Peer Review File

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Reviewer A

In the present article, the authors reported the excellent outcome of ABOi LDLT without DIHBS using tailored desensitization protocol. The topic is of interest. However, there are a few critical problems as follows.

1. The authors concluded that RTx-based tailored desensitization protocol for ABOi LDLT was feasible and acceptable. This might be true. However, this study could not demonstrate the usefulness of their tailored desensitization protocol including postoperative high dose IVIG and/or simultaneous splenectomy, because this study is a single arm study. The authors should perform a randomized controlled study or propensity score matching study to demonstrate the usefulness of their tailored desensitization protocol.

Ans) We agree the limitation of this study that the reviewer mentioned. We compared our outcome with those in the literature. The limitation is described in the discussion. However, to show the superiority of tailored protocol, the changes of isoaglutinine titer before- and after LT of different desensitization methods was added. There was no cases of rebound in additional IVIG+splenectomy group.

"However, additional randomized controlled study or PSM study between this tailored protocol and RTX based protocol without IVIG nor splenectomy among ABOi patients are needed to confirm the usefulness of this tailored protocol."

2. Moreover, the sample size in ABOi LDLTs is relatively too small to draw any definite conclusions.

Ans) We agree that the sample size of this study, 60 cases of ABOi would be not enough to draw definite conclusions. However, as mentioned in discussion, more than 100 cases of ABOi showed no clinically significant AMR associated DIHS. Therefore, we could report that the incidence of AMR was much reduced compared to other studies in virtue of tailored protocol.

3. The indication of simultaneous splenectomy is unclear.

Ans) The indication of simultaneous splenectomy is the same as that of the IVIG if ABO isoagglutinin titer just before liver transplantation (LT) after PE was higher than 1/16. As

shown in Table 1. However, there were 8 cases that only IVIG was used without splenectomy by the surgeon's decision. We added it in the Method section.

4. The authors show that they do routine biopsy at POD 7. I think it is not necessary to perform routine biopsy for all recipients at POD 7, because liver biopsy at POD 7 has a risk of bleeding.

Ans) We agree that there is a potential risk of complications associated with biopsy, especially in the immediate postoperative period. However, we have done the routine protocol biopsy at POD7 to evaluate the hidden ACR, AMR, and fatty liver of the graft. After experiencing a few cases of biopsy related complications, however, we recently started to limit the biopsy at POD7 except for study purposes or in cases with clinical abnormality.

5. Furthermore, AMR does not necessary occur around POD 7. Therefore, no evidence of AMR at POD 7 only could not demonstrate that their protocol could successfully prevent the onset of AMR.

Ans) We agree that the absence or presence of evidence of pathological AMR at biopsy at POD7 does not necessarily correlate with clinical outcome.

Reviewer B

General comments:

The authors performed a single center institutional review on their protocol for desensitization in ABO incompatible adult LDLT based on development of diffuse intrahepatic biliary stricture (DIHBS). The study is based on 60 cases of ABO incompatible LDLT that were matched by propensity score matching to 120 cases of ABO compatible LDLT. The study period was from 2012-2017. The authors used a tailored protocol of pre-transplant Rituximab, plasma exchange, intravenous immunoglobilin administration, and splenectomy. The authors did not find any case of DIHBS in both groups. The complication, rejection, and overall survival rates in both groups were similar. The authors concluded that RTx-based tailored desensitization protocol for ABOi LDLT was feasible and acceptable.

The use of Rituximab is not new in the management of ABO incompatible LDLT. Generation of protocols have been proposed to find the best strategy for its use (including dosage, timing of administration, route of administration, combination with other drugs, etc.). The authors alluded to this in their discussion referring several times to the Japanese

multicenter study on Rituximab. Having that said, this current report lacks novelty in its use of Rituximab and failed to emphasize the differences in their protocol with other protocols that may have probably resulted to their better outcome. Particularly it missed on the effects of IVIG and splenectomy.

Questions and comments to authors:

1. The protocol was to perform plasma exchanges at one week before LDLT. However, there is an outlier of 16x plasma exchanges. How was plasma exchange done in this patient at one week before LDLT? A brief detail or description of this case should be given for clarity.

Ans) In the initial period before stabilization of the desensitization protocol, a couple of cases with initially high titer (1:1024) received multiple times (>x10) during 2 weeks. A description was added in the result section.

However, two cases with initially high titer (1:1024) received 14 and 16 times of PE for 2 to 3 weeks among the initial series.

2. The indication for the addition of splenectomy in the protocol is not clear.

Ans) As we explained it in the above,

3. In the Methods section, it was mentioned that Rituximab was given at around 3 weeks pre-LDLT. In the Results section, the range was 4-33 days. An outlier of >3 weeks was present. Please reconcile the data. A brief detail of this outlier should be given to clarify the extended period.

Ans) There were 8 cases (12.5%) with longer days more than 3 weeks after Rtiuximab among 64 ABOi cases due to various reasons (multiple PE during more than 1 week, delayed due to donors' reasons, and so on) On the contrary, there were 3 emergent cases that the duration between RTx to LT was less than one week.

These brief details were added in the Result section.

4. In the ABO compatible group, the median length of follow-up was 59 (1-92) months. One month follow-up may be too short to determine the development of DIHBS.

Ans) We totally agree with the reviewer's comments. We reviewed all cases until the end of June, 2020. Therefore, the minimum follow-up was more than 3 years in all cases. There are very few cases of hospital mortalities in both groups. We didn't exclude these cases to analyze the risk of infectious complications or the changes of isoaglutinine antibody titer

5. What was the cause of intractable hyperbilirubinemia in the patient that required retransplant at POD 764? Please be certain that this is not due to DIHBS.
Ans) The patient showed mild dilatation of intrahepatic duct and small gas-containing biloma outside of the liver (around cut surface of the graf)t on CT scan at 8 months after LT. PTBD showed the anastomosis stricture, leakage, and common bile duct stones, however, all intrahepatic duct was normal appearance. Multiple PTBD procedures and frequent infection associated with the infected biloma containing the detached artificial graft in the graft for draining of MHV tributaries resulted in liver failure. This patient received deceased donor liver transplantation at POD 764. The pathologic findings of the explant liver were compatible with biliary cirrhosis.
The explanation was added in the result section.

6. The addition of HCC outcomes is superfluous as it is not included in the study objective nor alluded to in the title (in this study, we report the excellent outcome of ABOi adult LDLT without DIHBS using tailored desensitization protocol and compare it with that of ABO-compatible (ABOc) LDLT- from Introduction).
Ans) We agree your comments. Therefore, we removed the HCC associated results.

7. The discussion on CMV infection is also superfluous as it is not included in the study objective.
Ans) Even though infection issue was not directly mentioned in the title and objective, the safety issue is very important to advocate our tailored protocol because the infection is the most worrisome complication for the transplantation surgeon to apply Rx based protocols in ABOi patients.

8. In the Discussion section, the authors mentioned the importance of finding an additional strategy to reduce the risk of AMR (where DIHBS is one major manifestation) in ABO incompatible LT. Please define this high risk group. Also suggest to move this concern in the Introduction section rather than Discussion section to emphasize the importance of the study protocol.
Ans) Thank you for your good suggestion. We added some more paragraphs to emphasize the importance of the study protocol in the Introduction. Regarding the definition of high risk, we already discussed in the Discussion.
However, the risk factor of DIHBS has not been identified yet. Even though Song et al did not find any correlation between pre-LT or post-LT isoagglutinin titers and the

occurrence of DIHBS(5), there are several reports to show the relationship of treatment non-responsive high isoagglutinin titers and AMR(3, 15). We consider the patients with high titer even after RTx and PE therapy as a high risk group. We applied additional strategy including IVIG and splenectomy in this high-risk group.

9. Was there any patient with high isoagglutinin titers higher than 1/16 who did not receive IVIG with or without splenectomy and did not develop DIHBS? A comparison with this group could shed light on the actual efficacy of the protocol.

Ans) There are 6 cases with 1:32 or 1:64 of pre-LT IgG titer without IVIG nor splenectomy. No DIHBS was found in these patients. Also, there was no difference in terms of overall survival, graft survival, and biliary complication-free survival between these 6 cases without additional procedure and 20 cases with additional procedures. However, it is difficult to compare the two groups directly due to the higher proportion of high titer in the additional procedure group.

10 A table showing the serial changes in antibody/ isoagglutinin titers due to the effects of IVIG or splenectomy should be provided to clarity of effects of the additional intervention.
Ans) Thank you for your insightful comments. We added the changes in isoagglutinin titers of different groups (no treatment, IVIG only, and IVIG+splenectomy groups) in Figure 3. Also discussed in the Discussion.

11. A table comparing the results between conventional protocols (from literature review) and the present tailored protocol of the authors should be given to emphasize the acceptability of the current protocol.
Ans) We added a new table to compare the different outcomes in terms of AMR associated DIHBS of the previous studies from the literature review and this study (Table 4).

12. In the Conclusion, "RTx-based tailored desensitization protocol for ABOi LDLT was feasible and the outcome was acceptable". This statement should be qualified - in terms of what? in terms of DIHBS? Please note that HCC, infections, etc. are not in the study objectives.
Ans) We modified the conclusion as below.
Therefore, our RTx-based tailored desensitization protocol for ABOi LDLT was feasible and the outcome was acceptable in terms of overall survival and AMR associated

complications.

Reviewer C

1. The authors reported termination of DIHBC after ABO-I LDLT using Rituximab selectively combined by splenectomy and IVIG. Although the cohort was not large, their finding was encouraging.

2. Figures show changes of antibody titers of non-optimal treatment, splenectomy & high dose IVIG and high dose IVIG would be interesting. If possible, please show change of titer of individual patient in a figure of each group.

Ans) We appreciate your nice suggestion. We added figure 3 that shows the changes in titer of the individual patient by different desensitization regimens.

3. Their incidence of CMV infection was very low. Please show frequency of positive CMV IgG before transplantation in recipients and donors.

Ans) All donors & recipients are CMV IgG positive before transplantation. We added it in the Result section.

4. How often did they check antigenemia?

Ans) CMV antigenemia was followed up twice a week until discharge and then once a month until 3 months after LT. We added this protocol in the Method section.

5. Did they give patients VGCG or GCV prophylactically of preemptively?

Ans) We gave Ganciclovir preemptively if CMV antigenemia test shows equal or more than 5 positive cells/2x10^5 PMN. We added it in the Method section

6. Did ABO-I patients with IVIG have CMV infection?

Ans) Yes. IVIG, theoretically can reduce the risk of infection including CMV. But CMV infection is also related with other factors, such as degree of immunosuppression, liver function, general status. Fortunately, there is no CMV disease in ABOi patients.

7. Figure 4: There were ABO-I patients losing their grafts around 30 months after transplantation. Please show causes of graft loss, although I might miss it.

Ans) I answered it for the other reviewer's comments.

The patient showed mild dilatation of intrahepatic duct and small gas-containing biloma outside of the liver (around cut surface of the graf)t on CT scan at 8 months after LT.

PTBD showed the anastomosis stricture, leakage, and common bile duct stones, however, all intrahepatic duct was normal appearance. Multiple PTBD procedures and frequent infection associated with the infected biloma containing the detached artificial graft in the graft for draining of MHV tributaries resulted in liver failure. This patient received deceased donor liver transplantation at POD 764.
The explanation was added in the result section.

8. Figure 5: This is an important finding. But RFS seemed to be lower in ABO-I than ABO-C. They need to comment possibility of significance in the setting of large number cohort. On the contrary, although the number is small, the comparison of RFS between ABO-I and ABO-C within Milan criteria or without Milan criteria would be interesting.
Ans) We agree with the Reviewer B's comments that HCC issue is a little premature to be mentioned in this study. Furthermore, the main idea of this study is focusing on low incidence of AMR associated complications such as DIHBS. Therefore, we removed the result and discussion regarding HCC issues.

9. Small points: Please show references for "6.3 - 23.5 % in recent reports." on line 6 in page 2 and "A recent report showed ---- acute liver failure." on line 24-25 of page 6.
Ans) Thank you for your comments. We changed the percentage of DIHS in recent reports to 6.3-8% and added references. And also the paragraph "A recent report…. acute liver failure" was modified to "A recent report showed a trend that splenectomy is omitted with RTx B cell depletion except for the case of acute liver failure in that each center has different strategies (15)".