were significantly associated with HBV vaccination status. The importance of education during the perinatal period has been confirmed in other studies in which pregnant women who received prenatal educational sessions on immunization demonstrated an improved knowledge regarding various aspects of vaccination (Zuniga de Nuncio et al., 2003). Another study showed that incorporating immunization education into routine prenatal care increased maternal knowledge regarding infant vaccines and reduced delayed immunization (Navar et al., 2007). Additional studies are necessary to clarify the improvement in knowledge regarding immunization as it relates to improved vaccination rates.

Several factors need to be considered when analyzing the data in this study. First, the sample size was small, which decreased the likelihood of detecting potential differences. Second, the introduction of temporary budget during this study period increased the vaccination rates of the Hib vaccine and PCV7, which can confound the study results. Additionally, a television commercial for the PCV7 was aired during the study period in Japan, which emphasized the necessity of the PCV7 and may have reduced the impact of the intervention. Third, the study participants were well-educated and had a high socioeconomic status and had the ability to obtain information related to immunization on their own through a variety of resources, which might have affected the outcome of our intervention. Although we compared the resources about infant immunization, no difference was identified. Furthermore, since not only educational background and remuneration differs, but also immunization policy and practice vary between countries, the results of this study may not be generalizable. Fourth, attendance at antenatal classes was voluntary, and enrollment in the study was solely at the discretion of the participants. In addition, a few participants from the control group withdrew from the study. As such, it is possible that pregnant women who were uninterested in immunization, or less active in child health, did not enroll in the study. This group may be a more important target population for this study. Fifth, we need to consider the self-report bias (Adams et al., 1999), which can result in an inflation of behavioral intention. Sixth, during this study period, the media reported on an oral polio vaccine that can cause vaccine-associated polio paralysis, which led to increase the attention of parents toward the safety rather than the importance of immunization, which may have played a role indifferences in the attitudes and beliefs toward immunization. Lastly, the follow-up period of the study was only three months. A longer observation period may highlight differences in the immunization completion rates.

## Conclusions

Pre- and postnatal immunization education in pregnant women in Japan improved the immunization rates of infants and increased the intent to vaccinate infants and the maternal knowledge regarding immunization. Future research is necessary to investigate the optimal timing and content of immunization education and develop a standard education program with related materials to encourage the immunization of infants in Japan.

**Conflict of interest statement**

All authors have no conflict of interest.

## Acknowledgments

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## Appendix A

CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| Title and abstract | | | |
| | 1a | Identification as a randomised trial in the title | 1 |
| | 1b | Structured summary of trial design, menthods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 3 |
| Introduction | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 4 |
| | 2b | Specific objectives or hypotheses | 5 |
| Methods | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 6 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | none |
| Participants | 4a | Eligibility criteria for particaipants | 6 |
| | 4b | Settings and locations where the data were collected | 6 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 7 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 8-9 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | none |
| Sample size | 7a | How sample size was determined | 7 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | none |
| *Randomisation:* | | | |
| Sequence | 8a | Method used to generate the random allocation sequence | 6 |
| Generation | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | 6 |
| Allocation | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 6 |
| Concealment Mechanism | | | |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 6 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how | 7 |
| | 11b | If relevant, description of the similarity of interventions | none |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 9 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | none |
| Results | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome | 11 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | 11 |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | 6 |
| | 14b | Why the trial ended or was stopped | none |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | Table 1 |
| Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | 11, Figure 1 |
| Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 11-13 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | none |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | none |
| Harms | 19 | All importand harms of uninteded effects in each group (for specific quidance see CONSORT for harms) | none |
| Discussion | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and if relevent, multiplicity of analyses | 16-17 |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | 16 |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 14-16 |
| Other information | | | |
| Registration | 23 | Registration number and name of trial registry | 3 |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | none |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 18 |

CONSORT 2010 checklist Page 1

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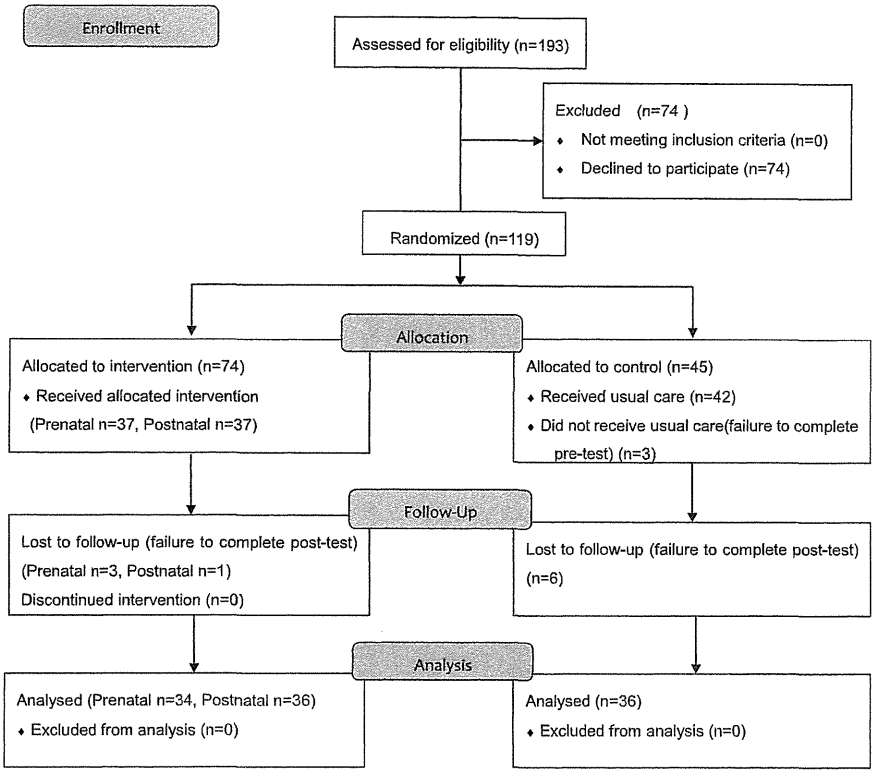


## Appendix B. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ypmed.2013.03.003.

## References

Adams, A.S., Soumerai, S.B., Lomas, J., Ross-Degnan, D., 1999. Evidence of self-report bias in assessing adherence to guidelines. Int. J. Qual. Health Care 11, 187–192.
Becker, M.H., 1974. The Health Belief Model and Personal Health Behavior. Slack, Thorofare, N. J.
Bjornson, G.L., Scheifele, D.W., Gold, R., 1997. Assessment of parent education methods for infant immunization. Can. J. Public Health 88, 405–408.
Center for Disease Control and Prevention (CDC), 1995. Guidelines for Assessing Vaccination Levels of the 2-year-old Population in a Clinic Setting. US Department of Health and Human Services. Public Health Service.
Daley, M.F., Crane, L.A., Chandramouli, V., et al., 2007. Misperceptions about influenza vaccination among parents of healthy young children. Clin. Pediatr. (Phila) 46, 408–417.
Davis, T.C., Fredrickson, D.D., Bocchini, C., et al., 2002. Improving vaccine risk/benefit communication with an immunization education package: a pilot study. Ambul. Pediatr. 2, 193–200.
Davis, T.C., Fredrickson, D.D., Kennen, E.M., et al., 2004. Childhood vaccine risk/benefit communication among public health clinics: a time-motion study. Public Health Nurs. 21, 228–236.
Diekema, D.S., 2012. Improving childhood vaccination rates. N. Engl. J. Med. 366, 391–393.
El-Mnohandes, A.A., Katz, K.S., El-Khorazaty, M.N., et al., 2003. The effect of a parenting education program on the use of preventive pediatric health care services among low-income, minority mothers: a randomized, controlled study. Pediatrics 111, 1324–1332.
Fielding, J.E., Cumberland, W.G., Pettitt, L., 1994. Immunization status of children of employees in a large corporation. JAMA 271, 525–530.
Glanz, K., Rimer, B.K., Viswanath, K., 2008. Health Behavior and Health Education: Theory, Research, and Practice. Jossey-Bass, San Francisco.
Gust, D.A., Kennedy, A., Shui, I., Smith, P.J., Nowak, G., Pickering, L.K., 2005. Parent attitudes toward immunizations and healthcare providers the role of information. Am. J. Prev. Med. 29, 105–112.
Gust, D.A., Kennedy, A., Wolfe, S., Sheedy, K., Nguyen, C., Campbell, S., 2008. Developing tailored immunization materials for concerned mothers. Health Educ. Res. 23, 499–511.
Japan Pediatric Society, 2012. Child immunization schedule. Available at www.jpeds.or.jp/saisin/saisin_110427.pdf (Accessed January 8, 2013).
Kennedy, A.M., Gust, D.A., 2005. Parental vaccine beliefs and child's school type. J. Sch. Health 75, 276–280.
Komatsu, H., Inui, A., Sogo, T., Hiejima, E., Kudo, N., Fujisawa, T., 2009. Source of transmission in children with chronic hepatitis B infection after the implementation of a strategy for prevention in those at high risk. Hepatol. Res. 39, 569–576.
Ministry of Health, Labor and Welfare, Japan, 2012. Immunization law. Available at http://www.mhlw.go.jp/topics/bcg/hourei/1.html (Accessed January 8, 2013).
Montano, D.E., 1986. Predicting and understanding influenza vaccination behavior. Alternatives to the health belief model. Med. Care 24, 438–453.
Murashige, N., Matsumura, T., Masahiro, K., 2011. Disseminating Japan's immunisation policy to the world. Lancet 377, 299.
Navar, A.M., Halsey, N.A., Carter, T.C., Montgomery, M.P., Salmon, D.A., 2007. Prenatal immunization education the pediatric prenatal visit and routine obstetric care. Am. J. Prev. Med. 33, 211–213.
Painter, J.E., Sales, J.M., Pazol, K., et al., 2010. Psychosocial correlates of intention to receive an influenza vaccination among rural adolescents. Health Educ. Res. 25, 853–864.
Saitoh, A., Okabe, N., 2012. Current issues with the immunization program in Japan: can we fill the "vaccine gap"? Vaccine 30, 4752–4756.
Vannice, K.S., Salmon, D.A., Shui, I., et al., 2011. Attitudes and beliefs of parents concerned about vaccines: impact of timing of immunization information. Pediatrics 127 (Suppl. 1), S120–S126.
Vora, S., Verber, L., Potts, S., Dozier, T., Daum, R.S., 2009. Effect of a novel birth intervention and reminder-recall on on-time immunization compliance in high-risk children. Hum. Vaccin. 5, 395–402.
Waterman, S.H., Hill, L.L., Robyn, B., et al., 1996. A model immunization demonstration for preschoolers in an inner-city barrio, San Diego, California, 1992–1994. Am. J. Prev. Med. 12, 8–13.
Zuniga De Nuncio, M.L., Nader, P.R., Sawyer, M.H., De Guire, M., Prislin, R., Elder, J.P., 2003. A prenatal intervention study to improve timeliness of immunization initiation in Latino infants. J. Community Health 28, 151–165.

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ELSEVIER

# Epstein-Barr Virus Infection after Pediatric Living-Related Liver Transplantation—Management and Risk Factors

T. Shigeta, K. Imadome, S. Sakamoto, A. Fukuda, T. Kakiuchi, N. Matsuno, H. Tanaka, A. Nakazawa, and M. Kasahara

ABSTRACT

Introduction. Posttransplant lymphoproliferative disorder (PTLD) is one of the severe complications after pediatric liver transplantation. Epstein-Barr virus (EBV) infection is a major risk factor developing PTLD. This study evaluates the risk factors, incidence, and clinical presentation of EBV infection at our institute.

Patients and Methods. This study examines 81 children who underwent living-related liver transplantation (LRLT) from November 2005 to December 2009. The immunosuppression protocol consisted of tacrolimus and low-dose steroids, which were withdrawn by 3 months after LRLT. Additional immunosuppression was indicated for the selected cases because of recurrent rejection or renal insufficiency. Fifteen ABO blood type incompatible LRLTs were enrolled into this study. EBV was periodically monitored by the use of a real-time quantitative polymerase chain reaction (cut-off value, $>10^2$ copies/$\mu$g DNA). The median follow-up period was 637 days (range, 85 to 1548 days). These patients were divided into two groups: EBV infection versus EBV noninfection, for analysis of risk factors by univariate analysis.

Results. The incidence of EBV infection was 50.6% (n = 41) with the mean onset of 276 ± 279 postoperative days (range, 7 to 1229 days). Nine cases (22.5%) presented clinical symptoms related to EBV infection, consisting of adenoid hypetrophy (n = 5), Evans's syndroms (n = 2), hemophagocytic syndrome (n = 1), and erythema nodosum (n = 1). There was no case of PTLD. The combination of a preoperative EBV seropositive donor and an EBV seronegative recipient was a high risk factor for postoperative EBV infection among the recipients (56.1% versus 26.8%, $P < .05$). The mean age at operation among the EBV infection group was younger than that of the EBV noninfection group (22 ± 30 months versus 62 ± 68 months; $P < .05$). The incidence of acute rejection episodes and cytomegalovirus infections; ABO blood type incompatible LRLT, and the length of steroid treatment and the additional immunosuppression were not significantly different between the two groups.

Conclusion. There were various clinical presentations related to EBV infection; however, none of our patients developed PTLD. Careful monitoring of EBV infection especially for cases with donor seropositivity is important to prevent disease progression.

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0041-1345/10/$–see front matter
doi:10.1016/j.transproceed.2010.09.134

**Table 1. Patient Demographics and the Analysis of the Risk Factors for EBV Infection**

| | EBV Positive | EBV Negative | *P* |
|---|---|---|---|
| n | 41 (50.6%) | 40 (49.4%) | NS |
| Mean age ± SD (months) | 20 ± 27 | 62 ± 68 | 0.001 |
| Median follow-up (postoperative days) | 618 (92–1548) | 693 (120–1544) | NS |
| Onset of EBV (postoperative days) | 276 ± 279 | — | |
| EBV serology (D-sero+ve R-sero−ve) | 24 (56.1%) | 11 (26.8%) | 0.009 |
| ABO-incompatible | 6 (14.6%) | 9 (22.5%) | NS |
| Cytomegalovirus | 12 (14.6%) | 19 (47.5%) | NS |
| Acute rejection | 13 (31.7%) | 15 (37.5%) | NS |
| Mycophenolate mofetil | 8 (19.5%) | 5 (12.5%) | NS |
| Steroid period (days; mean ± SD) | 217 ± 267 | 186 ± 15 | NS |

POSTTRANSPLANT lymphoproliferative disorder (PTLD) is characterized by uncontrolled B lymphocyte proliferation caused by the Epstein-Barr virus (EBV).[1] The most recent study reported the mortality rate of PTLD to be approximately 20%.[2] Risk factors for PTLD are EBV infection, high EBV load, and a high level of immunosuppression.[1,3] There is no effective drug to treat EBV infection. Therefore, the treatment strategy for PTLD is to stop the proliferation of EBV-infected cells. This study evaluates the incidence, clinical presentation, and management of as well as risk factors for EBV infection in our hospital.

## METHODS

This study examined 81 children who underwent living-related liver transplantation (LRLT) from November 2005 to December 2009. They were followed periodically after more than 3 months. Their ages ranged from 1 month to 21 years old. The median follow-up period was 637 days (range, 85 to 1548 days). The immunosuppression protocol consisted of tacrolimus and low-dose steroids, which were withdrawn by 3 months after LRLT. Mycophenolate mofetil (MMF) was added for selected cases with repeated acute rejection episodes, or renal dysfunction due to the calcineurin inhibitor.

EBV was monitored by the use of a real-time quantitative polymerase chain reaction (PCR) method. The cut-off value was more than $10^2$ copies/$\mu$g DNA. We performed the test once per week for the first 2 months after LRLT, continuing follow-up every 1 to 3 months in outpatient clinics. When persistent fever or diarrhea were present, we examined subjects by EBV-PCR.

The strategy to manage EBV infection consisted of prevention, early diagnosis, and early treatment. We controlled immunosuppression by monitoring the EBV-PCR values. If symptoms lead to suspicion of EBV infection or there were high values of EBV-PCR (more than $10^2$ copies/$\mu$g DNA), immunosuppression was withdrawn or held as long as possible with careful monitoring of liver function. The target trough level of the tacrolimus was decreased by 75%. If the values of EBV-PCR were persistently high (more than $10^4$ copies/$\mu$g DNA), or the symptoms were suspected to be due to PTLD, radiologic examinations and histological examinations of the suspected sites were performed to diagnose the disorder.

The number, mean age, median follow-up, and steroid periods were compared using Mann-Whitney tests. The values of risk factors were compared using chi-squared tests. A *P* values of less than .05 was considered statistically significant. All data analysis was performed using SPSS PASW Statics version 18.0 software (SPSS, Inc, Chicago, IL).

## RESULTS

The incidence of EBV infection, including asymptomatic patients, was 50.6% (n = 41). The incidence of EBV-related disease was 22.0% (n = 9).

### Risk Factors

Patients were divided into two groups: an EBV infection group and an EBV noninfection group (Table 1). The mean age at LRLT among the EBV infection group was younger than that of the EBV noninfection group. The mean onset was 276 ± 279 days after LRLT. The combination of a preoperative EBV seropositive donor and an EBV seronegative recipient was a significant risk factor for postoperative EBV infection in the recipient. Cytomegalovirus infection, acute rejection episode, ABO compatibility, the use of MMF, and the period of steroid use were not significantly different between the groups. The reduction rate of the through level of the tacrolimus was 26.6% (6.4 ng/mL to 4.7 ng/mL; Fig 1).

### EBV-Related Disease

The symptoms of EBV disease were adenoid hyperplasia (n = 5), Evans's syndrome (n = 2), virus associated

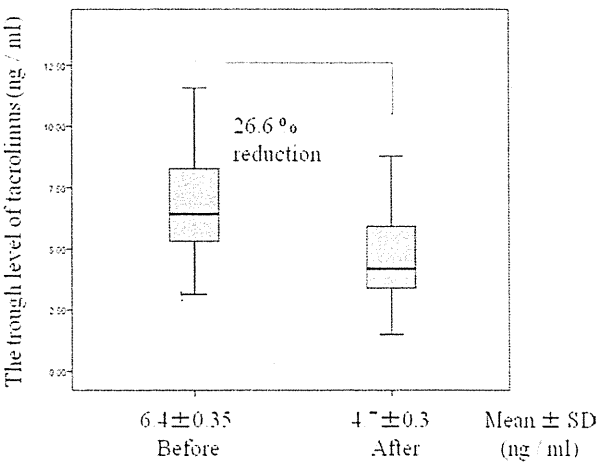


Fig 1. The change of the trough level of the tacrolimus before and after EBV infection. The reduction rate was 26.6%.

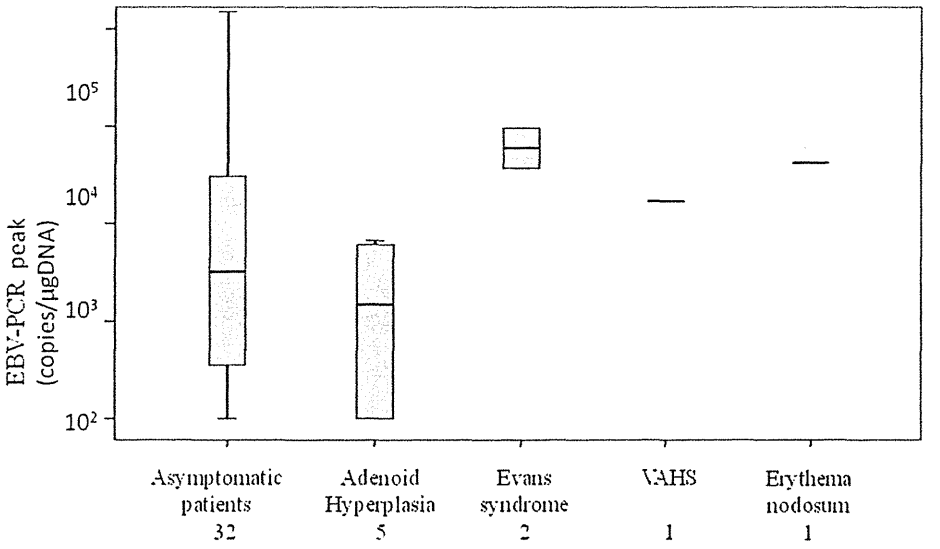


Fig 2. EBV-PCR peak values of the EBV-related disease.

hemophagocytic syndrome; (n = 1), and erythema nodosum (n = 1). In all cases, tacrolimus was withdrawn or ceased, leading to improvement in the symptoms Adenoidectomy was performed in all five cases. The severity of symptoms related to EBV diseases correlated with EBV load (Fig 2). There was no case of PTLD among our series.

## DISCUSSION

Herein we have reported the management of and risk factors for EBV infection among pediatric LRLT. In transplanted patients, EBV infection and the combination of EBV serology (donor positive and recipient negative) have been reported to be risk factors for developing PTLD.[1–4] As there is no evidence for antiviral therapy in the treatment of EBV infection, reduction of immunosuppression is the most effective way to prevent development of PTLD.[5,6] Therefore, the key to EBV infection management is frequent EBV-PCR monitoring and reduction of immunosuppression when the value is above the cut-off.[7,8] In our hospital, the target through level of tacrolimus was reduced by 25%. There was no case of PTLD, and the frequency of acute rejection episodes was not significantly increased between the two groups.

We previously reported two cases of EBV-related disease, associated with Evans's syndrome and erythema nodosum.[9,10] Both cases showed high EBV-PCR levels. The patient who had Evans's syndrome was treated by reduction of tacrolimus and immunoglobulin, resulting in a decreased EBV-PCR level with improvement in symptoms. In the patient who had erythema nodosum, EBV-encoded small RNA was detected in a skin biopsy specimen. This patient was also treated by reduction of tacrolimus, although the level of EBV-PCR decreased immediately, the skin lesion did not resolve completely. After switching from tacrolimus to cyclosporine, the skin lesion gradually disappeared. By monitoring EBV-PCR level carefully, we believe that we can prevent the development of PTLD. Early diagnosis and treatment are the most important factors to block development of PTLD among EBV-infected recipients.

In conclusion, there were various clinical presentations related to EBV infection; however, there was no development of PTLD among our series despite the short follow-up period. Careful monitoring of EBV infection with quantitative PCR method seems to be important to prevent disease progression.

## REFERENCES

1. Paya CV, Fung JJ, Nalesnik MA, et al: Epstein-Barr virus–induced posttransplant lymphoproliferative disorders. ASTS/ASTP EBV-PTLD Task Force and The Mayo Clinic Organized International Consensus Development Meeting. Transplantation 68:1517, 1999

2. Fernandez MC, Bes D, De Davila M, et al: Post-transplant lymphoproliferative disorder after pediatric liver transplantation: Characteristics and outcome. Pediatr Transplantation 13:307, 2009

3. Smets F, Sokal EM: Epstein-Barr virus related lymphoproliferation in children after liver transplant: role of immunity, diagnosis, and management. Pediatr Transplantation 6:280, 2002

4. Busuttil RW, Klintmalm GB. Transplantation of the Liver, 2nd Ed. Philadelphia: Elsevier Saunders, 2005; p 1247

5. Hurwitz M, Desai DM, Cox KL, et al: Complete immunosuppressive withdrawal as a uniform approach to posttransplant lymphoproliferative disease in pediatric liver transplantation. Pediatr Transplantation 8:267, 2004

6. Kerkar N, Morotti RA, Madan RP, et al: The changing face of post-transplant lymphoproliferative disease in the era of molecular EBV monitoring. Pediatr Transplantation 14:504, 2010

7. Stevens SJ, Verschuuren EA, Pronk I, et al: Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. Blood 97:1165, 2001

8. Lee TC, Savoldo B, Rooney CM, et al: Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. Am J Transplant 5:2222, 2005

9. Yokoyama S, Kasahara M, Fukuda A, et al: Evans syndrome after successful living-donor liver transplantation for neonatal giant cell hepatitis. Transplantation 84:798, 2007

10. Yokoyama S, Kasahara M, Fukuda A, et al: Epstein-Barr virus–associated erythema nodosum after living-donor liver transplantation: A case report. Liver Transpl 15:446, 2009

# 肝移植におけるサイトメガロウイルス感染症対策

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特集　臓器移植後のサイトメガロウイルス感染症対策

*Prevention and management of cytomegalovirus infection after liver transplantation*

肝移植後患者へのCMV感染症対策としてはprophylaxisとpreemptive therapyに大別されるが，一般的にはprophylaxisが主流である．当院での生体肝移植96例におけるpreemptive therapyを用いたCMV感染症対策について検討した．術後CMVアンチゲネミア陽性率は38.3%であった．発症は6例(6.4%)であり，その内訳はCMV syndromeが3例，肝炎・腸炎・肺炎がおのおの1例ずつであった．CMV陽性細胞個数は発症群が非発症群と比較して有意に多かった．術前CMVのserologic statusの組み合わせがD+/R−であること，原疾患が劇症肝炎であること，術前ICU管理であったことが，有意なCMV感染症関連因子であった．ほとんどの症例においてガンシクロビル静脈内投与にて治癒しえたが，一部の症例においてはガンシクロビル治療抵抗性を示しフォスカビルを使用した．

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***key words*** : prophylaxis, preemptive therapy, CMV antigenemia, ganciclovir, valganciclovir

本邦においては生体ドナーからの臓器提供を主として，肝移植は2008年末までの集計で5,250例に実施され，その累積生存率は術後5年で約75%，10年で約70%と良好な成績である[1]．その背景においては，サイトメガロウイルス(cytomegalovirus：CMV)感染症などの臓器移植後ウイルス感染症への対策が目覚ましく進歩したことが強く影響している．

近年，CMV感染症による直接的影響(direct effect)のみならず，間接的影響(indirect effect)が注目されており，拒絶反応やその他の日和見感染症(Epstein-Barr virus：EBV，その他)などとの関連が報告されている[2]．また，抗ウイルス薬においては，バルガンシクロビル(valganciclovir)が本邦においても2009年5月より臓器移植におけるCMV感染症に対して効能追加され，臓器移植後のCMV感染症対策が変遷しつつある．

本稿では，自験例を提示しながら，肝移植後のCMV感染症対策の現状について解説する．

## CMV感染症対策の必要性

肝移植において術後に有症状を呈するCMV感染症(CMV disease)の発症率は，術後の対策がなされなかった場合には約25%と報告されている[3]．CMV感染症の予後への悪影響は，CMV感染症の直接的影響により臓器浸潤性に炎症を引き起こす場合(tissue invasive CMV disease)のみならず，間接的影響により真菌感染症などの日和見感染症の危険性が高まることや，急性拒絶反応や慢性拒絶反応が惹起されうる可能性があることが関係している．したがって，肝移植後にCMV感染症を抑制することは大変重要である．

術後CMV感染症を引き起こす危険因子としては，CMV既感染ドナーよりCMV未感染レシピエントに臓器が提供される組み合わせ(D+/R−)の場合と，抗リンパ球抗体(anti-lymphocyte anti-

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body：ALA)などの免疫抑制薬を術直後の免疫誘導療法や急性拒絶反応の治療として使用される場合である．ドナーおよびレシピエントの術前IgG抗体によるserologic statusにより術後CMV感染症の発症率を分類すると，D+/R−の組み合わせでは44〜65%と高率となる[4]．

一方，京都大学における生体肝移植症例において術後CMV感染症の危険因子を解析した報告によると，D+/R−の組み合わせに加えて，原疾患が劇症肝炎であることと，ABO血液型不適合症例が新たに危険因子として判明した[5]．劇症肝炎にて肝移植となる症例においては，周術期に血漿交換や持続的血液濾過透析などの体外循環を施行していることや，肝腎症候群などを原因とする腎機能低下を呈していることが多く，また術後にステロイド不応性拒絶反応を発症することが多く他疾患と比較して免疫抑制過多となるために，術後CMV感染症に難渋することが多い．ABO血液型不適合症例においては，周術期の血漿交換やミコフェノール酸モフェチル(MMF)などの免疫抑制薬が追加され免疫抑制過多となることが関与しているものと考えられる．

CMVによる間接的影響として，急性拒絶反応や慢性拒絶反応，C型肝炎再発，真菌感染症などの日和見感染症，グラフト機能不全との関連性が報告されている[2]．これ以外にも，京都大学における生体肝移植症例での検討にて，CMV感染症と術後胆管合併症との関連性[6]や，原発性硬化性胆管炎(primary scrclosing cholangitis：PSC)症例における術後早期PSC再発との関連性[7]が報告されている．

## CMV感染症対策の現状

肝移植後患者へのCMV感染症対策は，予防(prevention)と治療(treatment)にわかれる．

### 1．予防(prevention)

CMV感染症の予防としては，術後CMV感染症のハイリスク群に対して，術直後より抗ウイルス薬を3〜6カ月間予防的に投与する方法(prophylaxis)と，術直後より定期的にCMV感染症に対するモニタリング検査を施行しながら，CMV感染症と診断された場合に抗ウイルス薬治療を行う方法(preemptive therapy)がある．

現行の米国移植学会によるCMV感染症対策のガイドライン[8]では，D+/R−の肝移植患者とALAやOKT3療法を行った患者には，3カ月間のガンシクロビルの経口投与あるいはバルガンシクロビル(なお，術後のバルガンシクロビルの予防的投与には注意を要する)の経口投与，もしくは1〜3カ月間のガンシクロビルの静脈内投与を推奨しており，preemptive therapyはローリスク群に行うべきとしている．また，preemptive therapyにてキーポイントとなるモニタリング方法においては，CMVの遺伝子検査あるいはアンチゲネミア法(CMV pp65 antigenemia assay)を術後3カ月間，1週間ごとに施行することを推奨している．また，北米における肝移植後患者へのCMV感染症対策に関する調査[9]によると，多くの移植施設がバルガンシクロビルを術後3カ月間投与する方法でprophylaxisを行っており，少数の施設のみがpreemptive therapyを施行しているという結果であった．CMVのモニタリング方法においては，CMVの遺伝子検査(CMV PCR法)をアンチゲネミア法よりも用いる施設が多かった．

Prophylaxisを施行した場合にはCMV感染症の発症率を，全体としては4.8%，特にD+/R−の組み合わせでは12〜30%まで減少させうる[4]．しかし，prophylaxis終了後にCMV感染症が発症(delayed-onset CMV感染症)する可能性があり，特に免疫抑制薬の投与量が通常よりも多い場合には注意を要する[10]．Delayed-onset CMV感染症の発症を抑制すべく，prophylaxisの期間を延長することが考えられるが，5年間のprophylaxisを施行したにもかかわらずCMV感染症の発症を抑えられなかったという報告[11]があることや，抗ウイルス薬に耐性を生じる一因となるために適当ではないと考えられる．

このように，肝移植後患者へのCMV感染症対策としてprophylaxisを施行することが有効な方法として一般的であるが，D+/R−の組み合わせ

であってもpreemptive therapyを施行している施設から良好な成績が報告されていることもまた事実である．最近の報告では，バルガンシクロビルによるpreemptive therapyをD+/R−の肝移植後患者に施行し，CMV diseaseの発症なく，また拒絶反応，日和見感染症，さらに生存率(いわゆるCMV感染症による間接的影響)において，その他の組み合わせの肝移植後患者群と比較し有意差がなかった[12]．

### 2．治療(treatment)

ガンシクロビルの静脈内投与がCMV感染症の治療法の基本となる．同様に，免疫抑制薬を可能な限り減量することも重要となる．

ガンシクロビルの静脈内投与に代わり，バルガンシクロビルを治療薬として使用し同等に有効であったとする報告[13]があるが，重篤なCMV感染症は除外されているなど，解釈に注意を要する．ガンシクロビルの静脈内投与により症状が軽快した段階で，維持治療としてバルガンシクロビルに変更することが一般的である．

治療が不完全に終了した場合には，再発するケースが多いことは周知の事実であり，最短でも2週間のCMV陰性という結果を受けてから治療終了とすべきである．

## 抗ウイルス薬の最近の知見

### 1．バルガンシクロビル

バルガンシクロビルはバリン基のついたガンシクロビルのプロドラッグで，経口投与後，主に腸管壁および肝臓で速やかに加水分解され，ガンシクロビルとして作用を発現する．成人の場合にはバルガンシクロビルの1日1回，900 mgの経口投与により，5 mg/kgのガンシクロビル静脈内投与と同等の血中濃度曲線下面積(AUC)が得られる．

前述したように，米国移植学会によるCMV感染症対策のガイドライン[8]では，肝移植後患者へのバルガンシクロビルの予防的投与には注意を促している．これは固形臓器移植患者を対象としたバルガンシクロビルを用いたprophylaxisの多施設のrandomized controlled trial(RCT)にて，肝移植後患者においてCMV diseaseの発症率がガンシクロビルの静脈内投与群と比較して高かったことが根拠となっている[14]．しかし，北米のほとんどの施設において，この事実を認識しているにもかかわらず，バルガンシクロビルを引きつづき使用しているとの調査結果であった[9]．

小児肝移植患者に対するバルガンシクロビルの投与についての報告は限られている．小児肝移植においてレシピエントはCMV未感染の可能性が高く，CMV既感染ドナーからの臓器提供を受ける頻度が高い．また，静脈内投与アクセスを得ることが非常に困難な場合もあり，経口投与剤はときに望ましい．小児向けにフルーツ味のバルガンシクロビルも開発されている[15]．小児の投与量は体表面積をもとに算出するが，成人に用いる場合と同様に腎機能(クレアチニンクリアランス)も考慮することにより，より安全で有効なAUCを得ることができる[16]．

### 2．フォスカビル

ガンシクロビルの静脈内投与にても，CMVのモニタリング検査にて上昇傾向が認められる場合には，第二選択としてフォスカビルが使用される．この場合にはガンシクロビル耐性のCMV感染症が疑われるために，耐性株の遺伝子検査を施行すべきである．また腎機能障害，電解質異常，貧血などの副作用を有することもあり，フォスカビルの使用中は厳重な水分バランスの管理が必要である．

## CMV感染症のモニタリング法

CMVの遺伝子検査で代表的なものはreal-time PCR法によるDNA定量であるが，CMV diseaseの病勢を反映する指標として最も鋭敏といえる検査法である．検体として全血，白血球，血漿のほかに，気管支肺胞洗浄液や脳脊髄液でも測定可能であるが，この検査法において検体の種類を把握しておくことは重要である．特に白血球中の

DNA 量は疾患の発症に先立って増加するが，検出自体で CMV disease と診断できるほど感度は高くない．血漿中 DNA 量は CMV disease の発症と相関するが，予知については白血球中の DNA 量のほうが早い場合がある．

一方，アンチゲネミア法は CMV disease の発症・活動性と相関すること，比較的簡便であり測定時間が短いことが利点である．一方，定量検査法ではなく，実際の臨床上ではアンチゲネミア法による CMV disease 発症の cut-off 値はどれぐらいであるか，どの程度の変動を有意差ありととるのかが問題となる[17]．

## 国立成育医療センターにおける CMV 感染症対策とその検討

### 1．当院における CMV 感染症対策

当院では，ドナーおよびレシピエントの術前 IgG 抗体による serologic status の組み合わせに関係なく，preemptive therapy を基本的に施行している．当院で扱う生体肝移植は小児症例が中心であるために，EBV 感染症対策として術後 3 カ月間のアシクロビル経口投与を施行しているが，CMV 感染症対策としては考慮していない．

CMV 感染症のモニタリングとして C7-HRP 抗体を用いたアンチゲネミア法(C7-HRP)を施行しており，術後 1 週間目より 1～2 週ごとに退院する日まで，退院後は約半年間をめどにモニタリングしている．入院中に一度でも CMV アンチゲネミア陽性が判明した患者においては，退院後も定期的にモニタリングを施行している．また，発熱，白血球減少，血小板減少，異型リンパ球の出現，肝機能障害を認める場合には，随時 C7-HRP を施行する．

C7-HRP はあくまでも定量法ではないことに注意している．つまり，上記に述べたような症状がある場合や，通常よりも免疫抑制過多になっているような場合，また肝機能異常の原因が明らかではない場合などでは，CMV 陽性細胞が白血球 5 万個中 1，2 個程度であってもガンシクロビルによる治療を開始する．逆に患者状態が安定している場合には厳重な経過観察としている．

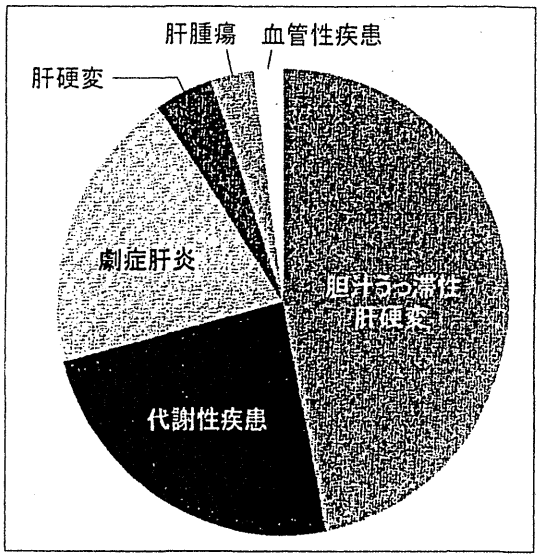


図 1 国立成育医療センターにおける生体肝移植症例：原疾患の内訳

いったん治療を開始した場合には，週 2 回の頻度で C7-HRP を測定し治療効果を判定する．もし，1 週間以上のガンシクロビルの静脈内投与にて効果を認めない場合には，CMV 高力価グロブリン製剤の投与とともにフォスカビルへの変更を考慮する．この際には，ガンシクロビル耐性株の遺伝子検査を同時に行い，陰性結果を得た場合には，ガンシクロビルに再度変更することとしている．バルガンシクロビルに関しては，上述した投与量によってガンシクロビル投与にて効果が確認されたあとに，維持療法として変更する場合もある．

### 2．対象と方法

当院において 2005 年 11 月～09 年 8 月までに，100 例に対して生体肝移植術を施行した．うち，術後 30 日以内に死亡した 2 例，術前 CMV の serologic status が不明であった 2 例を除外した 96 例を対象とした．移植時年齢は 1 カ月～21 歳 5 カ月(中央値 16.5 歳)，男性 44 例，女性 52 例であった．原疾患の内訳は，胆汁うっ滞性肝硬変 45 例(うち胆道閉鎖症 39 例，PSC 1 例)，代謝性疾患 23 例，劇症肝炎 19 例，肝硬変 4 例，肝腫瘍 3 例，

表1 CMV感染症の関連因子

| 因子 | C7-HRP陰性(58例) | C7-HRP陽性(36例) | P value |
|---|---|---|---|
| レシピエント年齢(yr) | 4.3±4.9 | 3.5±4.5 | NS |
| レシピエント体重(kg) | 15.6±12.9 | 15.1±12.4 | NS |
| 原疾患(劇症肝炎/その他) | 5/53 | 12/24 | 0.002 |
| 術前状態(ICU-bound/その他) | 12/46 | 16/20 | 0.014 |
| ドナー年齢(yr) | 35.0±6.8 | 36.8±7.6 | NS |
| CMV serologic status(D+/R−/その他) | 11/47 | 18/18 | 0.002 |
| GRWR(%) | 2.37±0.95 | 2.35±1.07 | NS |
| ABO血液型適合性(不適合/その他) | 8/50 | 6/30 | NS |
| 拒絶反応(あり/なし) | 19/39 | 16/20 | NS |
| EBV感染症(あり/なし) | 11/47 | 5/31 | NS |

血管性疾患2例であった．当院での生体肝移植症例では，代謝性疾患と劇症肝炎の比率が高い傾向にあった(図1)．

免疫抑制プロトコールは通常，タクロリムスと低用量の経口ステロイドにて行い，ステロイドを術後3カ月目に漸減・中止している．ABO血液型不適合症例(15例，15.6%)においては，5歳以上では成人と同様に，肝移植術の1カ月前のリツキシマブ(375 mg/BSA $mm^3$)投与と術直前の血漿交換，術後は門脈に挿入したカテーテルよりプロスタグランジン($PGE_1$)およびステロイドの投与を施行している．術後抗ドナー血液型抗体が上昇してきたときのみにMMFを追加投与している．急性拒絶反応に対しては，基本的に肝生検にて拒絶反応の有無および程度を組織病理学的に検索したうえでステロイドパルス治療を開始している．ステロイドパルス治療に反応が乏しい場合には，MMFあるいはシロリムスなどの免疫抑制薬の追加投与を行っている．

術前CMVのserologic statusの組み合わせを分類すると，D+/R+が46例(47.9%)，D+/R−が29例(30.2%)，D−/R+が9例(9.4%)，D−/R−が12例(12.5%)と，小児症例が大部分を占めるためにD+/R−(いわゆるハイリスク群)の比率が高かった．これらの症例において上記のCMV感染症対策を施行した．対象患者の術前・術後の臨床経過，CMVの検査結果を中心に，後方視的にカルテよりデータを抽出し検討を行った．

表2 術前CMVのserologic statusの組み合わせによる比較

| D/R CMV serologic combination | CMVアンチゲネミア陽性率：% |
|---|---|
| D+/R+ | 36.4(16/44) |
| D+/R− | 62.1(18/29) |
| D−/R+ | 11.1(1/9) |
| D−/R− | 8.3(1/12) |

なお，定義上，C7-HRPについては，白血球5万個中にCMV陽性細胞が1個でも認められた場合にすべて陽性とした．さらに，発熱，白血球減少，血小板減少，異型リンパ球の出現などの臨床症状を呈した場合にCMV syndromeとし，臓器特異的に症状，画像所見，組織学的所見を呈した場合にはCMV diseaseとした．

## 3．結　果

96例中8例が死亡した．死亡原因の内訳は，敗血症5例，移植肝不全2例(原病再発1例，血流不全1例)，腫瘍再発1例であったが，明らかなCMV感染症に起因する死亡症例はなかった．

### (1) CMVアンチゲネミア陽性率およびCMV感染症の発症率

劇症肝炎19例中2例において術前のC7-HRPが陽性であった．これらの症例においては，術前よりガンシクロビルの静脈内投与を施行し，術後もC7-HRPが陰性化するまで継続した．そのうち1例において，14日間のガンシクロビル投与に

てC7-HRPが増加傾向にあるためフォスカビルに変更し陰性化した.

術後CMVアンチゲネミア陽性率は94例(術前C7-HRP陽性2例を除く)中36例(38.3%)であり,陽性化の時期は術後13～56日(平均34.6±11.2日)であった.このうち発症は6例(6.4%)であり,その内訳はCMV syndromeが3例,肝炎・腸炎・肺炎がおのおの1例ずつであった.発症時期は術後26～47日(平均37.5±8.8日)であった.

CMV感染症の発症群と非発症群においてCMV陽性細胞個数を比較すると,発症群では76.1±62.0個であるのに対し,非発症群では13.7±17.7個と有意に発症群において多かった.

(2) CMV感染症の関連因子

CMV感染症の関連因子をCMVアンチゲネミア陰性群と陽性群にわけ比較した(表1).既存の報告と同様に,術前CMVのserologic statusの組み合わせがD+/R−である群において有意にCMVアンチゲネミア陽性率が高かった(表2).それ以外では,原疾患が劇症肝炎であることと,術前のICU管理の2項目において有意差を認めたが,ABO血液型不適合症例や拒絶反応の有無などについては有意差を認めなかった.

有意差を認めた理由としては,小児の劇症肝炎症例においては,術前の体外循環療法やステロイド治療による免疫抑制状態であることや,術後ステロイドパルス療法に難治性の拒絶反応が起こりやすく,他疾患と比較して免疫抑制過多となる症例が多いことなどが影響していると考えられた.一方,ABO血液型不適合症例において有意差を得なかった理由としては,成人症例とくらべて小児症例では,リツキシマブの術前投与や血漿交換を必要とする場合が少なく,また術後においてもMMFなどの追加の免疫抑制薬を必要としないことが影響していると考えられた.

(3) CMV感染症に対する治療

術後CMVアンチゲネミア陽性36例中31例(86.1%)に対してガンシクロビルの静脈内投与を行った.残りの5例に対しては,陽性細胞個数が少数であり,明らかなCMVに関連する症状を認めず,免疫抑制薬の減量を行い経過観察としたが,すべての症例においてC7-HRPは陰性化した.ガンシクロビルを投与した症例のうち4例において,ガンシクロビルに対する治療抵抗性を示したためにフォスカビルへと変更したが,この4例も含めすべての症例において最終的にC7-HRPは陰性化した.

C7-HRPが陰性化するまでの期間は全体で5～49日(平均16.5±9.3日)であったが,フォスカビルを使用した症例では14～49日(平均30.5±15.0日)と陰性化までに長期間を要した.フォスカビル投与症例におけるガンシクロビル投与開始時のCMV陽性細胞個数は平均27.9±29.7個であり,ガンシクロビル単剤による陰性化症例(平均29.5±43.1個)と比較して明らかな有意差を認めなかった.また最近の3例において,ガンシクロビル静脈内投与の2週間投与後にバルガンシクロビルによる維持療法を行い,CMV感染症再活性化を認めずに終了しえた.

当院におけるCMV感染症対策としてはpre-emptive therapyを基本として施行しており,術後CMVアンチゲネミア陽性率はprophylaxisを施行している報告と比較して高いものの,CMV感染症の発症率は6.4%であり,また重篤化することなくすべての症例において治癒しえた.

## おわりに

小児の生体肝移植症例におけるCMV感染症対策を中心に解説した.

本邦において肝移植が開始されてから約20年が経過し,ほぼ確立された医療として定着した.CMV感染症への対策においては,免疫抑制薬の適切な使用,CMV感染症に対するモニタリング方法の向上,および適切な治療により,ほぼ確立されたものになったといえる.

## 文 献

1) 日本肝移植研究会:肝移植症例登録報告.移植 44:559-571, 2009.
2) Paya CV: Indirect effects of CMV in the solid organ transplant patient. Transpl Infect Dis 1:8-12, 1999.

3) Sia IG, Patel R：New strategies for prevention and therapy of cytomegalovirus infection and disease in solid-organ transplant recipients. Clin Microbiol Rev　13：83-121, 2000.
4) Razonable RR：Cytomegalovirus infection after liver transplantation：Current concepts and challenges. World J Gastroenterol　14：4849-4860, 2008.
5) Saito T, Egawa H, Kudo T, Takakura S, Fujihara N et al.：Pre-transplant risk factors predicting post-transplant cytomegalovirus infection in liver transplant recipients. Transpl Int　20：419-424, 2007.
6) Kasahara M, Egawa H, Takada Y, Oike F, Sakamoto S et al.：Biliary reconstruction in right lobe living-donor liver transplantation：Comparison of different techniques in 321 recipients. Ann Surg　243：559-566, 2006.
7) Egawa H, Taira K, Teramukai S, Haga H, Ueda Y et al.：Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation：A single center experience. Dig Dis Sci　54：1347-1354, 2009.
8) Anon：Cytomegalovirus. Am J Transplant　4(Suppl 10)：51-58, 2004.
9) Levitsky J, Singh N, Wegener MM, Stosor V, Abecassis M, Ison WG：A survey of CMV prevention strategies after liver transplantation. Am J Transplant　8：158-161, 2008.
10) Arthurs SK, Eid AJ, Pedersen RA, Dierkhising RA, Kremers WK et al.：Delayed-onset primary cytomegalovirus disease after liver transplantation. Liver Transpl　13：1703-1709, 2007.
11) Kijpittayarit S, Deziel P, Eid AJ, Razonable RR：Primary cytomegalovirus disease after five years of antiviral prophylaxis. Transplantation　81：137-138, 2006.
12) Singh N, Wannstedt C, Keyes L, Mayher D, Tickerhoof L et al.：Valganciclovir as preemptive therapy for cytomegalovirus in cytomegalovirus-seronegative liver transplant recipients of cytomegalovirus-seropositive donor allografts. Liver Transpl　14：240-244, 2008.
13) Asberg A, Humar A, Rollag H, Jardine AG, Mouas H et al.：Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant　7：2106-2113, 2007.
14) Paya C, Humar A, Dominguez E, Washburn K, Blumberg E et al.：Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant　4：611-620, 2004.
15) Pescovitz M, Jain A, Robson R, Mulgaonkar S, Freeman R et al.：Establishing pharmacokinetic bioequivalence of valganciclovir oral solution versus the tablet formulation. Transplant Proc　39：3111-3116, 2007.
16) Vaudry W, Ettenger R, Jara P, Varela-Fascinetto G, Bouw MR et al.：Valganciclovir dosing according to body surface area and renal function in pediatric solid organ transplant recipients. Am J Transplant　9：636-643, 2009.
17) 吉田　敦：サイトメガロウイルスの検査法. 臨床病理　56：1034-1042, 2008.

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# Living-donor liver transplantation for propionic acidemia

Kasahara M, Sakamoto S, Kanazawa H, Karaki C, Kakiuchi T, Shigeta T, Fukuda A, Kosaki R, Nakazawa A, Ishige M, Nagao M, Shigematsu Y, Yorifuji T, Naiki Y, Horikawa R. Living-donor liver transplantation for propionic acidemia.
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Abstract: Propionic acidemia is a rare autosomal recessive disorder affecting the catabolism of branched-chain amino acids because of a genetic defect in PCC. Despite the improvements in medical treatment with protein restriction, sufficient caloric intake, supplementation of L-carnitine, and metronidazole, patients with the severe form of propionic acidemia have life-threatening metabolic acidosis, hyperammonemia, and cardiomyopathy, which results in serious neurologic sequelae and sometimes death. This study retrospectively reviewed three children with neonatal-onset propionic acidemia who received LDLT. Between November 2005 and December 2010, 148 children underwent LDLT, with an overall patient survival of 90.5%, in our center. Three patients were indicated for transplantation because of propionic acidemia. All recipients achieved a resolution of metabolic derangement and better quality of life with protein restriction and medication, although urine methylcitrate and serum propionylcarnitine levels did not decrease markedly. LT can reduce the magnitude of progressive cardiac/neurologic disability as a result of poor metabolic control. Further evaluation is therefore required to determine the long-term suitability of this treatment modality.

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Key words: living donor liver transplantation – liver transplantation – pediatric transplantation

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Propionic acidemia is a rare autosomal recessive disorder affecting the catabolism of branched-chain amino acids because of a genetic defect in PCC. PCC is a mitochondrial matrix enzyme that converts propionyl-CoA into D-methylmalonyl-CoA (1). Propionic acidemia affects approximately one in 465 000 live births in Japan (2). Despite the improvements in the medical treatment with protein restriction, sufficient caloric intake, supplementation of L-carnitine, and metronidazole, patients with the severe form of the disease have life-threatening metabolic acidosis, hyperammonemia, and cardiomyopathy, which results in serious neurologic sequelae and sometimes death. The prognosis of patients with propionic acidemia is generally poor, with survival rate of 41% (3). In addition, in spite of aggressive medical treatment, many of the patients who survive develop mental retardation.

LT may offer relief of the symptoms for genetically acquired errors in liver metabolism (4). Recent case studies have reported the benefits of LT in propionic acidemia, demonstrating that correcting hepatic enzyme deficiency by LT leads to clinical improvements, including better nutrition, better quality of life, and fewer

Abbreviations: CHDF, continuous hemo-diafiltration; DQ, developmental quotient; ECMO, extra corporeal membrane oxygenation; LDLT, living-donor liver transplantation; LT, liver transplantation; PCC, propionyl-CoA carboxylase.

ketoacidosis and cardiac insufficiency (ejection fraction 10%) prior to LDLT, which necessitated CHDF and ECMO for seven days. Thereafter, the patient was successfully weaned from CHDF and ECMO and listed for LT to avoid a fatal cardiac event (9). The ejection fraction was recovered to 69% with 30 days of medical treatment. The preoperative ejection fraction by ultrasonic cardiography was revealed to be 78.0%, 65.8%, and 69.0% in cases #1–3, respectively, which demonstrated normal cardiac function. All of the patients received nasogastric tube feeding because of feeding difficulties. The indications for LDLT were poor metabolic control in all of the patients. Pre-LDLT urinary methylcitrate concentrations in cases #1, #2, and #3 were 76.6, 316.0, and 64.1 mmol/molCr (range, 6.6 ± 1.9) (Fig. 1a).

The serum-free carnitine and propionylcarnithine levels were 35.2, 20.7, and 29.2 nmol/mL (range, 37.0 ± 6.0), and 50.3, 63.1, and 40.55 nmol/mL (range, 0.51 ± 0.25), respectively (Fig. 1b,c). The cerebrospinal fluid-free carnitine and propionylcarnithine levels were 32.9, 2.37, and 1.71 nmol/mL (range, 3.35 ± 0.43), and 27.6, 2.89, and 3.55 nmol/mL (range, 0.040 ± 0.005), respectively.

LDLT was indicated at seven months, two yr, and two yr two months of age. The duration and blood loss of the recipient operation ranged from 422 to 529 min and 170 to 760 g. Significant acidosis (−4.9 ± 0.8 nM), which was easily managed with sodium bicarbonate injection, was observed in the anhepatic phase of recipient operation. The histopathological examination of the explanted liver revealed microvesicular steatosis and mild fibrosis. A histological examination of the graft showed 5%, 5%, and 10% microvesicular steatosis, for the donors for cases #1–3, respectively. No metabolic decompensation was observed in the donor operation. All of the donors were discharged from the hospital within eight days after the operation, and they are currently doing well without any complications.

The postoperative course was uneventful in cases #2 and #3. Case #1 developed an intestinal perforation on postoperative day 7, which necessitated relaparotomy. Cases #1 and #2 experienced cytomegalovirus infection on postoperative days 48 and 39, which was successfully managed with ganciclovir administration. The pre- and postoperative DQ levels were 77 and 80, 81.3 and 83.3, and 54 and 59, showing no progressing developmental delays after transplantation.

Even after successful LDLTs, however, chronological changes in urinary methylcitrate, serum-free carnitine, serum acetylcarnitine, and serum propionylcarnitine did not decrease to normal ranges in any of the patients (Fig. 1).

All children are doing well, showing normal graft/cardiac function and mental development, with a better quality of life without hospitalization at a median follow-up after LDLT of 40 months.

## Discussion

The aim of this study was to evaluate the outcome in three patients who underwent LT for propionic acidemia. Despite intense conventional medical treatment for this condition, the accumulation of toxic metabolites usually results in death, especially in the patients of early-onset disease (3, 10). Our three patients with early-onset disease showed mild to moderate mental retardation prior to LDLT, despite successful management of metabolic acidosis and hyperammonemia with early induction of CHDF, and later dietary protein restriction, L-carnitine supplementation, and metronidazole. The patients who survive the disease without LDLT suffer from metabolic decompensation and numerous complications, particularly progressive cardiac insufficiency, such as was seen in our case #3, and severe neurologic sequelae (10). LT is now the preferred treatment modality for selected patients with propionic acidemia, and although the implanted liver graft produces PCC, the overall biochemical defect is only partially corrected, as PCC is expressed not only in the liver, but also expressed throughout the body (11). Illustrating this fact, the methylcitrate levels in urine and the propionylcarnitine levels in serum and even cerebrospinal fluid were not decreased markedly after successful LT in our three cases. None of the patients, however, have experienced life-threating metabolic acidosis and/or cardiac insufficiency after a median of 36 months of follow-up, providing them with a better quality of life.

The benefits of quality of life, and decreased cardiac and neurologic injury should be weighed against the risks of LDLT for the patient and donor (12). LT does not completely cure the disease but may decrease the disease severity in propionic acidemia. Given the risk of continued cardiac and neurologic compromise, the potential for improvement of quality of life represents a major benefit of performing early LT. As such, we recommend early LT for the patients with neonatal-onset propionic acidemia, because it appears that LT can reduce the magnitude of progressive cardiac/neurologic disability as a result of poor metabolic control.

In this study, no negative impacts of the use of potentially heterozygous carriers as donors on the postoperative course of either the donors or recipients have been observed to date. With respect to the use of heterozygous donors in the review of the patients, although the experience with LDLT in propionic acidemia is limited, there have been no descriptions of mortality or morbidity related to the use of heterozygous donors in the published literature (5, 13, 14).

In conclusion, the ultimate role of LT in the treatment of propionic acidemia will not be clear until the patients reach adulthood, because its effects on late complications such as cardiac and neurologic symptoms will not be clear until much later in life. A better understanding of the long-term suitability of this treatment modality will require the registration and ongoing evaluation of all patients considered for LT.

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## References

1. Rosenberg L, Fenton W. Disorders of propionate and methylmalonate metabolism. In: Scriver C, Beaudet A, Sly W, Valle D, eds. The Metabolic and Molecular Basis of Inherited Disease. New York: McGraw-Hill, 2007: pp. 821–844.
2. Yorifuji T, Kawai M, Muroi J, et al. Unexpectedly high prevalence of the mild form of propionic academia in Japan: Presence of a common mutation and possible clinical implications. Hum Genet 2002: 111: 161–165.
3. Van der Meer SB, Poggi F, Spada M, et al. Clinical outcome and long-term management of 17 patients with propionic academia. Eur J Pediatr 1996: 155: 205–210.
4. Todo S, Starzl TE, Tzakis A, et al. Orthotopic liver transplantation for urea cycle enzyme deficiency. Hepatology 1992: 15: 419–422.
5. Yorifuji T, Kawai M, Mamada M, et al. Living-donor liver transplantation for propionic academia. J Inherit Metab Dis 2004: 27: 205–210.
6. Romano S, Valayannopoulos V, Jais JP, et al. Cardiomyopathies in propionic aciduria are reversible after liver transplantation. J Pediatr 2010: 156: 128–134.
7. Hudon L, Moise KJ, Hegemier SE, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for the treatment of fetal hemolytic disease. Am J Obstet Gynecol 1998: 179: 858–863.
8. Tanaka K, Uemoto S, Tokunaga Y, et al. Surgical techniques and innovations in living related liver transplantation. Ann Surg 1993: 217: 81–92.
9. Sato S, Kasahara M, Fukuda A, et al. Liver transplantation in a patient with propionic academia requiring extra corporeal membrane oxygenation during severe metabolic decompensation. Pediatr Transplant 2009: 13: 790–793.
10. Surtees RA, Matthews EE, Leonard JV. Neurologic outcome of propionic academia. Pediatr Neurol 1992: 8: 333–337.
11. Sass JO, Hofmann M, Skladal D, Mayatepek E, Shwahn B, Sperl W. Propionic academia revisited: A workshop report. Clin Pediatr 2004: 43: 837–843.
12. Barshes NR, Vanatta JM, Patel AJ, et al. Evaluation and management of patients with propionic academia undergoing liver transplantation: A comprehensive review. Pediatr Transplant 2006: 10: 773–781.
13. Yorifuji T, Muroi J, Uematsu A, Nakahata T, Egawa H, Tanaka K. Living-related liver transplantation for propionic academia. J Pediatr 2000: 137: 572–574.
14. Morioka D, Kasahara M, Takada Y, et al. Living donor liver transplantation for pediatric patients with inheritable metabolic disorders. Am J Transplant 2005: 5: 2754–2763.

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Pediatric surgical image

# Living donor liver transplantation for multiple intrahepatic portosystemic shunts after involution of infantile hepatic hemangiomas☆

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**Key words:**
Infantile hepatic hemangioma;
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Living donor liver transplantation

**Abstract** We describe a 6-year-old girl presenting with multiple intrahepatic portosystemic shunts after the involution of infantile hepatic hemangiomas (IHHs), who successfully underwent living donor liver transplantation. The chronological changes of radiologic findings indicated that remnant portovenous shunts at the time of IHHs involution developed gradually on the background of atrophic intrahepatic portal veins. This suggests that patients should be carefully followed up for the late onset of intrahepatic portosystemic shunts after the involution of IHHs.


Infantile hemangioma is the most common pediatric tumor, affecting 4% to 5% of white infants. It is a benign tumor that exhibits rapid postnatal growth, followed by slow involution during childhood. Nevertheless, approximately 10% to 20% of infantile hemangiomas cause life-threatening or disfiguring complications [1]. Infantile hepatic hemangiomas (IHHs) share the same patterns of growth and regression as their more common cutaneous counterparts. Most are clinically silent; however, some IHHs become symptomatic, manifesting as cardiac failure secondary to high-volume shunting, hypothyroidism, or fulminant hepatic failure [2]. The initial management can be noninvasive because spontaneous regression occurs in many cases. Historically, the initial medical intervention for IHHs has been corticosteroids. The patients presenting with congestive heart failure are treated with digitalis and diuretics. In

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infants who fail medical management, surgical or radiologic intervention, such as excision of the tumors, hepatic arterial ligation or embolization, and liver transplantation, can be life saving [3].

An intrahepatic portosystemic shunt (IHPSS) is a rare anomaly that may lead to the development of pulmonary hypertension, hepatopulmonary syndrome, hepatic encephalopathy, and hypoglycemia [4]. In patients with IHPSS that show significant clinical manifestations in spite of conventional medical and dietary treatment, surgical or radiologic intervention may be required [5]. The patients, whose portal vein solely flows through the shunt vessel into a hepatic vein, can be treated by shunt ligation or embolization [6]. However, if the IHPSS is multifocal, liver transplantation

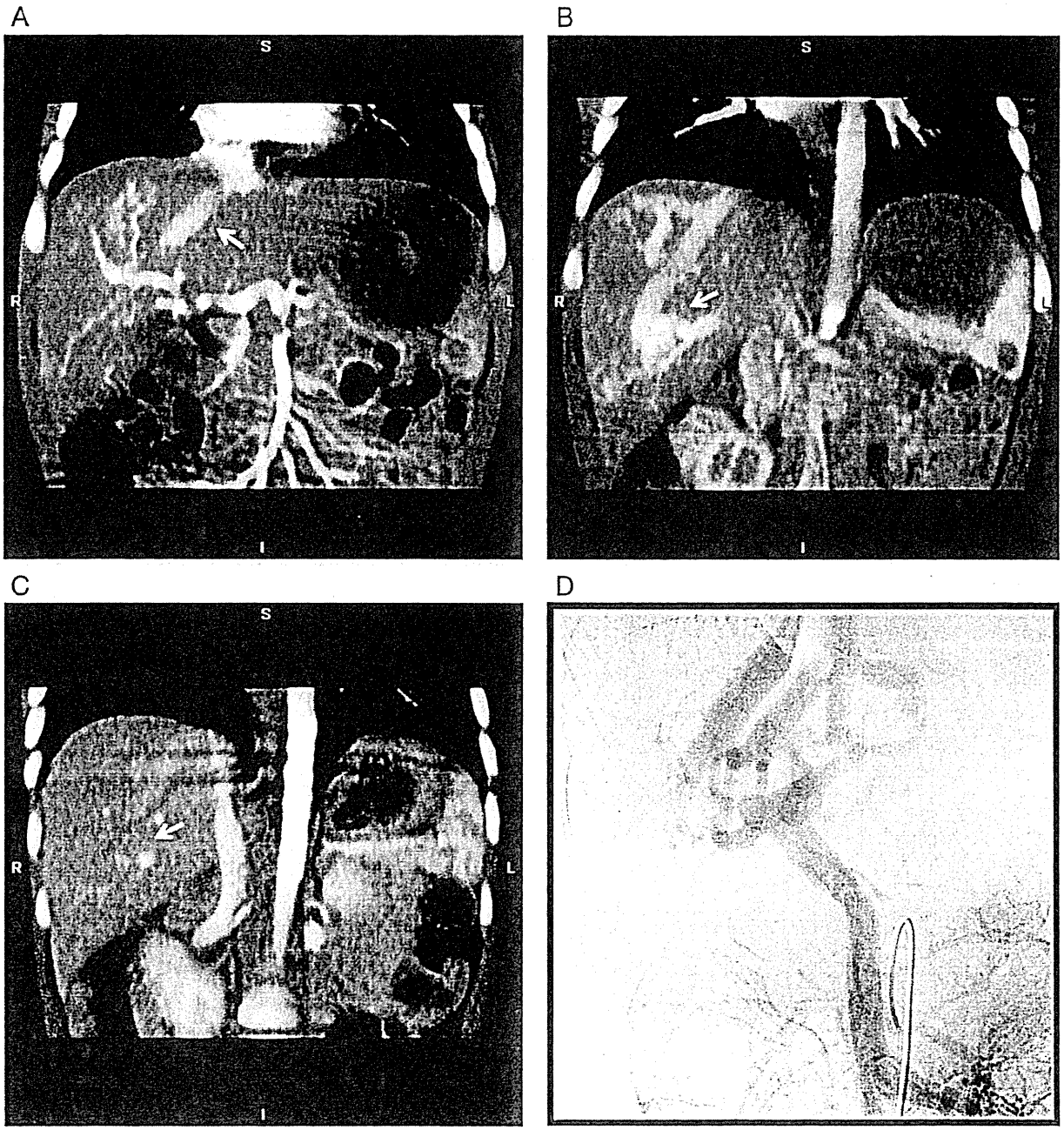


**Fig. 1** The chronological changes in radiologic findings. A, Abdominal enhanced CT (arterial phase) at the onset of IHHs revealed multiple tumors fed by hepatic arteries with early enhancement of the right hepatic vein (arrow). B, The abdominal enhanced CT (portal phase) at the onset of IHHs already revealed obvious portovenous shunts. The arrow indicates a huge shunt between the right portal vein and the right hepatic vein. C, The abdominal enhanced CT at the time of involution of IHHs revealed the disappearance of most of the portovenous shunts. The arrow indicates that the shunt pointed out in *B* had disappeared. D, The portography before LDLT revealed multiple IHPSS with tiny intrahepatic portal veins.

is indicated as a curative procedure. We describe our experience with living donor liver transplantation (LDLT) for multifocal IHPSS after successful treatment of IHHs.

## 1. Case presentation

An Asian female presented in the neonatal period (beginning at 4 days) with congestive heart failure owing to multiple IHHs (Fig. 1A, arterial phase; B, portal phase). Diuretics and corticosteroids were initiated as the initial treatment, which provided sufficient relief, and corticosteroid therapy was ceased within a month after confirming the regression of IHHs. The patient had been closely followed up for 3 years. An enhanced computed tomography (CT) of the abdomen showed nearly complete involution of the IHHs (Fig. 1C), and the patient's liver function tests were normal (serum bilirubin, 1.10 mg/dL; aspartate aminotransferase, 47 IU/L; alanine aminotransferase, 16 IU/L; gamma-glutamyl transpeptidase (GGT), 13 IU/L) at the final follow-up assessment. The patient was referred to our hospital at 6 years with hypoglycemia (serum fasting glucose level, 30 mg/dL) and hyperammonemia (serum $NH_3$ level, 150 $\mu$mol/L). At the time, a laboratory studies showed the following: serum bilirubin, 1.08 mg/dL; aspartate aminotransferase, 77 IU/L; GGT, 130 IU/L; total bile acid, 164 $\mu$mol/L; and prothrombin time - international normalized ratio (PT-INR), 1.39. Further imaging studies revealed multiple IHPSS *with tiny intrahepatic portal veins* (Fig. 1D). Per-rectal portal scintigraphy with technetium Tc 99m for the evaluation of the portal circulation showed a 60% portosystemic shunt ratio. Interventional treatment was not indicated because of the presence of multicentric portosystemic shunts. Because of recurrent hyperammonemia, hypoglycemia, progressive hyperintensity in the globuspallidus on magnetic resonance imaging of the brain, and multiple hepatic tumors despite medical treatment and protein restriction, the patient underwent LDLT. The donor was her 36-year-old mother with an identical blood type. The liver graft, a left lateral sector that weighed 227 g, thus representing 1.39% of the graft-to-recipient weight ratio, was implanted as a standard procedure. The recipient hepatectomy was uncomplicated. The macroscopic findings of the 333-g explanted native liver, which was 64.8% of the estimated standard liver volume, showed that portal veins directly drained through the hepatic veins (Fig. 2A).

Histopathologic examinations showed an atrophic portal vein in comparison with a normal bile duct and hepatic artery in the portal tract (Fig. 2B). The operative procedure lasted 6 hours and 20 minutes, and the patient blood loss was 298 mL. The postoperative course was uneventful. During the 3-months follow-up period, the patient has been doing well, with normal liver function and normal magnetic resonance imaging findings without either hyperammonemia or hypoglycemia.

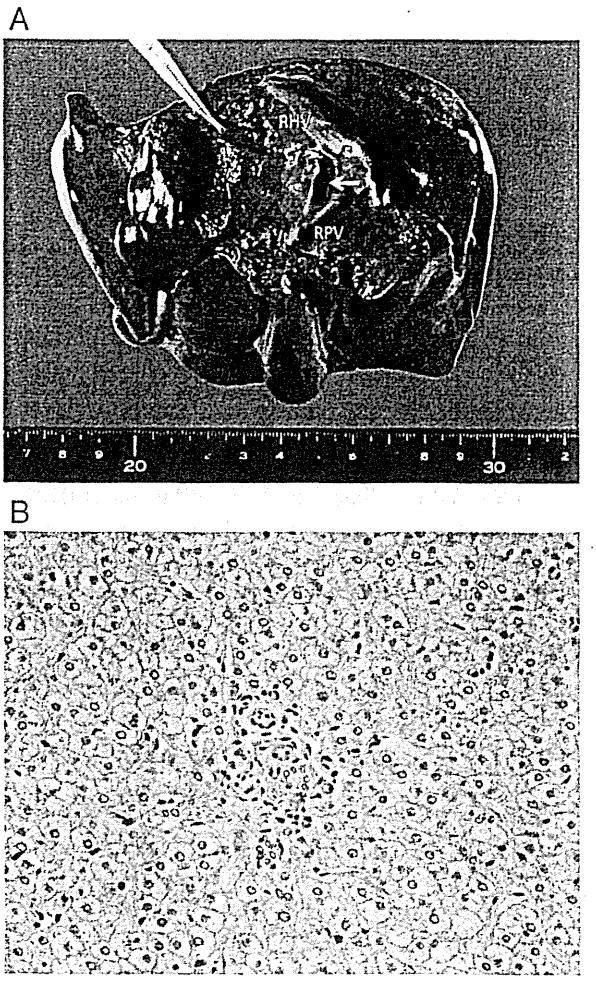


**Fig. 2** A, A gross specimen of the native liver showed a huge shunt (arrow) between the right portal vein (RPV) and the right hepatic vein (RHV). B, A histopathologic examination showed no structure of portal vein in comparison with a normal bile duct and hepatic artery in the portal tract.

## 2. Discussion

Infantile hepatic hemangioma is a rare mesenchymal tumor of the liver derived from endothelial cells that proliferate and form vascular channels. The hemangiomatous tissue is preferentially supplied by the hepatic artery, and therefore, ligation of the hepatic artery, one of the therapeutic options for IHHs, causes selective ischemia, which reduces the *microscopic or macroscopic* arteriovenous shunts and aids spontaneous involution [7]. The present case questions the pathogenesis of IHPSS after the involution of IHHs because the patient's clinical course had been uneventful without any signs related to IHPSS for several years after the involution of IHHs. One possible consideration would be the existence of IHPSS at the onset of IHHs. A previous study that reviewed the angiograms of patients with IHHs demonstrated that there can be a direct macroscopic,

angiographically visible arteriovenous and portovenous shunts, although a microscopic intralesional shunt is a characteristic of IHHs [8]. We retrospectively reviewed the chronological changes of the radiologic findings in our patient. Although the patient did not undergo an angiogram at the onset of IHHs, the enhanced CT showed portovenous shunts, which almost disappeared during the involution of IHHs. Unfortunately, no radiologic examinations had been performed after the involution of IHHs until she complained of new symptoms related to IHPSS at 6 years. We speculated that the remnant portovenous shunts likely developed gradually on the background of the atrophic intrahepatic portal veins, which was supported by the histopathologic examination of the explanted liver. The important point indicated by this case is that patients with IHH should be closely observed for clinical evidence of late-onset IHPSS.

The lack of complete involution of the primordial vessels, such as the ductus venosus, may give rise to abnormal vascular communications between any vein of the portal system and of the inferior vena cava system; these communications may exist inside or outside the liver, may be single or multiple, and vary in size [6]. Some small IHPSS disappear spontaneously by 1 to 2 years, but others, mostly the large shunts, persist throughout life and carry risks of complications [9]. Intrahepatic portosystemic shunts can be managed in a patient with pulmonary hypertension, hepatopulmonary syndrome, hepatic encephalopathy, and hypoglycemia. However, the risk of clinical manifestations increases if the shunt ratio exceeds 60%. The etiology of pulmonary hypertension/hepatopulmonary syndrome is an imbalance of vasoconstrictors and vasodilators, either produced or metabolized by the liver, which affects the pulmonary arterioles and capillaries by a portosystemic shunt [4].

In the present case, the girl had uncontrollable hypoglycemia, which was explained by defective liver uptake of glucose and secondary hyperinsulinism [10]. Hyperinsulinism (114.3 $\mu$U/mL) with a normal serum glucose level (118 mg/dL) at 30 minutes during the oral glucose tolerance test was observed. Late hyperinsulinism then led to the secondary hypoglycemia.

The treatment of IHPSS should be selected based on the number, size, and location of the portosystemic shunts. Surgical or angiographic intervention for shunt occlusion might be considered for cases where there are a small number of shunts that are small and located eccentrically [11]. Large and multiple portosystemic shunts located throughout the entire liver, such as noted in the present case, can be difficult to embolize with coils or other embolic agents because of the potential complications of coil embolization, which may include dislodgement of coils into the systemic circulation and exacerbation of portal hypertension caused by abrupt changes in the portal circulation [9]. Okada et al [9] previously reported a case that was successfully managed by staged coil embolization for multiple IHPSS; however, the authors mentioned that the catheterization into the complicated intrahepatic shunts was technically demanding and required better catheter maneuverability. Although we considered the use of the staged coil embolization, liver transplantation was selected for our case because of a scarcity of reports on the outcome of cases treated by staged coil embolization and the potential complications of its use.

We successfully performed liver transplantation for a child with IHPSS after IHHs. Our findings emphasize that patients should be closely monitored for late-onset IHPSS after the involution of IHHs. Early liver transplantation is indicated in symptomatic patients with IHH to prevent irreversible brain damage owing to hyperammonemia/hypoglycemia, even in patients without impaired liver function.

## References


[1] Mulliken JB, Fishman SJ, Burrows PE. Vascular anomalies. Curr Probl Surg 2000;37:17-84.

[2] Christison-Lagay ER, Burrows PE, Alomari A, et al. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. J Pediatr Surg 2007;42:62-8.

[3] Meyers RL. Tumors of the liver in children. Surg Oncol 2007;16: 195-203.

[4] Hoeper MM, Krowka MJ, Strassburg CP. Portopulmonary hypertension and hepatopulmonary syndrome. Lancet 2004;363:1461-8.

[5] Kanamori Y, Hashizume K, Kitano Y, et al. Congenital extrahepatic portocaval shunt (Abernethy type 2), huge liver mass, and patent ductus arteriosus—a case report of its rare clinical presentation in a young girl. J Pediatr Surg 2003;38:E15.

[6] Ikeda S, Sera Y, Yoshida M, et al. Successful coil embolization in an infant with congenital intrahepatic portosystemic shunts. J Pediatr Surg 1999;34:1031-2.

[7] Davenport M, Howard ER. Surgical disorders of the liver and bile ducts. In: Kelly DA, editor. Diseases of the liver and biliary system in children. London: Blackwell Science; 1999. p. 253-78.

[8] Kassarjian A, Dubois J, Burrows PE. Angiographic classification of hepatic hemangiomas in infants. Radiology 2002;222:693-8.

[9] Okada Y, Endo T, Kusano S, et al. Multiple intrahepatic portohepatic venous shunts: treatment with steel-coil embolization. AJR Am J Roentgenol 1991;157:971-3.

[10] Ishida T, Chap Z, Chou J, et al. Effects of portal and peripheral venous insulin infusion on glucose production and utilization in depancreatized, conscious dogs. Diabetes 1984;33:984-90.

[11] Lee SA, Lee YS, Lee KS, et al. Congenital intrahepatic portosystemic venous shunt and liver mass in a child patient: successful endovascular treatment with an Amplatzer Vascular Plug (AVP). Korean J Radiol 2010;11:583-6.