Research Article

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Assessment of chronic rejection in liver graft recipients receiving immunosuppression with low-dose calcineurin inhibitors

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Background & Aims: Calcineurin inhibitors represent the cornerstone immunosuppressants after liver transplantation despite their side effects. As liver graft is particularly well tolerated, low doses may be proposed. The aim of this study was to assess the prevalence of chronic rejection in patients with low calcineurin inhibitors regimen and to compare their characteristics with patients under standard doses.

Methods: All patients with liver transplantation between 1997 and 2004 were divided into two groups. Low-dose patients (n = 57) had tacrolimus baseline levels <5 ng/ml or cyclosporine levels <50 ng/ml at t0 or <100 ng/ml at t + 2 h and were prospectively proposed a liver biopsy, searching for chronic rejection according to Banff criteria. The remaining patients constituted the standard-doses group (n = 40).

Results: Among the low-dose group, 36 patients in the low-dose group were assessed by biopsy. No chronic rejection was found. Fifty-six percent had only calcineurin inhibitors and 8% received other immunosuppressants only. The median time between liver transplantation and biopsy was 90 months (64–157) and between IS regimen decrease and biopsy was 41 months (11–115). Liver tests were normal in 72% of the patients. Low-dose patients had more often hepatitis B ($p = 0.045$), less past acute rejection episodes ($p = 0.028$), and better renal function ($p = 0.040$). Decrease of calcineurin inhibitors failed in 15% of standard-dose patients without impacting the graft function. In the low-dose group, co-prescription of other immunosuppressants facilitated the decrease ($p = 0.051$).

Conclusions: The minimization, or even cessation, of calcineurin inhibitors may be an achievable goal in the long term for most of the liver graft recipients.

Keywords: Biopsy; Graft rejection; Immunosuppressive agents; Liver transplantation; Transplantation tolerance.

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Abbreviations: LT, liver transplantation; CNI, calcineurin inhibitors; LD, low-dose; SD, standard-dose; HCV, hepatitis C virus; LFT, liver function test; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; HBV, hepatitis B virus.

Introduction

Billingham *et al.* first introduced the concept of graft tolerance in 1953, defined by a state of non-response to foreign antigens without immunosuppression [1]. In a clinical setting of liver transplantation (LT), operational tolerance is the lack of acute or chronic rejection with a normal liver function and histology after a successful weaning of immunosuppression. Calne proposed the concept of *prope* tolerance in 1998, referring to patients with normal liver function and histology under minimal immunosuppression, usually with low blood levels of calcineurin inhibitors (CNI) [2,3]. Cortesini *et al.* have reported the alternative name of partial clinical tolerance in 2004 [4]. According to this definition, a minimal non-toxic maintenance immunosuppression could represent a safer option than uncontrolled attempts to completely wean the liver graft recipients from immunosuppressive drugs [3].

Although acute rejection is a widely described phenomenon [5,6], chronic rejection is less known and its underlying mechanisms remain unclear [7,8]. Chronic rejection of the liver graft was defined during the 5th Banff conference in 1999 [9] by "*an immunologic injury to the allograft, which usually evolves from severe or persistent acute rejection and results in potentially irreversible damage to the bile ducts, arteries, and veins.*" The prevalence of chronic rejection is around 4% [9]. Chronic rejection with graft failure can lead to retransplantation in 1.7% of LT [10].

Current CNI-based immunosuppressive regimens have considerably decreased the incidence of acute rejection. However, graft and patient survivals did not improve so dramatically after 1-year post-transplant: in more than half of the recipients, late

deaths were related to cardiovascular diseases, *de novo* malignancies, infections, and chronic renal failure [11]. All of them were favored by the administration of CNI, including hepatocellular carcinoma (HCC). More than 10% of the liver graft recipients have a severe or end-stage renal failure 10 years after LT; and a chronic renal disease was associated with mortality with an attributable risk of 13% [12]. Calcineurin inhibitors play a key role in the onset of post LT chronic renal disease, and diabetes mellitus, and nephroangiosclerosis. Their toxicity is dependent on the administered doses at least for renal dysfunction [13,14] and hepatocellular carcinoma recurrence [15]. Numerous clinical research protocols aimed at decreasing CNI doses in primary or secondary prevention [16], with new non-nephrotoxic immunosuppressive drugs like mycophenolate mofetil (MMF), mammalian target of rapamycin (mTOR) inhibitors or basiliximab.

A policy of CNI minimization was introduced for several years in the department according to the concept of *prope* tolerance. All patients with liver transplantation between 1997 and 2004 are reviewed retrospectively and compared according to their CNI regimen. The aims were: (i) to assess the risk of chronic rejection among low CNI patients, (ii) to evaluate which patients were the best candidates for CNI decrease.

Patients and methods

Type of study – End points

This is a monocentric descriptive study aiming at assessing the prevalence of asymptomatic chronic rejection by histology among liver-transplanted patients with long-term low-dose CNI. The primary end point (chronic rejection at histology) was prospectively assessed. The secondary end point was to determine the characteristics of patients receiving low-dose CNI compared with patients receiving standard-doses.

Patients

Included patients met the following criteria: alive, followed-up in the department, with a unique LT between 1997 and 2004, without any other organ transplantation or recurrence of cirrhosis. They were divided into two groups: low-dose (LD) and standard-dose (SD) groups according to their CNI blood levels.

Patients included in the LD group had tacrolimus baseline blood levels <5 ng/ml or cyclosporine blood levels <50 ng/ml at t0 or <100 ng/ml at t2 h for at least 6 months. Other patients were included in the SD group. Other immunosuppressive drugs may have been prescribed in both groups: steroids, azathioprine, MMF, and everolimus.

Immunosuppression protocol

Baseline immunosuppression was CNI with steroids, most of the time tacrolimus. Cyclosporine was used at the beginning of our experience (until 2003 for 2 patients), and tacrolimus was then the most widely prescribed CNI. Steroids were weaned during the first year after LT. Some patients were switched from tacrolimus to cyclosporine in case of hyperglycemia or hepatitis C virus (HCV) recurrence, according to the protocols. The patients with autoimmune diseases remained under CNI with other immunosuppressants. Decreasing CNI doses were preferred in young patients, patients with hepatocellular carcinoma, or with renal insufficiency or obvious side effects (induced diabetes mellitus, infections…). Mycophenolate mofetil was prescribed in case of renal dysfunction and everolimus in case of hepatocellular carcinoma. In case of HCV recurrence, standard CNI blood levels were maintained due to the difficulty of distinguishing liver function tests (LFTs) perturbation in relation to acute rejection or viral activity.

Liver biopsy

Percutaneous protocol liver biopsies were performed after patients' written consent in the absence of contraindications according to the guidelines [17] by trained hepatologists, under ultrasound guidance. Disposable automatic biopsy devices were used after providing local anesthesia. A protocol liver biopsy searching for chronic rejection was proposed to the patients in the LD group. For two patients, we had at our disposal results from biopsies performed in the past year before the start of inclusion and after 11 months and 7 years of CNI decrease. Specimens were re-analyzed but no new liver biopsy was performed. Two pathologists (SG and JC) analyzed independently all specimens.

Different parameters were assessed: presence of chronic rejection, fragment's size, number of portal spaces, interpretability (biopsy longer than 1 cm or encompassing 6 portal tracks), presence of ductopenia, non-specific inflammation, recurrence of the initial disease, and isolated central perivenulitis. Chronic rejection was defined according to Banff Criteria [9] and attested if the two pathologists agreed on the diagnosis. Ductopenia was defined as the loss of interlobular and septal bile ducts in at least 50% of portal tracks. Isolated central perivenulitis was defined by centrilobular necroinflammation without other features of chronic rejection [18]. Fibrosis was scored according to METAVIR system [19].

Renal function assessment

Creatinine clearance was assessed with the modification of the diet in renal disease glomerular filtration rate calculator [20]. Creatinine clearance at the time of inclusion was calculated as a percentage of creatinine clearance at the time of LT, according to the formula: Clearance ratio = 100∗Clearance at time of inclusion/Clearance at time of LT.

Statistical analysis

Census date was April 1, 2010. Statistical analysis was performed using Graph Pad Prism (GraphPad Software, Inc., La Jolla, CA, USA). p <0.05 was considered to be statistically significant. The results provided are the medians with the ranges in brackets and percentages. For two groups, qualitative variables were compared with the two-tailed Chi-square test, and quantitative variables with the unpaired t-test. For comparison of more than two groups, Chi-square test, one-way ANOVA, and Bonferroni's multiple comparison tests were performed.

Ethics

The study was approved by the local Ethics Committee and was allocated the ID RCB number 2009-A00614-53. As the center was not performing routine biopsies in LT recipients, the individual benefit of a liver biopsy was less in patients with standard CNI doses. Hence, proposal of a liver biopsy was done only to patients in the LD group.

Results

Repartition of the patients within groups

Between 1997 and 2004, 234 patients received an LT (Fig. 1). There had been no retransplantation for chronic rejection. Ninety-seven patients were included in the study, of whom 40 (41%) had standard CNI blood levels (SD group) and 57 (59%) had low CNI blood levels (LD group). Thirty-six patients (63%) in the LD group were assessed by liver biopsy. Of the remaining 21 patients in LD, 14 refused biopsy and 7 had contra-indications.

Primary end point: assessment of CR at liver biopsy in patients with low-dose CNI (n = 36)

Characteristics of the patients are summarized in Table 1.

Immunosuppressant regimen

Thirty-three patients received CNI (tacrolimus, n = 27 and cyclosporine, n = 6). As a result of hyperglycemia (n = 2) and drug toxicity (n = 1), 3 patients switched from tacrolimus to cyclosporine. Among patients under low-dose CNI with other immunosuppressants, patients received: MMF (n = 11), MMF with everolimus