

Mesenchymal stem cells in liver transplantation: assessment of long-term results

S.V. Korotkov[✉], E.A. Nazarova, E.G. Yurkina, V.V. Smolnikova,
V.Yu. Grinevich, E.A. Yanushevskaya, A.Yu. Startseva, A.E. Shcherba,
S.I. Krivenko, O.O. Rummo

*Minsk Scientific and Practical Center of Surgery, Transplantation and
Hematology,*

8 Semashko St., Minsk 220045 Republic of Belarus

[✉]Corresponding author: Sergey V. Korotkov, Assoc. Prof., Cand. Sci. (Med.), Head of the
Transplantation Department, Minsk Scientific and Practical Center of Surgery, Transplantation and
Hematology, skorotkov@tut.by

Abstract

Introduction. *Transplantation is an effective method of treating patients with end stage liver diseases. Long-term results are determined by two main factors: the development of immunological complications and calcineurin inhibitor nephrotoxicity. Application of mesenchymal stem cells (MSCs), which modulate the immune response, is a promising effective method to optimize the treatment results in patients after liver transplantation.*

*The **objective of the study** was to evaluate the long-term results of mesenchymal stem cells application in liver transplantation.*

Material and methods. *A retrospective study was performed, which included 186 patients after liver transplantation (2015-2023). The MSC group (n=93) received MSCs according to four protocols: local, systemic, combined administration, therapy for acute kidney injury; the*

control group (n=93) received the standard treatment. The median follow-up was 3 (2;5) years, the follow-up period being from 1-8 years. The patient survival, graft and renal function, depth of immunosuppressive therapy, anti-HLA antibody levels, and lymphocyte immunophenotype were assessed.

Results. In the MSC group the incidence of immunological dysfunction of the liver allograft was decreased (22% versus 40%, $p<0.05$), the development of stage 3 chronic kidney disease (23.4% versus 68.2%, $p<0.05$) and formation of anti-HLA antibodies (5% versus 20%, $p<0.05$) were reduced. The use of MSCs made it possible to reduce the Tacrolimus doses (4.15 vs. 5.2 ng/mL, $p=0.001$) without increasing the risk of rejection. Eight-year survival in MSC group was 87.7% versus 82.9% in the control group. Specific to immunological tolerance changes in the immunophenotype were identified.

Conclusion. Using MSCs in liver transplantation improves long-term outcomes by reducing the incidence of immunological complications, preserves the renal function, and reduces the need for high-dose immunosuppression.

Keywords: liver transplantation, mesenchymal stem cells, immunological tolerance, liver allograft rejection, immunosuppressive therapy

Conflict of interests: Authors declare no conflict of interest

Financing: The study was performed without external funding

For citation: Korotkov SV, Nazarova EA, Yurkina EG, Smolnikova VV, Grinevich VYu, Yanushevskaya EA, et al. Mesenchymal stem cells in liver transplantation: assessment of long-term results. *Transplantologiya. The Russian Journal of Transplantation*. 2025;17(3):232–245. (In Russ.). <https://doi.org/10.23873/2074-0506-2025-17-3-232-245>

AKI, acute kidney injury

Bm1, naive B lymphocytes
BMCP, biomedical cell product
CD3+ CD8+, cytotoxic T lymphocytes
CKD, chronic kidney disease
HLA, human leukocyte antigen
ISCT, International Society for Cellular Therapy
IST, immunosuppressive therapy
LT, liver transplantation
MSC, mesenchymal stem cell
MZB, marginal zone B cells
pDC, plasmacytoid dendritic cell
PRA, panel of reactive antibodies
TEMRA, terminally differentiated effector memory T cells

Introduction

Liver transplantation (LT) is the only definitive method of treating end-stage liver diseases. Despite significant progress in the field of transplantation, the long-term results of liver transplantation remain suboptimal because of developing immunological complications and side effects of immunosuppressive therapy (IST) [1-3]. The main problems include a chronic graft rejection and nephrotoxicity of calcineurin inhibitors, which incidence reaches 17% and 60%, respectively [4-6].

Mesenchymal stem cells (MSCs) have unique immunomodulatory properties and the ability to induce immunological tolerance [7-10]. In recent years, more and more data have emerged on the efficacy of using MSCs in transplantation [10-12]. However, the effect of cell therapy on the long-term results of liver transplantation remains insufficiently studied.

The objective was to evaluate the long-term results of using mesenchymal stem cells in liver transplantation.

Material and methods

Study design

The study using local and systemic administration of MSCs was approved by the Decision of the Ethics Committee of the State Institution “Minsk Scientific and Practical Center for Surgery, Transplantology and Hematology” (Protocol No. 6 of 09.08.2013, No. 8 of 15.10.2018). Patients’ consents to participate in the study were obtained in writing. A retrospective cross-sectional analytical comparative study was conducted that included 186 patients after LT operated on in the period 2015-2023. To evaluate the cell therapy efficacy patients were distributed into two groups: the main study group (n=93), in which the patients received various options for MSC therapy, and the control group (n=93) where the standard management was used [13]. In the main group, the following protocols for the MSC administration were used (Table 1): local administration (14 patients), systemic one (15 patients), combined administration for the immunosuppression induction (30 patients), and a systemic administration of MSCs to minimize IST in patients with acute kidney injury (AKI) (34 patients). The patient groups were comparable in terms of clinical and demographic data (Fisher's Exact test (F), $p>0.05$).

Table 1. Mesenchymal stem cell infusion strategies

Number of patients	Route of administration	Number of MSCs
14	Local (intraoperatively, into the portal vein)	20×10^6 cells
15	Systemic (0 and 4 days after surgery)	4×10^6 cells/kg
30	Combined administration	20×10^6 cells + 4×10^6 cells/kg
34	Systemic (0, 4, 8, 12 days of acute kidney injury development)	5.5×10^6 cells/kg

After excluding 37 patients who continued the follow-up at other centers, the final analysis included 73 patients in the MSC-therapy group and 76 patients in the control group.

The follow-up period ranged from 1 to 8 years. The median was 3 (2;5) years.

Characteristics of the cell product

Cell therapy was performed using the biomedical cell product (BMCP) “Human mesenchymal cells TU BY 100660677.001” (registration certificate No. IM-7.101480, registration number: Mn-7.117650-1402 dated 29.05.2014). BMCP was produced from allogeneic MSC of adipose tissue of brain-dead donors in accordance with the “minimum criteria for mesenchymal stem cells” (ISCT, 2006) [14].

Determination of anti -HLA antibodies

The determination of anti-HLA antibodies was performed in two stages. At the first stage, a qualitative analysis (screening) was carried out, during which IgG antibodies to HLA antigens were detected using the LIFECODES LifeScreen Deluxe (LMX, USA) test system on a Luminex 200 multiplex fluorescence analyzer. If the screening test result was positive, we proceeded to the second stage, which involved identifying anti-HLA antibodies using LIFECODES LSA kits (IMMUCOR, USA). The xPonent (LUMINEX, USA) and MatchIT Antibody (IMMUCOR, USA) programs were used to interpret the results. The patient's sensitization level was determined by the percentage of reactive antibodies (PRA).

Flow cytometry

The immunophenotype of peripheral blood cells was determined by multicolor flow cytometry using a FACSLytic Flow Cytometer (Becton Dickinson, USA) equipped with three lasers 488 nm, 633 nm, and 405 nm with the detection of 10 fluorescence channels. Data collection and analysis were performed in the FACSuite (v. 5.1) working software.

Histological examination of the graft

Histological examination of the graft was performed if and when the immunological dysfunction developed. Various histological stains were used to verify late cellular and chronic rejection: hematoxylin and eosin, MSB (Mallory, Sirius, and Blau), Masson and Van Gieson methods, Sudan red/black staining, and the PAS reaction. The diagnosis of antibody-mediated rejection was made by using the immunohistochemistry (IHC) technique with the identification of the C4d complement fragment associated with antibodies [15-17].

Statistical assessment of results

For statistical analysis, the Statistica 8.0 software package was used (StatSoft Inc., USA). The distribution type was assessed using the Shapiro-Wilk test. For non-normal distributions, the results were expressed as median with interquartile range (25th and 75th percentiles). Intergroup differences in quantitative parameters were assessed using the Mann-Whitney U test (MW), and qualitative parameters were assessed using the Fisher exact test (F). Spearman correlation analysis (Sp) was used to determine the degree of relationship between two quantitative parameters of the groups under study. Survival analysis and cumulative proportion of patients were determined using the Kaplan-Meier and Log-Rank tests.

Results

Analysis of postoperative mortality and patient survival

Analysis of postoperative mortality showed that 9 of 73 recipients, who received MSC therapy, died, which made 12.3%. In the control cohort of 76 patients on a standard patient management protocol, 13 deaths were recorded (17.1%) (F, $p>0.05$) (Table 2).

Table 2. Causes of fatal outcomes in liver recipients in the study groups

Parameter	MSCs (n=73)	Without MSCs (n=76)	P
Length of follow-up, years (min-max)	1–8	1–8	$p>0.05$
Median follow-up period, years	3 (2;5)	3 (2;5)	
Overall mortality, n (%)	9 (12.3)	13 (17.1)	$p>0.05$
One-year, n (%)	6 (8.2)	6 (7.89)	$p>0.05$
• Infectious complications, n (%)	4 (5.5)	4 (5.26)	$p>0.05$
• Acute pancreatitis, n (%)	1 (1.37)	0 (0)	
• Ischemic cholangiopathy, n (%)	1 (1.37)	0 (0)	
• Myocardial infarction, n (%)	0 (0)	1 (1.32)	
• Acute cerebrovascular accident, n (%)	0 (0)	1 (1.32)	
In the 2 nd year, n (%)	2 (2.74)	4 (5.26)	$p>0.05$
• Recurrence of cholangiocellular carcinoma, n (%)	1 (1.37)	1 (1.32)	$p>0.05$
• Ischemic cholangiopathy, n (%)	1 (1.37)	0 (0)	
• Cirrhosis of the graft, n (%)	0 (0)	2 (2.63)	
• Chronic heart failure, n (%)	0 (0)	1 (1.32)	
In the 3 rd year, n (%)	0 (0)	1 (1.32)	$p>0.05$
• Coronavirus infection, n (%)	0 (0)	1 (1.32)	
In the 4 th year, n (%)	0 (0)	0 (0)	$p>0.05$
In the 5 th year, n (%)	1 (1.37)	1 (1.32)	$p>0.05$
• Recurrence of hepatocellular carcinoma, n (%)	0 (0)	1 (1.32)	$p>0.05$
• Cirrhosis of the graft, n (%)	1 (1.37)	0 (0)	
In the 6 th year, n (%)	0 (0)	1 (1.32)	$p>0.05$
• Infectious complications, n (%)	0 (0)	1 (1.32)	

The study of mortality rates showed that the first postoperative year was the most critical: infectious complications were the main cause of

patient mortality. In the second year after transplantation, mortality rate decreased, with cases of the transplanted organ dysfunction predominating. In the long term (3-6 years after surgery), fatal outcomes were sporadic.

The analysis of survival rates demonstrated that 91.8% of patients survived one year after transplantation in the MSC group (6 of 73 died), while in the control group it was 92.1% (6 of 76 died). The three-year survival rate was 89% (8 deaths of 73) in the MSC group versus 85.5% (11 of 76) in the comparison group. When assessing the five-year survival rates, the figures were 87.7% (9 of 73) and 84.3% (12 of 76), respectively. The eight-year survival rates remained at 87.7% (9 of 73) in the MSC group and 84.3% (13 of 76) in the control group (Log-rank test, $p=0.39$; Fig. 1).

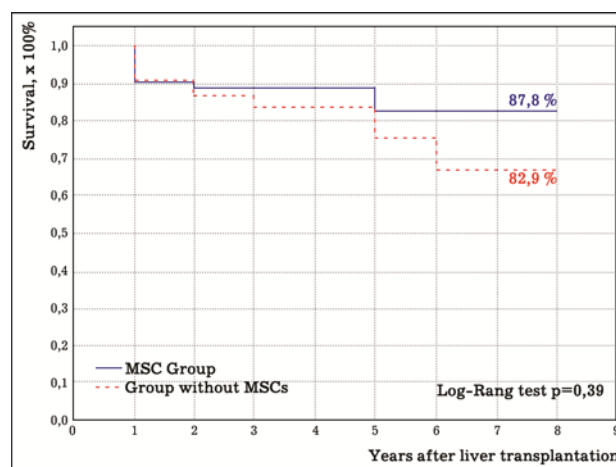


Fig. 1. Patient survival in the groups

Analysis of surgical complications

A comparative study of postoperative surgical complications showed that the similar incidence of their development from the arterial bed was observed: 9 cases (12%) in patients receiving MSCs and 10 cases (13%) in the control group ($F, p>0.05$) (Table 3). Arterial complications mainly occurred in the early postoperative period: 7 cases in each group,

while in the later period 2 and 3 cases were recorded, respectively (F, $p>0.05$).

Venous complications in the late period were minimal: one case of portal vein stenosis was recorded in each group, and no complications from the inferior vena cava were noted.

Biliary complications were the most frequent in the long-term follow-up period. The incidence of late anastomotic strictures reached 9.6% (7 patients) in the MSC group, and 6.6% (5 patients) in the comparison group (F, $p>0.05$). Special attention should be paid to the development of ischemic cholangiopathy, which in most cases manifested itself in the late period: 80% of all cases (4 patients) in the MSC group and 83% (5 patients) in the control group (F, $p>0.05$).

Table 3. Surgical complications after liver transplantation

Complications	MSCs (n=73)		Without MSCs (n=76)	
Arterial	9	11.8%	10	13.2%
• Early POP	7	9.6 %	7	9.2 %
• late	2	2.7 %	3	3.9 %
Venous	2	2.7 %	4	5.3 %
Portal	2	2.7 %	2	2.6 %
• early POP	1	1.4 %	1	1.3 %
• later	1	1.4%	1	1.3%
Caval	0	0 %	1	1.3 %
• early POP	0	0 %	1	1.3 %
• later	0	0%	0	0%
Biliary	16	21.9 %	14	18.4 %
• bile leak	2	2.7 %	2	2.6 %
Anastomotic strictures	13	17.8 %	12	15.8 %
• early POP	6	8.2 %	7	9.2 %
• later	7	9.6%	5	6.6%
Ischemic cholangiopathy	5	6.8 %	6	7.9 %
• early POP	1	1.4 %	1	1.3 %
• later	4	5.5%	5	6.6%
Combination of complications	6	8.2 %	4	5.3 %

Note: POP, postoperative period; the main categories of surgical complications are shown in bold

Graft function assessment

The graft function was assessed at two control time points: the first point was at the moment of the cross-sectional study; the second point was the maximum value of a parameter for the entire late postoperative period (Table 4).

The number of patients was 64 in the MSC group, and 63 in the standard IST group (data do not include deceased patients).

Table 4. Analysis of graft function in the late postoperative period

Parameter	Group	Current value	max
AST, U/L	MSCs	23 (19;27)	34* (25;48)
	Without MSCs	25 (18;34)	46 (30;96)
ALT, U/L	MSCs	23 (17;39)	45* (30;61)
	Without MSCs	27 (16;47)	59 (43;112)
Bilirubin, μ mol/L	MSCs	10 (7.4;15)	14* (11;19)
	Without MSCs	12 (8;17)	23 (13;25)
ALP, U/L	MSCs	80 (63;151)	98 (78;157)
	Without MSCs	105 (76;130)	120 (88;163)
GGTP, U/L	MSCs	31 (17.5;73)	83* (23;125)
	Without MSCs	30 (16;61)	169 (56;201)
INR	MSCs	0.91 (0.83;0.99)	0.97 (0.84;1.04)
	Without MSCs	0.89 (0.82;0.98)	1.01 (0.89;1.08)

Notes: * the difference is statistically significant versus the control group, $p < 0.05$. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGTP, gamma-glutamyl transpeptidase; INR, international normalized ratio

Analysis of biochemical parameters revealed significant intergroup differences. In the group of patients receiving MSC therapy, the maximum values of AST and ALT (max value) were statistically significantly lower compared to the control group: AST_{max} was 34 U/L versus 46 U/L, ALT_{max} was 45 U/L versus 59 U/L, respectively (MW, $p < 0.05$). The obtained results indicated a less pronounced cytolytic syndrome in patients receiving MSCs, which was confirmed by

maintained damage markers (AST, ALT) within the reference values, unlike the control group.

The incidence of immunological graft dysfunction in the long-term period was statistically significantly lower in patients receiving MSCs and made 22% (14 patients). In the standard immunosuppression group, this figure reached 40% (25 patients) (F, $p=0.02$) (Table 5). To exclude non-immunological causes of graft dysfunction, the patients were subjected to a comprehensive examination, including ultrasound, MSCT and MRI of the abdominal organs, and virological testing (for HBV, HCV, herpes group). The graft puncture biopsy was performed in cases of severe rejection and unclear etiology of dysfunction.

Table 5. Parameters of immunological graft dysfunction

Parameter	MSC Group		Group without MSCs	
Immunological dysfunction of the transplant	14*	22%	25	40%
Based on clinical data (without biopsy)	8	13%	15	24%
Late cellular rejection	4	6%	5	8%
Chronic rejection	2	3%	5	8%
Immunological dysfunction at the moment of transverse section	4	6%	6	9.5%

Note: * the difference is statistically significant versus the control group, $p<0.05$

At the time of the cross-sectional study, the signs of graft rejection were observed in 4 patients (6%) in the MSC group and in 6 (9.5%) in the standard IST group (F, $p>0.05$).

The immunological dysfunction was treated in accordance with the Clinical Protocol of “Liver transplantation (adult and pediatric population)” [13].

Renal function analysis

The renal function analysis demonstrated statistically significant differences between the study groups (Table 6).

Table 6. Patient distribution by chronic kidney disease stages

CKD stage by KDIGO (2012)	GFR mL/min	MSCs (n=64)		Without MSCs (n=63)	
C1 (normal)	>90	6	9.4%	3	4.8%
C2	60-89	36*	56.2%	13	20.6%
C3	30-59	15*	23.4%	43	68.2%
C3a	45-59	11*	17.2%	28	44.4%
C3b	30-44	4*	6.2%	15	23.8%
C4	15-29	6	9.4%	4	6.3%
C5	<15	1	1.6%	0	0
CNI nephrotoxicity		25*	39.1%	45	71.4%

Notes: * the difference is statistically significant versus, $p < 0.05$; the control group (without MSCs), CNI, calcineurin inhibitor; GFR, glomerular filtration rate

As can be seen from the table 6, in the MSC group, a lower incidence of stage 3 chronic kidney disease (CKD) was observed compared to the non-MSC group, the comparison making 23.4% (15 patients) versus 68.2% (43 patients), respectively (F, $p < 0.05$). The incidence of nephrotoxicity (NT) episodes was also statistically significantly lower in the group of patients receiving MSC.

Analysis of laboratory data revealed significant differences in renal function parameters (Table 7).

Table 7. Laboratory parameters of renal function in the late postoperative period

Parameter	Group	Current value	max;min
Blood urea, mmol/L	MSCs	6.6 (5.1;8.5)	8.8 (7.5;10.1)
	Without MSCs	7.2 (5.1;8.7)	9.6 (7.8;12)
Serum creatinine, $\mu\text{mol/L}$	MSCs	79 (71;91)	95 (82;105)
	Without MSCs	86 (63;94)	106 (84;115)
GFR, mL/min	MSCs	62* (49;73)	53* (42;60)
	Without MSCs	52 (44;70)	46 (39;57)

Note: * the difference is statistically significant versus the control group $p < 0.05$

When assessing the maximum values of azotemia, a tendency towards a lower level of urea_{max} was noted in the MSC group (8.8 mmol/L compared to 9.9 mmol/L in the control group, MW, p=0.08). The glomerular filtration rate (GFR) was statistically significantly higher in the main group both at the time of the study and during the periods of nephrotoxic action of calcineurin inhibitors (Tacrolimus) (MW, p<0.05).

To assess the kidney functional state, a urine biochemistry test was also performed to determine the damage markers (Table 8).

Table 8. Characteristics of the renal dysfunction and tubular damage markers in urine

Parameter	Group	Current value
Urine protein, g/L	MSCs	0.04 (0.01;0.12)
	Without MSCs	0.055 (0.02;0.09)
NGAL, ng/mL	MSCs	16 (3.8;18.9)
	Without MSCs	14 (3.6;16)
maU, mmol/L	MSCs	6 (3;12)
	Without MSCs	12 (2;22)
Uurea, mmol/L	MSCs	241 (165;312)
	Without MSCs	239 (154;379)
Ucreat, µmol/L	MSCs	7148 (6238;10297)
	Without MSCs	7551 (4364;11005)
UNa, mmol/L	MSCs	109 (85;138)
	Without MSCs	97 (62;133)

Notes: NGAL, Neutrophil gelatinase-associated lipocalin; maU, microalbuminuria; Uurea, urine urea; Ucreat, urine creatinine; UNa, urine sodium.

Analysis of urine biochemical parameters revealed a tendency towards a lower level of albuminuria in patients receiving MSCs, which was 6 (3;12) mmol/L compared to 12 (2;22) mmol/L in the control group (MW, p=0.07).

Immunosuppressive therapy

In the postoperative period, patients received both single-component and combined IST regimens (Table 9).

Table 9. Characteristics of immunosuppressive therapy regimens in the long-term postoperative period after liver transplantation

Immunosuppressive therapy regimen	MSCs (n=64)		Without MSCs(n=63)	
Tac	37	57.8 %	35	55.6 %
Tac + MMF	6	9.4 %	6	9.5 %
Tac + GCS	5	7.8 %	3	4.8 %
Tac + mTOR	9	14.1 %	6	9.5 %
Tac + MMF + mTOR	1	1.6 %	4	6.3 %
mTOR	1	1.6 %	0	0 %
MMF	1	1.6 %	0	0 %
MMF + mTOR	4	6.2 %	0	0 %
Tac + MMF + GCS	0*	0 %	7	11.1 %
Tac + GCS + mTOR	0	0 %	1	1.6 %
Tac + MMF + GCS + mTOR	0	0 %	1	1.6 %

Notes: * the difference is statistically significant versus the control group, $p < 0.05$. Tac, Tacrolimus; MMF, mycophenolate mofetil; GCS, glucocorticosteroid; mTOR, mTOR inhibitor (the mammalian target of rapamycin inhibitor).

It is important to note that patients in the control group did not receive monotherapy with either mycophenolate mofetil or mTOR inhibitors, nor their combination, which confirmed the need for a more intensive IST in this group of patients. Increased immunological activity in the control group is evidenced by the fact that 9 patients required enhanced immunosuppression: 7 patients received a combination of three agents (tacrolimus, MMF, and glucocorticosteroids) ($F, p=0.013$), another patient was administered a combination of tacrolimus, MMF, and mTOR inhibitors. In one more case, a combination of four drugs had to be used to achieve a sufficient immunosuppressive effect.

In order to study the effects of calcineurin inhibitor use in the long-term post-transplant period (3 months after surgery), blood levels of

Tacrolimus were monitored, taking into account the current concentration, peak values, and median values (Table 10).

Table 10. Comparative analysis of blood level of Tacrolimus

Parametr	Group	Current value	max	Median value
Tac, ng/mL	MSCs	4.15 (3.3;4.5)	5.9 (4.8;6.6)	4.6 (3.9;5.2)
	Without MSCs	5.2 (4.5;6.2)	8.2 (6.6;10.2)	6.1 (5.4;6.8)
MW, p		0.001	0.0001	0.001

Comparative analysis showed statistically significant higher Tacrolimus concentrations in patients in the control group at all study time-points ($p < 0.001$). This indicates that patients without MSC therapy require not only more intensive basic immunosuppression, but also additional administration of combination regimens of three and four drugs to maintain an adequate immunosuppressive effect.

A study of the clinical significance of the current blood level of Tacrolimus demonstrated a statistically significant relationship between the drug concentration and the kidney function state: blood levels of calcineurin were associated with a deterioration in the glomerular filtration rate (Sp, $p = 0.034$), (Fig. 2).

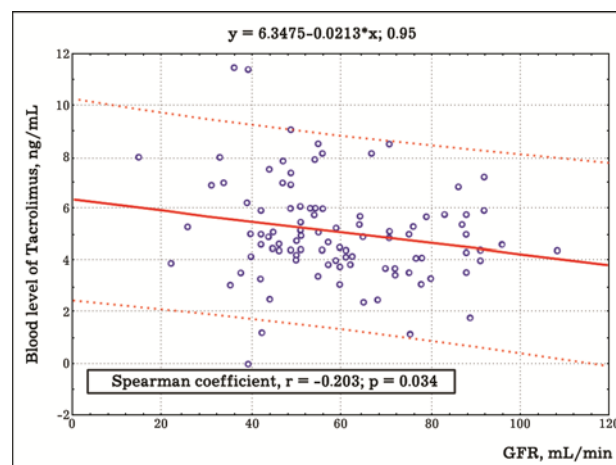


Fig. 2. Correlation between blood levels of Tacrolimus and eGFR

A correlation analysis did not reveal a statistically significant relationship between the low blood levels of Tacrolimus and the level of transaminase activity, which suggested the conclusion that low blood levels of the drug had no effect on the development of graft rejection (Sp, $p>0.05$) (Fig. 3).

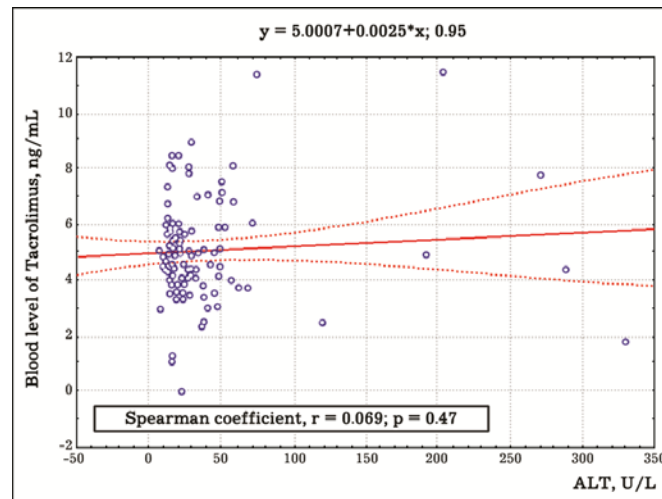


Fig. 3. Correlation between blood level of Tacrolimus and ALT activity

Analysis of the level of anti -HLA antibodies

In the cross-sectional study, the first step was the screening for anti-HLA antibodies. If the screening result was positive, the percentage of reactive antibodies (PRA) was determined.

The comparative analysis of the screening results showed significant differences between the groups. Antibodies were detected in only 3 patients (5%) among recipients who were administered MSCs, and in 13 (20%) in the group without MSCs (F, $p=0.007$).

Determining the PRA of anti-HLA antibodies revealed statistically significant higher titers of anti-HLA IgG in patients of the control group compared to the group receiving MSCs (MW, $p=0.029$, Table 11).

Table 11. Analysis of Anti-HLA antibodies in the long-term post-transplant period

Parameter	Group	Mean value	Median	Range	MW, p
PRA, %	MSCs	0.75	0	0-25	p=0.029
	Without MSCs	3.36	0	0-39	

Immunophenotype of peripheral blood lymphocytes

A study of the phenotypic characteristics of peripheral blood mononuclear cells revealed significant differences and certain patterns in the quantitative distribution of effector subpopulations when comparing between the groups (Table 12).

Table 12. Analysis of peripheral blood lymphocyte subpopulations

Parameter	MSC Group	Group without MSCs	MW, p
Terminally differentiated effector memory T cells (TEMRA, CD3+, CD8+, CD45RA+, CD62L-)			
Relative count, %	34.7 (24.2;47.5)	39.7 (28.2;46)	0.028
Absolute count, 10 ³ /μL	0.208 (0.11;0.387)	0.243 (0.151;0.385)	0.074
Marginal zone B cells (MZB cells, CD19+ CD27+ IgD+ IgM+)			
Relative count, %	7.4 (3.1;10.4)	9.5 (6.6;16.5)	0.011
Absolute count, 10 ³ /μL	0.0073 (0.0038;0.0199)	0.0118 (0.0071;0.0194)	0.016
Bm1 (naive B lymphocytes, IgD+/CD38-)			
Relative count, %	12.45 (8.65;19.5)	18.8 (12;25.15)	0.009
Absolute count, 10 ³ /μL	0.014 (0.011;0.029)	0.02 (0.01;0.043)	0.043
Plasmacytoid dendritic cells (pDCs CD11c- CD123br HLA-DR+)			
Relative count, %	0.07 (0.032;0.12)	0.048 (0.028;0.072)	0.047
Absolute count, 10 ³ /μL	0.0042 (0.0017;0.0063)	0.0028 (0.0016;0.0041)	0.041

Immunophenotype analysis showed that in the group of patients receiving MSCs, there was a statistically significant decrease in the relative number of CD3+CD8+ TEMRA cytotoxic T cells (MW, p=0.028) with a tendency to a decrease in their absolute number. A statistically significant decrease in the relative content and absolute count of MZB and Bm1 B cells (MW, p<0.05), the humoral rejection effectors involved in the development of chronic graft dysfunction, was revealed.

A statistically significant decrease in the relative and absolute count of plasmacytoid dendritic cells (MW, $p<0.05$) was also recorded, being a characteristic of a reactive immune response.

Discussion

The study demonstrates a number of significant clinical effects of the use of mesenchymal stem cells in liver transplantation.

The key result was a decrease in the immunological graft dysfunction incidence in patients receiving MSCs (22% versus 40%, $p<0.05$). The mechanism of this effect is confirmed by the identified changes in the immunophenotype of lymphocytes: the decreases in the count of effectors of the cellular immunity link, namely CD3+CD8+ TEMRA cells, the cells-participants in humoral rejection, namely the marginal zone B cells and naive B lymphocytes; and the distribution of antigen-presenting dendritic cell population typical to immunotolerant immunophenotype.

The obtained data are consistent with our previously obtained results in kidney transplantation [18], demonstrating a decrease in the level of effector T lymphocytes in patients with a stable course of the long-term postoperative period, and a lower level of dendritic cells in patients with chronic rejection associated with the migration of plasmacytoid dendritic cells (pDCs) into the graft to implement the antigen-presenting function. The obtained data also correlate with the results of N. Perico et al. (2013) [19] and Y. Peng et al. (2013) [20], indicating the ability of MSCs to induce immune tolerance through the regulation of T- and B-cell activation.

The decreased frequency of detecting anti-HLA antibodies in the MSC group (5% versus 20%, $p<0.05$) and their lower level (range from 0 to 25% compared to 0-39%, $p<0.05$) indicates effective suppression of

the alloimmune response. The obtained data are consistent with the studied by Y. Peng et al. (2013) [20] on the ability of MSCs to modulate B-cell immunity.

A significant outcome of our research was the preserved renal function demonstrated in subjects administered with MSCs. The lower incidence of stage 3 CKD (23.4% versus 68.2%, $p < 0.05$) and calcineurin inhibitor nephrotoxicity (39.1% versus 71.4%, $p < 0.05$) are associated with the ability of MSCs to reduce Tacrolimus doses (current concentration: 4.15 ng/mL versus 5.2 ng/mL, $p = 0.001$) without the risk of liver graft rejection. These data correlate with the results of studies by G. Pan et al. (2016) [21] demonstrating the nephroprotective effect of low concentrations of Tacrolimus in combination with MSCs in renal transplant patients.

Despite the absence of statistically significant differences in overall patient survival ($p = 0.39$), the reduction in mortality in the MSC group (12.3% versus 17.1%) indicates a potential protective effect of cell therapy.

We should note that the first postoperative year, where infectious complications predominated in both groups, emphasizes the necessity to optimize immunosuppressive therapy using MSCs and requires further research.

Conclusion

The use of mesenchymal stem cells in liver transplantation demonstrates a multifactorial positive effect, including a decreased immunological graft dysfunction, the preservation of renal function and a decreased dependence on high doses of Tacrolimus. A promising trend is the development of individualized MSC therapy regimens taking into account the immunological, nephrological and infectious status of the recipient.

Based on the study results we can make the following conclusions:

1. The use of various protocols of cell therapy with mesenchymal stem cells in the early postoperative period of liver transplantation has a beneficial effect on the long-term treatment outcomes of patients.

2. Using mesenchymal stem cells in the early stages of liver transplantation promotes the induction of immunological tolerance, which has a positive effect on the graft function and reduces the incidence of rejection by 18% (from 40% in the standard patient management group to 22% when using mesenchymal stem cells, ($p=0.02$)).

3. The formation of a stable immunotolerant phenotype is associated with a decrease in the effectors of the humoral immune response link (MZB and Bm1), a decrease in the number of terminally differentiated T-cytotoxic lymphocytes and the corresponding distribution of antigen-presenting plasmacytoid dendritic cells.

4. A decreased intensity of the humoral response to the graft alloantigens when using mesenchymal stem cells is confirmed by a statistically significantly lower rate of formation of anti-HLA antibodies (5% compared to 20%, $p=0.007$) and their lower titer (range from 0 to 25% compared to 0-39%, $p=0.029$).

5. The decrease in immunological reactivity in patients after mesenchymal stem cell therapy contributes to the optimization and maintenance of adequate depth of immunosuppressive therapy with achieving lower blood levels of Tacrolimus (4.6 (3.9;5.2) ng/mL compared to 6.1 (5.4;6.8) ng/mL, $p=0.001$) and a reduction in the need for multicomponent immunosuppressive regimens.

6. Minimizing the doses of calcineurin inhibitors with their nephrotoxic properties is an effective long-term nephroprotective strategy. In the long-term period after liver transplantation, patients who

received mesenchymal stem cells had better renal function: the glomerular filtration rate was 62 (49;73) compared to 52 (44;70) mL/min, $p<0.05$), the number of renal damage episodes associated with Tacrolimus nephrotoxicity was lower (25 versus 45 cases, $p=0.015$), the incidence of stage 3 chronic kidney disease was statistically significantly lower (22.5% versus 65%, $p=0.001$).

7. The use of cell therapy in the early postoperative period contributed to the improvement of long-term outcomes of liver transplantation: 8-year survival in the mesenchymal stem cell therapy group was 87.7% compared to 82.9% in the control group.

References

1. EASL Clinical Practice Guidelines on liver transplantation. *J Hepatol.* 2024;81(6):1040–1086. PMID: 39487043
<https://doi.org/10.1016/j.jhep.2024.07.032>

2. *Annual report on liver transplantation*. Report for 2023/2024 (1 april 2014 – 31 march 2024). Available at: [https://www.odt.nhs.uk/search/?search=Annual%20report%20on%20live%20transplantation.%20Report%20for%202023/2024%20\(1%20april%202014%20%e2%80%93%2031%20march%202024\)](https://www.odt.nhs.uk/search/?search=Annual%20report%20on%20live%20transplantation.%20Report%20for%202023/2024%20(1%20april%202014%20%e2%80%93%2031%20march%202024)) [Accessed June 26, 2025].

3. Millson C, Considine A, Cramp M, Holt A, Hubscher S, Hutchinson J, et al. Adult liver transplantation: UK clinical guideline – part 2: surgery and post-operation. *Frontline Gastroenterol.* 2020;11(5):1–12. PMID: 32879722
<https://doi.org/10.1136/flgastro-2019-101216>

4. Choudhary N, Saigal S, Bansal R, Saraf N, Gautam D, Soin A. Acute and chronic rejection after liver transplantation: what a clinician

needs to know. *J Clin Exp Hepatol*. 2017;7(4):358–366. PMID: 29234201 <https://doi.org/10.1016/j.jceh.2017.10.003>

5. Tovikkai C, Sawetwanichakul J, Kositamongkol P, Mahawithitwong P, Dumronggittigule W, Sangserestid P, et al. Incidence and risk factors associated with chronic kidney disease after liver transplantation: a review of a 20-year experience at a single center. *Transplantation Proceedings*. 2024;56(3):613–619. PMID: 38388291 <https://doi.org/10.1016/j.transproceed.2023.11.036>

6. López Panqueva R. Most relevant pathology issues in the late post liver transplant period. *Rev Col Gastroenterol*. 2016;31(3):292–304. <https://doi.org/10.22516/25007440.104>

7. Vandermeulen M, Grégoire C, Briquet A, Lechanteur C, Beguin Y, Detry O. Rationale for the potential use of mesenchymal stromal cells in liver transplantation. *World J Gastroenterol*. 2014;20(44):16418-16432. PMID: 25469010 <https://doi.org/10.3748/wjg.v20.i44.16418>

8. Akabane M, Imaoka Y, Kawashima J, Endo Y, Schenk A, Sasaki K, et al. Innovative strategies for liver transplantation: the role of mesenchymal stem cells and their cell-free derivatives. *Cells*. 2024;13(19):1–13. PMID: 39404368 <https://doi.org/10.3390/cells13191604>

9. Wen F, Yang G, Yu S, Liu H, Liao N, Liu Z. Mesenchymal stem cell therapy for liver transplantation: clinical progress and immunomodulatory properties. *Stem Cell Research and Therapy*. 2024;15(320):1–12. PMID: 39334441 <https://doi.org/10.1186/s13287-024-03943-6>

10. Khubutiya MSh, Gulyaev VA, Khvatov VB, Lemenev VL, Kabanova SA, Novruzbeikov MS, et al. Immunological tolerance in organ transplantation. *Transplantologiya. The Russian Journal of*

Transplantation. 2017;9(3):211–225. (In Russ).
<https://doi.org/10.23873/2074-0506-2017-9-3-211-225>

11. Basok YuB, Ponomareva AS, Grudinin NV, Kruglov DN, Bogdanov VK, Belova AD, et al. Use of mesenchymal stem cells in solid organ transplantation: challenges and prospects. *Russian Journal of Transplantology and Artificial Organs.* 2025;27(1):114–134. (In Russ).
<https://doi.org/10.15825/1995-1191-2025-1-114-134>

12. Detry O, Vandermeulen M, Delbouille M, Somja J, Bletard N, Briquet A, et al. Infusion of mesenchymal stromal cells after deceased liver transplantation: a phase I–II, open-label, clinical study. *Journal of Hepatology.* 2017;67(1):47–55. PMID: 28284916
<https://doi.org/10.1016/j.jhep.2017.03.001>

13. *Transplantatsiya pecheni (vzrosloe i detskoe naselenie): Klinicheskiy protokol. (utverzhdyon MZ RB 13.02.2023 № 31).* Available at: <http://minzdrav.gov.by> [Accessed June 26, 2025]. (In Russ).

14. Dominici M, Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8(4):315–317. PMID: 16923606 <https://doi.org/10.1080/14653240600855905>

15. Demetris A, Bellamy C, Hübscher S, O'Leary J, Randhawa P, Feng S, et al. 2016 Comprehensive update of the banff working group on liver allograft pathology: introduction of antibody-mediated rejection. *Am J Transplant.* 2016;16(10):2816–2835. PMID: 27273869
<https://doi.org/10.1111/ajt.13909>

16. Borbat AM, Dubova EA, Gainullina ER, Lishchuk SV. Protocol for histological examination of liver transplant dysfunction. *Arkhiv patologii.* 2019;81(6):71–73. (In Russ).
<https://doi.org/10.17116/patol20198106171>

17. Shkalova LV, Mozheyko NP, Ilyinskiy IM, Moisyuk YaG, Tsirulnikova OM, Gautier SV. The diagnosis of liver allograft acute rejection in liver biopsies. *Russian Journal of Transplantology and Artificial Organs*. 2011;13(3):15–19. (In Russ). <https://doi.org/10.15825/1995-1191-2011-3-15-19>

18. Nosik AV, Korotkov SV, Smolnikova VV, Grinevich VYu, Efimov DYU, Dmitrieva MV, et al. Effector memory CD4⁺ T cells and dendritic cells are noninvasive biomarkers of late cellular rejection after kidney transplantation. *Transplantologiya. The Russian Journal of Transplantation*. 2018;10(3):207–216. (In Russ). <https://doi.org/10.23873/2074-0506-2018-10-3-207-216>

19. Perico N, Casiraghi F, Gotti E, Inrona M, Todeschini M, Cavinato R, et al. Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. *Transpl Int*. 2013;26(9):867–878. PMID: 23738760 <https://doi.org/10.1111/tri.12132>

20. Peng Y, Ke M, Xu L, Liu L, Chen X, Xia W, et al. Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. *Transplantation*. 2013;95(1):161–168. PMID: 23263506 <https://doi.org/10.1097/TP.0b013e3182754c53>

21. Pan G, Chen Z, Xu L, Zhu J, Xiang P, Ma J, et al. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study. *Oncotarget*. 2016;7(11):12089–12101. PMID: 26933811 <https://doi.org/10.18632/oncotarget.7725>

Information about the authors

Sergey V. Korotkov, Assoc. Prof., Cand. Sci. (Med.), Head of the Transplantology Department, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0002-8536-6911>, skorotkov@tut.by

40%, development of the concept and design of the study, collection of material, statistical data processing, analysis of the obtained data, preparation of the text, editing

Ekaterina A. Nazarova, Assoc. Prof., Cand. Sci. (Biol.), Doctor of Clinical Diagnostic Laboratory of the Laboratory for Cell Biotechnology, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0001-7147-4834>, k.nazarova-86@mail.ru

5%, collection and processing of material, analysis and interpretation of the obtained data

Ekaterina G. Yurkina, Doctor of Clinical Diagnostic Laboratory of the Laboratory for Cell Biotechnology, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0002-0966-7456>, ekatherina999@mail.ru

5%, collection and processing of material, analysis and interpretation of the obtained data

Victoria V. Smolnikova, Cand. Sci. (Biol.), Leading Researcher of the Scientific Department, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0001-5947-8285>, vsmolnikova2603@mail.ru

5%, collection and processing of material, analysis and interpretation of the obtained data

Victoria Yu. Grinevich, Head of the Clinical Diagnostic Laboratory of the Bone Marrow Transplant Service, Minsk Scientific and Practical

Center of Surgery, Transplantation and Hematology,
<https://orcid.org/0000-0002-4505-4884>, grinevich.viktorija@gmail.com

5%, collection and processing of material, analysis and interpretation of the obtained data

Ekaterina A. Yanushevskaya, Doctor of Clinical Diagnostic Laboratory of the HLA-typing Laboratory, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology,
<https://orcid.org/0009-0003-1174-4179>, 9gkbhla@mail.ru

5%, collection and processing of material, analysis and interpretation of the obtained data

Anna Yu. Startseva, Head of the HLA-typing Laboratory, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0009-0004-5040-9768>, 9gkbhla@mail.ru

5%, collection and processing of material, analysis and interpretation of the obtained data

Aleksey E. Shcherba, Prof., Dr. Sci. (Med.), Deputy Director for Surgery, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0003-0569-6150>, aleina@tut.by

5%, development of the concept and design of the study, analysis of the obtained data, editing

Svetlana I. Krivenko, Prof., Dr. Sci. (Med.), Deputy Director for Research, Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0002-6813-4465>, svtl_kr@tut.by

5%, development of the concept and design of the study, analysis of the obtained data, editing

Oleg O. Rummo, Academician of the National Academy of Sciences of the Republic of Belarus, Prof., Dr. Sci. (Med.), Director of

Minsk Scientific and Practical Center of Surgery, Transplantation and Hematology, <https://orcid.org/0000-0001-7023-4767>, oleggrumm@tut.by

20%, development of the concept and design of the study, analysis of the obtained data, editing, final approval of the manuscript for publication

The article was received on May 5, 2025;

Approved after reviewing on May 23, 2025;

Accepted for publication on June 25, 2025