Journal of Positive Psychology & Wellbeing http://journalppw.com
2022, Vol. 6, No. 1, 2059 – 2064 ISSN 2587-0130

FEATURES OF THE BONE MARROW

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ABSTRACT

The use of bone marrow for transplantation is used all over the world to help patients who were previously doomed to die. The possibilities of the bone marrow have been studied by less than 50%. The new functions of the bone marrow have yet to be learned and studied, and most importantly, to unlock the full potential of bone marrow cells. In the last decade, cell therapy has been actively developing and being introduced into clinical practice. In this regard, the bone marrow is becoming increasingly important as the main "niche" of stem cells. For normal life and performance of basic functions, which include self-renewal and differentiation, stem cells (SC) need a microenvironment with constant parameters and properties. Among the tissue in which stem cells are located, the presence of various types of auxiliary cells is necessary, only when these conditions are present, stem cells can function. The accumulated data made it possible to create a hypothesis about the existence of two different stem cell niches in the bone marrow: mesenchymal and hematopoietic. To maintain cells in an undifferentiated state, it is necessary to maintain the constancy of the microenvironment, which includes the extracellular matrix, a certain pH, the concentration of electrolytes, certain cellular elements. The constancy of homeostasis creates optimal conditions for stem cells and excludes early differentiation.

Key words: limb amputation, bone marrow, autogenous cell therapy, stem cells, cell transplantation, therapeutic strategy

1. INTRODUCTION:

Cellular therapy is becoming increasingly important in medicine. It is based on bone marrow functions. The study of bone marrow is insufficient. We can use stem cells as efficiently as possible when we study the bone marrow completely. Bone marrow is a special tissue that is just discovering its potential. It is necessary to study the bone marrow not only from the extraction site, and these may be various bone marrow cavities of flat and tubular bones, but also in liquid media with which the bone marrow comes into contact and where bone marrow derivatives migrate (Ding L et al., 2013; Greenbaum et al., 2013). The functions of the bone marrow are diverse, it is possible to determine them, and, consequently, to get to know the bone marrow closer by comparing the composition of the media that come into contact with the bone marrow. First of all, blood, which not only connects all organs and systems, but also transports cellular elements and compounds from the bone marrow (2017; Николаева et al., 2015).

Purpose of research: to conduct a comparative characterization of the bone marrow of flat and tubular bones. To evaluate the biochemical parameters of bone marrow and blood.

2. MATERIALS AND METHODS

2.1 Patient characteristics

The study included 40 patients who underwent lower limb amputation after signing an informed consent. Indications for amputation were: gangrene of the lower extremity (type 2 diabetes mellitus with the development of mixed diabetic foot syndrome - 39 patients) and frostbite (1 patient). The age of patients is 50-75 years (on average 63 years), 25 patients are men (62.5%), 15 are women (37.5%). It should be borne in mind that flat bone marrow punctate was taken from patients of the hematology department, where patients were examined with suspected cancer, if this disease was excluded, the result was taken for research. The puncture was performed once, when it was necessary to study the myelograms and at the same time part of the punctate was taken for research. Sternal puncture was not performed in patients who underwent amputation due to the threat of deterioration of the patient's condition and ethical standards. The blood counts of patients who underwent amputation were obtained from the medical history during the period of normalization of the condition.

2.2 Obtaining bone marrow samples

The studied bone marrow samples were obtained in the operating room, immediately after amputation of the lower limb. The bone marrow was removed from the lumen of the femur with a Volkmann spoon into a sterile test tube and transported to the laboratory. The level of amputation in the present study was determined individually. The volume of the extracted bone marrow depended on the level of amputation (upper, lower or middle third of the thigh) and was equal to 10-100 ml. The largest amount of bone marrow (up to 100 ml) was obtained during limb amputation at the level of the upper third of the thigh. The number of cells in the bone marrow of the femur depended on the volume of the obtained bone marrow and ranged from about 500 thousand to 7 million cells per sample. To destroy cell conglomerates and transfer cells to a suspension state, the bone marrow was gently homogenized with the addition of phosphate buffered saline. Removal of adipose tissue was carried out after 10 minutes. sedimentation of the sample, while the bone marrow fat rises to the upper layer. Without affecting the upper layer, the lower phase of nucleated bone marrow cells was taken into a new tube and washed twice in phosphate buffer, followed by centrifugation at 400g for 5 min. The cell pellet was resuspended in 300 µl of phosphate buffer; their number was counted in a Goryaev chamber. The cell suspension was diluted with phosphate buffer to a concentration of 1 × 107 cells / ml. Quantification of hematopoietic stem cells (HSC) and multipotent mesenchymal stromal cells (MMSC) in bone marrow samples. The absolute amount of HSC and MMSC in bone marrow samples was calculated using the formula: number of cells = cell concentration * sample volume. BD Trucount ™ Tubes were used to determine the concentration of HSC and MMSC in the bone marrow, which contain a known concentration of microparticles released into a separate area by forward and lateral light scattering on a flow cytometer. The concentration of cells in the sample was determined from the ratio of microparticles to cells. Determination of electrolytes and biochemical parameters of the bone marrow was determined on a hospital analyzer. The use of a hospital analyzer was a high risk due to the possibility of being taken out of operation [7,8]. Removal of conglomerates of cells and adipose tissue was performed by allowing the sample to stand for 10 minutes, while the bone marrow fat rises to the upper layer. Without affecting the upper layer, the lower phase containing the nuclear cells of the bone marrow was taken into a new test tube. Additionally, to exclude damage to the analyzer, the bone marrow was passed twice through four layers of gauze. The ion content was determined on a blood acid-base and gas composition analyzer (Radiometer Medical Aps, Akandevej 21 DK-2700 Bronshoj, Denmark).

2.3 Statistical analysis

Statistics are presented as absolute values, percentages and arithmetic means with standard deviation. Spearman's correlation coefficient was used to determine the presence of links between accounting signs

3. RESULTS AND DISCUSSION:

Table 1. *Indicators of cellular composition and biochemical data of bone marrow and blood.*

Indicators	**Observation groups**		**p**
	Femoral bone marrow (n = 40)	**Sternal puncture (n=40)**	
	1	**2**	
Age	59,37±8,63	62,13±7,47	p=0,373
GSK	0,040 (0,030; 0,060)	0,007(0,006; 0,008)	p<0,001
MSC	0,08 (0,05; 0,51)	0,0 (0,00; 0,00)	p<0,001
pH	6,88±0,13	7,43±0,14	p<0,001
pCO2	14,88±2,77	23,00±8,36	p<0,001
pO2	113,86±26,68	104,94±15,31	p=0,003
K	4,10±0,78	4,99±0,43	p<0,001
Na	130,54±27,37	137,87±2,4	p=0,027
Ca2	1,37±0,46	0,99±0,081	p<0,001
CL	131,22±15,35	105,57±1,75	p<0,001
cHCO3P	2,80±1,22	22,98±0,39	p<0,001
SBE	0,0 (0,0; 0,0)	3,1(2,8; 3,2)	p<0,001
Urea	0,20 (0,00; 0,30)	7,9(7,2; 8,3)	p<0,001
Creatinine	3,0 (2,0; 7,0)	104,0 (97,0; 109,0)	p<0,001
ALT	21,0(0,7; 67,6)	54,4(51,0; 59,0)	p<0,001
AST	10,3(0,9; 20,3)	65,0(45,0; 92,0)	p<0,001
Alkaline phosphatase	24,8(9,2; 34,7)	285,0(262,0; 316,0)	p<0,001
GLUC	0,02(0,00; 0,05)	5,40(5,2; 5,6)	p<0,001
Cholesterol	0,0(0,0; 0,04)	2,9 (2,7; 3,1)	p<0,001
Triglycerides	0,05(0,00; 0,07)	1,70(1,6; 1,8)	p<0,001

1. Analysis of the data obtained shows [Table 1] that in the flat bones are found mainly hematopoietic stem cells (HSC), and in the tubular bones, both HSC and mesenchymal stem cells (MSC). Mesenchymal stem cells are found in greater numbers than hematopoietic ones.

2. The percentage of MMSC in the bone marrow of the femur was 0.08%, HSC 0.04%. The biological value of the femur bone marrow is significantly higher than from other sources.

3. Biochemical composition and acid-base indices of sternal puncture practically coincide with those of blood.

4. The pH of the femoral bone marrow is 6.88 ± 0.13, where mesenchymal and hematopoietic stem cells are found, and the pH of sternal puncture is 7.43 ± 0.014, where only hematopoietic stem cells are predominantly found. In the blood, the pH is 7.35-7.45. We can make an unambiguous conclusion that the pH of sternal puncture and blood are the same.

5. Indicators pCO2 and pO2, which affect the acid-base state, in the bone marrow of the femur are 14.88 ± 2.77 and 113, 86 ± 26.68, respectively. These data are outside the blood norm indicators. In the bone marrow, sternal puncture pCO2 and PO2 have numbers 23.00 ± 8.36 and 104.94 ± 15.31, respectively, which is within the range of normal blood fluctuations.

6. Data on electrolytes are ambiguous: K and Na in all three samples coincide with blood values (fluctuations within normal limits). But Ca2 ions in the bone marrow of the femur are slightly higher than in the blood and equal to 1.37 ± 0.46, while in sternal puncture, on the contrary, it is lower and has a value of 0.99 ± 0.081. These data affect the functional state of cells and are maintained at a certain level in all samples. Without the presence of this element, the blood coagulation process is disrupted, the elasticity of the vessels is lost and their permeability increases. The higher the metabolism of an organ and the faster biochemical processes occur in it, the more calcium the tissue will need.

7. The indicator of calcium in the bone marrow of the femur is higher than in the sternal puncture and blood, it can be assumed that metabolic processes in the bone marrow of the femur are more accelerated.

8. Higher than blood data on CL - which in the bone marrow of the femur is 131.22 ± 15.35, but in sternal puncture this indicator is 105.57 ± 1.75 as in blood. Cl assists in maintaining acid-base balance. The main part of Cl anions is concentrated in the intercellular space, in cells their content is several times less. To establish the water-salt balance, chlorine "monitors" that the volume of the available liquid has constant indicators. In the bone marrow of the femur, there is slightly more chlorine than in the blood and bone marrow of the sternal bone, it can be assumed that the load to maintain the acid-base balance in the femur is greater and the requirements for the constancy of pH are higher.

9. Urea and creatinine in sternal puncture 7.9 and 104.0, respectively, as in the range of blood counts. In the bone marrow of the femur, urea is 0.20 and creatinine is 3.00, which is significantly lower than the parameters of blood and bone marrow obtained with sternal function. Urea is formed by the breakdown of proteins into amino acids. Creatinine is a breakdown product that is formed in muscle tissue, after being broken down into its constituent parts of creatine. Creatine is part of the energy metabolism that the body uses to contract muscles. Most often, these indicators are associated with protein metabolism and a high metabolic rate.

10. The level of alkaline phosphatase in the bone tissue is 24.8, and in the sternal punctate 285.0, which is significantly higher than in the marrow tissue of the femur. And in the blood. Alkaline phosphatase is a hydrolase enzyme that cleaves phosphate from many types of molecules, such as nucleotides, proteins, and alkaloids. The enzyme is most active in an alkaline environment. Alkaline phosphatase is relatively resistant to inactivation, denaturation and degradation. Perhaps one of the functions of phosphatase is the cleavage of phosphates from organic molecules, since many phosphorylated compounds cannot penetrate the plasma membrane. Alkaline phosphatase is an enzyme belonging to the subgroup of hydrolases (cleaves bonds with the participation of a water molecule). Its main function is to carry out a dephosphorylation reaction at the molecular level, in which phosphate is cleaved from organic substances. Normally, alkaline phosphatase has a constant activity, participating in the transfer of phosphorus across the cell membrane. The enzyme is a marker of the course of phosphorus-calcium metabolism. The level of alkaline phosphatase in the bone marrow of the femur is significantly lower than in the sternal function and in the blood. This low level indicates a decreased requirement of the bone marrow for alkaline phosphatase functions.

11. Other biochemical parameters of the femur: glucose 0.02; cholesterol 00; triglycerides of 0.05, much lower than in sternal puncture and blood, where they coincide.

4. CONCLUSIONS:

Research data show that during bone marrow transplantation, it is necessary to take into account the indicators of the "niche" of the bone marrow and, accordingly, the indicators of the environment into which the bone marrow cells will be (Chee et al., 2015; Papadea et al., 2002). If these parameters do not coincide, bone marrow cells can differentiate chaotically and a therapeutic effect may not be obtained (Klimczak et al., 2016; García-Prat et al., 2017; Gattazzo et al., 2014). When cultivating cell cultures to obtain cells of the desired differentiation, it is necessary to take into account the obtained biochemical parameters to create optimal conditions for the vital activity of cells (Stzepourginski et al., 2017; Pires et al., 2016).

The presence of HSCs and MMSCs in the bone marrow of tubular bones makes it possible to use them in autogenous cell therapy of a patient. (Rumman et al., 2015; Visvader et al., 2016). Cell therapy is becoming a reality in surgical practice. The need to use the bone marrow obtained during amputation is an objective reality and it is unacceptable to lose this opportunity.

Bone marrow from an amputated limb is a unique opportunity to replenish the bone marrow bank.

The study of bone marrow requires more in-depth study, and perhaps new data will change our understanding of the functions and role of bone marrow in the regeneration of organs and systems.

5. ETHICS:

To conduct this study, the permission of the ethics committee was obtained (protocol No. 56/2014).

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