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Development of a clinical protocol for personalized minimization of immunosuppression in liver recipients in the long-term period after transplantation

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Abstract

Background. *Long-term immunosuppression in standard regimens, though preventing a rejection, simultaneously increases morbidity and mortality in liver transplant recipients. Minimization or discontinuation of immunosuppressive drugs may reduce this burden; however, the permissible limits, safety, tolerability, clinical outcomes, and long-term results of this strategy remain undefined and require thorough study and clarification.*

Objective. *To develop a clinical protocol and investigate the feasibility and clinical safety of personalized immunosuppression minimization in the long-term period after liver transplantation.*

Materials and methods*. Forty stable liver recipients, at 74±7.21 months (range: 6–182 months) post-transplantation, were included in a retrospective single-center study on tacrolimus*

minimization/discontinuation. Mean age at enrollment was 56.3±1.4 years. Twenty-two (55%) patients were on tacrolimus monotherapy, and 18 (45%) on a combination tacrolimus-everolimus therapy. Tacrolimus dose was gradually reduced over 8 (4;11) months.

Results. *Graft dysfunction developed in 32.5% of cases (n=13) with dose reductions of 25–95%. Rejection was diagnosed in 7 (17.5%) patients based on laboratory and morphological data, 3–9 months after starting minimization. All dysfunction episodes were reversible. The median duration of subsequent dysfunction-free graft period in all intolerant patients was 28 (26;33) months. Twenty-seven (67.5%) patients tolerated minimization: 15 (37.5%) completely discontinued tacrolimus, and 12 (30%) achieved a 40–87.5% dose reduction (mean 62.7%). Median follow-up for tolerant patients after completing the minimization was 26 (20;30) months. Control biopsies performed 1–33 months (median 15 months) after completing the minimization showed no negative dynamics in histological patterns. Comparison between the everolimus group and the monotherapy group revealed significant differences: tolerant patients to immunosuppression minimization accounted for 83% vs. 55% (p=0.053); tacrolimus discontinuation was achieved in 72.2% vs. 9.1% (p<0.001); the dose reduction extent was 100 (90.3;100) % vs. 56 (42.5;78.8) % (p<0.001). In the whole cohort, the estimated GFR increased from the baseline of 62 (50;70) mL/min/1.73 m² to 66.5 (54;80) mL/min/1.73 m² by the end of the follow-up (p=0.011*

Conclusion. *Controlled immunosuppression minimization in the long-term post-transplant period is successful in a significant proportion of carefully selected recipients, being safe with strict protocol adherence. A maximal tacrolimus dose reduction is achieved more frequently and with lower rejection risk in patients on everolimus. A positive effect of minimization on kidney function has been confirmed.*

Keywords: liver transplantation, minimization of immunosuppression, tacrolimus, everolimus, liver transplant rejection, tacrolimus nephrotoxicity

Conflict of interest The authors declare no conflict of interest.

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ALP, alkaline phosphatase
ALT, alanine aminotransferase
AST, aspartate aminotransferase
CCC, cholangiocellular carcinoma
CNI, calcineurin inhibitor
E, everolimus
GFR, glomerular filtration rate
GGT, gamma-glutamyl transpeptidase
HBV, hepatitis B virus
HCC, hepatocellular carcinoma
HCV, hepatitis C virus
IST, immunosuppressive therapy
LC, liver cirrhosis
LFTs, liver function tests
LT, liver transplantation
MSD, minimum sufficient dose
RAI, rejection activity index
T, tacrolimus
ULN, upper limit of normal

Introduction

Liver transplantation (LT) as a treatment technology involves the use of lifelong immunosuppressive therapy (IST) to prevent graft rejection. In a significant proportion of patients, these drugs, primarily the calcineurin inhibitor (CNI) tacrolimus (T), cause serious side effects. These include nephrotoxicity, cancer, metabolic disorders, cardiovascular disease, and opportunistic infections. infections, which largely cause increased morbidity and mortality in liver transplant recipients [1–8].

In the transplant community, there is an opinion among some experts that a significant proportion of stable patients receive excessive IST for a long time, and therefore, a strategy to minimize chronic immunosuppressive effects may become a way to solve these problems and a very promising therapeutic target [9–11]. However, there are no standardized criteria for a personalized determination of the minimum sufficient dose (MSD) of T for a specific recipient. Current international clinical protocols and recommendations are focused on drug monitoring and are limited to specifying the range of Tacrolimus (T) concentrations for a certain period after surgery, taking into account the use of other immunosuppressants, the nature of the underlying disease, and comorbid risks. However, they do not take into account the individual characteristics of the patient and the severity of his/her immune response to the graft (alloreactivity), and, consequently, the required degree of its suppression [12–16].

It is well known that some kidney and liver transplant recipients can discontinue taking conventional immunosuppressive drugs without developing rejection. This phenomenon, referred to as spontaneous “operative tolerance” in the scientific literature, is defined as successful cessation of IST for at least 12 months with preserved graft function and no histopathological signs of rejection [17].

A number of early studies, beginning in the last decade of the 20th century, described observations of successful complete withdrawal of immunosuppression in individual liver transplant recipients. These were retrospective observations or small prospective series that lacked standardized inclusion criteria and withdrawal algorithms. Important clinical aspects of the withdrawal protocol, such as achieving surgical tolerance, histopathological results, and clinical and morphological predictors of successful withdrawal, were not adequately defined and quantified [17–21].

Subsequently, prospective studies were undertaken, in which the average rate of successful targeted discontinuation of IST in liver transplant recipients was 20%, reaching 40% in isolated observations [22–29]. However, different approaches to patient selection, processing, and presentation of data in published materials do not allow for an accurate comparison or generalization of the results of different centers, an assessment of the overall effectiveness of the method, and its recommendation for routine clinical practice.

We initiated our own a retrospective single-center interventional clinical trial to evaluate the feasibility and safety of minimizing/discontinuing the main immunosuppressant drug, Tacrolimus, in stable adult recipients late after transplantation and to identify factors influencing the success of this procedure. All patients signed a written informed consent to participate in the study. The study protocol was approved by the local ethics committee (Minutes No. 14 of the NEC Meeting at the Moscow Regional Research and Clinical Institute n.a. M.F. Vladimirskiy, Moscow, October 12, 2023).

The aim of the study was to develop a clinical protocol and to study the feasibility and safety of personalized minimization of immunosuppression in patients in the later period after liver transplantation.

Material and methods

Patient enrollment

The study cohort was selected from 172 patients undergoing outpatient follow-up after LT in the Department of Surgery and Liver Transplantation of the Moscow Regional Research and Clinical Institute n.a. M.F. Vladimirskiy. The inclusion period was from January 2022 to December 2024.

***Inclusion criteria for the prospective study**:*

- Recipient aged 18 years or older;
- Liver transplantation from a deceased donor with follow-up for at least 3 years;
- Immunosuppression: monotherapy with T or combination T + Everolimus (E) therapy;
- Absent of autoimmune and cholestatic liver diseases that could nave served as an indication for liver transplantation;
- Absent episodes of rejection and/or graft dysfunction during the latest 12 months;
- Liver function test (LFT) level: aspartate aminotransferase (AST), alanine aminotransferase (ALT) not exceeding the upper limit of normal (ULN); alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) not exceeding 1.5 ULN;
- patients who signed informed consent

Exclusion criteria:

- Presence of viral hepatitis B and/or C (a positive test result for the hepatitis B virus surface antigen, a positive test result for the hepatitis C virus (HCV) RNA in case of a positive test result for HCV antibodies)
- Pregnancy;

- Alcohol consumption;
- Patient non-compliance;
- Long distance from the investigator center, making impossible to keep the schedule of visits and examinations;
- Patients with previous or unstable comorbidities or clinical conditions that prevent the inclusion of the patient in the study.
- Negative result of screening liver biopsy: 1) signs of acute (RAI more than 3) or chronic rejection according to the Banff criteria [30]; 2) histological activity of inflammation more than 7 points according to Kneel and (or) more than A1 according to METAVIR; 3) fibrosis more than F 2 according to Kneel and (or) according to Isaac.

Minimization protocol

The T dose was gradually reduced according to the established protocol. With an initial daily dose of at least 5 mg, it was reduced by 1 mg every 4 weeks to reach 3 mg/day, then by 0.5 mg every 4 weeks to reach 0.5 mg/day. Then, a dose of 0.5 mg/day was prescribed for administration every other day (4 weeks), twice a week (4 weeks), and once a week (4 weeks), after which T was discontinued. A biochemical LFT assay was performed before each dose reduction (Fig. 1).

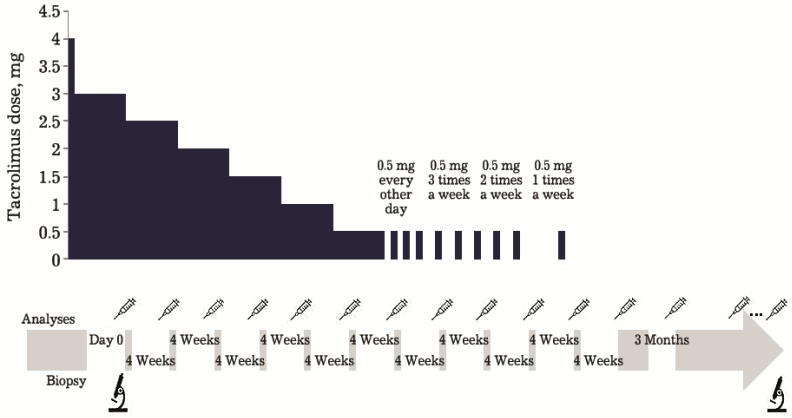

Fig. 1 Tacrolimus tapered dose reduction protocol

Blood levels of Tacrolimus concentrations were measured at baseline and after minimization, as well as if dysfunction or rejection developed. In patients receiving Everolimus, the dose was adjusted as needed to maintain blood drug concentrations within the range of 3–8 ng /mL.

Laboratory and instrumental monitoring

Liver function tests were performed every 4 weeks during the dose tapering period, every 2 weeks or more frequently if dysfunction developed, and at least every 3 months during follow-up after the completion of the dose reduction. Renal function was assessed using the estimated glomerular filtration rate (GFR) using the CKD-EPI 2021 formula.

Liver biopsies were performed in 26 patients at study entry, in 10 patients as indicated (for graft dysfunction), and in 22 patients, follow-up biopsies were performed at various points during the observation period after completion of liver minimization. Liver biopsies were performed under ultrasound guidance using the PLURI-GUN automated system (MDLSRL, Italy) with a 16-gauge needle. All histological specimens were analyzed by an expert pathologist, with quantitative assessment using the Banff (RAI), Knodell, and Ishak scores. A total of 62 biopsies were subjected to morphological assessment.

Definition and treatment of graft dysfunction

Graft dysfunction was defined as an isolated or synchronous increase in AST, ALT, GGT, and ALP levels by more than 1.5 times the baseline. If AST and/or ALT levels did not exceed the ULN by twice (borderline dysfunction), a further dose reduction was discontinued, and biochemical testing was repeated after 7 to 14 days. In case of a more pronounced increase in biochemical parameters (a severe dysfunction), the previous T dose was reinstated, and monitoring was also repeated

after 7 to 14 days. If LFTs returned to normal, the minimization was discontinued. Further progression or persistence of dysfunction served as an indication for liver biopsy and termination of minimization with a return to the previous or even the baseline dose. Isolated increases in GGT levels were not considered an indication for liver biopsy.

Definition of the "rejection" concept

Rejection was diagnosed based on the combination of severe dysfunction and characteristic liver biopsy findings according to the Banff criteria [30]. A severe dysfunction occurring during the minimization period and rapidly responding to an increase in the T dose and hormonal pulse therapy was also considered a sign of rejection even in the absence of a liver biopsy, provided that other etiologic causes (vascular and/or biliary complications, reactivation of a viral infection) were excluded.

Study endpoints

The primary endpoints of the study were the MSD achievement or a successful complete T discontinuation. Secondary endpoints were the following: incidence and severity of rejection episodes, time from the onset of minimization to rejection, the minimization rate at the time of rejection, reversibility and timing of normalization of graft function after a rejection episode, long-term changes in LFTs, changes in renal function and histopathological characteristics.

Statistical processing and data analysis

The study materials were statistically processed using parametric and nonparametric analysis methods. The initial data were systematized and clinical observations were recorded in Microsoft Office Excel 2016

format. Data analysis was performed using the Jamovi software statistical package (version 2.3.28.0). Descriptive statistics determined by the type of statistical parameter, were used to characterize the study cohort for all statistical parameters. Normally distributed parameters were represented by the following values: sample mean, standard error of the mean, 95% confidence interval, and minimum and maximum values of the sample data (introduced by the authors to detail the characteristics of the analyzed group). To describe quantitative parameters with a distribution different from normal, the median, 25% and 75% quartiles, and the minimum and maximum values of the sample data were used. The Shapiro–Wilk test or the Kolmogorov–Smirnov test was used to assess the normality of the distribution of quantitative data. Statistical comparison of the mean values of continuous quantitative variables between two independent groups was performed using Student's t-test (for normally distributed parameters). To compare independent populations in cases where the data were not normally distributed or when comparing by ordinal parameter, the Mann–Whitney U test was used. Differences in values were considered statistically significant at a $p<0.05$ level. Nominal data were compared using the Pearson χ^2 test and Fisher's exact test. The odds ratio was used as a quantitative measure of effect when comparing relative parameters.

Results

General characteristics of patients

Between January 2022 and December 2024, 172 outpatient liver transplant recipients were screened to determine their eligibility for inclusion in the prospective study of a minimization study according to the adopted protocol. A total of 35 patients were selected for the

prospective study (Fig. 2), and screening liver biopsy was performed in 26 (74.3%) patients.

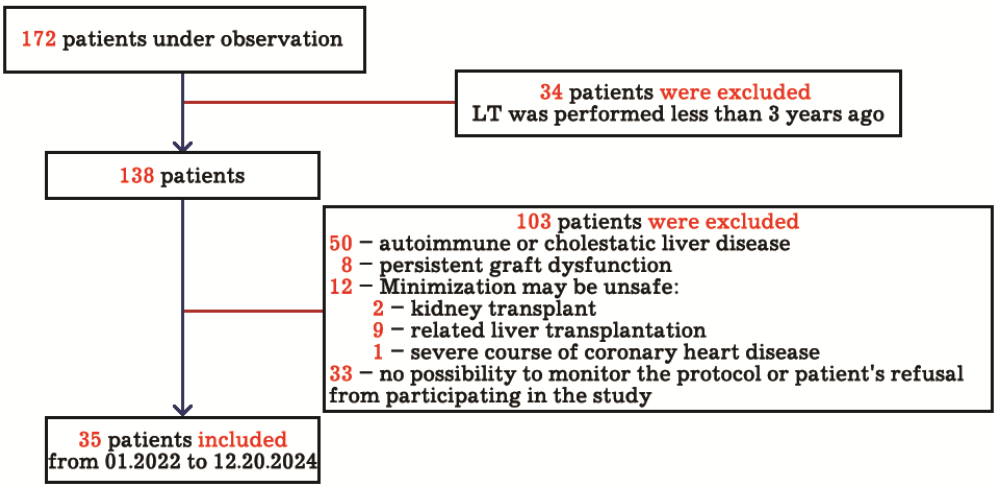

Fig. 2. Selection of patients for inclusion in the prospective study

In addition, the final data analysis retrospectively included one patient who completely self-discontinued the prescribed combination IST 20 months after LT and 4 patients whose T was aggressively (in accelerated manner) discontinued while receiving E due to the development of hepatocellular carcinoma (HCC) relapse at 6, 20, and 66 months and cholangiocellular carcinoma (CCC) at 12 months after LT.

As a result, the study cohort included 40 liver recipients. Their indications for transplantation are presented in Fig. 3. In one patient operated on for HCC, CCC was detected during histological examination of the removed liver specimen.

The mean age at the time of transplantation was 50±1.5 [47–53] (27–67) years, 56.3±1.4 [53.5–59] (37–69) years at the time of starting minimization. Males predominated, 32 (80%). The mean time after LT at the minimization start was 74±7.21 [60–88] (6–182) months. Twenty-two

(55%) patients were receiving T monotherapy at inclusion, and 18 (45%) were receiving a combination of T+E. All patients received extended-release T once daily. Blood levels of drugs were within the recommended ranges according to the time after LT.

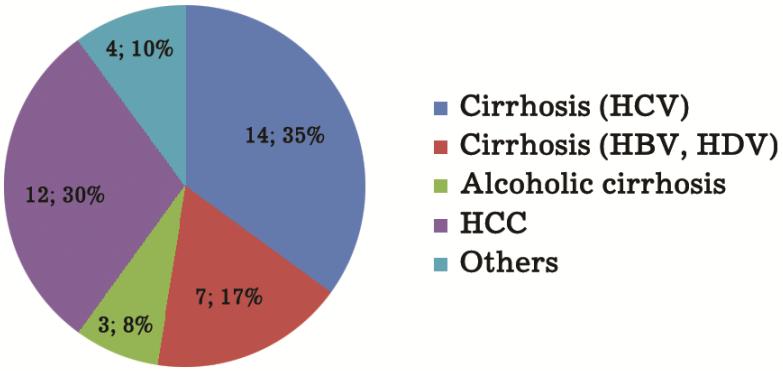

Fig. 3. Indications for transplantation in patients included in the study

The median T dose at baseline was 2 (2;4) (1–8) mg/day, E dose was 2 (2;3) (1–3) mg/day. The mean baseline blood level of T was 4.4±0.3, 95% CI [3.9–5] (2–8.5) ng/mL, that of E was 5.62±0.4, 95% CI [4.7–6.5] (3–9) ng/mL.

Tolerance to minimization

Depending on the stepwise T dose minimization tolerability, which was assessed by the LFT changes over time, the intervention phase of the study proceeded according to different scenarios (Fig. 4). Fifteen (37.5%) patients completely discontinued T as a result of the stepwise dose reduction according to the protocol without deterioration of biochemical parameters during the minimization period and the entire subsequent follow-up. The majority of them (13; 86.6%) received E during the withdrawal period and continues to receive E. In 12 (30%) patients, the

successfully continued minimization according to the protocol was discontinued: in 8 cases due to their refusal for subjective reasons, in 4 patients due to the development of events not associated with a decrease in immunosuppression, such as the reactivation of viral hepatitis B/D (N=2), biliary complications (N=2). In this subgroup, however, a significantly reduced T dose was recorded at 12.5% (N=1), 17% (N=1), 20% (N=2), 25% (N=2), 30% (N=1), 33% (N=1), 37.5% (N=1), 50% (N=2), and 60% (N=1) of the baseline and continued to take it unchanged throughout the follow-up. Thus, in this subgroup, a dose reduction of 40–87.5% was achieved, with an average dose reduction of 62.7%.

Ultimately, of the 40 patients included in the analysis, 27 (67.5%) were **tolerant** to T dose reduction to varying rates without biochemical manifestations of graft dysfunction.

The group of **intolerant** patients included 13 (32.5%) patients, in whom biochemical abnormalities were observed during the minimization process, with a stepwise T dose reduction to 5–75 % of the initial dose. Six patients experienced borderline dysfunction as a manifestation of insufficient immunosuppression, which completely regressed after cessation of minimization or return to the previous dose.

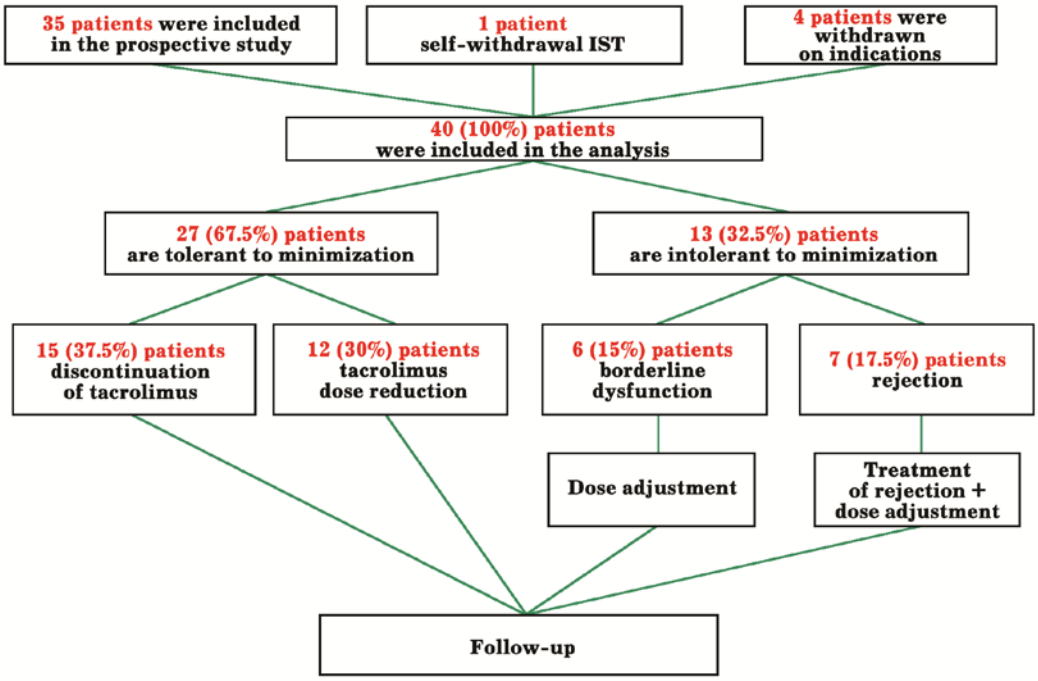

Fig. 4. Study progress and minimization scenarios

In 7 (17.5%) patients with more significant biochemical abnormalities after a dose reduction of 33–95 %, a **rejection** was suspected when, during the next control, they revealed an increase in the level of aminotransferases to 2–40 ULN, GGT to 7 ULN, and ALP up to 4.5 ULN. Only one patient had an increase in bilirubin up to 10 ULN (Table 1).

Histological verification of rejection was obtained in 6 patients; meanwhile, the rejection severity according to histological examination, as shown in the table, did not correlate with the severity of the biochemical response. According to the RAI index, the rejection was assessed as indefinite in 1 (16.6%), mild in 2 (33.3%), and moderate in 3 (50.0%). All 7 patients required an increase in the T dose and additional steroid treatment to stop rejection. However, only 3 (43%) of them returned to the baseline dose.

Table 1. Clinical, laboratory and histological characteristics of rejection episodes

Parameter	Pat. K.	Pat. F.	Pat. P.	Pat. B.	Pat. Kh.	Pats. R.	Pats. T.
Age, years	59	57	69	56	39	63	56
Gender	F	f	m	F	M	M	f
Period after T, months	191	127	109	91	89	39	103
Tacrolimus concentration at the time of rejection, ng/mL	3.1	0.7	1.1	1.5	2.4	4.2	3
Tacrolimus dose at the time of rejection, mg/day (% of the initial dose)	3 (37.5%)	0.5 (17%)	0.14 (5%)	0.25 (12.5%)	1 (50%)	1 (33%)	1 (50%)
Everolimus dose, mg/day	0	0	0	0	0	1.5	2
Period of dose minimization before rejection, months	9	8	9	5	3	7	7
Histological assessment, RAI scores	7	3	No	6	4	6	4
Peak AST, U/L	86	234	171	173	106	1093	1461
Peak ALT, U/L	90	278	336	271	257	1594	1061
Peak bilirubin, µmol/L	15.8	35.3	19.2	17.0	20.6	191	18.1
Peak GGT, U/L	116	181	321	55.7	36	224	372
Peak ALP, U/L	136	167	216	283	103	534	124
Treatment: intravenous methylprednisolone (pulse therapy) mg	500x3	250x3	250x3	250x3	500x3	500x3*	500x3
Return to Tacrolimus dose, mg/day (% of initial)	5 (62.5%)	3 (100%)	1.5 (50%)	1 (50%)	2 (100%)	2 (67%)	2 (100%)
Normalization time (weeks)	2	12	2	2	3	12	10
Period of relapse-free follow-up after treatment for rejection, months	31	36	34	36	32	24	19

*** In patient R.**, methylprednisolone pulse therapy was supplemented by oral methylprednisolone at a dose of 24 mg/day with tapering to 4 mg/day over three months due to persistent cholestasis. GGT and ALP levels were noted to be normalized 1.5 years after the rejection. Steroids were discontinued.

The time to LFT normalization ranged from 1 to 12 weeks. All rejection episodes were successfully treated and had no long-term biochemical or histological sequelae during follow-up periods of 19 to 36 months. Immunosuppressant doses remained unchanged during the follow-up period.

To identify the potential predictors of tolerability of LFT, we compared groups of tolerant and non-tolerant patients. As shown in Table 2, the comparison groups did not differ in age at the time of LT and the start of minimization, or in time since LT. The proportion of males was higher in the tolerant group, but the difference did not reach statistical significance. No significant differences were observed in the etiology of the liver disease that served as an indication for transplantation.

Table 2. Comparative characteristics of the baseline clinical data of tolerant and intolerant patients

Parameter	All patients (n=40)	Tolerant (n=27)	Intolerant (n=13)	p
Age at the time of LT, years M±m [95%CI] (min–max)	50±1.5 [47–53] (27–67)	50.4±1.9 [47–54] (27–67)	49.5±2.4 [45–54] (32–64)	0.791
Age at the start of minimization, years M±m [95% CI] (min–max)	56.3±1.4 [53.5–59] (37–69)	56±1.8 [52.5–59.5] (37–69)	56.8±2.2 [52.5–61] (39–69)	0.977
Period after LT to the start of minimization, months M±m [95% CI] (min-max)	74±7.21 [60–88] (6–182)	66±8.83 [49–83.4] (6–182)	90±11.6 [67.4–113] (32–181)	0.118
Male gender, n (%)	32 (80.0%)	24 (88.9%)	8 (61.5%)	0.109
Indications for LT, n (%):				
Viral LC (HCV)	14(35%)	9(33.3%)	5(38.5%)	0.426
Viral LC (HBV, HDV)	7(17%)	3(11.1%)	4 (30.8%)	
HCC	12(30%)	9 (33.3 %)	3 (23.1 %)	
Alcoholic LC	3(8%)	3 (1 1.1 %)	0 (0.0 %)	
Other	4(10%)	3(11.1 %)	1 (7.6 %)	

Note: LC, liver cirrhosis

When considering the immunosuppression at baseline, a significant predominance of the combined T+E regimen and a lower baseline T blood level in the group of tolerant patients were noted, however, these differences did not reach the level of statistical significance, most likely due to insufficient sample size (Table 3, Fig. 5).

Baseline biochemical parameters, including LFT, creatinine, and estimated GFR values, did not differ between groups, ether.

Table 3. Immunosuppression in tolerant and intolerant patients before the start of minimization

Parameter	All patients (n=40)	Tolerant (n=27)	Intolerant (n=13)	p
Tacrolimus (monotherapy), n (%)	22 (55%)	12 (44.4 %)	10 (76.9 %)	0.090
Tacrolimus + Everolimus, n (%)	18 (45%)	15 (55.6%)	3 (23.1%)	
Tacrolimus dose, mg/ day, Me (Q_1;Q_3) (min–max)	2 (2;4) (1–8)	2 (2;4) (1–6)	2 (2;3) (2–8)	0.865
Everolimus dose, mg/day, Me (Q_1;Q_3) (min–max)*	2 (2;3) (1–3)	2 (2;3) (1–3)	2 (1.75;2) (1.5–2)	0.310
Blood level of Tacrolimus, ng/mL, Me (Q_1;Q_3) (min–max)	4.3 (3;5.5) (2–8.5)	4.1 (3.2;4.9) (2–8.5)	4.9 (2.9;6.6) (2–7.4)	0.495
Blood level of Everolimus, ng/mL, Me (Q_1;Q_3) (min–max)*	5.2 (4.3;6.7) (3–9)	5, 2 (4,2;6,45) (3–8.9)	5.5 (5.3;7.3) (5–9)	0.353

* Calculation was made for 15 tolerant and 3 intolerant patients

Thus, none of the studied parameters demonstrated statistically significant predictive value in assessing the tolerability of T dose reduction, or, in other words, tolerance to tapering. However, it is noteworthy that 83.3% (15 of 18) of patients in the combination IST group were tolerant, compared to only 54.5% (12 of 22) in the monotherapy group. Furthermore, 13 of 15 (86.6%) tolerant patients successfully discontinued T, continuing E monotherapy.

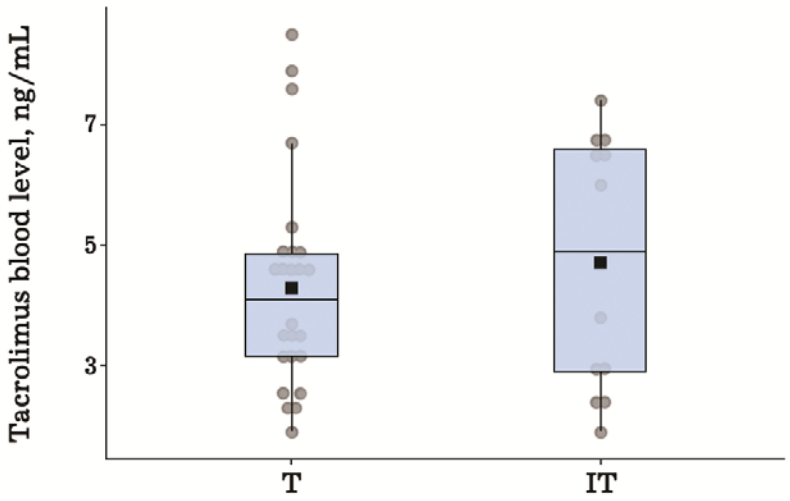

Fig. 5. Baseline tacrolimus blood level (ng/mL) in the groups of tolerant (T) and intolerant (IT) patients (p=0.495)

Effect of everolimus on tolerance to minimization

To clarify the E role in the success and rate of minimization, a comparative analysis was conducted between the T monotherapy and T+E combination therapy groups. Certain differences were observed between the groups in terms of baseline characteristics (Table 4). In the T+E group, men of older age significantly predominated, and the primary indication for transplantation was HCC. In this group, patients received significantly lower T doses before minimization and, accordingly, had lower blood levels of the drug (Fig. 6).

Minimization in the combination therapy group followed a more favorable scenario and was characterized by a statistically significant more frequent successful T discontinuation, safe achievement of the maximum dose reduction to the MSD and blood level of the drug: the proportion of tolerant patients was 83% vs. 55% (p=0.053); the dose reduction rate was 100 (90.3;100)%. vs. 56 (42.5;78.8)% (p<0.001) (Table 5). T discontinuation was possible in 72% of cases in the combination IST group, while only in 9.1% of cases in the monotherapy group (p<0.001). The chances of T discontinuation while taking E were 26 times higher than with monotherapy (95% CI [4.4–154.5]).

Table 4. Baseline characteristics of monotherapy and combination therapy groups

Parameter	All patients (n=40)	Tacrolimus (monotherapy) (n=22)	Tacrolimus + Everolimus (n=18)	p
Age at the time of LT, years, M±m [95% CI] (min–max)	50±1.5 [47–53] (27–67)	46.9±2.1 [42.8–51] (27–61)	54.1±1.79 [50.5–57.6] (35–67)	**0.016**
Age at the start of minimization, years, M±m [95% CI] (min–max)	56.3±1.4 [53.5–59] (37–69)	54.6±2.1 [50.6–58.6] (37–69)	58.3±1.75 [54.8–61.7] (40–69)	0.190
Time after LT to the	74±7.21 [60–88]	91±9.21 [73–109]	53±9.49 [34.4–71.7]	**0.008**

start of minimization, months, M±m [95% CI] (min–max)	(6–182)	(34–182)	(6–134)	
Male gender, n (%)	32 (80 %)	15 (68.2 %)	17 (94.4 %)	**0.039**
Indications for LT, n (%):				
Viral CP (HCV)	14(35%)	8 (36.4 %)	6 (33.3 %)	0.230
Viral CP (HBV, HDV)	7(17.5%)	4 (18.2%)	3 (16.7%)	
HCC	12(30%)	4 (18.2%)	8 (44.4%)	
Alcoholic CP	3(7.5%)	2 (9.1%)	1 (5.6%)	
Other	4(10%)	4 (18.2 %)	0 (0%)	–
Tacrolimus dose, mg/day, Me (Q_1;Q_3) (min–max)	2 (2;4) (1–8)	3 (2;4) (1–8)	2 (2;2) (1–5)	**0.027**
Everolimus dose, mg/day Me (Q_1;Q_3) (min–max)	–	–	2 (2;3) (1–3)	–
Tacrolimus concentration, ng/mL, Me (Q_1;Q_3) (min–max)	4.3 (3;5.5) (2–8.5)	4.9 (3.7;6.7) (1.9–6.7)	3.2 (2.5;4.4) (1.9–6.4)	**0.002**
Everolimus concentration, ng/mL, Me (Q_1;Q_3) (min–max)	–	–	5.2 (4.3;6.7) (3–9)	–
GFR, mL/min/1.73m^2, Me (Q_1;Q_3) (min–max)	62 (50;70) (34–96)	66 (52.5;73.8) (43–96)	53 (49;67) (34–96)	0.196

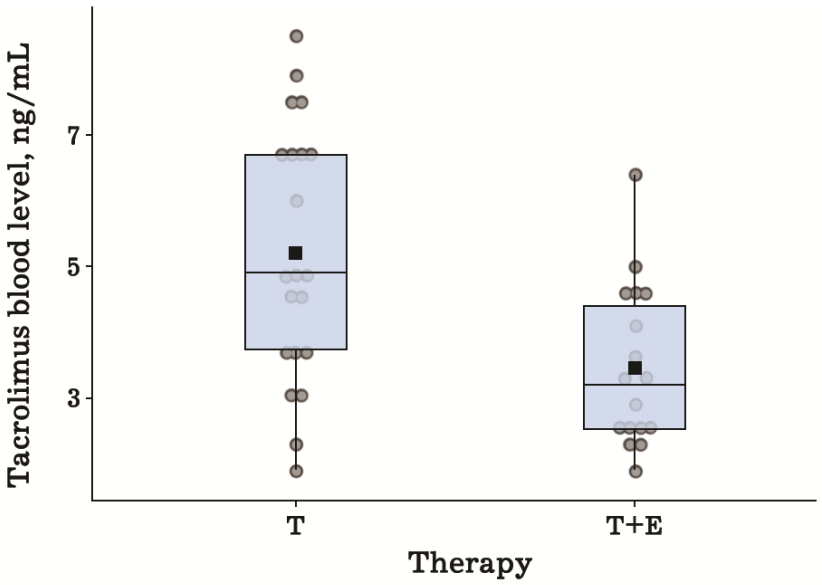

Fig. 6. Baseline tacrolimus blood level (ng/mL) in the monotherapy (tacrolimus) and combination therapy (tacrolimus + everolimus) groups, p=0.002

Table 5. Comparative characteristics of the minimization results in patients on monotherapy and on combination therapy at baseline

Parameter	All patients (n=40)	Tacrolimus (monotherapy) (n=22)	Tacrolimus + Everolimus (n=18)	p
Period of minimization, months, Me (Q_1;Q_3) (min–max)	8 (4;11) (1–33)	7.5(5;10) (2–30)	9.5 (3;12) (1–33)	0.712
Tolerant, n (%)	27 (67.5%)	12(55%)	15(83%)	0.053
Tacrolimus discontinuation, n (%)	15 (37.5%)	2 (9.1%)	13 (72.2%)	**<0.001**
Rejection, n (%)	7 (17.5%)	5 (23%)	2 (11%)	0.427
Dysfunction (including rejection), n (%)	13 (32.5%)	10 (45%)	3 (16.7%)	0.197
Period of minimization, months, Me (Q_1;Q_3) (min–max)	8 (4;11) (1–33)	7.5(5;10) (2–30)	9.5 (3;12) (1–33)	0.712
Tolerant, n (%)	27 (67.5%)	12 (55%)	15 (83%)	0.053
Dose reduction rate, %, Me (Q_1;Q_3) (min–max)	77.5 (50; 100) (0 –100)	56 (42.5; 78.8) (0–100)	100 (90.3;100) (0–100)	**< 0.001**
Tacrolimus final tapered dose, mg/day, Me (Q_1;Q_3) (min–max)	1 (0;1.5) (0–5)	1.25 (1;2) (0–5)	0 (0;0.2) (0–2)	**< 0.001**
Blood level of Tacrolimus, ng/mL, Me (Q_1;Q_3) (min–max)	2.25 (0;4) (0–6.3)	3.4 (2.4;4.4) (0–5.7)	0 (0;0.38) (0–6.3)	**< 0.001**
Follow-up period, months, Me (Q_1;Q_3) (min–max)	27 (22.8;31.3) (3–103)	30 (26.3;32) (13–41)	24 (10.8;29.3) (3–103)	0.013
GFR at the end of follow-up, mL/min/1.73 m^2, Me (Q_1;Q_3) (min–max)	66.5 (54;80) (34–117)	74 (59.3;81) (44–100)	59 (48;78.8) (34–117)	0.316

Summarized study results

The length of the minimization/withdrawal period varied greatly, from 1 to 33 months, depending on the initial T dose, the dysfunction or rejection development, patient adherence to the protocol, and refusal to continue treatment for subjective or objective reasons. It should be clarified that in 13 intolerant patients, the minimization period included not only the period of T dose reduction but also the period of its return to normalization until the LFT was normalized and the MSD was fixed. The shortest withdrawal period was observed in four retrospective cases

where an aggressive minimization was performed for urgent indications (Fig. 7).

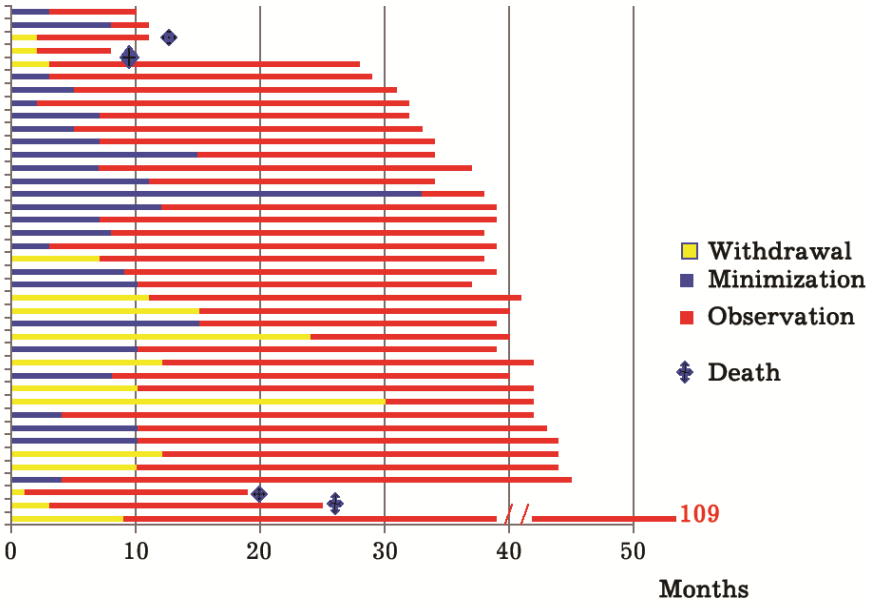

Fig. 7. Tacrolimus dose minimization/withdrawal periods and follow-up in 40 recipients

The median period of minimization was 8 months, and the median follow-up period exceeded 2 years, with a minimum of 3 months and a maximum of 109 months. The follow-up period lasted from the end of the intervention phase until database closure (October 2025) or patient death. These parameters did not differ between the tolerant and non-tolerant groups (Table 6).

To provide a summarized assessment of the pharmacological results of minimization and its clinical effects in general, we compared the parameters that we considered significant at the beginning of the study and after its completion (Table 7).

Table 6. Time period of tacrolimus dose minimization and follow-up

Parameter	All patients (n=40)	Tolerant (n=27)	Intolerant (n=13)	p
Period of minimization, months, Me (Q_1;Q_3) (min–max)	8 (4;11) (1–33)	8 (3.5;11.5) (1–33)	8 (5;10) (3–15)	0.954
Follow-up period, months, Me (Q_1;Q_3) (min–max)	27 (22.8;31.3) (3–103)	26 (20;30) (5–103)	28 (26;33) (3–38)	0.284

Table 7. Immediate and long-term results of tacrolimus dose minimization

Parameter	Outcome	End of follow-up	p
Do not receive immunosuppression	0	3 (7.5%)	
Tacrolimus (monotherapy)	22 (55%)	20 (50%)	
Tacrolimus + Everolimus	18 (45%)	5 (12.5)	
Everolimus (monotherapy)	0	12 (30%)	
Tacrolimus dose, mg/day, Me (Q_1;Q_3) (min–max)	2 (2;4) (1–8)	1 (0;1.5) (0–5)	**<0.001**
Everolimus dose, mg/day, Me (Q_1;Q_3) (min–max)	2 (2;3) (1–3)	2 (1.13;2.88) (0–3)	0.164
Blood level of Tacrolimus, Me (Q_1;Q_3) (min–max)	4.3 (3;5.5) (2–8.5)	2.25 (0;4) (0–6.3)	**<0.001**
Blood level of Everolimus, Me (Q_1;Q_3) (min–max)	5.2 (4.3;6.7) (3–9.1)	6.05 (4;7.7) (0–14)	0.661
AST, U/L, Me (Q_1;Q_3) (min–max)	22 (18;26) (12–38)	27 (22;33) (12–53)	**0.002**
ALT, U/L, Me (Q_1;Q_3) (min–max)	19 (15;27) (10–47)	24 (20;30) (10–52)	0.072
GGT, U/L, Me (Q_1;Q_3) (min–max)	23 (16;31) (8.2–63)	29 (20;41) (11–95)	0.08
ALP, U/L, Me (Q_1;Q_3) (min–max)	94 (65;117) (38–129)	107 (89;126) (44–146)	**0.002**
Total bilirubin in blood, mmol/L, Me (Q_1;Q_3) (min–max)	12.3 (10:15) (5–27)	11 (9;15) (6–37)	0.902
GFR , mL/min/ 1.73 m^2, Me (Q_1;Q_3) (min–max)	62 (50;70) (34–96)	66.5 (54;80) (34–117)	**0.011**

As a result, T dose minimization of varying degrees was successful in 35 (88%) patients, and only 5 (12%) patients returned to the baseline dose of the drug. The dose was reduced by 50% or more in 32 (80%)

patients, and by 75% or more in 23 (58%) patients (Fig. 8). T was successfully discontinued in 15 (37.5%) patients. As a result of this discontinuation, 12 (30%) patients receiving a combination IST switched to E monotherapy, and three have not been receiving IST for 18, 36 months, and 9 years.

Four tolerant patients who received E after T discontinuation died with normal graft function at 6, 9, 18, and 22 months after conversion due to recurrence and progression of HCC and CCC.

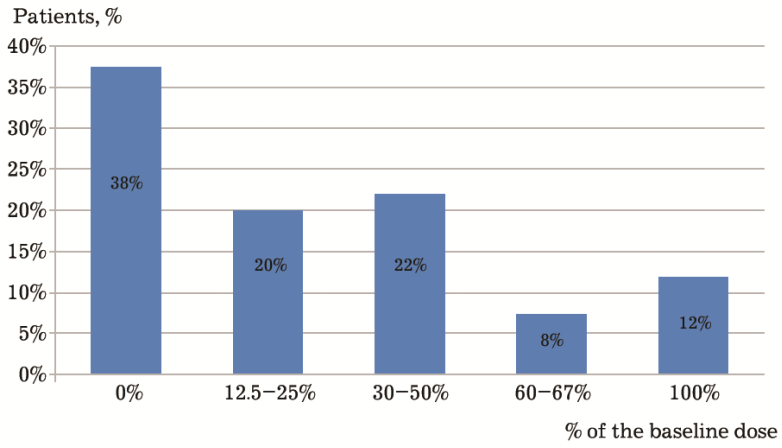

Fig. 8. Patient distribution with respect to the achieved degree of minimization

The median T daily dose in the cohort as a whole was reduced by 2-fold, and the median blood level of the drug was reduced by 1.9-fold ($p<0.001$). During the follow-up period, regardless of the scenario and outcome of the interventional phase of the study, no patient required a change in the achieved T dose, and no episodes of graft rejection or dysfunction were observed.

Biochemical parameters of LFT did not undergo significant changes, remaining within limits not exceeding 1.5 ULN in all patients.

The most important positive result of minimization was a significant improvement in renal function by the end of the follow-up

period: the median GFR statistically significantly increased by 4.5 mL/min (p=0.011).

Follow-up biopsies were performed in 22 patients, including twice in three patients, at intervals ranging from 1 to 25 months (median 16 months) after completing the minimization. No patient demonstrated worsened histopathological findings in their biopsy specimens compared to screening data. In four patients who experienced a rejection during minimization, no signs of rejection, fibrosis progression, or inflammation were detected at 7, 10, 18, and 25 months after the resolution of the episode.

Discussion

Optimal long-term IST aims to achieve the following objectives: 1) minimize the risk of acute and chronic rejection; 2) limit the side/toxic effects of immunosuppressants; 3) reduce infectious and cancer morbidity and mortality; 4) reduce the incidence and severity of metabolic, cardiovascular, and renal complications; 5) maintain maximum possible adherence to treatment; 6) improve the recipient's life expectancy and quality of life.

To address these issues, combinations of various immunosuppressive drugs and drug monitoring for some of them are typically used. At the same time, it is undeniable that extrahepatic causes predominate among the causes of long-term morbidity and mortality in liver transplant recipients, and these are largely associated with the cumulative side effects of immunosuppressants, the length of their use, and the total drug load.

On the one hand, IST deficiency can lead to an acute rejection or chronic immune-mediated graft dysfunction, up to and including graft loss and patient death. At the same time, excessive IST is often

accompanied by the manifestation and progression of serious side effects, such as cardiovascular diseases, metabolic disorders, renal dysfunction, obesity, diabetes mellitus, infectious complications, and oncopathology, which reduce the life expectancy and quality of life. In liver recipients who survive more than a year after transplantation, mortality remains significantly higher than in the general population and has not changed significantly over the recent 20 years. The main causes of late mortality in the population of adult recipients are malignant neoplasms, metabolic and cardiovascular complications, and renal failure [3, 4, 6–8, 12, 16, 31–36].

Clinicians must find a balance between an excessive IST, which unnecessarily increases the risks and likelihood of complications, and the risk of rejection due to insufficient immunosuppression. Maintaining this balance requires personalized approaches in selecting the baseline and maintenance IST regimen for each individual patient based on their initial clinical profile and existing guidelines for immunosuppression and recipient management [12–16]. IST protocols and regimens vary significantly in their drug load across different transplant centers, while the immediate and long-term outcomes are generally similar.

Glucocorticosteroids and antiproliferative drugs such as mycophenolic acid derivatives and azathioprine, as well as mTOR inhibitors can be used as adjuvants to reduce the dose and toxicity of CNI therapy, primarily T, or for patients with a higher risk of rejection with a standard dose of T. Optimal T blood concentrations for liver transplant recipients in the late period have not yet been precisely determined. Current guidelines and clinical randomized trials generally indicate fairly wide and high ranges of T blood levels for the early (up to 12 months) and late (more than 1 year) periods. In the absence of precise tools for personalized determination of the minimum sufficient level of immunosuppression, in routine practice the T dose remains unchanged,

usually lifelong, for many years in the late period. Thus, there is every reason to believe that a large proportion of liver transplant recipients receive excessive IST [9, 10]. Currently, evidence-based recommendations on how to personalize the long-term immunosuppression management to improve clinical outcomes have not been sufficiently developed.

The minimization strategy is one of the current clinical strategies in the management of recipients aimed at identifying of those who may require less immunosuppression. The clinical potential for minimization in LT is greater than in other types of transplantation due to the known ability of the liver graft to withstand the damaging effects of alloimmune reactions, which determines its immunoprivileged status. Unfortunately, there have not yet been established reliable criteria or biomarkers of tolerance that would allow an objective determination of the minimum sufficient immunosuppression, although intensive work in this area is under way [37–40]. Therefore, from the standpoint of the clinical practice, the biochemical response of the graft, immunological and morphological monitoring remain acceptable safety criteria for personalized minimization to date [41, 42].

While T is the main component of IST after LT, it is its side effects that account for a significant rate of long-term morbidity and mortality. T causes a dose-dependent nephrotoxicity; high exposure to T increases the risk of developing malignant neoplasms de novo and HCC recurrence; and T dose reduction or discontinuation can reduce the risk and severity of most complications associated with a long-term IST [43–49]. However, the potential benefits of minimizing or discontinuing IST must be balanced against the risks and consequences associated with a possible rejection provocation. It is important to consider that individual recipient

immunoreactivity and the required IST intensity to prevent rejection decrease over time and vary greatly among individuals.

Over the past two decades, numerous studies have appeared in the liver transplant literature on minimizing/withdrawing the IST. While some results have been promising; however, their heterogeneity and relatively small sample size preclude the generation of truly generalized information for the creation of evidence-based recommendations. Therefore, long-term management of IST remains an empirical practice.

J. Lerut et al. first randomized 156 patients to T monotherapy and to T plus a short course of steroids. There were no differences between the two groups in the overall incidence of acute rejection, although steroid-resistant rejection was statistically significantly more common in recipients receiving monotherapy (12.8% versus 3.8%, p=0.04). No differences in metabolic complications, infections, or 1-year survival were also observed. Taken together, the obtained results allowed us to state that the clinical outcomes with T monotherapy are completely comparable to those with the use of “traditional” combination IST regimens [50]. Despite this, the use of T as monotherapy has not become widespread within the framework of the current IST paradigm. This is probably due to the fact that the current focus is on the prevention of renal dysfunction by reducing the T dose while taking mycophenolates or mTOR inhibitors. Later, Professor Ya.P. Leruth became a leading and recognized global ideologist not only of T monotherapy, but also of its minimization, even to the point of its cancellation.

In the absence of precise tools to determine the optimal level of immunosuppression required for each individual patient, it is difficult to provide an objective definition of the concept of "minimization." The historical definition is to administer the least amount of immunosuppression compatible with a non-rejection state [51, 52]. Thus,

to meet this definition, drug doses must be reduced in recipients until rejection occurs. Another, more flexible, moderate definition of minimization is to gradually achieve a state in which the immunosuppressive load is reduced in a controlled and gradual manner to a level that does not cause clinically significant impairment of graft function while preventing a graft rejection.

In 2013, C. Benítez et al. presented the results of the first European multicenter prospective study on complete IST discontinuation in stable liver transplant recipients [27]. A total of 102 recipients were included in the study, and discontinuation was possible in 41.8%. Although rejection in response to immunosuppression minimization was observed in 58.2% of recipients, the rejection episodes were generally mild and resolved in all cases. The median time after LT prior to the inclusion in the discontinuation protocol was significantly higher in the tolerant group. Successful IST discontinuation was also associated with male gender (OR 3.53; 95% CI [1.34–9.3]; p=0.009), older recipient age at transplantation (OR 1.041; 95% CI [1–1.084]; p=0.05), and the absence of CNIs in the IST regimen. In multivariate analysis, only time since LT, age at surgery, and male gender were independently associated with the likelihood of achieving tolerance. Time since LT was identified as the strongest predictor of successful weaning from IST: in patients enrolled in the study 10.6 years after transplantation, the weaning success rate was 79%, while in those who underwent minimization 5.7 years after LT, weaning was successful in 38% of cases. In these two subgroups of recipients with high and intermediate tolerance probabilities, the inclusion of age or gender did not improve the predictive ability of the model. In contrast, among recipients enrolled in the study 3–5.7 years after transplantation, age at transplantation helped to distinguish subgroups of recipients with high and intermediate tolerance probabilities. After 36 months of follow-

up, there were no significant changes in the prevalence and severity of comorbidities. In conclusion, the authors noted that the proportion of adult liver transplant recipients able to discontinue IST was higher than previously estimated, particularly among carefully selected recipients without hepatitis C or autoimmune diseases, in whom the drug withdrawal was performed long after LT. In this group of recipients, IST discontinuation is a safe procedure if performed under close medical supervision and is not associated with clinically adverse histological changes for at least the first 3 years after the drug discontinuation. It is emphasized that baseline histological characteristics did not determine the success of minimization/discontinuation.

Thus, the time from LT to the onset of minimization proved to be the most powerful clinical predictor of successful IST discontinuation. This finding is consistent with the well-known phenomenon of host-graft adaptation, which explains the decrease in graft immunogenicity and the reduced risk of rejection that occur over time. The finding that older recipients have a higher likelihood of tolerance is consistent with the lower rejection rates observed in older kidney and liver transplant recipients. A higher likelihood of achieving tolerance has also been established in men compared to women.

In another large study by T. M. Manzia et al. [29], clinical operational tolerance was achieved in more than 40% of carefully selected recipients. Seventy-five liver transplant recipients were included in the IST discontinuation protocol. The study population included 32 (42.7%) patients with tolerance, 41 (54.7%) patients requiring maintenance of minimized IST after rejection, and two (2.7%) patients who developed early rejection after the first dose reduction. The primary endpoint of the study was the assessment of long-term outcomes. The secondary endpoint was the assessment of cost savings in the context of

IST discontinuation. The median follow-up was 95 (22.5;108.5) months. IST discontinuation did not result in patient or graft death and provided significant cost savings of approximately €630,000. In conclusion, it has been noted that long-term discontinuation of IST brings significant savings in medical and drug costs for liver recipients without exposing them to the risk of graft loss.

The North American ITN030ST A–WISH trial, a 7-center study, evaluated immunosuppression withdrawal in recipients with hepatitis C and nonautoimmune liver disease [53]. Ninety-five recipients enrolled in the study were randomized in a 4:1 ratio to discontinue (n=77) or continue IST (n=18) 1–2 years after transplantation. Eligibility criteria for randomization included stable immunosuppression, adequate liver and renal function, Ishak fibrosis score 2 or less, and absence of rejection based on biopsy. IST withdrawal was performed using an 8-step dose reduction algorithm with an interval of at least 8 weeks for each dose level. In 52 patients (67.5%), the IST dose was reduced to no more than 50% of the initial dose, and 10 (13.0%) discontinued IST at a mean of 2.8 years after LT for more than 1 year. Acute rejection and/or dysfunction were treated with increased immunosuppression, and only 5 of 32 rejection episodes required bolus methylprednisolone. The authors conclude that early minimization of immunosuppression is possible in selected liver transplant recipients, while a complete withdrawal is successful in only a small proportion of patients. This study demonstrated that clinically guided minimization can be performed in selected patients early after transplantation with manageable risk and acceptable safety. Such minimization during the first two years after transplantation only rarely leads to complete withdrawal of immunosuppression. However, if biomarkers can be identified to select patients to expand the population of

potentially tolerant individuals, the restrictive approach to early discontinuation may be reconsidered.

Despite certain limitations, our own data confirm the reported results of international studies in the adult liver transplant population and challenge the existing paradigm that lifelong standard immunosuppression is necessary for the health and well-being of all liver transplant recipients. Future studies on minimizing IST should determine the appropriate early time after transplantation when such attempts do not pose a threat to the graft and the patient.

Our results have also demonstrated that, with careful monitoring, graft dysfunction associated with individual immunosuppression failure during minimization can be reversed by reversible T dose adjustment. Graft dysfunction and clinically suspected rejection, with or without biopsy confirmation, were managed by T dose adjustment, with only a few intolerant patients requiring steroid bolus therapy. Our study and most immunosuppression withdrawal studies have shown that even those who develop rejection ultimately receive lower doses of immunosuppressive agents than at enrollment. In our cohort of patients, liver function at the end of the follow-up period did not differ from baseline and was similar in the withdrawal and minimization groups of varying degrees, confirming the absence of irreversible damage associated with attempts to minimize immunosuppression. We found no evidence of worsening morphological changes in liver biopsies during the two-year follow-up period after T minimization or complete withdrawal.

Overall, the clinical benefits of T minimization late after transplantation remain unclear. Although a nephro-sparing effect has been observed in some cases, such as in our study, there are virtually no data on whether late IST minimization affects other clinical endpoints. Furthermore, the potential impact of IST minimization protocols on

subclinical histological graft damage (chronic rejection, idiopathic chronic hepatitis, and/or progressive fibrosis) also remains poorly understood. This is important given that most protocol biopsy studies have found significant histological abnormalities in long-term liver transplant recipients even with intact LFTs while maintaining standard IST regimens [54]. The likelihood of an improved renal function and more favorable course of other comorbidities is likely to be higher if T minimization is implemented early (i.e., within the first 2 years after transplantation). However, the risk of rejection in the early stages after transplantation is higher than in the later stages.

Using mTOR inhibitors deserves close attention in the context of the minimization problem. P. De Simone et al. reported the results of a large prospective international study in which recipients who underwent LT de novo, were randomized into three groups according to IST regimens: 1) T withdrawal after E administration, 2) E and T at a reduced dose, and 3) T at a standard dose with mycophenolates. In the 2nd group, an improvement in kidney function was observed compared with the standard dose of T. However, recruitment to the T elimination group was terminated early due to a high incidence of an acute rejection [55, 56].

Studies by J. Levitsky group have shown that conversion from T to mTOR inhibitors (sirolimus) at a later stage may have an additional positive effect, promoting an increase in the number of immunoregulatory T cells, which potentiates the development of tolerance [57, 58]. Moreover, achieving tolerance by conversion from T to sirolimus followed by withdrawal is associated with a reduced risk of cancer development, as well as of the graft fibrosis and steatosis [59].

A recent review of studies on the issue of tolerance and IST withdrawal suggests new promising clinical approaches, molecular and

histological markers that might help predict tolerance and manage immunosuppression in LT [60].

Conclusion

Personalized minimization of immunosuppression late after transplantation was successful in a significant proportion of carefully selected recipients. The combination of baseline clinical and morphological parameters used is essential for selecting patients with predictable outcomes and the safety of this strategy. Tacrolimus reduction to the minimum dose required is significantly more frequently achieved and with a lower risk of rejection in patients receiving everolimus. A positive effect of minimization on renal function has been established. There is an urgent need for long-term prospective clinical trials of minimization initiated earlier after liver transplantation and focused on assessing clinically significant endpoints (liver and kidney function, cardiometabolic effects, oncopathology, histological changes in the graft). Further research is needed to confirm the universality of this approach, determine its advantages compared to lifelong maintenance immunosuppression, and refine and validate candidate Tolerance biomarkers. Identifying clinical parameters associated with a high probability of successful discontinuation of immunosuppressive therapy is crucial for selecting patients in whom the potential benefit outweighs the risk of rejection. The benefits of T minimization may not be evident unless it is performed early after transplantation — before the aforementioned adverse effects of chronic immunosuppressive therapy develop.

Based on the study results and the literature analysis we have made the following conclusions:

1.Proposed protocol for personalized tacrolimus dose minimization allowed us to reduce it to the minimum sufficient dose in 88% of recipients, without regard to its blood concentration, and to discontinue the drug completely in 37.5%. The effectiveness and safety of dose minimization is significantly increased with the simultaneous administration of everolimus: the proportion of tolerant patients was 83% vs. 55% ($p=0.053$); the dose reduction rate was 100 (90.3;100)% vs. 56 (42.5;78.8)% ($p<0.001$), statistically significant in both cases.

2.Tacrolimus dose reduction procedure is safe when the protocol is strictly followed, clinical and laboratory monitoring is performed, and any development of graft dysfunction is addressed promptly. Mild or moderate rejection occurs in 17.5% of patients and is easily managed by increasing the tacrolimus dose or pulse therapy without long-term consequences.

3.Inclusion of patients in the minimization protocol contributes to the preservation and improvement of kidney function: the glomerular filtration rate by the end of the follow-up period statistically significantly increases compared to the baseline: 62 (50;70) mL/min/1.73 m^2 vs. 66.5 (54;80) mL/min/1.73 m^2 ($p=0.011$).

4.When followed-up for two years after the tacrolimus dose minimization, no graft function impairment or negative histopathological changes were observed in either tolerant or intolerant recipients. Follow-up biopsies performed in 22 patients (7 non-tolerant and 15 tolerant) at 1 to 25 months (median 16 months) after completion of tacrolimus dose minimization revealed no deterioration in the histopathological pattern of the biopsy specimens compared to the initial screening data.

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