Manuscript title

The impact of chronic Epstein–Barr virus infection on the liver graft of pediatric liver transplant recipients: A retrospective observational study

Running title

Chronic pediatric EBV and liver graft

Key words

Epstein–Barr virus, graft fibrosis, rejection, pediatric liver transplantation

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Abstract

Background:

Chronic high Epstein–Barr virus loads (CHEBV) are commonly observed in pediatric liver transplant patients. However, it is unclear how CHEBV impacts the liver graft. The aim of this study was to clarify the clinical and pathological impacts of CHEBV on the liver graft.

Methods

From 2012 through 2020, we retrospectively investigated 46 pediatric liver transplant patients (under 16 years old) who survived ≥ 6 months. The patients were divided into two groups: CHEBV group (EBV DNA >10,000 IU/mL of whole blood for ≥ 6 months) and NCHEBV group (patients who did not meet CHEBV criteria). Tacrolimus was reduced to < 3.0 ng/mL in patients with EBV DNA > 5,000 IU/mL. Blood biochemistry date and pathological findings, obtained at the time of protocol and episodic biopsy, were compared between the two groups.

Results

Out of 46 patients, 28 CHEBV and 18 NCHEBV patients were enrolled. The blood biochemical examination did not show a significant difference between the two groups. In addition, no significant differences between the two groups were found in the pathological findings, including frequency of late acute rejection and the progression of fibrosis at the time of both protocol and episodic biopsy. Appropriate adjustment of immunosuppression for CHEBV management may have contributed to the prevention of the progression of fibrosis.

Conclusion

CHEBV had little adverse effect on the liver graft. Graft fibrosis might have been avoided through optimal dose modification of tacrolimus. Further long-term monitoring is necessary because CHEBV may affect the pediatric liver graft in the long term.

List of abbreviations

Alb, albumin; ALT, alanine transaminase; AST, aspartate transaminase; CHEBV, chronic high EBV loads; EBER-ISH, Epstein–Barr encoding region in situ hybridization; EBV, Epstein–Barr virus; GRWR, graft-to-recipient weight ratio; IS, immunosuppressants; LAR, late acute rejection; LDH, lactate dehydrogenase; Lymph, lymphocyte; NCHEBV, nonchronic high EBV loads; Plt, platelet; PT, prothrombin time; PT-INR, prothrombin time–international normalized ratio; PTLD, posttransplant lymphoproliferative disorders; RAI, rejection activity index; T-bil, total bilirubin; WBC, white blood cell.

Social media

Chronic high EBV loads after liver transplantation had little clinical and pathological impact on the liver graft, in pediatric liver transplantation patients.

Introduction

Epstein–Barr virus (EBV) is one of the causes of posttransplant lymphoproliferative disorders (PTLD) in solid organ transplantation. PTLD is associated with morbidity and mortality [1,2] and is often diagnosed in children [3]. Moreover, EBV DNAemia and chronic high loads are very common and persistent in children after solid organ transplantation [4]. Therefore, monitoring and management of EBV after solid organ transplantation are considered to be extremely important, particular for children.

There are many reports on the risk factors for the development of chronic high-load carriage and therapy for chronic high-load EBV carriers. Our group previously reported that long warm ischemic time, high graft-to-recipient weight ratio (GRWR), and preoperative EBV seronegativity were risk factors for the development of chronic high-load carriage [5]. Regarding therapy for EBV DNAemia, it has been reported that antivirals did not prevent EBV infection or chronic high-load carriage, moreover, reduction/discontinuation of immunosuppressants (IS) might not also improve persistent EBV DNAemia [4]. In any case, close serial monitoring of EBV DNA is considered useful for early diagnosis and prevention of PTLD and EBV-related diseases [6-8].

Although EBV is one virus causing hepatitis (infectious mononucleosis), most pediatric recipients with chronic high EBV loads (CHEBV) are asymptomatic and the high viral load resolves spontaneously [9]. Some studies proposed reduced IS for the treatment of EBV infection after transplantation to prevent the development of PTLD [10,11], while it has been reported that such dose reduction of IS leaded to the progression of graft fibrosis [12]. Unfortunately, there have been no reports focused on the impact of CHEBV on the liver graft. In particular, liver biopsy in children has not been considered an easy task because of the requirement for sedation. Nevertheless, we recognize that long-term management is important. If the impact of CHEBV on the liver graft can be clarified, it may serve as useful information for the management of the IS dose and the decision of whether to perform liver biopsy, as well as the appropriate interval for serial EBV DNA monitoring.

The aim of this study was to clarify the clinical and pathological impact of CHEBV on the liver grafts. In this study, we focused on evaluation of the following two points: (1) the blood biochemical impact of CHEBV on liver function, and (2) the pathological impact of CHEBV on the liver graft.

Methods

Patient population

We performed a retrospective observational study of 48 patients who were under 16 years old and underwent liver transplantation at Nagoya University Hospital from January 2012 through December 2020. We evaluated the patients who survived more than 6 months after liver transplantation. Because two patients expired less than 6 months after liver transplantation, 46 other pediatric patients were enrolled in this study.

Immunosuppressive protocol

Tacrolimus and steroid were administered to all patients after liver transplantation. Oral steroid was usually tapered during the 3–6 months after liver transplantation. The target trough levels of tacrolimus were gradually lowered to about 5.0 ng/mL by 6 months after liver transplantation.

Mycophenolate mofetil was administered in nine out of 46 patients because of ABO blood type–incompatible transplants or requirement for stronger immunosuppression. A long-term maintenance oral steroid was needed in four of the 46 patients.

We quantitated EBV DNA every week during hospitalization and at each outpatient visit every 4–6 weeks for ≥ 6 months after liver transplantation. If EBV DNA load > 5,000 IU/mL was detected, the target trough level of tacrolimus was decreased to < 3.0 ng/mL for those patients [13]. No patients were administered antiviral acyclovir for detected EBV DNA.

Quantitative analysis of EBV and the definition of CHEBV

Quantitative analysis of EBV DNA in whole blood samples was performed as previously described [14]. The detection limit was 25 IU/mL. A clinically significant high load of EBV DNA was defined as a quantified viral load ≥10,000 copies/mL [15].

We allocated patients to the CHEBV group if the continuous presence of EBV loads in whole blood >10,000 IU/mL was detected for at least 6 months. The others were allocated to nonchronic high EBV loads (NCHEBV) group.

The evaluation of impact on liver graft

We performed an annual protocol biopsy for all asymptomatic patients, in addition to episodic liver biopsy for symptomatic patients (e.g., hepatobiliary dysfunction). We evaluated the most significant findings when patients underwent several biopsies. Moreover, we also collected blood biochemical samples from those patients at the same times as liver biopsy. With these samples, we investigated the impact on the liver graft,

in terms of the blood biochemical and pathological findings. Once the definition of CHEBV was met, the data was treated as the data of the CHEBV group, regardless of the time obtained. Of note, we evaluated blood biochemical samples and liver biopsy specimens obtained 6 months after liver transplantation in this study because we considered that the impact of perioperative factors including biliary complications, cholangitis, or acute cellular rejection caused by an insufficient adjustment of immunosuppressants, was greater than the impact of EBV, especially within 6 months after liver transplantation.

CHEBV impact on blood biochemical examination

With the samples obtained at the protocol or episodic liver biopsy, we evaluated the following in the CHEBV and NCHEBV groups: white blood cell (WBC) count, lymphocyte (Lymph) count, and platelet **(**Plt) count, as well as concentrations of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), albumin (Alb), total bilirubin (T-bil), and prothrombin time–international normalized ratio (PT-INR), and prothrombin time (PT).

CHEVB impact on pathological findings

In this study, with the liver biopsy specimen obtained 6 months after liver transplantation, we evaluated the pathological findings in the CHEBV and NCHEBV groups. If late-onset acute rejection (LAR, diagnosed as acute rejection ≥ 6 months after liver transplantation) was identified [16], we evaluated the findings based on Banff's classification and rejection activity index (RAI). We investigated the frequency of LAR, which was moderate (RAI ≥ 5) or mild (RAI ≥ 3). The pathologist assessed the histopathological findings including graft fibrosis, by hematoxylin–eosin staining and azan staining based on the METAVIR score [17]. As for the evaluation of graft fibrosis, we included specimens obtained within 6 months after liver transplantation.

Statistical analysis

Categorial variables were compared using Fisher's exact test or χ^2 test. Normality of distribution was evaluated by the Shapiro–Wilk test. If the data did not show a normal distribution, the Mann–Whitney U-test was used. For a normal distribution, homogeneity of variance was evaluated by Levene's test. If the data showed homogeneity of variance, Student's t-test was used. The estimate of the cumulative incidence of fibrosis ≥ 2 was evaluated by the Kaplan–Meier method. *P*-values < 0.05 were regarded as statistically significant. All statistical analyses were performed using EZR (Easy R; Division of

Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [18].

Ethical approvement and informed consent

The study protocol was performed in accordance with the Declaration of Helsinki and was approved by the ethics review committee of Nagoya University Graduate School of Medicine (Approval No. 2017-0431). All participants provided written informed consent to take part in the study. This study was fully supported by a Grant-in-Aid for Scientific Research (C, No. 17K10509) from the Japanese Ministry of Education, Culture, Sports, Science and Technology and by a grant from the Japanese Society for the Promotion of Science.

Results

Patient characteristics

The comparison of patient characteristics between the CHEBV and NCHEBV groups are summarized in Table 1. Of the 46 patients investigated in this study, the number of patients in the CHEBV and NCEBV groups were 28 and 18, respectively. Older age and higher body weight at liver transplantation were statistically significant parameters in the NCHEBV group. Three patients in the NCHEBV group died because of graft failure; however, there were no statistically significant differences in frequency of graft failure between the two groups. Large GRWR and long follow-up period were statistically significant in the CHEBV group. The low tacrolimus trough level in the CHEBV group was statistically significant. Although one patient in the CHEBV group developed PTLD surrounding the rectum, the condition was improved by the administration of rituximab.

The changes in EBV DNA numbers and tacrolimus trough level in the CHEBV group are shown in Figure 1. The median of the first day showing EBV DNA ≥ 10,000 IU/mL was 88 days (range, 26–596 days) after liver transplantation. Twenty-four of 28 patients in the CHEBV group (85.7%) showed EBV DNA ≥ 10,000 IU/mL within 180 days after liver transplantation. Only two of 28 patients (7.1%) in the CHEBV group had decreased to undetectable level during the study period.

Blood biochemical impact on liver function

Comparisons of blood biochemical results between the CHEBV and NCHEBV groups at the time of the protocol and episodic biopsies are shown in Table 2.

The samples collected at protocol biopsy showed no significant differences between the two groups. The episodic biopsies included one patient who showed T-bil 44.0 mg/mL and died as a result of graft failure. We excluded this data because the patient was an obvious outlier. With this data excluded, there were no differences between the two groups.

Data obtained within 6 months after liver transplantation is shown as supplementary data in Table 3. The number of lymphocytes in the CHEBV group and T-bil in the NCHEBV group were significantly high.

Pathological impact on liver grafts

Results of the comparison between the CHEBV and NCEBV groups of pathological findings of the liver graft biopsies are shown in Table 4. At protocol biopsy, 11 of 25 patients (44.0%) in the CHEBV group were normal on histopathological examination, whereas three of nine patients (33.3%) in the NCHEBV group were normal. Eight patients

(32.0%) had LAR of RAI ≥ 3 (mild) in the CHEBV group and three patients (33.3%) in the NCHEBV group. At episodic biopsy, LAR of RAI ≥ 3 (mild) and RAI ≥ 5 (moderate) were three of 10 patients (30.0%) and three of 10 patients (30.0%) in the CHEBV group, but none of four patients (0%) and one of four patients (25.0%) in the NCHEBV group, respectively. As a result, although overall LAR showed a tendency to develop in CHEBV group more frequently, none of the parameters showed statistically significant differences between the two groups.

In evaluating the progression of fibrosis in the biopsy samples, the cumulative incidences of fibrosis ≥ 2 showed no significant difference between the CHEBV and NCHEBV groups (Figure 2).

Discussion

PTLD associated with EBV leads to high morbidity and mortality after solid organ transplantation in children [19,20]. Therefore, serial monitoring of EBV DNA is important for the early diagnosis and treatment of EBV-associated diseases [6]. Moreover, persistent EBV infection is often observed in children who received transplantation not only of liver, but also of heart and kidney [4,9]. Our previous study identified the risk factors for chronic high EBV load carriage: long warm ischemic time, high GRWR, and preoperative EBV seronegativity [5]. However, there are no reported studies on how CHEBV impacts the liver graft, even though EBV is one of the viruses that causes hepatitis. To our knowledge, the present study is the first to focus on this point. In this study, we demonstrated that CHEBV did not impact liver graft functionally or pathologically. Our results also indicated that the appropriate adjustment of IS might contribute to management of chronic EBV infection and the prevention of the progression of graft fibrosis.

We showed changes in EBV DNA levels in the CHEBV group after liver transplantation and tacrolimus trough level (Figure 1). EBV DNA tended to decrease gradually over time. We reduced the tacrolimus dose for EBV DNA > 5,000 IU/ml patients in this study. There may be correlation between the decrease of EBV DNA and lower tacrolimus level, but we did not investigate this correlation in this study. To date, there have been no reports on an association between a reduction of IS and a decrease in EBV load. Therefore, we considered that the reduction of IS was not directly associated with the trend shown in Figure 1. We assume that the development of the immune system or lymphocytes may have impacted this result, although there is no clear evidence for this.

Blood biochemical examination for clinical impact on liver function was compared between the CHEBV and NCHEBV groups. In this study, we investigated 49 blood samples of 25 patients in the CHEBV group and 19 blood samples of 10 patients in the NCHEBV group (some patients underwent several liver biopsies). As shown in Table 2, there were no significant differences in blood samples collected at the time of protocol and episodic biopsies. Previous studies showed that AST and ALT increased with rejection [21,22]. Although AST and ALT at the time of episodic biopsy increased compared with those at protocol biopsy, there were no statistically significant differences in blood biochemical examination between the two groups. In this study, many patients in the CHEBV and NCHEBV groups were stable 6 months after liver transplantation and did not need to receive episodic biopsy, compared with patients within 6 months after liver transplantation. Although this result can be for reference, caution is warranted in interpreting these results because of the small number of patients, especially in episodic biopsy.

Data obtained within 6 months after liver transplantation are shown as supplementary data (Table 3). The data obtained in this period are probably not related to the impact of EBV but rather perioperative factors including biliary complications, cholangitis, or acute cellular rejection related to the insufficient adjustment of IS. T-bil levels were significantly higher in the NCHEBV group, possibly because three patients died related to graft failure. The significantly higher number of lymphocytes in the CHEBV group might be associated with EBV, but the clinical implications are unclear.

Regarding the pathological impact, we demonstrated that CHEBV had also little impact on the liver graft. To exclude the perioperative factors, we evaluated only the specimens that were obtained ≥ 6 months after liver transplantation. We showed the most significant findings when patients underwent several biopsies. As shown in Table 4, the normal findings at protocol biopsy were 11 (44.0%) of 25 patients in the CHEBV group and three (33.3%) of nine patients in the NCHEBV group. In the protocol biopsy, there was no significant difference in the incidence of more than mild (RAI ≥ 3) between the two groups [eight of 25 patients (32.0%) in the CHEBV group, three of nine patients (33.3%) in the NCHEBV group]. In the episodic biopsies, although there were small number of patients showed more than moderate (RAI ≥ 5) [three of 10 patients (30.0%) in the CHEBV group and none of four patients (0%) in the NCHEBV group], there were no significant differences between the two groups.

The association of fibrosis ≥ 2 and time after liver transplantation are shown in Figure 2. There were no statistically significant differences in the progression of fibrosis between the CHEBV and NCHEBV groups, although fibrosis in the CHEBV group showed a tendency for progression compared with that in the NCHEBV group. Previous studies have reported that IS reduction might be associated with liver fibrosis [12,23]. Therefore, we thought the CHEBV group was more likely to progress to fibrosis than the NCHEBV group before this study was initiated because tacrolimus levels were significantly lower in the CHEBV group (Table 1). However, there was no significant difference in the progression of fibrosis between the two groups. Some studies have reported that IS should be reduced or withdrawn in patients with EBV infection to avoid the development of PTLD [10,11]; therefore, we adjusted the IS dose for liver transplant patients infected with EBV based on these studies. Our results suggest that the strict management of IS which previously reported, such as discontinuation or reduction of IS dose to one-quarter or one-half dose, may be unnecessary in terms of fibrosis progression.

EBV is one of the viruses that causes hepatitis as well as infectious mononucleosis. However, it is unknown whether EBV is detected in liver tissue in patients with chronic EBV infection. Although we performed Epstein–Barr encoding region in situ

hybridization (EBER-ISH) in some samples (> 10 samples) that were collected at the time that EBV DNA was > 10,000 IU/mL, all specimens showed negative staining. There may be biopsy-associated sampling errors, but the findings support our results that CHEBV do not impact the liver graft.

One limitation is the relatively small sample size, especially in episodic biopsy, because many patients were stable 6 months after liver transplantation and did not need to receive episodic biopsy. Therefore, it is difficult to draw definitive conclusion, particularly from the episodic biopsy. Another limitation of this study is that the follow-up period for evaluation of the liver graft was short; we have only shown the short- to middle-term outcomes, as shown in Table 1. Liver transplantation for children is often performed during a growth phase; therefore, long-term monitoring is necessary. To validate our results, large-scale and continuous studies are required.

In conclusion, chronic high EBV infection did not impact the liver graft functionally or pathologically. Furthermore, we have shown the possibility that optimal management of the trough level of tacrolimus might have contributed to the management of CHEBV without the progression of graft fibrosis. These results provide meaningful information for the management of pediatric liver transplant patients with CHEBV.

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Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

Author contribution

M. Shizuku: study design, data collection, data analysis and manuscript writing.
H. Kamei, A. Yoshizawa, Y. Ito: study design, data collection and manuscript editing.
Y. Ogura: data collection and manuscript editing.
J. Yoshikawa, N. Kurata. K. Jobara: data collection.
Y. Kodera: critical review of the manuscript.

References

1. Mynarek M, Schober T, Behrends U, Maecker-Kolhoff B. Posttransplant Lymphoproliferative Disease after Pediatric Solid Organ Transplantation. *Clin Dev Immunol.* 2013:14.
2. Cacciarelli TV, Reyes J, Jaffe R, et al. Primary tacrolimus (FK506) therapy and the long-term risk of post-transplant lymphoproliferative disease in pediatric liver transplant recipients. *Pediatric Transplantation.* 2001;5(5):359-364.
3. Green M, Michaels MG. Epstein-Barr Virus Infection and Posttransplant Lymphoproliferative Disorder. *American Journal of Transplantation.* 2013;13:41-54.
4. Kullberg-Lindh C, Saalman R, Olausson M, Herlenius G, Lindh M. Epstein-Barr virus DNA monitoring in serum and whole blood in pediatric liver transplant recipients who do or do not discontinue immunosuppressive therapy. *Pediatric Transplantation.* 2017;21(5).
5. Kamei H, Ito Y, Kawada J, et al. Risk factors and long-term outcomes of pediatric liver transplant recipients with chronic high Epstein-Barr virus loads. *Transplant Infectious Disease.* 2018;20(4).
6. Seo E, Kim J, Oh SH, Kim KM, Kim DY, Lee J. Epstein-Barr viral load monitoring for diagnosing post-transplant lymphoproliferative disorder in pediatric liver transplant recipients. *Pediatric Transplantation.* 2020;24(4):10.
7. Kullberg-Lindh C, Ascher H, Saalman R, Olausson M, Lindh M. Epstein-Barr viremia levels after pediatric liver transplantation as measured by real-time polymerase chain reaction. *Pediatric Transplantation.* 2006;10(1):83-89.
8. Chen HS, Ho MC, Hu RH, et al. Roles of Epstein-Barr virus viral load monitoring in the prediction of posttransplant lymphoproliferative disorder in pediatric liver transplantation. *J Formos Med Assoc.* 2019;118(9):1362-1368.
9. Bingler MA, Feingold B, Miller SA, et al. Chronic high Epstein-Barr viral load state and risk for late-onset posttransplant lymphoproliferative disease/lymphoma in children. *American Journal of Transplantation.* 2008;8(2):442-445.
10. Ganschow R, Schulz T, Meyer S, Broering TC, Burdelski M. Low-dose immunosuppression reduces the incidence of post-transplant lymphoproliferative disease in pediatric liver graft recipients. *Journal of Pediatric Gastroenterology and Nutrition.* 2004;38(2):198-203.
11. Narkewicz MR, Green M, Dunn S, et al. Decreasing Incidence of Symptomatic Epstein-Barr Virus Disease and Posttransplant Lymphoproliferative Disorder in Pediatric Liver Transplant Recipients: Report of the Studies of Pediatric Liver

Transplantation Experience. *Liver Transplantation.* 2013;19(7):730-740.
12. Koshiba T, Li Y, Takemura M, et al. Clinical, immunological, and pathological aspects of operational tolerance after pediatric living-donor liver transplantation. *Transplant Immunology.* 2007;17(2):94-97.
13. Orii T, Ohkohchi N, Satomi S, Hoshino Y, Kimura H. Decreasing the Epstein-Barr virus load by adjusting the FK506 blood level. *Transplant International.* 2002;15(11):529-534.
14. Ito Y, Suzuki M, Kawada J, Kimura H. Diagnostic values for the viral load in peripheral blood mononuclear cells of patients with chronic active Epstein-Barr virus disease. *J Infect Chemother.* 2016;22(4):268-271.
15. Bakker NA, van Imhoff GW, Verschuuren EAM, van Son WJ. Presentation and early detection of post-transplant lymphoproliferative disorder after solid organ transplantation. *Transplant International.* 2007;20(3):207-218.
16. Nacif LS, Pinheiro RS, Pecora RAD, et al. LATE ACUTE REJECTION IN LIVER TRANSPLANT: A SYSTEMATIC REVIEW. *ABCD-Arq Bras Cir Dig-Braz Arch Dig Surg.* 2015;28(3):212-215.
17. Bedossa P, Bioulacsage P, Callard P, et al. INTRAOBSERVER AND INTEROBSERVER VARIATIONS IN LIVER-BIOPSY INTERPRETATION IN PATIENTS WITH CHRONIC HEPATITIS-C. *Hepatology.* 1994;20(1):15-20.
18. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplantation.* 2013;48(3):452-458.
19. Webber SA, Naftel DC, Fricker FJ, et al. Lymphoproliferative disorders after paediatric heart transplantation: a multi-institutional study. *Lancet.* 2006;367(9506):233-239.
20. Green M, Michaels MG, Katz BZ, et al. CMV-IVIG for prevention of Epstein Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. *American Journal of Transplantation.* 2006;6(8):1906-1912.
21. Woo YS, Lee KH, Lee KT, et al. Postoperative changes of liver enzymes can distinguish between biliary stricture and graft rejection after living donor liver transplantation A longitudinal study. *Medicine.* 2017;96(40):7.
22. Wiesner RH, Demetris AJ, Belle SH, et al. Acute hepatic allograft rejection: Incidence, risk factors, and impact on outcome. *Hepatology.* 1998;28(3):638-645.
23. Yoshitomi M, Koshiba T, Haga H, et al. Requirement of Protocol Biopsy Before and After Complete Cessation of Immunosuppression After Liver Transplantation. *Transplantation.* 2009;87(4):606-614.

Table 1 Patient characteristics

	CHEBV (n = 28)	NCHEBV (n = 18)	*p*
Age at LTx, years; median (range)	0 (0.3–2.0)	1 (0.4–16.0)	**0.0083**
Sex, male/female	10/18	6/12	0.87
Body weight at LTx, kg; median (range)	7.3 (4.7–13.3)	8.5 (4.9–66.0)	**0.017**
Follow-up period, years; median	5.7	4.2	**0.044**
Underlying disease			
Biliary atresia	19	13	0.97
Fulminant hepatic failure	3	2	
Alagille syndrome	3	1	
Hepatoblastoma	2	1	
PFIC2	1	1	
PELD score, median	14	12	0.39
ABO-incompatible	3	1	0.49
Pretransplant EBV serostatus			
D−/R−	1	1	0.38
D+/R−	19	12	
D−/R+	1	0	
D+/R+	7	3	
Incomplete data	0	2	
Death after 6 months of LTx	0	3	0.054
Living/deceased donor	28/0	16/2	0.15
Graft type			
Lateral	27	13	0.070
Left	1	1	
Right	0	3	
Whole liver	0	1	
Graft weight, g; median	234	263	0.072
GRWR, % median	3.2	2.4	**0.013**
Development of PTLD	1	0	0.42
Tacrolimus trough level at biopsy, ng/mL; mean	2.0	3.4	**0.0073**

Mycophenolate mofetil, number of cases	6	3	1.00
Maintenance oral steroid, number of cases	1	3	0.28
Biopsy			
LTx - 6 months			
Protocol, number of patients (number of samples)	0	0	n.a
Episodic, number pf patients (number of samples)	20 (51)	13 (27)	1.00
6 months -			
Protocol, number of patients (number of samples)	25 (49)	11 (18)	**0.033**
Episodic, number pf patients (number of samples)	10 (19)	4 (7)	0.51

CHEBV, chronic high EBV loads; EBV, Epstein–Barr virus; GRWR, graft-to-recipient weight ratio; LTx, liver transplantation; NCHEBV, nonchronic high EBV loads; PELD, Pediatric End-stage Liver Disease (score); PFIC, progressive familial intrahepatic cholestasis; PTLD, posttransplant lymphoproliferative disorders.

Table 2 Clinical impact on graft function by protocol/episodic liver biopsy

	Protocol biopsy			Episodic biopsy		
	CHEBV (n=25)	NCHEBV (n=11)	p	CHEBV (n=10)	NCHEBV† (n=4)	p
Sample number	49	18		19	7	
WBC	7700	7900	0.83	9874	10314	0.81
Lymph	3575	3739	0.73	5062	3746	0.33
Plt	235	204	0.59	195	164	0.42
AST	39	44	0.32	149	69	0.10
ALT	21	24	0.28	125	32	0.27
LDH	335	352	0.45	396	380	0.53
Alb	4.1	4.2	0.40	3.8	3.6	0.36
PT-INR	1.03	1.04	0.59	1.07	1.21	0.21
PT	94.3	93.8	0.90	85.6	66.9	0.25
T-Bil	0.5	0.6	0.30	0.6	0.8	0.072

WBC, White blood cell; Lymph, Lymphocyte; Plt, Platelet; AST, Aspartate transaminase; ALT, Alanine transaminase; LDH, Lactate dehydrogenase; Alb, Albumin; PT-INR, Prothrombin time-international normalized ratio; PT, Prothrombin time; T-bil, Total bilirubin;

† One patient excluded (data at the time of graft failure).

Table 3 The Supplementary data: Clinical impact on graft function by protocol/episodic liver biopsy obtained within 6 months after liver transplantation

	Episodic biopsy LTx – 6 months		
	CHEBV (n=20)	NCHEBV (n=13)	p
Sample number	51	27	
WBC	8700	8800	0.75
Lymph	3550	1700	**< 0.01**
Plt	160	155	0.41
AST	125	113	0.29
ALT	168	122	0.22
LDH	351	363	0.38
Alb	3.2	3.2	0.58
PT-INR	1.21	1.31	0.34
PT	69.3	54.5	0.24
T-Bil	0.9	2.0	**< 0.01**

LTx, Liver transplantation; WBC, White blood cell; Lymph, Lymphocyte; Plt, Platelet; AST, Aspartate transaminase; ALT, Alanine transaminase; LDH, Lactate dehydrogenase; Alb, Albumin; PT-INR, Prothrombin time-international normalized ratio; PT, Prothrombin time; T-bil, Total bilirubin;

Table 4　Pathological impact on liver graft by protocol/episodic liver biopsy

	Protocol biopsy				Episodic biopsy		
	CHEBV (n = 25)	NCHEBV (n = 9)	*p*		CHEBV (n = 10)	NCHEBV (n = 4)	*p*
Normal/almost normal	11	3	0.70	Normal/almost normal	1	0	1.00
LAR				LAR			
Number of RAI ≥3	8	3	1.00	Number of RAI ≥3	3	0	0.51
Number of RAI ≥5	0	0	n.a.	Number of RAI ≥5	3	1	1.00
Steatosis	2	3	0.10	Steatosis	1	1	1.00
Other	4	0	0.55	Cholangitis	0	1	0.29
				Other	2	1	1.00

CHEBV, chronic high EBV loads; EBV, Epstein–Barr virus; LAR, late acute rejection; NCHEBV, nonchronic high EBV loads; RAI, rejection activity index.

Figure legends

Figure 1: Change in EBV DNA and tacrolimus trough level in 28 patients of the CHEBV group after liver transplantation.

In most patients in the CHEBV group, levels of EBV DNA ≥10,000 IU/mL were reached within 180 days after liver transplantation. EBV DNA showed a tendency to decrease over time.

CHEBV, chronic high EBV loads; EBV, Epstein–Barr virus.

Figure 2: Comparison of the cumulative incidence rate (%) of fibrosis ≥2 between the CHEBV and NCHEBV groups.

The incidence of fibrosis over grade 2 was not statistically significantly different between the CHEBV and NCHEBV groups.

CHEBV, chronic high EBV loads; EBV, Epstein–Barr virus; FK, tacrolimus; NCHEBV, nonchronic high EBV loads.

Figure 1

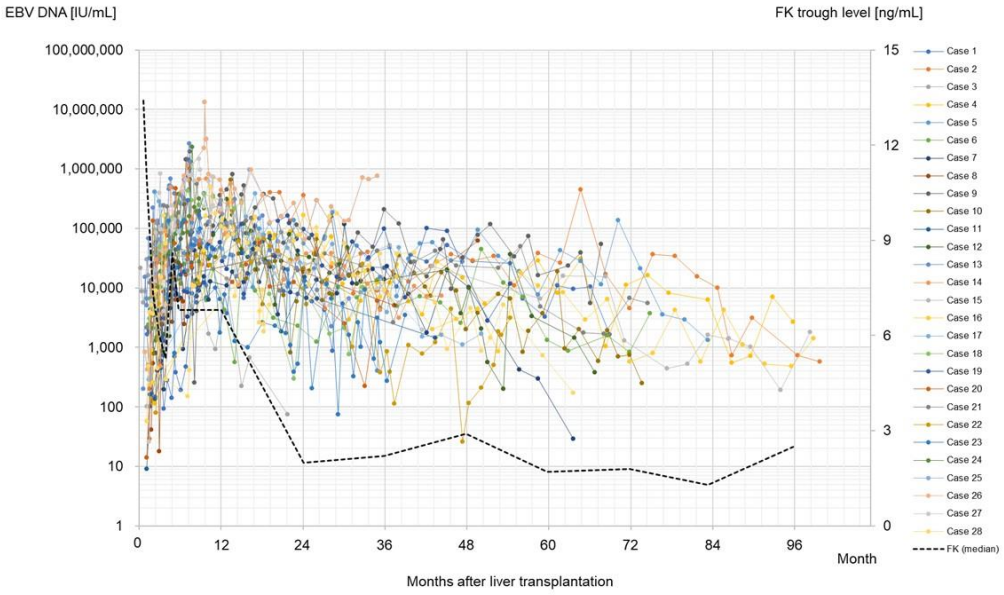
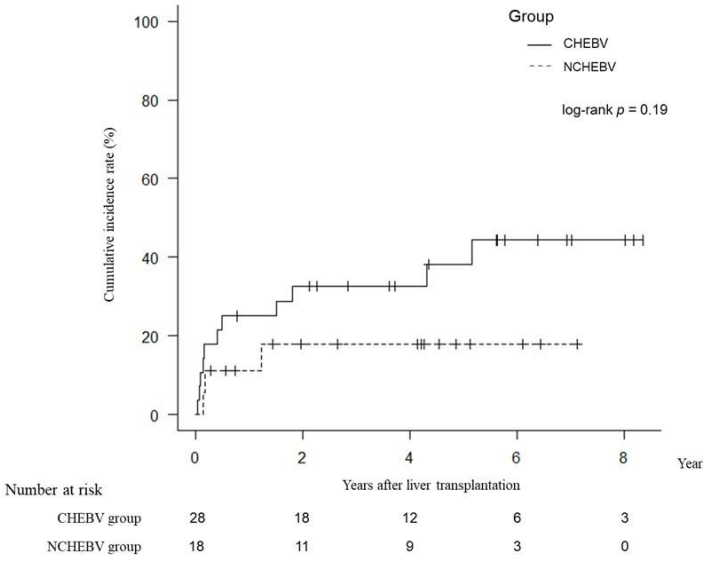

EBV DNA [IU/mL]
FK trough level [ng/mL]
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
15
12
9
6
3
0
0
12
24
36
48
60
72
84
96
Month
Months after liver transplantation
Case 1
Case 2
Case 3
Case 4
Case 5
Case 6
Case 7
Case 8
Case 9
Case 10
Case 11
Case 12
Case 13
Case 14
Case 15
Case 16
Case 17
Case 18
Case 19
Case 20
Case 21
Case 22
Case 23
Case 24
Case 25
Case 26
Case 27
Case 28
FK (median)

Figure 2


Group
CHEBV
NCHEBV
log-rank p = 0.19
Cumulative incidence rate (%)
100
80
60
40
20
0
0
2
4
6
8
Year
Years after liver transplantation
Number at risk
CHEBV group 28 18 12 6 3
NCHEBV group 18 11 9 3 0