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INDUCTION OF CIRCULATING CD133+ STEM CELLS COMMITTED TO CIRRHOTIC LIVERS IN WAITLISTED PATIENTS

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Studies on the regenerative capabilities of tissues have shown that damaged liver can recover using hematopoietic stem cells (HSCs), which are able not only to replace cells in the target organ, but can also deliver trophic factors that support endogenous liver regeneration. There is practically no data on how organ-derived humoral signals involve such morphogenic/trophic cells in circulation. **Objective:** to investigate the role of non-invasive vibromechanical percutaneous action on the liver in cirrhosis by quantification of CD133+ lymphoid HSCs with specific hepatic marker alpha-fetoprotein (AFP) in patients awaiting liver transplantation. **Materials and methods.** In order to increase the number of AFP+ part of CD133+ stem lymphoid cells in the blood, the patient's cirrhotic liver was mechanically activated by transcutaneous microvibration using electromagnetic vibrophones in contact with the skin. This generated mechanical impulses with a 10 μm amplitude and a smoothly varying frequency from 0.03 kHz to 18 kHz and back to within one cycle lasting 1 minute. The amount of AFP+ lymphocyte fraction in the total content of CD133+ HSCs in lymphocytes of potential recipients was monitored by flow cytometry before and during daily 15-minute sonication of the skin zone corresponding to the liver projection for three weeks with eight synphased vibraphones. **Results.** Sonication of the liver projection zone significantly increased the number of liver-specific CD133+ AFP+ lymphocytes by 2–3 times compared to the baseline values. Repeated similar sonication of the same site after a three-week break showed a statistically insignificant increase from the initial level. With a similar effect on the spinal projection in the control group of waitlisted patients with cirrhosis, there was no increase in CD133+ AFP+ lymphocytes. **Conclusion.** Mechanical stress prompts the organ to secrete specific humoral signals that provoke the bone marrow to produce additional lymphoid stem cells committed to the liver and recruit them into circulation.

Keywords: hematopoietic stem cells, cirrhosis, regeneration, waiting list, mechanical microvibration.

INTRODUCTION

Hematopoiesis and normal tissues

Most primitive mononuclear cells in the bone marrow and blood of adults are represented by CD133+CD34–, CD133+CD34+, and CD133–CD34+ markers, with the CD133 marker being earlier than CD34. Intensively proliferating CD133+ cells are capable of differentiating into cells with meso-, endo- and neurodermal layer characteristics, namely: endothelial progenitor cells, neural progenitor cells, astrocytes, oligodendrocytes, renal proximal tubule cells, mammary duct cells, cells of the prostate gland, skin, lungs, intestines, hepatocytic cells and skeletal muscle cells, expressing basic tissue proteins [1, 2]. Primitive bone marrow cells migrate through the blood to various tissues and organs, especially after damage [3]. There is abundant evidence of increased tissue regeneration resulting from stimulation of primitive bone marrow cells or their introduction into the body [4–6]. So, the idea of bone marrow as a source of circulating tissue-committed morphogenic stem cells is confirmed. Vascular endothelium is renewed with the help of circulating CD133+ progenitor cells from the bone marrow [7]. Even if primitive bone marrow cells do not transdifferentiate, as some researchers suggest, they aggregate with other host cells (the fusing phenomenon), or release regenerative cytokines and nutrients [8], thus supporting target tissue regeneration. The earliest lymphocytes, as well as mononuclear stem and progenitor cells, penetrate the capillary walls into the interstitial non-lymphoid tissues, including the liver in order to maintain dynamic proliferative balance, that is, homeostasis under normal conditions [9]. Such cells sacrifice themselves to support the function of surrounding cells with a different phenotype. For example, TDT+ prolymphocytes, γδ-T cells (CD4−CD8–) [10] and CD3+CD31+CXCR4+ angiogenic T lymphocytes [11] are directly involved in tissue cellular renewal by producing growth factors and nutrients, and maintaining angiogenesis processes. All these cells, according to Fiedler's prediction [12], are not immunocytes, but rather trophocytes feeding the feeder lymphocytes.

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Stem cells and liver

It is known that chronic liver disease leads to non-functional regeneration, which is controlled by interaction between liver tissue cells (hepatocytes, hepatic stellate cells, inflammatory cells, biliary epithelial cells, sinusoidal endothelial cells), in the end fibrosis-cirrhosis is formed [13]. Along with the actual liver cells, recruited from the circulation of hematopoietic stem cells (HSCs) from the bone marrow, they can also promote liver regeneration by fusion with damaged hepatocytes or by differentiation into hepatocyte-like cells, or through their morphogenic and pro-angiogenic growth factors [14–16]. Lymphocyte colony-stimulating factors, modulating hematopoiesis, are used to mobilize HSCs into the bloodstream in order to reverse induced chronic liver damage. However, the approach has standard limitations on the number of cycles and hematological toxicity of agents [17]. Intraportal infusions of autologous HSCs isolated from a pre-aspirated bone marrow volume represent another invasive approach to artificial enrichment of the cirrhotic liver microenvironment with cellular elements of autologous HSCs [18].

A liver transplant in patients with cirrhosis may itself be a natural long-term stimulus for recruitment of additional HSCs into the peripheral blood [19], confirming the existence of a humoral information pathway (axis) between these two tissues. Interestingly, HSCs produced during embryonic liver development can traffic between different tissues, just like HSCs in adults, and in both cases, they are provided with humoral signals, regulated tightly enough to reach their final destinations [20, 21].

While waiting for liver transplantation, it seems extremely interesting to find an opportunity to induce pro-regenerative paracrine signals emanating from the organ parenchyma in the liver-bone marrow axis.

Having had a positive experience in remote non-invasive recruitment of HSCs into the blood by means of moderate percutaneous mechanical microvibration of cancellous bones containing active ("red") bone marrow [22–24], we suggested that a similar mechanical effect (stress) on altered liver tissue would probably open additional up additional opportunities for a positive interaction between AFP+ morphogenic HSCs and liver cells.

The **aim** of the study was to study the effect of percutaneous mechanical microvibration on mobilization of liver-committed HSCs from the bone marrow via humoral signals.

MATERIALS AND METHODS

The study included 9 patients aged 53 to 61 years with cirrhosis awaiting liver transplantation. Six out of the 9 patients received mechanical vibration courses through skin-contact vibraphones on the projection area of the liver. Three patients received vibration in the spine projection in a similar manner. Certified serial device Vitafon-5 (GOST 50444-92 and TU9444-009-23138557-2009 RF) was used as the electromechanical vibration source for vibroacoustic impact. In addition, data obtained from 6 healthy volunteers, whose age did not differ from the subjects, were used for comparison. produced synchronous and in-phase mechanical vibrations in cyclic mode. During one cycle (60 seconds), the vibration frequency varied from 0.03 Hz to 18 kHz and in reverse sequence at about 10 μm constant vibration amplitude. The block of vibraphones (45 mm diameter each) consisted of two rows (four in a row) with 75 mm distance between the centers of the rows and 55 mm distance between the centers of vibraphones within each row. Thus, the block formed an active rectangular field of mechanical microvibrations 12 × 22 cm, held by a flat fabric frame with eight fastening sockets and soft fasteners for tight fixation on the human body.

Before exposure, all patients had blood sampling for clinical and biochemical analysis. Vibration exposure was performed once a day for 15 minutes while sitting in an armchair (reclining position). Two exposure modes were used: the first (I) included 15 minutes of exposure daily for three weeks, the second (II) – 15 minutes daily for three weeks, then a break for three weeks and repeated exposure.

The control scheme (III) consisted of simultaneous mechanical microvibrations on 8 pairs of equidistant points along the spine with 0.5 cm distance between vibraphones in each pair, for 3 weeks (2nd, 3rd, and 4th) 15 minutes daily.

Once a week, we determined the proportions of circulating lymphocytes in the synthetic phase of S+, CD133+, CD31+ cells in the lymphoid fraction, the proportion of double positive cells AFP+CD133+, AFP+CD31+, and the derived ratios (AFP+CD133+)/CD133+, (AFP+CD31+)/CD31+ and AFP+CD133+/AFP+CD31+. For this purpose, a mononuclear cell (MNC) fraction was isolated from peripheral blood by classical separation on a Ficoll density gradient, omitting the final enrichment stage. [25]. Viability of MNCs was assessed using the trypan blue exclusion test. Cells from two equal parts of the MNC fraction were stained for analysis on an LSR Fortessa flow cytometer (Becton-Dickinson). Hoechst 33342 staining was used for cell cycle analysis as previously described [26] with slight modifications.

The phenotypes of circulating cells in the lymphocytic part of MNCs were assessed using monoclonal antibodies to markers CD133/2, CD31, AFP (α-fetoprotein) conjugated with allophycocyanine (APC), fluorescein isothiocyanate (FITC), and phycoerythrin (PE), respectively.

The parameters were evaluated statistically by calculating the mean (M) and standard error (SE). M values were compared using the t-test and p probability. Kinetic tendencies of the parameters before and after scoring were characterized by mathematical functions generated automatically using nonlinear approximations in Excel. The R-squared was used as a statistical goodness-of-fit

measure of the regression line to the data. Satisfactory R-squared values were confirmed using the t-parameter equation: t = R2 × (n – 2) (1 – R2) [27].

RESULTS

A relative decrease in S+, CD31+ cells and a minor increase in CD133+ cells are characteristic of patients awaiting liver transplantation as compared to healthy volunteers (Table, Fig. 1).

As a result of the acoustic effect on the liver area according to two schemes, the S+, CD31+, and CD133+ content before treatment was normalized by 4–5 weeks (Fig. 2). In addition, the number of double-positive liver-committed CD133+AFP+ cells increased. This effect can be considered specific for the effect on the liver area, since only the CD133+ population increased in the spine projection when using the third sound exposure scheme (Fig. 3). Spine vibration stress significantly increased the count of uncommitted CD133+ cells in circulation, exceeding not only their number before exposure, but also the level of these cells after the first course of exposure to the liver (Fig. 3).

The real concentrations corresponding to Fig. 2 are 0.07 ± 0.022 ± 0.0125 (Scheme 1) and 0.079 ± 0.037 ± 0.0099 (Scheme 2). These mean values increased 1.95 times (p = 0.006) and 1.75 times (p = 0.008), respectively for spine exposure based on Scheme 3.

No specific changes were found at week 10–12 of exposure compared to the data before it (Fig. 4). The increase in the CD133+AFP+ cell count and the CD133+AFP+/CD133 ratio obtained at week 4–5 according to scheme 1 (Fig. 2) lost its statistical significance by week 10–12 (Fig. 4). After repeated exposure to the liver area according to scheme 2, the specific increase in CD133+AFP+ cell count disappeared at week 10–12, but the cells were replaced by nonspecific CD133+ cells, whose increased level remained (Fig. 1, dashed line, left column for CD133+).

Table

Baseline indicators of circulating lymphocytes in healthy volunteers

Parameters	CD133+	CD133+ AFP+	CD31+	CD31+ AFP+	s	CD133+/ CD31+	CD133+AFP+/ CD31+AFP+	CD133+AFP+/ CD133+	CD31+AFP+/ CD31+
M	0.037	0.0052	40.1	0.39	0.95	0.101*	1.64*	12.36*	0.97*
SD	0.012	0.0044	9.2	0.36	0.98	0.037	1.25	8.22	0.74
SE	0.004	0.0015	3.1	0.12	0.28	0.013	0.44	2.90	0.24
KV	0.32	0.84	0.23	0.92	1.03	0.37	0.76	0.66	0.76

Note. * Obtained by averaging personal odds.

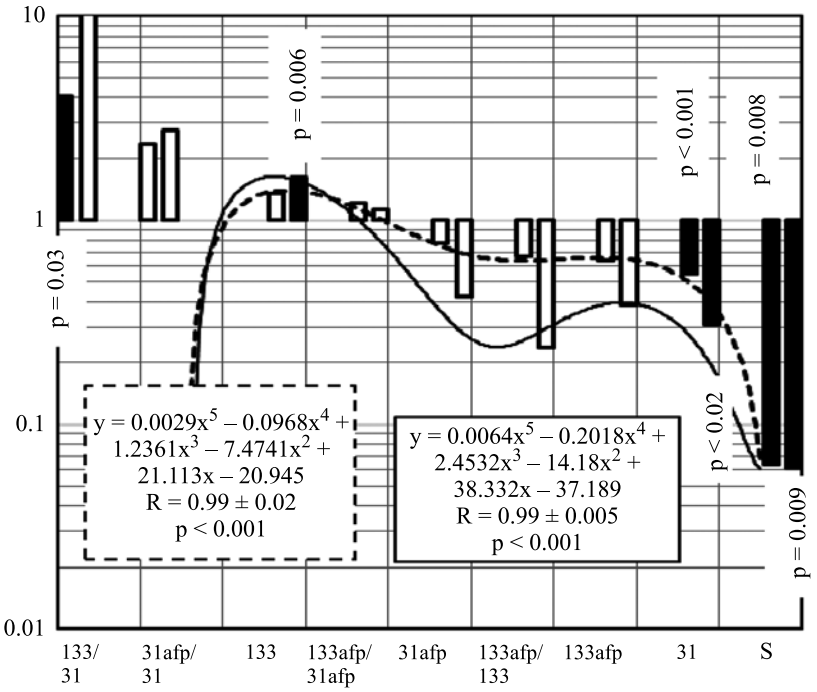

Fig. 1. Changes in mean parameters (M) in cirrhosis relative to mean parameters for healthy volunteers, taken as 1.0. The right-hand columns represent the data obtained in the first week before sonication based on Scheme 1 (solid approximation line). The left-hand columns represent the data obtained before sonication based on Scheme 2 and Scheme 3 (dashed line). Statistically significant deviations are shown in black

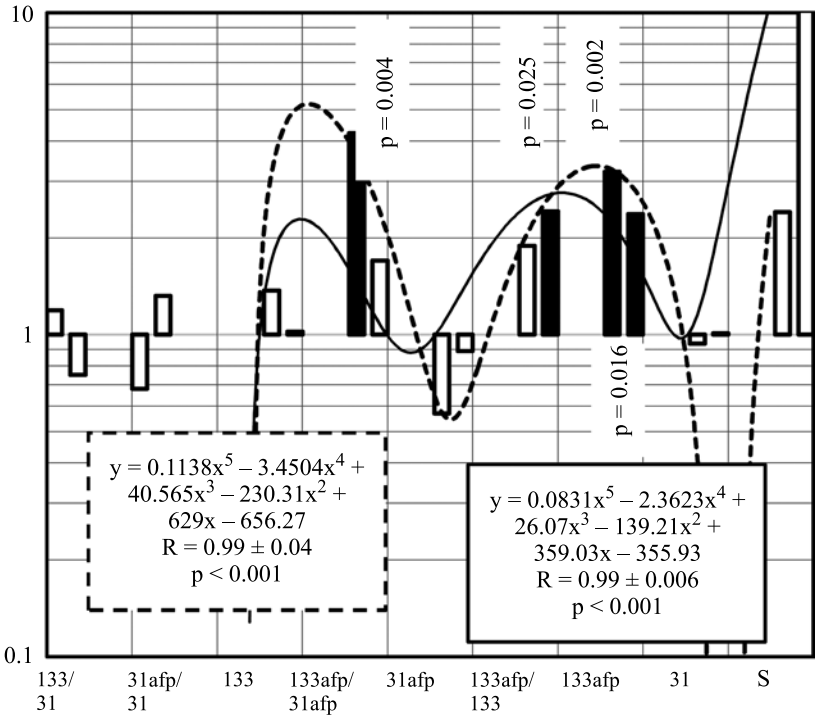

Fig. 2. Changes in mean parameters (M) by 4–5 weeks of sonication compared to the liver area relative to mean parameters for patients in Fig. 1, taken as 1.0. The right and left columns represent the relative data obtained by 4–6 weeks after the start of sonication based on Scheme 1 (solid line, right columns) and Scheme 2 (dashed line, left columns). Statistically significant deviations are shown in black

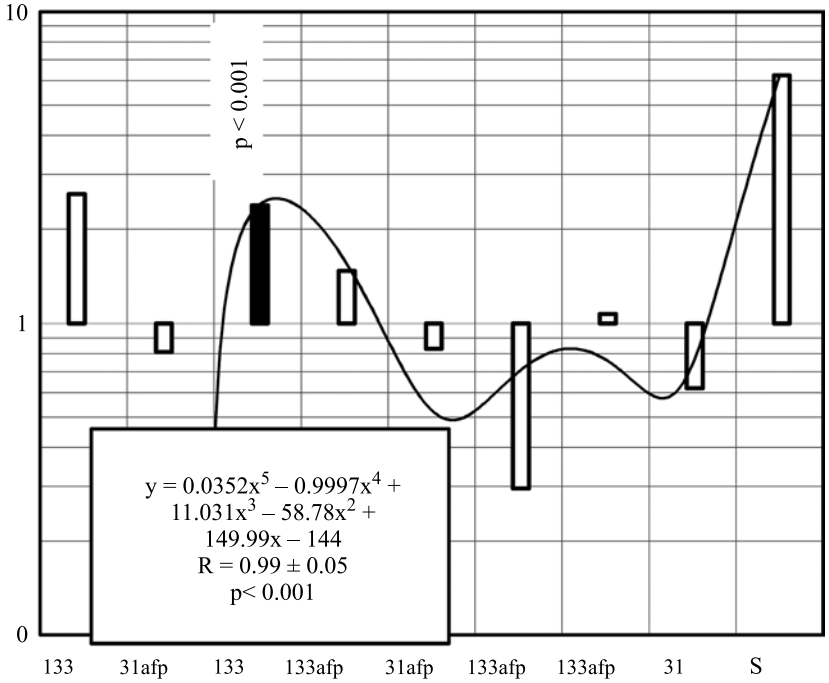

Fig. 3. Changes in mean cell parameters after sonication of the spine (Scheme 3) relative to the parameters in healthy people (Fig. 1), taken as 1.0. The columns show the relative data obtained at 4–6 weeks

Thus, indirect percutaneous mechanical mobilization of liver-committed CD133+AFP+ stem cells in the lymphocyte pool occurs within week 4–6 of exposure and is weakened at week 10–12, regardless of continuation of exposure (scheme 2) or its termination (scheme 1).

DISCUSSION

Physical factors obviously play an important role in biological processes. Application of tissue tensile stress, shear stress, electromagnetic fields, and ultrasound induces variants of enhancement of osteogenesis and chon-

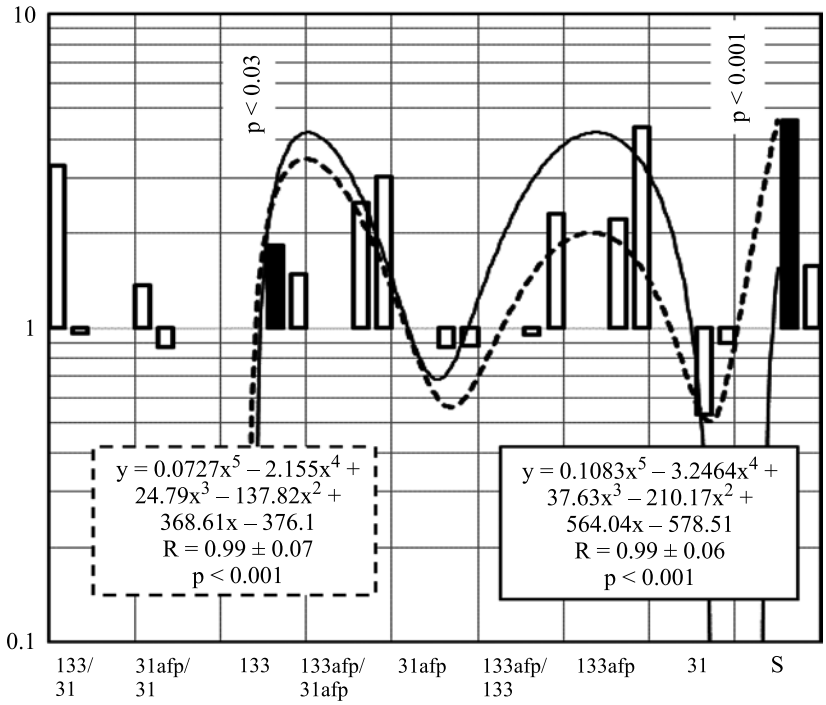

Fig. 4. Changes in mean cell parameters (M) over 10–12 weeks compared to baseline (right-hand columns with solid approximation line). The columns show the relative data obtained at 10–12 weeks after the start of sonication based on Scheme 1 (solid line, right columns) and Scheme 2 (dashed line, left columns)

drogenesis, involving human stem cells in the process. Therefore, direct physical intervention appears to be a potentially attractive approach and can be used to support tissue regeneration.

Application of acoustic non-invasive effect on the liver in compensated cirrhosis seems to be a promising method. Some researchers have previously reported that the liver exhibits mechanical resonance within a registered frequency of 30–400 Hz, depending on the odd harmonics of 1–3 orders [28]. The liver tissue itself has its own natural frequency of about 55–60 MHz [29]. For non-invasive effect on this complex system with little-known physical properties, mechanical vibrations seemed promising, as they can essentially produce stochastic resonance (SR) in nonlinear biological systems, amplifying subthreshold stimuli in metabolic pathways [30, 31]. We expected that humoral signals of a still unknown nature from artificial liver tissue tension would reach the lymphopoietic niches of the bone marrow and intensify either the natural production of morphogenic liver-committed CD133+AFP+ lymphocytes, or enhance their recruitment into circulation, against the background of initial suppression in cirrhosis. If this assumption is correct, we could get a new approach for non-invasive and long-term support of liver function in waitlisted patients. To increase the likelihood of resonance processes in liver tissues, we chose a source of mechanical vibrations in a wide frequency range [24].

Circulating lymphocytes were chosen as objects of this study for several reasons. First, lymphoid cells have the most developed and flexible mechanisms of navigation to various tissues or tropism, and contain fractions of morphogenic/trophic cells, such as CD133+, CD34+ stem cells, lymphoid TdT+ stem cells, angiogenic CD31 T+ cells, and other trophic lymphocytes of intermediate degree of differentiation, which are usually called regulatory cells. Secondly, lymphocytopoiesis is the most damaging process, and lymphoid tissue is characterized by the highest depreciation, that is, loss of actual and functional mass during the lifetime of the organism [32]. Thirdly, in various organ diseases, including liver diseases, the worst forecasts are associated with a high ratio of neutrophil count to blood lymphocyte count (the so-called neutrophil-lymphocyte ratio), which emphasizes the role of weakening of lymphopoiesis in the loss of vitality of the organism as a whole [33].

A study of blood samples from cirrhotic patients revealed a deficiency of naive CD31+ lymphocytes with angiogenic properties [11, 34], as well as a deficiency of lymphocytes in the S-phase of DNA synthesis. The first course of action on the liver or spine normalized both deficiencies. Thus, this was an argument against the specificity of these two effects.

On the contrary, the first exposure to the liver increases the number of committed CD133+AFP+ cells, which seems to be quite specific for liver tissue. We interpret the increasing number of CD133+AFP+ cells as a distant result of specific paracrine stimulation occurring remotely as a result of mechanical resonance strained liver parenchyma. A humoral stimulus targeted on the bone marrow reproductive system specifically activates it, recruiting trophic/morphogenic lymphoid bone mar-

row stem cells for targeted maintenance of the function of the damaged liver [35]. Signals can be either easily soluble molecular substances in plasma, or circulating extracellular vesicles measuring tenths of microns, recently discovered [36]. In any case, mechanical vibration is likely to simulate liver injury perceived as real by the physiological "lymphopoiesis – liver" axis effector.

Several liver cell types can be targets for such interaction with CD133+AFP+ lymphocytes. Among the parenchymal cells of the organ, there is a CD133+ oval cell population with the function of primitive, "bipotent" liver stem cells [37, 38], but they do not carry the AFP marker [39]. That is why the youngest type of hepatic stem cells (HepSCs) are hardly a target for AFP+CD133+ lymphocytes. Immature "unipotent" hepatoblasts arising during regenerative processes in the liver, have an antigenic profile with strongly positive expression of both CD133 and the liver-specific AFP marker [40, 41]. Thus, they are probably more likely to be considered as targets for CD133+AFP+ migrants [5].

This agreement may be an additional argument backing the fact that hepatocytes are the most likely inducer of humoral stimuli, as well as a target for committed lymphoid cells. Notably, the second course of exposure to the liver area (scheme 2) did not alter the attenuation effect of the first course in the CD133+AFP+ subpopulation. The reasons for this are not completely clear, but the general inhibition of lymphocytopoiesis in cirrhotic patients, and the associated instability/turbulence of hematopoiesis [5, 20], may be one of the possible causes. On the other hand, the initially depleted/amortized natural ability of the cirrhotic parenchyma to produce paracrine signals may also be responsible for altered sensitivity to mechanical stress as compared to an intact organ.

CONCLUSION

The study confirms that the number of trophic lymphoid stem cells committed to the liver in patients with cirrhosis can be increased indirectly and non-invasively. The new technique proposed by us is specific and has no restrictions in re-use. We consider our results only as a therapeutic roadmap for non-invasive support of liver regeneration in cirrhosis in patients waitlisted for liver transplantation. Further studies are needed to evaluate the clinical effects.

The authors declare no conflict of interest.

REFERENCES

1. *Drapeau C*. Cracking the Stem Cell Code: Demystifying the most dramatic scientific breakthrough of our times. Sutton Hart Press, Hillsboro; 2010.
2. *Kucia M, Ratajczak J, Ratajczak MZ*. Bone marrow as a source of circulating CXCR4+ tissue-committed stem cells. *Biol Cell*. 2005; 97 (2): 133–146. doi: 10.1042/BC20040069.
3. *Stroo I, Stokman G, Teske GJ, Florquin S, Leemans JC*. Haematopoietic stem cell migration to the ischemic damaged kidney is not altered by manipulating the SDF-1/CXCR4-axis. *Nephrol Dial Transplant*. 2009; 24: 2082–2088. doi: 10.1093/ndt/gfp050.
4. *Kolvenbach R, Kreissig C, Cagiannos C, Afifi R, Schmaltz E*. Intraoperative adjunctive stem cell treatment in patients with critical limb ischemia using a novel point-of-care device. *Ann Vasc Surg*. 2010; 24: 367–372. doi: 10.1016/j.avsg.2009.07.018.
5. *Shoutko AN, Gerasimova OA, Ekimova LP, Zherebtsov FK, Mus VF, Granov DA*. Long-term activation of circulating liver-committed mononuclear cells after OLT. *Jacobs Journal of Regenerative Medicine*. 2016; 1 (3): 1–9. https://jacobspublishers.com/.
6. *Halin C, Mora J, Sumen C, von Andrian UH. In vivo* imaging of lymphocyte trafficking. *Ann Rev Cell Dev Biol*. 2005; 21: 581–603. doi: 10.1146/annurev.cellbio.21.122303.133159.
7. *Wassmann S, Werner N, Czech T, Nickenig G*. Improvement of endothelial function by systemic transfusion of vascular progenitor cells. *Circ Res*. 2006; 99: 74–83. doi: 10.1161/01.RES.0000246095.90247.d4.
8. *Burchfield JS, Dimmeler S*. Role of paracrine factors in stem and progenitor cell mediated cardiac repair and tissue fibrosis. *Fibrogenesis Tissue Repair*. 2008; 1: 1–11. doi: 10.1186/1755-1536-1-4.
9. *Strick-Marchand H, Masse GX, Weiss MC, Di Santo JP*. Lymphocytes support oval cell dependent liver regeneration. *J Immunol*. 2008; 181: 2764–2771. doi: 10.4049/jimmunol.181.4.2764.
10. *Roullet M, Gheith SMF, Mauger J, Junkins-Hopkins JM, Choi JK*. Percentage of {gamma} {delta} T cells in panniculitis by paraffin immunohistochemical analysis. *Am J Clin Pathol*. 2009; 13: 1820–1826.
11. *Hur J, Yang H-M, Yoon C-H, Lee C-S, Park K-W et al*. Identification of a novel role of T cells in postnatal vasculogenesis. Characterization of endothelial progenitor cell colonies. *Circulation*. 2007; 116: 1671–1682. doi: 10.1161/CIRCULATIONAHA.107.694778.
12. *Shoutko A., Shatinina N*. Chronic cancer could it be? *Coherence Int J Integrated Med*. 1998; 2: 36–40.
13. *Ding B-S, Cao Z, Lis R, Nolan DJ, Guo P, Simons M et al*. Divergent Angiocrine Signals from vascular niche balance liver regeneration and fibrosis. *Nature*. 2014; 505: 97–102. doi: 10.1038/nature12681.
14. *Woo DH, Kim SK, Lim HJ, Heo J, Park HS, Kang GY et al*. Direct and indirect contribution of human embryonic stem cell-derived hepatocyte-like cells to liver repair in mice. *Gastroenterology*. 2012; 142: 602–611. doi: 10.1053/j.gastro.2011.11.030.
15. *Ratajczak J, Kucia M, Mierzejewska K, Marlicz W, Pietrzkowski Z, Wojakowski W et al*. Paracrine proangiopoietic effects of human umbilical cord blood-derived purified CD133+ cells – implications for stem cell therapies in regenerative medicine. *Stem Cells and Dev*. 2013; 22: 422–430. doi: 10.1089/scd.2012.0268.
16. *Liu W-H, Ren L-N, Wang T, Navarro-Alvarez N, Tang L-J*. The involving roles of intrahepatic and extrahepatic stem/progenitor cells (SPCs) to liver regeneration. *Int J Biol Sci*. 2016; 12: 954–963. doi: 10.7150/ijbs.15715.
17. *Tsolaki E, Athanasiou E, Gounari E, Zogas N, Siotou E, Yiangou M et al*. Hematopoietic stem cells and liver re-

generation: differentially acting hematopoietic stem cell mobilization agents reverse induced chronic liver injury. *Blood Cells Mol Dis*. 2014; 53: 124–132. doi: 10.1016/j.bcmd.2014.05.003.

18. *Mohamadnejad M, Vosough M, Moossavi S, Nikfam S, Mardpour S, Akhlaghpoor S et al.* Intraportal infusion of bone marrow mononuclear or CD133+ cells in patients with decompensated cirrhosis: a double-blind randomized controlled trial. *Stem Cells Transl Med*. 2016; 5 (1): 87–94, doi: 10.5966/sctm.2015-0004
19. *Lemoli RM, Catani L, Talarico S, Loggi E, Gramenzi A, Baccarani U et al.* Mobilization of bone marrow-derived hematopoietic and endothelial stem cells after orthotopic liver transplantation and liver resection. *Stem Cells*. 2007; 24: 2817–2825. doi: 10.1634/stemcells.2006-0333.
20. *Shoutko AN, Gerasimova OA, Ekimova LP, Zherebtsov FK, Mus VF, Matyurin KS et al.* Lymphocyte reproductive activity normalized to numbers of hematopoietic stem cells in blood and rate of death in fatal diseases. *Int J of Genetics and Genomics*. 2017; 5: 54–62. doi: 10.11648/j.ijgg.20170505.12.
21. *Heinig K, Sage F, Robin C. Sperandio M.* Development and trafficking function of haematopoietic stem cells and myeloid cells during fetal ontogeny. *Cardiovasc Res*. 2015; 107 (3): 352–363. doi: 10.1093/cvr/cvv146.
22. *Karamullin M, Baback A, Ekimova L, Phedorov V, Kireeva E, Sosukin A et al.* The blood stem cell's pool modulation in remote period improved health status of Chernobyl clean-up workers. *Radioprotection*. 2008; 43: 89. doi: 10.1051/radiopro:2008667.
23. *Baback A, Karamullin M, Ekimova L, Phedorov V, Kireeva E, Sosukin A et al.* Exercise performance vs. growth of haemopoietic stem cells amount in CUWs blood after nonivasive modulation. *Radioprotection*. 2008; 43: 161. doi: 10.1051/radiopro:2008724.
24. *Shutko AN, Fedorov VA.* Sposob obogashcheniya krovi stvolovymi krovetvornymi kletkami. Patent RF № 2166924, 20.07.2008. http://ru-patent.info/21/65-69/2166924.html.
25. *Eaker ShS, Hawley TS, Ramezani A, Hawley RG.* Detection and enrichment of hematopoietic stem cells by side population phenotype. *Hawley TS and Hawley RG, Eds., Methods in molecular biology: flow cytometry protocols*, 2nd Edition, Humana Press Inc., Totowa, 2008: 161–180. doi: 10.1385/1-59259-773-4:161.
26. *Sales-Pardo I, Avendaño A, Martinez-Muñoz V, García-Escarp M, Celis R, Whittle P et al.* Flow cytometry of the side population: tips and tricks. *Cellular Oncology*. 2006; 28: 37–53. https://www.ncbi.nlm.nih.gov/pubmed/16675880.
27. *Loveland JL.* Mathematical justification of introductory hypothesis tests and development of reference materials. All graduate plan B and other reports. Utah State University, Logan, 14. 2013.
28. *Oldenburg AL, Boppart SA.* Resonant acoustic spectroscopy of soft tissues using embedded magnetomotive nanotransducers and optical coherence tomography. *Physics in Medicine & Biology*. 2010; 55: 1189–1201. doi: 10.1088/0031-9155/55/4/019.
29. *Sharma A, Maurya AK.* Aggregate frequencies of body organs. *Int J of Electrical, Electronics and Data Communication*. 2017; 5: 94–98. http://iraj.in.
30. *Arredondo LT, Perez CA.* Spatially coincident vibrotactile noise improves subthreshold stimulus Detection. *PLoS One*. 2017; 12 (11): e0186932. doi: 10.1371/journal.pone.0186932.
31. *Milanese C, Cavedon V, Sandri M, Tam E, Piscitelli F, Boschi et al.* Metabolic effect of bodyweight whole-body vibration in a 20-min exercise session: a crossover study using verified vibration stimulus. *PLoS One*. 2018. 31; 13 (1): e0192046. doi: 10.1371/journal.pone.0192046.
32. *Richardson RB, Allan DS, Lea Y.* Greater organ involution in highly proliferative tissues associated with the early onset and acceleration of ageing in humans. *Experimental Gerontology*. 2014; 55: 80–91. doi: 10.1016/j.exger.2014.03.015.
33. *Agiasotelli D, Alexopoulou A, Vasilieva L, Kalpakou G, Papadaki S, Dourakis SP.* Evaluation of neutrophil/leukocyte ratio and organ failure score as predictors of reversibility and survival following an acute-on-chronic liver failure event. *Hepatology Research*. 2016; 46: 514–520. doi: 10.1111/hepr.12582.
34. *Ashman LK, Aylett GW.* Expression of CD31 epitopes on human lymphocytes: CD31 monoclonal antibodies differentiate between naïve (CD45RA+) and memory (CD-45RA–) CD4-positive T cells. *Tissue Antigens*. 1991; 38: 208–212. doi: 10.1111/j.1399-0039.1991.tb01899.x.
35. *Huch M, Dolle L.* The plastic cellular states of liver cells: are EpCAM and Lgr5 fit for purpose? *Hepatology*. 2016; 64: 652–662. doi: 10.1002/hep.28469.
36. *Teixeira JH, Silva AM, Almeida MI, Barbosa MA, Santos SG.* Circulating extracellular vesicles: their role in tissue repair and regeneration. *Transfus Apher Sci*. 2016; 55 (1): 53–61. doi: 10.1016/j.transci.2016.07.015.
37. *Rountree CB, Barsky L, Ge SH, Zhu J, Senadheera SH, Crooks GM.* A CD133-expressing murine liver oval cell population with bilineage potential. *Stem Cells*. 2007; 25: 2419–2429. doi: 10.1634/stemcells.2007-0176.
38. *Corbeila D, Fargeasa CA, Jászaib J.* CD133 might be a pan marker of epithelial cells with dedifferentiation capacity. *PNAS*. 2014; 111: E1451–E1452. doi: 10.1073/pnas.1400195111.
39. *Turner R, Lozoya O, Wang Y, Cardinale V, Gaudio E, Alpini G et al.* Human hepatic stem cell and maturation liver lineage biology. *Hepatology*. 2011; 53: 1035–1045. doi: 10.1002/hep.24157.
40. *Haruna Y, Saito K, Spaulding S, Nalesnik MA, Gerber MA.* Identification of bipotential progenitor cells in human liver development. *Hepatology*. 1996; 23: 476–481. doi: 10.1002/hep.510230312.
41. *Liang OD, Korff T, Eckhardt J, Rifaat J, Baal N, Herr FT et al.* Oncodevelopmental alphafetoprotein acts as a selective proangiogenic factor on endothelial cell from the fetomaternal unit. *J of Clin Endocrinology & Metabolism*. 2004; 89: 1415–1422. doi: 10.1210/jc.2003-031721.

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