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BRIEF REPORT

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Donor des-gamma-carboxy prothrombin positivity is a risk factor for poor early graft function in liver transplantation

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Abstract Des-gamma-carboxy prothrombin (DCP) is an abnormal prothrombin that lacks coagulating activity. The aim of this study was to determine if the presence of DCP in the donor could be used as a marker of post-transplant graft function. We collected data and serum samples on 90 organ donors. DCP level was correlated with donor-specific factors and with graft function intraoperatively and in the early post-transplant period. Twenty-seven donors (30.0%) had positive DCP levels before harvesting. Although recipients were similar in demographics, preoperative liver function, and primary disease distribution, patients transplanted with livers from DCP-positive donors needed significantly more intraoperative transfusion. Furthermore, donor DCP positivity was identified as a preoperative risk factor for poor early graft function based on multivariate analysis (odds ratio = 6.58, $P = 0.0032$). Our findings suggest that DCP is another valuable marker for evaluating the quality of donor livers.

Key words Liver transplantation, des-gamma-carboxy prothrombin · Graft function, des-gamma-carboxy prothrombin, liver transplantation · Des-gamma-carboxy prothrombin, liver transplantation

Introduction

Prothrombin is a vitamin K-dependent blood coagulation protein that is synthesized in the liver. In the serum of patients deficient in vitamin K or receiving vitamin K antagonists, an abnormal prothrombin appears that lacks coagulant activity [1]. This abnormal prothrombin is called des-gamma-carboxy prothrombin (DCP) or protein induced by vitamin K absence or antagonist-II (PIVKA-II). It is known that vitamin K deficiency is responsible for the bleeding disorders observed in patients with malabsorption, biliary obstruction, biliary fistula, and hemorrhagic disease in newborns. In 1984, Liebman et al. reported that DCP levels were increased in patients with hepatocellular carcinoma (HCC) [5]. Since then, DCP has been used as a tumor marker for HCC [13]. Severe liver disease can also be associated with this dysfunctional prothrombin and with significant bleeding disorders [1]. Recently, we found that increased plasma DCP levels were associated with severe acute cellular rejection after liver transplantation [12]. In this study, we report that DCP may be a useful marker for donor liver evaluation.

Patients and methods

Study population

Ninety organ donors were evaluated (58 men, 32 women). Their ages ranged from 7 to 73 years (mean 36 years). Peak and last AST, ALT, bilirubin, prothrombin time (PT), protein, albumin, cholesterol, and platelet levels during intensive care unit (ICU) stay of the donor were recorded. The livers were flushed and preserved in University of Wisconsin (UW) solution at 4 °C for variable periods (mean: 563 ± 138 min) and transplanted into 90 adult recipients. Livers with more than 50% macrovesicular fat (as

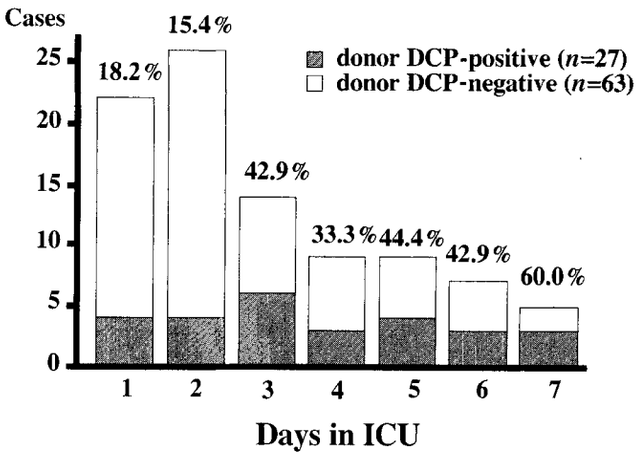

Fig. 1 Prevalence of donor DCP positivity according to the length of stay in ICU. Twenty-seven of 90 donors showed positive DCP levels before harvesting. The number of DCP-positive donors increased with the time spent in the ICU

shown by biopsy) are routinely discarded and were excluded from this study. Blood samples were obtained from organ donors before crossclamp and centrifuged at 3000 rpm for 10 min.

DCP assay

The plasma DCP concentration was measured by an EIA method using a monoclonal antibody specific for DCP (Eitest MONO P-II, Eisai Co., Tokyo) [7]. A DCP level of 0.0625 arbitrary unit(AU)/ml is the lowest detectable value with this assay. The linear curve from which the assay sensitivity is extracted ranges from 0.06 to 8.0 AU/ml. Levels greater than 0.0625 AU/ml were considered positive. Coefficient values of variances (CV) for samples repeated in the same assay were found below 3%. CV values were below 10% from run to run. All samples, including standards, were run in duplicate.

Early graft function

In recipients, peak AST and ALT levels for the first 3 postoperative days (POD) and prothrombin time on POD 2 were also recorded. Poor early graft function (PEGF) was defined as peak AST or ALT above 2500 U/l and PT above 16 s. Retransplantation or death within 7 days because of PEGF was considered primary graft nonfunction (PNF).

Statistics

Statistical evaluation was performed using the Mann-Whitney test, the Spearman rank correlation coefficient test, the Yates' continuity-corrected chi-square test, and McNemar's chi-square test. To evaluate the prognostic influence of potential risk factors, a stepwise logistic regression model was used. All statistical results were considered significant at a P value below 0.05.

Table 1 The relationship between donor DCP positivity and donor characteristics

	Donor DCP		P value
	Positive (n = 27)	Negative (n = 63)	
Days in ICU	3.74 + 1.95	2.54 + 1.55	0.0026
Age	36.6 + 17.9	32.5 + 20.6	0.3751
Male/female	16/11	42/21	0.5011
AST peak (U/l)	99.0 + 189.2	104.2 + 83.85	0.8572
AST last (U/l)	78.7 + 141.4	72.5 + 57.6	0.7965
ALT peak (U/l)	57.5 + 90.68	66.6 + 83.8	0.6598
ALT last (U/l)	45.5 + 70.47	47.8 + 48.7	0.8658
Bilirubin peak (mg/dl)	1.20 + 1.01	1.90 + 5.93	0.5699
Bilirubin last (mg/dl)	0.77 + 0.63	1.60 + 5.86	0.4664
PT first (s)	13.14 + 1.64	13.35 + 2.07	0.6518
PT last (s)	13.61 + 1.78	13.71 + 2.14	0.8265
Albumin first (g/dl)	3.74 + 0.94	3.47 + 0.87	0.1438
Albumin last (g/dl)	3.18 + 0.69	3.04 + 0.75	0.2594
Total prothrombin (mg/dl)	56.98 + 24.08	69.15 + 28.76	0.4804
Cardiac arrest	8 (29.6%)	4 (6.3%)	0.2250
Cause of death:			
Intracranial bleeding	13 (48.1%)	31 (49.2%)	
Other	1 (3.7%)	2 (3.2%)	
Blood type: 0	16 (59.3%)	32 (50.8%)	0.2173
A	6 (22.2%)	24 (38.1%)	
B	5 (18.5%)	5 (7.9%)	
AB	0 (0%)	2 (3.2%)	
Rh-positive	23 (85.2%)	60 (95.2%)	0.1027
CMV-positive	16 (59.3%)	35 (55.6%)	0.7452
Ischemic time: Cold (min)	575.7 + 162.4	549.7 + 149.9	0.4639
Warm (min)	51.9 + 9.9	48.7 + 8.3	0.1241
Total (min)	627.5 + 164.7	598.4 + 152.1	0.4190
Arterial pH	7.39 ± 0.13	7.43 ± 0.08	0.6806

Results

Twenty-seven donors (30.0%) showed positive (> 0.0625 AU/ml) DCP levels before harvesting (mean 0.78 ± 2.159 AU/ml; range, 0.062 to 10.9 AU/ml). The number of DCP-positive donors increased with the time spent in the ICU (Fig. 1).

Table 1 demonstrates the relationship between donor DCP positivity and donor characteristics. No correlation was found between donor age and DCP levels (data not shown). DCP-positive donors had longer ICU stays than DCP-negative donors (3.74 ± 1.95 vs 2.54 ± 1.55 days, P = 0.0026). No significant difference in liver function was observed between donors with and without positive DCP levels. Ischemic times (cold, warm, and total) and donor liver weight were also similar.

Table 2 shows the relationship between donor DCP positivity and recipient characteristics. Although recipient characteristics were similar, recipients transplanted with livers from DCP-positive donors needed significantly more intraoperative transfusions [packed red

Table 2 The relationship between donor DCP positivity and recipient characteristics

	Donor DCP		*P* value
	Positive ($n = 27$)	Negative ($n = 63$)	
Age	49.7 + 13.0	47.9 + 13.2	0.5604
Male/female	17/10	35/28	0.5144
Chronic cholestatic disease	6 (22.2%)	8 (12.7%)	0.6788
Chronic hepatocellular disease	16 (59.3%)	40 (63.5%)	
Other chronic liver disease	2 (7.4%)	3 (4.8%)	
Fulminant hepatitis	2 (7.4%)	2 (3.2%)	
Retransplantation	1 (3.7%)	10 (15.9%)	
UNOS 3	9 (33.3%)	18 (28.6%)	0.1241
UNOS 2	11 (40.7%)	32 (50.8%)	
UNOS 1	7 (25.9%)	13 (20.6%)	
Child's B	7 (29.2%)	18 (35.3%)	0.4190
Child's C	17 (70.8%)	33 (64.7%)	
Positive crossmatch	5 (18.5%)	3 (4.7%)	0.33705

Table 3 Intraoperative transfusion

	Donor DCP		*P* value
	Positive ($n = 27$)	Negative ($n = 63$)	
Packed red blood cells (U)	20.9 + 20.9	6.9 + 4.5	0.0001
Fresh-frozen plasma (U)	23.7 + 21.4	9.6 + 4.8	0.0001
Platelets (U)	16.7 + 13.3	7.5 + 8.1	0.0001

Table 4 Post-transplant graft function

	Donor DCP		*P* value
	Positive ($n = 27$)	Negative ($n = 63$)	
AST peak for first 3 PODs	1538 + 1447	775 + 858	0.0031
ALT peak for first 3 PODs	1664 + 1682	972 + 1160	0.0303
PT on POD 2	17.7 + 4.8	15.2 + 4.1	0.0142
Poor early graft function	10 (37.0%)	2 (3.2%)	0.0001
Primary graft nonfunction	3 (11.1%)	2 (3.2%)	0.1320

Table 5 Risk factors for poor early graft function

Univariate analysis	Early graft function		*P* value
	Poor ($n = 12$)	Good ($n = 78$)	
Donor days in ICU	4.25 + 2.63	2.81 + 1.91	0.0235
Cold ischemic time	643.4 + 155.0	544.3 + 149.7	0.0362
Warm ischemic time	54.6 + 11.4	48.9 + 8.3	0.0365
Total ischemic time	698.0 + 159.4	593.2 + 151.3	0.0290
Donor DCP value	2.51 + 3.98	0.75 + 2.16	0.0279
Donor DCP positivity	10 (83.3%)	17 (21.8%)	0.0001
Multivariate analysis			
	Odds ratio		*P* value
Donor DCP positivity	6.58		0.0032

blood cells (PRBC), platelets (PLT), and fresh-frozen plasma (FFP)] (Table 3). Furthermore, livers from DCP-positive donors showed a significantly higher rate of PEGF (10/27 vs 2/63, $P = 0.0001$, Table 4). Higher absolute DCP levels, however, were not associated with a greater likelihood of graft dysfunction than a borderline positive value.

We also performed univariate and multivariate analyses for the risk factor for PEGF. The results are shown in Table 5. Univariate analysis revealed that days in ICU, cold and warm ischemic time, and donor DCP were risk factors for PEGF. By multivariate analysis, we found that donor DCP positivity was the only independent risk factor for PEGF (odds ratio = 6.58, $P = 0.0032$).

Discussion

Successful liver transplantation depends on accurate donor and recipient evaluation [3, 9]. Because of the shortage of donor organs, use of "marginal" quality livers is becoming more common [6, 14]. Despite modified criteria for graft acceptance, and despite increasing severity of liver disease in the recipient population at our center, our 2-month survival rate is over 90% [2]. The incidence of PEGF in this study was 18%; in 3 cases (5%), patients required retransplantation.

It has been reported that many factors affect graft function, and the cause of poor early graft function is still unclear. Ploeg et al. retrospectively analyzed data from 323 orthotopic liver transplantations and identified fatty donor liver, older donor age, retransplantation, renal insufficiency, and prolonged cold ischemic time as risk factors [7]. Gonzalez et al. examined donor and recipient variables by multivariate analysis and found four independent risk factors for poor early graft function: donor serum sodium concentration, total ischemic time, platelet transfusion during surgery, and recipient prothrombin activity [8]. Strasberg et al. reviewed the literature and identified relative risk factors (moderate steatosis, cold ischemic time > 12 h, donor age > 50 years, warm ischemic time > 90 min, retransplantation, UNOS status 1, and kidney failure) and absolute risk factors (severe steatosis, cold ischemic time > 30 h, and warm ischemic time > 150 min) [11]. Based on these findings, one can conclude that poor early graft function is caused by multiple factors.

Several donor tests are thought to predict graft function after transplantation; in particular, arterial ketone body ratio (AKBR) and lecithin cholesterol acyltransferase (LCAT) might be reliable markers. Shimada et al. measured LCAT in 39 organ donors. They found LCAT activity to be an independent risk factor for PEGF but could not establish a cut-off value because LCAT activity was significantly lower in organ donors

than in healthy controls [10]. Yamaoka et al. measured AKBR in 40 cadaveric donors and 13 living-related liver donors and found that grafts obtained from donors with AKBR below 0.7 have a significantly increased risk of PEGF [15]. The DCP assay offers an additional means for evaluating the quality of donor livers. DCP levels are easily and quickly measured, and results can be obtained within 4 h. This abnormal PT is a functional marker, undetectable in healthy individuals [4]. The presence of nonfunctional PT predicts a high risk of intraoperative bleeding complications in recipients, as well as compromised early postoperative graft function.

The causes of increased DCP production in the donor need to be clarified. Since prolonged ICU stay resulting in malnutrition and an acquired defect in hepatic vitamin K-dependent carboxylation are likely explanations, the effect of preventive vitamin K administration in the donor on DCP levels also needs to be analyzed.

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