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OPTIMIZING MAINTENANCE IMMUNOSUPPRESSIVE THERAPY AFTER LIVER TRANSPLANTATION

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Objective: to justify the rational selection of maintenance immunosuppressive therapy following liver transplantation (LT). **Materials and methods.** The study included 42 recipients of deceased donor liver grafts, observed for periods ranging from 1 month to 15 years LT. The mean age at transplantation was 49.4 ± 7.0 years. All patients received everolimus in combination with low-dose extended-release tacrolimus. Indications for everolimus therapy were tacrolimus-induced nephrotoxicity (n = 13), history of hepatocellular carcinoma (HCC, n = 21), and development of *de novo* malignancies at non-hepatic sites (n = 8). Target trough concentrations were 2–3 ng/mL for tacrolimus and 3–8 ng/mL for everolimus. Adverse events of everolimus and serum cholesterol dynamics were assessed at 12, 36, 60, and 120 months after conversion to this regimen, and compared with data from 20 randomly selected recipients maintained on tacrolimus monotherapy. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation at the same time points. Liver stiffness (kPa) was measured by transient elastography once at study completion. In patients with a history of HCC, baseline alpha-fetoprotein (AFP) levels were also taken into account. **Results.** Long-term use of everolimus with low-dose extended-release tacrolimus did not impair renal function (baseline GFR: 84.13 ± 16.70 mL/min/1.73 m^2; final GFR: 84.99 ± 21.30 mL/min/1.73 m^2). However, serum cholesterol levels were consistently higher compared with tacrolimus monotherapy (12 months: 5.7 ± 0.91 *vs* 4.01 ± 1.21 mmol/L; 120 months: 5.52 ± 1.51 *vs* 4.58 ± 0.72 mmol/L). Among 21 patients with a history of HCC, recurrence or progression occurred in 6 patients (30%), which was associated with elevated baseline AFP levels prior to LT (429.2 ± 306.9 U/mL; Z = 4.2, p = 0.0001). Liver stiffness, assessed once at the endpoint of the retrospective study, averaged 4.8 ± 1.8 kPa, corresponding to F0–1 by the METAVIR scale. **Conclusion.** Long-term maintenance therapy with everolimus combined with low-dose extended-release tacrolimus after LT is safe and helps mitigate calcineurin inhibitor (CNI) nephrotoxicity. Nevertheless, this regimen does not prevent recurrent HCC, which depends on the biological activity of the tumor.

Keywords: liver transplantation, everolimus, extended-release tacrolimus, glomerular filtration rate, hypercholesterolemia, liver stiffness, alpha-fetoprotein.

INTRODUCTION

For decades, progress in transplantation has been closely linked to the development of new immunosuppressive drugs, such as cyclosporine, tacrolimus, sirolimus, mycophenolate mofetil, thymoglobulin, and interleukin-2 receptor antagonists. In recent years, however, clinical trials of new molecules have faced setbacks, while the growth of the generic market has provided economic benefits but also introduced challenges in selecting and alternating between brands, some of which remain insufficiently studied [1]. This does not apply to everolimus and mycophenolic acid, which have undergone rigorous clinical trials and are recognized as fully effective immunosuppressants [2].

Calcineurin inhibitors (CNIs), particularly tacrolimus, remain the cornerstone of maintenance immunosuppression following liver transplantation (LT) [3, 4]. Nevertheless, the optimal regimen for long-term maintenance remains uncertain, as meta-analyses have not shown clear superiority of any single drug combination [5].

A major limitation of CNIs is the well-documented risk of chronic nephrotoxicity with prolonged use, which negatively affects long-term survival [3, 6]. One preventive strategy is minimizing tacrolimus exposure in combination with the mammalian target of rapamycin (mTOR) inhibitors [3, 7]. In addition, everolimus, due to its antiproliferative properties, is often prescribed for LT recipients with a history of hepatocellular carcinoma (HCC) or *de novo* extrahepatic malignancies [8]. As a result, a distinct cohort of patients is emerging for whom a maintenance regimen combining low-dose CNIs with mTOR inhibitors is preferred.

Based on these findings and previous studies confirming the efficacy of extended-release tacrolimus after LT, the authors analyzed their single-center experience with

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the long-term use of low-dose extended-release tacrolimus in combination with everolimus. As such studies remain limited [9, 10], evaluating the effectiveness of this regimen may provide important evidence for improving long-term survival after LT. The aim of this study was to substantiate a rational approach to maintenance immunosuppression in the late post-transplant period.

MATERIALS AND METHODS

A retrospective analysis was conducted on 42 LT recipients under follow-up at an outpatient transplant center, with observation periods ranging from 1 to 15 years post-transplant. The cohort included 16 men and 26 women, with a mean age at LT of 49.4 ± 7.0 years. Data collection was completed by the end of 2023. Indications for everolimus initiation included early signs of nephrotoxicity in 13 patients, a history of HCC in 21 patients, and the presence of *de novo* extrahepatic malignancies in 8 patients (Fig. 1). Everolimus was introduced no earlier than 1 month after LT. The initial daily dose was 3 mg, titrated to maintain a target trough concentration of 3–8 ng/ml. Tacrolimus trough levels were maintained at approximately 2 ng/ml. Tacrolimus and everolimus concentrations were monitored using an automated biochemistry analyzer.

The effectiveness of immunosuppressive therapy was evaluated using key biochemical parameters: bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, gamma-glutamyltransferase (GGT), creatinine, and urea. Proteinuria was assessed, and glomerular filtration rate (GFR) was calculated using the CKD-EPI formula (ml/min/1.73 m^2).

Total blood cholesterol levels were retrospectively analyzed and compared with a random sample of 20 LT recipients who received standard-dose extended-release tacrolimus monotherapy without evidence of graft dysfunction. Alpha-fetoprotein (AFP) levels were determined preoperatively in patients with HCC.

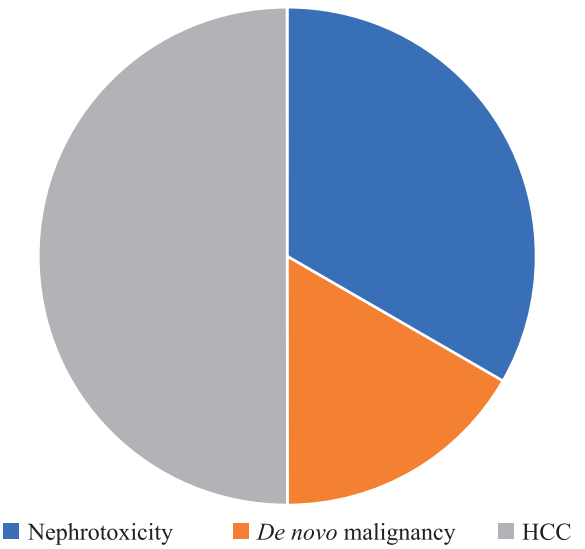

Fig. 1. Distribution of patients according to the indication for conversion to everolimus

At the end of follow-up, liver stiffness was measured in kilopascals (kPa) using a Fibroscan-430Mini transient elastography device.

Statistical analysis was performed using Statistica for Windows, version 14. Both descriptive and nonparametric methods were applied. Intergroup differences were evaluated using the Mann–Whitney U test, and correlations within the study group were assessed using Spearman's rank coefficient. Results were considered statistically significant at $p < 0.05$.

RESULTS

At target concentrations of everolimus (3–8 ng/mL) and tacrolimus (≤3 ng/mL), the mean maintenance doses at the end of the follow-up period were 2.75 ± 0.4 mg/day and 2.1 ± 0.9 mg/day, respectively (Table 1).

Long-term monitoring of liver graft function showed no significant deviations from normal values for bilirubin, ALT, or AST at 12, 36, 60, and 120 months of therapy with this immunosuppressive regimen. Recorded adverse events were not life-threatening for either the graft or recipients and were effectively managed with supportive measures, such as iron supplementation for anemia and dose adjustments of immunosuppressive agents (Fig. 2).

The most frequent complication was hypercholesterolemia, observed in 28.5% of patients. Mean total cholesterol did not significantly change over time, amounting to 5.69 ± 1.19 mmol/L at baseline and 5.52 ± 1.51 mmol/L after 10 years of combined therapy (Fig. 4). However, when compared with a random control group of 20 LT recipients maintained on long-term tacrolimus monotherapy with normal graft function, cholesterol levels were consistently and significantly higher in the study group throughout the entire follow-up (Table 2), thus confirming the negative effect of everolimus on blood cholesterol levels.

All patients with hypercholesterolemia were advised long-term statin therapy. Nevertheless, some declined treatment, which contributed to persistently unsatisfactory overall cholesterol levels. In contrast, in a subgroup of 10 patients who adhered to statin therapy, cholesterol remained within acceptable limits, averaging 4.73 ± 0.31 mmol/L after 10 years.

Table 1

Average maintenance doses and target immunosuppressant concentrations

Drug	Starting dose	Average maintenance dose	Target concentration
Everolimus	3 mg	2.75 ± 0.40 mg/day	3–8 ng/mL
Tacrolimus		2.10 ± 0.90 mg/day	2–3 ng/mL

Over a 12–120 month follow-up, mean creatinine levels and estimated GFR (eGFR) remained within acceptable limits (Fig. 3). After one year of combined everolimus and low-dose extended-release tacrolimus therapy, the mean eGFR was 84.13 ± 16.70 mL/min/1.73 m^2. At 3 and 5 years, values were 91.15 ± 14.17 and 84.92 ± 17.72 mL/min/1.73 m^2, respectively; among patients reaching the 10-year threshold, mean eGFR was 84.99 ± 21.30 mL/min/1.73 m^2.

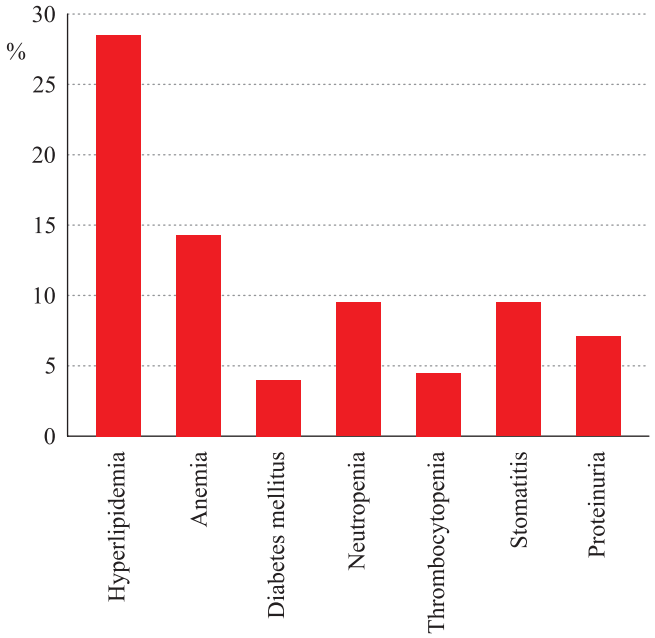

Fig. 2. Proportion of adverse events during long-term therapy with everolimus combined with low-dose extended-release tacrolimus

Among the 21 patients with a history of HCC treated with this regimen, recurrence or progression occurred in 6 patients (30%) at various time points post-LT. The mean pre-LT AFP level in this subgroup was 429.2 ± 306.9 IU/mL (range 3.6–1500 IU/mL). Recurrence was significantly associated with baseline AFP concentration (Z = 4.2, p = 0.0001).

Transient elastography performed at the end of follow-up provided indirect evidence of regimen effectiveness. The mean liver stiffness was 4.8 ± 1.8 kPa, corresponding to METAVIR stages F0–F1, indicating no significant fibrosis progression.

Table 2

Comparative dynamics of blood cholesterol levels in liver transplant recipients under different immunosuppressive regimens (p < 0.05*, p < 0.01)**

Regimen / Duration	12 months	36 months	60 months	120 months
Tacrolimus + Everolimus	5.7 ± 0.91*	5.52 ± 1.32**	5.4 ± 1.38*	5.52 ± 1.51**
Tacrolimus alone	4.01 ± 1.21	4.11 ± 0.82	4.34 ± 0.90	4.58 ± 0.72

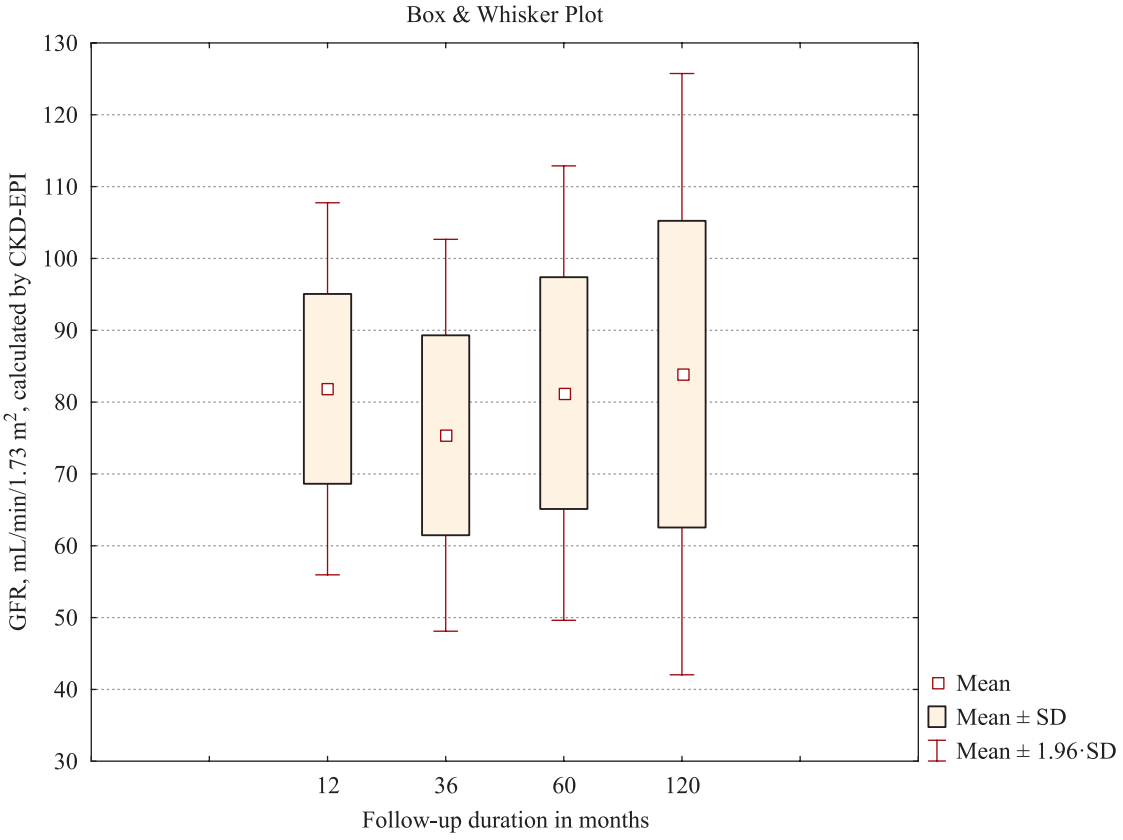

Fig. 3. Dynamics of renal function in patients receiving low-dose extended-release tacrolimus with everolimus

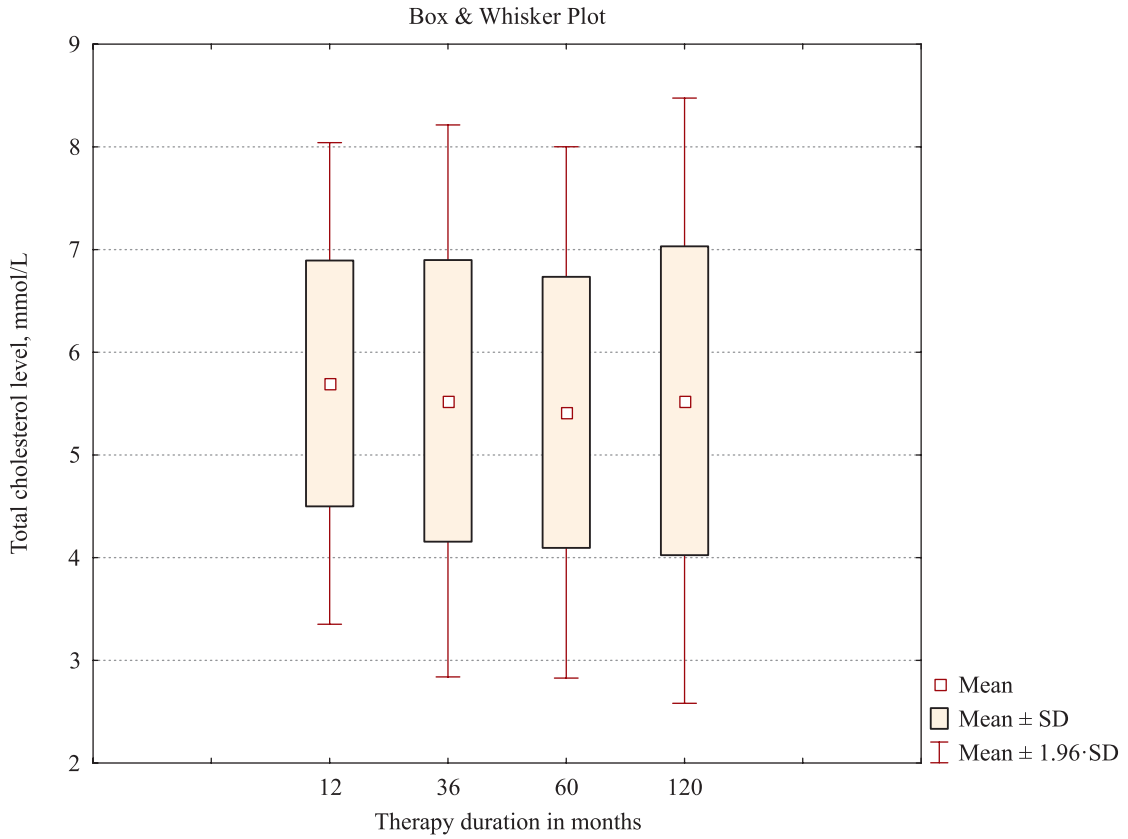

Fig. 4. Dynamics of total serum cholesterol in patients receiving long-term therapy with everolimus and low-dose extended-release tacrolimus

DISCUSSION

In a review study, Pavel Trunecka [2] analyzed publications addressing late conversion from twice-daily tacrolimus to its extended-release formulation. The findings showed that switching to extended-release tacrolimus is justified, as it improves dosing convenience, enhances adherence, and contributes to better patient survival. In the present cohort, however, everolimus was used as the second immunosuppressive component, necessitating twice-daily dosing, which diminished the compliance-related advantage of once-daily tacrolimus. Nevertheless, long-term use of the extended-release formulation contributed to more stable blood concentrations, consistent with observations reported by other investigators [11].

Based on data from two randomized trials [12] that included 772 liver transplant recipients – 488 from deceased donors (H2304) and 284 from living-related donors (H2307) – pooled analyses were conducted to evaluate the efficacy and safety of everolimus with low-dose tacrolimus versus standard-dose tacrolimus over 24 months of follow-up. The two regimens demonstrated comparable overall efficacy when assessed by the incidence of biopsy-confirmed rejection, graft loss, or death (9.8% vs. 10.8%; p = 0.641). Importantly, renal outcomes favored the everolimus regimen, with a smaller decline in eGFR at 24 months (–8.37 vs. –13.40 mL/min/1.73 m^2; p = 0.001).

In our study, which featured a considerably longer follow-up period but included a smaller cohort, no decline in renal function was observed. Twelve months after conversion, mean eGFR was 84.13 ± 16.70 mL/min/1.73 m^2, and after ≥120 months on reduced-dose extended-release tacrolimus plus everolimus, mean eGFR remained stable at 84.99 ± 21.30 mL/min/1.73 m^2. This favorable outcome is most likely attributable to the small patient sample and the benefit of close, individualized monitoring.

Regarding HCC recurrence, previous studies in patients outside the Milan criteria suggested a trend toward lower recurrence with everolimus (5.9% [1/17] vs. 23.1% [6/26], p = 0.215), though the difference did not reach statistical significance. For patients within the Milan criteria, recurrence rates were comparable, regardless of pre-transplant AFP levels [12]. The authors of this comparative study concluded that further long-term studies are needed.

In our cohort, recurrence was clearly influenced by AFP concentration prior to LT. While values ranged widely – from normal to 1500 IU/mL – the mean AFP was 429.2 ± 306.9 IU/mL. Importantly, AFP levels >400 IU/mL, a threshold generally recognized as conferring a high risk of HCC recurrence [13], were strongly associated with post-LT relapse (Z = 4.2, p = 0.0001).

A recent review by S. Poudel et al. [14] summarized current approaches to maintenance immunosuppression

after LT. To minimize toxicity, monotherapy with either CNIs or mTOR inhibitors is recommended in clinically stable patients. In the presence of renal dysfunction or proteinuria, modification of therapy is advised – most often by combining an mTOR inhibitor with a low-dose CNI. In the late post-transplant period, efforts are increasingly directed toward minimizing or even discontinuing immunosuppression, particularly to reduce the risk of HCC recurrence. However, this strategy carries a substantial risk of irreversible rejection and graft loss. Against this background, the regimen applied in our study can be regarded as a rational choice.

CONCLUSION

Our experience with a maintenance immunosuppressive regimen combining low-dose extended-release tacrolimus with everolimus suggests that this approach is safe and effective, supporting stable graft function and preserving renal function in long-term LT recipients. The most frequent complication associated with prolonged everolimus therapy was hypercholesterolemia, which can be successfully managed with modern lipid-lowering drugs.

However, this regimen does not reliably prevent HCC recurrence after LT. It is evident that the biological activity of the tumor and adherence to transplant center selection criteria are decisive prognostic factors. The possibility that higher prophylactic doses of everolimus may provide additional benefit remains an open question and warrants further investigation.

The authors declare no conflict of interest.

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