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ОЦЕНКА ТРАНСПЛАНТАЦИИ МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК ИЗ КОСТНОГО МОЗГА У ПАЦИЕНТОВ С ЦИРРОЗОМ ПЕЧЕНИ, ВЫЗВАННЫМ ВИРУСОМ ГЕПАТИТА С (ПИЛОТНОЕ ИССЛЕДОВАНИЕ)

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Evaluation Transplantation of Bone-Derived Mesenchymal Stem Cell in the Patients with Hepatitis C-Related Liver Cirrhosis (Pilot Study)

Резюме

Введение. Цирроз печени является конечной стадией прогрессирования хронических диффузных заболеваний печени. Поздние стадии цирроза печени, как правило, не поддаются консервативному лечению, и единственным эффективным методом помощи пациентам на данной стадии является трансплантация печени. Однако широкое применение последней в клинической практике сопряжено с серьезными препятствиями: нехваткой донорских органов, отторжением трансплантата, осложнениями в ходе операции и послеоперационном периоде, а также высокой стоимостью такого вмешательства. Трансплантация стволовых клеток костного мозга, особенно трансплантация мезенхимальных стволовых клеток, может быть потенциальным средством лечения цирроза печени и применяться после проведения дополнительных клинических исследований по эффективности и безопасности. **Цель исследования** — оценить эффективность и безопасность интрапаренхимальной трансплантации аутологичных мезенхимальных стволовых клеток из костного мозга для лечения

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пациентов с циррозом печени, вызванным вирусом гепатита С (ВГС). **Материалы и методы.** Проведено пилотное открытое нерандомизированное проспективное исследование с включением 6 пациентов с циррозом печени, вызванным вирусом гепатита С. Аутологичные мезенхимальные стволовые клетки трансплантировали внутрипаренхимально в ткань печени из расчета 1×10^6/кг массы тела — по 1 мл в 5 точек. **Результаты.** К 6 мес. после трансплантации наблюдалось снижение уровня билирубина (с 36,4 мкмоль/л до 27 мкмоль/л, p=0,03), баллов по показателю MELD (с 11,5 до 8, p=0,035), повышение уровней тромбоцитов к 3 мес. (с 83×10^9/л до $124,6\times10^9$/л, p=0,031) и 6 мес. (до $119,5\times10^9$/л, p=0,031). Не было отмечено влияния к 6 мес. после трансплантации на баллы по шкале Чайлд-Пью (p=0,181), показатели цитолиза (сохранение повышенных уровней аланинаминотрансферазы (p=0,062) и аспартатаминотрансферазы (p=0,844)), репликативную активность вируса (сохранение РНК ВГС в крови) (p=0,219). Введение мезенхимальных стволовых клеток к 6 мес. после трансплантации не приводило к разрешению цирроза печени и воспалительной инфильтрации по данным световой микроскопии, а также к разрешению капилляризации синусоидов (p=0,586) и трансдифференцировки звездчатых клеток Ито в миофибробласты (p >0,99) по данным иммуногистохимического исследования. Ни у кого из пациентов после проведения трансплантации не было отмечено повышения температуры тела, увеличения лабораторных показателей, изменений со стороны жизненно важных функций. У одного пациента при госпитализации через 6 мес. после трансплантации мезенхимальных стволовых клеток был диагностирован тромбоз глубоких вен правой голени. **Выводы.** Отмечено положительное влияние мезенхимальных стволовых клеток на улучшение функции печени, при отсутствии их влияния на репликативную активность вируса и сохраняющуюся активность воспалительного процесса. Использованная методика трансплантации мезенхимальных стволовых клеток является безопасной процедурой для пациентов с циррозом печени, вызванным вирусом гепатита С классов тяжести А и В и может быть применена в клинической практике.

Ключевые слова: *вирус гепатита С, цирроз, мезенхимальные стволовые клетки, трансплантация*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

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Abstract

Introduction. Liver cirrhosis (LC) is the final stage in the progression of chronic diffuse diseases. As common, late stages of LC do not respond to conservative treatment methods, so liver transplantation is the most effective method at this stage. Widespread use of transplantation in clinical practice is due to serious obstacles: a shortage of donor organs, transplant rejection, complications during the operation and the postoperative period, as well as the high cost of such an intervention. We consider bone marrow stem cell transplantation as a potential treatment for liver cirrhosis and additional clinical trials for efficacy and safety. The aim of the study was to assess the efficacy and safety of intraparenchymal transplantation of autologous MSCs from the bone marrow for the treatment of patients with cirrhosis of the liver caused by the hepatitis C virus (HCV-LC). **Materials and methods**. A pilot open-label non-randomized prospective study with the inclusion of 6 patients with HCV-LP. Autologous MSCs were transplanted intraparenchymally into the liver tissue at the rate of $1x10^6$/kg body weight — 1 ml at 5 points. **Results**. By 6 months after transplantation, there has been a decrease in the level of bilirubin (from 36,4 μmol/L to 27 μmol/L, p=0.03), MELD scores (from 11,5 to 8, p=0.035), and an increase in platelet levels by 3 months (from 83x109 / l to $124,6x10^9$/l, p=0,031) and 6 months (up to $119,5x10^9$/l, p=0,031). By 6 months after transplantation, there has been no statistically significant result in changing on points on the Child-Pugh scale (p=0,181), cytolysis indicators (maintaining elevated levels of ALT (p=0,062) and AST (p=0,844)), replicative activity of the virus (preservation of HCV RNA in the blood) (p=0,219). Moreover, introduction of MSCs by 6 months after transplantation did not lead to resolution of liver cirrhosis and inflammatory infiltration according to light microscopy data, as well as to resolution of sinusoidal capillarization (p=0,586) and PCI transdifferentiation into myofibroblasts (p>0,99) according to immunohistochemical studies. None of the procedures after the transplantation had an increase in body temperature, an increase in laboratory parameters, or changes in vital functions. One patient was admitted to hospital after 6 months. after MSC transplantation, deep vein thrombosis of the right leg was diagnosed. **Conclusion**. The positive effect of MSCs on the improvement of liver function was noted. There was no effect on the replicative activity of the virus. The continuing activity of the inflammatory process was observed. The used MSC transplantation technique is a safe procedure for patients with HCV-LC severity classes A and B and can be applied in clinical practice.

Key words: *hepatitis C virus, cirrhosis, mesenchymal stem cells, transplantation*

Conflict of interests

The authors declare no conflict of interests

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α-SMA — alpha smooth muscle actin, AE — adverse event, AFP — alpha-fetoprotein, ALP — alkaline phosphatase, ALT — alanine aminotransferase, AST — aspartate aminotransferase, BM — bone marrow, CD — cluster of differentiation, FBS — fetal bovine serum, FITC — fluorescein isothiocyanate, GGTP — gamma-glutamyl transpeptidase, HCV — hepatitis C virus, HSCs — hematopoietic stem cells, IMDM — Iscove's modified Dulbecco's medium, ICs — Ito cells, LC — liver cirrhosis, MCA — monoclonal antibodies, Me (min; max) — median (minimum; maximum), MELD — model for end-stage liver disease, MSCs — mesenchymal stem cells, NLB — needle liver biopsy, US — ultrasound.

Liver cirrhosis (LC) is the terminal stage in the progression of chronic diffuse liver diseases and is characterized by impaired liver architecture with the formation of regenerative nodules. Late stages of LC usually do not respond to conservative treatment. Hence, liver transplantation is the only effective way of helping patients at this stage. However, the widespread use of the latter in clinical practice faces significant obstacles: lack of donor organs, transplant rejection, complications during surgery and in the postoperative period, and the high cost of such an intervention.

Cell-based therapy, which seems a minimally invasive procedure, may complement the management of advanced LC. Bone marrow is a reservoir of various stem cells, including hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Although MSCs can differentiate into mesoderm- and neuroectoderm-derived cells, [1] they have the potential for endodermal differentiation and differentiation into functional hepatocyte-like cells [2]. HSCs and MSCs can trans-differentiate into hepatocytes *in vivo*; however, MSCs have the highest potential among bone marrow cells for differentiation in the liver [3]. This is confirmed by experimental and clinical trials. For example, there are studies demonstrating that human embryonic stem cells can trans-differentiate into hepatocytes in 2- and 3D *in vitro* culture systems [4, 5]. Other studies have shown that circulating adult stem cells can differentiate into mature hepatocytes or cholangiocytes in the human body [6, 7]. Animal studies demonstrated that MSCs injected in rats through the tail vein can protect them from liver fibrosis during the experiment [8]. Moreover, injection of non-hematopoietic bone marrow stem cells can lead to fibrosis regression in mice [9]. There are clinical trials that have demonstrated the safety and positive effect of MSCs on the course of chronic liver diseases of various etiology: absence of prooncogenic potential, improved biochemical parameters, decreased inflammation in the liver parenchyma, and decreased collagen production [10, 11]. In a controlled clinical study with 20 participants with decompensated liver cirrhosis after MSCs transplantation, the parameters of MELD (Model For End-Stage Liver Disease) ($p = 0.0001$), INR ($p = 0.012$), bilirubin ($p < 0.0001$) and total albumin ($p < 0.0001$) improved significantly [12]. At the same time, there are studies that demonstrated no effect of MSCs on the reduction of liver fibrosis [13].

The objective of this study was to evaluate the efficacy and safety of intraparenchymal transplantation of autologous MSCs from bone marrow for the treatment of patients with liver cirrhosis caused by hepatitis C virus (HCV LC).

Characteristics of patients with HCV LC

A pilot, open-labeled, non-randomized, prospective trial was conducted. Subject recruitment was carried out at the State Institution «Belarusian Research Center for Pediatric Oncology, Hematology and Immunology», Minsk, Belarus from 23.02.2009 (date of enrollment of the first patient) to 29.12.2009 (date of enrollment of the last patient).

This study included patients who signed the provided informed consent, males and females, aged over 18 and up to 53 years with HCV LC of class A and B according to the Child — Pugh score [14–16], with anti-HCV (total antibodies to HCV) and HCV RNA detected in blood. The patients had previously experienced failed treatment with standard interferon, and at least a year had passed since the end of this treatment.

Exclusion criteria were concomitant HIV infection, viral hepatitis B, hepatocellular carcinoma, tumors of other localizations, severe comorbidity, pregnancy and breastfeeding. Patients under the age of 18 and patients after liver and kidney transplantation were not included.

Study procedures

Enrollment in the study was consecutive.

Primary hospitalization was carried out for a comprehensive examination and sampling MSCs from the bone marrow. Demographic, clinical, laboratory, and biological history was taken.

Diagnostic tests and instrumental methods included blood biochemistry to determine the levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), cholesterol, total protein, albumin, alpha fetoprotein (AFP), as well as common blood count, common urinalysis and ultrasound of abdominal organs. Information about the

patients was added to an electronic database. The above laboratory tests and instrumental examinations were used for monitoring the status of patients over time during the post-transplantation period and for evaluating its effectiveness.

A morphological study of liver biopsy was added to the efficacy assessment scale. Biopsy samples were assessed by light microscopy. Methods for immunohistochemical assessment of liver changes were also used, allowing to assess the activation of myofibroblasts by the expression of alpha-smooth muscle actin (α-SMA) and the phenomenon of sinusoid capillarization by the expression of CD34+. For immunohistochemical tests, liver biopsy samples were fixed in 10% neutral formalin solution and embedded in paraffin according to the standard method. Subsequently, commercial antibodies to CD34 and α-SMA antigens (Dako, USA) were used. For the morphometric study, microscope slides were photographed in 5–6 fields of vision (objective 40), as well as in 10 fields of vision (objective 100) with a resolution of 1,798*1,438 pixels using a Leica microscope with a Leica digital camera (Leica Microsystems, Germany). The area of the analyzed fields of vision was 298.47 × 238.71 = 71,247.77 μm^2 (magnification ×40) and 113.53×98.29 = 11,158.86 μm^2 (magnification ×100), respectively. The prevalence of fibrotic changes (CD34, α-SMA) was assessed semi-quantitatively: 1 point — poorly expressed (immunoreactivity of cells in the separate sinusoids of lobules); 2 points — moderately expressed (immunoreactivity of cells to approximately half of the sinusoids of lobules); 3 points — significant (immunoreactivity of the cells of most sinusoids of lobules).

Cirrhosis was diagnosed based on the results of a comprehensive clinical and laboratory examination of patients and liver biopsy results [17]. To clarify LC etiology, we used data from the epidemiological history (indication of past acute viral hepatitis, previous blood transfusions, surgical interventions, dental care, etc.), history of present disease, blood test results for markers of viral hepatitis (HBsAg, anti-HCV, HCV RNA).

MSC graft preparation

Bone marrow was sampled in a volume of 40–60 ml by needle biopsy (under anesthesia) 35–45 days before the planned injection of MSCs. The mandatory requirement was testing MSCs from each passage for sterility across the entire spectrum of possible bacterial and viral contamination.

To obtain an autograft of MSCs from the bone marrow of patients with HCV LC, the method developed by Ya. I. Isaikin et al. was used [18] after modification, which consisted of washing of cells three times, 48 hours after the removal of the non-adherent fraction in order to minimize possible contamination of the viral infection with blood cells. Several passages were performed where MSCs were grown *in vitro* in IMDM (Iscove's modified Dulbecco's medium) with 10% fetal bovine serum (FBS) (Sigma, USA), 2 mM of L-glutamine and 10^{-4} M of 2-mercaptoethanol to the required volume depending on patient's body weight. Cells removed from the surface of culture flasks during the last passage were washed twice with saline and transferred to a 10 ml syringe for further injection to a patient. The classification of cells obtained by this method as MSCs was confirmed by the presence of CD105, CD90, CD44, CD140 surface markers.

Immunophenotypic analysis of MSCs. Cell staining with monoclonal antibodies (MCA) CD105, CD90, CD44, CD34, CD14 labeled with phycoerythrin and CD45 labeled with FITC (Beckman Coulter Inc., USA) was performed according to the standard technique. Nonspecific binding of MCA was assessed by isotype control. 20 μl of specific MCA and isotype controls were added to the sample (100–200 thousand cells) and incubated in the dark at room temperature for 25–30 min. After incubation with antibodies, cells were washed twice in phosphate buffer by centrifuging for 5 min at 300 g. The analysis was performed on a Becton Dickinson FACSCan flow cytometer (BioLine, Finland) using CellQuestPro software. At least 10 thousand cells were analyzed for each sample. In addition to the evaluation of MCA binding, the values of forward and side scatter were recorded.

Assessment of the viability of MSCs. For the analysis of viability, cells were stained with 0.4% trypan blue solution. At least 100 stained (dead) and unstained (live) cells were visually counted in a Goryaev chamber using a light microscope. Cell viability coefficient was calculated (as a percentage of the total number of counted cells).

Injection of MSCs

Intraparenchymal MSC transplantation was performed under laparoscopic or ultrasound control via sequential percutaneous liver punctures in the 5-7-cm region of previously performed needle biopsy (5 ml of the MSC suspension calculated as 1×10^6/body weight, 1 ml for one of five injection points, to a depth of 2–2.5 cm).

Study design

During the first hospitalization, a comprehensive laboratory and diagnostic examination of patients was carried out, needle liver biopsy (NLB) was performed, and bone marrow was sampled.

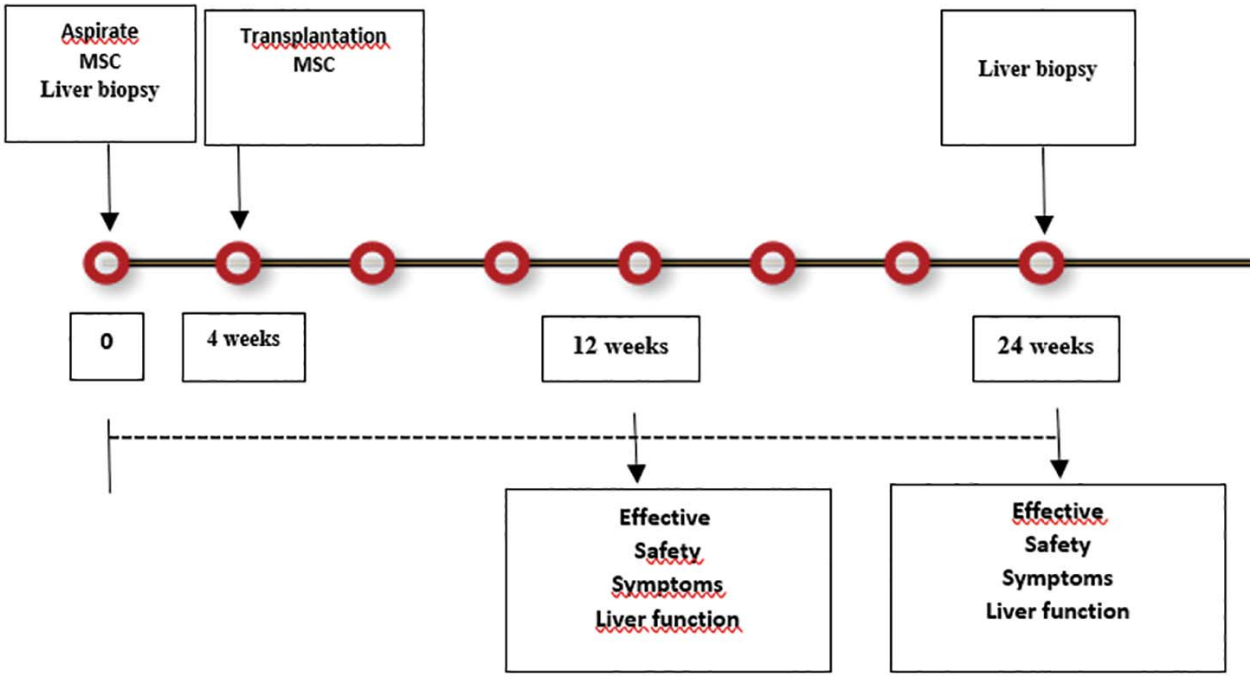


***Figure 1**. Scheme (control points) of patient observation*

During each visit, patients underwent medical examination, information about the presence/absence of such symptoms as fever, general weakness, nausea, vomiting, and abdominal pain was recorded.

Upon re-hospitalization of patients in 1 month, intraparenchymal transplantation of MSCs was performed.

Laboratory tests (ALT, AST, bilirubin, GGTP, urea, creatinine, alkaline phosphatase, cholesterol, total protein, albumin), MELD score [19] and viral load were performed 12 and 24 weeks after transplantation. If a patient had the same biochemical index several times at the same control point, the mean value was taken for analysis. NLB was additionally performed in 24 weeks (Fig. 1). During the entire follow-up period, patients received no drug treatment.

Primary efficacy endpoint: some of the patients who achieved a decrease in the MELD and Child — Pugh score and laboratory test results in 6 months after MSC transplantation.

Secondary efficacy endpoint: some of the patients who achieved cirrhosis regression in 6 months after MSC transplantation.

Safety assessment

The safety of MSC transplantation was assessed in all patients. The assessment included recording adverse events (AEs) from the moment of MSC transplantation and up to 6 months after or after withdrawal from the study, changes in vital signs, and clinical laboratory test results.

Compliance with ethical standards

This study was approved by the Human Research Ethics Committee and was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki, as well as with the principles of the International Council for Harmonization Good Clinical Practice. Before enrollment in the study, the patient was provided with information about the goals and methods of the study and the possible risks associated with participation in the study. Written informed consent was obtained from each patient. Data obtained during the study were processed in accordance with the principles of confidentiality of patient information.

Statistical analysis

Descriptive statistics of quantitative parameters are represented by median and range in the form of Me (min; max), taking into account the small sample size. Comparisons of baseline and post-transplantation parameters (after 3 and 6 months) were carried out using

the Wilcoxon test for paired data without taking into account corrections for multiple testing.

Differences were considered significant at $p < 0.05$. Calculations were carried out in the R statistical package (The R Project for Statistical Computing. R version 3.6.3., Austria) [20].

Results

The analysis included 6 patients with HCV LC (Fig. 2) who fully complied with the study protocol.

Mean age of patients in the study population was 44 ± 6 years (from 37 to 53 years); there were three male and three female patients. Hepatitis C virus (HCV) was the etiological factor in all patients.

In four patients, LC severity according to the Child — Pugh classification corresponded to class A, in two patients — to class B. The data are shown in Table 1.

All patients at the time of enrollment had signs of asthenia (general weakness, fatigue) and dyspepsia (recurrent pain or a feeling of heaviness in the right hypochondrium, nausea, loss of appetite), as well as clinical signs of portal hypertension: splenomegaly (n = 6), esophageal varices (n = 6). Also, two patients had a single episode of ascites in history, which was immediately reversed; there was no ascites at the time of enrollment. Three patients had yellowing of skin and sclera. One female patient had signs of cryoglobulinemic vasculitis. All patients had no clinical signs of hepatic encephalopathy. Results are shown in Table 2.

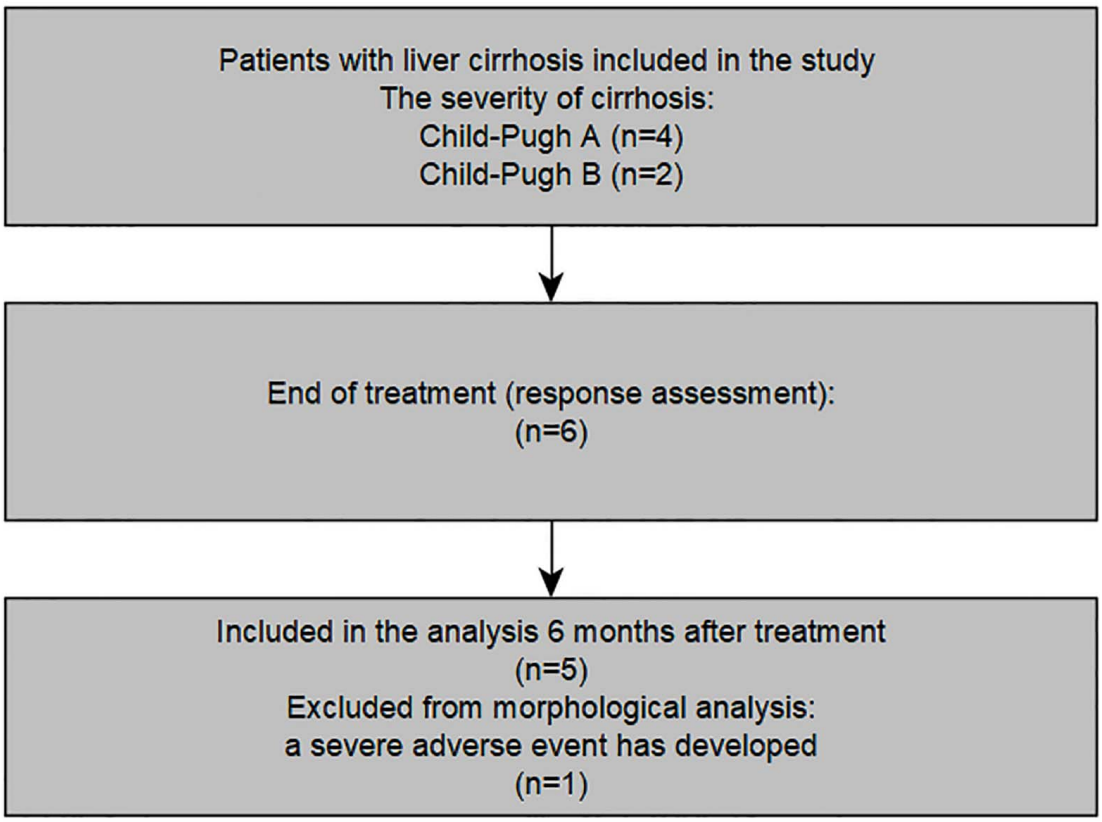


Figure 2. *Scheme of distribution of patients included in the study*

Table 1*. Demographic characteristics of HCV-LC patients included in the study*

The patients	Age	Gender	Etiology	Child-Pugh severity class
P1	51	Female	HCV	A
P2	39	Female	HCV	A
P3	37	Male	HCV	A
P4	41	Female	HCV	A
P5	46	Male	HCV	B
P6	53	Male	HCV	B

Table 2. Clinical characteristics of HCV-LC patients included in the study

Clinical data	P1	P2	P3	P4	P5	P6
Dyspeptic syndrome	Yes	Yes	Yes	Yes	Yes	Yes
Asthenovegetative syndrome	Yes	Yes	Yes	Yes	Yes	Yes
Jaundice	No	No	Yes	No	Yes	Yes
Ascites (previously)	No	No	No	No	Yes	Yes
Varicose veins of the esophagus	1st degree	1st degree	1st degree	1st degree	2nd degree	2nd degree
Encephalopathy (clinical manifestations)	No	No	No	No	No	No
Splenomegaly	Yes	Yes	Yes	Yes	Yes	Yes
Vasculitis	No	No	No	Yes	No	No

Follow-up results

All patients maintained compliance throughout the entire period after transplantation of MSCs; in six months, they were hospitalized to assess treatment results.

All of them reported subjective improvement six months after transplantation of MSCs: decrease and then disappearance of clinical signs of asthenia and dyspepsia. None demonstrated increased signs of portal hypertension.

Patients demonstrated improved liver function during follow-up period. A significant decrease in the MELD score from 11.5 (9; 17) to 8 (6; 10) (p = 0.035) was observed six months after transplantation. However, there was no significant decrease in the Child — Pugh score. The data are shown in Figure 3 and Table 3.

Table 3. Dynamics of HCV-LC severity according to Child-Pugh scale and MELD 6 months after MSC transplantation in patients included in the study

Index	До начала Before the start of transplantation (points) Me (min; max)	After 6 months after transplantation (points) Me (min; max)	p
Child-Pugh scale	6 (5; 10)	5,5 (5; 8)	0,181
MELD scale	11,5 (9;17)	8 (6; 10)	0,035

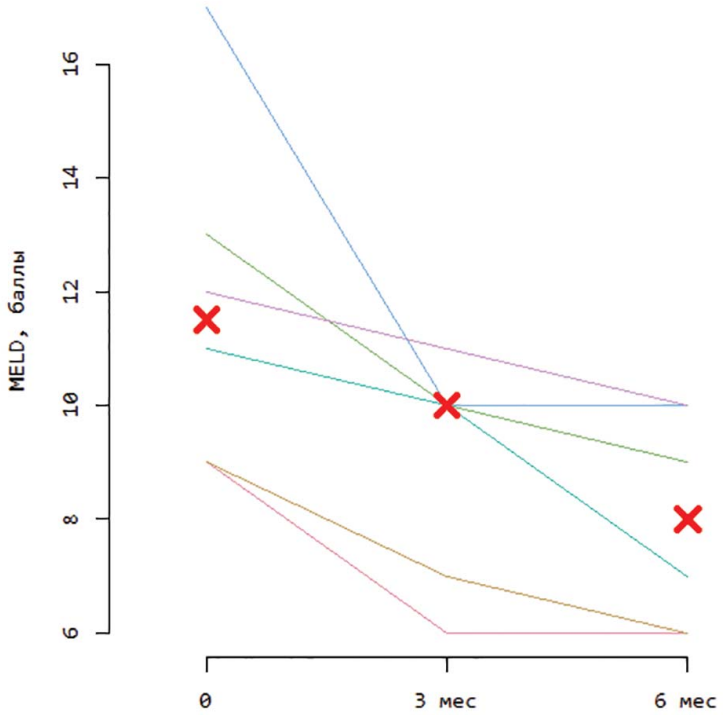


Figure 3. *Dynamics of MELD (points) in HCV-LC patients after 3 months and 6 months after MSC transplantation*

A decrease in bilirubin level from 36.4 μmol/l to 27 μmol/l (p = 0.03) was observed six months after transplantation (Fig. 4 and Table 4).

Patients demonstrated a trend towards decreasing ALT levels from 110.5 U/l to 82.7 U/l (p = 0.062) during the follow-up period six months after transplantation. In three and six months, an increase in platelet level was registered: from 83 x 10^9/l to 124.6 x 10^9/l in three months (p = 0.031) and 119.5 x 10^9/l (p = 0.031) (Table 4). There were no changes in the severity of portal hypertension (spleen dimensions and grade of esophageal varices) during the follow-up period.

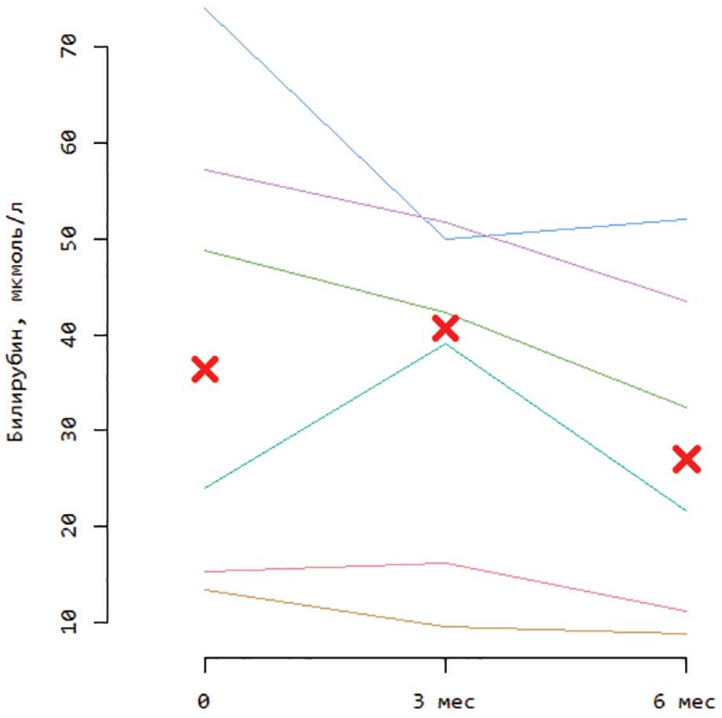


Figure 4. *Dynamics of bilirubin levels in patients with HCV-CP after 3 months and 6 months after MSC transplantation*

Table 4. *Dynamics of laboratory parameters in patients with HCV-CP after 3 months. and 6 months. after MSC transplantation*

Index	Before the start of transplantation Me (min; max)	After 3 months after transplantation Me (min; max)	After 6 months after transplantation Me (min; max)	p_{0-3}	p_{0-6}
Total bilirubin, μmol/l	36,4 (13,5; 74)	40.6 (9,6; 51,7)	27 (8,9; 52)	0,437	0,03
ALAT, units/l	110,5 (79; 212)	80,3 (54,8; 159,8)	82,7 (41; 173)	0,562	0,062
ASAT, units/l	104,5 (52,9; 232)	100,8 (20; 140,3)	96 (55; 275)	0,312	0,844
ALF, IU/l	217,5 (80,1; 590)	206 (96,7; 260)	228 (101; 312)	0,437	0,844
GGTP, IU/l	84,8 (27,4; 317)	103.5 (14,0; 541,6)	77,6 (12,8; 342,6)	0,437	>0,99
Cholesterol, mmol/l	3,9 (3,03; 6,6)	4,2 (3,2; 5,7)	3,6 (2,75; 5,08)	>0,99	0,177
Urea, mmol/l	3,9 (2,09; 5,3)	3,1 (2,4; 5,0)	3,6 (2,8; 5,7)	0,094	0,844
Creatinine, μmol/L	55,5 (52; 70)	66,5 (57,7; 94)	60,5 (54; 69)	0,094	0,292
Albumin, g/l	39,05 (33,98; 44,3)	39 (32; 48,44)	40,3 (31,4; 45)	0,752	0,787
Total protein, g/l	76,15 (71,1; 85)	72,5 (68; 82,5)	79,8 (75; 86,1)	0,031	0,562
AFP, IU / ml	3,17 (2,07; 6,2)	----	4,5 (2,2; 12,18)	N/A	0,562
Platelets, ×10^9/l	83 (38; 140)	124,6 (85,8; 213)	119,5 (54,5; 205)	0,031	0,031
Leukocytes, ×10^9/l	4,5 (3,3; 6,4)	4,6 (2,8; 9,6)	4,7 (1,8; 8,2)	>0,99	0,916

Note: ALAT — alanine aminotransferase, ASAT — aspartate aminotransferase, ALF — alkaline phosphatase, GGTP — gamma glutamyl transpeptidase, AFP — alpha-fetoprotein

Table 5. Dynamics of viral load in patients with HCV-LC after 6 months after MSC transplantation

Index	Before the start of the transplant Me (min; max)	After 6 months. after transplant Me (min; max)	p
Viral load (IU/ml)	286000 (42000; 630000)	155650 (4030; 637000)	0,219

Table 6. Dynamics of α-SMA and CD34 in patients with HCV-LC after 3 months and 6 months after MSC transplantation

Index	Before the start of the transplant Me (min; max)	After 6 months. after transplant Me (min; max)	p_{0-6}
α-SMA, баллы	2,5 (2; 3)	3 (1,5; 3)	>0,99
CD34, баллы	2 (1; 3)	2 (1; 2)	0,586

No effect of MSCs on the level of viral load was observed six months after transplantation (p = 0.219) (table 5).

According to the morphological analysis carried out via light microscopy, signs of LC persisted in all patients.

There were no significant changes in the parameters of IC transdifferentiation (by α-SMA, points) (p > 0.99) and sinusoid capillarization (by CD34, points) (p = 0.586) six months after transplantation according to immunohistochemical analysis (table 6).

According to the morphological analysis carried out via light microscopy, signs of LC with inflammatory infiltration of liver parenchyma persisted in all patients.

Safety assessment

None of the patients had increased body temperature, increased AFP or other laboratory parameters, changes in vital functions, as well as developed severe complications of portal hypertension (encephalopathy, hepatorenal syndrome, gastrointestinal bleeding) after transplantation.

During hospitalization six months after MSC transplantation, one patient was diagnosed with deep vein thrombosis of the right leg, possibly associated with impaired venous outflow or inherited predisposition and MSC transplantation six months prior. Due to the developed AE, no repeated NLB was performed and the patient was excluded from the analysis by the morphological parameter.

The following effects were revealed **as a result** of transplantation of autologous MSCs from the bone marrow (BM) into the liver parenchyma in the amount of 10^6/kg body weight in patients with HCV LC of A and B severity classes according to Child — Pugh.

There was a significant decrease in bilirubin level (from 36.4 μmol/l to 27 μmol/l, p = 0.03) and the MELD score (from 11.5 to 8, p = 0.035) in six months, and an increase in platelet count in three months (from 83 × 10^9/l to 124.6 × 10^9/l, p = 0.031) and six months (up to 119.5 × 10^9/l, p = 0.031) after transplantation.

Six months after MSC transplantation, there was no significant decrease in virus replicative activity (HCV RNA in blood) (p = 0.219), levels of ALT (p = 0.062) and AST (p = 0.844), Child — Pugh score (p = 0.181), parameters of sinusoid capillarization (based on the expression of CD34+, p = 0.586) and trans-differentiation of IC into myofibroblasts (based on the expression of α-SMA, p > 0.99) according to immunohistochemical tests; according to light microscopy, morphological signs of inflammatory cell infiltration and liver cirrhosis persisted.

The MSC transplantation technique caused no signs of decompensated cirrhosis in patients with Child — Pugh classes A and B: there was no deterioration of laboratory parameters and no clinical signs of severe complications of portal hypertension (encephalopathy, hepatorenal syndrome, gastrointestinal bleeding).

Discussion

The lack of drugs that can radically affect the formation of fibrous tissue and significantly improve the functional state of parenchyma necessitates the search for alternative ways of treating patients with LC, especially during the pre-transplantation period. In this regard, using stem cells is becoming an important method of management for the terminal stage of liver diseases [10].

Studies revealed that the injection of human MSCs can reduce liver fibrosis in rats and humans [8, 10]. Also, it was reported that the transplantation of MSCs contributes to a significant improvement in liver function: the authors demonstrated increased albumin and cholesterol levels in patients, a decreased MELD score and a higher patient survival rate [10, 11, 21]. Our study also revealed improved liver function: a decrease in bilirubin level and MELD score.

The question of possible mechanisms of MSC action leading to the improved functional reserve of the liver is widely discussed in the literature. One of these mechanisms is the ability of MSCs to differentiate into hepatocytes *in vitro* and *in vivo* [2–7]. There is a strong probability that MSCs transplanted into the liver can fulfill

their potential in this way. At the same time, animal studies have shown that only a small percentage of donor MSCs (1–3%) can differentiate into hepatocytes [6, 7]. This suggests that the improvement of liver function also occurs due to other additional mechanisms of action of donor cells. Another possible explanation for the described effect is that MSCs, according to the literature, can significantly enhance the functional state of resident hepatocytes. They can secrete a wide range of bioactive molecules (growth factors and cytokines), thereby enhancing hepatocyte proliferation and liver revascularization. MSCs can prevent hepatocyte apoptosis; there are indications of their immunosuppressive properties [7, 10]. It is very likely that under conditions of significant liver damage observed in cirrhosis, MSCs have the potential to have an effect on several pathogenetic links at once. These multiple effects should be clarified and proven through further research.

In our study, a complex morphological approach was chosen for more thorough control of the effects of MSCs. Despite that we found no signs suggesting that MSCs can drastically eliminate the activity of the pathological process (which is quite reasonable taking into account the persisting replicative activity of the virus), the regularity we identified allows us to develop an important algorithm of action for clinical practice — firstly, to eliminate the etiological factor that constantly contributes to the active inflammatory process in the liver, and simultaneously or sequentially start pathogenetic treatment with MSCs in order to improve the functional reserve of the liver tissue with an intact structure.

We demonstrated that intraparenchymal transplantation of MSCs could not eliminate the morphological signs of liver cirrhosis. At the same time, we believe that constant processes of fibrogenesis and fibrolysis in liver tissue during cirrhosis are very deep and dynamic, and they cannot be fully defined by the conventional METAVIR morphological scale. For a more detailed analysis, other additional methods are required, such as the electron microscopy method, which helped us earlier to describe the positive changes in the liver at the microstructural level during MSC transplantation [22].

Figure 5-9. *Dynamics of morphological data according to the results of light microscopy in patients with HCV-LC after 6 months MSC transplantation*

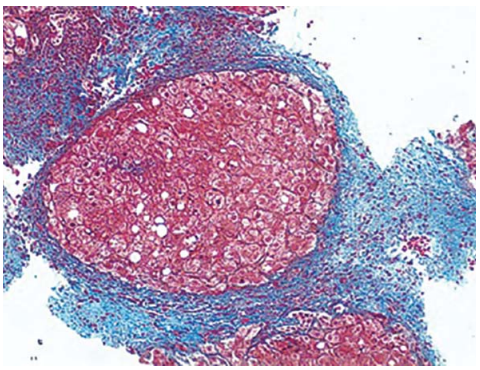
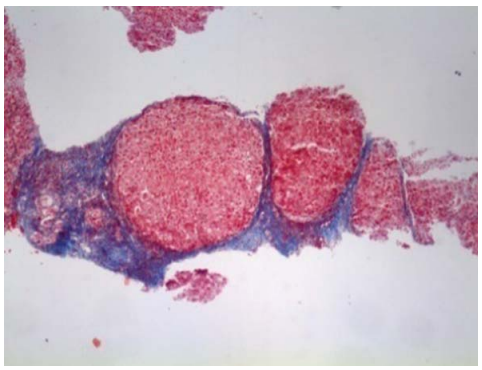
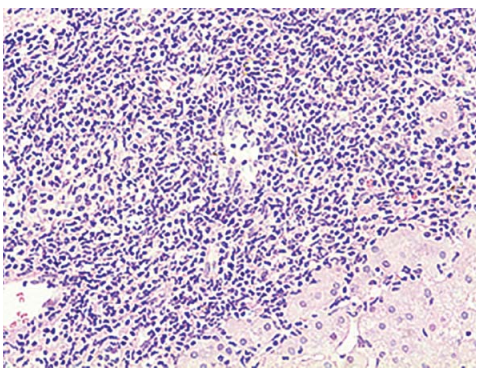
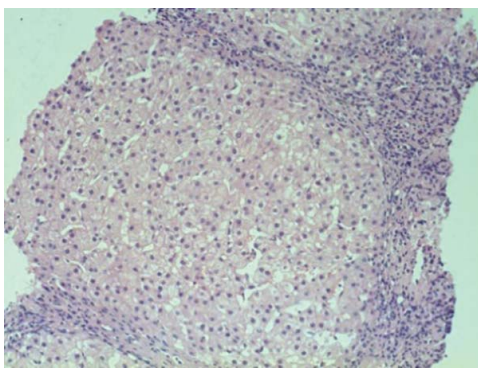
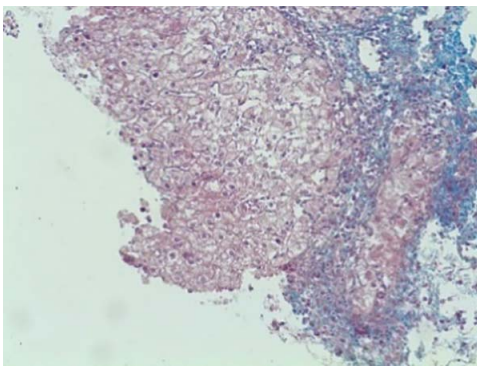
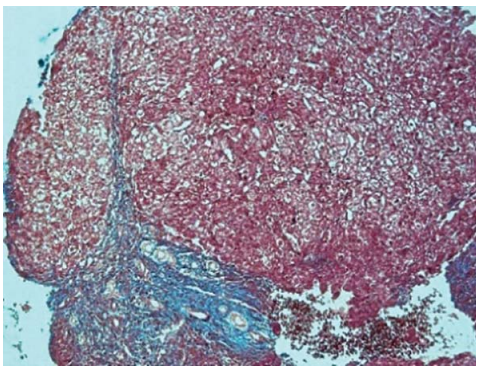
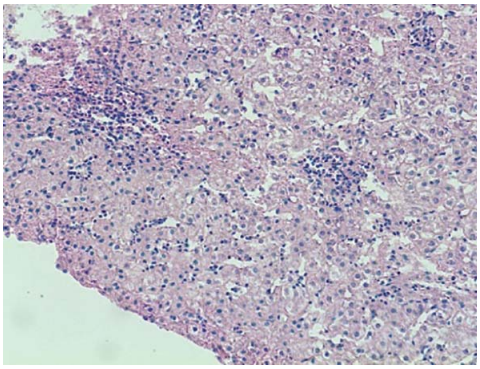
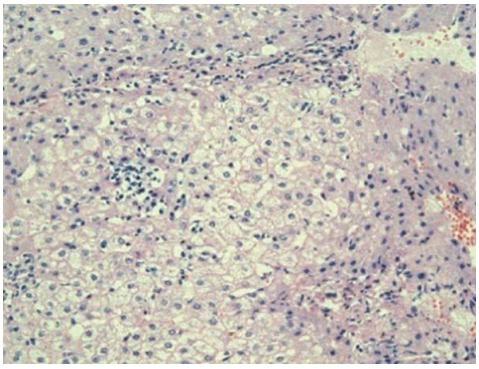
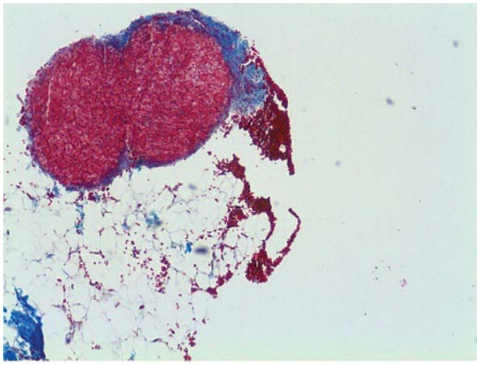
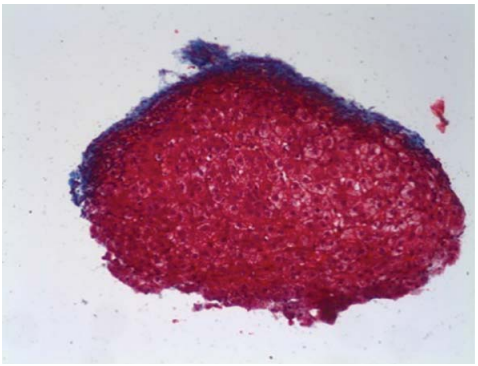
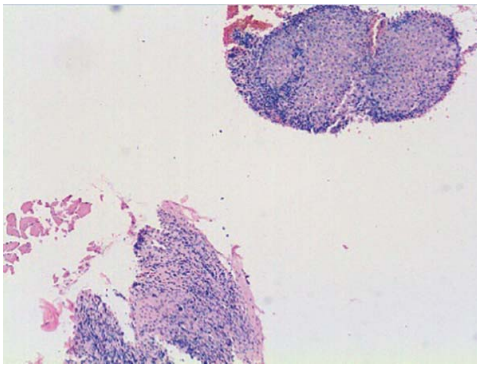
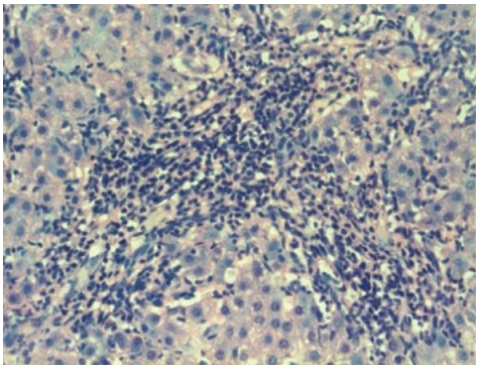
Patients	Before the start of the transplant	After 6 months. after transplant
П1 Р1		
		

Figure 5a. *Micronodular cirrhosis. Masson's staining, ×63*

Figure 5b. *Micronodular cirrhosis. Masson's staining, ×63*

Figure 5c. *Severe infiltration in fibrous septa and periportally. Staining with hematoxylin and eosin, ×126.*

Figure 5d. *Weak infiltration in fibrous septa and periportally. Staining with hematoxylin and eosin, ×126.*

Patients	Before the start of the transplant	After 6 months. after transplant
П2 Р2		
	Figure 8a. *Micronodular cirrhosis. Masson's staining, ×63*	***Figure 8b.*** *Micronodular cirrhosis. Masson's staining, ×63*
		
	Figure 6c. *Lymphoplasmacytic infiltration, ×63*	***Figure 7b.*** *Micronodular cirrhosis. Masson's staining, ×63*
П3 Р3		
	Figure 7a. *Micronodular cirrhosis. Masson's staining, ×63*	***Рисунок 7b.*** *Микронодулярный цирроз. Окраска по Массону, ×63* ***Figure 7b.*** *Micronodular cirrhosis. Masson's staining, ×63*
		
	Figure 7c. *Lymphocytic infiltration, in many areas penetrating deep into the lobule, ×63*	***Figure 7d.*** *Severe inflammatory lymph-macrophage infiltration in fibrous septa and periportally. Staining with hematoxylin and eosin, ×126.*

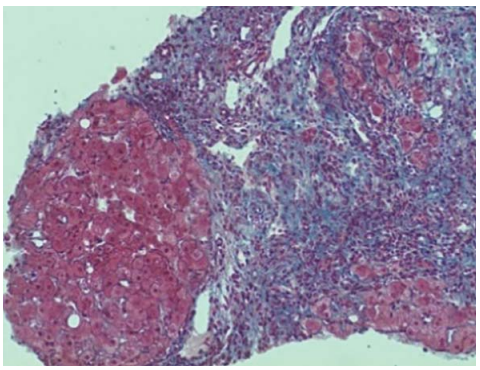
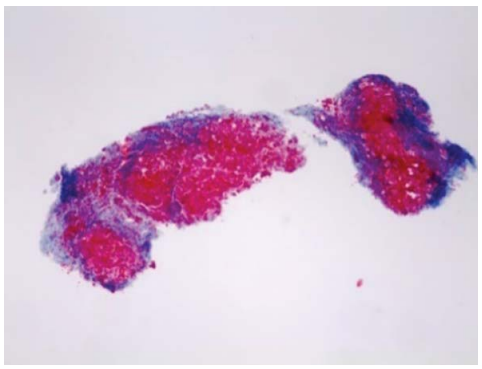
Patients	Before the start of the transplant	After 6 months. after transplant
П4 Р4		

Figure 8a. *Micronodular cirrhosis. Masson's staining, ×126*

Figure 8b. *Micronodular cirrhosis. Masson's staining, ×126*

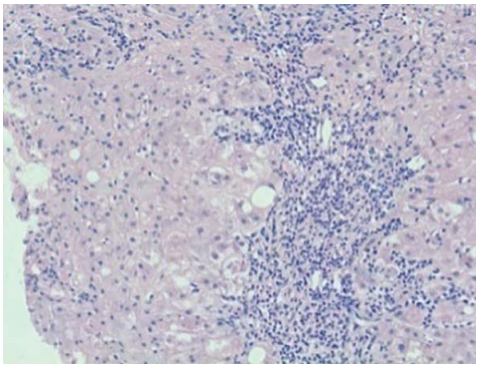


Figure 8c. *Lymphocytic infiltration, in some areas penetrating deep into the lobule. Staining with hematoxylin and eosin, ×126*

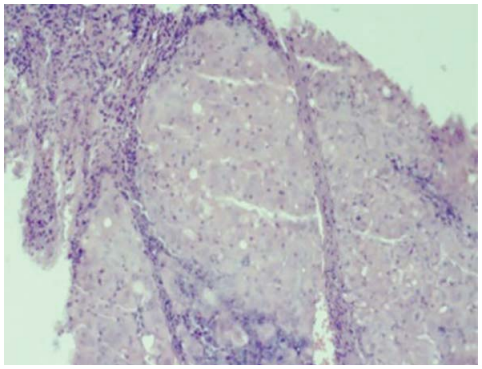


Figure 8d. *False lobule. Lymphocytic infiltration, in some areas penetrating deep into the lobule. Staining with hematoxylin and eosin, ×63*

П6
Р6

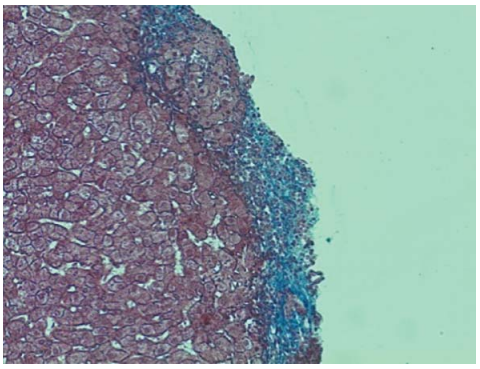


Figure 9a. *Macronodular cirrhosis. Masson's staining, ×63*

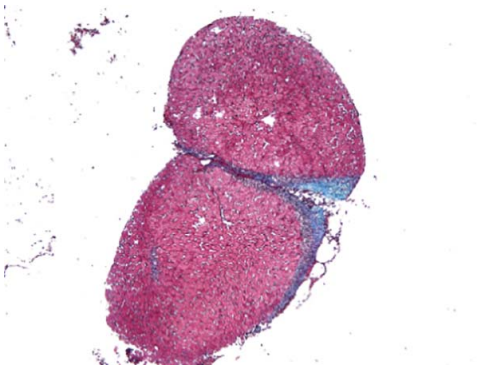


Figure 9b. *Macronodular cirrhosis with inflammatory lympho-macrophage infiltration Masson's stain, ×63*

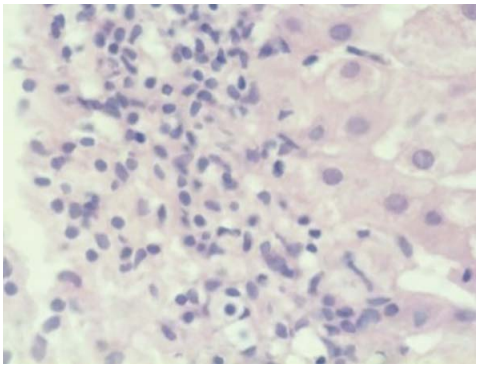


Figure 9c. *Infiltrate that penetrates deep into the lobule. Staining with hematoxylin and eosin, ×65*

There are other methods that are not yet available for clinical practice but can be used in experimental and clinical trials. This aspect should be taken into consideration when planning such trials.

Critically, our results demonstrate the safety of intraparenchymal transplantation of MSCs from the bone marrow and its applicability in clinical practice for the management of liver cirrhosis. At the same time, the protocol for MSC transplantation requires further development; its effectiveness should be further evaluated in randomized trials.

This study also highlights several key issues that should be considered when designing future clinical trials: determining the best cell type for this technique, the minimum effective amount of MSCs for transplantation, and improving the optimal method of transplantation in terms of efficacy and safety taking into account new data.

Conclusion

1. Transplantation of BM MSCs in patients with HCV LC of A and B severity classes improves liver function six months after transplantation as evidenced by a significant decrease in bilirubin level ($p = 0.03$) and the MELD score ($p = 0.035$), and an increase in platelet level ($p < 0.05$) three and six months after transplantation.
2. At the same time, MSC transplantation does not lead to a significant decrease in HCV replicative activity ($p = 0.219$), cytolytic activity (acc. to ALT ($p = 0.062$) and AST ($p = 0.844$) levels), immunohistochemical parameters of fibrogenesis (acc. to expression of CD34+ ($p = 0.586$) and α-SMA ($p > 0.99$)), as well as Child — Pugh score ($p = 0.181$).
3. The used MSC transplantation technique is safe (with no changes in laboratory parameters and no severe complications such as encephalopathy, hepatorenal syndrome, bleedings) for patients with HCV LC of A and B severity classes.
4. Considering the safety of intraparenchymal transplantation of BM MSCs and its effects (improved liver function, no effect on HCV replicative activity, retained virus-related active liver disease), further research is recommended in order to improve approaches to the treatment of patients with HCV LC. One of these methods could be an integrated approach with the primary prescription of direct-acting drugs for managing HCV infection and subsequent transplantation of MSCs as one of the pathogenetic treatment methods that would allow eliminating the virus, stopping the active inflammatory process, and improving the function of the remaining parenchyma.

Вклад авторов

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

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Исайкина Я.И.: написание текста статьи, перевод резюме на английский язык

Красько О.В.: статистическая обработка данных

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

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Aleinikova O.V.: research concept and design, verification of critical intellectual content, article editing

Tsyrkunov V.M.: research concept and design, verification of critical intellectual content, article editing

Karpov I.A.: research concept and design, verification of critical intellectual content, article editing.

Isaykina Y.I.: writing article, translation of the resume in English

Krasko O.V.: statistical processing of materials for the article

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