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ESPS manuscript NO: 32217
Title: Impact of interferon-free antivirus therapy on lipid profiles in patients with chronic hepatitis C genotype 1b
Reviewer's code: 03647107
Reviewer's country: Thailand
Science editor: Yuan Qi
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COMMENTS TO AUTHORS

This study is very interesting and informative regarding the changes of serum lipids during and after DAA therapy. Thus, the sample size and the frequency of lipid monitoring are quite impressive to me. However, I do have a major comment that needs to be addressed, and also few minor comments. My major comment is that since the included patients were quite old (mean age of 67-68 years), they should have some comorbidities and co-medications. The authors did not mention any of these data in the manuscript. I noticed that the mean LDL levels of the patients (83-89 mg/dL) were quite low, in which perhaps they were taking lipid-lowering agents. I think it is important to state their comorbidities (that may involve in lipid levels) as well as their medication, particularly lipid lowering agents, in the manuscript (or in the table). My minor comments are (1) As this is a retrospective analytic study, the author should mention the word "retrospective" in the Study Design; (2) At the end of the 2nd paragraph in the Discussion, the authors state that "the difference in antiviral efficacy between the two regimens was not involved in the extent of the increase in serum cholesterol" I think this statement may be a bit too strong. Perhaps "…was not likely to involve…." May be better, since this is the assumption and HCV-RNA at week 2 was not tested; (3) In the 4th paragraph in the Discussion, the authors state that "the therapy regimen was not associated with the difference in virological efficacy". This statement seems questionable to me since the SVR12 was 86% versus 98% (p-value not provided). Perhaps SOF-LDV was associated with slightly better SVR.

Reply to the reviewer 1:
Thank you very much for reviewing our manuscript.

First we changed the group names to DCV+ASV-SVR group and SOF+LDV-SVR group because we made clear that in this study, we chose an SVR case and examined it. Next, we added the data of the DCV+ASV-nSVR group as requested by reviewer 2. Because the article became lengthy, we omitted the comparison of HDL-C as we considered it to be less important.

We would like to reply to your comments one by one as follows.

・Reply to major comment
As you suggested, LDL-C level is fairly low in our patients. However, as shown in our past articles and other articles from Japan, LDL-C tends to be low in patients, especially Japanese, who are infected with hepatitis C genotype 1b, (Kenichi Satoh *et al*. *World Journal of Hepatology* 2015; 8: 291-300. Hashimoto S *et al*. *PLoS One* 2016; 11: 1-12). Therefore, we do not consider it strange that mean LDL-C in our patients is as low as around 83 mg/dL. We thoroughly investigated the following medical history of our patients: oral intake of cholesterol-lowering drug, glucose-lowering drug for type 2 diabetes, past history of coronary disease, and coinfection of HBV or HIV as well as complication of hepatocellular carcinoma and decompensated cirrhosis. Moreover, addition or withdrawal of drugs that potentially affect the lipid metabolism was not performed during the study period.
We have added these data in the third paragraph of the *Patient population*.

・Reply to minor comments
(1) In order to avoid misleading that this may be a prospective study, we have added the sentence "In this retrospective study, serum lipid profiles of the patients who achieved SVR were examined" in the first paragraph in Study design. In addition, in order to emphasize that this is a retrospective study, we have added the word "retrospective" in the last paragraph of Introduction.

(2) According to your comment, we revised the following sentence: "the difference in antiviral efficacy between the two regimens was not likely to be involved in the extent of the increase in serum cholesterol."

(3) We agree with your opinion that SOF-LDV was associated with slightly better SVR.

However, in our study, we compared the patients who achieved SVR. Therefore, we have revised the sentence (now moved to paragraph 5) as follows: "As mentioned above, the therapy regimen (DCV+ASV *vs* SOF+LDV) was not associated with the difference in the final virological efficacy in these patients because we selected only the SVR patients in this retrospective study." In order to avoid the misunderstanding that SOF-LDV group contained all the patients who were treated with SOF+LDV, we have changed the nomenclature of SOF-LDV group to SOF+LDV-SVR group, etc.

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COMMENTS TO AUTHORS

The manuscript entitled "Impact of interferon-free antivirus therapy on lipid profiles in patients with chronic hepatitis C genotype 1b" shows the influence of interferon-free antivirus therapy on lipid profiles in chronic hepatitis C patients infected with HCV genotype 1b. Between DCV/ASV therapy and SOF/LDV therapy, there is a quite enormous difference in the changes of serum lipid profile which are delivered by the 2 regimen. The data shown in this manuscript are very important to take measures against it. There are comments that are needed to be addressed to the authors. Major comments: 1. The authors did not mention in detail about exclusion criteria in Materials and Methods section. Chiefly, current medication and past medical history associated with lipid profiles are necessary. Please add the description of exclusion criteria. 2. In Table 1, p-values for each items are not provided. Please add the description of p-values. Especially, in the group of patients treated with SOF/LDV, there seem to be more number of females than that of males. 3. In the Discussion, the authors argue that "This finding suggested that DCV-ASV therapy somewhat inhibited the increase in serum cholesterol." To confirm the consideration, lipid profiles of the patients whom treatment with DCV/ASV failed in and whose HCVs were still detected after treatment should be shown. Furthermore, if possible, lipid profiles of the patients whose treatment with DCV/ASV was given up within 24 weeks because of side effect and whose HCV could be lost are needed. 4. In the Discussion, the authors state that "the difference in antiviral efficacy between the two regimens was not involved in the extent of the increase in serum cholesterol." This statement sounds too durable. If the authors want to discuss the influences of each drug, the mechanisms of each drug should be suggested. Minor comments: 1. In this manuscript, references are overly abundant. Please choose only the articles which are related to the contents of your manuscript closely. 2. In "Detection of the factors affecting the change in TC, LDL-C, and HDL-C at 4 weeks of therapy by multiple linear

regression analysis" subsection, one of the therapy protocol names is wrong. In the subsection, the expression "SOF/DCV" is found. It should be correct. 3. In this manuscript, the unit used for measurement of HCV-RNA is wrong. It is not "log copy/mL" but "log IU/mL." Please correct it. 4. In the footnote of Table 1, misspellings are found: "Alubmin" and "ribonucleic asid." Please correct misspellings. 5. In the footnote of Table 2, a misspelling is found: "tryglyceride." Please correct it. 6. In the figure legend of Figure 3, the authors display "SOF-LDV: Sofosbuvir plus LDV therapy." Please change it to the correct notation.

Reply to reviewer 2

Thank you very much for reviewing our manuscript.

First, we have changed the group names to DCV+ASV-SVR group and SOF+LDV-SVR group because we made clear that in this study, we chose an SVR case and examined it. Next, we have added the data of the DCV+ASV-nSVR group as requested by reviewer 2. Because the article became lengthy, we omitted the comparison of HDL-C as we considered it to be less important.

We would like to reply to your comments one by one as follows.

・Major comment

(1) We re-investigated the medical history of our patients, especially for intake of oral cholesterol-lowering drugs. In this study, coinfection of HBV or HIV as well as complication of hepatocellular carcinoma or decompensated cirrhosis was not included. Addition or withdrawal of drugs that potentially affect the lipid metabolism was not performed during this study. This point is mentioned in the third paragraph of the *Patient population*.

(2) We have added the P-value in Table 1. There was the tendency about the sex ratio, but it did not reach the significance. This has been mentioned in Patient characteristics.

(3) Of the 20 patients who were treated with DCV+ASV but did not achieve SVR 12, 9 dropped out. Various reasons and periods were associated with these dropout patients. Therefore, it is difficult to treat these patients as one statistical group. Whereas, the remaining 11 patients who accomplished 24 weeks of DCV+ASV therapy but not achieved SVR could be treated as one statistical group. We named this group as DCV+ASV-nSVR group. In this group, TC and LDL-C did not change during the treatment, but they transiently increased after the end of treatment. This finding further supported our hypothesis that DCV+ASV therapy suppressed the increase in serum cholesterol. We added the data of this group in the Results. In addition, longitudinal changes in cholesterol level in this group are shown in Figure 4. The significance of the longitudinal changes in cholesterol level in this group is freshly discussed in the fourth paragraph in the Discussion. We think that we can partially confirm our following hypothesis: “This finding suggested that DCV-ASV therapy somewhat inhibited the increase in serum cholesterol.”

(4) Although the mechanisms affecting serum cholesterol cannot be elucidated for each drug, we have discussed the possibility of a mechanism in the sixth paragraph of the Discussion. The difference in the potency between DCV+ASV and SOF+LDV on very early virus kinetics of HCV may influence the degree of increase in cholesterol. However, the difference in the changes in cholesterol during the treatment between DCV+ASV and SOF+LDV in whom achieved SVR is difficult to explain by this mechanism alone. This issue is freshly discussed in the seventh and eighth paragraphs of the Discussion. Finally, we have revised the sentence as follows: "the difference in antiviral efficacy between the two regimens was not likely to be involved in the extent of the increase in serum cholesterol".

・Minor comment

(1) We agree with your comment. We have reduced the number of references to 30 from 45.

We apologize for the errors and have corrected them as follows:

(2) Revised "SOF/DCV" to "SOF/LDV"

(3) Revised "log copy/mL" to "log IU/mL"

(4) Revised "Alubmin" to "Albumin" and "ribonucleic asid" to "ribonucleic acid"

(5) Revised "tryglycerid" to "triglyceride"

(6) Revised "SOF-LDV: Sofosbuvir plus LDV therapy" to "SOF-LDV: Sofosbuvir plus Ledipasvir therapy"

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COMMENTS TO AUTHORS

This paper is an interesting paper and offers an insight in a phenomenon related to plasma lipids changes during and after the HCV treatment with two DAA combinations, DCA-ASV and SOF-LDV. These two combinations have one drug in each arm in common, the 5A inhibitor. The other drug in each group is different in inhibitory sites, protease and polymerase enzymes, which might affect the viral kinetic during the early phase of treatment in different way. In addition, the potency of each drug make different inhibitory effects. SOF has been used as a backbone in many regimens and shown to be stronger in viral inhibition. This clinical trial also confirms that SOF-LDV had better efficacy interm of SVR and shorter time of treatment.

As the authors stress in the discussion that SOF-LDV itself, not the viral inhibition effect alone, accelerates the increase in serum cholesterol. This trial showed the same viral inhibitory effect of both combinations at 4 week of treatment. I would like to propose the other idea that should be in the paper which may require more works to prove the theory, not for the setting in this study.

The other possible explanations are

1. The viral kinetic of each combination might not be the same during the initial phase of treatment. As there is no evaluation of viral reduction during the first few days in this trial. The author could not commit that these two combinations have the same inhibitory potency by using the viral responses at 4 week. The viral kinetic of DCV was demonstrated as a biphasic decline of virus starting from the first 12 hours of treatment [Anushree Chatterjee *et al.* Clin Liver Dis. 2013; 17: 13–26]. An also SOF-combination which suppressed virus at 2 days of therapy could result in shorter time of therapy around 4 – 6 week [Harel Dahari *et al.* J of Hepatology 2016; 64:1232-1239]. The data from a Chinese study using triple DAA regimen demonstrated the same 2-day viral detection less than 500 IU as an indicator that could shorten the treatment to 3 weeks [Lau KG *et al.* Hepatology 2015; 6:1394A]. So, it may not be the drugs, but it may be the potency of drugs could rapidly eradicate the virus.

2. The earlier the viral reduction, the better reduction of hepatic inflammation could be seen in

clinical practice using these new interferon free regimens. Cholesterol is an indicator of improving or decreasing function of the liver. Cholesterol levels become lower in progressive liver impairment. So, the increase in TC, LDLC shown in this trial might represent the improvement in liver pathology or inflammation in the SOF-LDV group faster than the DCV-ASV group.

Overall, this paper is good in term of careful design and shows a clinical phenomenon with some interesting scientific explanations. **It would be better if the clinical or scientific implications of this finding being added to the final part of the discussion.**

Reply to reviewer 3

Thank you very much for your kind advice.

First we have changed the group names to DCV+ASV-SVR group and SOF+LDV-SVR group because we made clear that in this study, we chose an SVR case and examined it. Next, we added the data of the DCV+ASV-nSVR group as requested by reviewer 2. Because the article became lengthy, we omitted the comparison of HDL-C as we considered it to be less important.

We have gratefully accept your proposal and have added these scientific and clinical implications regarding the possible mechanism of different TC levels between DCV+ASV and SOF+LDV in the sixth and seventh paragraphs of the Discussion. Thank you again for your excellent opinion.