Author's response to reviews

Title: Prevalence and clinical consequences of Hepatitis E in patients who underwent liver transplantation for chronic Hepatitis C in the United States

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Author's response to reviews: see over
Concerning: Submission manuscript “Prevalence and clinical consequences of Hepatitis E in patients who underwent liver transplantation for chronic hepatitis C in the United States”

Dear Sir/Madam,

We would like to resubmit our manuscript, entitled “Prevalence and clinical consequences of Hepatitis E in patients who underwent liver transplantation for chronic hepatitis C in the United States”. First, we would like to kindly thank the referees for their time and efforts to review our manuscript. We will answer their concerns and comments with a point-by-point response given below.

Referee 1:

1. The paper doesn’t include any clinical descriptions of the patients—only lab results. What immunosuppressive drugs were the pts receiving?? Were they getting interferon, ribavirin?? If so in what doses?? Were there episodes of clinical hepatitis—elevated liver enzymes rejection etc etc?? If so—how were they managed

A lot of patients transplanted at the Mayo Clinic return to their local hepatologists after transplantation for follow up. Most of them will come back at yearly intervals for a check-up. Data is then collected on whether patients were treated for hepatitis C. However, exact duration and dose of medications is often not registered. The same applies to the episodes of elevated liver enzymes. Considering the immunosuppressive drugs: the immunosuppressive regimen often changed throughout time and with a follow up of five year and the uncertainty around seroconversion (possible missed HEV infections) given in the discussion section, we decided not to include this information in the manuscript since any interpretation of these data would be at high risk of bias. For the same reason we chose to omit the immunosuppressive regimen in the seroconverters (who were all on tacrolimus at the time of seroconversion).

2. What were the causes of death in the many patients who died prior to the last follow up??

Not all patients died between follow up points. Some were lost to follow up (but may have also died of unknown causes). Some patients also missed one follow up, but came back for a later follow up.

3. How many transfusions did the patients receive. Were any transfusions followed by clinical evidence of hepatitis?

Unfortunately we don’t have data available on transfusions in this cohort.

4. Many references are incomplete lacking page numbers, volume numbers etc

Thank you for pointing this out. We changed them accordingly.
Referee 2:

This study by Koning and colleagues reports on the HEV seroprevalence in patients who underwent liver transplantation for chronic hepatitis C in the US. They found a high anti-HEV IgG prevalence in this population (around 40 %) but no HEV viremia. They also underline the need for close monitoring of HEV infection in these patients particularly in the context of new ribavirin and interferon free anti-HCV therapies. This topic is important and the design of this study is interesting. The manuscript is well written.

Comments: 1. The high prevalence of anti-HEV IgG antibodies in this US population is surprising. Recent studies from this country (Ditah Hepatology 2014) suggested a seroprevalence of 6 % based on testing 2009-2010 National Health and Nutrition Examination Survey sera. Could this difference be linked to difference in the limit of detection of serologic assays?

There may be several reasons for this difference. First of all, the subjects in the NHNES study were younger than the patients in our study. It has been repeatedly shown that seroprevalence increases with age, as the data in the NHNES study confirms. Also, our study population (immunocompromised patients with hepatitis C) is quite different from the general population in the NHNES study and one could argue that they are at increased risk of contracting HEV due to blood transfusions, but also through the same ways which they have been infected with hepatitis C. The DSI serologic test used in the NHNES study underperforms in terms of specificity and sensitivity in immunocompetent as well as immunocompromised patients (Pas Journal of Clinical Virology 2013). We included your reference in the introduction.

2. The lack of control group in the same age class (immunocompetent individuals) and the absence of data on food habits are limitations of this study.

We agree with the reviewer, we added a limitation section in the discussion.

3. Several factors explaining the high incidence of HEV infection in this liver transplant population are discussed. Another possibility could be the systematic monitoring for HEV markers as previously performed by others (Abravanel J Infect Dis 2014).

We agree with the reviewer that the awareness of HEV has increased while more patients are tested.

4. HEV seroprevalence of 22 % has been reported in US blood donors but the prevalence of HEV viremia in donors is unknown (none in 1939 donors as reported by Xu Transfusion 2013).

Nonetheless, several European blood donor cohorts have been screened for HEV RNA and consistently shown HEV viremia. A recent study confirmed that transmission of HEV through infected blood donors occurred in patients receiving infected blood components. Immunocompromised patients who were infected through blood products showed indeed longer duration of viremia and abberant seroconversion patterns (Hewitt Lancet 2014).
Referee 3:

*Minor essential revisions please revise the introduction to include published papers and abstract on hepatitis E in organ transplant recipients and seroprevalence in the US*

We updated the reference list as requested.

*Provide information on the study subjects including clinical progression and consequences in the 145 persons.*

Due to the nature of the study and the follow up course in these patients where many of them return to their hometown and have their regular checkups there, it is not possible to give a comprehensive overview of the clinical progression in these patients.

*What is the characteristics of these persons—indication for transplant proportion by indication, region of residence, country of birth, etc.*

All patients were transplanted for hepatitis C related liver cirrhosis. The majority of the 145 patients were Caucasian (N=117; 81%), other ethnicities include Hispanic (N=5; 4%), American-Indian (N=3; 2%), Asian (N=3; 2%) and in 9 cases the ethnicity was unknown. No data on country of birth of region of residence was collected.

*As the article is written it appears that the study is based on serological testing alone without any information on the clinical status of cases at or around the time of possible seroconversion. Were there organs rejections, how frequent were elevated ALTs recorded in the seroconverts, how does this compare to the non seroconverters? what type of immunosuppressive treatment was used? difference by transfusion status?*

There is no official ‘non-seroconverter’-group, since patients may have lost seroconversion between follow up times and sensitivity of serology testing is lower in immunocompromised patients. Comparing non-seroconverters with seroconverters will therefore lead to false conclusions.

*was any attempt made to compare with persons without HCV as a reason for liver transplant?*

Unfortunately the cohort used consisted of only HCV patients

*In the post LT follow up protocol how often was serum collected and stored?*

At 1, 3 and 5 years

*what is the reason that very few of them had one or more follow up? Are those with more follow up the same as those for whom no follow up sample was available?*

This is due to patients transferring their care to their local hospital, but also due to deaths and lost to follow up.

*What is the evidence that post LT patients were taking HCV treatment during the “seroconversion”?*
We had data available on HCV treatment in all seroconverters (see also table 2)

*Discretionary revisions line 72: caution with the interpretation of seroprevalence estimates among blood donors--test performance should be considered. A recent study among young PWIDs found much less prevalence.*

This indeed has to be taken into account. The NHNES study has been added to the references.

*line 80: data on acute on CLD in areas where genotype 3 is the predominant cause is still unclear. There was a poster presented by I think NIH group at AASLD 2013.*

We agree with the reviewer

*line 116: please describe the population characteristic line 130: do you have the titers of those post LT seroconverters who lost IgG*

Yes, we indeed have the titers of all serologic patients. The predetermined thresholds have been used to mark a result as positive or negative.

*line 158: revise the sentence, not very clear line*

We revised the sentence

*162: sentence ending with "have been in contact with HEV" needs clarification. How is contact defined? by serostatus?*

Indeed contact is defined by positive HEV serology, since the specificity and sensitivity of the Wantai test in immunocompetent patients (pre-transplant) is very high.

*line 171: we do not have US population estimate with the Wantai assay. In general prevalence is higher among older persons. In PWID the most recent published study (EID 2013) doesn't show an increase prevalence.*

We found one study on PWID in EID 2013 from Mahajan and colleagues where in a cohort of 508 people aged 18-40 years, HEV IgG seropositivity was seen in 14 patients. They conclude in their cohort that an age of >=30 years is associated with an increase in HEV IgG seropositivity. The average age in our cohort is even higher. The latest NHNES study also shows that HEV seropositivity increases with age.

*line 189: may need to mention the probability of spontaneous clearance in post LT. Also mention probability of symptomatic vs asymptomatic HEV infection and risk of progression to chronic hepatitis/ there is a need to list limitations of this study: lack of clinical and biochemical data, lack of data from patients with graft rejection and death after LT. No liver enzyme at or around the seroconversion, information on immunosuppressive region, ecologic association of HCV treatment and seroconversion and finally lack of comparison group without HCV treatment (LT due to other causes).*

We have added these comments to the discussion section. However, it was a conscious choice to refrain from adding data such as liver enzymes and immunosuppression in the current manuscript. We believe
elaborating on these data in a retrospective cohort may lead to unnecessary bias, due to the patients that were lost to follow up or had their care transferred. Especially since no active HEV infection was found and the exact time of infection can therefore not be determined, considering the fact that in immunocompromised patients seroconversion of HEV is often delayed, aberrant or short-durated.

Sincerely yours,

Ludi Koning, MD  Michael Charlton, MD  Annemiek van der Eijk, MD PhD