

Supplementary Materials and Methods

Study population

A total of 121 adult patients (≥ 18 years) who underwent primary liver transplantation (LT) from donation after circulatory death (DCD) at the authors' center from January 2014 to January 2016 was included in this study. Exclusion criteria were multiple-organ transplantation, split LT or missing pretransplant biopsy specimens. With the application of the same inclusion and exclusion criteria, a total of 37 patients receiving LT from DCD between November 2016 and June 2017 composed an independent validation group to verify the predictive capability of the proposed model of this study.

Data on surgical technique, perioperative management, immunosuppression and follow-up protocol are provided elsewhere.^{1,2} Patient demographics and clinical characteristics, and biochemistry parameters were obtained from the hospital's electronic medical records. Baseline serum biochemical indexes of hepatorenal function were defined as the first recorded intraoperative laboratory values or values measured immediately before operation.³

Protocol liver biopsy and allograft weighting

Procurement of DCD livers and pretransplant graft biopsy have been described in our previous studies.⁴ Briefly, all DCD donors received intravenous heparin before artificial life support was withdrawn. Three physicians unrelated to the

surgical procurement or the transplant team were assigned to declare cardiac death following a 2-minute mandatory waiting period from the time of cardiac asystole. Donor livers were flushed with preservation solution via both the abdominal aorta and inferior mesenteric vein and stored at 4°C for transport.

During the back-table procedure after cold preservation and before implantation, a wedge biopsy in the left lateral liver lobe was routinely performed for DCD livers; the obtained tissues were formalin-fixed and embedded in paraffin. Moreover, the “back-table” of our center systematically includes a precise measurement of the allograft weight (in grams). Just before the start of the case, all intended DCD liver grafts were weighed after they were flushed with preservation fluid, drained of biliary fluids, and underwent the removal of ligaments attached and all tissues surrounding the vessels. Of note, values of graft biopsy histology in predicting the development of EAD have been carefully investigated in our and others’ prior studies without useful features identified.^{4,5} Hence, histological parameters were not considered in the present investigation.

Immunohistochemical analysis

The immunohistochemistry (IHC) staining procedure has been described previously according to a standard protocol.⁶ Paraffin-embedded tissues, obtained from liver wedge biopsy, were sliced into 5-µm-thick sections, deparaffinized, and rehydrated. A 3% H₂O₂ solution was used to block

endogenous peroxidase activity. After the sections were washed with phosphate-buffered saline (3 times, 5 minutes for each time), they were immersed in 10 mmol/L citrate buffer solution (pH 6.0, heated by a 750-W microwave) for 10–15 minutes. After cooling down, the sections were washed with phosphate-buffered saline and then blocked with normal foetal bovine serum (37°C) for 10 minutes; the serum was then decanted. Subsequently, the sections were incubated with a panel of 7 primary antibodies overnight at 4°C, which targeted vascular cell adhesion molecule-1 (VCAM-1; AB134047, Abcam; 1:500), intercellular adhesion molecule (ICAM-1; BA0406, Boster; 1:100), endothelin-1 (AB2786, Abcam; 1:250), cytochrome C (AB13575, Abcam; 1:1000), vascular endothelial growth factor (VEGF; PB0084, Boster; 1:100), NADPH oxidase-1 (NOX-1; BA3335, Abcam; 1:50) and NADPH oxidase-2 (NOX-2; AB139371, Abcam; 1:250). Negative controls consisted of omission of the primary antibody. Afterwards, the sections were washed with phosphate-buffered saline (3 times, 5 minutes for each time), and secondary antibodies were then applied (37 °C, 30 minutes). The slides were rinsed in phosphate-buffered saline again (3 times, 5 minutes for each time), treated with diaminobenzidine for 2 minutes, rinsed in distilled water, and finally counterstained with hematoxylin.

The extent of antibody staining on every slide was semiquantitatively assessed in a dichotomized fashion (high or low expression) and considered both the staining intensity and area.⁷ Specifically, staining intensity, which was

tailored for specific IHC biomarkers, was scored as follows: 1 (weak), 2 (moderate), and 3 (strong). Examples of the intensity grading are depicted in Figure S1. Staining area was expressed by the positively stained percentage. For NOX-1, VEGF and Cytochrome C, the staining area was assigned scores of 0 (positivity of less than 10% of hepatocytes), 1 (positivity of 10%–30% of hepatocytes), 2 (positivity of 30%–60% of hepatocytes) and 3 (positivity of more than 60% of hepatocytes). Likewise, the staining areas of ICAM-1, VCAM-1, Endothelin-1 and NOX-2 were also assigned scores of 1, 2, and 3 when approximately 10%–30%, 30%–60%, and >60% of the sinusoidal liver cells demonstrated immunoreactivity, respectively, and 0 if less than 10% of the cells were positive. At least five randomly chosen and non-overlapping fields at a magnification of $\times 200$ were required, with avoidance of inflammation infiltrate or peripheral regions. Then, a synthesized score was calculated as “the staining intensity score \times the area score”. Protein expression was regarded as low if the synthesized score was <4 ; otherwise, it was categorized as high (score ≥ 4). The slides were reviewed by two experienced pathologists who were blinded to the study design. If there was substantial disagreement between these 2 pathologists, the slide was evaluated by a third experienced pathologist who was also blinded to the study design.

Definitions

Early allograft dysfunction (EAD) was defined as the presence of at least 1

of the following: 1) bilirubin ≥ 10 mg/dL (171 μ mol/L) on postoperative day (POD) 7; 2) international normalized ratio ≥ 1.6 on POD 7; or 3) alanine aminotransferase or aspartate transaminase >2000 IU/L within the first postoperative week.⁸ Acute kidney injury (AKI) was defined as a $>50\%$ rise in the post-LT serum creatinine from the baseline level.^{9,10}

Patient and graft survival

Patient and graft survival was calculated from the date of transplant to death or last follow-up, and graft loss was defined as either recipient death or re-transplantation. During a median follow-up of 22.1 months, the overall 6-, 12- and 18-month survival of the 121 recipients was 93.4%, 90.1% and 87.6%, respectively. The graft 6-, 12-, and 18-month probabilities of graft survival were 92.6%, 89.3%, and 86.8%, respectively. Fifteen recipients in this cohort (15/121) died, 7 because of hepatocellular carcinoma (HCC) recurrence, four because of sepsis, three because of multiple-organ failure and one because of HBV-related liver failure. One patient underwent re-transplantation due to graft failure.

Statistical analysis

The Shapiro-Wilk test was performed to determine the normality. Quantitative variables are expressed as the mean \pm standard deviation or median (25th–75th percentile) in tables and text, and categorical variables are described as numbers (percentages). For univariate comparisons, Student's *t*-test was used

for normally distributed variables, Mann-Whitney test when variables were skewed, and Pearson's Chi-square test or Fisher's exact test for categorical variables. Bivariate correlation was analysed using Spearman correlation analysis.

Variables with $P < 0.10$ in the univariate analysis were entered into a multivariate logistic regression analysis to identify independent predictors of EAD and establish the predictive model.¹¹ The method of Hosmer and Lemeshow was used to evaluate goodness of fit. A receiver operating characteristic (ROC) curve was created to examine the diagnostic efficacy and determine the optimal cut-off value.

The survival analysis was conducted using the Kaplan-Meier method, and the log rank test was used for comparisons between groups. Univariate and multivariate Cox analyses were used to identify independent prognostic factors. Statistical analysis was carried out using IBM SPSS Statistics 19.0 (SPSS Inc., Chicago, IL). All P -values were two-tailed, and $P < 0.05$ was considered statistically significant.

Supplementary References

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Staining Intensity Grading (x 200 magnification)

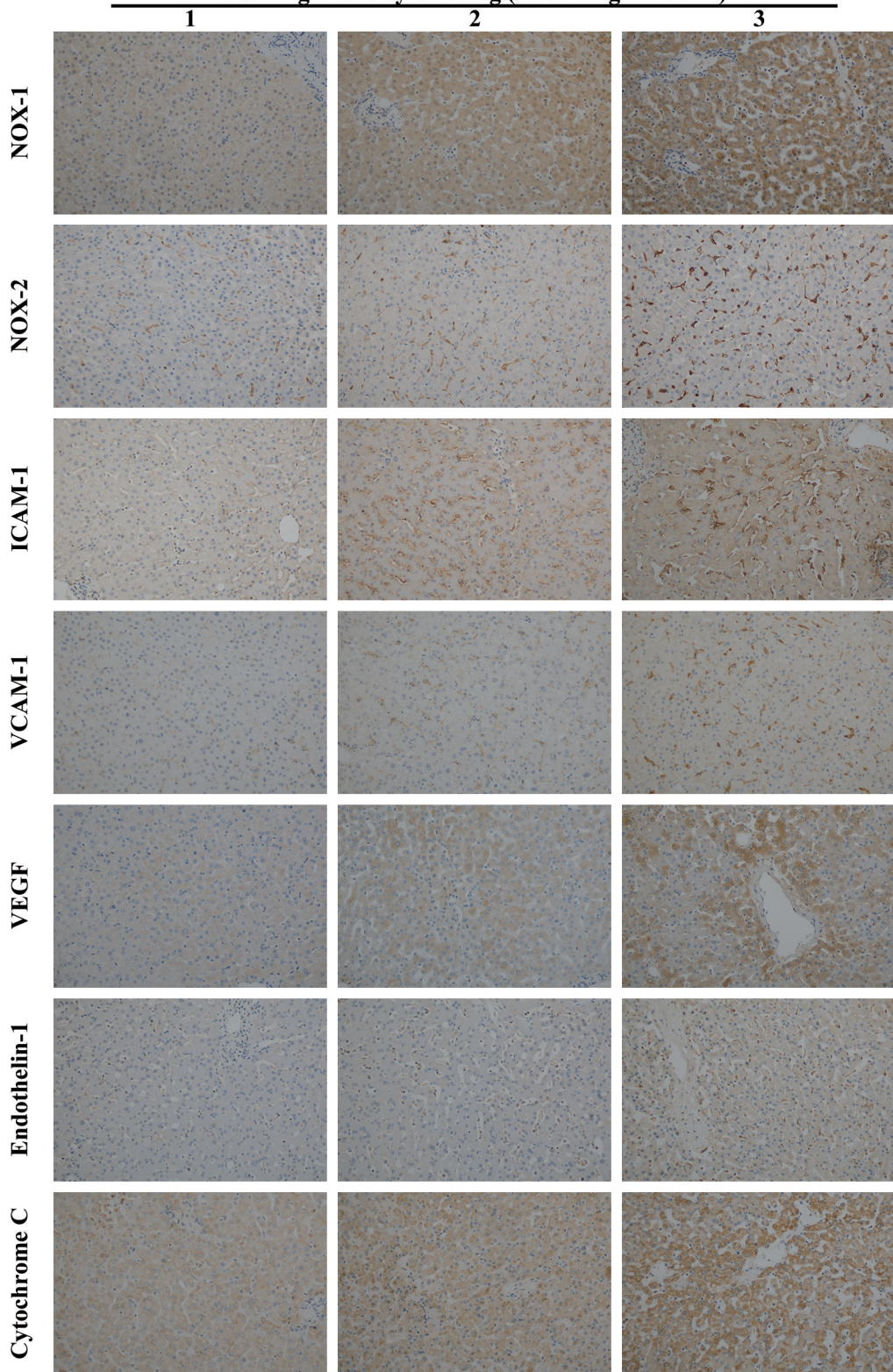


Figure S1. Representative photos for three staining intensity grades (1/2/3) of the expression levels of seven studied protein biomarkers in pretransplant graft biopsy specimens (Original Magnification: $\times 200$). One image in Figure 1(Non-EAD group, ICAM-1, $\times 200$ Magnification) was used here (ICAM-1, grade 1)

Table S1. Univariate and multivariate Cox analyses of variables related to the recipient survival

Variables	All subjects				D-MELD <1600			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i> values	OR (95% CI)	<i>P</i> values	OR (95% CI)	<i>P</i>
EAD	2.883 (0.985- 8.436)	0.053			2.865 (0.863- 9.516)	0.086		
RRT requirement	3.819 (1.214- 12.012)	0.022			2.115 (0.463- 9.654)	0.334		
Recipient MELD	1.063 (1.012- 1.116)	0.014			1.039 (0.985- 1.095)	0.162		
Macrovesicular steatosis ≥30%	2.168 (0.489- 9.611)	0.308			2.708 (0.593- 12.364)	0.198		
Allograft weight, per g	1.002 (1.001- 1.002)	0.007	1.003 (1.001- 1.005)	0.001	1.002 (1.000- 1.004)	0.025	1.003 (1.001- 1.005)	0.004

	1.004)		1.004)		1.004)		1.004)
High NOX-1	2.969 (1.057-	0.039			2.673 (0.848-	0.093	
expression	8.344)				8.422)		
High VEGF	4.226 (1.444-	0.009	5.730 (1.894-	0.002	6.088 (1.647-	0.007	8.369 (2.186-
expression	12.368)		17.338)		22.498)		32.041)

CI, confident interval; EAD, early allograft dysfunction; MELD, model for end-stage liver diseases; NOX, NADPH oxidase; OR, odds ratio; RRT, renal replacement therapy; VEGF, vascular endothelial growth factor.