatitis A.

Response:

The reviewer's observation is correct. We intended to present the possible scenarios (acute vs. chronic) without detailing in which case each occurs, since this is detailed in Table 1. However, in order to make this sentence in the introduction clearer, it was slightly modified. (page 5 in the version of active change track).

5) It is stated in the Hepatitis A section discussing circulation patterns "a) in high endemicity areas from low- and middle-income countries, where the incidence varies from low to high, there is a peak age of infection in early childhood, the transmission pattern is person-to-person, and outbreaks are uncommon". This sentence is a bit confusing. What does "where the incidence varies from low to high" mean? Does it mean the incidence varies over time? Or between different regions of the country? Similarly, why would outbreaks be uncommon in a region of high endemicity, and common in regions of low endemicity?

Response:

The expression "where the incidence varies from low to high" means variations over the time and the different regions within a country.

Regarding the question "why would outbreaks be uncommon in a region of high endemicity, and common in regions of low endemicity?": in areas with high viral circulation (high endemicity), infections usually occur in childhood, with high rate of asymptomatic cases. So, people reach youth and adulthood with high levels of immunization, which reduces the frequency of outbreaks. The opposite would occur in areas with low viral circulation (low endemicity).

6) It is stated that "Because of HAV pediatric immunization, in addition to the improvement of socio-economic, hygienic and sanitation measures, young adults are now becoming more susceptible to HAV infections, so the prevalence of symptomatic cases in this age group has increased." I don't understand the link between the two clauses of this sentence. Why would better immunization and improvement of hygiene lead to young adults becoming more susceptible to infection, not the other way round?

Response:

Improvements in the economic and sanitation situation, as well as the introduction of vaccination programs in children in the last years, may translate into an increase in the number of adults who have never been infected and who lack immunity (because they were not naturally infected and/or because they were not included into current vaccination programs). In order to make this sentence clearer, it was slightly modified (page 7 in the version of active change track).

7) The HAVNET should be defined.

Response:

In the original version of the manuscript, an explanation of what is the HAVNET have already been included in page 8: This is an international HAV network of scientists who work in reference laboratories of hepatitis A and share molecular and epidemiological data on this virus, information that is really useful for the scientific community. The HAVNET aims to increase the knowledge about HAV infections and map the worldwide distribution of HAV strains. However, we added the following clarification of HAVNET: "Hepatitis A Virus Network" (page 8 in the version of active change track).

8) In the hepatitis B section, it is stated "from HBeAg-negative infection (formerly called inactive carrier state) to chronic hepatitis with different degrees of severity". I think this definition of inactive carrier state is inadequate. Not all HbeAg-negative infection is an inactive carrier state, which was defined as (as per Kumar et al, Virol J. 2005; 2: 82) "absence of HBeAg and presence of anti-HBe, undetectable or low levels of HBV DNA in PCR-based assays, repeatedly normal ALT levels, and minimal or no necroinflammation, slight fibrosis, or even normal histology on biopsy".

Response:

Nomenclature for the phases of chronic HBV infection has evolved over time and varies slightly between different international societies.
In their latest guideline, the European Association for the Study of the Liver have proposed to base the stages of the natural history of HBV on the description of the two main characteristics of chronicity: infection vs. liver disease [European Association for the Study of the Liver. Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017; 67: 370-398. doi: 10.1016 / j.jhep.2017.03.021].
According to the latest update, the different stages were identified as:
1- HBeAg-positive chronic HBV **infection** (previously known as ''immune tolerant").
2- HBeAg-positive chronic HBV **hepatitis** (previously termed "Immune clearance" or "HBeAg-positive chronic active hepatitis").
3- HBeAg-negative chronic HBV **infection** (previously termed 'inactive carrier' or "low replicative chronic HBV infection").
4- HBeAg-negative chronic HBV **hepatitis** (previously termed "Immune escape", "Negative HBe Chronic hepatitis" or "Reactive phase").

For this reason, due to recent changes in the nomenclature, we have pointed out in the text that "HBeAg-negative chronic HBV infection" refers to the previously called inactive carriers.

On the other hand, the Asian Pacific Association for the study of the liver guideline suggests that the term "*inactive carrier*'' *should be avoided, as HBV infection is a dynamic interaction between the host and the virus, and the inactive state could change at different time points and gives the individual an undue false sense of security*", and they propose to use the term "Low replicative chronic HBV infection" [Sarin SK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016; 10:1-98. doi: 10.1007/s12072-015-9675-4].
Anyway, to avoid misunderstandings, we modify the text, using the previously accepted nomenclature. (page 10 in the version of active change track).

9) It is stated regarding hepatitis B chronicity that "gender, alcohol intake, obesity, and comorbidities were also reported to play a role"- in what way? Can the effect of these factors be described in more detail?

Response:

Several studies have identified non-genetic host factors associated with the evolution of the course of chronic infection.
Regarding gender, different studies have identified a worse evolution in males than in females. Moreover, heavy alcohol consumption contributes to the development of end-stage

liver disease, as well as the presence of comorbidities, in particular coinfections with other hepatotropic viruses. To clarify this paragraph, the text was modified, these factors were described in more detail and additional references were included (page 11 in the version of active change track).

10) The authors quote a study by Liu et al stating "Nonetheless, qHBsAg has become a useful diagnostic tool to discriminate low replicative chronic HBeAg-negative infections from HBeAg-negative chronic hepatitis" in a paragraph that is discussing the correlation of qHBsAg with cccDNA. However, the study by Liu et al does not analyse cccDNA levels at all. Furthermore, its findings were not exactly that once-off qHBsAg measurement helps to "discriminate low replicative chronic HBeAg-negative infections from HBeAg-negative chronic hepatitis". In fact, "The correlation between baseline HBsAg and HBV DNA levels was moderately low, with a correlation coefficient $R = 0.369$ ($P < 0.001$)." Instead, it was useful in predicting an inactive carrier state over an 18 month follow up period, wherein the viral load remained persistently <2000 IU/mL. The practical applicability of qHBsAb measurement was in obviating the need for regular viral load measurements over time.

Response:

As the reviewer points out, the original version was inaccurate, therefore the entire paragraph has been rewritten (pages 12 and 13 in the version of active change track).
Regarding the use of qHBsAg as a diagnostic tool in the context of chronic HBV infections, in recent years several studies have characterized qHBsAg levels at different stages of the natural history of chronic HBV infection, in order to determine its usefulness in clinical practice. Particularly, in the HBeAg negative stage that encompasses two contrasting clinic-pathological conditions, inactive carriers and patients with HBeAg negative chronic hepatitis. Inactive carriers were found to show significantly lower qHBsAg levels than HBeAg-negative chronic hepatitis patients. In this way, single-point combined HBsAg and HBV-DNA quantification provides the most accurate identification of inactive carriers, comparable with that of long-term tight monitoring.
Consistent with these findings, guidelines from the European Association for the Study of Liver Disease and the Asia Pacific Association for the Study of the Liver endorsed this algorithm for identifying inactive carrier patients.
The references of the paragraph were also modified.

11) The authors state "NAs rarely achieves functional cure and have high chances of HBV reactivation when therapy is discontinued, implying lifelong therapy". However, there is an increasing body of literature discussing the prevalence of sAg loss and functional cure with a "therapeutic flare" after NA discontinuation. See Hadziyannis et al Gastroenterology 2012, 143, 629–636.e1; Jeng et al Gastroenterology 2012, 143, 629–636.e1; Papatheoridis et al Antivir. Ther. 2018, 25, 25; Liu et al Hepatology 2019, 70, 1045–1055; Hall et al Viruses 2020 Aug 25;12(9):934;. This could be discussed in brief.

Response:

As noted by the reviewer, there is a growing body of literature addressing the issue of duration of treatment for chronic B infection with nucleo(s)tide analogs (NA); however, this topic remains controversial.

It is still debatable whether treatment with NAs can be discontinued in patients who have, particularly in HBeAg-negative chronic hepatitis patients, since rebound rates go up to 50% during the first year after therapy.

The main reasons for discontinuing NAs therapy are risk of side effects (reduction of renal function and bone mineral density), increasing cumulative cost and the decline adherence. While the main argument against discontinuation of long-term NAs therapy is the risk of severe HBV reactivation and clinical exacerbation following the frequent virological and often biochemical relapses.

No uniform approach is currently available among international scientific associations regarding when and how to stop the administration of NAs without compromising optimal management in the majority of patients with chronic hepatitis B.

On the one hand, the EASL [EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017; 67:370-398] and APASL [Sarin SK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016; 10:1-98] suggest a "stopping rule" in patients without cirrhosis before HBsAg seroclearance, with an undetectable HBV DNA level on three occasions, 6 months apart after at least 2 years of treatment. On the other hand, the AASLD indicate treatment withdrawal can be exercised only in patients who achieve HBsAg seroclearance [Terrault NA et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018; 67:1560-1599]. Unfortunately, the clearance of HBsAg is an uncommon event during NAs therapy. In fact, according to mathematical models, HBsAg sustained clearance could only be attained after a period of approximately 30-50 years of therapy with NAs.

For these reasons, several attempts are being made to develop more effective therapies to obtain a functional cure of chronic infection.

Following the suggestions of the reviewer the text of the manuscript was modified (pages13 and 14 in the version of active change track).

12) The paragraph opening the Hepatitis C section, detailing its historical discovery, could be shortened to a sentence or two. At present it seems unnecessarily long and hagiographic.

Response:

According to the reviewer's suggestion this paragraph was shortened (page 17 in the version of active change track).

13) It is stated "When cured patients eventually are re-infected with HCV, it is still not clear if they will need to be DAA re-treated or if they could spontaneously control HCV infection since their immunity will be restored after a successful DAA treatment". I am unaware of any international guidelines, let along empirical evidence, that suggests that reinfected patients could "spontaneously control HCV infection". All guidelines advocate for retreatment of this patient population to reduce further morbidity, transmission and mortality (especially as many HCV patients may be re-infected with different genotypes). The authors cite an article by Maticic et al after this sentence. However, the article does not mention anything about this...

Response:

The reviewer's observation is correct. The bibliography cited in that paragraph is not correct. The sentence "When cured patients eventually are re-infected with HCV, it is still not clear if they will need to be DAA re-treated or if they could spontaneously control HCV infection since their immunity will be restored after a successful DAA treatment" was written according to the following publication: Wedemeyer H, Khera T, Strunz B and Björkström NK (2020) Reversal of Immunity After Clearance of Chronic HCV Infection—All Reset? Front. Immunol. 11:571166. doi: 10.3389/fimmu.2020.571166 (page 2). However, it is true what the reviewer indicates when he mentions that all guidelines advocate for retreatment of this patient population to reduce further morbidity, transmission and mortality (especially as many HCV patients may be re-infected with different genotypes). Taking this into account and in order not to be confusing, we decided to remove that sentence from the manuscript. (page 18 in the version of active change track).

14) The authors state "transplanting organs from HCV-infected donors into infected or uninfected recipients is now a reality." However, I think this paragraph presents view of this situation that is a bit too premature and optimistic. AASLD 2020 guidelines by Ghany et al state "Although early outcome data are encouraging, the overall experience is limited, and many ethical issues and scientific questions remain, such as avoidance of selection bias, the optimal timing of DAA therapy, detailed evaluation of drug–drug interactions between DAAs and immunosuppressants, and long-term graft and patient outcomes... there are no data on possible long-term hepatic and extrahepatic adverse effects associated with HCV exposure, even among those cured of the infection." As such, transplanting livers from HCV-infected donors into uninfected recipients requires special approval from governing bodies in the USA and in nearly all countries around the world. It is by no means a mainstream reality.

Response:

This sentence was written on the basis of the recent revisions made by Kahn JA. The use of organs from hepatitis C virus-viremic donors into uninfected recipients. Curr Opin Organ Transplant 2020; 25(6): 620-625 [PMID: 33105203 DOI: 10.1097/MOT.0000000000000826] and by Kappus MR, Wolfe CR, Muir AJ. Direct-Acting Antivirals and Organ Transplantation: Is There Anything We Can't Do? J Infect Dis 2020; 222(Supplement_9): S794-S801 [PMID: 33245347 DOI: 10.1093/infdis/jiaa420]. However, we agree with the reviewer that it is not a mainstream reality and, therefore, has many points that still need to be discussed. This is why we added the paragraph mentioned by the reviewer to the manuscript and cited the article Ghany MG, Morgan TR, Panel A-IHCG. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America (pages 21 and 22 in the version of active change track).

15) The authors describe that "DAA therapy reports informed a potential high risk of HCC occurrence and recurrence after treatment". However, this paragraph should be deemphasised. As the authors state, "it is likely that the impact of DAAs on HCC risk may be an old tale"- this is truly the case, expert opinion and large prospective studies/meta-analyses show that the previous fears raised by the articles of Reig et al and Conti et al from around 2016 are unfounded; and were likely affected by selection bias of older, more

cirrhotic patients compared to previous IFN-based studies. See studies by Guarino et al, Liver Cancer International, July 2020, 1:1 pp 12-2; Waziry et al J Hepatol 2017;67:1204-12; Saraiya et al Aliment Pharmacol Ther 2018;48:127-37; Ioannou et al J Hepatol 2018, 68, 25–32. Essentially, I believe that this matter is no longer under debate and doesn't need to be highlighted in this review article. The implications of those previous fears (making clinicians reluctant to offer HCV treatment to those with previous HCC/ those at perceived high HCC risk) are clinically detrimental to the patient population at large.

Response:

Although we agree with the reviewer and, as we mentioned in the manuscript, the impact of DAAs on HCC risk is an old tale, we consider that it is important, and it should be included in our manuscript. However, to make the idea clearer, we modify some parts of this section and include the bibliography mentioned by the reviewer (pages 22 and 23 in the version of active change track).

16) The hepatitis E section has no paragraph about treatment of hepatitis E. While the vast majority of cases are of course treated conservatively, there are worthwhile discussions to be had about the role of ribavirin or IFN treatment in certain scenarios, especially post transplant (see Shrestha et al Euroasian J Hepatogastroenterol. 2017 Jan-Jun; 7(1): 73–77; Goel et al Expert Rev Gastroenterol Hepatol. 2016 Sep;10(9):1065-74., Lhomme et al J Clin Med. 2020 Feb; 9(2): 331; Horvatits et al Viruses. 2019 Jul; 11(7): 617.)

Response:

The reviewer's observation is correct. We included a new paragraph regarding hepatitis E treatment including a reference (page 34 in the version of active change track).

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.

Response:

The grammar, sentence structure, spelling, capitalization, punctuation, format, and the manuscript's language were reviewed.

EDITORIAL OFFICE'S COMMENTS

Science editor:

Scientific quality: The manuscript describes a Review of the viral hepatitis update. The topic is within the scope of the WJG. (1) Classification: Grade C; (2) Summary of the Peer-Review

Report: This is a comprehensive review. An additional table/box would be helpful. Some sentence need to be further clarified. The questions raised by the reviewers should be answered; (3) Format: There is 1 table; (4) References: A total of 175 references are cited, including 84 references published in the last 3 years; (5) Self-cited references: There are 10 self-cited references; and (6) References recommendations (kindly remind): The authors have the right to refuse to cite improper references recommended by the peer reviewer(s), especially references published by the peer reviewer(s) him/herself (themselves). If the authors find the peer reviewer(s) request for the authors to cite improper references published by him/herself (themselves), please send the peer reviewer's ID number to editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

Language evaluation: Classification: Grade B. 3 Academic norms and rules: No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by National Agency for Scientific and Technology Promotion, and National Research Council. The topic has not previously been published in the WJG. 5 Issues raised: (1) The "Author Contributions" section is missing. Please provide the author contributions; (2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); and (3) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. PMCID is not required. Please revise throughout. 6 Recommendation: Conditional acceptance.

Response:

As detail above, in the revised version of the manuscript we included boxes summarizing the most relevant aspects addressed in the manuscript. Regarding the references, some of the references suggested by the reviewer that we considered appropriate were added in the new version of the manuscript. Moreover, the Author Contributions section was also included, and the paragraph related to the grant support was removed. Finally, the references were reviewed and modified.

ROUND 2

Lian-Sheng Ma, Science Editor, Company Editor-in-Chief, Editorial Office World Journal of Gastroenterology Please find attached the revised manuscript entitled “Viral hepatitis update: progress and perspectives” (ID 63182) to be considered for publication as a review in the World Journal of Gastroenterology. Here after I include the second point-by-point response to reviewer. Sincerely yours, Pamela Valva, PhD. Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP- CONICET-GCBA) Laboratorio de Biología Molecular, División Patología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. Gallo 1330, CABA, Buenos Aires, C1425EFD, Argentina.

Response to reviewers All the authors and I would like to acknowledge the editorial board and the reviewer the suggestions performed to our manuscript, as well as the interest in our work. We have made a point-by-point list with all the changes performed to the manuscript. Reviewer #1: To the authors Thank you very much for taking most of my suggestions on board. I am really honoured to have been involved in such an substantial piece of work. Where you have argued against my suggestions, your reasoning was very clear and sensible, and I do agree with your positions. I would please ask for a bit more clarification in the revised manuscript of the following two paragraphs which remain a little bit confusing to the casual reader, incorporating the excellent responses that you have given me: 1) "Three circulation patterns have historically been described for HAV: a) in high endemicity areas from low- and middle-income countries, where the incidence varies from low to high, there is a peak age of infection in early childhood, the transmission pattern is person-to-person, and outbreaks are uncommon; b) in moderate endemicity areas, from middle-income countries (regions where sanitary conditions are variable), the incidence is high, the peak age of infection is in the late childhood/adolescence or in young adults, the transmission pattern is also from person-to-person, food and water, and therefore outbreaks are common; and c) in low endemicity

areas from high income-countries, the incidence is low, the peak age of infection is in young adulthood, the transmission pattern is from person-to-person, and also by food and water; and outbreaks are common[3]." Perhaps it could be (my suggested additions in CAPITAL LETTERS): "Three circulation patterns have historically been described for HAV: a) in high endemicity areas from low- and middle-income countries, where the incidence varies from low to high OVER TIME AND BETWEEN DIFFERENT REGIONS, there is a peak age of infection in early childhood WHICH IS FREQUENTLY ASYMPTOMATIC, the transmission pattern is person-to-person, and outbreaks are uncommon DUE TO HIGH RATES OF IMMUNITY FROM PREVIOUS CHILDHOOD INFECTION; b) in moderate endemicity areas, from middle-income countries (regions where sanitary conditions are variable), the incidence is high, the peak age of infection is in the late childhood/adolescence or in young adults WHICH IS FREQUENTLY SYMPTOMATIC, the transmission pattern is also from person-to-person, food and water, and therefore outbreaks are common DUE TO LOW RATES OF IMMUNITY FROM PREVIOUS CHILDHOOD INFECTION; and c) in low endemicity areas from high income-countries, the incidence is low, the peak age of infection is in young adulthood WHICH IS FREQUENTLY SYMPTOMATIC, the transmission pattern is from person-to-person, and also by food and water; and outbreaks are common DUE TO LOW RATES OF IMMUNITY FROM PREVIOUS CHILDHOOD INFECTION[3].

Response: In accordance with the reviewer's comment, we have modified the paragraph to include his suggestions (page 6 in the final version of the manuscript).

2) "Because of HAV pediatric immunization, as well as the improvement of socio-economic, hygienic and sanitation measures, young adults are now becoming more susceptible to HAV infections, so in areas of low and middle-endemicity, the prevalence of symptomatic cases in this age group has

increased[6]." Perhaps it could be (my suggested additions in CAPITAL LETTERS): "RECENT improvement of socio-economic, hygienic and sanitation measures MAY TRANSLATE INTO AN INCREASE IN THE NUMBER OF ADULTS WHO HAVE NEVER BEEN INFECTED IN CHILDHOOD AND THEREFORE LACK IMMUNITY. FURTHERMORE, DESPITE PEDIATRIC IMMUNIZATION PROGRAMS, MANY YOUNG ADULTS MAY HAVE BEEN ABOVE THE CUT-OFF AGES TO BE INCLUDED WHEN SUCH SOCIAL PROGRAMS WERE INTRODUCED. Therefore, young adults are now becoming more susceptible to HAV infections in areas of low and middle-endemicity, and the prevalence of symptomatic cases in this age group has increased[6]."

Response: In accordance with the reviewer's comment, we have modified the paragraph to include his suggestions to make it clearer (page 7 in the final version of the manuscript).