Research Article

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Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma

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Background & Aims: Recurrence of hepatocellular carcinoma (HCC) is a major complication after liver transplantation (LT). The initial immunosuppression protocol may influence HCC recurrence, but the optimal regimen is still unknown.

Methods: 219 HCC consecutive patients under Milan criteria, who received an LT at 2 European centres between 2000 and 2010, were included. Median follow-up was 51 months (IQR 26-93). Demographic characteristics, HCC features, and immunosuppression protocol within the first month after LT were evaluated against HCC recurrence by using Cox regression.

Results: In the explanted liver, 110 patients (50%) had multinodular HCC, and largest nodule diameter was 3 ± 2.1 cm. Macrovascular invasion was incidentally detected in 11 patients (5%), and microvascular invasion was present in 41 patients (18.7%). HCC recurrence rates were 13.3% at 3 years and 17.6% at 5 years. HCC recurrence was not influenced by the use/non-use of steroids and antimetabolites ($p = 0.69$ and $p = 0.70$ respectively), and was similar with tacrolimus or cyclosporine ($p = 0.25$). Higher exposure to calcineurin inhibitors within the first month after LT (mean tacrolimus trough concentrations >10 ng/ml or cyclosporine trough concentrations >300 ng/ml), but not thereafter, was associated with increased risk of HCC recurrence (27.7% *vs.* 14.7% at 5 years; $p = 0.007$). The independent predictors of HCC recurrence by multivariate analysis were: high exposure to calcineurin inhibitors defined as above (RR = 2.82; $p = 0.005$), diameter of the largest nodule (RR = 1.31; $p < 0.001$), microvascular invasion (RR = 2.98; $p = 0.003$) and macrovascular invasion (RR = 4.57; $p = 0.003$).

Conclusions: Immunosuppression protocols with early CNI minimization should be preferred in LT patients with HCC in order to minimize tumour recurrence.

Keywords: Liver transplantation; Hepatocellular carcinoma; Recurrence; Calcineurin inhibitors; Trough concentrations; Immunosuppression.
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Abbreviations: LT, liver transplantation; HCC, hepatocellular carcinoma; CNI, calcineurin inhibitors; TC, trough concentrations.

Introduction

Despite an optimal selection of candidates with hepatocellular carcinoma (HCC) for liver transplantation (LT) using Milan criteria, tumour recurrence still affects 15–20% of patients, and therapeutic options are very limited in this situation [1,2]. Although the immune system plays a critical role in preventing malignancy and tumour spreading [3], therapeutic immunosuppression is needed to avoid rejection and graft loss. The way in which immunosuppression influences HCC recurrence in transplanted patients remains unclear.

In vitro studies and animal models have shown that calcineurin inhibitors (CNI), currently the mainstay of immunosuppression protocols in LT, are able to stimulate tumour growth and progression through overexpression of transforming growth factor β [4]. In HCC transplanted rats, specific pathways, such as Rho/Rho associated kinase, are also triggered by the use of CNI, leading to increased HCC recurrence after LT [5]. However, this evidence is not applicable to clinical practice since the dosing of tacrolimus used to treat these animals was much higher (2–10 mg/kg) than in humans. In liver transplant patients, the studies evaluating the influence of immunosuppression on HCC recurrence are scarce, due to the heterogeneity in the immunosuppression protocols used, which vary widely between centres. Two retrospective studies from the same group with 69 and 139 patients respectively, reported higher trough concentrations (TC) of CNI in patients experiencing HCC recurrence [6,7].

There is growing evidence suggesting that LT patients may be overimmunosuppressed, particularly within the first month after LT when exposure to immunosuppressive drugs is higher: acute

cellular rejection rates are unnecessarily reduced to very low levels while adverse effects, particularly renal impairment, are increased [8–10]. Indeed many LT centres in Europe have empirically implemented protocols with CNI minimization, but the actual impact of this strategy on HCC recurrence is unknown. Therefore, we aimed at exploring the influence of the initial immunosuppression protocol and exposure to immunosuppressants on HCC recurrence after LT, and whether reduced immunosuppression is able to influence this outcome.

Materials and methods

This study was a retrospective evaluation of a prospectively collected LT database performed at 2 European LT centres: Royal Free Hospital, London, UK and Reina Sofía University Hospital, Córdoba, Spain. 219 consecutive HCC patients listed within the Milan criteria, who received a LT between 2000 and 2010, and survived longer than 1 month were included. Exclusion criteria were: donor-recipient blood group incompatibility, HIV positive or combined transplantation. Demographic and clinical data, including pre-LT HCC features were recorded. The initial evaluation of HCC included abdominal triple phase computed tomography or dynamic magnetic resonance, chest computed tomography, bone scan and serum α-fetoprotein. From listing to LT, patients were evaluated every 3 months with serum α-fetoprotein and an imaging technique of the liver (computed tomography or magnetic resonance). Histological analysis of the explanted liver included tumour differentiation (according to Edmonson scale [11]), microvascular invasion (defined as cluster of tumour cells within a portal or hepatic vein branch and contacting with the vessel wall [12]), number of nodules, and diameter of the largest nodule. Immunosuppressive drugs used, dosage, and the mean whole blood TC of CNI within the first month after LT were also recorded. Median follow-up after LT was 51 months (IQR 26-93). Patient surveillance ensured at least one visit every 3 months within the first year and every 6 months thereafter, including a complete clinical evaluation, an imaging technique (liver ultrasound, magnetic resonance or computed tomography), and α-fetoprotein serum levels.

Immunosuppression protocol

At both centres, immunosuppression was started immediately after LT, and oral medication given once gut function was restored. The immunosuppression protocol was based on CNI. Dosing was adjusted to whole blood TC (determined on at least alternate days excepting week-ends) and occurrence of renal impairment and other adverse effects. In most cases, tapering corticosteroids and/or antimetabolites were used, with some differences at each institution: at the Royal Free Hospital, the initial dose of oral prednisolone was 20 mg/day with progressive reduction thereafter, and the preferred antimetabolite was azathioprine (1 mg/kg/day). At the Reina Sofía University Hospital, the initial corticosteroid was intravenous methylprednisolone 120 mg/day, rapidly tapered and then switched to oral deflazacort 30 mg at day +5, with progressive reduction thereafter. The preferred antimetabolite was mycophenolate (1000–2000 mg/day). mTOR inhibitors (everolimus or sirolimus) were not used before 2005 and seldom thereafter, except for patients with histological features of aggressive tumour behaviour. Thus the use of mTOR inhibitors could not be analyzed with respect to HCC recurrence because of high risk of bias, but it was controlled in the multivariate analysis as a possible confounding factor.

Sample size calculation

The sample size required was calculated with EPIDAT 3.1, aiming to correlate CNI exposure within the first month with HCC recurrence. Taking into account data of CNI exposure after LT [8] and data of HCC recurrence [13], we made the following assumptions: HCC recurrence rate with low CNI exposure (7%), HCC recurrence with high CNI exposure (23%), prevalence of high exposure to CNI (25%), statistical power (80%), α error 5%. Under these premises, 219 patients were needed.

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (Chicago, USA). Variables were displayed in frequency Tables or expressed as means and standard deviations, except for those with asymmetric distribution in which median and interquartile range were used. Chi square test was used for frequencies, Student's *t* or ANOVA tests for quantitative variables, and Mann-Whitney's U or Kruskal-Wallis for asymmetric distributions. Kaplan Meier curves (Log rank test) and Cox regression were used to explore the influence of immunosuppression protocol and dosage on HCC recurrence. Thresholds for continuous variables were obtained from ROC curves. Those variables with $p \leqslant 0.20$ in the univariate analysis were included in the multivariate model. Previously known factors predicting HCC recurrence after LT, the use of mTOR inhibitors, and the institution in which LT was performed were controlled as possible confounding factors. Every hypothesis tested was two tailed and considered significant if $p < 0.05$.

Results

From 219 patients included, 190 (86.8%) were male and mean age was 55.2 ± 7.4 years. All patients had cirrhosis and major etiologies were: hepatitis C (n = 80; 36.5%), alcoholic liver disease (n = 51; 23.3%), their combination (n = 23; 10.5%), and hepatitis B (n = 48; 22%). The remaining patients had cryptogenic cirrhosis (n = 6; 2.7%), hepatitis B and C (n = 5; 2.3%), and others (n = 6; 2.7%). Descriptive values depending on the institution in which LT was performed are shown in Table 1. Although there was a male predominance in both cohorts, this was more pronounced at the Reina Sofía University Hospital (91.4% *vs.* 81.6%; $p = 0.03$). At the Royal Free Hospital, the time in waiting list was shorter (2.9 *vs.* 5.9 months; $p < 0.001$), and local treatment of the tumour before transplantation was used more frequently (88% *vs.* 66.7%; $p < 0.001$). Transarterial therapies (embolization or chemoembolization) were the preferred techniques: 86% at the Royal Free and 80% at the Reina Sofía University Hospital ($p = 0.35$). There was an imbalance in some patient and tumour characteristics, suggesting that patients at the Royal Free had earlier disease: reduced diameter of the largest nodule (2.6 *vs.* 3.4 cm; $p = 0.006$), and less incidental macrovascular invasion (0.9% *vs.* 8.7%; $p = 0.02$) and lymphatic permeation rates (0.9% *vs.* 12.2%; $p = 0.001$). The number of nodules in the explant was similar in both centres ($p = 0.45$).

Overall the initial regimen of immunosuppression was based on CNI: tacrolimus in 198 patients (90.4%) and cyclosporine in 21 patients (9.6%). Number of TC measurements within the first month after LT were 10.3 ± 3.6 for tacrolimus and 10.7 ± 3.8 for cyclosporine. In 102 patients (46.5%), CNI were used with concomitant antimetabolites: mycophenolate in 74 patients (33.8%) and azathioprine in 28 patients (12.7%). Dose of mycophenolate was 2000 mg/day in 50 cases (68%) and 1000 mg/day in 24 cases (32%). Dose of azathioprine was 75 mg/day in all patients but 1 (receiving 100 mg/day). Tapering corticosteroids were given in 158 cases (72.1%). Significant acute rejection requiring boluses of steroids within the first month after LT occurred in 68 patients (31.1%). The immunosuppression protocol had also some differences between institutions (Table 1): at the Reina Sofía University Hospital, CNI with steroids, with or without antimetabolites accounted for the vast majority of patients (97.4%); at the Royal Free hospital, protocols without steroids (CNI alone or combined with antimetabolites) were used in approximately half of the patients (51.5%). Despite this, the rate of tumour recurrence at 5 years after LT was similar in both cohorts: 16.7% at the Royal Free and 20% at the Reina Sofía University Hospital ($p = 0.21$).

Predictors of HCC recurrence

HCC recurrence occurred in 36 patients (16.4%) after a median follow-up of 51 months (IQR 26-93). Sites of recurrence were liver (n = 12; 33.3%), bone (n = 6; 16.6%), lung (n = 5; 14%),