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ORIGINAL ARTICLE

The clinical significance of early histological rejection with or without biochemical abnormality in adult living donor liver transplantation for hepatitis B virus related end stage liver disease

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Summary

There is no agreement regarding the treatment of early allograft rejection (EAR) in adult living donor liver transplantation (LDLT). A protocol biopsy was performed in 62 adult LDLT recipients. Twenty-one patients (33.9%) had histological evidence of EAR. Of these, 14 patients had biochemical abnormalities and seven patients had no associated biochemical abnormalities. None of the seven patients with subclinical EAR (11.3% of the entire study population) were treated, and no subsequent rejection was observed. Gender mismatch (female-to-male) was the single independent risk factor for histological EAR [odds ratio (OR) = 13.458; 95% confidence interval (CI), 1.836–98.649] and the cumulative probability for a subsequent rejection was higher in patients with EAR (OR = 11.085; 95% CI, 1.221–100.654). However, the actuarial 1 year patient and graft survival rate in patients with EAR (81.0% and 85.5%) were similar to those without EAR (92.7% and 97.25%; $P = 0.127$ and 0.302, respectively). The presence of an initial biochemical abnormality was an independent risk factor for both a decreased patient survival (OR = 5.827; 95% CI, 1.095–31.017; $P = 0.039$) and graft loss (OR = 20.646; 95% CI, 2.044–208.524; $P = 0.010$). Subsequent rejection developed more frequently in patients with EAR. However, the survival is not determined by the presence of EAR but by the presence of a biochemical abnormality.

Introduction

Acute cellular rejection (ACR) was the most relevant obstacle to organ transplantation in the early era, but the development of modern immunosuppressive drugs has significantly reduced its disastrous effects. However, hepatic allograft rejection remains an important problem after liver transplantation (LT), and is the major reason why immunosuppressive therapy is essential. Although there is evidence suggesting that early allograft rejection (EAR) episodes may have a negative impact on long-term graft survival of renal transplants [1], this association is not evident in LT [2]. Nevertheless, inadequately treated ACR can progress to steroid-resistant rejection and graft failure from chronic rejection [3].

A mild to moderate degree of biochemical abnormality is common on the first 7–10 days after a living donor liver transplantation (LDLT), and has many causes, including pre-existing donor abnormalities in the form of steatosis, ACR, small-for-size syndrome, and surgical complications associated with blood vessels or the biliary tree [4]. However, it is difficult to make a precise differentiation of EAR from technical complications or a small-for-size graft dysfunction. Moreover, there is no

agreement regarding both the outcome and treatment of histological ACR with or without a biochemical abnormality early after LDLT. From this viewpoint, this study examined the results of a first single-center study, using protocol liver biopsies performed on the 10th postoperative day after LDLT to determine the incidence, severity and factors contributing to the development of EAR as well as to assess impact on the outcomes of EAR in adult LDLT recipients.

Patients and methods

Patients

Sixty-seven consecutive adult LDLT recipients, who were diagnosed with hepatitis B virus (HBV) related end stage liver disease, underwent a liver biopsy on the 10th postoperative day at our institution between September 2002 and August 2004. Of these, 62 patients, who also underwent a multiphase computed tomography (CT) scan on the same day to precisely detect technical problems, were finally enrolled in this study. The patients were followed up for a median of 23.5 months (range, 0–39 months). Hepatocellular carcinoma (HCC) was diagnosed in 25 patients (40.3%). There were 41 men and 21 women, ranging in age from 24 to 65 years of age (median, 49 years). The United Network for Organ Sharing (UNOS) status was I or IIA in nine patients, and IIB or III in 53 patients. The Child–Pugh classification was class A or B in seven patients and class C in 55 patients. The calculated Model for End Stage Liver Disease (MELD) scores immediately before LDLT ranged from 8 to 42 (median, 23). Lymphocytotoxic crossmatch and flow cytometry were performed routinely before surgery in all cases, as previously described [5]. Three patients (4.8%) were positive for the cytotoxic test.

Donors and grafts

The relevant donors consisted 47 men and 15 women, ranging in age from 19 to 52 years old (median, 27 years) and in body mass index from 18.3 to 32.0 kg/m^2 (mean, 23.6 kg/m^2). All grafts were blood type ABO identical ($n = 49$) or were compatible ($n = 13$) with the relevant recipients. The type of graft consisted of 50 right livers and 12 left livers. Of the 50 right liver grafts, five grafts included the middle hepatic vein. The graft to recipient weight ratio ranged from 0.60% to 1.67% (mean, 1.05%). The mean operation time was 545 ± 108 min, and the cold and war ischemic times were 79 ± 30 and 40 ± 13 min, respectively. Of the 62 grafts, 36 grafts (58.1%) were gender matched and 26 grafts (41.9%) were gender mismatched.

Liver biopsy and histopathological analysis

Protocol liver biopsies were routinely performed on the 10th postoperative day after obtaining informed consent from each patient. All biopsy specimens were obtained using a uniform procedure using an 18-gauge percutaneous biopsy needle under ultrasound guidance. Fresh liver sections were embedded in paraffin, sectioned, and routinely stained with hematoxylin and eosin, Masson–Trichrome, and reticulin staining. The sections were analyzed by experienced hepatopathologists who were blinded to the laboratory parameters and clinical data. During the follow-up, an additional liver biopsy was also carried out if a biochemical abnormality developed and clinical ACR was suspected. A follow-up CT scan was also performed if technical complications were suspected. ACR was graded according to the Banff schema [6]. The rejection activity index (RAI) was also calculated by adding the portal tract, bile duct, and venous endothelial inflammation scores.

Immunosuppression, antirejection therapy, and antiviral treatment of HBV

Immunosuppression was based on a flexible double-drug protocol. The maintenance immunosuppressive agents used during the study period consisted primarily of a calcineurin inhibitor and a corticosteroid. The primary immunosuppressant was tacrolimus for 39 patients and cyclosporine for 23 patients. Patients initially receiving tacrolimus were treated with a dose of 0.05 mg/kg/day orally twice a day within the first 48 h after surgery, with target trough whole blood concentration of 13–17 ng/ml for the first 2 weeks after the transplant. This was followed by 8–13 ng/ml for the first 3 months, and 5–8 ng/ml thereafter. Patients initially receiving cyclosporine were treated with a dose of 2–4 mg/kg/day orally twice a day within the first 48 h after surgery, with target trough whole blood concentrations of 300–450 ng/ml for first 2 weeks after the transplant. This was followed by 200–300 ng/ml for first 3 months, and 100–200 ng/ml thereafter. Methylprednisolone was given before the portal and arterial reperfusion, twice at a bolus of 0.5 g, and then tapered for 6 days. Oral prednisone (20 mg/day) was initiated at the 7th postoperative day and was tapered out over 6 months.

If ACR occurred, it was treated with high-dose steroid pulse therapy, a switch of immunosuppression (e.g. from cyclosporine to tacrolimus), or an augmentation of immunosuppression.

All patients received postoperative combination prophylaxis with hepatitis B immune globulin and lamivudine.

Definition of rejection and biochemical abnormality, and patients grouping

Histological EAR was defined as a RAI ≥3 based on the results of the protocol biopsy regardless of the presence of biochemical abnormalities. The patients studied were categorized into EAR− group (RAI < 3; $n = 41$) or EAR+ group (RAI ≥3; $n = 21$) according to the RAI. A biochemical abnormality was defined when the transaminase elevations were greater than twice the upper limit of normal (reference value, 0–40 IU/l) and/or the total bilirubin elevations were greater than twice the upper limit of normal (reference value, 0.2–1.2 mg/dl) [7]. Morphological EAR was detected using a biopsy routinely performed and confirmed by histology when there was no biochemical abnormality, and was defined as subclinical EAR. Subsequent ACR was diagnosed by an additional biopsy during the follow-up when a subsequent biochemical abnormality was observed.

Statistical methods

The continuous normally distributed variables are reported as means ± standard deviation and the discontinuous variables are expressed as the median (range). A Fisher's exact test was used to determine if there was a difference in the distribution of nonparametric variables between the groups. The group means were compared using a Mann–Whitney *U*-test. Binary logistic regression was used to assess the relative influence of the variables on the categorical data. An enter method was used to remove any nonsignificant variables and to determine the most parsimonious model including both fixed factors and covariates.

The survival rates were calculated using the Kaplan–Meier method and the differences between the groups were assessed using a log-rank test. The Cox proportional hazards model was used to assess the effects of the prognostic factors. All analyses were carried out using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA) and a P-value < 0.05 was considered significant.

Human subjects

This study was performed on archival histologic material that had been obtained as part of routine clinical practice.

Results

Incidence of early clinical and subclinical rejection after LDLT

According to the definition used, 21 (33.9%) out of the 62 patients enrolled in this study had histological evidence of EAR on biopsy. Of these 21 patients with histological EAR, 14 had biochemical abnormalities (clinical EAR) and seven had no associated biochemical abnormalities (subclinical EAR) on the 10th day after LDLT. This subclinical EAR composed 11.3% of the entire study population (Fig. 1).

Overall, 18 (29.0%) patients experienced a biochemical abnormality on the 10th day after LDLT. Biochemical abnormalities were observed more frequently in the EAR+ group (66.7%) than in the EAR− group (9.8%; $P < 0.001$). Interestingly, four patients with a biochemical abnormality without histological EAR eventually experienced biliary complications (Fig. 2).

Factors that influence developing EAR

Table 1 summarizes the preoperative characteristics of the patients, donors, and grafts. The gender distribution of

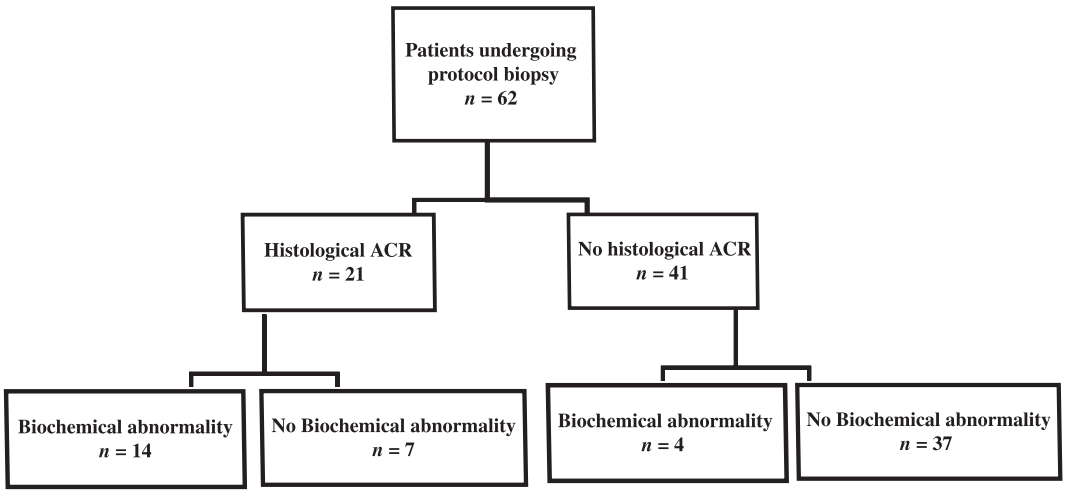

Figure 1 Histologic findings of the 62 patients undergoing a protocol biopsy.

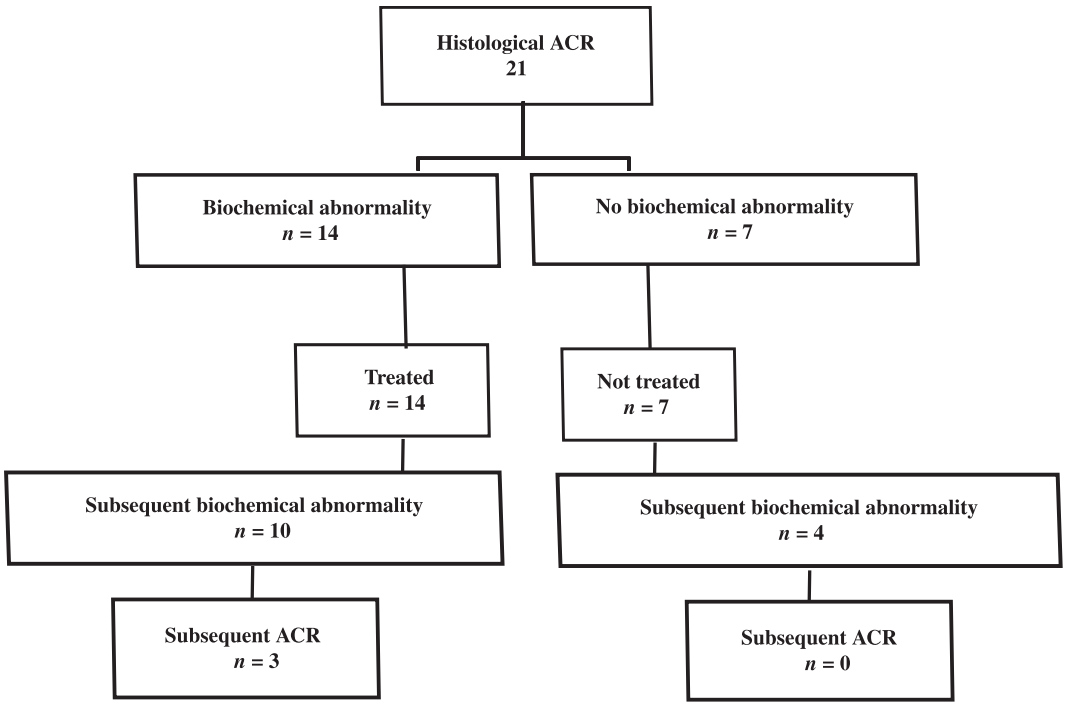

Figure 2 Outcome of patients with histological early allograft rejection with or without biochemical abnormalities.

Table 1. Recipients, donor, and graft characteristics of the patients.

	EAR– N = 41	EAR+ N = 21	*P*-value*
Recipient age	50.4 ± 8.4	45.9 ± 9.3	0.034
Recipient gender (M/F)	27/14	14/7	1.00
UNOS status (I + IIA/IIB + III)	5/36	4/17	0.472
Calculated Model for End-stage Liver Disease score	23.5 ± 8.5	24.4 ± 9.7	0.930
Donor age	28.2 ± 8.3	33.1 ± 9.7	0.048
Relation (Related/Un-related)	36/5	18/3	1.00
Donor gender (M/F)	34/7	13/8	0.115
Gender match (Matched/Mismatched)	29/12	7/14	0.007
Graft-to-recipient weight ratio (%)	1.09 ± 0.23	0.98 ± 0.23	0.045
Type of graft (Right/Left)	34/7	16/5	0.520
Operation time (min)	546.3 ± 96.9	542.4 ± 128.9	0.447
Cold ischemic time (min)	76.3 ± 27.2	85.6 ± 34.9	0.451
Warm ischemic time (min)	40.6 ± 11.9	39.86 ± 15.5	0.548
ABO match (Identical/Compatible)	31/10	18/3	0.514
Cytotoxic Ab (Negative/Positive)	39/2	20/1	1.00
Immunosuppression (Tacrolimus/Cyclosporine)	25/16	14/7	0.784

*Statistical tests were Mann-Whitney for continuous variables, and Fisher's exact test for discontinuous variables.

both recipients and donors was similar in the patients with or without EAR. There was no difference in the severity of disease immediately before the LDLT in terms of the UNOS status or the calculated MELD score. The type of graft, operation time and ischemic time were similar in the two groups. The proportion of positive cytotoxic antibody and the type of primary immunosuppressant used were also similar. Patients who experienced EAR were younger and their grafts were smaller than those did not experienced EAR ($P = 0.034$ and 0.045, respectively). In the EAR+ group, the donor age was older and the proportion of gender mismatch was higher ($P = 0.048$ and 0.007, respectively). Multivariate analysis showed that gender mismatch (female donor to male recipient) was the only independent risk factor for the development of EAR with an odds ratio (OR) of 13.458 [95% confidence interval (CI), 1.836–98.649; $P = 0.011$].

The natural history of clinical and subclinical EAR

Of the 14 patients with clinical EAR, all patients were managed using pulse steroid ($n = 4$), the addition of mycophenolate mofetil ($n = 5$), and the augmentation of immunosuppression ($n = 5$). After treating the clinical EAR, a subsequent biochemical abnormality developed within 1 year after LDLT in 10 patients. Of them, a subsequent ACR was observed in three patients, a recurrence

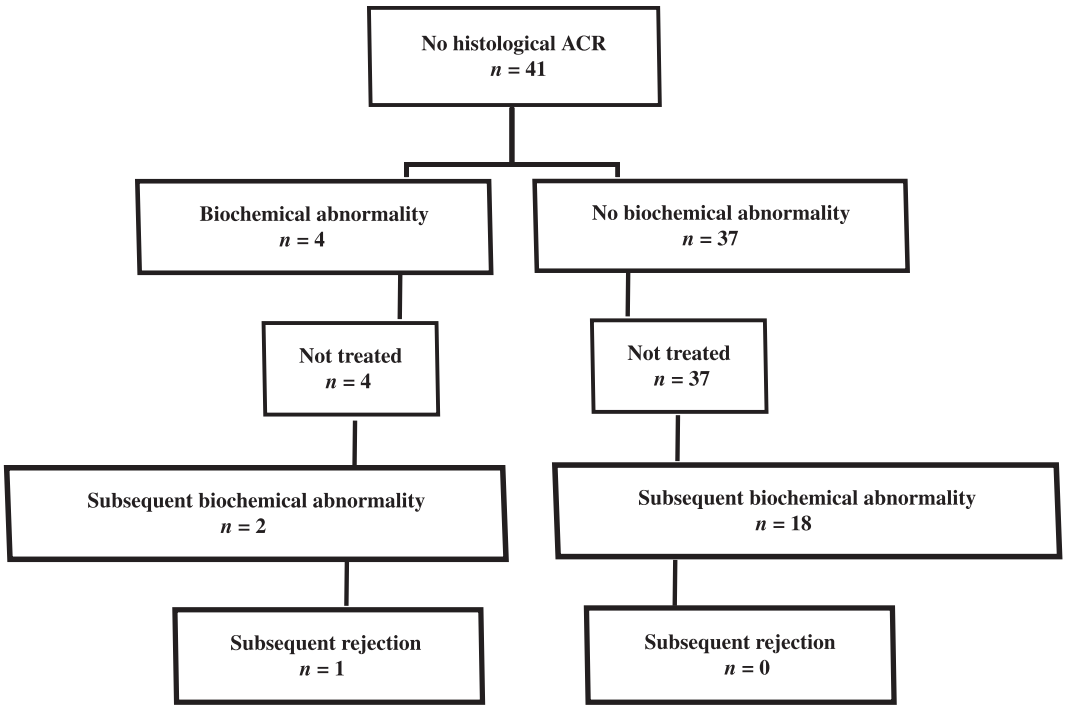

Figure 3 Outcome of patients without histological early allograft rejection with or without biochemical abnormalities.

of the HBV was encountered in one, a recurrence of the HCC in one, and biliary complications in five patients. The incidence of a subsequent ACR was similar irrespective of the types of treatment for clinical EAR ($P = 0.239$). A subsequent ACR was treated by switching the immunosuppression from cyclosporine to tacrolimus ($n = 2$) or augmenting tacrolimus ($n = 1$), and all patients showed a response to each antirejection therapy.

None of the seven patients with subclinical EAR were treated. Of them, a subsequent biochemical abnormality developed in four patients but no subsequent ACR was observed. These four patients were eventually found to have biliary complications ($n = 2$), post-transplant diabetes mellitus ($n = 1$), or a recurrence of a HBV infection ($n = 1$).

Impact of EAR or biochemical abnormalities on the patient outcome

Overall, four subsequent ACR (22.2%) were encountered in 18 patients with initial biochemical abnormalities, while no ACR developed in the 44 patients without an initial biochemical abnormality ($P = 0.005$) (Fig. 3). During the follow-up, the overall proportion of patients who developed a subsequent biochemical abnormality was similar between the EAR+ groups and EAR− groups ($P = 0.281$). Within the first 12 months after LDLT, 82.4% of at least one episode of subsequent ACR developed with a median time of 5.5 months after LDLT. The overall cumulative probability for the occurrence of subsequent ACR within 12 months was 6.9% (Fig. 4a). The cumulative probability for the occurrence of subsequent ACR within 12 months in the EAR+ group was higher than that in the EAR− group (OR = 11.085; 95% CI, 1.221–100.654; $P = 0.033$; Fig. 4b). However, there was no difference in the severity or the time of occurrence of a subsequent ACR after LDLT between the patients with or without EAR ($P = 0.564$ and 0.180, respectively).

Survival analysis using the Kaplan–Meier survival function was performed to determine if the patient's survival was affected by the presence of EAR. The actuarial 1-year patient and graft survival rate of the EAR− group (92.7% and 97.25%, respectively) were higher than those in the EAR+ group (81.0% and 85.5%, respectively) but this was not statistically significant ($P = 0.1265$ and 0.3022, respectively; Fig. 5a and b). The severity of EAR also did not affect the patients' survival ($P = 0.812$). After adjusting by Cox regression analysis, the presence of biochemical abnormalities remained an independent risk factor for decreased patient survival (OR = 5.827; 95% CI, 1.095–31.017; $P = 0.039$). Moreover, the presence of biochemical abnormalities was an independent risk factor for graft loss (OR = 20.646; 95% CI, 2.044–208.524; $P = 0.010$). The 1-year patient and graft survival rate of the patients without biochemical abnormalities (93.0% and 100%, respectively) were significantly higher than in those in

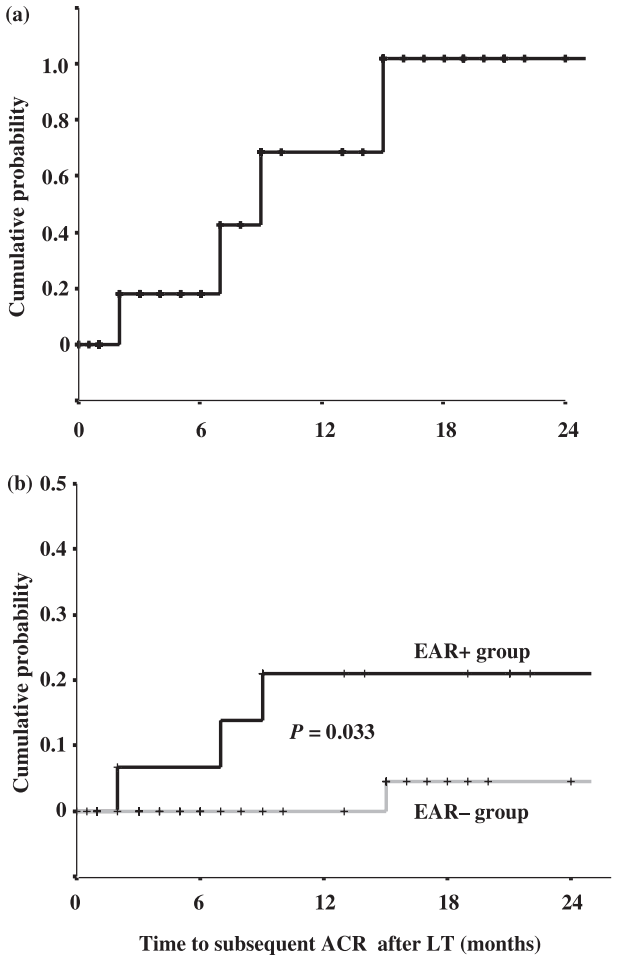

Figure 4 Cumulative overall (a) probability of developing subsequent acute rejection within 12 months and cumulative probability between patients with or without early allograft rejection (b).

patients with biochemical abnormalities (65.0% and 77.0%, respectively; $P = 0.0051$ and 0.0012, respectively; Fig. 5c and d).

Biopsy related complications

Of the 62 patients who underwent a protocol liver biopsy on the 10th day after LDLT, 11 (17.7%) experienced procedure-related complications. The incidence of complications was similar in terms of the presence of EAR or biochemical abnormalities ($P = 0.485$ and 0.271, respectively). Mild transient transaminase elevations ($n = 7$) were observed in most patients. Two cases of an intraperitoneal hematoma were encountered and percutaneous catheter drainage was needed in one of them. Two patients experienced potentially life-threatening intrahepatic hemorrhage and transarterial embolization was performed. This patient survived without any long-term complication.

Discussion

Acute cellular rejection has a peak incidence at 1–2 weeks post-LT and is usually accompanied by an increase in the serum transaminase and bilirubin levels [8]. However, none of the biochemical or clinical features of ACR are specific, and a liver biopsy is generally needed to confirm the diagnosis [9,10]. Because EAR does not develop in all patients after LT, it is possible that identifying patients with a greater risk of rejection could be useful for modifying the immunosuppressive regimen. Some centers routinely perform protocol biopsies 1–2 weeks post-LT to search for evidence of EAR even in the absence of biochemical abnormalities. The implicit assumption underlying this practice is that an early diagnosis and treatment of EAR at the subclinical stage is beneficial [11]. Subclinical rejection is an entity defined by the presence of morphological signs of rejection but a lack of any signs of clinical rejection. Therefore, this condition can only be detected with a routine biopsy obtained from grafts with normal function or with a dysfunction not attributed to rejection [12].

By the definition of this study, approximately one-third of LDLT recipients experienced histological EAR after LDLT, and approximately one-third of histological EAR patients had no accompanying biochemical abnormalities. The incidence of EAR was greater than in those reported in centers performing a liver biopsy only when clinically indicated [13]. This is presumably because the criteria used to diagnose ACR include portal infiltrates, but these are not always associated with target organ damage [14]. The development of subclinical EAR is relatively rare (11.3% of the study population in this study). The proportion of accompanying biochemical abnormalities in LDLT recipients with histological EAR was similar to that in deceased donor liver transplant recipients, but the incidence of histological EAR itself (33.9%) after LDLT found in this study was lower than that reported for the protocol biopsy based incidence after deceased donor LT (67%) [15]. Patients with hepatitis B related cirrhosis are known to develop ACR during the follow-up less frequently than those with other diseases, indicating that the immune system in patients chronically infected by hepatitis B is disturbed [16,17]. In addition, hyperimmune immunoglobulin administered to these patients prophylactically to avoid graft reinfection also could have contributed to the decreased incidence of EAR because of its immunosuppressive effects [18,19].

After LDLT, approximately one-third of recipients experienced a biochemical abnormality in the early post-LDLT period. Interestingly, none of the patients without a biochemical abnormality on the 10th post-LT day experienced clinical ACR. However, the presence of EAR

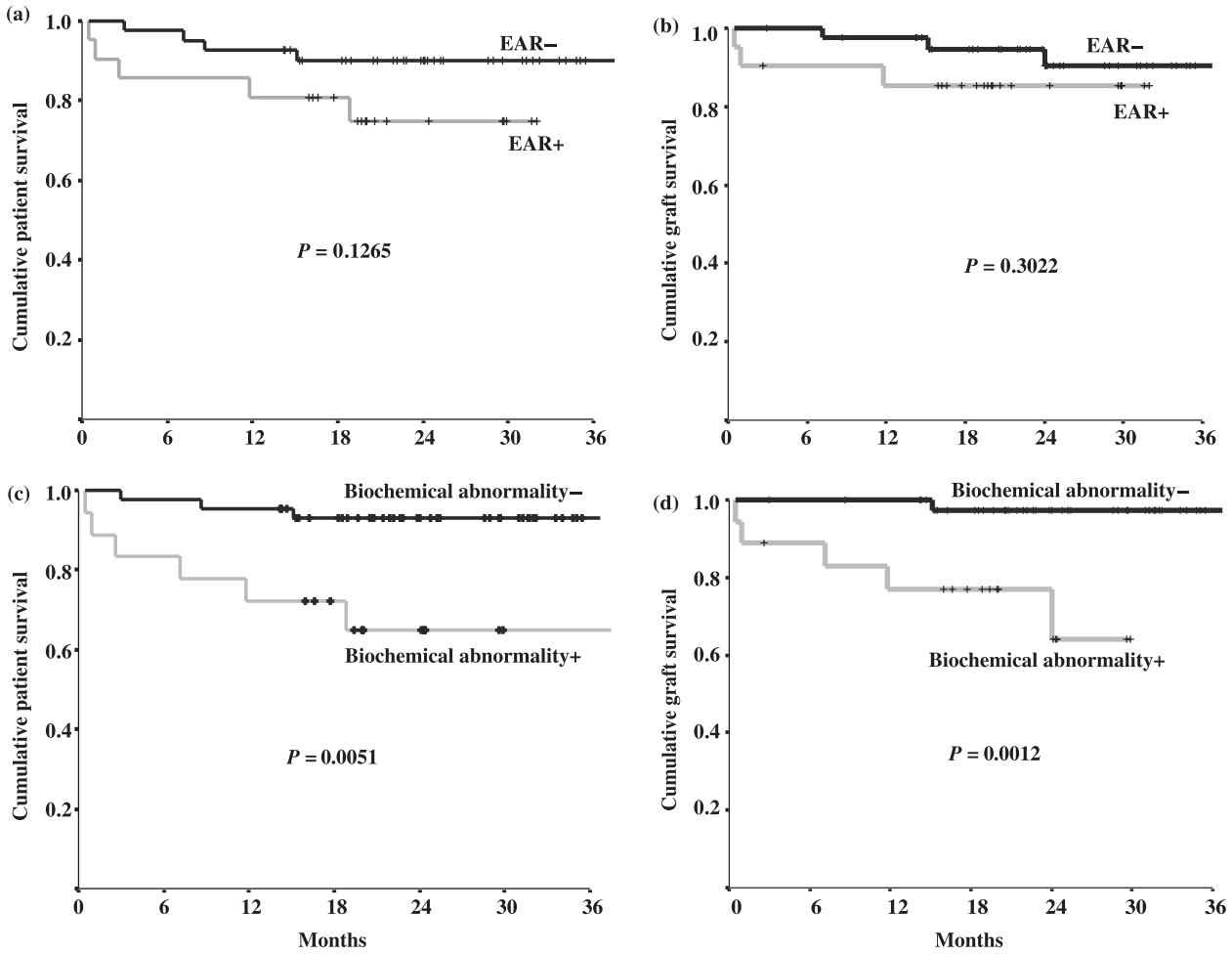

Figure 5 Cumulative patient (a) and graft (b) survival rates of the patients with or without histological early allograft rejection, and the cumulative patient (c) and graft (d) survival rates of the patients with or without a biochemical abnormality.

increased the risk of a subsequent development of treatment-required ACR within 12 months more than 11-fold, even though the presence of EAR did not affect the patient or graft survival. The patient and graft survival were determined not by the presence of EAR but by the presence of biochemical abnormalities. It undoubtedly is true that a sizeable group of LT recipients do not require treatment for EAR without biochemical abnormalities and possibly even in the presence of biochemical abnormalities given that tolerance is probably characterized by a graft infiltration that is indistinguishable from that defined as cellular rejection [20]. Based on these observations, there does not appear to be any disadvantage in withholding treatment in patients with histological EAR until they develop a biochemical abnormality. By taking this approach, unnecessary adjuvant immunosuppression can be safely avoided in patients with histologic ACR and a normal graft function.

The justification for performing protocol biopsies is based on the following grounds: the liver biochemistry tests have a poor sensitivity and specificity in the diagnosis of a graft dysfunction; liver tests provide little information on the severity of graft damage; the graft function is better preserved if the liver damage is diagnosed and treated early; and knowledge of the histological changes in the allograft under different clinical situations results in a better management of the overall patient population [11]. On the other hand, the liver biopsy itself is associated with small but definite risks, including hemorrhage, bile leakage, and bacterial sepsis. These risks are increased post-LT by factors such as thrombocytopenia and platelet dysfunction, ascites, biliary obstruction, and Roux-en-Y biliary reconstruction [9,21]. In this study, adverse events occurred in 17.7% of patients undergoing a liver biopsy after LDLT with the possibility of life-threatening complications. Therefore, it appears prudent to avoid liver biop-

sies that do not have a potentially therapeutic indication in the absence of biochemical abnormalities.

In conclusion, the incidence of subclinical EAR was relatively low but subsequent ACR developed more frequently in those patients with EAR than in those without. However, the presence of EAR did not affect the patients' survival after LDLT. A protocol liver biopsy is not recommended only as a therapeutic indication in patients without biochemical abnormalities because there is no obvious therapeutic benefit but definite biopsy-related risks.

Conflict of interest

Nothing to declare.

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