TABLE 3. EBV-Induced FHF Treated With LT

Age (Years)/Sex	Type of Transplant	Timing of Transplant	EBV Load (Plasma; Copies/g of DNA)	EBER-in situ hybridization (ISH) in Liver	Outcome After LT	Reference
1.6/female	Living donor	Day 15	5.1×10^4	+	Alive at 2 years 4 months	Present study
1.8/female	Cadaveric	Day 15	Positive for EBV DNA by PCR	+	Alive at 2 years	Feranchak et al.[2]
5/male	Living donor	Day 33	1.2×10^4	+	Died on day 93	Present study
8/male	Living donor	Not recorded	8.83×10^4	+	Alive at 8 days	Tohyama et al.[10]
9/female	Cadaveric	Day 11	3.4×10^5	+	Alive at 3 years 7 months	Present study
18/female	Cadaveric	Day 9	2.6×10^5	+	Alive at 1 year 0 months	Dumortier et al.[11]
19/male	Cadaveric	Day 10	Not recorded	Positive by PCR	Alive at 26 days	Park et al.[12]
44/female	Not recorded	Not recorded	Not recorded	Positive by PCR	Alive at 18 months	Mellinger et al.[3]

venulitis was not identified. Lymphocytes were positive for cytoplasmic CD3, CD8, granzyme B, perforin, and EBERs, but they were negative for CD4, CD20, and CD56. EBER-positive lymphocytes were positive for CD45RO but were negative for CD20 (Fig. 3B). Immunostaining of cytoplasmic CD3, CD8, and EBV by in situ hybridization revealed a sinusoidal distribution of the lymphocytes. In case 4, CD20 staining highlighted several large B cells with scattered CD4-positive T cells that were observed. T cell clonality studies revealed monoclonal proliferation in cases 4 and 5.

EBV DNA was detected with a real-time quantitative PCR assay in the CD8-positive T cell component in all 5 liver tissues. The pathological findings in all cases are summarized in Table 2.

DISCUSSION

EBV infection is an extremely rare cause of ALF and accounted for only 0.21% of ALF cases in a cohort of 1887 adult patients.[3] To date, there have been 25 reported cases of EBV-induced FHF or severe ALF, and they have presented with a mortality rate of 68% (17 of 25).[2,3,10-12] Among the 8 survivors, emergency LT was performed in 5.[2,3,10-12] Characteristics of the 8 EBV-induced FHF patients who received LT, including the 3 patients in the present study, are summarized in Table 3. All examined cases were positive for EBERs in liver tissues, which showed extremely high levels of EBV DNA (10^4-10^5) in plasma. Currently, specific pathological findings for EBV-induced FHF are not yet available in the literature.

Notably, all but 1 patient survived and remained healthy after emergency LT. The patient who died at 93 days after LT (case 2) underwent surgery 33 days after the initial presentation. The timing of LT in this case was clearly much later than that in the other cases (9-15 days from the initial presentation).

We re-evaluated each of our patients with the KCC and the ALFED model. According to the KCC and the ALFED model, only 1 patient (case 2) was regarded as a candidate for LT. Previously, the KCC were found to be of limited usefulness for patients with FHF in Japan. The predictive accuracy of the criteria, as adopted for the patients seen between 1993 and 1995, was found to be only 55% for the assessment conducted at the onset of hepatic encephalopathy and 53% for the assessment conducted on day 5 after the onset of encephalopathy.[13] Hence, a new set of criteria for use in Japan was established by the ALFSG in 1996 and updated in 2011.[6] When the prognosis of patients with a total score of 5 or more was judged to be death, the predictive accuracy was 0.80, with sensitivity, specificity, positive predictive, and negative predictive values greater than 0.70 even in the validation cohort.

According to the ALFED model, case 3 was at low risk on day 3 but was at high risk on day 5. The ALFED assessment on day 3 seems to be in line with the JALFSG criteria on day 5 in Japan where LDLT is

predominant because the waiting time for a deceased donor from listing to LT is 2 to 3 days in most countries. Hence, strategies for patients with a moderate risk in the ALFED model should be determined on a case-by-case basis. In case 1, severe coagulopathy and a low platelet count persisted on day 5, and LDLT was performed without liver biopsy. On the other hand, liver biopsy was critical for the determination of the treatment strategy for cases 4 and 5. Notably, case 5 was diagnosed with EBV-T-LPD by liver biopsy, which is a contraindication for LT.

A primary EBV infection often causes infectious mononucleosis, whereby B cells are infected with EBV and polyclonal B cell expansion is accompanied by oligoclonal or monoclonal proliferation of CD8-positive cytotoxic T cells.[14] EBV also triggers secondary HLH. In EBV-induced HLH, the targets of EBV infection are CD8-positive T cells and oligoclonally or monoclonally proliferated T cells, which induce hypercytokinemia and, in turn, lead to hemophagocytosis and dysfunction of various organs.[15,16] EBV-T-LPD may develop in association with acute or chronic EBV infections and EBV-induced HLH. However, differentiating reactive T cell lymphocytosis from EBV-T-LPD can be a diagnostic challenge, especially in EBV-induced HLH, because monoclonality analysis alone is unable to support the diagnosis. Upon the initiation of steroid or immunosuppressive treatment for EBV-induced HLH, it can also be difficult to predict if the patient with EBV-induced FHF will require LT, conservative treatment for HLH, or aggressive chemotherapy. Hence, liver biopsy is important not only for the evaluation of EBV-T-LPD in EBV-induced HLH but also for the assessment of potential massive or submassive hepatocellular necrosis, although there are limitations of biopsy in terms of the sampling artifact. In cases 4 and 5, the liver biopsies showed CD8-positive T cell proliferation without massive necrosis, which suggested that LT was not necessary. In these 2 patients, a high level of EBV DNA was detected in PBMNCs but not in the plasma, possibly because of EBV-infected T cells not yet lysed by cytotoxic T cells. On the other hand, in the 3 patients who received LT, high levels of EBV DNA were detected in both PBMNCs and the plasma.

Patients with EBV-induced FHF usually have severe coagulopathy, which makes biopsy difficult because of bleeding. As such, many physicians tend to avoid liver biopsy. To that end, we opted for open liver biopsy rather than percutaneous and transjugular liver biopsy because of the limitation of the substantial sampling artifact regarding the extent of hepatic necrosis. We agree that plural or serial transjugular liver biopsy would be helpful as an alternative.[17] However, transjugular liver biopsy is not covered by the national insurance system in Japan. Donaldson et al. reported that the percentage of necrosis appeared to have a significant discriminatory prognostic value, and significantly greater hepatocellular necrosis was seen in nonsurvivors versus survivors regardless of the etiology of FHF. Despite that, there were a few survivors who had liver necrosis greater than 70% in that study. Because acute hepatocellular injury and early rapid regeneration are investigated in some cases, massive necrosis does not necessarily mean a poor prognosis. In such cases, a model such as the ALFED model is necessary to decide the treatment strategy.

In conclusion, an early and precise assessment of the pathogenesis of EBV-induced FHF is critical to determine the next course of treatment. We found that a serial assessment of the severity of FHF based on the ALFED score during the first 5 days of commencement of artificial liver support helped to determine the necessity for LT. Liver biopsy should be mandatory to assess the pathogenesis of EBV-induced FHF.

ACKNOWLEDGMENT

The authors thank Julian Tang from the Department of Clinical Research Education, National Center for Child Health and Development, for proofreading, editing, and rewriting part of this article.

REFERENCES

1. Papatheodoridis GV, Delladetsima JK, Kavallieros L, Kapranos N, Tassopoulos NC. Fulminant hepatitis due to Epstein-Barr virus infection. J Hepatol 1995;23:348-350.
2. Feranchak AP, Tyson RW, Narkewicz MR, Karrer FM, Sokol RJ. Fulminant Epstein-Barr viral hepatitis: orthotopic liver transplantation and review of the literature. Liver Transpl Surg 1998;4:469-476.
3. Mellinger JL, Rossaro L, Naugler WE, Nadig SN, Appelman H, Lee WM, Fontana RJ. Epstein-Barr virus (EBV) related acute liver failure: a case series from the US Acute Liver Failure Study Group. Dig Dis Sci 2014; 59:1630-1637.
4. O'Grady JG, Alexander GJ, Thick M, Potter D, Calne RY, Williams R. Outcome of orthotopic liver transplantation in the aetiological and clinical variants of acute liver failure. Q J Med 1988;68:817-824.
5. Kumar R, Shalimar, Sharma H, Goyal R, Kumar A, Khanal S, et al. Prospective derivation and validation of early dynamic model for predicting outcome in patients with acute liver failure. Gut 2012;61:1068-1075.
6. Naiki T, Nakayama N, Mochida S, Oketani M, Takikawa Y, Suzuki K, et al. Novel scoring system as a useful model to predict the outcome of patients with acute liver failure: Application to indication criteria for liver transplantation. Hepatol Res 2012;42:68-75.
7. Imadome K, Fukuda A, Kawano F, Imai Y, Ichikawa S, Mochizuki M, et al. Effective control of Epstein-Barr virus infection following pediatric liver transplantation by monitoring of viral DNA load and lymphocyte surface markers. Pediatr Transplant 2012;16:748-757.
8. Langerak AW, Groenen PJ, Brüggemann M, Beldjord K, Bellan C, Bonello L, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia 2012;26:2159-2171.
9. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124–131.
10. Tohyama T, Takada Y, Watanabe J, Hatano M, Kushihata F, Inoue H, et al. ABO-incompatible living

donor liver transplantation saved a child with fulminant hepatic failure caused by primary EBV infection [abstract]. Liver Transpl 2011;17(suppl 1):S146.

11. Dumortier J, Mekki Y, Rimmelé T, Ber C, Maillard E, Fafi-Kremer S, et al. EBV-induced fulminant hepatic failure: favorable outcome after liver transplantation [in French]. Gastroenterol Clin Biol 2007;31:725-728.
12. Park HW, Lee SG, Hwang S, Kim KH, Park YH. Epstein-Barr virus associated fulminant hepatitis requiring liver transplantation (case report) [abstract]. HPB 2001;13 (Suppl.3):187.
13. Sugihara J, Naito T, Ishiki Y, Murakami N, Naiki T, Koshino Y, et al. A multicenter study for liver transplantation in Japanese Acute Hepatic Failure Study Group (1996) [in Japanese]. Acta Hepatol Jpn 2001;42:543-557.
14. Callan MF, Steven N, Krausa P, Wilson JD, Moss PA, Gillespie GN, et al. Large clonal expansions of CD8+ T cells in acute infectious mononucleosis. Nat Med 1996;2: 906-911.
15. Kawaguchi H, Miyashita T, Herbst H, Niedobitek G, Asada M, Tsuchida M, et al. Epstein-Barr virus-infected T lymphocytes in Epstein-Barr virus-associated hemophagocytic syndrome. J Clin Invest 1993;92:1444-1450.
16. Okano M, Gross TG. Acute or chronic life-threatening disease associated with Epstein-Barr virus infection. Am J Med Sci 2012;343:483-489.
17. Donaldson BW, Gopinath R, Wanless IR, Phillips MJ, Cameron R, Roberts EA, et al. The role of transjugular liver biopsy in fulminant liver failure: Relation to other prognostic indicators. Hepatology 1993;18:1370-6374.

Pediatr Transplantation 2015: 19: 144–152

Pediatric Transplantation
DOI: 10.1111/petr.12402

Evaluation of the immune function assay in pediatric living donor liver transplantation

Fukuda A, Imadome K-I, Sakamoto S, Shigeta T, Uchida H, Matsunami M, Sasaki K, Kanazawa H, Kawano F, Nakazawa A, Fujiwara S, Kasahara M. (2015) Evaluation of the immune function assay in pediatric living donor liver transplantation. *Pediatr Transplant*, 19: 144–152. DOI: 10.1111/petr.12402.

Abstract: The immune function (ImmuKnow) assay is a measure of cell-mediated immunity based on the peripheral CD4+ T cell ATP activity. The efficacy of ImmuKnow in pediatric LDLT is not well documented. The aim of this study was to assess the correlations between the ImmuKnow and the clinical status in pediatric LDLT recipients. A total of 716 blood samples were obtained from 60 pediatric LDLT recipients (one month to 16 yr of age). The recipient's status was classified as follows: stable, infection, or rejection. The ImmuKnow values in the pediatric LDLT recipients with a clinically stable status had a lower immune response (IQR 85–297 ATP ng/mL) than that previously reported in adults. Meanwhile, the ImmuKnow values of the stable patients were not correlated with age. Furthermore, a significant difference was found in the ImmuKnow values between the bacterial or fungal infection and stable groups, but not between the CMV or EBV infection and stable groups. The ImmuKnow levels in the pediatric LDLT were lower than those observed in the adult LDLT. The proposed reference value is between 85 and 297 ATP ng/mL in pediatric LDLT recipients. We conclude that the ImmuKnow assay could be helpful for monitoring pediatric LDLT recipients with bacterial or fungal infections.

Akinari Fukuda[1], Ken-Ichi Imadome[2], Seisuke Sakamoto[1], Takanobu Shigeta[1], Hajime Uchida[1], Masatoshi Matsunami[1], Kengo Sasaki[1], Hiroyuki Kanazawa[1], Fuyuko Kawano[2], Atsuko Nakazawa[1], Shigeyoshi Fujiwara[2] and Mureo Kasahara[1]

[1]Transplantation Center, National Center for Child Health and Development, Tokyo, Japan, [2]Department of Infectious Diseases, National Research Institute for Child Health and Development, Tokyo, Japan

Key words: pediatric liver transplantation – living donor liver transplantation – immune responses – T lymphocytes – infectious risk – rejection

Akinari Fukuda, MD, Department of Transplant Surgery, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
Tel.: +81 3 3416 0181
Fax: +81 3 3416 2222
E-mail: fukuda-a@ncchd.go.jp

Accepted for publication 29 October 2014

The main goal of treatment of transplant recipients is to provide sufficient immunosuppression to prevent rejection without causing over immunosuppression that may result in opportunistic infections (1, 2). Monitoring the efficacy of immunosuppressive treatment is based on the analysis of liver enzyme measurements and liver function tests along with assessments of the blood drug levels (3–6). Not all immunosuppressive drug levels are measurable in routine clinical work-ups, and the development of a reliable and comprehensive immune function test for immune monitoring is essential, regardless of the regimen of immunosuppression.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; ATP, adenosine triphosphate; AUROC, area under the receiver operator characteristic curve; EBV, Epstein–Barr virus; GCV, ganciclovir; IQR, interquartile range; LDLT, living donor liver transplantation; LT, liver transplantation; PHA, phytohemagglutinin; ROC, receiver operator characteristic; s.d., standard deviation.

The ImmuKnow assay, which was approved by the Food and Drug Administration in 2002, belongs to the new generation of tests that directly measure the immune function of T cells and has been shown to reliably be used to distinguish between an immune profile of over immunosuppression and that of under immunosuppression in adults (7). The ImmuKnow assay has also been reported to be a user-friendly, non-invasive, *in vitro* assay with demonstrated effectiveness as an immune monitoring tool for use in organ transplantation recipients (8). Comprehensive reports have described the use of this test in the immune monitoring of adult transplant recipients (9), while smaller scale studies have reported its efficacy in pediatric renal transplant recipients (10). However, there have been few reports regarding the application of the ImmuKnow assay in pediatric LDLT patients, most of which relied on a small sample size. The aim of this study was therefore to clarify the reference value of the ImmuKnow assay in

pediatric LDLT recipients and to assess the usefulness of this test for distinguishing between recipients with infection and those with rejection.

Patients and methods

Patients

This study reports the results of an analysis of 60 pediatric LDLT recipients treated at the National Center for Child Health and Development, Tokyo, Japan, between December 2009 and February 2013. The characteristics of the liver transplant patients are presented in Table 1. The mean patient age at LT was 3.1 ± 4.0 yr (range: one month–16 yr). The median post-LDLT follow-up period was 2.8 yr (range: 0.9–6.7 yr). The patients included 18 males and 42 females. A total of 31 patients (51.7%) were <1 yr of age at the time of transplantation. The indications for LT included cholestatic disease (n = 35: biliary atresia, congenital hepatic fibrosis, Alagille syndrome, primary sclerosing cholangitis, and progressive familial intrahepatic cholestasis type 2), metabolic liver disease (n = 15: ornithine transcarbamylase deficiency, carbamoylphosphate synthetase I deficiency, glycogen storage disease type Ib, methylmalonic acidemia, propionic acidemia, and primary hyperoxaluria), acute liver failure (n = 7), vascular disease (n = 2: congenital absence of the portal vein), and cryptogenic liver cirrhosis (n = 1).

Indications for the immune function assay

There were two indications for performing an immune function assay in this study: programmed tests (pre-LDLT, every week until one month after LDLT, then two, three, six and nine months, and one yr after LDLT; n = 41) and event-driven tests. The reasons for ordering an additional immune function assay included the presence of infectious symptoms (fever, diarrhea, and coughing), signs of rejection, or significant elevation of liver function parameters (the levels of AST or ALT were elevated above 100 IU/L) in the recipient.

Immunosuppression

The immunosuppression protocol consisted of tacrolimus and low-dose steroids. The target whole-blood trough level of tacrolimus was 10–12 ng/mL during the first two wk, then approximately 10 ng/mL and 5–10 ng/mL starting the second month after LDLT. Methylprednisolone (1 mg/kg/day administered intravenously) was given on postoperative days 1–3, followed by 0.5 mg/kg/day on postoperative days 4–6. The steroid treatment was then switched to oral prednisolone (0.3 mg/kg/day) on postoperative day 7, and the dose was reduced to 0.1 mg/kg/day one month after LDLT. If the liver function was stable, the recipient was weaned off steroids at 3–6 months after LDLT. We did not use immunosuppressive agents for induction based on our immunosuppression protocol.

Table 1. Characteristics of the 60 pediatric LDLT recipients

Characteristics	Data at transplantation (n = 60)
Age [yr, mean ± s.d. (range)]	3.1 ± 4.0 (0.1–16)
0–5 months	7 (11.7%)
6–11 months	24 (40.0%)
1–4 yr	14 (23.3%)
5–9 yr	9 (15.0%)
10–17 yr	6 (10.0%)
Male/female	18/42
Blood type combination identical/compatible/incompatible	37/18/5
Follow-up period [yr, mean ± s.d. (range)]	2.8 ± 1.4 (0.9–6.7)
Primary diagnosis of recipient	
Cholestatic disease	35 (58.3%)
Metabolic disease	15 (25.0%)
Acute liver failure	7 (11.7%)
Vascular disease	2 (3.3%)
Cryptogenic liver cirrhosis	1 (1.7%)

Immune function assay

The sodium-heparinized peripheral blood samples obtained from the transplant patients were submitted for an analysis of the T cell immune function (ImmuKnow assay; Cylex, Columbia, MD, USA). All blood samples were processed on the day of sample collection. Briefly, 250 μL of anticoagulated whole blood was diluted with the provided sample diluent to a final volume of 1000 μL. The samples were added to wells and incubated for 16 h with PHA in an incubator (37 °C, 5% CO_2). Following enrichment in CD4+ T cells with the addition of magnetic particles coated with antihuman CD4 monoclonal antibodies, the blood cells were washed and lysed to release intracellular ATP. The released ATP was measured with a luciferin/luciferase assay using a luminometer. The patient's immune response was expressed as the amount of ATP (ng/mL).

Evaluation of the blood concentration and concentration/dose (C/D) ratio of tacrolimus and the ImmuKnow ATP levels

The daily dose of tacrolimus was recorded, and its weight-adjusted dose (mg/kg/day) was calculated. The blood tacrolimus concentration was normalized according to the corresponding dose per body weight blood sampling to obtain the concentration/dose (C/D) ratio. The correlation between the tacrolimus C/D ratio and the ImmuKnow ATP levels was evaluated.

Patient monitoring and clinical status

The patient's clinical status was monitored, including assessments of the complete blood cell count, liver function tests, and trough levels of immunosuppressants. The patients were divided retrospectively as having a status of stable, bacterial, fungal, or viral (cytomegalovirus and EBV) infection or rejection based on clinical information in their medical chart. Post-transplant bacterial and fungal infections were diagnosed based on clinical features, positive microbiologic tests, and imaging findings. The patients were routinely screened for antigenemia due to cytomegalovirus (CMV) in addition to blood polymerase chain reaction for EBV. CMV-pp65 antigenemia was measured weekly for the first three months postoperatively, while the recipient was hospitalized, and then monthly in the outpatient setting until six months after LT. We have used the cutoff for a positive CMV-pp65 antigenemia as 5/50 000 cells. Measurements of the EBV-DNA load were obtained every two wk while the patient remained in the hospital, then every 1–3 months thereafter until one yr after transplantation, followed by testing at the physician's discretion. Quantification of EBV-DNA was performed using a real-time quantitative PCR assay. A peripheral blood EBV-DNA

load of more than 2.4×10^3 copies/μg DNA (1.6×10^4 copies/mL) was considered to indicate significant elevation of the DNA load (11, 12). In cases of CMV-pp65 antigenemia or a positive EBV-DNA load, the dose of tacrolimus was reduced to 75% of the regular dose (13). Treatment with GCV (5 mg/kg/dose, every 12 h) was initiated in the CMV-positive patients for the first two wk, followed by the administration of a maintenance dose of IV GCV (5 mg/kg/dose, every 24 h). The treatment was continued until CMV-pp65 antigenemia became negative. Rejection was identified based on the results of liver function tests, a pathological biopsy analysis, or clinical suspicion. A stable post-transplant condition was defined as a normal liver function without any episodes of infection or rejection. The ImmuKnow ATP values obtained within three days before and after a clinical event were selected for the analysis. Throughout the study period, no clinical intervention protocol was implemented based upon the ImmuKnow ATP assay results, and the clinicians were discouraged from intervening in the immunosuppression regimen based on these results. This research was approved by the Institutional Review Board of the National Center for Child Health and Development (#410).

Statistical analysis

The Mann–Whitney *U*-test and Kruskal–Wallis test were used to compare continuous variables between two groups and three groups or more, respectively. The Pearson correlation coefficient was used to determine the relationship between the blood concentrations of tacrolimus and the ImmuKnow ATP levels. Estimates of the thresholds of the ImmuKnow ATP values for rejection and infection were determined using ROC curves. The AUROC was calculated. An AUROC of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value. The results were considered to be statistically significant for p-values of <0.05. All statistical analyses were performed using the SPSS 19.0 statistical software program (SPSS, Inc., Chicago, IL, USA).

Results

A total of 716 blood samples in 60 patients were available for the analysis. The mean patient age at the time of the ImmuKnow assay was 3.9 ± 3.8 yr. Among the samples, 157 were collected at the time of infection, 91 at the time of rejection, and 468 while in stable condition (without infection or rejection). Among the patients with infections, 52 examinations (in 22 recipients) were associated with bacterial infections, nine examinations (in six recipients) were associated with fungal infections, 14 examinations (in 11 recipients) were associated with cytomegalovirus infections, and 107 examinations (in 28 recipients) were performed in patients with EBV infection (including coinfections) (Table 2). All cases of EBV infections were classified as asymptomatic EBV viremia. There were 37 episodes of biopsy-proven acute cellular rejection in 14 recipients (23.3%). The grade of rejection included four episodes of severe rejection, 17 episodes of moderate rejection, and 16 episodes of mild rejection, according to the Banff criteria (14). There were no significant differences in the severity of rejection or values of the ImmuKnow assay (p = 0.663, Kruskal–Wallis test).

Table 2. The infections detected within three days before and after the sampling points of the immune function assay were reported. There were several patients with contained coinfections

Type of infection	Number of examinations
Bacterial	52 (in 22 recipients)
Escherichia coli	10
Enterobacter cloacae	7
Klebsiella pneumoniae	5
Pseudomonas aeruginosa	5
Enterococcus faecalis	4
Klebsiella oxytoca	3
Enterobacter aerogenes	2
Enterococcus faecium	2
Haemophilus influenzae	2
Methicillin-resistant Staphylococcus aureus	2
Staphylococcus epidermidis MRS	2
Streptococcus pneumoniae	2
Group A Streptococcus	1
Moraxella catarrhalis	1
Mycoplasma	1
Pseudomonas putida	1
Staphylococcus epidermidis	1
Streptococcus oralis	1
Fungal	9 (in 6 recipients)
Candida albicans	6
Candida spp.	2
Pneumocystis pneumonia	1
Viral	126 (in 33 recipients)
EBV (EBV-DNA loads ≥2400 copies/μg DNA)	107
Cytomegalovirus (C7-HRP ≥5/50 000 WBC counts)	14
Adenovirus	2
Influenza virus	2
Herpes simplex virus	1

Evaluation of the blood concentration and concentration/dose (C/D) ratio of tacrolimus and the ImmuKnow ATP level

The average tacrolimus trough level and C/D ratio were 5.6 ± 3.3 ng/mL and 63.5 ± 47.5 ng/mL per mg/day, respectively, in the stable group, 4.9 ± 3.7 ng/mL and 107.7 ± 170.7 ng/mL per mg/day, respectively, in the infection group, and 7.8 ± 3.7 ng/mL and 111.0 ± 109.4 ng/mL per mg/day, respectively, in the rejection group. A scatterplot was drawn to evaluate the correlation between the tacrolimus blood concentration or C/D ratio and the ImmuKnow level at each examination point (Fig. 1a,b). The tacrolimus blood C/D ratio ranged from 2.5 to 963.1 ng/mL per mg/day. There were no correlations between the tacrolimus blood concentration or C/D ratio and the ImmuKnow level ($R = 0.051$ or $R = -0.092$). There was no significant difference

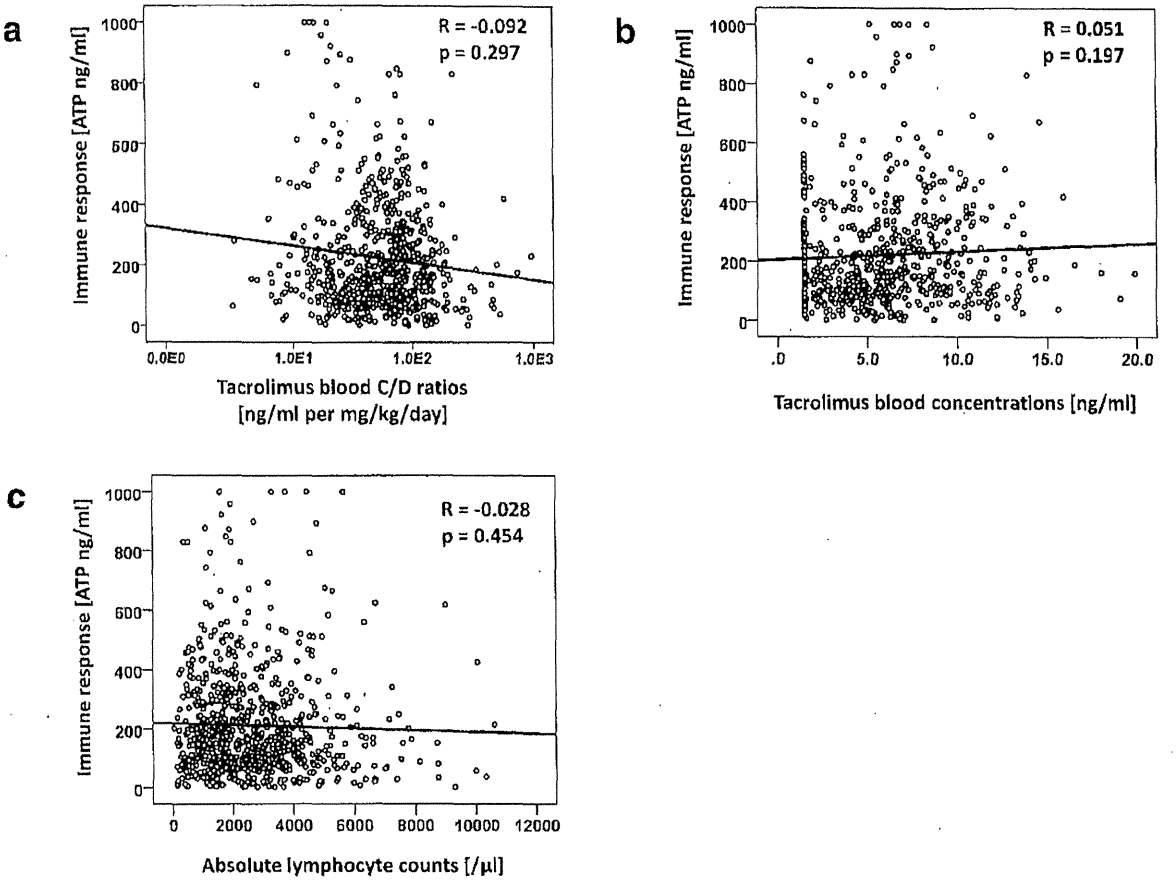

Fig. 1. The relationship between the tacrolimus blood concentration (a) or C/D ratios (b) and the ImmuKnow ATP values. The Pearson correlation coefficients were $R = 0.051$ ($p = 0.297$) and $R = -0.092$ ($p = 0.197$). (c) The correlation between the absolute lymphocyte count (ALC) and the ImmuKnow results was analyzed, but there were no significant correlations ($R = -0.028$, $p = 0.454$).

in the ImmuKnow values during high-dosage steroid bolus therapy compared to conventional treatment ($p = 0.281$, Mann–Whitney *U*-test).

Correlations between the ImmuKnow measurements and a stable immune function

A total of 468 of the 716 (65.4%) samples were collected from recipients in a clinically stable state. The median ImmuKnow level in the stable state LDLT recipients was 162 ATP ng/mL. Half of the measurements were between 85 and 297 ATP ng/mL, between the 25th and 75th percentiles (Fig. 2).

ImmuKnow values and age distribution in the pediatric LDLT recipients

The ImmuKnow values according to the age of the pediatric LDLT recipients at examination were plotted using the locally weighted regression smoother values of the stable state recipients (Fig. 3a). There were no significant differences in the distribution of the ImmuKnow values between infants and children older than one yr old at the time of the transplant ($p = 0.109$). The ImmuKnow values of the stable pediatric LDLT patients showed no evidence of age dependence. The median ImmuKnow value was 162 ATP ng/mL among the 468 stable state patients.

Impact of the liver transplant operation on the ImmuKnow values in pediatric LDLT recipients

The ImmuKnow values at each time point after LDLT for the pediatric LDLT recipients were plotted using the locally weighted regression smoother of the stable state recipients (Fig. 3b). There was an inflection point three months after LDLT. The median ImmuKnow level within the first three months after LDLT was

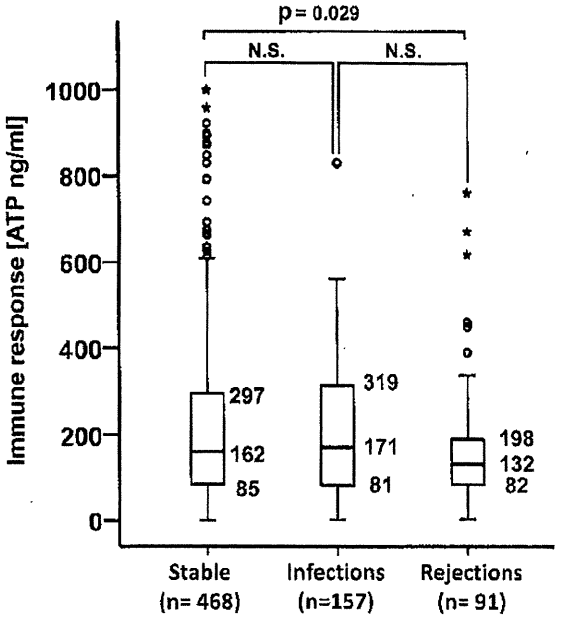

Fig. 2. ImmuKnow measurements grouped according to clinical patient status on the day of sample collection. The immune response measurements collected during events of rejection or infection did not differ between the groups. The ImmuKnow levels were low in the rejection group compared with that observed in the stable group ($p = 0.029$; Kruskal–Wallis test). The horizontal line indicates the median ImmuKnow level in each group, the vertical lines indicate the s.d., and the boxes describe the range of the central 50% of the measurements in that group.

196 ATP ng/mL. The median levels decreased significantly after three months to 139 ATP ng/mL ($p = 0.001$).

Comparison of the ImmuKnow values according to the type of infection

The ImmuKnow values in the patients with bacterial or fungal infections were significantly lower than those observed in the stable patients (Fig. 4a,b). To determine a reference value for the ImmuKnow level for diagnosing infection, a ROC curve analysis was performed. When the ImmuKnow value was set at 102.5 ATP ng/mL and 91.5 on the ROC curve for the patients diagnosed with bacterial and fungal infections, the sensitivity was 68.2% and 72.7%, the specificity was 45.5% and 66.7%, and the AUROC was 0.602 and 0.798, respectively (Fig. 5a,b).

Fourteen episodes of CMV infection and 107 episodes of EBV infection were diagnosed. There were no significant differences in the ImmuKnow values in the patients with CMV infection (Fig. 4c). There was a paradoxical significant difference in the ImmuKnow values in the patients with EBV infection in that the ImmuKnow values in the patients with EBV viremia were higher than those observed in the non-infected patients (Fig. 4d).

Discussion

The T-cell immune function assay can be used to categorize adult individuals as low, moderate, and strong immune responders, and the correlation with clinical quiescence in adults is defined as an ATP measurement within the

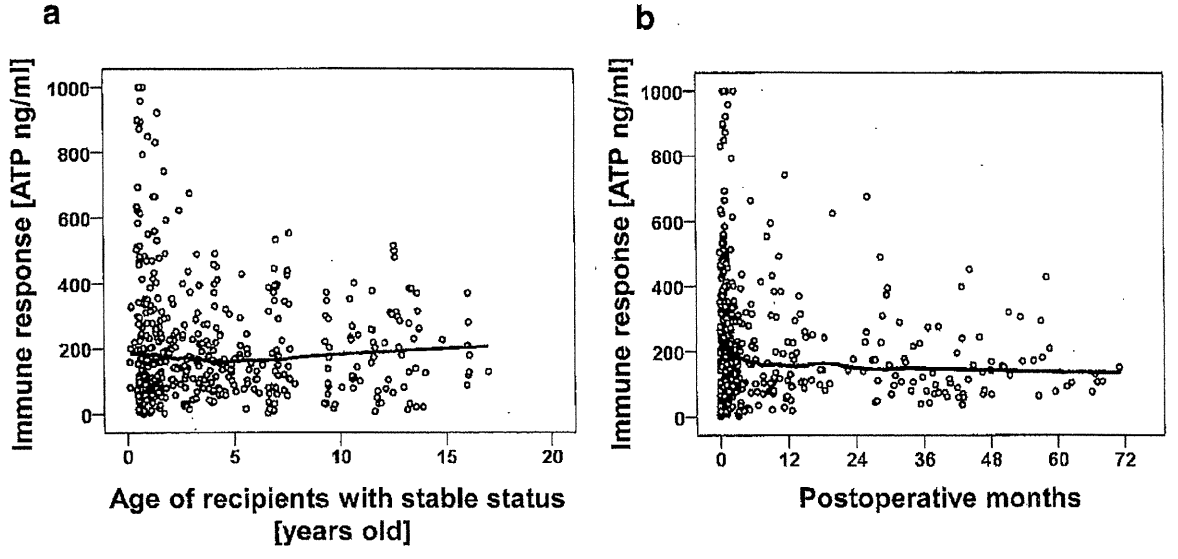

Fig. 3. A scatterplot comparing the immune responses according to the age in years (a) and the time after operation (b) among the stable liver transplant recipients. The solid line was calculated using the locally weighted regression of the ImmuKnow values. (a) There were no points of inflection in the solid line. (b) There was an inflection point three months after LDLT.

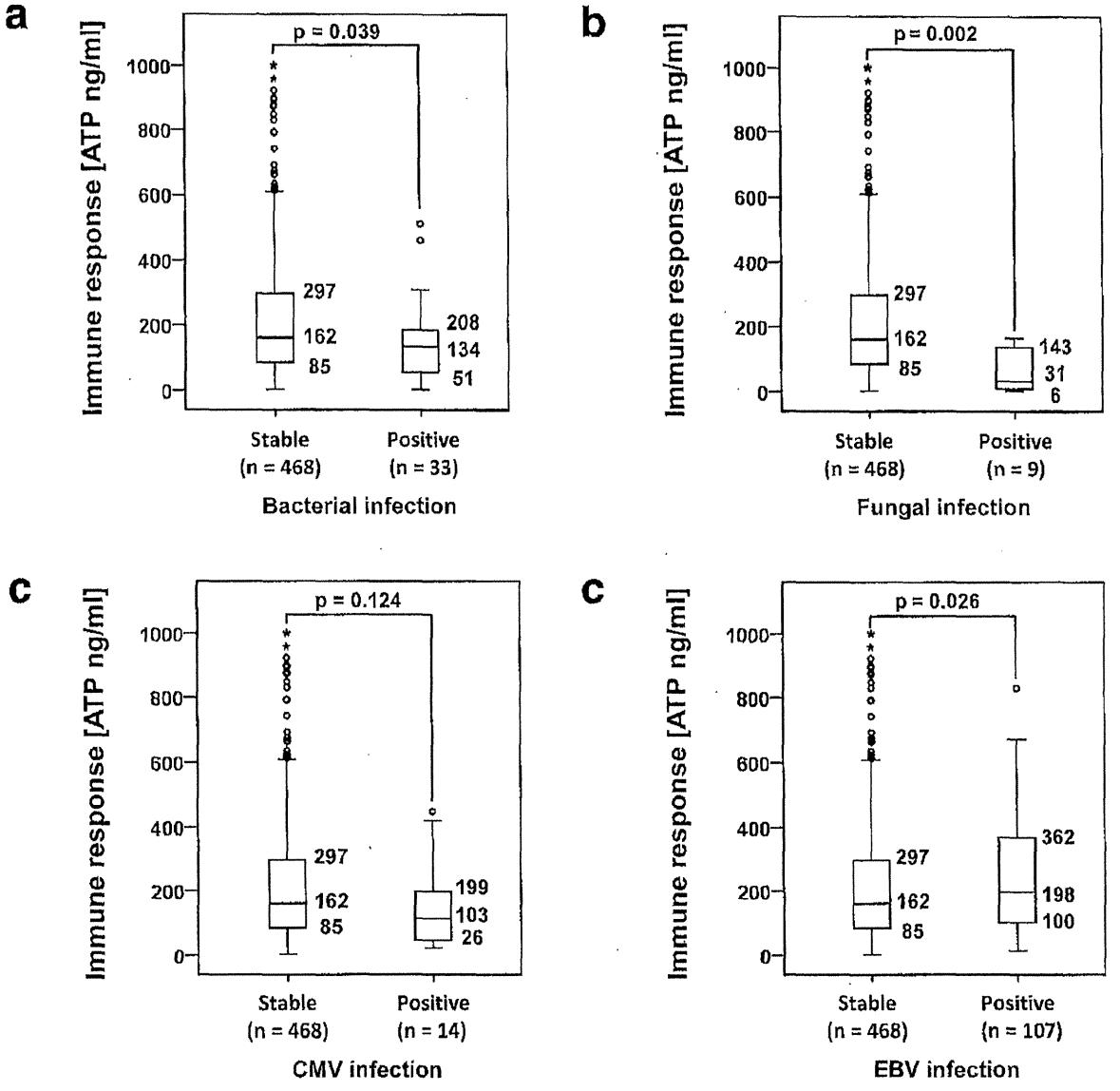

Fig. 4. ImmuKnow measurements grouped according to (a) bacterial infections, (b) fungal infections, (c) CMV infections, and (d) EBV infections, compared with the stable group.

established "moderate" range, between 225 and 525 ATP ng/mL (7). However, our study showed that the ImmuKnow ATP levels are lower in the pediatric LDLT population than in the adult LDLT population. We propose that the "moderate" range of the ImmuKnow levels in the pediatric LDLT population is between 85 and 297 ATP ng/mL based on the results of our stable state pediatric LDLT recipients analysis.

In the present study, low values on the T-cell immune function assay were associated with susceptibility to infection, although this issue is controversial (15–17). In addition, we found a significant difference in the distribution of the ImmuKnow values between the patients with a stable status and those with infection, with the exception of the patients with asymptomatic EBV viremia. Furthermore, a low ImmuKnow value was more helpful for identifying patients at higher risk of bacterial and fungal infection, and the ROC analysis showed that the sensitivity and specificity for diagnosing fungal infection episodes were 72.7% and 66.7%, respectively. Therefore, the possibility of a fungal or bacterial infection should be considered in patients in

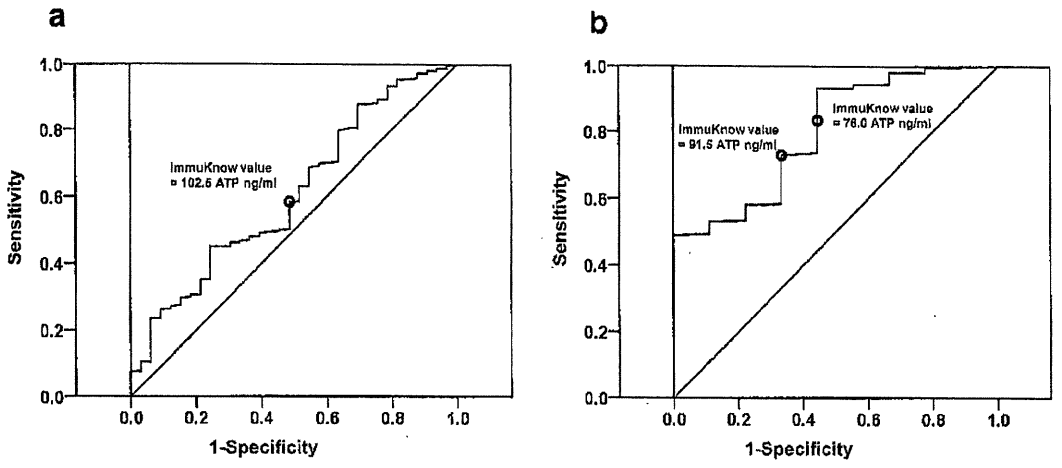

Fig. 5. Receiver operating characteristic (ROC) curve for predicting bacterial and fungal infections. (a) ROC curve: stable vs. bacterial infection. When the cutoff ImmuKnow value was set at 102.5 ATP ng/mL, the sensitivity and specificity were 68.2% and 45.5%, respectively (AUROC: 0.602). (b) ROC curve: stable vs. fungal infection. When the cutoff ImmuKnow value was set at 91.5 ATP ng/mL, the sensitivity and specificity were 72.7% and 66.7%, respectively (AUROC: 0.798). When the cutoff ImmuKnow value was set at 76.0 ATP ng/mL, there were significant differences between fungal infections and a stable status (the sensitivity was 0.803 and specificity was 0.556, p = 0.008, Mann–Whitney *U*-test).

whom the ImmuKnow value is lower than 91.5 ATP ng/mL. When the ImmuKnow value is <90 ATP ng/mL, it is better to increase the monitoring or to introduce prophylactic treatments for bacterial and/or fungal infections.

Regarding the ImmuKnow values in the patients with bacterial or fungal infection, there were no significant differences in the ImmuKnow values between the patients with CMV and EBV infections. Pediatric transplant recipients are generally dosed at higher concentrations of immunosuppressants per kilogram of body weight than adults. We investigated the relationship between the ATP levels and the pharmacokinetics of tacrolimus and found no correlations between the tacrolimus blood C/D ratios and the ImmuKnow ATP levels. Typically, measuring the peripheral blood trough levels of immunosuppressive drugs after transplantation is considered a critical step in therapeutic management to prevent toxicity and to provide effective immunosuppression. These data imply that measuring the immunosuppressive drug levels remains an important aspect of therapeutic management; however, such measurements have limited clinical value in monitoring for over immunosuppression and/or infection (18).

High values are associated with the risk of rejection in kidney (9), heart (19), liver (20–23), and pancreas (24) transplant patients, although other studies have shown no significant associations (16, 25–28). Kowalski et al. reported the results of a meta-analysis of 504 adult patients treated with a variety of transplant procedures, including LT, that recipients with an immune response of 700 ATP ng/mL were 30-fold more likely to develop rejection than those with a lower immune response value (8). The present study results differ from those of a prior study of adult solid organ transplant patients in that our pediatric recipients in the rejection group did not exhibit high ImmuKnow levels. It is important to note that the adaptive immune system is immature in children compared to adults and that a lower response to mitogen stimulation is observed in children (29). The factors which were not related with ATP levels of the T-cell immune function might be mainly associated with acute cellular rejection in pediatric liver transplant population, such as antibody-mediated rejection (30).

In the present study, we found that pediatric liver transplant recipient immune responses are age-independent. Within the pediatric patient population (age <12 yr), we found no age-related effects on the immune function. Similar findings have been reported in pediatric renal and liver transplant recipients (29, 31). In addition, Hooper et al. assessed the mean ImmuKnow values among 50 healthy children (32 children <12 yr of age and 18 children ≥12 yr of age) and 155 healthy adults and found that the mean ImmuKnow value remained constant around 327 ng/mL until 10 yr of age, after which it progressively became elevated at 10–12 yr of age and plateaued at the level observed in adults (around 433 ng/mL) after 12 yr of age (29).

We analyzed the relationship between the height/weight and the ImmuKnow value pre-transplant. There were no correlations between the clinical status of recipients' development and the ImmuKnow values (the Pearson correlation coefficients were $R = 0.131$ for height and 0.231 for weight, data not shown). A subgroup analysis based on the preoperative ImmuKnow values was performed; however, we did not find any significant differences in the incidence of developing infections.

We did not observe an adequate, absolute cutoff value for the ImmuKnow level for predicting EBV infection. A previous study reported that infectious episodes are accompanied by low of ImmuKnow values. In the present study, the ImmuKnow values were significantly higher in the patients with EBV infection than in those with a stable status, a paradoxical change. PHA activation of CD4+ T cells usually necessitates co-stimulation by other cells, such as B cells (32, 33). EBV-transformed B-cell lines are approximately 4–20 times as efficient on a per cell basis as non-T cells in stimulating the amplitude of the co-stimulation function (15, 32). Consequently, in cases of EBV infection, transformed B cells enhance the activation process, resulting in the overproduction of ATP, thus inducing an elevated ImmuKnow level (34). The unpredictably high ImmuKnow results observed in our EBV-infected recipients may be explained by this phenomenon, in contrast to the expected low values observed in the cases of viral infections (9, 10, 35).

The independent risk factors associated with the clinical status (stable vs. bacterial, fungal, CMV or EBV infection, and acute cellular rejection) were the recipients' age, time since LDLT, and ImmuKnow values, as determined by a logistic regression test. The ImmuKnow values were associated with bacterial infections ($p = 0.031$, $OR = 0.997$), fungal infections ($p = 0.014$, $OR = 0.987$), and acute cellular rejection ($p = 0.005$, $OR = 0.998$). It can be concluded that the ImmuKnow values were a useful tool for diagnosing fungal infections in our study. When the cutoff ImmuKnow value was set at 76.0 ATP ng/mL, there were significant differences between fungal infections and a stable status (the sensitivity was 0.803 and specificity was 0.556, $p = 0.008$, Mann–Whitney U-test). Ling et al. reported that the ImmuKnow value was not able to determine individuals at risk for infection or rejection in their relatively large meta-analyses in cases of adult solid organ transplantation using a ROC curve analysis (35). They concluded that it was not a useful test for diagnosing infections, because the AUROC for infection was 0.77. We intended to validate the cutoff ImmuKnow value for diagnosis rejection and infection using a ROC curve analysis in pediatric LDLT recipients. However, we also could not set an adequate cutoff value for the ImmuKnow assay for diagnosing a bacterial infection, because the AUROC was 0.602.

There were a few possible limitations associated with this study. We have used CMV-pp65 in the PCR era, because our previous study revealed the effectiveness of a universal pre-emptive therapy for CMV infection based on the cutoff for a positive CMV-pp65 antigenemia of ≤5/50 000 cells (36). However, this is not the standardized value. Further studies are necessary to determine the optimal cutoff value for CMV-pp65 antigenemia and to compare it with the results of CMV-DNA PCR. There was another limitation, that this study was retrospective study and we need to validate the cutoff values using an independent dataset in another prospective study.

Conclusions

The ImmuKnow ATP levels are lower in the pediatric LDLT population than in the adult LT population. We herein proposed a reference value between 85 and 297 ATP ng/mL in pediatric LDLT recipients, although we did not identify any age-related effects on the immune function in the pediatric LDLT recipients. The ImmuKnow assay could be helpful for monitoring pediatric LDLT recipients with infection, particularly those with fungal infections. Meanwhile, the correlations between the ImmuKnow values and the status of rejection or EBV viremia were limited.

Acknowledgments

This study was supported by Japanese Society for the promotion of Science, Grant-in-Aid for Scientific Research (C) Grant Number 24591593 and the Grant of National Center for Child Health and Development (H21-4 and H24-8). The authors also thank Chihiro Yamada and Sayumi Ichikawa at Department of Infectious Diseases (National Research Institute for Child Health and Development, Japan) for running the immune function assay.

Disclosure

All authors declare no conflict of interests.

Authors' contributions

Akinari Fukuda, Mureo Kasahara, Ken-Ichi Imadome, and Seisuke Sakamoto participated in the design, data analysis, research, and writing of the manuscript. Takanobu Shigeta,

Hajime Uchida, Masatoshi Matsunami, Kengo Sasaki, Hiroyuki Kanazawa, Fuyuko Kawano, Atsuko Nakazawa, and Shigeyoshi Fujiwara participated in the data analysis and research.

References

1. Patel J, Kobashigawa JA. Minimization of immunosuppression: Transplant immunology. Transpl Immunol 2008: 20: 48–54.
2. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med 2007: 357: 2601–2614.
3. Hwang S, Lee SG, Ahn CS, et al. A clinical assessment of mycophenolate drug monitoring after liver transplantation. Clin Transplant 2010: 24: E35–42.
4. Monchaud C, Marquet P. Pharmacokinetic optimization of immunosuppressive therapy in thoracic transplantation: Part I. Clin Pharmacokinet 2009: 48: 419–462.
5. Masuda S, Inui K. An up-date review on individualized dosage adjustment of calcineurin inhibitors in organ transplant patients. Pharmacol Ther 2006: 112: 184–198.
6. del Mar Fernandez De Gatta M, Santos-Buelga D, Dominguez-Gil A, Garcia MJ. Immunosuppressive therapy for paediatric transplant patients: Pharmacokinetic considerations. Clin Pharmacokinet 2002: 41: 115–135.
7. Kowalski R, Post D, Schneider MC, et al. Immune cell function testing: An adjunct to therapeutic drug monitoring in transplant patient management. Clin Transplant 2003: 17: 77–88.
8. Kowalski RJ, Post DR, Mannon RB, et al. Assessing relative risks of infection and rejection: A meta-analysis using an immune function assay. Transplantation 2006: 82: 663–668.
9. Israeli M, Yussim A, Mor E, Sredni B, Klein T. Preceeding the rejection: In search for a comprehensive post-transplant immune monitoring platform. Transpl Immunol 2007: 18: 7–12.
10. Gautam A, Morrissey PE, Brem AS, et al. Use of an immune function assay to monitor immunosuppression for treatment of post-transplant lymphoproliferative disorder. Pediatr Transplant 2006: 10: 613–616.
11. Macedo C, Zeevi A, Bentlejewski C, et al. The impact of EBV load on T-cell immunity in pediatric thoracic transplant recipients. Transplantation 2009: 88: 123–128.
12. Rowe DT, Qu L, Reyes J, et al. Use of quantitative competitive PCR to measure Epstein-Barr virus genome load in the peripheral blood of pediatric transplant patients with lymphoproliferative disorders. J Clin Microbiol 1997: 35: 1612–1615.
13. Imadome K, Fukuda A, Kawano F, et al. Effective control of Epstein-Barr virus infection following pediatric liver transplantation by monitoring of viral DNA load and lymphocyte surface markers. Pediatr Transplant 2012: 16: 748–757.
14. Ormonde DG, de Boer WB, Kieratii A, et al. Banff schema for grading liver allograft rejection: Utility in clinical practice. Liver Transpl Surg 1999: 5: 261–268.
15. Ben-Youssef R, Baron PW, Sahney S, et al. The impact of intercurrent EBV infection on ATP levels in CD4+ T cells of pediatric kidney transplant recipients. Pediatr Transplant 2009: 13: 851–855.
16. Gupta S, Mitchell JD, Markham DW, et al. Utility of the Cylex assay in cardiac transplant recipients. J Heart Lung Transplant 2008: 27: 817–822.
17. Bennett WM, Meyer L, Ridenour J, Batiuk TD. Surveillance and modification of immunosuppression minimizes BK virus nephropathy. Am J Nephrol 2010: 32: 10–12.
18. Xue F, Zhang J, Han L, et al. Immune cell functional assay in monitoring of adult liver transplantation recipients with infection. Transplantation 2010: 89: 620–626.
19. Israeli M, Ben-Gal T, Yaari V, et al. Individualized immune monitoring of cardiac transplant recipients by noninvasive longitudinal cellular immunity tests. Transplantation 2010: 89: 968–976.
20. Cabrera R, Ararat M, Soldevila-Pico C, et al. Using an immune functional assay to differentiate acute cellular rejection from recurrent hepatitis C in liver transplant patients. Liver Transpl 2009: 15: 216–222.
21. Hashimoto K, Miller C, Hirose K, et al. Measurement of CD4+ T-cell function in predicting allograft rejection and recurrent hepatitis C after liver transplantation. Clin Transplant 2010: 24: 701–708.
22. Dong JY, Yin H, Li RD, et al. The relationship between adenosine triphosphate within CD4(+) T lymphocytes and acute rejection after liver transplantation. Clin Transplant 2011: 25: E292–296.
23. Rodrigo E, Lopez-Hoyos M, Corral M, et al. ImmuKnow as a diagnostic tool for predicting infection and acute rejection in adult liver transplant recipients: A systematic review and meta-analysis. Liver Transpl 2012: 18: 1245–1253.
24. Thai NL, Blisard D, Tom K, et al. Pancreas transplantation under alemtuzumab (Campath-1H) and tacrolimus: Correlation between low T-cell responses and infection. Transplantation 2006: 82: 1649–1652.
25. Helantera I, Koskinen P. Association of immune cell function assay with protocol biopsy findings and viral infections in well matched kidney transplant recipients. Clin Nephrol 2010: 74: 123–131.
26. Kobashigawa JA, Kiyosaki KK, Patel JK, et al. Benefit of immune monitoring in heart transplant patients using ATP production in activated lymphocytes. J Heart Lung Transplant 2010: 29: 504–508.
27. Rossano JW, Denfield SW, Kim JJ, et al. Assessment of the Cylex ImmuKnow cell function assay in pediatric heart transplant patients. J Heart Lung Transplant 2009: 28: 26–31.
28. Huskey J, Gralla J, Wiseman AC. Single time point immune function assay (ImmuKnow) testing does not aid in the prediction of future opportunistic infections or acute rejection. Clin J Am Soc Nephrol 2011: 6: 423–429.
29. Hooper E, Hawkins DM, Kowalski RJ, et al. Establishing pediatric immune response zones using the Cylex ImmuKnow assay. Clin Transplant 2005: 19: 834–839.
30. Markiewicz-Kijewska M, Kalicinski P, Kluge P, et al. Antibody-mediated rejection in pediatric liver transplant recipients. Ann Transplant 2014: 19: 119–123.
31. Israeli M, Klein T, Sredni B, et al. ImmuKnow: A new parameter in immune monitoring of pediatric liver transplantation recipients. Liver Transpl 2008: 14: 893–898.
32. Halvorsen R, Leivestad T, Gaudernack G, Thorsby E. Role of accessory cells in the activation of pure T cells via the T cell receptor-CD3 complex or with phytohaemagglutinin. Scand J Immunol 1988: 27: 555–563.
33. Bekoff M, Kubo R, Grey HM. Activation requirements for normal T cells: Accessory cell-dependent and -independent stimulation by anti-receptor antibodies. J Immunol 1986: 137: 1411–1419.
34. Grimm P. Use of an immune function assay to monitor immunosuppression. Pediatr Transplant 2006: 10: 533–535.
35. Lee TC, Goss JA, Rooney CM, et al. Quantification of a low cellular immune response to aid in identification of pediatric liver transplant recipients at high-risk for EBV infection. Clin Transplant 2006: 20: 689–694.
36. Saitoh A, Sakamoto S, Fukuda A, et al. A universal preemptive therapy for cytomegalovirus infections in children after live-donor liver transplantation. Transplantation 2011: 92: 930–935.

Transplantation. 2014 Dec 23. [Epub ahead of print]

Luminal Injection of Hydrogen-Rich Solution Attenuates Intestinal Ischemia-Reperfusion Injury in Rats.

Shigeta T[1], Sakamoto S, Li XK, Cai S, Liu C, Kurokawa R, Nakazawa A, Kasahara M, Uemoto S.

Author information

Abstract

BACKGROUND: Luminal preservation of the intestine is an attractive method to locally mitigate preservation **injury** and ischemic-reperfusion **injury** in small bowel transplantation (SBT) because this method has a potential to maintain the **intestinal** graft integrity. Hydrogen is noted as an antioxidant material by reducing hydroxyl radicals. We hypothesized that hydrogen-containing **solution** can be an optimum material for **luminal** preservation method in SBT.

METHODS: Ischemic reperfusion was induced in Lewis **rats** by occlusion of the supramesenteric artery and vein for 90 min. Experimental protocols were divided into four groups: sham operation group, no **luminal injection** (control) group, **luminal injection** of 5% glucose saline (GS) **solution** group, and **luminal injection** of **hydrogen-rich** GS (HRGS) group. Two milliliters of experimental **solution** was locally injected into the lumen of the intestine before declamping of vessels. Oxidative stress markers, proinflammatory cytokines, apoptosis in the crypt cells, and morphologic changes of the intestine were assessed.

RESULTS: The production of malondialdehyde and 8-hydroxydeoxyguanosine, as oxidative stress markers, were markedly suppressed in HRGS group. The level of proinflammatory cytokines, such as inducible nitric oxide synthase and interleukin-6, was significantly inhibited in HRGS group. Crypt apoptosis was also significantly suppressed in HRGS group. Histopathologically, integrity of villus in intestine was maintained in HRGS group in comparison to the other groups.

CONCLUSION: Luminal injection of **hydrogen-rich solution** can reduce oxidative stress and consequently ameliorate ischemic-reperfusion **injury**. Hydrogen-containing **solution** can be a novel and promising **luminal** preservation material in SBT.

PMID: 25539463 [PubMed - as supplied by publisher]

■ 原 著

小児肝移植におけるオンライン登録システムの構築

田中久子[1]，瀧本哲也[1]，福田晃也[2]，阪本靖介[2]，高木保子[3]，岡田昌史[3]，中澤温子[4]，笠原群生[2]

Construction of the online registration system in a pediatric liver transplantation

[1]*Clinical Research Center, National Center for Child Health and Development,*
[2]*Transplantation Center, National Center for Child Health and Development,*
[3]*Tsukuba Critical Path Research and Education Integrated Leading Center (CREIL), University of Tsukuba,*
[4]*Department of Pathology, National Center for Child Health and Development*

Hisako TANAKA[1], Tetsuya TAKIMOTO[1], Akinari FUKUDA[2], Seisuke SAKAMOTO[2], Yasuko TAKAGI[3], Masafumi OKADA[3], Atsuko NAKAZAWA[4], Mureo KASAHARA[2]

【Summary】

【Objective】 Different characteristics are found between adult and pediatric recipients of liver transplantation. However, a detailed nationwide database of pediatric liver transplantations has not been fully elucidated in Japan. Therefore it is necessary to accumulate such a nationwide registry of data on pediatric liver transplants. The primary objective of the database is to characterize the trends in indications, outcomes, and developments in pediatric liver transplantations in Japan.

【Methods】 In 2013, the on-line registration system was opened at several Japanese pediatric liver transplantation centers in Japan. This registry is designed to collect data online, and the subjects will be donors and recipients aged less than 18 years at the time of transplantation. The composition of this registry is classified into 3 categories, and 436 items.

【Results】 We added some evaluation items of growth and development to LITRE-J (Liver Transplantation REgistry in Japan). We will collect prospective data on children receiving liver transplantations at the pediatric liver transplant centers in Japan and accumulate comprehensive data on pre-and posttransplant outcomes of pediatric liver transplantations.

【Conclusion】 That the data accumulated by this registration system are expected to serve as basic histories and statistics data for the evaluation and development of pediatric liver transplantations in Japan, such as improvements in results by standardized medical treatment through decisions of the guidelines.

Keywords: liver transplantation, pediatric, registry

I. はじめに

1. 背 景

日本肝移植研究会・肝移植症例登録報告によると，小児（18歳未満）の肝移植は，全国で年間120～140例前後が施行されている[1]。しかし，報告されているレシピエントの年齢は，原疾患・移植肝・累積生存率で「18歳未満」，性別・累積生存率で「0～9歳/10～19歳……」，ABO血液型不適合群の累積生存率で「0～2歳/3～17歳……」のように大きく区分されており，現在のところ，これ以上の詳細な年齢別の結果は不明である。

このような現状に加えて，われわれは先の研究で，小児に特有な肝移植後の成長・発達および長期予後等を評価するには，それらに関する項目の充実が必要であることを指摘した[2]。すなわち，1989年11月に肝移植が初めて施行されて[3]から20年以上が経過し，移植医療末期肝不全に対する治療として確立されてきた

[1]国立成育医療研究センター臨床研究センター臨床研究推進室，[2]国立成育医療研究センター臓器移植センター移植外科，[3]筑波大学次世代医療研究開発・教育統合（CREIL）センター，[4]国立成育医療研究センター病理診断部
（2013・9・26受領；2013・11・21受理）

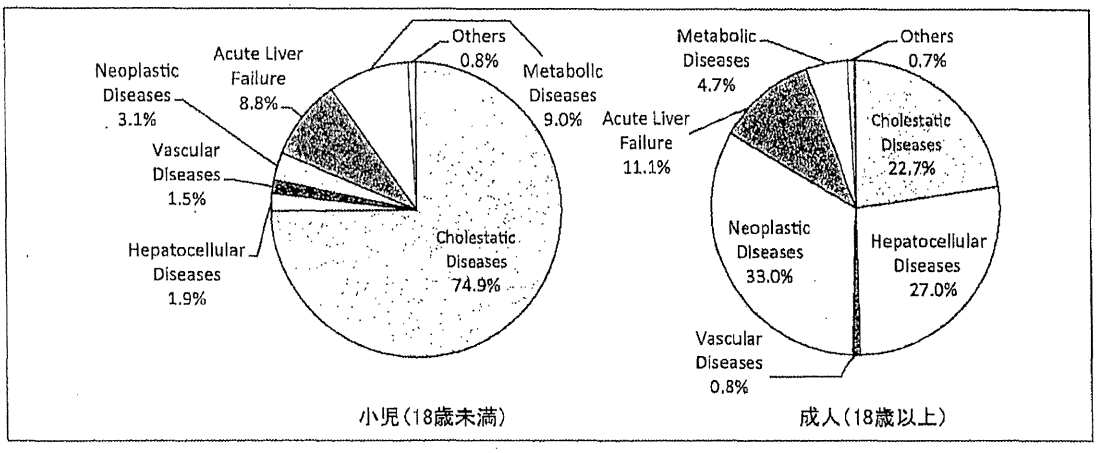

図1 レシピエントの原疾患（生体肝移植，初回移植：日本肝移植研究会集計）

現在，肝移植による長期的な影響を明らかにする必要性が高まってきている。

本研究は，小児肝移植の症例数の推移や移植後の患者の予後，移植臓器の予後等のデータを詳細に解析し，本邦における小児肝移植医療の実態把握だけでなく，肝移植が小児の成長・発達に及ぼす影響の解明を行い，日本における小児の肝移植医療の発展に寄与することを目的としている。そのためには，長期にわたって詳細なデータを集積していくことが不可欠であり，本報では，2010年5月に検討を始めた小児肝移植のオンライン登録システムが，2013年1月1日より開始されたので報告する。

2. 小児用の登録システムの必要性

肝移植を必要とする原疾患の内訳（生体肝移植，初回移植）は，成人（18歳以上）と小児（18歳未満）で大きく異なることが知られている[1]。図1に示すように，成人は腫瘍性疾患が33.0%，肝細胞性疾患が27.0%，胆汁うっ滞性疾患が22.7%であるのに対して，小児は胆汁うっ滞性疾患が多く74.9%，ついで代謝性疾患9.0%，急性肝不全が8.8%である。また，表1に示すように疾患ごとの内訳も大きく異なり，中でも胆汁うっ滞性疾患と代謝性疾患の内訳は小児に特異的である。しかしながら，肝移植症例登録システム（以下，LITRE-J）における原疾患の内訳の選択肢は小児特有の疾患には対応していない。われわれが小児肝移植のオンライン登録システムの項目検討を始めた2010年7月当時，LITRE-Jはテスト運用されていたが，成人にはまれな疾患である「胆汁うっ滞性疾患」のPFIC（progressive familial intrahepatic cholestasis），先天性肝線維症，「代謝性疾患」のglycogen storage disease，CPS 1欠損症（carbamyl phosphate synthase I deficiency），ASS欠損症（argininosuccinate synthase deficiency），propionic academia，「血管性病変」の項目はなかった。その2年後の登録スタート時には，PFIC-Ⅰ，PFIC-Ⅱ，glycogen storage disease，CPS 1欠損症，propionic academiaが追加されたが，それ以外の項目については，LITRE-Jでは「その他」を選択せざるを得ない。また，「その他」を選択した場合にはテキスト入力となり，入力自体に負担が増えるうえ，詳細なデータ解析の際に手作業の集計が増えるためエラーが増加することが予想され，データマネジメントの観点からも効率的ではない。したがって，小児に多い疾患の選択項目が網羅されているほうが，入力者およびデータ解析者双方に有益であると考えられる。

また，原疾患構成が異なれば予後に関連するリスク因子や生存率などにも違いが生じてくることが予想されるが，年齢ごとの原疾患別累積生存率やリスク因子などの詳細は不明である。さらに，小児の場合は成人と異なり，成長・発達への影響が懸念されるが，移植のタイミングや術後経過などが移植後のレシピエントの成長・発達にどのような影響を与えるのかについては十分に解明されていない[4]。さらに今後，生体肝移植後20年以上経過した症例が増えてくると，妊娠出産の問題，代謝性疾患の挙児の問題，悪性腫瘍の問題など，新たな課題に直面することが予想される[5]。例えば妊娠・出産・育児等の体への負担は大きく，これを機に病状が悪化する可能性や，移植後の免疫抑制状

表1　レシピエントの原疾患（生体肝移植，初回移植：日本肝移植研究会集計）

	Age of Recipient		Total
	<18 y.o.	≧18 y.o.	
Cholestatic Diseases	1,608	860	2,468
Biliary Atresia	1,471	145	1,616
Primary Biliary Cirrhosis	0	535	535
Primary Sclerosing Cholangitis	20	141	161
Alagille Syndrome	70	2	72
Byler's Disease	33	2	35
Congenital Bile Duct Dilatation	5	7	12
Caroli Disease	3	9	12
Others	6	19	25
Hepatocellular Diseases	41	1,025	1,066
HCV	1	461	462
HBV	0	236	236
Alcoholic	0	134	134
Autoimmune Hepatitis	3	64	67
NASH	2	28	30
Cryptogenic Cirrhosis	27	98	125
Others	8	4	12
Vascular Diseases	32	30	62
Budd-Chiari Syndrome	7	26	33
Congenital Absence of Portal Vein	21	2	23
Others	4	2	6
Neoplastic Diseases	66	1,253	1,319
Hepatocellular Carcinoma	6	1,219	1,225
HCV	0	739	739
HBV	0	375	375
Alcoholic	0	44	44
Primary Biliary Cirrhosis	0	11	11
Others	6	50	56
Hepatoblastoma	52	1	53
Liver Metastatis	1	17	18
Others	7	16	23
Acute Liver Failure	190	422	612
HBV	7	134	141
Drug-induced	2	30	32
Autoimmune Hepatitis	2	22	24
Viral（≠HBV）	11	12	23
Unknown	163	222	385
Others	5	2	7
Metabolic Diseases	194	179	373
Wilson Disease	59	50	109
Familial Amyloid Polyneuropathy	0	72	72
Citrullinemia	6	39	45
OTC Deficiency	40	2	42
Glycogen Storage Disease	15	6	21
Methylmalonic Acidemia	20	0	20
Primary Hyperoxaluria	9	5	14
Tyrosinemia	13	0	13
Others	32	5	37
Others	17	27	44
Total	2,148	3,796	5,944

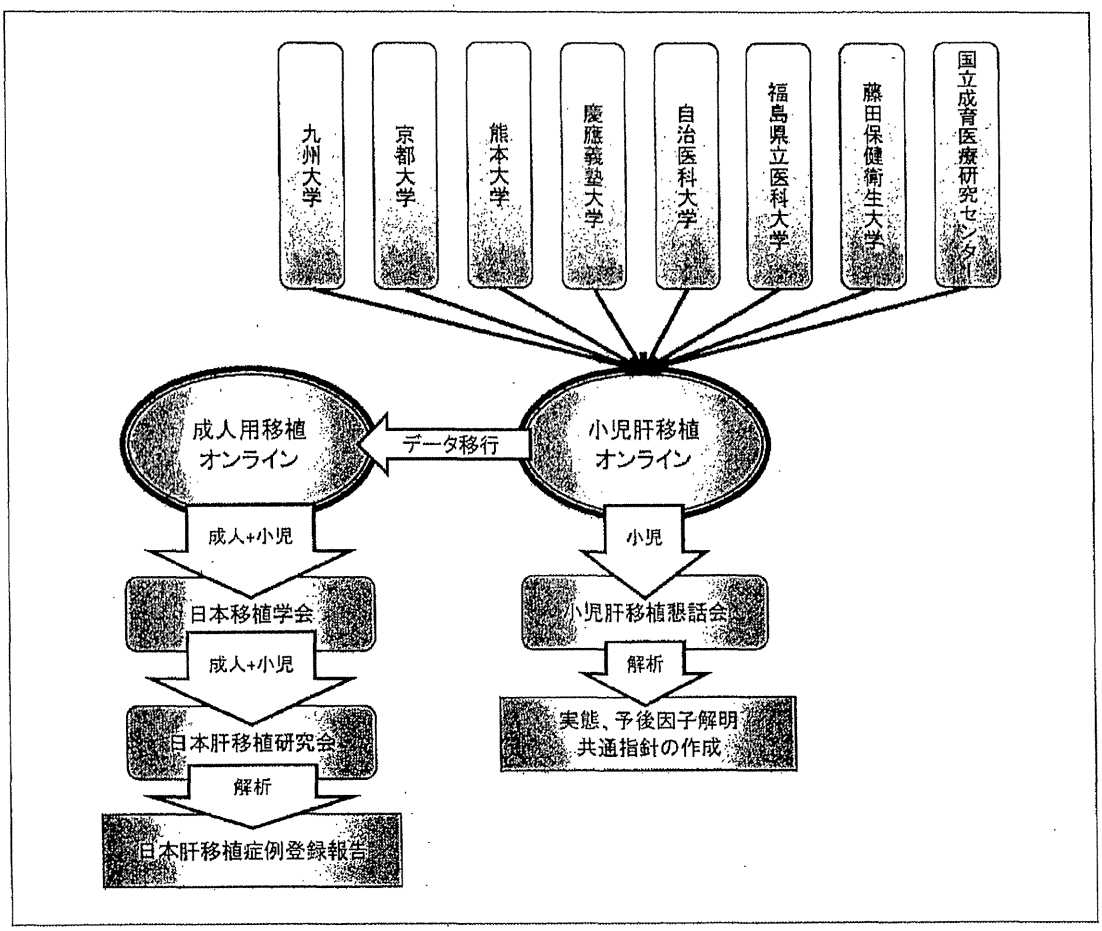

図2　小児肝移植オンライン登録の流れ

態により悪性腫瘍の発症が増加することが危惧される[9]。これらの問題の解明には長期のフォローアップを要するため，小児肝移植症例の詳細なデータを集積し，継続的なフォローアップを行っていくことが必要不可欠であると考えられる。一方で今後，小児のデータベースを効率的かつ永続的に構築するためには，LITRE-Jとの連携が重要である。

本システム構築の試みは2010年5月に開始され，日本移植学会によってLITRE-Jが作成中であったことを受けて，相互の連携を図るため，LITRE-Jを基に小児用のオンライン登録システム作成を行った。2012年1月からLITRE-Jのオンライン登録が開始され，小児用の登録システムもほぼ完成したため，6月より具体的な両システムの連携のあり方について協議を行った。この結果，小児の肝移植症例のデータは，小児用の登録システムに入力し，定期的にLITRE-Jのデータベースに移行することとした（図2）。

II. 対象と方法

1. 登録参加施設（施設責任者）

参加施設は，2010年6月の第28回日本肝移植研究会・小児移植懇話会において，本研究の提案に賛同していただいた以下の8施設で登録を開始することとした。九州大学（田口智章），京都大学（上本伸二），熊本大学（猪股裕紀洋），慶應義塾大学（黒田達夫），国立成育医療研究センター（笠原群生），自治医科大学（水田耕一），福島県立医科大学（後藤満一），藤田保健衛生大学（鈴木達也），施設名50音順，敬称略。

2. 登録対象者

研究参加施設において実施された小児（18歳未満）の肝移植症例およびそのドナー。

3. 登録システムの構築

1）登録項目の決定

2010年7月より，LITRE-Jの登録項目をベースに，小児特有の項目や成長発達の評価に必要であると考えられる項目を追加し，原案を作成した。2011年7月に研究計画書案（登録項目を含む）を参加施設に送付し，同月開催された第29回日本肝移植研究会・小児移植懇話会にて意見交換を行った。その後，同意を得られた案に基づき，システムのサンプル画面を作成した。2011年10月に開催された第47回日本移植学会総会で，「小児肝移植オンライン登録データベース構築に関する研究」についての検討会を開催し，実際にオンライン画面を操作するなどして，参加施設で最終確認を行った。

2）小児肝移植オンライン登録の構成

項目を「症例登録」，「初回調査」，「追跡調査」の3つに大別した（図3）。このうち症例登録は，グラフト，レシピエント，ドナーに分類され，さらに基本情報や原疾患に分かれている。初回調査も，レシピエント，ドナーに分類され，それぞれ手術前後のデータを分類した構成である。同様に追跡調査もレシピエント，ドナーに分類され，追跡期間中のデータを分類した構成となっている。今後のデータ移行を考慮して，LITRE-Jに準じている。

3）日本移植学会データベースLITRE-Jとの連携

日本移植学会データベースLITRE-Jと小児肝移植オンライン登録との間で，データファイルの移行時期，移行方法などの運用を協議した結果，参加施設担当者は小児肝移植症例のデータを小児肝移植オンライン登録へのみ入力作業を行い，小児肝移植オンライン登録事務局が入力されたデータを3カ月ごとにLITRE-Jへと移行する以下の手順による連携方法を確立した。

①小児肝移植オンライン登録事務局で症例データをダウンロード，LITRE-Jとの連携用データを作成する。

②小児肝移植オンライン登録事務局からLITRE-Jにパスワード付データファイルを送付する。

③LITRE-Jに小児症例データを取り込む（インポート）。

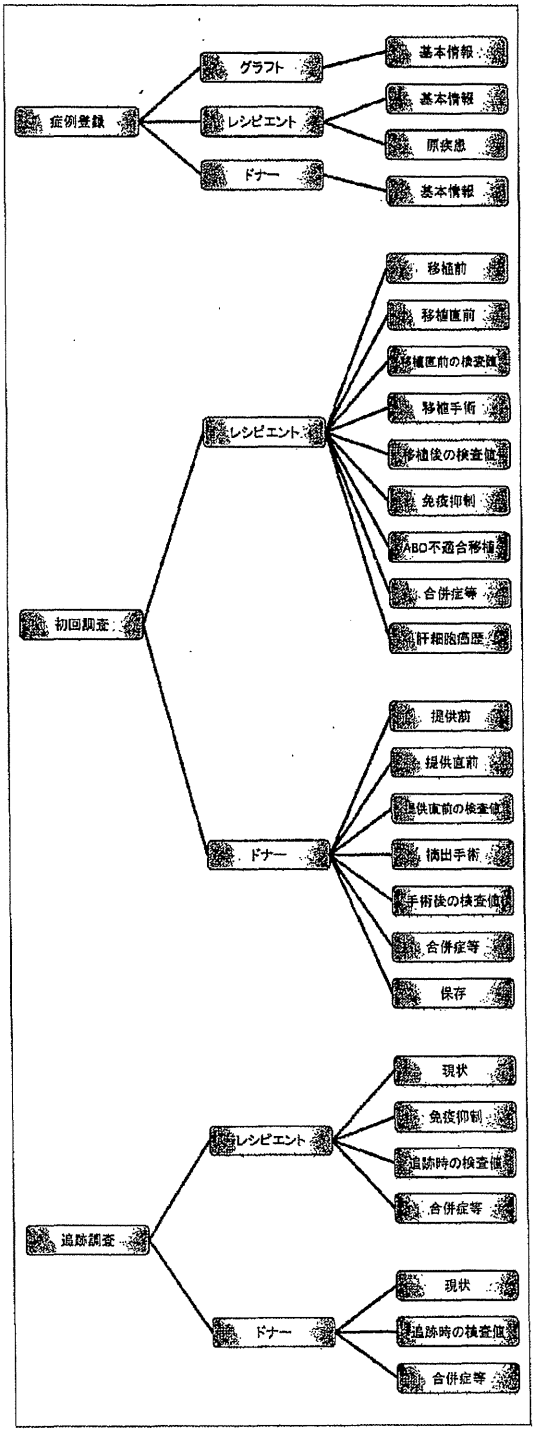

図3　小児肝移植オンライン登録システムの構成

④LITRE-J で症例 ID を付番されたファイルを小児肝移植オンライン登録事務局へ送付する。
⑤小児肝移植オンライン登録事務局で LITRE-J 番号症例 ID を取り込む。

4）倫理的配慮

①同意の取得方法

研究対象者の尊厳に十分に配慮するため，本研究では，研究の目的，方法，同意および拒否の自由，同意撤回の自由，個人情報保護等を含めた情報につき，口頭および文書で説明し，対象者から文書により同意を得る。

研究対象者が未成年の場合，および研究対象者が死亡例でその生前における明示的な意思に反していない場合等の際には，代諾者等からインフォームドコンセントを得る。また，拒否・撤回の申し出がある時には，研究者等は本人からの申し出と同じく扱う。研究対象者が 16 歳以上の場合には，代諾者とともに，研究対象者本人からのインフォームドコンセントを得る。16 歳未満の研究対象者が 16 歳になった時には，本人からインフォームドコンセントを得る。16 歳以前でも，直接本人への説明の希望があればインフォームドコンセントを行う。

②個人情報の保護に関する措置

個人情報を保護するために，登録を行う施設ごとに個人情報管理者を設置し，登録対象者に新たに付された符号（コード）と対象者氏名との対応表を管理する連結可能匿名化を行う。

III. 結　果

オンライン上に各施設と登録事務局のページを作成し，参加施設は自施設のデータを入力，閲覧可能で，事務局は全施設の登録状況を確認できる。

1. 登録項目

全 436 項目を**表 2** に示す。これは LITRE-J の登録項目をベースに，小児特有の項目や成長発達の評価に必要であると考えられる項目を追加したものである。

1）原疾患に関する追加項目

「胆汁うっ滞性疾患」の選択肢に，「Byler」，「PFIC 3」，「PFIC その他」，「先天性肝線維症」を追加した。「代謝性疾患」の選択肢には，成人ではまれな「ASS 欠損症」を追加した。

2）成長・発達に関する追加項目

IQ，DQ，身長 SD スコア，体重 SD スコア，骨密度，骨年齢，TSH，Free-T 4，LH，FSH，IGF-1，testosterone，estradiol，プロテイン C，プロテイン S，ヒアルロン酸，血清鉄，プレアルブミン，レチノール結合蛋白，トランスフェリン，総コレステロール，月経の有無，月経開始日等を，小児の成長・発達を評価するための指標として追加した。

2. オンライン登録システムの概要

本システムは，筑波大学次世代医療研究開発・教育統合センター（以下，CREIL データセンター）と共同で，FileMaker Server Advanced を使用して構築された。データの精度の保持と品質管理を考慮して，自動計算，自動表示，入力欄の空欄対策などの措置を講じた。

1）自動計算、自動表示

本登録の項目数は 400 を超え，必須項目も多いため，入力者・データ管理者の負担軽減のために，可能な限り自動表示や自動計算を組み込んだ。**表 2** の項目名の横に「@」で示したが，施設情報や一度入力したイニシャル，生年月日，性別，移植時年齢等は自動表示とし，年齢，日数，SD 等は自動計算とした。身長・体重の SD 自動算出には，2000 年度版『標準身長・体重表』[7]を用いた。PS や門脈血栓の Grade はカーソルを項目上に持っていくと説明書きがポップアップされるようにした。

2）入力欄の空欄対策

入力欄が空欄の際，回答が選択肢の場合には「選択忘れ」，「選択肢以外の答えのため答えることができなかった」，「不明」のいずれか，検査値等の数値入力の場合は「入力忘れ」，「不検」，もしくは「不明」のいずれかの場合が考えられる。いずれにしても，登録事務局からの問い合わせが必要となる。このような事態を予め回避するためにも，「不明」の場合は「-（ハイフン）」を入力するなどの記述を添え，空欄では入力が完了しないようにした。

3）リアルタイム集計の設定

登録総数，レシピエント性別，血液型適合度，原疾患分類，移植時年齢，施設別登録数については，症例登録画面にて送信ボタンが押された時点で登録完了となり，リアルタイム集計が行われる（初回移植症例のみ）。単年の集計だけでなく，累積集計も可能である。リアルタイム集計を設定したのは，最新の自施設のレシピエントの基本情報や，他施設の最新登録症例数を，いつでも把握可能にするためである。自ら集計す

表2　小児オンライン登録システムの登録項目

			項目
症例登録	グラフト	基本情報	施設名◎
			診療科◎
			TEL番号◎
			FAX番号◎
			移植回数
			生体肝/脳死肝の別
			脳死肝分割
			移植肝の形状
			移植肝重量
			移植肝/標準肝容積
			移植日
			レシピエントとの関係
			ドミノ移植
	レシピエント	基本情報	イニシャル
			性別
			生年月日
			移植時年齢◎
			入院日
			移植回数
			ABO血液型
			ABO血液型適合度◎
			Rh血液型
			HLA Type
		原疾患	原疾患（初回移植の場合）
			胆汁うっ滞性疾患
			代謝性疾患
			急性肝不全
			血管性病変
			肝細胞腫瘍（肝腫瘍）
			腫瘍性疾患
			その他の疾患
			移植適応（再移植以降の場合）
			Primary non function
			慢性拒絶反応
			肝炎の再発
			血管系合併症
			その他
	ドナー	基本情報	イニシャル
			性別
			生年月日
			移植時年齢◎
			入院日
			ABO血液型
			Rh血液型
			HLA Type
初回調査	レシピエント	移植前	イニシャル◎
			生年月日◎
			性別◎
			移植時年齢◎
			入院
			身長
			身長計測日
			身長SDスコア◎
			体重
			体重計測日
			体重SDスコア◎
			発達障害
			IQ
			DQ
			知能検査実施日
		移植直前	腹水
			脳症
			消化管出血
			肝肺症候群
			透析治療
			肝腎症候群
			呼吸管理
			Quality of Life
			PS
		移植直前の検査値	血小板
			AST
			ALT
			γGTP
			ChE
			T.Bil
			D.Bil
			Alb
			Creatinine
			PT
			INR
			Na
			骨密度
			骨年齢
			TSH
			Free-T4
			LH
			FSH
			IGF-1
			testosterone
			estradiol
			プロテインC
			プロテインS
			ヒアルロン酸
			胆汁酸
			プレアルブミン
			トランスフェリン
			レチノール結合蛋白
			総コレステロール
		移植直前の検査値	HBsAg
			HBsAb
			HBeAg
			HBeAb
			HBcAb
			HBV-DNA
			HCVAb
			HCV-RNA
			HIV
			ATLA
			CMV（IgG）
			CMV（IgM）
			EBV-VCA（IgG）
			EBV-VCA（IgM）
			EBV-EA（IgG）
			EBV-EA（IgM）
			EBV-EBNA
			EBV-DNA
			MELD/PELDスコア
			CTPスコア
初回調査	レシピエント	移植手術	肝動脈（再建）の移植の状況
			胆道系（再建）の同時移植
			手術時間
			出血量
			手術法
			分割（split）移植
			移植肝重量◎
			GV/SLV◎
			GRWR◎
			門脈血栓
			特殊な門脈再建
			肝動脈再建
			出血量
			輸血量
			輸血製剤
			移植手術後の状態
		移植後の検査値	退院前後（2週間）
			検査日
			血小板
			AST
			ALT
			γGTP
			ChE
			T.Bil
			D.Bil
			Alb
			Creatinine
			PT
			INR
			Na
		免疫抑制	導入期（移植後1カ月以内）
			ステロイド
			カルシニューリン阻害剤
			mTOR阻害剤
			核酸合成阻害剤
			抗体製剤
			その他
		ABO不適合	抗体価（IgG）
			抗体除去療法
		合併症等	退院日
			術後入院日数◎
			合併症
			複合併症（有）の場合のみ
			出血
			Primary non-function
			免疫学的合併症
			血管系合併症
			一血栓症
			一狭窄
			胆道合併症
			感染症
			免疫抑制剤に伴う合併症
			その他
			合併症による再手術
		肝細胞癌	治療歴
			移植直前の状態
			viableな肝細胞癌
			腫瘍の個数
			腫瘍の最大径
			移植直前の腫瘍マーカー
			AFP
			PIVKA-II
	ドナー	提供時	イニシャル◎
			生年月日◎
			性別◎
			移植時年齢◎
			入院
			身長
			体重
			BMI◎
		提供直前（脳死ドナー）	死因
			入院中の心停止
			心停止前の処置
			喫煙歴
			アルコール歴
			糖尿病
			脂肪肝
			入院中のピーク値
			AST
			ALT
			T.Bil
			(Na)ピーク値
			Na
			HBsAg
			HBsAb
			HBeAg
			HBeAb
			HBcAb
			HCVAb
			HIV
			ATLA
			CMV（IgG）
			細菌培養
			血液型
		生体ドナー	既往歴
			脂肪肝
			併存症
			術前的問題
		提供直前の検査値	AST
			ALT
			T.Bil
			D.Bil
			Alb
			Creatinine
			PT
			INR
			APTT
			HBsAg
			HBsAb
			HBeAg
			HBeAb
			HBcAb
			HCVAb
			ATLA
			CMV（IgG）
			CMV（IgM）
			EBV-VCA（IgG）
			EBV-VCA（IgM）
			EBV-EA（IgG）
			EBV-EA（IgM）
			EBV-EBNA
			EBV-DNA
初回調査	ドナー	摘出手術直前（生体ドナー）	摘出手術日
			肝の摘出部位
			手術担当チーム
			摘出術式
		摘出手術（生体ドナー）	手術時間
			摘出肝
			摘出肝重量
			残肝率
			肝の摘出部位：切離
			出血量
			輸血
			摘出手術後の状態
		摘出手術後の検査値	摘出手術後（1週間）
			AST
			ALT
			T.Bil
			D.Bil
			Alb
			Creatinine
			PT
			INR
			APTT
			血清T.Bil最高値
			T.Bil 5.0以上の持続期間
			T.Bil 2.0以上の持続期間
			血清AST最高値
			血清ALT最高値
			PT最低値
			血中アンモニア最高値
		合併症等	退院日
			術後入院日数◎
			合併症
			複合併症（有）の場合のみ
			出血
			心血管系
			呼吸器系
			胆道系
			その他の消化器系
			手術部位感染（SSI）
			それ以外の感染症
			その他
			合併症による再手術
		保存	初期灌流
			保存液
			リンス液
			冷阻血時間
			温阻血時間
			全阻血時間◎
追跡調査	レシピエント	現状①	現状
			最終生存確認日または死亡日
			死亡の場合のみ
			主な死因
			出血
			Primary non-function
			拒絶反応
			血管系合併症
			一血栓症
			一狭窄
			胆道合併症
			感染症
			原疾患の再発
			PTLD
			悪性腫瘍
			免疫抑制剤に伴う合併症
			その他
		現状②	生存の場合のみ
			Quality of Life
			PS
			向精神薬の継続的使用
			就学の有無
			最終通院日
			入院回数
			服薬回数
		免疫抑制（現在）	ステロイド
			カルシニューリン阻害剤
			mTOR阻害剤
			核酸合成阻害剤
			その他
		追跡時の検査値①	身長
			身長計測日
			身長SDスコア◎
			体重
			体重計測日
			体重SDスコア◎
			発達障害
			IQ
			DQ
			血小板
			AST
			ALT
			γGTP
			ChE
			T.Bil
			D.Bil
			Alb
			PT
			Creatinine
			INR
			Na
			骨密度
			骨年齢
			TSH
			Free-T4
			LH
			FSH
			IGF-1
			testosterone
			estradiol
			プロテインC
			プロテインS
			胆汁酸
			ヒアルロン酸
			プレアルブミン
			レチノール結合蛋白
			トランスフェリン
			総コレステロール
追跡調査	レシピエント	追跡時の検査値②	HBsAg
			HBsAb
			HBeAg
			HBeAb
			HBcAb
			HBV-DNA
			HCVAb
			HCV-RNA
			HIV
			ATLA
			CMV（IgG）
			CMV（IgM）
			EBV-VCA（IgG）
			EBV-VCA（IgM）
			EBV-EA（IgG）
			EBV-EA（IgM）
			EBV-EBNA
			EBV-DNA
			原肝細胞癌の場合のみ
			AFP
			PIVKA-II
		前回調査以降の合併症等	前回調査以降のもののみ
			出血
			Primary non-function
			免疫学的合併症
			血管系合併症
			一血栓症
			一狭窄
			胆道合併症
			感染症
			原疾患の再発
			PTLD
			悪性腫瘍
			診断年月（悪性腫瘍）
			免疫抑制剤に伴う合併症
			その他
			合併症による再手術
			合併症による再入院
	ドナー	現状①	現状
			最終生存確認日または死亡日
			死亡の場合のみ
			死因
		現状②	生存の場合のみ
			術前状態への完全復帰
			否の場合、その理由
			就労者の場合のみ
			休職の場合：その理由
			休職期間
			術前生活への復帰
			就学/就労の場合のみ
			経済状況：術前に比べて
			Quality of Life
			PS
			向精神薬の継続的使用
			入院回数
			通院回数
		追跡時の検査値	検査日
			AST
			ALT
			T.Bil
			D.Bil
			Alb
			Creatinine
			PT
			INR
			APTT
		合併症等	疾患の有無
			心血管系
			呼吸器系
			胆道系
			その他の消化器系
			手術部位感染（SSI）
			それ以外の感染症
			その他
			合併症による再手術
			合併症による再入院
転院届	レシピエント	転院先	イニシャル
			性別
			生年月日（西暦）
			転院確認日
			施設名
			主治医名
			郵便番号
			住所
			TEL番号
			FAX番号
	ドナー	転院先	イニシャル
			性別
			生年月日（西暦）
			転院確認日
			施設名
			主治医名
			郵便番号
			住所
			TEL番号
			FAX番号

◎：自動表示または自動計算