24th April, 2022

Manuscript ID 76322

Title: **Hepatocellular Carcinoma, Decompensation, and Mortality Based on Hepatitis C Treatment: A Prospective Cohort Study**

Dr. Subrata Ghosh & Dr. Andrzej S Tarnawski

Editors-in-Chief

World Journal of Gastroenterology

Dear Drs. Ghosh & Dr. Tarnawski;

We thank you for giving us the opportunity to revise our paper (76322) titled "Hepatocellular Carcinoma, Decompensation, and Mortality Based on Hepatitis C Treatment: A Prospective Cohort Study" and the helpful comments from the reviewers.

We have attached a revised version showing the changes in red font and separately listed our point-by-point responses. We feel that the comments have allowed us to improve the paper and hope you convey our gratitude to the reviewers for their time and effort in reviewing our manuscript.

Yours sincerely,

Sook-Hyang Jeong, M.D., Ph.D.
Professor, Department of Internal Medicine
Seoul National University Bundang Hospital, Seoul National University College of Medicine
82, Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 13620, South Korea
Tel: +82-031-787-7034; Fax: +82-31-787-4052; E-mail: jsh@snubh.org

Point-to-point responses to the specific comments of the editor and reviewers

Reviewer: 1

General comments:

The Authors of the paper "Hepatocellular carcinoma, decompensation, and mortality based on hepatitis C treatment: a prospective cohort study" performed a prospective analysis of the long-term outcomes for patients with chronic hepatitis C infection untreated and treated with antiviral therapy, IFN- or DAA- based. The manuscript is well-written and provides valuable data from clinical practice. It should be emphasized that the observed population consists of patients with different degrees of severity of liver disease at baseline, which increases its value. However, below I present some questions and critical comments to the paper.

1. Here is information in the "Methods" about the exclusion of 62 patients with positive HBV serology from the analysis. What tests are they about? HBsAg(+) only? Or anti-HBc(+) also? Since 62/2485 gives only 2,5% and the analysis concerns the Asian population with high endemicity of HBV infection (according to reference cited below the rate of HBsAg(+) among the population in South Korea was 3%), I assume that the Authors mean HBsAg positivity only (doi: 10.3904/kjim.2019.007.). Therefore, I have a question about the percentage of patients with positive anti-HBc antibodies. Was it assessed? How many anti-HBc positive patients were diagnosed with liver cirrhosis? Did they receive nucleos(t)ide analogues as prophylaxis of HBV reactivation? Were they monitored for the HBV reactivation during treatment and follow-up period? It is known that the risk of HBV reactivation following antiviral therapy in HBsAg(-)/anti-HBc(+) patients, exists and cannot be omitted especially in cirrhotics. It is a very important issue due to the impact of HBV reactivation on the risk of HCC, decompensation, death, and liver transplantation in HCV-infected patients with liver cirrhosis, especially but not only, in DAA treated patients. This issue should be included and discussed in the manuscript (doi: 10.1007/s12072-019-09988-7.). If this parameter has not been analyzed, it should be listed among limitations in "Discussion".

Response 1: Thank you for your valuable comments. As you expected, we only exclude HBsAg (+) patients, and this is clearly mentioned in the Method, study subjects.(see page 7, paragraph 2)

As the reviewer pointed out, HBcIgG positivity can be an important confounding factor, which potentially related to worse clinical outcomes. Therefore, the proportion of HBcIgG positive patients is additionally presented in the revised Table 1, Table 4, and Supplementary Table S1. Because HBcIgG testing was not mandatory in the protocol of this prospective study, there were considerable

missing values (n=612/2054, 29.8%), and previous propensity-score (PS) matching 218 pairs (SVR cohort) showed a significant difference in HBcIgG positivity (5.6% versus 12.3%, p=0.042). Therefore, we redo PS matching that includes HBcIgG positivity, and new PS matching 213 pairs showed a similar proportion of HBcIgG positivity (7.8% versus 9.7%, p=0.546). However, they showed similar outcomes compared to the previous results of 218 pairs (see Figure 3).

When we compared the HBcIgG positivity between cirrhosis group (12.4%, 49/395) and non-cirrhosis group (9.1% , 95/1047; p=0.062), it was not statistically significant. In addition, HBcIgG was not a significant risk factor for the development HCC (HR 0.39, 95% CI 0.05–2.89, p=0.358) and occurrence of death/transplantation (HR 1.27, 95% CI 0.37–4.33, p=0.700) using univariate Cox regression analysis among SVR cohort. Therefore, HBcIgG positivity was not a significant factor for the development of outcomes in this study. This result was added in the method and result section of the revised paper.(see page 15, paragraph 2)

In terms of HBV reactivation, there was no ALT flare during DAA treatment in the HBcIgG positive group, though we did not test HBV DNA in them. Therefore, we added a comment in the discussion as a limitation of our study. (see page 21, paragraph 3).

Changes in the text:

Page 7, Paragraph 2.

Patients who met any of the following criteria were excluded: positive serology for hepatitis B virus surface antigen (n=62)

Page 15, Paragraph 2.

However, HBcIgG (HR 0.39, 95% CI 0.05–2.89, p=0.358) and presence of fatty liver disease (HR 0.16, 95% CI 0.02–1.18, p=0.072) were not a significant risk factor for the development HCC.

Page 16, Paragraph 1.

However, after PS matching, the DAA-SVR group showed no significant differences in the risks of HCC (HR, 2.72; 95% CI, 0.63–11.81; *P*=0.179), decompensation (HR, 9.74; 95% CI, 0.42–224.81; *P*=0.155), and death/LT (HR, 2.18; 95% CI, 0.47–10.15; *P*=0.318) compared with the IBT-SVR group (Figure 3).

Page 21, Paragraph 3.

Fourth, presence of anti-HBc IgG in HCV patients has been implicated in HCC development (Mak LY, et al. *J Hepatol*. 2020;73(4):95), and non-negligible risk of HBV inactivation during DAA treatment (0.91%).(Kanda, T. et al. *Hepatology International* 2019:13(6):649). However, approximately 30% of our patients did not test anti-HBc IgG, and none of anti-HBc IgG positive patients tested HBV DNA during DAA treatment. Likewise, the role of fatty liver in the

outcomes of HCV patients was not completely evaluated due to missing data.

Figure 3. Cumulative incidences of HCC, decompensation, and death/transplantation in entire cohort.

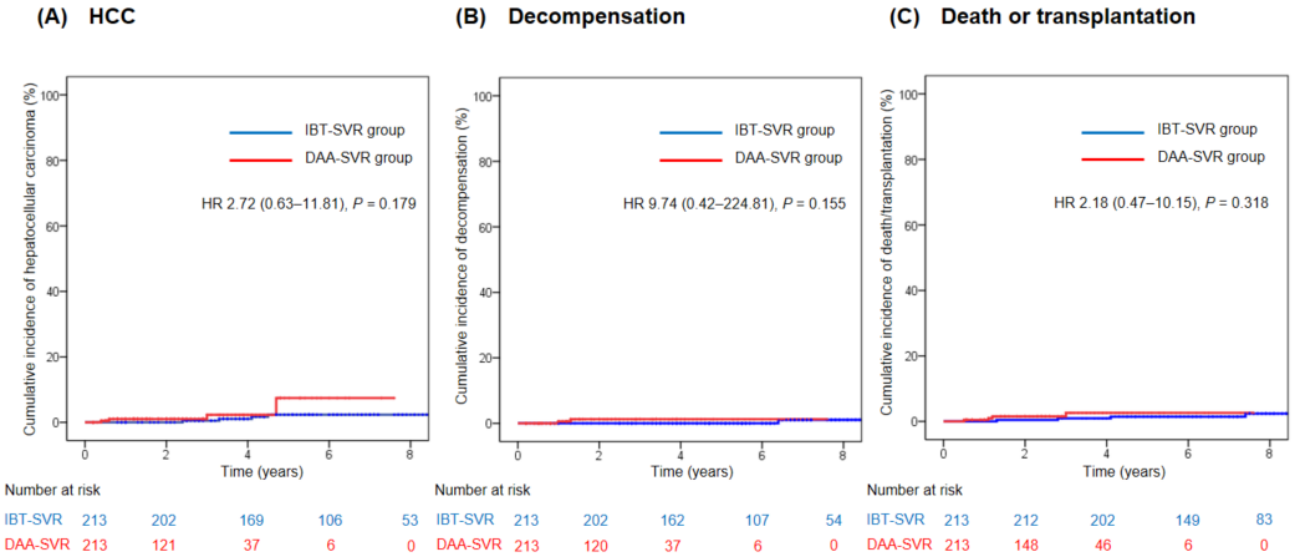

Table 1. Baseline Characteristics of the Entire HCV Cohort according to the Treatment

Variable, n (%)	Untreated group (n=619)	IBT group (n=578)	DAA group (n=857)	*P* (3 Gr.)	*P* (U vs I)	*P* (U vs D)	*P* (I vs D)
Fatty liver disease on USG, n=1389	71 (19.3), n=367	86 (21.1), n=408	140 (22.8), n=614	0.435	0.549	0.202	0.516
Anti-HBcIgG (+), n=1442	47 (11.0), n=429	33 (8.4), n=391	64 (10.3), n=622	0.460	0.225	0.730	0.330

Table 4. Comparison of Characteristics and Outcomes Between IBT-SVR and DAA-SVR Groups: Unadjusted and PS matching Groups

Variable	Before adjust				PS matched			
	IBT-SVR group (n=451)	DAA-SVR group (n=817)	*P*	SMD	IBT-SVR group (n=213)	DAA-SVR group (n=213)	*P*	SMD
Fatty liver disease on USG	77 (22.8), n=337	131 (22.4), n=586	0.863	0.039	34 (24.3)	35 (24.8)	0.917	0.013
HBcIgG positivity	25 (8.6), n=290	62 (10.5), n=592	0.386	0.062	12 (7.8)	15 (9.7)	0.546	0.062

Supplementary Table 1. Clinical Characteristics at the Time of Treatment Initiation and Clinical Outcomes Among IBT and DAA Groups According to SVR Status

Variable	IBT group			DAA group		
	IBT-SVR group (n=451)	IBT-No-SVR group (n=217)	*P*	DAA-SVR group (n=817)	DAA-No-SVR group (n=40)	*P*
Fatty liver disease on USG	77 (22.8), n=337	22 (15.6), n=141	0.075	131 (22.4), n=586	9 (32.1), n=28	0.228
HBcIgG positivity	25 (8.6), n=290	14 (8.7), n=161	0.978	62 (10.5), n=592	2 (6.7), n=30	0.503

2. The second question is about fatty liver disease. Have patients been assessed for this condition and its impact on the long-term outcomes?

Response 2: Thank you for your valuable comments. As the reviewer pointed out, presence of fatty liver disease can be another confounding factor, which potentially related to worse clinical outcomes. Therefore, the proportion of fatty liver disease based on ultrasonography or CT scan is additionally presented in the revised Table 1, Table 4, and Supplementary Table S1. There were considerable missing values (missing data=32.4%, 665/2054), however, the proportion of fatty liver disease was not significantly different among the 3 groups, and presence of fatty liver disease was not a significant risk factor for the development HCC (HR 0.16, 95% CI 0.02–1.18, p=0.072) or occurrence of death/transplantation (HR 0.92, 95% CI 0.34–2.44, p=0.860) using univariate Cox regression analysis among SVR cohort.

We addressed this point as a limitation of this study in the Discussion section (see page 21, paragraph 3).

Changes in the text:

Page 15, Paragraph 2.

However, HBcIgG (HR 0.39, 95% CI 0.05–2.89, p=0.358) and presence of fatty liver disease (HR 0.16, 95% CI 0.02–1.18, p=0.072) were not a significant risk factor for the development HCC.

Page 21, Paragraph 3.

Fourth, presence of anti-HBc IgG in HCV patients has been implicated in HCC development (Mak LY, et al. *J Hepatol*. 2020;73(4):95), and non-negligible risk of HBV inactivation during DAA treatment (0.91%).(Kanda, T. et al. *Hepatology International* 2019:13(6):649). However, approximately 30% of our patients did not test anti-HBc IgG, and none of anti-HBc IgG positive patients tested HBV DNA during DAA treatment. Likewise, the role of fatty liver in the outcomes of HCV patients was not completely evaluated due to missing data.

3. And the last comment concerns the other studies evaluating long-term outcomes in HCV-infected patients. I suggest discussing paper reporting the most prolonged follow-up in a DAA-cured real-world population observed for 5 years after treatment (doi: 10.3390/cancers13153694.) and manuscript on the long-term outcomes in a population of two clinical trials TOPAZ-I and TOPAZ-II after treatment with O/P/r/D±RBV (doi: 10.1111/jvh.13261.).

Response 3: Thank you for the recommendation of important papers. We cited the references, and added a comment addressing long-term follow-up study after DAA treatment in the Discussion section (see page 17, paragraph 3).

Changes in the text:

Page 17, Paragraph 3.

Recent 5-year follow-up studies after DAA treatment showed that SVR is associated with a gradual but significant reduction in liver fibrosis in terms of FIB-4, METAVIR, transient elastography, and Child-Pugh score.(Flisiak R, et al. Cancers. 2021;13(15):3694. & Poordad F, et al. J Viral Hepat. 2020;27(5):497), while induced SVR was associated with a reduced risk of clinical disease progression in patients with Child-Pugh A cirrhosis but not in patients with Child-Pugh B/C cirrhosis.(Krassenburg L, et al. J hepatol 2021;74(5):1053). In this context, this study showed approximately 85% of risk reduction of decompensation after IBT or DAA treatment in Child-Pugh A patients, while decompensated cirrhotic patients were excluded in this study.

Reviewer: 2

General comments:

The authors explored the associations between the long-term outcomes for patients with chronic HCV infection and treatment with IBT or DAA, and outcome-determining factors after SVR. The design and methods used in this study are basically reasonable. Only several minor points needs to be revised.

1. In the entire cohort, 619 (30.1%) patients were left untreated, but the authors did not explain why they did not receive treatment, for ethical principle, they should be explained. If the patients did not receive treatment because of contraindications, these contraindications must be taken into consideration, as they also lead to poor prognosis.

Response 1: Thank you for your valuable comments. During the follow-up period of this study, 78.8% (457/619) of the untreated patients were enrolled before 2015 when DAA was introduced, and 39.7% (200/619) were lost to follow-up before 2015. Though DAA therapy has been covered by a National Health Insurance system, 30% of the drug price is out-of- pocket expense of the patients, which is approximately 3000 US dollars. Therefore, the main reasons for non-treatment are the relatively high price of DAA drugs, old age, and extrahepatic or advanced hepatic malignancy. Therefore, we added these comments in the Discussion section (see page 20, paragraph 3).

Changes in the text:

Page 20, Paragraph 3.

To meet the 2030 HCV elimination target of WHO, active testing and enhanced linkage to treatment is important. In this study, 30.1% of patients remained untreated, and it should be reduced. Indeed, the untreated group in this study showed a higher mortality rate of 23.7% during median follow-up of 5.6 years (88 liver related deaths and 59 non-liver related deaths). In the untreated group, 3 patients received IBT after HCC diagnosis, and 19 patients received DAA treatment after HCC diagnosis or decompensation event (3.6%, 22/619). Majority of the untreated patients (78.8%, 457/619) were enrolled in the Korean HCV cohort before June 2015, when the first DAA was introduced, and 46.7% (289/619) were expired or lost to follow-up before June 2015. Though DAA therapy has been covered by a National Health Insurance system, 30% of the drug price is out-of-pocket expense of the patients, which is approximately

3000 US dollars. Even in DAA era, the main reasons for non-treatment are the relatively high price of DAA drugs, old age, and extrahepatic or advanced hepatic malignancy.

2. For accuracy, throughout the manuscript, “chronic HCV” should be “chronic HCV infection”.

Response 2: Thank you for your important comments. We changed “chronic HCV” to “chronic HCV infection” throughout the manuscript.

Changes in the text:

Page 6, Paragraph 1.

In 2019, the World Health Organization estimated that 58 million people worldwide had chronic HCV infection.

Page 6, Paragraph 4.

Using these data, we aimed to elucidate the clinical outcomes, including HCC, hepatic decompensation, and all-cause death, among Korean patients with chronic HCV infection.

Page 17, Paragraph 1.

We analyzed the incidence rates of HCC, decompensation, and all-cause death/LT in a large, prospective, Asian cohort including 2,054 patients with chronic HCV infection.

3. In the first sentence of the second paragraph in Introduction, “HCC” may be “HCV”.

Response 3: Thank you for your detailed review. We changed “HCC” to “HCV” in the first sentence of the second paragraph in the Introduction.

Page 6 Paragraph 2.

From the identification of HCV to the introduction of DAA therapy, interferon (IFN)-based therapy (IBT) was the only option for HCV treatment, with an approximate SVR rate of 50%.

4. In the section “Follow-up evaluations”, “unless there were no contraindications” should be “unless there were contraindications”.

Response 4: Thank you for the detailed review. This sentence is corrected as follows.

Changes in the text:

Page 9, Paragraph 2.

All patients underwent regular clinical assessments every 3 to 12 months, and HCV treatment was recommended by their attending physicians according to the treatment guidelines for HCV unless there were ~~no~~ contraindications or patients’ refusal.

5. One-way analysis of variance (ANOVA) was also used for categorical variables, is that reasonable?

Response 5: We corrected Table 1 the following sentence in the Method section using Chi-square test. For the revision of this paper, all the statistical methods were checked by the statistical support team in our institution.

Changes in the text:

Page 10, Paragraph 3.

Baseline characteristics of the patients were compared using the chi-square test ~~or one-way analysis of variance (ANOVA)~~ for categorical variables, and an one-way analysis of variance (ANOVA) or *t*-test was used for continuous variables.

Reviewer: 3

General comments:

Results of the present study were reasonable, and confirmed the previous studies. A few new findings were added in the present long followed-up observation.

Comment 1. First, guideline has recommended only DAA therapy since 2015. So, there were several differences in supporting liver therapies, imaging modalities for HCC between IBT and DAA groups. The time of patient enrollment into to study should be added Table 1. Also, authors had better add limitations in details.

Response 1: Thank you for your valuable comments. We totally agree with your comments. We added the time of cohort entry in Table 1. And we additionally discussed improvement of imaging modality and treatment options can affect the clinical outcomes between the IBT and DAA groups. (see page 22, paragraph 1)

Changes in the text:

Page 22, Paragraph 1.

Finally, compared to the IBT era, there is an improvement in diagnostic imaging modality and treatment options for HCC in the DAA era. Therefore, these points can affect the clinical outcomes between IBT and DAA Groups.

Table 1. Baseline Characteristics of the Entire HCV Cohort according to the Treatment

Variable, n (%)	Untreated group (n=619)	IBT group (n=578)	DAA group (n=857)	*P (3 Gr.)*	*P (U vs I)*	*P (U vs D)*	*P (I vs D)*
Cohort entry time				<0.001	<0.001	<0.001	<0.001
Jan.2007–Jun.2015	488 (78.8)	561 (97.1)	49 (5.7)				
Jul.2015–Jun.2019	131 (21.2)	17 (2.9)	808 (94.3)				

Comment 2. Page 10; Follow-up evaluations At baseline of patients treated with IBT or DAA was not clear. The initiation of anti-viral therapy or the end of the therapy or others? Authors should clearly mention the start of observation in each group; untreated, IBT, and DAA. How about outcome of non-SVR patients? Were there any differences between untreated, IBT, and DAA? Comparisons of outcome between IBT and DAA were interesting.

Response 2: Thank you for your important comment. The index date of the entire cohort was defined as the date of cohort entry when HCV RNA positivity was confirmed to avoid bias from immortal time periods. To compare the outcomes of patients with SVR induced by IBT and DAA (SVR cohort), the index date for the SVR cohort was defined as the initiation day of the antiviral treatment. We addressed the “index date” of the entire cohort and the SVR cohort in the

"Measurement of liver-related outcomes" in the Methods section.(See page 10, paragraph 2)

Changes in the text:

Page 10, Paragraph 2.

To calculate the outcomes of the entire cohort, the index date of the entire cohort was defined as the date of cohort entry when HCV RNA positivity was confirmed by the referred clinics. To compare the outcomes of patients with SVR induced by IBT and patients with SVR induced by DAA, the index date for the SVR cohort was defined as the initiation day of the antiviral treatment.

Comment 3. Figure 1 showed that 147 of 186 untreated patients died during observational period. This was high mortality. Cause of death should be shown in results; add data describing at least the hepatic or extrahepatic.

Response 3: Thank you for your comment. In untreated group (n=619), 186 patients experienced a composite endpoint during the observational period and 147 deaths were observed (mortality rate 23.7%). And as you recommended, we additionally addressed the cause of death as follows.

Changes in the text:

Page 13, Paragraph 1.

During this period, 113 patients developed HCC, 69 patients experienced hepatic decompensation, 202 patients died (119 liver related and 83 non-liver related), and four patients underwent LT.

Page 20, Paragraph 3.

Indeed, the untreated group had a higher mortality rate of 23.7% (88 liver related deaths and 59 non-liver related deaths) during median follow-up of 5.6 years.

Comment 4. Finally, the HCV elimination campaign is going by WHO. Why were 619 patients observed without anti-HCV therapies for 3.4-8.2 years? Some comments had better be added in Discussion.

Response 4: Thank you for your important comment. During the follow-up period of this study, 78.8% (457/619) of the untreated patients were enrolled before 2015 when DAA was introduced, and 39.7% (200/619) were lost to follow-up before 2015. Though DAA therapy has been covered by a National Health Insurance system, 30% of the drug price is out-of- pocket expense of the patients, which is approximately 3000 US dollars. Therefore, the main reasons for non-treatment are the relatively high price of DAA drugs, old age, and extrahepatic or advanced hepatic malignancy.

Therefore, we added these comments in the Discussion section (see page 20, paragraph 3).

Changes in the text:

Page 20, Paragraph 3.

To meet the 2030 HCV elimination target of WHO, active testing and enhanced linkage to treatment is important. In this study, 30.1% of patients remained untreated, and it should be reduced. Indeed, the untreated group in this study showed a higher mortality rate of 23.7% during median follow-up of 5.6 years (88 liver related deaths and 59 non-liver related deaths). In the untreated group, 3 patients received IBT after HCC diagnosis, and 19 patients received DAA treatment after HCC diagnosis or decompensation event (3.6%, 22/619). Majority of the untreated patients (78.8%, 457/619) were enrolled in the Korean HCV cohort before June 2015, when the first DAA was introduced, and 39.7% (246/619) were lost to follow-up and 6.9% (43/619) were expired before June 2015. Though DAA therapy has been covered by a National Health Insurance system, 30% of the drug price is out-of-pocket expense of the patients, which is approximately 3000 US dollars. Even in DAA era, the main reasons for non-treatment are the relatively high price of DAA drugs, old age, and extrahepatic or advanced hepatic malignancy.

Comment 5. Minor; Page 8; METHODS, Study subjects Patients who met any of the following criteria were excluded: None of the patients had a history of HCC. These sentences were misread. Authors had better revise them.

Response 5: Thank you for your comment. We change the sentence as follows.

Changes in the text:

Page 7, Paragraph 2.

Also, Patients who had HCC before or at the time of cohort entry were excluded.