Author's response to reviews

Title: Discontinuation of Antiviral Prophylaxis Correlates with High Prevalence of Hepatitis B Virus (HBV) Reactivation in Rheumatoid Arthritis Patients With HBV carrier statea Real-world Clinical Practice

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Author's response to reviews: see over
Dear Editor Angel:

We would like to accept the comments from you and referees. We addressed the comments to revise the manuscript and provide a point-by-point response to the concerns (more detailed can be found at the base of this letter). The revision was highlighted in yellow in the revised manuscript.

We hope that the revised manuscript meets the standards of your journal. We are looking forward to receiving a favorable response from you regarding the acceptance of our manuscript. Thank you and best regards.

Sincerely yours,

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Point-by-point Response to the Concerns

Referee 1:
This article is in a relatively neglected area in rheumatology. The data contained here is interesting and worthy of publication. This is essentially a large case series, as such I think the information described is adequate and have no additional suggestions in regard to methodology or reporting.

However there are major issues with the quality of the writing, most evident in the discussion but present throughout the manuscript. It is virtually unreadable at points. A great deal of effort is necessary to understand what the authors are trying to convey. This article requires a major English language/style revision before being published.

——Thank you for your kind suggestions! H. Ralph Schumacher, MD (one co-author in the manuscript) is a native-English speaker and an expert at rheumatology, who is Professor of Medicine at the University of Pennsylvania School of Medicine, Director of the Arthritis-Immunology Center at the Philadelphia VA, and former Chief of Rheumatology at the VA Medical Center, Philadelphia. He has revised the English language/style of the manuscript.

Referee 2:
In this study, the prevalence of hepatitis B reactivation in rheumatoid arthritis patients and the used of anti-viral prophylaxis has been evaluated. The study analyzed 36 patients affected by rheumatoid arthritis in immunosuppressive therapy having HbsAg positive: 20 patients were HBV-DNA negative and 16 HBV-DNA positive. Patients HbsAg positive, with HBV-DNA > 2000 copies/L should be considered affected by chronic hepatitis B (active carrier of HBV). The authors didn't make the difference between chronic hepatitis patients and
inactive carriers of HBV. This is essential because antiviral therapy instead of prophylaxis should be considered in the first group.

——Thank you for your kind suggestions! Chronic HBV infection is generally classified into chronic hepatitis B (with fluctuant ALT) and HBV carrier state (with persistent normal ALT). According to 2012 EASL clinical practice guidelines, classifying a patient as inactive HBV carrier required a minimum follow-up of 1 year with ALT levels at least every 3-4 months and serum HBV DNA levels. ALT levels should remain persistently within the normal range and HBV DNA should be below 2000 IU/ml (10^4 copies/mL). Some inactive carriers, however, may have HBV DNA levels greater than 2000 IU/ml (usually below 20,000 IU/ml or 10^5 copies/mL) accompanied by persistently normal ALT levels. So the cutoff value of inactive carrier is still controversial and it is difficult to divided patients into active carriers and inactive carriers.

Also according to 2012 EASL clinical practice guidelines, indications for antiviral therapy include patients who have HBV DNA levels above 2000 IU/ml, serum ALT levels above the upper limit of normal and severity of liver disease assessed by liver biopsy (or non-invasive markers once validated in HBV infected patients) showing moderate to severe active necroinflammation and/or at least moderate fibrosis using a standardized scoring system. In patients who fulfill the above criteria for HBV DNA and histological severity of liver disease, treatment may be initiated even if ALT levels are normal. That is to say, HBV carriers with HBV DNA levels above 2000 IU/ml are not always considered for antiviral therapy.

Chronic hepatitis is characterized by chronic necroinflammatory liver diseases. ALT levels can be elevated intermittently, so at least two determinations, 6 month a part, are recommended. Also liver biopsy might be important to decide to initiate antiviral therapy in chronic hepatitis therapy. Biopsy data are not reported by the authors.

——We agreed with you! Since none of patients received liver biopsy, we used ALT levels (normal ALT ≥ 6 months) to define HBV carriers (See Methods 1 “RA Patients with HBV carrier state who had positive HBsAg, normal ALT ≥ 6 months and normal total bilirubin (TBil) were included”). Lack of liver biopsy data was one of limitations in this observational study.

The title is confusion. The study population doesn’t include only inactive carriers of HBV but also active. In the active carrier of HBV antiviral therapy and not lamivudine prophylaxis should be considered. This might justify the high prevalence of HBV reactivation.

——We agreed with you! We had divided our patients into inactive carriers or active carriers of HBV according to the cutoff value of HBV-DNA ≥ 2000 IU/mL or 10^4 copies/mL, 9 patients were active carriers of HBV and only one of them developed HBV reactivation in the absence of antiviral therapy.

Our study showed 71% of patients who discontinued antiviral prophylaxis developed HBV reactivation 3~21 months after discontinuation. Both survival curve and regression analysis proved that discontinuation of antiviral prophylaxis was risk factor of HBV reactivation for RA patients with HBV carrier state during DMARDs therapy. Therefore, discontinuation of antiviral prophylaxis may be important reason for high prevalence of HBV reactivation in our observational study. To make the title more clear, we changed to “Discontinuation of Antiviral Prophylaxis Correlates with High Prevalence of Hepatitis B Virus (HBV) Reactivation in Rheumatoid Arthritis Patients With HBV carrier state: a Real-world Clinical Practice”.

Moreover only 18/36 patients didn’t accepted lamivudina treatment and 7/18 discontinued lamivudina treatment for the high cost. It’s not reported which of these patients were HBV negative and positive.
Thank you for your kind suggestions! We made a new table (Table 2) for this data and described in the Results.

“Sixty-three percentage (10/16) of patients with detectable baseline HBV-DNA accepted antiviral prophylaxis, which tended to be higher than 40% (8/20) of patients with undetectable baseline HBV-DNA (Table 2), but no statistical significance between these two groups was found.” (Page 6 line 114–116)

### Table 2 Antiviral prophylaxis, HBV reactivation and HBV hepatitis between RA patients with undetectable and detectable baseline HBV-DNA

<table>
<thead>
<tr>
<th>Baseline HBV DNA</th>
<th>Antiviral prophylaxis</th>
<th>HBV reactivation</th>
<th>HBV hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes* Discontinuation No</td>
<td>12 (60%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Undetectable (n=20)</td>
<td>4 (20%)</td>
<td>4 (20%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Detectable (n=16)</td>
<td>7 (44%)</td>
<td>3 (19%)</td>
<td>6 (37%)</td>
</tr>
</tbody>
</table>

* Patients who discontinued antiviral prophylaxis were not included.

△ all P > 0.05

See the following reference:


Thank you for your kind recommendations! We read the reference and cited in the Background and Discussion.

“International associations for the study of liver disease recommended antiviral prophylaxis should be used before immunosuppressive therapy and continued minimal 6–12 months after suspension of immunosuppressant for chronic HBV infection patients........” (Page 3 line 55–57)

“Entecavir and tenofovir, which are nucleotide analogs with high antiviral potency and a high barrier to resistance, are both recommended as first line antiviral drugs for patients who have a high HBV-DNA level and/or may receive a lengthy and repeated cycles of immunosuppression (e.g. long-term DMARDs therapy) by European........” (Page 9 line 202–208)

According to the table 2 were 8 the patients which had a switch of serum HBV-DNA from undetectable to detectable instead of 5. Correct also the number of patients which had a 10 fold rise in serum HBV-DNA.

Thank you for pointing out the mistakes! We corrected as follows:

“Among patients with undetectable baseline HBV-DNA, 40% (8/20) had a switch of serum HBV-DNA from undetectable to detectable. Among patients with detectable baseline HBV-DNA, 31% (5/16) had a 10-fold rise in serum HBV-DNA (Table 2).” (Page 6 line 120–122)

Make a table showing characteristic of HBV-positive vs HBV negative patients.

Thank you for your kind suggestions! We added the data in table 1 and table 2.

“There was no significant difference of baseline characteristics between patients with undetectable and detectable HBV-DNA, except that ALT was significantly higher in the latter than in the former (P<0.05, Table 1) and both ALT were not exceeding normal range.” (Page 5 line 108–110)

This is an observational study. Nevertheless, according to the international guidelines lamivudina should not used in patients under immunosuppressive therapy and chronic hepatitis (HbsAg posivite, HBV-DNA > 2000 copies/L).
In these patients it is recommended to use entecavir and tenofovir as first line antiviral agents. So, increasing of HBV-DNA copies and developing of acute hepatitis in some patients are expected. Authors need to address these observations and make clear these points in the discussion.

Thank you for your kind suggestions! In this study, entecavir and tenofovir were recommended to the included patients. However, only lamivudine was accepted by the patients for economic reason (stated in the Results).

We also discussed these points in the Discussion:

“Entecavir and tenofovir, which are nucleotide analogs with high antiviral potency and a high barrier to resistance, are both recommended as first line antiviral drugs for patients who have a high HBV-DNA level and/or may receive a lengthy and repeated cycles of immunosuppression (e.g. long-term DMARDs therapy) by European, American and Asian-Pacific associations for the study of liver disease. However, in this real-world study, although all patients were recommended entecavir or tenofovir, only 50% of patients accepted lamivudine as antiviral prophylaxis. The most important reason was the cost of antiviral drug, which is much more expensive than conventional DMARDs causing high economic burden for self-paid RA patients, not only in developing countries but also in developed countries.” (Page 9 line 202~208)

It would be useful to compare in the discussion with patient affected by HCV and rheumatic diseases (Clin Rheumatol. 2014 Long-term safety of anti-TNF-# in PsA patients with concomitant HCV infection: a retrospective observational multicenter study on 15 patients. Costa L).

Thank you for your kind suggestions! We compared with patient affected by HCV in the Discussion.

“One of limitations in this study was lack of data about biologic DMARDs, since only 4 patients receiving TNF-α antagonist for 4–6 weeks were included. HBV reactivation occurred 21–24 months after discontinuation of TNF-α antagonist in 3 patients, which might be attributed to subsequent conventional DMARDs, rather than TNF-α antagonist. Recently, Costa et al. reported long-term use of TNF-α antagonist alone was safe for 15 psoriatic arthritis patients with chronic hepatitis C virus (HCV) infection in the absence of specific therapy for HCV. Giannitti et al. reported tocilizumab combined with cyclosporine-A was effective and safe for a RA patient with chronic HCV infection. Cyclosporine-A could control HCV replication by inhibition of cyclophilin-B (while the inhibition of calcineurin causes immunosuppressive effect) and may be safe in patients with autoimmune disorders and concomitant HCV infection. However, litter is known about the safety of cyclosporine-A in patients with RA and concomitant HBV infection.” (Page 12 line 256~264)

Referee 3:
Major Compulsory Revisions:

The paper should not be accepted for many reasons: the methodology is poor, the patients are scarcely characterized both from the hepatic point of view and for the rheumatoid arthritis. You should specify how many patients had chronic HBV infection and how many were only potential occult carriers,

Thank you for your kind comments! Chronic HBV infection is generally classified into HBV hepatitis (with fluctuant ALT) and HBV carrier state (with persistent normal ALT). This study only included “RA Patients with HBV carrier state who had positive HBsAg, normal ALT ≥6 months and normal total billirubin (TBil)”.

Potential occult carriers (HBsAg-negative anti-HBc-positive) or patients with HBV hepatitis were excluded.

you should specify the HBV serological markers for each patient both at baseline and at the end of follow-up.

Thank you for your kind suggestions! We added the data in Supplement 1.
There are also poor details about the rheumatological treatments. In table 1 we see that anti TNF therapy has been prescribed to 4 patients, but in the text there is no mention about it and above all about the follow up of these 4 patients.

Thank you for your kind suggestions! We added the detail about the rheumatological treatments in the Results:

“Therapeutic regimens for RA during follow-up were shown in Table 1. Low-dose MTX ($\leq 15$mg/w) or MTX+LEF therapy was prescribed for patients responding insufficiently to the original DMARD(s) therapy, intolerant to other DMARDs, in moderate to high disease activity or with prognostically unfavourable factors******Four patients have received TNF-α antagonist for 4–6 weeks. After receiving TNF-α receptor: IgG Fc fusion protein (50mg/w) for 4 weeks, one of two patients changed to MTX+LEF therapy for economic reason and developed HBV reactivation 21 months later (Patient 1 in Table 3); and the other changed to MTX+HCQ+SSZ+Lamivudine therapy and kept undetectable HBV-DNA and normal ALT. After receiving infliximab for 3 times, one of two patients changed to MTX+HCQ+SSZ therapy and developed HBV reactivation 22 months later (Patient 2 in Table 3); and the other changed to MTX+LEF therapy and developed HBV reactivation 24 months later (Patient 3 in Table 3).” (Page 6 line 127–137)

The Discussion and Conclusions are not adequately supported by the literature data, the authors don’t compare their results with those previously published (Efficacy and safety of tocilizumab combined with cyclosporine A in a patient with rheumatoid arthritis and concomitant chronic hepatitis C virus infection. Giannitti C, et al. Clin Exp Rheumatol. 2013; Safety of anti-tumor necrosis factor agents in rheumatic potential carriers of occult hepatitis B virus. Giannitti C, et al. J Rheumatol. 2011)

Thank you for your kind suggestions! We revised the Discussion.
One of limitations in this study was lack of data about biologic DMARDs, since only 4 patients receiving TNF-α antagonist for 4~6 weeks were included. HBV reactivation occurred 21~24 months after discontinuation of TNF-α antagonist in 3 patients, which might be attributed to subsequent conventional DMARDs, rather than TNF-α antagonist. Recently, Costa et al. reported long-term use of TNF-α antagonist alone was safe for 15 psoriatic arthritis patients with chronic hepatitis C virus (HCV) infection in the absence of specific therapy for HCV. Giannitti et al. reported tocilizumab combined with cyclosporine-A was effective and safe for a RA patient with chronic HCV infection. Cyclosporine-A could control HCV replication by inhibition of cyclophilin-B (while the inhibition of calcineurin causes immunosuppressive effect) and may be safe in patients with autoimmune disorders and concomitant HCV infection. However, litter is known about the safety of cyclosporine-A in patients with RA and concomitant HBV infection. Additionally, RA patients who were HBsAg-negative anti-HBc-positive potential occult carriers should also be emphasized.”

Minor Essential Revisions: English Language should be revised, the flow chart should have more details.

Thank you for your kind suggestions! H. Ralph Schumacher, MD (one co-author in the manuscript) is a native-English speaker and an expert at rheumatology, who is Professor of Medicine at the University of Pennsylvania School of Medicine, Director of the Arthritis-Immunology Center at the Philadelphia VA, and former Chief of Rheumatology at the VA Medical Center, Philadelphia. He has revised the English language/style of the manuscript. The flow chart has been added more details.