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The effect of early everolimus administration on the renal function while reducing the dosage of calcineurin inhibitors in liver transplant recipients in a long-term follow-up

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Abstract

Introduction. *The lifelong use of calcineurin inhibitors in liver transplant recipients leads to an increased incidence of chronic kidney disease.*

Objective. *To compare the changes in glomerular filtration rate over five years in liver transplant recipients between those on everolimus with a reduced exposure to calcineurin inhibitors and those on standard doses of calcineurin inhibitors.*

Material and methods. *Fourteen liver transplant recipient switched to everolimus with a minimization of calcineurin inhibitors exposure in the first months after liver transplantation from February 2009 to February 2015 who had received that therapy continuously for at least 60 months were included in the case-control study. Twenty eight liver transplant recipients (matched by sex, etiology of the underlying disease, calcineurin inhibitors) who were followed-up for at least 60 months after liver transplantation, who had received no dose of everolimus, in whom the glomerular filtration rate could be calculated at all points of analysis were selected as a comparison group (1:2). Glomerular filtration rate*

was calculated immediately before liver transplantation; 12, 24, 36, 48, and 60 months after liver transplantation. The glomerular filtration rate after liver transplantation was also calculated for liver transplant recipients from the main group immediately before the conversion to everolimus.

Results. *Before liver transplantation, the median of glomerular filtration rate in the main group of liver transplant recipients was lower (81.2 ml/min) than in the comparison group (97.5 ml/min, p=0.01). After liver transplantation, the renal function worsened in both groups of patients. In a pairwise comparison, the medians of glomerular filtration rate were statistically significantly lower after 12 months, 24 months, 36 months, 48 months after liver transplantation, than before liver transplantation. The median of glomerular filtration rate at the time of immunosuppression conversion was 44.3 ml/min. After the conversion of immunosuppression, the median of glomerular filtration rate gradually increased, and after 36 months the differences in glomerular filtration rate reached statistical significance compared with the level before conversion (69.4 ml/min; p=0.048). These differences still increased after 60 months after conversion (72.3 ml/min; p=0.041).*

Conclusion. *Long-term administration of everolimus with minimization of calcineurin inhibitors exposure with the early conversion to this immunosuppression regime provides a steady improvement in renal function in liver transplant recipients with a low glomerular filtration rate in the preoperative and early post-transplant period.*

Keywords: liver transplantation, immunosuppression, calcineurin inhibitors, proliferative signal inhibitors, everolimus

Conflict of interests Authors declare no conflict of interest

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AVT, antiviral therapy
BMI, body mass index
CI, confidence interval
CKD, Chronic Kidney Disease
CN, calcineurin inhibitors
EVE, everolimus
GFR, glomerular filtration rate
HCC, hepatocellular carcinoma
IS, immunosuppression
LT, liver transplantation
Me, median
MPA, mycophenolic acid

Introduction

After liver transplantation (LT), the recipients need lifelong maintenance immunosuppression (IS), which standard for the latest decades included calcineurin inhibitors (CI): cyclosporine and tacrolimus. One of the serious complications of their long-term use is the development of chronic kidney disease (CKD). According to the results of investigators from France, before LT, the glomerular filtration rate (GFR) of lower than 60 mL/min was observed in 11% of patients on the

Waiting List, but at 1 year after LT, a decrease in GFR of lower than 60 mL/min occurred in 51% of recipients [1]. Minimization of exposure to CIs (as per area under the concentration-time curve) has been one of the promising interventions aimed at slowing the progression of CKD and preserving kidney function in liver recipients. Obviously, a decrease in CI exposure below a certain level is accompanied by an increase in the risk of rejection. An important method to correct insufficient IS in this case is the use of proliferative signal inhibitors (mTOR): sirolimus or everolimus. The renoprotective properties of everolimus in combination with reduced doses of tacrolimus have been demonstrated in a clinical trial CRAD2304, which compared GFR levels between the groups of recipients who received everolimus in combination with reduced tacrolimus exposure, and recipients who received doses that provided standard tacrolimus exposure. After randomization, the recipients of both groups were followed-up for two years after LT [2]. The results obtained in this study were confirmed in further observation of a cohort of recipients who agreed to continue the study for the third year [3].

However, both groups of recipients in this study had relatively high GFR at the time of randomization (80 mL/min/1.73 m^2). Statistically significant differences in GFR were detected after 24 months of therapy (mean GFR 77.6 mL/min/1.73 m^2 in the group of recipients who received everolimus with the reduced tacrolimus exposure, compared to 66.1 mL/min/1.73 m^2 in the comparison group). The significant difference between the mean GFR values is all the more impressive, since the overlap of tacrolimus concentration levels between the studied groups of patients turned out to be greater than expected [2]. An important result of the study is that the differences in GFR between the groups were detected very quickly, as early as at one month after randomization, meanwhile, a complete differentiation between the groups had been achieved by the 4th

month of therapy. That is, the kidney function of the recipients included in this study remained normal or deteriorated in the early post-transplant period. In this context, it is important to study the effect of minimizing CI exposure while taking everolimus on the renal function in the recipients with initially impaired renal function who currently comprise a significant proportion of liver recipients.

The study objective was to compare the GFR changes during 60 months of everolimus administration in combination with reduced CI exposure and the GFR changes in liver recipients who received CIs at standard doses, in early IS conversion.

Material and methods

The main study group. From February 2009 to February 2020, 215 liver recipients from a deceased donor received one or more doses of everolimus as part of routine clinical practice. Twenty of these recipients received everolimus as one of the components of maintenance IS continuously, for more than 60 months. From the further analysis we excluded 3 recipients who underwent simultaneous liver and kidney transplantation, 2 recipients in whom everolimus was administered in the long-term post-transplant period (52 and 36 months after LT), and a female patient who received everolimus after liver retransplantation surgery performed 76 months after her first LT. Thus, for the further analysis we selected 14 recipients in whom everolimus was administered in the first month after LT (n=8), in the 2nd month (n=3), in the 3rd month (n=2), and at 4 months after LT (n=1).

Principles of forming the comparison group

The comparison group consisted of liver recipients who, by the time of analysis, had been followed-up for at least 60 months after LT

and did not receive a single dose of everolimus. From September 2000 to February 2015, 320 LTs were performed at the Moscow Liver Transplantation Center. After excluding those who died after liver retransplantation and dropped from follow-up earlier than 60 months after surgery, as well as the recipients who received everolimus during any period of follow-up, patients with a transplanted kidney, patients who received chemotherapy for the progression of hepatocellular cancer (HCC), there remained a group of 135 liver transplant recipients. Patients who did not have information about body weight and serum creatinine at least at one of the analysis time points were excluded from the considering them as candidates for the comparison group.

To enhance the statistical power of the study, pairs were selected in a ratio of 1:2. Thus, for the comparison group, we selected 28 recipients, most comparable to those in the main study group in gender, etiology of the underlying disease, and the CI exposure. In a retrospective review, the recipient groups were comparable in age, body mass index, the presence of diabetes mellitus, and the incidence of post-transplant hepatitis C.

The clinical and demographic characteristics of the compared recipient groups are presented in Table 1.

Table 1. Clinical and demographic characteristics of the compared groups of recipients

Parameters		**Main group, n=14**	**Comparison group, n=28**
Gender (M/F), % M		11/3, 78.6%	21/7, 75%
Age at the time of LT, M (95% CI), years		56.1 (53.0; 59.2)	51.1 (48.2; 54.0)
Calcineurin inhibitor: Tacrolimus / Cyclosporine, % tacrolimus		10/4 (71.4%)	20/8 (71.4%)
Etiology of	Viral, n (%)	10 (71.4%)	19 (67.9%)
	Alcoholic, n (%)	4 (28.6%)	6 (21.4%)

cirrhosis	Autoimmune, n (%)	—	3 (10.7%)
BMI, M (95% CI), kg/m^2		26.9 (24.0; 29.7)	26.8 (25.2; 28.4)
HCV infection, n (%)		5 (35.7%)	14 (50%)
Diabetes mellitus	Before LT	1 (7.14%)	5 (17.9%)
	After LT	4 (28.6%)	10 (35.7%)

Notes: CI, confidence interval; BMI, body mass index; M, mean

Liver recipients from the main study group were administered everolimus at an initial dose of 2 mg/day; the dose was adjusted, if necessary, to reach a trough concentration of 2-6 ng/mL. In parallel, the CI dose was reduced until the target trough concentrations of tacrolimus 2-5 ng/mL, and cyclosporine 50-80 ng/mL had been reached.

Recipients from the comparison group received one of the ICs in doses that provided the standard target trough blood levels of the drug with regard to the time elapsed after LT [4].

In all liver recipients included in the analysis, GFR was calculated according to the Cockcroft-Gault formula at the time periods: immediately before LT, at 12, 24, 36, 48, and 60 months after LT [5]. The acceptable time deviation from the corresponding time point was considered to be 1 month. Recipients from the main study group were also evaluated for GFR after LT immediately before everolimus administration.

Statistical processing of the study results was performed using the Statistica 8.0 software. The Kolmogorov-Smirnov test was used to check the normality of the data distribution. Statistical significance of differences between the compared parameters in case of a normal distribution was determined using the Student's t-test. In the absence of a normal data distribution, nonparametric criteria were used, namely, Wilcoxon test for pairwise comparisons of dependent variables, the Mann-Whitney U-test for comparison of independent variables. Differences between the compared parameters were considered

statistically significant if the error probability was lower than 0.05 ($p<0.05$).

Results

At the time of data analysis, all recipients were alive.

The indications to the use of everolimus in 5 patients included an impaired renal function in the early post-transplant period. In other 5 cases, everolimus was administered to prevent the HCC recurrence. Both those indications were seen in 4 patients. No HCC progression in any case was observed during the follow-up period.

IS conversion (reduction of CI exposure with concomitant administration of everolimus) took place shortly after LT. The median follow-up from the time of LT to IS conversion was 0.52 months (interquartile range 0.13-1.8 months). The median follow–up period for recipients from the main study group was 73.4 months, the minimum was 63.6 months, and the maximum was 100.5 months.

By the 60th month of follow-up, the maintenance immunosuppressive therapy in 14 recipients of the main group included everolimus at a mean daily dose of 2.1 mg (mean C0 (trough concentration was 5.0 ng/mL; 95% CI: 3.9; 6.0 ng/mL) and either IC tacrolimus (n=9) at a mean daily dose of 2.3 mg (mean C0 3.6 ng/mL, 95% CI 2.5; 4.6 ng/mL), or cyclosporine (n=3) at doses of 75-100 mg/day (C0 40 ng/mL; 95% CI: 40.6 ng/mL, 42.7 ng/mL). In 2 liver recipients, CI was completely canceled at 7 and 33 months after everolimus administration, and then they continued to receive monosuppression with everolimus in combination with 4 mg of methylprednisolone until the end of the follow-up period. All recipients received mycophenolic acid (MPA) preparations in the first 3 months after LT, which had been discontinued by the 4th month according to the protocol established at the

Center. Two recipients from the comparison group resumed taking MPA in the long-term period after LT.

In the comparison group, 20 patients received tacrolimus at a mean daily dose of 5.7 mg (95% CI: 4.7; 6.7 mg) by the 12th month after LT, with the mean blood level of tacrolimus being 7.6 ng/mL (95% CI: 5.8; 9.3 ng/mL) before taking the morning tacrolimus dose. At 60 months after LT, the mean daily dose of tacrolimus in this group was 4.4 mg (95% CI: 3.6; 5.1 mg), with a mean trough tacrolimus concentration of 5.9 ng/mL (95% CI: 5.0; 6.9 ng/mL). Eight patients in the comparison group received cyclosporine as the main component of maintenance IS. By the 12th month of follow-up, the mean daily dose of cyclosporine was 212 mg (95% CI: 159; 266 mg) at a mean trough concentration of 159 ng/mL (95% CI: 109; 205 ng/mL). By the 60th month after LT, the mean daily dose of cyclosporine was 150 mg (95% CI: 105; 195 mg) with a mean trough concentration of 96 ng/mL (95% CI: 59; 132 ng/mL).

During the follow–up period, a histologically confirmed episode of an acute cellular rejection was observed in a single (female) patient from the comparison group, and in no case in the main study group (including those 2 recipients who received monosuppression with everolimus). The episode of an acute cellular rejection was completely coped with by using a pulse therapy with methylprednisolone.

Recurrent hepatitis C was observed after LT in 5 of 14 recipients (35.7%) from the main study group and in 14 of 28 (50%) recipients from the comparison group ($p>0.3$). HCV replication persisted for more than a half of the follow-up period (>30 months) in one patient from the main study group and in 5 patients from the comparison group. The median start of antiviral therapy (AVT) in the main study group was 9 months (Q25% 6.5 months; Q75% 27 months). In the comparison group, the median start of AVT was 22.5 months. (Q25% 7 months.; Q75% 38.5

months, p=0.056). After AVT, a stable virological response was obtained in all patients.

The results of GFR calculation during the follow-up period, depending on the scheme of the maintenance IS, are presented in Table. 2 and in the Figure.

Table 2. Glomerular filtration rate depending on the type of maintenance immunosuppression

Glomerular filtration rate	Group of liver transplant recipients	
	Main study group (n=14)	Comparison group (n=28)
before LT (ml/min), M (Q25%; Q75%)	81.2 (57.0; 96.9)	97.5 (86.5; 140.9)
before starting EVE (ml/min), M (Q25%; Q75%)	44.3 (36.3; 74.0)	—
after 12 months (ml/min), M (Q25%; Q75%)	65.4 (51.3; 78.0)*	74.9 (65.9; 84.8)†
after 24 months (ml/min), M (Q25%; Q75%)	63.3 (52.0; 77.3)*	79.1 (70.0; 107.0)†
after 36 months (ml/min), M (Q25%; Q75%)	69.4 (48.0; 79.5)*	84.1 (63.5; 101.9)†
after 48 months (ml/min), M (Q25%; Q75%)	67.1 (46.8; 73.5)*	79.0 (57.3; 101.0)†
after 60 months (ml/min), M (Q25%; Q75%)	72.3 (49.7; 80.0)	79.7 (60.0; 97.1)†

Notes: EVE, everolimus.

† p <0.002 compared to GFR before LT;

* p <0.05 compared to GFR before LT.

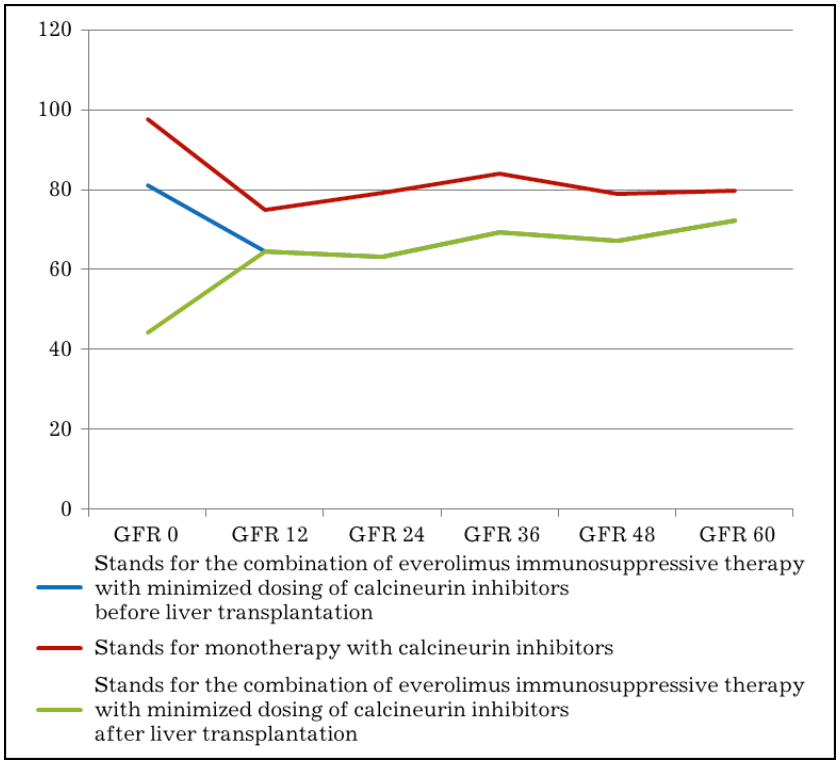

Figure. Changes in the median glomerular filtration rate in patients from the comparison group (red line) and the main group compared to the glomerular filtration rate before orthotopic liver transplantation (blue line) and glomerular filtration rate at the time of immunosuppressive therapy conversion (green line)

None of the patients who received everolimus in our group for a long time displayed any significant proteinuria. In 3 patients from the comparison group, diabetic nephropathy was identified, in one case it was manifested by massive proteinuria.

Discussion

We analyzed the routine clinical practice of our Liver Transplantation Center in order to identify the effect of early conversion of immunosuppressive therapy (reducing the CI exposure and administering everolimus) on kidney function in the long-term period after LT. At the time of the analysis (February 2020), only 14 recipients

met the criteria for inclusion in the data analysis. IS conversion was performed in the early post-transplant period, followed by continuous administration of everolimus for at least 60 months. The comparison group was selected in such a way as to balance the main possible risk factors for CKD progression in liver recipients (CIs, BMI, diabetes mellitus, recurrent hepatitis C). Despite all the disadvantages inherent in retrospective studies with a relatively small patient sample size, the statistical power of our study seems to be sufficient to solve the tasks set.

Before LT, the median GFR in the main study group of recipients was significantly lower (81.2 mL/min) than in the comparison group (97.5 mL/min, p=0.01), which could be explained by the study design. After LT, the renal function deteriorated in both groups of patients. In pairwise comparison, GFR medians were significantly lower at 12 months, 24 months, 36 months, and 48 months than before LT. After 60 months of follow-up, statistically significant differences in GFR compared to the level of pre-LT persisted in liver recipients from the comparison group who received monosuppression with CI. It should be noted that in this group of recipients, GFR having initially deteriorated, then remained stable throughout the entire follow-up period.

The group of patients who underwent early conversion of immunosuppressive therapy mainly included recipients whose renal function was initially decreased. Only 5 of 14 recipients operated on for HCC had GFR more than 60 mL/min at the time of immunosuppressive therapy conversion (to everolimus with reduced CI exposure), but none of them had GFR more than 90 mL/min. The median GFR at the time of immunosuppressive therapy conversion was 44.3 mL/min. After IS conversion, the median GFR gradually increased, and after 36 months, the differences in GFR reached statistical significance compared to the pre-conversion level (69.4 mL/min; p=0.048). These differences, also

statistically significant, increased even more at 60 months after conversion (72.3 mL/min; p=0.041).

I. Bilbao et al. (2015) found that in the recipients with an impaired renal function who started taking everolimus in the long term (after the first year post-LT), GFR did not improve, or, having initially improved, GFR deteriorated again at 12 months after the everolimus administration. If the IS conversion in such recipients was undertaken in the first year after LT, the improvement in kidney function was more significant [6]. However, during the first year, earlier IS conversion (the first 3 months after LT) did not lead to such pronounced differences in GFR changes compared to a later administration of everolimus [7].

Another group of investigators emphasized the importance of timely detection of renal dysfunction in liver recipients and an immediate immunosuppressive therapy conversion with administering everolimus [8]. Thus, in the group of liver recipients whose GFR continued to decrease after IS conversion, the follow-up from the moment of recording the renal failure to the moment of IS conversion was significantly longer (26 ± 14 months), than in the group of recipients whose GFR improved (7 ± 10 months; p = 0.04, statistically significant).

The results of our study have confirmed the possibility of early (in the first 3 months) conversion of immunosuppressive therapy, which is safe from the point of the risk of liver graft rejection and effective from the point of improving glomerular filtration rate. Such a change in immunosuppressive therapy leads to a lasting improvement of the renal function in liver recipients having a low glomerular filtration rate in the preoperative and early post-transplant period, and this improvement retains for 5 or more years. The results obtained give us the grounds to recommend the earliest possible administration of everolimus with concomitantly reducing the calcineurin inhibitor exposure in the patients

with a marked decrease in glomerular filtration rate (no more than 60 mL/minute), especially in the presence of other risk factors for deterioration of the renal function.

Conclusions

1. A long-term use of calcineurin inhibitors in standard doses in liver transplant recipients with the normal renal function leads to its deterioration by the mean of 18.3% after 60 months from orthotopic kidney transplantation.

2. An early administration of everolimus in combination with minimizing the dose of calcineurin inhibitors in liver transplant recipients with an initially impaired renal function leads to its rapid improvement, which retains for a long time in 39% of patients.

References

1. Karie-Guigues S, Janus N, Saliba F, Dumortier J, Duvoux C, Calmus Y, et al. Long-term renal function in liver transplant recipients and impact of immunosuppressive regimens (calcineurin inhibitors alone or in combination with mycophenolate mofetil): the TRY study. *Liver Transpl.* 2009;15(9):1083–1091. https://doi.org/10.1002/lt.21803

2. Saliba F, De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, et al. Renal function at two years in liver transplant patients receiving evero-limus: results of a randomized, multicenter study. *Am J Transplant.* 2013;13(7):1734–1745. https://doi.org/10.1111/ajt.12280

3. Fischer L, Saliba F, Kaiser GM, De Carlis L, Metselaar HJ, De Simone P, et al. Three-year outcomes in de novo liver transplant patients receiving everolimus with reduced tacrolimus: Follow-up results from a randomized, multicenter study. *Transplantation.* 2015;99(7):1455–1462. https://doi.org/10.1097/TP.0000000000000555

4. Lucey MR, Terrault N, Ojo L, Hay J, Neuberger J, Blumberg E, et al. Long-term management of the successful adult liver transplant: 2012 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Liver Transpl.* 2013;19(1):3–26. https://doi.org/10.1002/lt.23566

5. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16(1):31–41. https://doi.org/10.1159/000180580

6. Bilbao I, Salcedo M, Gómez MA, Jimenez C, Castroagudín J, Fabregat J, et al. Renal function improvement in liver transplant recipients after early everolimus conversion: A clinical practice cohort study in Spain. *Liver Transpl.* 2015;21(8):1056–1065. https://doi.org/10.1002/lt.24172

7. Lopez FN, Alfocea PA, Ortega Suazo EJ, López Garrido MA, Massare BA, Gila Medina AM, et al. Impact of everolimus-based immunosuppression on renal function in liver transplant recipients. *Transplant Proc.* 2020;52(2):556–558. PMID: 32035673 https://doi.org/10.1016/j.transproceed.2019.12.012

8. Cholongitas E, Goulis I, Theocharidou E, Antoniadis N, Fouzas I, Giakoustidis D, et al. Everolimus-based immunosuppression in liver transplant recipients: a single-centre experience. *Hepatol Int.* 2014;8(1):137–145. PMID: 26202415 https://doi.org/10.1007/s12072-013-9492-6

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