Response to Reviewers and the Editorial Team

Dear Editor and Reviewers:

We appreciate your encouragement and kind comments and are happy to provide a r evised document. Few studies exist regarding BSI after pediatric LT, and risk factors are not well-known. We believe that this report will be a useful reference, especially for clinicians in gastroenterology and transplantation fields. We have enjoyed receivin g comments from Editorial Team and Reviewers and if another revision is needed, w e will provide it as soon as possible. Our responses to reviewer are presented herein.

Reviewer #1

Comment 1: As there might be nonlinear relationship between continuous variables (e. g., age, height z-score, weight z-score, volume of RBC transfusion, and post LT hospi tal day) and BSI in pediatric LT, this strategy might couldn't reflect the real associati on for this issue. I suggest using restricted cubic splines to explore the best cut-off v alues for these variables, which is more powerful for dealing with continuous variabl es. The model should be reconstructed by this strategy.

Answer 1: Thank you for your kind comments; we agree with your recommendation. There were non-linear relationships between continuous variables and risk metrics of BSI. As you recommended, we conducted restricted cubic spline regression (RCS) to evaluate the relationship between continuous variables and their ORs, using the rms package in R. The four knots were positioned in percentiles 10, 25, 75, and 90 of the empirical distribution of variables. First, probability density from logistic regression of raw continuous variables was evaluated according to the BSI events (Figure R1-A). Nagelkerke's R^2, Brier score, and Hosmer–Lemshow *p*-value were calculated to evaluate goodness of fit. Then, if there was evidence of non-linear association, we examined the RCS curve to identify inflection points that could be used to categorize ranges of values (Figure R1-B), and the optimal cut-off point for each continuous variable was selected on the maximum AUC. Detailed methodologies were described in previous literature[1]. Using selected cut-off values, we then evaluated clinical usefulness in terms of discrimination (sensitivity, specificity, positive predictive

value, negative predictive value, and diagnostic accuracy) (Figure R1-C). Finally, the clinical usefulness of new cut-off values from RCS analysis was compared to our previous values from ROC curve analysis (Table R1).

In results, there were non-linearity relationships between some continuous variables (age, height z-score, weight z-score, operation time, PELD score, RBC transfusion, and donor BMI) and their own ORs of BSI. However, there was no natural cut-off point for hospital stay in RCS analysis (Figure R1-D), whereas ROC optimal cut-off values for hospital stay were defined by the Youden index [maximum (sensitivity + specificity − 1)] in ROC curve analysis (Figure R1-F). Table R1 shows summarized AUCs of cut-off values from RCS analyses and ROC analyses; overall AUCs from RCS analysis seem to be improved compared to those of our previous ROC analysis. Using newly categorized variables, we conducted univariate and multivariate logistic regression analyses (Table R2). In univariate analysis, age, weight z-score, growth failure, biliary atresia, liver support system, RBC transfusion, post-LT hospital stay, reoperation, and portal vein complication were risk factors of BSI. In multivariate analysis, age, growth failure, ventilator use, liver support system, and post-LT hospital stay were independent risk factors of BSI. The profiles of risk factors were roughly similar to those of our previous results.

The new Nagelkerke's R^2 was 0.201, which was the same value of our previous logistic regression analysis using cut-off values from ROC curve analysis. However, *p*-value of Hosmer–Lemshow test was 0.032, suggesting that goodness-of-fit of this analysis was insufficient. To evaluate the discriminatory ability of the new model, two AUCs (previous analysis vs. new analysis) were compared (Figure R2), and that of the new analysis (0.75) was almost identical to that of the previous analysis. Overall, the authors think that RCS analysis for these data improved each AUCs slightly by providing new cut-off values but did not improve the performance of multivariate logistic regression model. Then, we discussed whether the newly reconstructed model was more clinically meaningful compared to the previous one. We believe that there is no difference in terms of clinical meaning. In conclusion, we decided to continue using the previous logistic regression model based on cut-off values from the conventional ROC analysis.

Selection of variables and their cut-off values is critical in statistical modeling based on the purpose of the modeling: prediction, estimation, and hypothesis testing. By this point of view, the priority of our study is hypothesis testing to identify the presence of interpretable and simple clinical factors. We believe that parsimony and interpretable modeling are of

virtue in this analysis. Therefore, we adopted dichotomization of prognostic variables in this clinical study. However, this may have resulted in some lost information, and estimated values may have been reduced in precision. RCS analysis can be a good method for data exploration; unfortunately, it was not particularly helpful in this dataset. We are grateful for the opportunity to revise our manuscript and are willing to add RCS analysis to the manuscript if you feel it would strengthen the study.

Figure R1. Examples of RCS analysis to evaluate BSI risk and continuous variables. A~C: RCS for age, D~F: RCS for hospital stay.

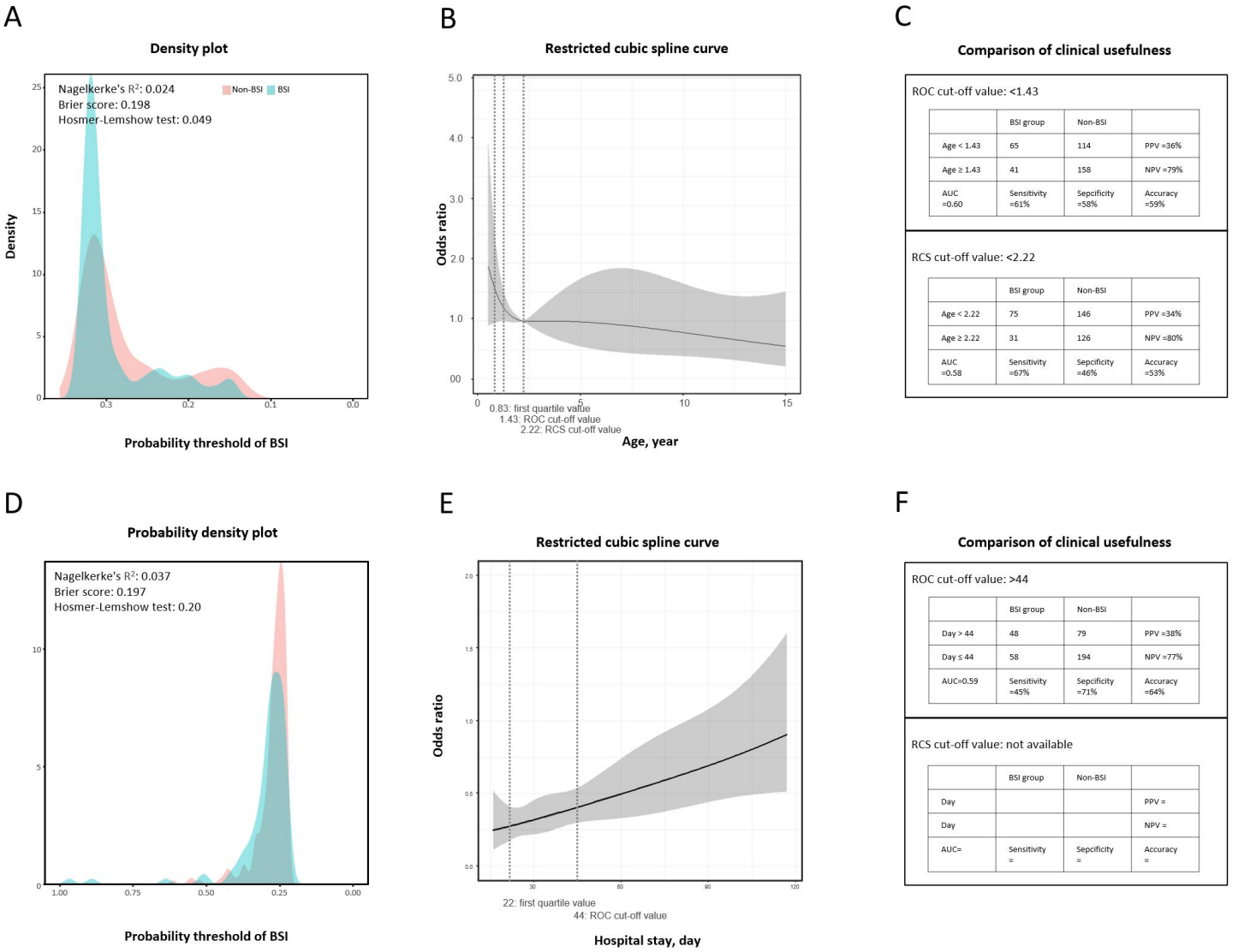


Table R1. Comparison of discrimatory ability among cut-off variables according RCS and ROC analyses.

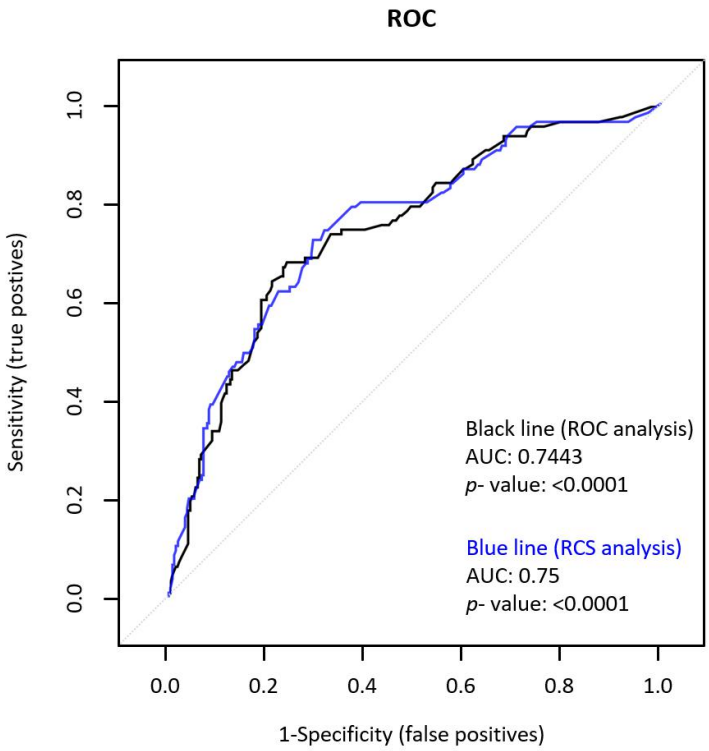
	Non-BSI (n=272)	**BSI (n=106)**	**RCS analysis (new analysis)**		**ROC analysis (previous analysis)**	
Variables	Median (IQR) or n (%)	Median (IQR) or n (%)	AUC	Cut-off value	AUC	Cut-off value
Age, year	1.83 (0.86~6.0)	1.17 (0.75~3.08)	0.58	<2.22	0.60	≤1.33
Height, z score	-0.66 (-1.60~0.34)	-1.23 (-2.17~0.0)	0.54	<0.15	0.59	≤-1.22
Weight, z score	-0.07 (-1.04~0.71)	-0.53 (-1.7~0.4)	0.64	<-0.11	0.60	≤-0.11
PELD	16.2 (6.40~23.7)	15.5 (10.6~24.3)	0.58	>25	0.54	>8.99
Operation time, hour	417 (357~536)	411 (350~507)	0.54	<7 or >11	0.52	> 8.25
RBC transfusion, cc/kg	17.37 (7.55~29.54)	25.7 (9.8~42.1)	0.64	>20.5	0.61	>21.51
Post-LT hospital day, day	36 (26~50)	41 (28~67)	NA	NA	0.59	>44
Donor, body mass index	22.3 (20.3~24.4)	22.7 (21.1~24.5)	0.55	>22	0.54	>20.58

NA: not available due to linear relation between variable and BSI outcome.

Table R2. Univariate and multivariate logistic regression analyses using new cut-off v alues.

	Univariate analysis			**Mutivariate analysis**		
Variables	ORs	95% CI	*p*-value	ORs	95% CI	*p*-value
Age	2.09	(1.29~3.38)	0.003	2.11	(1.14~3.9)	0.017
Sex, male	1.149	(0.733~1.801)	0.544			
Height, z score	1.61	(0.95~2.7)	0.075	1.04	(0.53~2.03)	0.90
Weight, z score	1.80	(1.12~2.89)	0.015	1.46	(0.79~2.70)	0.228
Growth failure	2.776	(1.696~4.545)	<0.001	2.36	(1.30~4.29)	0.005
Diagnosis: biliary atresia	1.699	(1.074~2.689)	0.024	1.23	(0.67~2.22)	0.499
Ventilator	2.134	(0.936~4.864)	0.071	2.65	(1.02~6.89)	0.046
Renal replacement	1.937	(0.757~4.958)	0.168			
Liver support system	2.729	(1.102~6.761)	0.03	3.51	(1.14~10.7))	0.028
PELD	1.30	(0.74~2.28)	0.35			
MELD	1.038	(0.946~1.139)	0.432			
LT, DDLT	0.658	(0.377~1.149)	0.141			
ABO mismatch	0.961	(0.250~3.694)	0.954			
Operation time	0.63	(0.34~1.17)	0.144			
RBC transfusion, cc/kg	2.116	(1.34~3.35)	0.001	1.48	(0.88~2.48)	0.136
Post-LT hospital stay (days)	2.022	(1.27~3.21)	0.003	2.21	(1.31~3.77)	0.003
Donor, male	0.798	(0.507~1.257)	0.331			
Donor, body mass index	1.07	(0.61~1.87)	0.804			
Reoperation	2.070	(1.121~3.823)	0.02	1.41	(0.69~2.85)	0.35
Hepatic artery complication	2.596	(0.361~18.672)	0.343			
Hepatic vein complication	1.703	(0.856~3.390)	0.129			
Portal vein complication	2.656	(1.452~4.859)	0.002	1.93	(0.99~3.75)	0.052
Bile duct complication	0.792	(0.283~2.219)	0.657			
Cytomegalovirus infection	0.935	(0.593~1.473)	0.771			
Epstein-Barr virus infection	0.951	(0.587~1.541)	0.839			
Acute cellular rejection	1.433	(0.913~2.250)	0.118			

Figure R2. Comparison of AUCs between previous ROC analysis and new RCS analysis.



Comment 2: Results: In the last paragraph, the authors stated that, “The predictive performance of the model was internally validated through 10-fold cross-validation and the average validation-corrected AUC was 0.701 (95% CI: 0.641~0.762) (Supplementary Table 4), suggesting overfitting of apparent performance in the analysis.” Do you mean the model was overfitted? If so, the model may cannot be applied to other cohorts. -Discussion: Just as you stated that, “this nomogram needs an external validation”.

Answer 2: Thank you for your insightful comment. You are correct that this model cannot be applied to other cohorts. There are risks of overfitting in our logistic regression model and decreased performance in the internal validation. If we develop a model to predict future event, we are primarily interested in the validity of the predictions for new subjects, outside of the current study group, and overfitting threatens validity. Clinical practice and features in a single center may differ from those of other centers. Shrinkage (reducing the estimated regression coefficient to less extreme value) by performing a multicenter study can be a statistical solution[2]. If a multicenter study is not possible, 'bootstrap resampling' can be another reasonable technique to correct overfitting. Therefore, we added a bootstrap resampling method in the internal validation.

Our retrospective study was not to designed to develop a prediction model. Description of pathogens of BSI after pediatric LT and identification of independent risk factors were the primary aims of our study. Our data may be helpful to pediatric transplant hepatologists, and infectionists who encounter difficulties in post-LT care. In addition, growth failure as one of independent risks implies the importance of nutritional care in the preparation of LT in children and our identification of this and other risks such as young age, liver support device, and long hospital stay, can help clinicians identify high-risk patients.

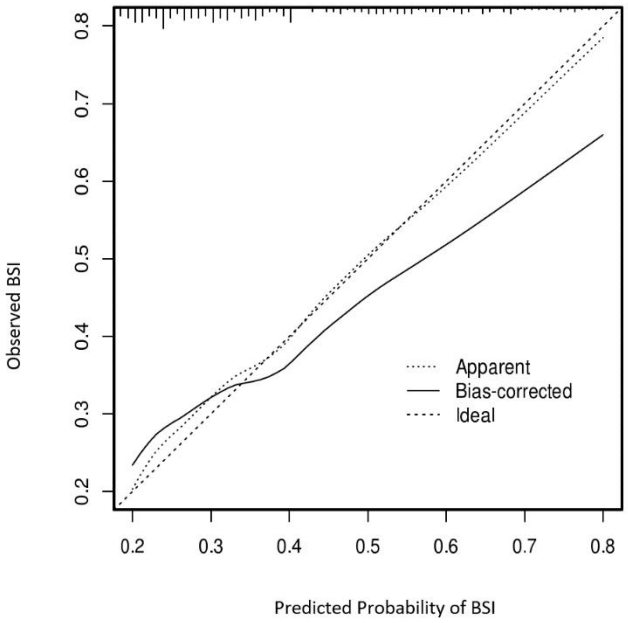
It should be noted that the identification of these risks is merely based Wald statistics, and the logistic model should be evaluated in terms of overall performance. The Chi square test and Hosmer–Lemshow test are commonly used for simple evaluation of a model's fitness. Many risk studies provide only ORs and p-value and do not provide detailed true aspects of overall performance of their models. That is why 'estimation bias' prevails in the medical field, in our opinion. Therefore, detailed evaluati

on should include at least the model AUC for discrimination and calibration of slope for precision, according to the TRIPOD guideline[2]. In addition, cross-validation or bootstrapping is essential in determining the reproducibility of modeling[2].

Let's come back to the point at the 'overfitting' issue. Unfortunately, additional internal validation by bootstrap resampling showed optimism in this logistic regression analysis. Bootstrap-corrected AUC was 0.71 and calibration slope was decreased to 0.8 (Figure R3). Because the criteria of validation included obtaining an optimism of ≤0.02 in AUC and slope ≥0.9[3], this study failed to show the reproducibility in the internal validation. Herein, we concluded that this dataset is not 'ready' to be applied to other cohorts, as reviewer #1 mentioned. Therefore, we deleted our nomogram. Also, we revised our descriptions for RESULTS and DISCUSSION. In the beginning, the purpose to provide this nomogram was only to visualize the impact of risk factors on the BSI event. We appreciate your comments.

Correction 2: [METHOD] The predictive performance of the model was also internally validated through 10-fold cross-validation and the bootstrap resampling method. Based on the TRIPOD statement, the mean difference between the 200 bootstrapping re-samples is defined as the optimism. [RESULTS] Bootstrap-corrected AUC was 0.71 and bootstrap-corrected calibration slope was decreased to 0.8 (Supplementary Figure 2). [DISCUSSION] In addition, internal validation showed optimism about the apparent performance. To develop a solid prediction model, multicenter studies are needed.

Figure R3 (Supplementary Figure 2). Bootstrap-corrected calibration slope assessed by the bootstrap resampling method.



Reviewer #2

Comment 1: please answer the questions raised by reviewer and correct the mistakes pointed by reviewer.

Answer 1: We appreciate your kind comment. We have revised the paper, as per the reviewer's request.

Reviewer #3

Comment 1: Better not to repeat the number of the patients, both in methods and results.

Answer 1: Thank you for your kind comments. We revised sentences as you mentioned.

Correction 1: [METHOD] A total of 378 cases were reviewed, including 287 (76%) living donor LTs and 91 (24%) deceased donor LTs. Among them, 27 patients required a second transplant. Medical records of the patients were reviewed for the period up to 36 months after the transplant, or until death. [RESULT] ~~During the study period, 378 children had primary LTs, consisting of 287 (76%) living donor LTs and 91 (24%) deceased donor LTs. Among them, 27 children experienced re-LTs.~~ The median recipient age of the 378 children with LTs was 1.58 years (interquartile range [IQR]: 0.83~5.42 years)

Comment 2: The study results contributed to the design of a nomogram to estimate the risk for bloodstream infections.

Answer 2: As we mentioned to Reviewer 1, the study was not to develop a prediction model and nomogram. We think that this nomogram caused some misunderstanding of our real motives: nomogram can be used to visualize the impact of risk factors for BSI. To develop a solid prediction model, we believe at least 1,000 samples are needed, and this study just meets the minimal requirements of the logistic regression analysis. Therefore, the nomogram is not ready to predict BSI and we deleted from the manuscript.

Correction 2: [Supplementary data] Supplementary Figure 2 was deleted.

Comment 3: I would include a paragraph on the strengths of the study.

Answer 3: We agree, as the study has several unique strengths compared to findings from other studies (Supplementary Table 2). First above all, this is the largest retrospective study to describe detailed pathogens of BSI. Second, this is the first study to

reveal the growth failure as an independent risk factor of BSI. Only a few studies have identified risks of BSI in pediatric LT patients (Supplementary Table 2), and have specified low body weight, blood loss, etiology (biliary atresia), young age, bile duct complication, long hospital stay, and long operation time as risk factor. Growth failure in children with end-stage liver disease generally indicates the severity of liver disease and increased mortality. Therefore, the PELD score system, which predicts waiting list mortality before LT in children, includes growth failure as poor prognostic factor. PELD score system is also known to predict mortality after LT. Therefore, the finding between post-LT BSI and growth failure suggests the clinical importance of nutritional care before LT.

Correction 3: [DISCUSSION] This large-scale retrospective study aimed to assess prevalence and risk factors of pediatric post-LT BSI based on extensive statistical analysis.

This is the first study to reveal growth failure as an independent risk factor of BSI, suggesting the clinical importance of nutritional care before LT.

Comment 4: Few minor corrections should be made. The authors must verify the punctuation and some English language errors again.

Answer 4: We apologize for the language issues. We made some modifications after English correction service and have repeated the revision service.

Reviewer #4

Comment 1: The title needs verb as (evaluation or assessment ---)to be more conclusi ve

Answer 1: We appreciate your kind comments. Your recommendation is apt, and the title was changed to "Assessment of pathogens and risk factors associated with bloo dstream infection in the year after pediatric liver transplantation".

Correction 1: [TITLE] Assessment of pathogens and risk factors associated with blood stream infection in the year after pediatric liver transplantation

Comment 2: Introduction need definition first of blood stream infection.

Answer 2: Thank you for your precise comment. We added a summary on BSI. Than k you.

Correction 2: [INTRODUCTION] BSI is infection present in the bloodstream, which is normally a sterile environment[4,5]. BSI is diagnosed when bacteria or fungi are detect ed in blood cultures, and sepsis is the common inflammatory immune response. Cons idering the perioperative complexity of LT, children may be exposed to BSI as a seve re complication of localized infection in the abdomen, contamination during surgery, or from catheters or invasive procedures.

Comment 3: What the number of cases have dual infections.

Answer 2: Thank you for the important question. There were 12 cases of dual infecti on and 1 case of triple infection.

Comment 4: What is effect of CMV infection (59 %) and EBV on the survival. you fo cus only on bacterial infections and neglect these viral infections during discussing yo ur results

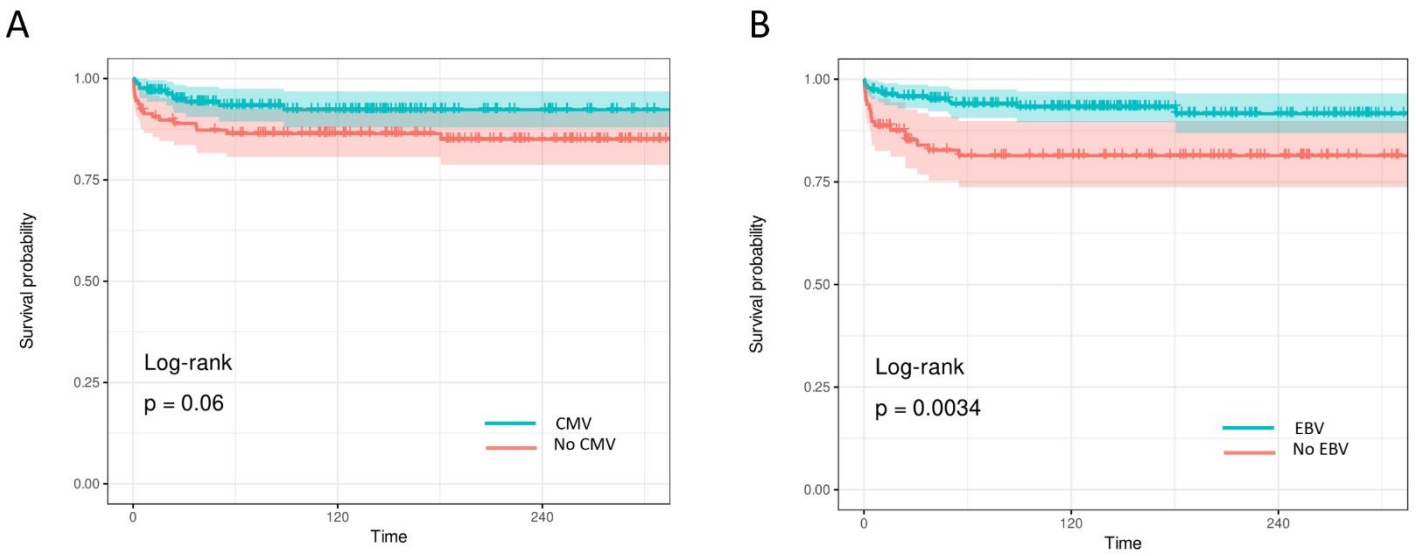
Answer 4: Thank you for your comments. This study is about bloodstream infection after pediatric LT. By the general definition of BSI, viral infection is not included, alt hough viral infections such as CMV and EBV spread through the bloodstream. Theref ore, we described CMV and EBV as risks for BSI. It is our mistake to omit the defin ition of BSI in the INTRODUCTION. We realized your suggestion (definition of BSI i n INTRODUCTION) is important.

However, the topic of CMV and EBV is very important in survival after pediatric LT, although these are not BSIs. CMV and EBV are well-known independent poor p rognostic factors to patient and graft survival. CMV directly invades grafts and vario us organs, and CMV infections may be related with graft failure and mortality[6]. In a ddition, CMV infection is thought to indirectly increase the risk of acute rejection and opportunistic infections after LT[7,8]. Similarly, post-transplant lymphoproliferative diso rder is a fatal complication mainly evoked by chronic exposure to EBV[9]. Because pe diatric patients are likely to be naive to these viral infections before LT, they are gen erally at greater risk than adults. We published studies titles 'CMV infection and its hybrid strategy' and 'EBV and risks for PTLD'[10,11]. In those reports, CMV viremia wa s frequent. No CMV disease was noted, and CMV was not related to survival in our cohort. Contrarily, we observed that the mortality of children with PTLD was 14.3%. PTLD mortality has been decreasing recently in our cohort, as seen through extensiv e viral surveillance and prevention strategy. This decrease of PTLD mortality and the effective strategy behind it were recently published in our LDLT report[12]. In this co hort, we demonstrated the effect of viral infections on patient survival (Figure R3). T here was no survival difference between the CMV infection group and non-CMV gro up (Figure R3-A). Furthermore, the EBV-positive group had higher survival than non-EBV group (Figure R3-B). We believe that this phenomenon cannot be understood si mply because viral quantitative PCR monitoring had been used since 2003 and univer sal application began in 2005 in our center. Therefore, the EBV monitoring was missi ng for patients receiving LT during the early period of our transplant center on non-EBV group. To deal with the missing data and exclusion criteria, we should analyze subgroups.

However, the scope of the present study is about BSI and the study does not inc lude viral infections in the beginning. In addition, the analysis about virus was alrea

dy included in our recent report this year[12]. We request your kind understanding re garding this, as we are concerned about data duplication. We will add further viral d ata in the next revision if you deem it necessary.

Figure R3. Patient survivals after LT according to viral infection. A: CMV infection. B: EBV infection.



Comment 5: The discussion better be started by time line of infection after transplant ation and accordingly a table better be added to classify these infections according to timeline after transplantation.

Answer 5: We agree with your recommendation. We added a timeline for infection af ter LT and a TABLE in the supplementary data to discuss time line of BSI.

Correction 5: [SUPPLEMENTARY FIGURE 3.] Causative pathogens contributing to BSI according to the time after transplantation. Gram-positive bacteria predominated in t he first month, but after that, the proportion of gram-negative bacteria increases. Fun gal infections were identified only in the first month. Line thickness indicates the nu mber of BSI episodes.

[DISCUSSION] Most of the first episodes of BSI in our cohort occurred in the early phase after LT; about 60% of BSI episodes within a year after LT developed in the fi rst month after surgery (Supplementary Figure 3).

Gram-positive bacteria predominated as pathogens in the first month, but after that, t he proportion of Gram-negative bacteria increased (Supplementary Figure 3).

Comment 6: References not coping with the style of the journal(e.g where the DOI P MID)

Answer 6: We revised the references according to the journal style.

Correction 6: [REFERENCE]

Science Editor

Comment 1: There are few concerns regarding the possibility of overfitting the model, and validation of the nomogram in other systems in the country and outside the co untry. These concerns should be address in the discussion paragraph.

Answer 1: Thank you for your insightful comments. This was an important issue and we agree with Reviewer #1. There are risks of overfitting in our logistic regression model and also of decreased performance in the internal validation. We performed an additional bootstrap resampling method to check the overfitting in this model, resulti ng in a failure of internal validation. As reviewer #1 suggested, we deleted the nomo gram. Our retrospective study was not to designed to develop a prediction model. D escription of pathogens of BSI after pediatric LT and identification of independent ris k factors are the primary aims of our study.

Correction 1: Please read 'response to reviewer #1'.

Comment 2: Finally, the references have to be adjusted to respect the journal style.

Answer 2: We revised references according to the journal style.

Correction 2: [REFERENCE]

Company editor-in-chief

Comment 1: Please provide decomposable Figures (in which all components are mova ble and editable), organize them into a single PowerPoint file.

Answer 1: Yes, we have included the PowerPoint file in our re-submission.

Comment 2: Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing spe cifications, and the lines of each row or column of the table should be aligned. Do n ot use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

Answer 2: We have revised the tables accordingly.

References

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