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Soluble tumor necrosis factor receptors as predictors of 1-year mortality and renal dysfunction after liver transplantation☆,☆☆

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ABSTRACT

Background: Several risk factors influence survival after liver transplantation (LT). Some research has demonstrated a relationship between soluble tumor necrosis factor receptors (sTNFRs) and worse clinical liver disease outcomes, but there are no data showing an association between sTNFRs and outcomes after LT. The primary aim of this study was to determine whether an association exists between perioperative sTNFRs and renal dysfunction or mortality after LT.
Methods: Data were collected prospectively from 122 patients submitted to deceased-donor orthotopic LT. Blood samples were collected at seven different perioperative times and analyzed by ELISA. The statistical analysis included univariate analysis followed by logistic regression. The predictive value of significant variables was assessed using ROC curves.
Results: One-month and 1-year LT survivals were 91% and 81%, respectively. Increased levels of soluble tumor necrosis factor receptor 1 (sTNF-R1) after 24 h of graft perfusion were associated with postoperative Renal Replacement Therapy (RRT) (OR 1.25) and 1-year mortality (OR 1.1). RRT was associated with 30-day and 1-year LT mortality, with OR 19.78 and 45.45, respectively.
Conclusion: A higher sTNF-R1 level measured 24 h after graft perfusion is an independent predictor of RRT and 1-year mortality after LT.

1. Introduction

Liver transplantation (LT) is a life-saving intervention for patients with end-stage liver disease. Despite an increasing survival rate, LT is a complex procedure in patients with multi-systemic dysfunction and poor functional reserve. Currently, patient survival rates at 1 and 5 years post-LT approach 90% and 70%, respectively [1]. Activation of the inflammatory system plays a relevant role in liver disease. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine that has been implicated to play a role in a large number of inflammatory conditions [2]. TNF-α also seems to play a central role in the immune response to alloantigen, with several studies demonstrating an increase in TNF-α levels in liver, kidney, and pancreas transplant rejection [3]. Moreover, elevated levels of TNF-α have been associated with acute and chronic liver diseases [4,5]. Higher levels of TNF-α are a risk factor for renal impairment and mortality in cirrhotic patients [6,7].

Assessing TNF-α blood levels is difficult due to the short half-life and considerable variability of this molecule in the blood. In contrast, the levels of soluble TNF receptors (sTNFRs) have shown greater stability in peripheral circulation and a longer half-life by approximately 24 h, and they have been proposed to be more accurate in assessing activation of the TNF system [4,8]. Soluble TNF-R is a cleaved product of the membrane-bound TNF receptor. Two different types of sTNFRs, a 55-kd protein (sTNF-R1) and a 75-kd protein (sTNF-R2), can be detected [4]. There is an association between higher levels of sTNFRs and worse liver cirrhotic outcomes and mortality [4,8–10]. In a previous study, our group also demonstrated that preoperative sTNF-R1 levels can predict adverse events in cardiac surgery [11]. Kaufmann et al. showed that sTNF-R1 concentrations measured within 24 h of admission to the hospital correlate with the prognosis of multiple organ failure in acute pancreatitis [12].

The aim of this study was to determine if there is an association between perioperative sTNFRs levels and renal dysfunction or mortality after LT.

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2. Material and Methods

2.1. Patients

Data were collected prospectively from 122 patients submitted to deceased-donor orthotopic LT. Research was conducted in the Clinics Hospital of the Federal University of Minas Gerais (UFMG) over 39 consecutive months, following approval by the local ethics committee (Statement No. ETIC 0244.0.203.000-08). The patients were invited to participate in the study during the preoperative consultation and gave a written informed consent. Patients were excluded if they had a retransplant or fulminant hepatitis, were less than 18 years old, or refused to participate in the study.

Preoperative renal dysfunction was defined as a creatinine clearance (CCr) lower than 70 mL/min or receiving RRT in the week before surgery. Postoperative renal dysfunction was defined as needing RRT in the 30 days following LT. RRT indication criteria were severe metabolic acidosis (pH <7.2), hypervolemia with hydric overload or hyperkalemia (K > 6 mE/l) that did not respond to other forms of therapy. Patients who died up to 2 days post-transplantation were excluded from the postoperative renal dysfunction analysis.

All patients received the same immune suppression treatment with tacrolimus and methylprednisolone according to our institution protocol.

2.2. Sample collection and soluble receptor determination

Whole blood samples were collected between anesthesia induction and surgery incision (T1); before the portal vein clamp (T2); 5 min before graft reperfusion (T3); 15 min after graft reperfusion (T4); 2 h after graft reperfusion (T5); 8 h after graft reperfusion (T6); and 24 h after graft reperfusion (T7). Blood samples (2 mL) were obtained from a radial arterial line in sterile tubes containing heparin (Becton–Dickinson, Franklin Lakes, NJ, USA). The blood was centrifuged at 3000 rpm for 10 min at 4 °C. After that, the plasma was stored at −80 °C. The samples for the sTNF-R1 and sTNF-R2 measurements were thawed at room temperature in order to determine the plasmatic levels using the sandwich-type ELISA method (R&D Systems, Minneapolis, MN-USA).

2.3. Statistical analysis

Continuous variables were compared using Student's *t*-test or the Mann–Whitney test and summarized as the means or medians. Categorical variables were compared using the Chi-square statistics test or Fisher's exact test and summarized as percentages and frequencies. For multivariate analyses, all variables found to be significant at $p < 0.2$ in univariate analysis and those thought to be important on clinical grounds were entered into a backward step-down Cox proportional hazards regression analysis. A p value <0.05 was considered to be significant. The predictive value of significant variables was assessed by means of the receiver operating characteristic (ROC) curve. The cut-off point, which is defined as the ratio between the true positives and negatives over the total number of patients, was set such that a greater acuity could be found. The statistical program SPSS 18.0 (SAS, Chicago, IL) was used for analysis.

3. Results

From September 2008 to December 2011, 122 patients (84 males) met the inclusion criteria. Their ages ranged from 18 to 71, with a mean of 52.04 (SD ± 11.51) years. Their MELD scores ranged between 6 and 41, with a mean of 17.68 (SD ± 5.68). The RRT incidence at 30 days was 17.2% (21 patients). Five patients were excluded from postoperative renal dysfunction analyses. The 30-day and 1-year survival rates were 91% and 81.1%, respectively. Twenty-three patients died in the first postoperative year: 22% due to surgical causes, 56% due to infectious causes, 13% due to cardiovascular causes and 9% due to other causes.

For the sake of comparison, we also measured sTNF-R1 and sTNF-R2 levels in 30 healthy controls: sTNF-R1 levels were 1025 (139–2618)pg/ml and sTNF-R2 1772 (119–7067)pg/ml. It is worth emphasizing that these control values were significantly below the values obtained for patients at different time points ($p < 0.001$).

Univariate and multivariate 30-day survival analyses are presented in Tables 1 and 2. Liver disease etiology, body mass index (BMI), platelet transfusion, ICU stay, hospital stay, postoperative RRT, postoperative alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate, sTNF-R1 (T3, T5, T6, T7) and sTNF-R2 (T4, T6) had $p < 0.2$ and were included in the multivariate analyses.

Univariate and multivariate assessments of the predictors for 1-year survival are presented in Tables 3 and 4. BMI, red blood cell transfusion,

Table 1
Comparison of clinical and laboratory parameters between patients who survived and patients who did not survive 30 days after liver transplantation.

	Died (n = 11)	Survived (n = 111)	p-Value
Age (y)[b]	51(24–69)	53 (18–71)	0.93
Gender (male)[a]	8(72.7%)	76(68.5%)	1
Liver disease etiology[a]	–	–	0.14+
Autoimmune	1(9.1%)	6(5.4%)	
Alcoholic	1(9.1%)	37(33.4%)	
Parenchymal disease	5(45.5%)	22(19.8%)	
Biliary disease	0(0%)	12(10.8%)	
Viral	4(36.4%)	34(30.6%)	
Preoperative renal function (normal)[a]	9(81.8%)	79(71.2%)	0.73
Body mass index (≤25)[a]	8(72.7%)	110(99.1%)	<0.01+
MELD score[b]	16.88(6–30)	17.81(6–41)	0.56
Anhepatic phase (min)[b]	118.5(30–183)	117(50–295)	0.93
Cold ischemia (min)[b]	530(292–750)	527 (255–832)	0.55
Operative duration (min)[b]	379(180–495)	355(225–605)	0.83
Red blood cell transfusion (ml)[b]	1500(0–4200)	600(0–4800)	0.35
Fresh frozen plasma transfusion (ml)[b]	0(0–1200)	0(0–2400)	0.63
Platelet transfusion (units)[b]	8(0–20)	0(0–23)	0.02+
Cryoprecipitate transfusion (units)[b]	0(0–10)	0(0–15)	0.22
ICU stay (days)[b]	7.5(6–39)	4(1–61)	<0.01+
Hospital stay (days)[b]	46(34–88)	17.5(7–84)	<0.01+
Postoperative RRT[a]	6(54.5%)	15(13.5%)	<0.01+
Postoperative infection[a]	4(36.4%)	30(27.3%)	0.50
sTNF-R1 (pg/ml)[b]			
T1	3799(507–15,943)	3488(336–23,007)	0.53
T2	4002(631–14,025)	3825(318–21,902)	0.45
T3	7153(422–12,814)	4363(764–21,547)	0.14+
T4	12,680(1148–17,176)	11,337(3155–21,775)	0.92
T5	15,477(1341–22,760)	11,374(2516–21,970)	0.05+
T6	11,613(857–14,103)	6367(885–21,176)	0.07+
T7	14,637(1245–179,382)	7095(1052–23,172)	0.03+
sTNF-R2 (pg/ml)[b]			
T1	4085(1370–8284)	4142(2185–15,954)	0.38
T2	3688(1390–7822)	4293(1781–19,508)	0.55
T3	5268(2332–7424)	5146(1807–13,113)	0.58
T4	4952(3286–9147)	6314(2344–12,398)	0.17+
T5	6445(3224–9672)	6815(2920–15,851)	0.64
T6	6415(3344–8026)	7131(3012–20,372)	0.198+
T7	6164(4677–14,195)	6816(2634–20,133)	0.94

T1 Preoperative, T2 Portal vein clamping, T3 5 min before reperfusion, T4 15 min after reperfusion, T5 2 h after reperfusion, T6 8 h after reperfusion, T7 24 h after reperfusion.
[a] Fisher exact test.
[b] Mann–Whitney test.
+ Significant variable ($p < 0.20$).