Format for ANSWERING REVIEWERS

October 22, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: responses to reviewers.doc).

Title: A protocol liver biopsy is the only examination that can detect mid-term graft fibrosis after pediatric liver transplantation

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
Response to reviewers' comments:

Reviewed by 02456611

It is a good study, but I have several comments:

Thank you for your favorable comment.

1. Could the authors comment of why no acute cellular rejection was an independent risk factor for ≥A1 at two years after LDLT.

We described the following sentences in Discussion. "We believe that no acute cellular rejection was found to be an independent risk factor for ≥A1 at two years after LDLT because acute cellular rejection may give rise to an immune response due to the use of a lower number of immunosuppressants."

2.What was the indication for intensifying immunosuppression, why some patients in A0 or F0 increased the immunosuppressants after PLB?

We described a strategy of our immunosuppressant therapy in Methods (Strategy of increasing the dose of immunosuppressants after LDLT). In the outpatients, when the serum level of ALT or hyaluronic acid was high, we increased the dose of immunosuppressants if the suspected causes of elevation of these levels were an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. When the grade of PLB was A0 and F0, we gradually decreased the dose of immunosuppressants. Therefore, some patients in A0 or F0 increased the immunosuppressants after PLB because the serum level of ALT or hyaluronic acid was high. However, these increasing the dose of immunosuppressants was not concerned with the results of PLB, and therefore, these results was deleted.

We described the following sentences in Discussion based on the present study. "In our department, we initially defined a histopathological abnormality as a Metavir score of ≥A2 or ≥F2. However, among 21

patients who underwent PLB at both two and five years after LDLT, the activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT exhibited worse a score of ≥A1 or ≥F1, respectively. Three patients with a score of A1 or F1 at two years after LDLT exhibited worse a score of ≥A2 or ≥F2, respectively. Therefore, we currently define a histopathological abnormality as a Metavir scoring system of ≥A1 or ≥F1 and consider such scores to indicate the need for treatment because liver fibrosis is reversible if early treatment is initiated."

3. There are some grammatical errors that should be very carefully reviewed and fixed to improve readability and flow.

The revised paper has been proofread for grammar and style by a native English-speaking pathologist.

Reviewed by 00054001

This manuscript described small series of protocol liver biopsy (PLB) after pediatric liver transplantation. Issues dealt with in this manuscript were very important. However, many self-satisfied descriptions spoiled the significance of this manuscript. If the following concerns were addressed, this manuscript will be worth enough publication.

Thank you for your favorable comment.

Major concerns

1. When did the authors' PLB program start? If not since a beginning of their liver transplantation program, they must specify why your PLB program was started.

We began to perform PLB in pediatric patients at two and five years after LT in July 2008 because we experienced cases of a normal LFT coexistent with histopathological portal inflammation and fibrosis, including the following Cases 4 and 5. This is described in Methods.

2. The authors must specify criteria for performing either PLB or episode biopsy.

As to the criteria for performing either PLB or episode biopsy, we described in Methods. We began to perform PLB in pediatric patients at two and five years after LT in July 2008. In cases in which the dose of immunosuppressants was increased after PLB, we generally performed a follow-up liver biopsy between six months and one year after PLB. In addition to PLB, an episode biopsy was performed when a recipient with a high serum level of ALT or hyaluronic acid was refractory to an increase in immunosuppressants.

3. Since this is small case series, the authors must summarize clinical course of all patients transplanted during this study period regardless of whether they underwent PLB or not. After that, they should summarize results of PLB.

As to the clinical course of PLB patients, we described in Results. PLB was performed at both two and five years after LDLT in 21 cases; the results are summarized in Table 6. The activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient, respectively. Seven patients with A0 and F0 at two years after LDLT maintained scores of A0 and F0 at five years; however, the remaining patients exhibit worse a score of ≥A1 or ≥F1. Three patients with a score of A1 or F1 at two years after LDLT maintained a score of A1 or F1 at five years; however, the remaining patients exhibited worse a score of ≥A2 or ≥F2.

4. Criteria for intensifying or weakening immunosuppression must be specified. Was immunosuppression always intensified if PLB showed A1/F1 or worse?

As to the criteria for performing either PLB or episode biopsy, we described in Methods (Strategy of

increasing the dose of immunosuppressants after LDLT). In the outpatients, when the serum level of ALT or hyaluronic acid was high, we increased the dose of immunosuppressants if the suspected causes of elevation of these levels were an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. When the grade of PLB was A0 and F0, we gradually decreased the dose of immunosuppressants.

In our department, we initially defined a histopathological abnormality as a Metavir score of ≥A2 or ≥F2. However, among 21 patients who underwent PLB at both two and five years after LDLT, the activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT exhibited worse a score of ≥A1 or ≥F1, respectively. Three patients with a score of A1 or F1 at two years after LDLT exhibited worse a score of ≥A2 or ≥F2, respectively. Therefore, we currently define a histopathological abnormality as a Metavir scoring system of ≥A1 or ≥F1 and consider such scores to indicate the need for treatment because liver fibrosis is reversible if early treatment is initiated.

5. Clinical course after PLB must be described/ summarized. Minor concerns English editing is required. I consider any definitive conclusion(s) could not be gained in this series because this was too small. The authors should focus on describing clinical course before and after PLB. That is enough for revealing some important suggestion(s)/ proposal(s).

As to the English editing, the revised paper has been proofread for grammar and style by a native English-speaking pathologist.

As to the Conclusions, we changed the following sentences. “PLB performed at two years after LDLT is an unnecessary examination because the serum ALT level reflects the degree of portal inflammation. In addition, immunosuppressive therapy should be modulated with preservation of the ALT level below 20 IU/l. PLB at five years is an excellent examination for detecting early reversible graft fibrosis, as no serum markers reflect the degree of graft fibrosis.”

As to the clinical course of PLB patients, we described in Results. PLB was performed at both two and five years after LDLT in 21 cases; the results are summarized in Table 6. The activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient, respectively. Seven patients with A0 and F0 at two years after LDLT maintained scores of A0 and F0 at five years; however, the remaining patients exhibit worse a score of ≥A1 or ≥F1. Three patients with a score of A1 or F1 at two years after LDLT maintained a score of A1 or F1 at five years; however, the remaining patients exhibited worse a score of ≥A2 or ≥F2.

Reviewed by 00013146

MAJOR COMMENTS:

1. **Assessment of fibrosis**

   The METAVIR system was mainly designed for the assessment of chronic viral hepatitis (particularly hepatitis C) in the native liver, where fibrosis is mainly periportal in location. There have been a number of recent studies suggesting that different patterns of late fibrosis (centrilobular and sinusoidal) occur in the paediatric liver allograft (Egawa, Hepatol Res. 2012 Sep; 42(9):895-903; Miyagawa-Hayashino. Liver Transpl. 2012 Nov;18(11):1333-42; Venturi C. Am J Transplant. 2012 Nov;12(11):2986-96; Yamada H. Pediatr Transplant. 2012 Dec;16(8):858-65). These are not referred to in the current study. The authors should:

   (a) State why they have chosen the METAVIR system.

   We described the following sentences in Discussion based on your comment, and added the above paper in References. "As to the concrete assessment methods for evaluating graft liver fibrosis, portal fibrosis–based liver fibrosis staging systems, such as those reported by Ishak16) and the Metavir Study Group,11) are widely used, even in studies of pediatric liver transplant recipients.7)-8),17) Therefore, we applied histopathological assessments using the Metavir score in the present study. Recent reports have indicated that centrilobular perisinusoidal fibrosis occurs in pediatric liver transplant recipients in association with tacrolimus withdrawal or the presence of donor-specific anti-human leukocyte antigen antibodies.18)-19) Venturi et al.17) recently developed a novel histopathological scoring system based on the detection of fibrosis in three areas: portal tracts, sinusoids, and centrilobular veins. However, the significance of these histopathological findings with respect to morbidity has yet to be clarified, and is the most important issue that should be addressed in the future."

   (b) Provide further information (re-analysing the biopsies if necessary) regarding the additional patterns of fibrosis referred to above.

   Recent reports have indicated that centrilobular perisinusoidal fibrosis occurs in pediatric liver transplant recipients in association with tacrolimus withdrawal or the presence of donor-specific anti-human leukocyte antigen antibodies.18)-19) Venturi et al.17) recently developed a novel histopathological scoring system based on the detection of fibrosis in three areas: portal tracts, sinusoids, and centrilobular veins. However, the significance of these histopathological findings with respect to morbidity has yet to be clarified, and is the most important issue that should be addressed in the future. Therefore, we applied histopathological assessments using the Metavir score in the present study, and thought that Metavir scores were alternative for other scores at present.

2. **Treatment with increased immunosuppression (IS)**

   (a) The reason for increasing IS is not clearly stated in the Methods or Results. The final paragraph of the Discussion suggests that this was carried out on the basis of a METAVIR score of $\geq$ A1 or $\geq$ F1. Please confirm if this is the case.

As to the strategy of increasing the dose of immunosuppressants after LDLT, we described in Methods (Strategy of increasing the dose of immunosuppressants after LDLT). In the outpatients, when the serum level of ALT or hyaluronic acid was high, we increased the dose of immunosuppressants if the suspected causes of elevation of these levels were an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. When the grade of PLB was A0 and F0, we gradually decreased the dose of immunosuppressants. In our department, we initially defined a histopathological abnormality as a Metavir score of ≥A2 or ≥F2. However, among 21 patients who underwent PLB at both two and five years after LDLT, the activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT exhibited worse a score of ≥A1 or ≥F1, respectively. Three patients with a score of A1 or F1 at two years after LDLT exhibited worse a score of ≥A2 or ≥F2, respectively. Therefore, we currently define a histopathological abnormality as a Metavir scoring system of ≥A1 or ≥F1 and consider such scores to indicate the need for treatment because liver fibrosis is reversible if early treatment is initiated.

(b) The nature of the increased immunosuppression used is not specified. The two case reports refer to increasing the dose of Tac and adding MMF. Was this done for all cases?

As to the clinical course of PLB patients who increased immunosuppression, we described in Results. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. The incidence of ≥A2 or ≥F2 at two years after LDLT was 3.4% (three cases), and all patients had an absolute score of ≥A2 (Table 4). In all cases, the dose of immunosuppessants was increased after PLB, and two patients who underwent a follow-up liver biopsy improved to below A1 and F1. The incidence of ≥A2 or ≥F2 at five years after LDLT was 20.0% (11 cases), and all patients had an absolute score of ≥F2 (Table 4). In all cases, the dose of immunosuppressants was increased after PLB, and all eight patients who underwent a follow-up liver biopsy improved to below A1 and F1.

(c) Follow-up data regarding the effects of immunosuppression are incomplete. It would be helpful to know the outcome after five years in those patients who were A0 and F0 at year 2 (and presumably therefore were not given increased immunosuppression – see point 2a above) versus the other patients in whom immunosuppression was increased.

As to the clinical course of PLB patients, we described in Results. PLB was performed at both two and five years after LDLT in 21 cases; the results are summarized in Table 6. The activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient, respectively. Seven patients with A0 and F0 at two years after LDLT maintained scores of A0 and F0 at five years; however, the remaining patients exhibit worse a score of ≥A1 or ≥F1. Three patients with a score of A1 or F1 at two years after LDLT maintained a score of A1 or F1 at five years; however, the remaining patients exhibited worse a score of ≥A2 or ≥F2.

3. **Assessment of inflammation**

A METAVIR score of A1 can be derived by portal inflammation with interface hepatitis (piecemeal necrosis) and/or by the presence of lobular inflammation. It would be helpful to have more detailed

information concerning the patterns of inflammation present in these patients. In particular, did any cases have features of central perivenulitis?

Recent reports have indicated that centrilobular perisinusoidal fibrosis occurs in pediatric liver transplant recipients in association with tacrolimus withdrawal or the presence of donor-specific anti-human leukocyte antigen antibodies.18)-19) Venturi et al.17) recently developed a novel histopathological scoring system based on the detection of fibrosis in three areas: portal tracts, sinusoids, and centrilobular veins. However, the significance of these histopathological findings with respect to morbidity has yet to be clarified, and is the most important issue that should be addressed in the future. Therefore, we applied histopathological assessments using the Metavir score in the present study, and thought that Metavir scores were alternative for other scores at present.

OTHER POINTS:

4. **Serum ALT as a predictor of inflammation at 2 years**

A previous study by Gelson, Transplantation. 2010 Mar 27;89(6):739-48 also demonstrated an association between transaminase levels and histological inflammatory activity in late protocol biopsies for patients with normal LFTs. This study was carried in an adult population, but should still be cited.

As to the association between ALT and histopathological findings, we described in Discussion based on your comments. In the present study, PLB performed two years after LDLT was found to be an unnecessary examination because the serum ALT level reflects the degree of portal inflammation, and immunosuppressive therapy should be modulated with preservation of below the ALT level of 20 IU/l. Gelson et al.20) reported that the histological inflammatory index is correlated with the ALT level. PLB performed at five years is an excellent examination for detecting early reversible graft fibrosis, as no serum markers reflect the degree of graft fibrosis. Therefore, PLB performed at two years after LDLT is an unnecessary examination because the serum ALT level reflects the degree of portal inflammation. In addition, immunosuppressive therapy should be modulated with preservation of the ALT level below 20 IU/l. However, PLB at five years is an excellent examination for detecting early reversible graft fibrosis, as no serum markers reflect the degree of graft fibrosis.

5. Why should cold ischaemia time and no acute cellular rejection be risk factors for inflammation ≥ A1 at two years?

We described the following sentences in Discussion. “We believe that ≥2hrs of cold ischemic time was found to be an independent risk factor for ≥A1 at two years after LDLT because a prolonged cold ischemic time may induce an immune response by affecting graft liver dysfunction. In addition, we believe that no acute cellular rejection was found to be an independent risk factor for ≥A1 at two years after LDLT because acute cellular rejection may give rise to an immune response due to the use of a lower number of immunosuppressants.”

6. **Page 12**.

Case I had a biopsy showing A2 F2 and was classified histologically as “borderline abnormal”. What does “borderline” mean? Case 2 with the same METAVIR scores was simply classified as “abnormal”.

We deleted the “borderline” to avoid confusion on your comment.

7. In the Discussion (page 13) the authors state that the necessity of protocol liver biopsies is controversial in paediatric allograft recipients, because of the risk of complications related to liver biopsy. The same controversy applies to the use of protocol liver biopsy in adult patients.

PLB suffers from a disadvantage both pediatric and adult patients just like your comments. Therefore, we changed the following sentences. “PLB is an invasive procedure that is potentially associated with severe complications (incidence: 0.57%).21) In the present study, although the rate

of PLB-associated complications was only 0.7%, this may nevertheless be considered high. Non-invasive examinations, such as imaging, may be used in place of PLB if such examinations become more effective than PLB in the future. Acoustic radiation force impulse and transient elastography imaging have been reported to exhibit good accuracy in the noninvasive diagnosis of liver fibrosis in the setting of pediatric liver transplantation.22)-23)"

8. **Figure 2**

The legend is incomplete. In addition to stating the METAVIR scores, the patterns of inflammation and fibrosis should be described.

(a) For the H & E section in Figure 2A, it is not clear if the inflammation is portal or perivenular (central perivenulitis) in distribution.

This is portal inflammation.

(b) The Azan-stained section for Figure 2A appears to show some fibrosis in centrilobular regions, although this is difficult to make out at the low magnification provided. Please comment if this is present? (see also point 1B above).

This is portal and pericellular fibrosis.

(c) The Azan-stained section for Figure 2B shows a delicate septum associated with possible bridging fibrosis. How is this classified as F0?

Our pathologist diagnosed that the Azan-stained section for Figure 2B was F0.

9. **Figure 3**

The figure legend is incomplete (as per Figure 2 – see above).

In Figure 3, the Metavir scores were abnormal: A2 (portal inflammation) and F2 (portal fibrosis) (a). A follow-up liver biopsy was performed at 20 months after PLB, at which time, the scores were A1 (portal inflammation) and F0 (b).

Reviewed by 00073425

The paper prestentsthe the clinical significance of histopathological findings in liver biopsy performed at 2 and 5 years after liver transplantation in pediatric patients. The problem is interesting, since graft liver fibrosis promotes liver cirrhosis. I have few questions:

Thank you for your favorable comment.

1. What was the cause of liver transpalantation in described padiatric patients?

Almost all of the original disease was biliary atresia. The original disease rate of biliary atresia was 70.9% and 78.2% at two and five years after LDLT, respectively. We showed on Table 1 in detail.

2. Why was 20 IU/L of ALT used as cut off value? It should be explained in detail. There are some redactional errors: aspartate amino transferase instead of alanine amino transferase (in the Result section).

As to the cut off value of ALT, we perceived as a predictive factor because a multivariate analysis including these variables identified a ALT level of ≥20 IU/l to be independent risk factors for ≥A1 at two years after LDLT (p=0.012) (Table 3). As a result of the ROC curve analysis of the ALT level at two years after LDLT in the patients with a score of ≥A1, the recommended cutoff value for diagnosing ≥A1 was set at 20 IU/l (sensitivity: 50.0%, specificity: 76.1%, area under the curve: 0.685 and 95% confidence interval:

0.557-0.813) (Figure 1).

As to grammatical and spell errors, the revised paper has been proofread for grammar and style by a native English-speaking pathologist.

Reviewed by 02446778

Good work , needs few grammatical corrections.

Thank you for your favorable comment. The revised paper has been proofread for grammar and style by a native English-speaking pathologist.

Reviewed by 00005191

The paper's assumption is that it remains hard to predict the occurrence of graft liver fibrosis or portal inflammation with the standard liver function test. It has recently been reported that histopathological assessments using protocol liver biopsy (PLB) could be useful. However, the indication of treatment for abnormal PLB is controversial, especially in the pediatric patient population. The paper is a retrospective study investigating the clinical significance of PLB in pediatric living donor liver transplantation (LDLT). Between July 2008 and August 2012, 89 and 55 PLBs were performed for pediatric patients at two and five years after liver transplantation at the Authors' institution in Japan. After analyzing and comparing the reports, the Authors conclude that: - PLB at 2 years after LDLT is an excellent examination for early detection of graft liver fibrosis because it is possible to predict the occurrence of portal inflammation by the serum ALT, but none of the serum markers reflects the graft fibrosis; - PLB at 5 years after LDLT is an excellent examination for early detection of portal inflammation and graft liver fibrosis because none of the serum markers reflects the portal inflammation and graft fibrosis.

The Authors are obviously well aware that PLB is an invasive examination with a risk of complications and that a very problematic aspect ot it is the obscure definition of abnormal hosto èathology, but they should probably stress it further and extend this portion of their Discussion with more data.

Thank you for your favorable comment.

Style should be reviewed here and there. See for example on page 11 where the verbal expression should be corrected in the sentence "The ratio of patients who were increased the immunosuppressants after PLB...". Or again on page 12, where the sentence "The immunosuppression was strengthened by increasing the does

of Tac....", where "does" is clearly mispelled ("dose").

The revised paper has been proofread for grammar and style by a native English-speaking pathologist.

Reviewed by 00506409

In this manuscript the authors report on the value of a protocol liver biopsy in pediatric patients after a living-donor liver transplant. The post-transplant monitoring includes convential parameters such as liver function tests. A protocol liver biopsy was conducted at 2 and 5 years after transplantation, and abnormalities were scored following the Metavir scoring system regarding activity (that means, the amount of inflammation (specifically, the intensity of necro-inflammatory lesions)) and fibrosis. A retrospective evaluation was conducted in a reasonable large data set, comprising 89 biopsies taken at 2 years after transplantation, and 55 biopsies taken at 5 years after transplantation from a total number of 144 transplants in a 4-year period. The authors document that serum alanine amino transferase can function as a predictor for activity in the 2-year biopsy, but none of the parameters investigated has predictive value for the presence of fibrosis. The relevance of detecting activity or fibrosis is that increased immunosuppression can restore these abnormalities as shown in two illustrative case reports. The design of the study and the message is clear. There are a number of najor and minor comments that need to be addressed in the revision of the manusvript. There are a number of comments. ?

Thank you for your favorable comment.

A major point is that the manuscript is difficult to read because of use of english language. The paper should go through editing the english language before being accepted for publication. ?

The revised paper has been proofread for grammar and style by a native English-speaking pathologist.

It is advised to include a table describing the various scores for activity and fibrosis following the Metavir analysis. Why is the term ‘activity’ used, when it describes ‘inflammation’: would it ne easier to use the term ‘inflammation’? ?

We think that ‘activity’ is synonymous with ‘inflammation’. We used in terms of ‘activity’ because we applied histopathological assessments using the Metavir score in the present study.

The conventional follow-up of patients after transplantation should be described. ?

As to the strategy of immunosuppressive therapy after LDLT, we described in Methods (Immunosuppressive therapy). Tacrolimus (Tac) and methylprednisolone (MP) were used as the standard

postoperative immunosuppressive resimen. The target trough level of Tac was 15-20 ng/ml during the first week, 8-12 ng/ml during the first month, 5-8 ng/ml during the first six months, 3-5 ng/ml during the first year, and 2-4 ng/ml thereafter. MP was administered at an initial dose of 20 ml/kg intravenously on the morning of the operation and before graft reperfusion. The MP dose was thereafter decreased gradually to 3 mg/kg/d on postoperative day (POD) 1, to 0.5 mg/kg/d on POD 7 and 0.25 mg/kg/d at one month after LDLT, then discontinued within one year, except in patients in whom immunosuppression could not be maintained at the lower dose. Mycophenolate mofetil (MMF) was used when more potent immunosuppression was required, for example, in ABO-incompatible recipients older than five years, patients with steroid-resistant acute rejection episodes and patients with liver dysfunction following the cessation of MP therapy.

As to the strategy of increasing the dose of immunosuppressants after LDLT, we described in Methods (Strategy of increasing the dose of immunosuppressants after LDLT). In the outpatients, when the serum level of ALT or hyaluronic acid was high, we increased the dose of immunosuppressants if the suspected causes of elevation of these levels were an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. When the grade of PLB was A0 and F0, we gradually decreased the dose of immunosuppressants.

Following this it needs to be stated how acute cellular rejection was diagnosed, and what was the consequence of this diagnosis. ?

We described the following sentences in Methods (Diagnosis of acute cellular rejection). “All episodes of acute cellular rejection were diagnosed based on the histopathological findings of a liver biopsy. In all specimens, the diagnosis of acute cellular rejection was evaluated by highly experienced pathologists and graded into four classes according to the Banff scheme.10) The degree of portal infiltration of lymphocytes (P0-3), bile duct inflammation or damage (B0-3) and venous endothelial inflammation (V0-3) in the Banff scheme was evaluated. A liver biopsy was indicated when all liver function data (aspartate amino transferase, alanine amino transferase (ALT), gamma-glutamyl transpeptidase, and total bilirubin) were elevated compared with the previous data.”

It is not clear which parameters are included in the univariate and multivariate statistical analysis. This major point is quite relevant as different series of parameters are presented in the results. ?

We performed the multivariate analysis for the parameter which was $p<0.1000$ in the univariate analysis.

It is advised to state already in the Introduction , or in the Discussion why a protocol biopsy is performed, namely to have the possibility to adapt immunosuppression? ?

We divided the liver biopsy into episode and protocol, and described the following sentences in Methods. “In the outpatients, when the serum level of ALT or hyaluronic acid was high, we increased the dose of immunosuppressants if the suspected causes of elevation of these levels were an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. When the grade of PLB was A0 and F0, we gradually decreased the dose of immunosuppressants.”

Following this point the statement “The ratio of patients who were increased the immunosuppressants after PLB was 28.1% (25/89). The items were 14 patients (20.9%: 14/67) in A0 and 11 patients (50.0%: 11/22) in ≥A1 ($p=0.008$), or the items were 15 patients (20.9%: 14/67) in F0 and 10 patients (45.5%: 11/22) in ≥F1 ($p=0.013$)” on page 10 needs explanation. Why was immunosuppression increased after a biopsy in patients with a biopsy score A0 or F0? This question is relevant because case reports present only patients with a

biopsy score A2 and F2. On the other hand, not in all patients with a score ≥A1 or ≥F1 the immunosuppression was increased, and this needs an explanatation, i.e., why was immunosuppression increased in some and not in others? ? A similar statement is made on page 11, with the same comment: “The ratio of patients who were increased the immunosuppressants after PLB was 36.4% (20/55). The items were 5 patients (17.2%: 5/29) in A0 and 15 patients (57.7%: 15/26) in ≥A1 (p=0.002), or the items were 10 patients (27.8%: 10/36) in F0 and 10 patients (52.6%: 10/19) in ≥F1 (p=0.068).”. ?

We described a strategy of our immunosuppressant therapy in Methods (Strategy of increasing the dose of immunosuppressants after LDLT). In the outpatients, when the serum level of ALT or hyaluronic acid was high, we increased the dose of immunosuppressants if the suspected causes of elevation of these levels were an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. When the grade of PLB was A0 and F0, we gradually decreased the dose of immunosuppressants. Therefore, some patients in A0 or F0 increased the immunosuppressants after PLB because the serum level of ALT or hyaluronic acid was high. However, these increasing the dose of immunosuppressants was not concerned with the results of PLB, and therefore, these results was deleted.

We described the following sentences in Discussion based on the present study. “In our department, we initially defined a histopathological abnormality as a Metavir score of ≥A2 or ≥F2. However, among 21 patients who underwent PLB at both two and five years after LDLT, the activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT exhibited worse a score of ≥A1 or ≥F1, respectively. Three patients with a score of A1 or F1 at two years after LDLT exhibited worse a score of ≥A2 or ≥F2, respectively. Therefore, we currently define a histopathological abnormality as a Metavir scoring system of ≥A1 or ≥F1 and consider such scores to indicate the need for treatment because liver fibrosis is reversible if early treatment is initiated.”

It is advised to present more data on the patients in the case reports. For instance, the demographic data presented in Table 1 should be given for the patients, which could be done in a table. For instance, it is not clear whether the abnormalities in the liver biopsy were due to insufficient exposure to immunosuppressants. If this is the case, the beneficial effect of increasing the immunosuppressant dose is logical. ?

As to the clinical course of PLB patients, we described in Results. PLB was performed at both two and five years after LDLT in 21 cases; the results are summarized in Table 6. The activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient, respectively. Seven patients with A0 and F0 at two years after LDLT maintained scores of A0 and F0 at five years; however, the remaining patients exhibit worse a score of ≥A1 or ≥F1. Three patients with a score of A1 or F1 at two years after LDLT maintained a score of A1 or F1 at five years; however, the remaining patients exhibited worse a score of ≥A2 or ≥F2.

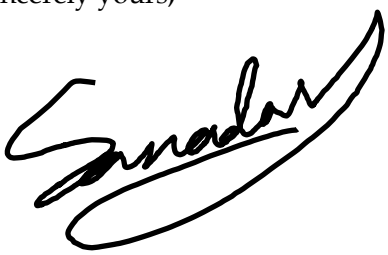
As to the clinical course of PLB patients who increased immunosuppression, we described in Results. In the recipients of PLB, when the grade of PLB was ≥A2 or ≥F2, we increased the dose of immunosuppressants in order to provide early treatment for portal inflammation or fibrosis. The incidence of ≥A2 or ≥F2 at two years after LDLT was 3.4% (three cases), and all patients had an absolute score of ≥A2 (Table 4). In all cases, the dose of immunosuppessants was increased after PLB, and two patients who underwent a follow-up liver biopsy improved to below A1 and F1. The incidence of ≥A2 or ≥F2 at five years after LDLT was 20.0% (11 cases), and all patients had an absolute score of ≥F2 (Table 4). In all cases, the dose of immunosuppressants was increased after PLB, and all eight patients who underwent a follow-up liver biopsy improved to below A1 and F1.

We described the following sentences in Discussion based on the present study. “In our department, we initially defined a histopathological abnormality as a Metavir score of ≥A2 or ≥F2. However, among 21 patients who underwent PLB at both two and five years after LDLT, the activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥A2 or ≥F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT exhibited worse a score of ≥A1 or ≥F1, respectively. Three patients with a score of A1 or F1 at two years after LDLT exhibited worse a score of ≥A2 or ≥F2, respectively. Therefore, we currently define a histopathological abnormality as a Metavir scoring system of ≥A1 or ≥F1 and consider such scores to indicate the need for treatment because liver fibrosis is reversible if early treatment is initiated.”

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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