Author’s response to reviews

Title: Chronic Hepatitis Infection Is Associated with Extrahepatic Cancer Development: A Nationwide Population-Based Study in Taiwan

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Author’s response to reviews:

Reviewer #1:

The authors present a large linkage study of site-specific cancer incidence by HCV and HBV infection status. It is a large and powerful epidemiological analysis, profiting from nationwide medical databases in Taiwan. The validity of the approach is confirmed by the strong findings for a causal effect of HCV and HBV on liver cancer risk, and also with the weaker, but now accepted association of both viruses (particularly HCV) with NHL.

However, the key messages in terms of associations with other extrahepatic sites (some of which I suspect are statistical chance findings) would be somewhat improved by a more structured prioritisation in terms of strength of evidence, also given the totality of the scientific literature. Certainly the concluding statement that "comprehensive clinical surveillance is required for ALL cancers" is clinically unhelpful and also unjustified given the evidence. The increased risk for ALL cancer is largely driven by liver cancer.

Reply: Thank you for your comment and thoughtful suggestions. We have revised the Conclusion provided after the Discussion section of the revised manuscript.
Perhaps a good starting point for discussion of findings in terms of strength and consistency of evidence is the IARC monograph 100B, where a group of experts judged upon all available evidence in 2009, and for which the summary evaluations were the following:

"There is sufficient evidence in humans for the carcinogenicity of chronic infection with HBV. Chronic infection with HBV causes hepatocellular carcinoma. Also, positive associations have been observed between chronic infection with HBV and cholangiocarcinoma and non-Hodgkin lymphoma."

"There is sufficient evidence in humans for the carcinogenicity of chronic infection with HCV. Chronic infection with HCV causes hepatocellular carcinoma and non-Hodgkin lymphoma. Also, a positive association has been observed between chronic infection with HCV and cholangiocarcinoma."

Of course there have also been many more individual studies published since this date (most relevantly, nationwide linkage studies of HCV/HBV infection and cancer mortality in Taiwan which should be carefully discussed in context), but the IARC monograph is the most up to date consensus picture to build upon.

Hence, I would suggest to discuss cancer sites in the order liver, NHL, cholangiocarcinoma (bile duct and gallbladder), for which the picture is quite clear and proves the robustness of the current approach, and then move to the more novel associations (thyroid, pancreatic), with some caveats that there may be chance findings. Signals for kidney, colorectal, ovarian and skin cancer are the least convincing in this respect.

Reply: Thank you for your thoughtful comments and suggestions. We have discussed site-specific cancers in the order of liver, non-Hodgins lymphoma, bile duct and gallbladder, thyroid, pancreas, kidney, colorectal, and ovarian cancer. However, the presence of thyroid and skin cancers were nonsignificant after a reanalysis of the data. Hence, these cancers are not discussed in the revised manuscript.

It would also be relevant to discuss where negative associations in the present study conflict somewhat with important studies that show positive ones (e.g. HCV and head and neck, Mahale P et al, J Natl Cancer Inst. 2016; HCV and prostate + esophageal cancer, Lee MH, JID, 2012) (Need to discuss the above mentioned papers)

Reply: Thank you for your thoughtful suggestion. In the revised manuscript, we have discussed the noted studies that conflict with our findings in paragraph 5 of the Discussion section.

Other important concerns:
1. I do not see any evidence of interaction between HCV and HBV on cancer risks. Risks in co-infected patients are generally not even additive and so do not suggest anything more than what would be expected from the two viruses acting entirely independently. Hence I would suggest not to report risks, at least not in the abstract, as if this is a particularly special group of patients, but rather suggest a general absence of interaction.

Reply: Thank you for your suggestion. We completely agree with your opinion. Hence, in the revised manuscript, we have deleted the mentions of these risks in the Abstract and the Discussion section. In Taiwan, it is highly likely that HBV and HCV infection are acquired through two different mechanisms. For example, vertical (maternal) transmission is likely for HBV, whereas HCV is transmitted horizontally through contaminated blood and blood product. Therefore, we have deleted the HBV/HCV coinfection analysis, and have redistributed the coinfection group back into the individual HBV and HCV categories in the revised manuscript.

2. In fact, given such evidence of independency, the power of the study may even be improved by a secondary analysis that re-distributes the HCV/HBV co-infected patients back into the individual HCV and HBV categories. This may clarify some of the more borderline and unexplainable associations (e.g. colorectal and ovarian cancer).

Reply: Thank you for your suggestion. We have redistributed the HBV/HCV coinfection into the individual HBV and HCV categories.

3. The utility of age stratified HR analysis shown in Table 4 is unclear and I would suggest to drop it. Different cancers have different age-specific incidence patterns (so that a standard <60 vs 60+ is questionable) and the number of cases in the different strata are not shown. I think that this type of analysis should only be done in an ad hoc manner if there is an a priori reason to believe that it is informative for any given cancer site (and I'm not sure if this is the case).

Reply: Thank you for your suggestion. In the revised manuscript, we have not included the findings of the age stratified HR analysis in the Results section.

Reviewer #2:

The present article includes the findings of an extraordinary record linkage study in a population that is unique in terms of high burden of HBV and/or HCV infection. Unfortunately, statistical analyses are not adequate and are inconsistent across tables. Some findings, I suspect, are
distorted by insufficient adjustment or standardization by age, sex, and, possibly, region of living. By the way, some tables are wrongly embedded in the text.

Major issues

Page 7, lines 15-20: authors state that "The index date for patients with chronic HBV or HCV infection was the first instance the chronic HCV or HBV". In which way were individuals with dual infection dealt with? You should mention, if possible, how often the two infections were detected at the same time and comment on whether of either HBV or HCV had been diagnosed the earlier, possibly before the availability of record linkage, of subsequently to the other infection.

Reply: Thank you for your thoughtful comment and suggestion. In Taiwan, it is highly likely that HBV and HCV infection are acquired through two different mechanisms. For example, vertical (maternal) transmission is likely for HBV, whereas HCV is transmitted through blood. Approximately 50% of HBV infections are acquired in the neonatal stage through vertical transmission, and other HBV infections are acquired in early childhood. This HBV transmission route is distinct from that commonly observed in Western countries. Thus, we prefer using the term “identified” HBV or HCV rather than “diagnosed,” because patients might have had such a diagnosis before entering universal health coverage. Therefore, we have deleted the HBV/HCV coinfection analysis and redistributed the coinfection group back into the individual HBV and HCV categories in the revised manuscript. A data reanalysis was conducted, as suggested by Reviewer 1.

Figure 1: individual matching was made but I understand that the three groups of CHI were eventually all compared with the pool of controls. Is it so? Then the substantial unbalance in sex and age, and, possibly, region, across the three group of CHI and the controls should be always adjusted for in order to compare like with like.

Reply: The nonhepatitis control cohort (n = 98,872) was frequency matched to the chronic hepatitis cohort, which consisted of patients with HBV infection (n = 15,888) and HCV infection (n = 8,830), at a 4:1 ratio by age, sex, and index date and year. (Please see the last nine lines of the first paragraph in the “Study sample” subsection on page 7).

Moreover, the data were adjusted for sex, age, geographical region, occupation, level of urbanization, monthly income, and the presence of comorbidities, as seen in Tables 3 and 4 of the revised manuscript.
Table 1: findings are widely discussed and many different comparisons across the four columns are described in the Results. However the only statistic is a p-value for chi-square that does not even indicate degrees of freedom. P-values are obviously highly significant on account of the large study population but the direction of the associations is not given and, most important, comparisons should be adjusted at least for sex and age group that vary substantially between HBV-positive and HCV-negative individuals.

Reply: Thank you for your suggestion. We have indicated the chi-square value and degree of freedom in Table 1 of the revised manuscript. We did not adjust for any variable in Table 1, because this table presents a univariate analysis. However, such adjustments were made in the multivariate analysis, as outlined in Tables 3 and 4.

Table 2: IRR should be standardized for a standard population (like in cancer incidence studies). The standard should be the sex and age distribution of the control group if the three CHI group are all compared with the same pool of controls.

Reply: Thank you for your suggestion. We have revised Table 2, and the incidence rate ratio was standardized on the basis of the general adult population in the LHID2000 database, which contains claims data of one million beneficiaries randomly selected from all residents enrolled in the National Health Insurance program in 2000 in Taiwan.

Table 3: I do not see any reason to adjust for urbanization, hypertension, and hyperlipidemia in Table 3 and also 4. I actually think that this is actually a dangerous complication. In addition, sex- and age- adjustment is adequate to compute HRs in each of the three groups of CHI but it does not allow to compare HRs across the three groups of CHI as they are too different. Notably HCV-positive are older than HBV-positive and less often male. Age and sex are, obviously, important modifiers of cancer risk as it clearly shown by the differences in HR between individuals < and >= 60 year-old (Table 4).

Reply: In Table 1, geographical region, occupation, level of urbanization, monthly income, the presence of comorbidities, and number of outpatient visits were all significantly different between the CHI and nonhepatitis cohorts. Hence, we adjusted for these cofactors to prevent their confounding effects on our findings. We also adjusted for sex and age because they are key modifiers of cancer.

Last but not least, it is wrong to state that certain extrahepatic cancer are uniquely associated with one of the two infections when HR ratios are of similar size but only one is statistically significant, e.g., for thyroid cancer in Table 3 the Hrs are similar (1.67 and 1.51) and the 95%
confidence intervals are overlap. To state a difference you should test for heterogeneity in the association (Wald chi-square).

Reply: Thank you for your comment. However, we did not compare the two HRs of site-specific cancer. Moreover, we separately investigated the association of HBV or HCV infection with the cancer risk in the revised manuscript.

Other issues

Abstract, line 8: add "hepatic" before "extrahepatic"

Reply: Thank you for your correction. In the revised manuscript, we have added “hepatic” before “extrahepatic” on line 8 of the Abstract section.

Page 7 and Figure 1: the sentence "Insurants without hepatitis or missing information on age and sex" is ambiguous. Please change into "Insurants without hepatitis and with information on age and sex"

Reply: Thank you for correction. We have revised the statement in Figure 1 and in the Methods section on page 7 of the revised manuscript.

Page 8, line 12: please find a "label" to identify dual infection, e.g., HBV/HCV, and use it all over the text

Reply: Thank you for suggestion. In the revised manuscript, we have used “HBV/HCV” to identify dual infection throughout the text.

Page 12, line 27: correct 192 into 1.92

Reply: Thank you for the correction. The HR of 1.92 was corrected after reanalysing the data set in the revised manuscript.


Reply: Thank you for suggestion. We have replaced reference 21 with Plummer et al., 2016, and have noted that the global cancer burden is attributed to infection in the revised manuscript.
Discussion: authors make enormous efforts to provide biological explanations of the associations of HBV or HCV and some extrahepatic cancers. They neglect, however, the high probability of surveillance bias especially for cancers like thyroid, colorectal, kidney, skin, or ovary that can be very easily be over-diagnosed if HBV- or HCV positive people receive special medical attention, e.g., ultrasonography.

Reply: Current clinical practice recommends screening patients diagnosed with CHI for liver cancer, particularly those with fibrosis. However, such a recommendation is not applicable for extrahepatic cancers. Hence, surveillance bias can only exist for liver cancer, for which the relationship with hepatitis is more clear and more documented than for other site-specific cancers. In addition, we further adjusted for the number of outpatient visits of study patients in the multivariate analysis to reduce bias, as summarized in Tables 3 and 4.

Page 14, lines 49-51: interferon-alfa is mentioned in relation to HBV as a possible disruptor of the thyroid but then: 1) information on the frequency of use of antiviral treatment in HBV-positive patients should be reported; and 2) till recently interferon was also used to treat HCV infection.

Reply: Thank you for your thoughtful comment. However, HBV and HCV infections were not significantly associated with thyroid gland cancer after a reanalysis of the data. Therefore, this discussion has been removed from the revised manuscript.

Table 1: indicate "lowest" and "highest" at the side of 1st and 4th category of urbanization

Reply: Thank you for your suggestion. In the revised manuscript, we have indicated “highest” and “lowest” as subcategories of the first and fourth categories of urbanization in Table 1, respectively.