Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 1775 - 1780
Received 20 January 2021; Accepted 08 February 2021.

Tubular Bone Marrow Electrolytes

Nikolaeva Lyudmila Petrovna[1*]
[1*]PhD in Medicine, Krasnoyarsk, Russia. E-mail: lpnikolaeva@yandex.ru

ABSTRACT

Determination of bone marrow ions is of certain difficulty because of the reliability of the obtained results. But under strict conditions it is possible. Bone marrow ion structure has not practically been studied by anyone. This is due to the fact that, for research, bone marrow is usually obtained by puncture of the iliac crest or puncture of the sternum. As a rule, during this procedure a quantity of blood that distorts the indicators gets to punctate. Recently cell therapy associated with the therapeutic effect of stem cells has been actively studied and introduced into medical practice. One of the sources of stem cells is bone marrow. The content of ions in biological tissue, their constant quantitative structure, provide a certain functional state of cells, therefore, the study of bone marrow electrolytes is important. In this work, for the first time, quantitative indicators of bone marrow ions are determined, a comparative analysis of ions is carried out depending on the place of receipt, electrolyte norms for tubular bone marrow are determined. Quantitative indicators of bone marrow electrolytes of the tubular bones were established: K 4.1 ± 0.12 mmol / l; Na 130.5 ± 4.3 mmol / L; Ca 1.37 ± 0.07 mmol / l; Cl 131 ± 2.4 mmol / l. Studying electrolytes and conditions surrounding the stem cells in the bone marrow it is possible to create a medium for the cultivation of stem cells as close as possible to natural ones.

KEYWORDS

Ionic Structure, Bone Marrow, Sternal Puncture of the Brain, Ions, Bone Marrow Stem Cells.

Introduction

Bone marrow transplantation and cell therapy are inextricably connected with the production and cultivation of stem cells. Studying stem cells microenvironment gives us information that allows to cultivate stem cells in an artificial environment. The bone marrow has its own special niche, it is located inside the bone cavities, both tubular and flat bones. Immature stem cells are located in the area of the endosteum of large bones, which are comparable in physiological effect to embryonic cells. Stem cells are found in various tissues and organs, but the most significant stem cells are found in large number in the bone marrow. Bone marrow stem cells are the most undifferentiated cells that are ready to be converted into cells necessary for the body. These cells can produce various hematopoietic lines. It is from stem cells that red blood cells, white blood cells, platelets and other cellular elements arise.In the early stages of embryo development, bone marrow already arise, blood vessels and sinusoids are formed almost simultaneously. Blood vessels and foci of haematopoiesis develop almost simultaneously. From this period bone marrow already controls blood formation and hematopoietic foci arise in it. Bone marrow volume and mass increase rapidly, especially in the area of the epiphyses. In the tubular and flat bones, especially in large ones, bone marrow cavities are formed. Already in the early stages of development, red bone marrow fills in the bone marrow space. Before birth, the baby does not have yellow bone marrow, which appears in the first months of life. As the body grows, yellow bone marrow mass increases, which is localized mainly in the diaphysis of the tubular bones. The older the person, the more yellow bone marrow he has that displaces the red bone marrow.

Percentage of red and yellow bone marrow changes periodically during different periods of life. Yellow bone marrow develops in greater amount at an older age. Views on the bone marrow of the tubular bones, which is called yellow due to the high content of adipose tissue, have changed. It is already obvious that this bone marrow has a valuable cellular composition and plays an important role in the process of haematopoiesis. The presence of a large amount of adipose tissue can be explained by the necessity of the energy reserve and the feature of bone marrow cells of the tubular bones functioning. High mobility of this bone marrow, that is, the ability to perform the function of replenishing haematopoiesis when losing a large amount of blood, shows a wide range of functional capabilities of the yellow bone marrow.

Study of the ionic composition of the bone marrow is of particular interest, since it is of a certain difficulty. This is due to the fact that, for the study, red bone marrow is usually obtained by puncture of the iliac crest or sternal puncture. As a rule, during this procedure a certain amount of blood enters the sample, which distorts the ion composition. To get intravital yellow bone marrow is impossible, only in the case of the necessary amputation of the lower limb, when it is not possible to save it, from the amputated limb, namely, from the femur, it is possible to get the yellow bone marrow. In recent years, cell therapy, which is connected with the therapeutic effect of stem cells,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 1775 - 1780
Received 20 January 2021; Accepted 08 February 2021.

has been actively studied and introduced into medical practice. One of the sources of stem cell is bone marrow. The study of the bone marrow microenvironment is a very important factor in assessing the function of haematopoiesis. The content of ions in biological tissue, their constant quantitative composition, provide a certain functional state of stem cells, therefore, the study of bone marrow electrolytes is relevant. The constancy of the electrolyte composition is important for assessing the pathological condition in disturbance of electrolyte metabolism. Quantitative indicators of electrolytes in the blood may not coincide with the indicators of electrolytes in the body. This is due to the higher content of intracellular fluid than extracellular and the constant movement of electrolytes through the cell membrane. Different magnesium and calcium concentrations are not the same in cells and intercellular fluid, especially it concerns calcium, which is much more prevalent in bone tissue. The constant potassium content inside the cell is provided by intracellular proteins with a negative charge on the surface, which are constantly inside the cell, but amino acids formed in the process of proteolysis pass through the cell membrane. In the case of the "sick cell" syndrome, the sodium content inside and outside the cell is sharply disturbed, inside the cell sodium content is much more than in a normally functioning cell, this is found in severe diseases.

Liquid introduced to the patient is distributed depending on the content of electrolytes. Saline solution enters the extracellular fluid due to the osmotic pressure produces by sodium. Saline enters the plasma (25%) and extracellular space (75%). Sodium provides the osmotic pressure of the extracellular space, and potassium controls negatively charged proteins inside the cell. The constancy of microelements and their distribution is controlled by a sodium-potassium pump. The physiological balance of cations and anions makes the cell electro-neutral.

There are many works evaluating the capabilities of stem cells, both hematopoietic and mesenchymal stem cells derived from bone marrow [1]. In all cases, it refers to the bone marrow of the flat bones. The bone marrow is found not only in the flat but also in the tubular bones. Studying the bone marrow of the tubular bones will supplement the information on stem cells of the bone marrow and will provide an opportunity to reinterpret the niches of stem cells and their differentiation [2,3]. The study of bone marrow of the tubular bones obtained in the operating room makes it possible to exclude blood from getting into the resulting bone marrow. The data obtained in such a sample can be considered not distorted. In the surgical department, situations arise when it is not possible to save the lower limb, and lower limb is amputated up to the hip. From the amputated lower limb, namely from the femur, bone marrow can be obtained. The obtained bone marrow is predominantly yellow. There are practically no studies of the yellow bone marrow. The study of the ionic composition of the bone marrow of the tubular and flat bones is of scientific interest. Biological processes are entirely determined by the zone of electrolyte location [4,5]. The study of bone marrow electrolytes is of great importance, because data on the rate of electrolytes and changes in the ionic composition of the bone marrow in various conditions have not been studied. Considering new ideas about the functions of the bone marrow, and mainly the fact that bone marrow is a niche of stem cells, the study of ionic composition is of great importance [6,7]. Everything related to the bone marrow, even a minor detail, can be important. Now, medicine is actively introducing cell therapy into clinical practice. Studying the bone marrow and its ionic balance will make it possible to influence the stem cell. To change the functional state of the cell, including the stem cell, means to learn how to manage it.

Purpose of the study: 1. Determination of the ionic composition of the bone marrow of the tubular and flat bones, to conduct a comparative description of the quantitative composition of bone marrow ions, depending on the place of extraction.

2. The possibility of using acid-base and gas composition of the blood (Radiometer Medical Aps, Akandevej 21 DK-2700 Bronshoj, Denmark) analyser to determine indicators of bone marrow electrolytes.

Materials and Methods

To perform the study, permission was obtained from the ethics committee of the State budgetary educational institution Krasnoyarsky State Medical University Protocol No. 56/2014Characteristics of the patients. Bone marrow samples were studied in 30 patients after the informed consent. The age of the patients was from 50 to 75 years, mean age 63 years, 18 patients were men, 12 - women. Most patients (96%) had type 2 diabetes mellitus with the development of FDS of the mixed type. As a result of foot gangrene, lower limb was amputated in all patients. Bone marrow was taken from the femur into a sterile tube and transported to a laboratory.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 1775 - 1780
Received 20 January 2021; Accepted 08 February 2021.

Bone marrow from flat bones was obtained by sternal puncture, which was performed in haematological patients and in patients with a diagnostic purpose. Data on the ionic composition in haematological patients and in patients in whom the diagnosis was not confirmed were similar. The results of the ionic composition of the bone marrow of patients in whom the diagnosis of haematological disease was excluded were taken for the study. Before the puncture of the sternum, the patient was carefully examined. Conducted a study of Blood coagulation and general tests were performed a few days before the sternal puncture. Medicines were limited, all drugs were excluded, except for those vital for the patient. On the day of the manipulation, all other procedures were excluded. Local anaesthetics (lidocaine, novocaine) were used, since the sternal puncture procedure is quite painful, especially at the time of needling. Puncture was performed along the midline at the junction of the third or fourth rib with the sternum. When the needle pierces the sternum (feeling of failure) and enters the medullary canal, using the attached syringe, vacuum was created and the bone marrow was quickly aspirated in a volume of 0.5-1 ml. The extracted from the sternum cavity bone marrow was immediately sent to the laboratory.

Tubular bone marrow was obtained by amputation of the lower limb, usually at the level of the femur (upper or lower third of the thigh). Amputation of the lower extremity was performed according to the vital indications, the great majority of patients had type II diabetes mellitus. Bone marrow sampling during limb amputation was performed immediately in the operating room to exclude bone marrow infection. To confirm the sterility of the bone marrow, the obtained material was sent for inoculation. In all cases, inoculate showed the absence of microflora. Depending on the level of amputation 5–50 ml of bone marrow was extracted from the femoral cavity using Volkman's spoon. Before bone marrow extraction, the edges of the femur were carefully processed to exclude small bone fragments from entering the tube. Then the edge of the femur was isolated from the surrounding tissues so that blood from the surgical wound did not enter the test sample. The obtained bone marrow was sent for investigation.

The state of the bone marrow was of interest in regards of tissue density. In some cases, the bone marrow was in the form of a liquid yellowish mass, in others it is dense jelly. Different densities were most likely associated with different functional states of the bone marrow. The obtained bone marrow samples sometimes have weak pinkish areas, foci of haematopoiesis, but in most cases it was a homogeneous yellow tissue. In the area the of the tubular bones epiphyses, where flat tissue predominates, the bone marrow is red. It can be concluded that different parts of the femur are responsible for certain hematopoietic functions. The study of this feature is of interest for a more complete understanding of the haematopoiesis process.

The use of a hospital analyser was a great risk due to the possibility of withdrawal from the operating mode [8]. Conglomerates of cells and adipose tissue were removed by 10-minute sedimentation of the sample, the bone marrow fat rose to the upper layer. The lower phase containing the bone marrow nuclear cells was selected into a new tube; the upper layer was not affected. To prevent damage to the analyser, bone marrow was additionally passed twice through four layers of gauze. The ion content was determined on an analyser of acid-base and gas composition of the blood (Radiometer Medical Aps, kandevej 21 DK-2700 Bronshoj, Denmark).

Statistical Processing

Statistical data processing was performed using the IBM SPSS Statistics v.19 statistical package. To describe the quantitative data, the arithmetic mean (M), the arithmetic mean error (m), and the minimum and maximum values were calculated. For visual presentation of the data, the Box plot diagram was used, which shows the arithmetic mean, standard deviation, and the minimum and maximum values of the analysed parameters.

Results

When studying the ionic composition of sternal puncture, the data ranged within the blood parameters: K 3.5 - 4.3 mmol / L; Na 126 - 146 mmol / L; Ca^{2+} 1.15-1.2 mmol / L; Cl 98 - 107 mmol / L. pH and gas composition of sternal puncture were identical to those of blood: pH 7.35 - 7.45 mg / L; pCO_2 32 - 78 mg / L; pO_2 83 - 108 mg / L. [9]. The ionic composition of bone marrow obtained from the tubular bone was quite different. Potassium content in the bone marrow of the tubular bones was 4.1 ± 0.12. Potassium content in the bone marrow of the tubular bones approached the upper limit of the normal potassium content in the blood, 4.3 mmol / L. In sternal puncture, the electrolyte content corresponds to the electrolyte content in the blood, the data of quantitative indicators of electrolytes can be distorted due to blood clots in the test sample. In the bone marrow of the tubular bones a foreign

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 1775 - 1780
Received 20 January 2021; Accepted 08 February 2021.

impurity, including blood, was excluded. The bone marrow of the tubular bone was taken in the operating room from an amputated limb in compliance with all the rules of asepsis and antiseptics. Sodium content of 130.5 ± 4.3 mmol / L, also approached the lower limit of sodium content in the blood and sternal puncture, 126 mmol / L [8]. As to calcium and chlorine the situation was different. Mean calcium content in the bone marrow of the tubular bones was 1.37 ± 0.07 mmol / L, approximately 16% higher than normal comparing with blood content of 1.15 - 1.2 mmol / L. Chlorine content in the bone marrow was 131.2 ± 2.4 mmol / L, 27% higher than chlorine content in the blood and bone marrow punctate of flat bones (sternum), 98-107 mmol / L. K and Ca content in the bone marrow of the tubular bones was comparable with the upper limit of norm of K and Ca in blood.

Higher need of bone marrow of tubular bones in electrolytes, and especially calcium and chlorine ions, is explained by their function in cells and tissues. Potassium provides maintaining of water balance in an optimal level, has an antihypoxic effect, and removes toxins. High level of potassium in the bone marrow of tubular bones shows high functional activity of bone marrow cells. Calcium is an electrolyte responsible for the regulation of metabolism, ensuring the strength of bone tissue.

Electrolytes perform many functions in the body, but in the bone marrow their activity at the cellular level is more specific. The opinion that the bone marrow is a “depot” of stem cells is not entirely reliable. In the bone marrow there is a dynamic activity of cells and the consumption of electrolytes is rather high, the content of which has a certain constant range. Osteogenic cells are another type of bone marrow cell. They are also capable of producing growth factors and are involved in the differentiation of hematopoietic cells. Stem cells differentiating into hematopoietic cells are located on the bone tissue of the endostoma the cells of which are located on the inner surface of the tubular bones [10,11]. The number of stem cells is the greatest on the endosteum, their concentration is three times higher comparing with the centre of the bone marrow cavity.

Bone marrow tissue contains a large number of fat cells (adipocytes). The large surface of sinusoidal vessels has adventitious cells, which can contract under the influence of haematopoietins and other cytokines. Their activity promotes penetration of cells into the bloodstream. Another element of the bone marrow is endothelial cells. They take part in the development of stroma, blood cells, as well as in the synthesis of collagen and haematopoietins. Endothelial cells that are in sinusoids interact with stromal and haematopoietic cells (this is due to the special structure of the basement membrane). Endotheliocytes push blood cells into the capillaries and support the flow of new haematopoietic cells. Endothelial cells produce fibronectin, which ensures the convergence of cells and production of CSF. Ionic composition, forming the microenvironment of these important bone marrow structures, can affect the functional activity of these cells and bone marrow in general.

Discussion

The data obtained show that in order to ensure the viability of stem cells, a specific medium with a constant composition of ions is necessary. Electrolytes influence acid-base indicator, which is the main factor for stem cells differentiation. The acid-base balance ranged from 6.7 to 6.9 [12,13,14]. If pH of the bone marrow differs in the flat and tubular bones, it is necessary to find other differences to which a certain pH value leads to. This is primarily a significant predominance in the flat bones of haematopoietic stem cells, where pH varies within the blood parameters. But there are also mesenchymal and haematopoietic cells in the tubular bones. If hematopoietic cells of the tubular and flat bones in the microenvironment have different pH and different quantitative composition of ions, it can be assumed that these haematopoietic cells are significantly different. Haematopoietic cells of the tubular bones are less differentiated and retain greater pluripotency and are intermediate cells, which, as the pH approaches the blood parameters, completely transfer to haematopoietic cells. Quantitative indicators of the ionic composition of the bone marrow also create optimal conditions for the functioning of stem cells. Creating the optimal environment for haematopoietic or mesenchymal stem cells it is possible to get the necessary amount of cell mass for treating a patient.

Conclusion

pH control or change can cause a shift in the cellular composition of the bone marrow in the required direction, affect the differentiation of cells and their functional state. The obtained data should be further analysed and information on the acid-base balance of the bone marrow should be received. This study revealed the following facts:

1. Bone marrow (both of tubular and flat bones) has its own constant ionic composition. The ionic composition of the bone marrow of the tubular bones has different indicators compared to the ionic composition of flat bones (sternal puncture). The ionic composition of the bone marrow of the tubular bones has potassium and sodium ions indicators similar to those of the upper limit of the blood norm, and calcium and chlorine ions are higher than in the blood, i.e. calcium ions 1.37 mmol / L, and chlorine ions 131, 2 mmol / L, which is higher than the blood content by 16% and 27%, respectively.
2. Possibility of using an analyzer of acid-base and gas composition of the blood (Radiometer Medical Aps,Akandevej 21 DK-2700 Bronshoj, Denmark) to determine the ionic composition of the bone marrow of the tubular bones.

All these data allow to make important conclusions:

- Bone marrow has its permanent ionic composition.
- Ionic composition of the flat and tubular bones is different.
- Ionic composition of flat bones and blood coincide.

The study of bone marrow continues. Determination of the ionic composition of the bone marrow of the tubular and flat bones may be important for evaluating the function of haematopoiesis. Considering development of cellular technologies, it is possible to study the microenvironment of the stem cell niche, acid-base balance, and ion composition of the bone marrow in time and relevantly.

References

[1] Manual on laboratory haematology / B. Sisla: translated from English /ed. A.I. Vorobyova. – Moscow: Practical Medicine, 2011, 352.

[2] Veldhoen, E.S., De Vooght, K.M., Slieker, M.G., Versluys, A.B., & Turner, N.M. (2014). Analysis of bloodgas, electrolytes and glucose from intraosseous samples using an i-STAT® point-of-care analyser. *Resuscitation*, *85*(3), 359-363.

[3] Paladino, L., Sinert, R., Wallace, D., Anderson, T., Yadav, K., & Zehtabchi, S. (2008). The utility of base deficit and arterial lactate in differentiating major from minor injury in trauma patients with normal vital signs. *Resuscitation*, *77*(3), 363-368.

[4] Shirakabe, A., Hata, N., Kobayashi, N., Shinada, T., Tomita, K., Tsurumi, M., & Mizuno, K. (2012). Clinical significance of acid–base balance in an emergency setting in patients with acute heart failure. *Journal of cardiology*, *60*(4), 288-294.

[5] Rudkin, S.E., Kahn, C.A., Oman, J.A., Dolich, M.O., Lotfipour, S., Lush, S., & Langdorf, M.I. (2012). Prospective correlation of arterial vs venous blood gas measurements in trauma patients. *The American journal of emergency medicine*, *30*(8), 1371-1377.

[6] Nicoll, S.J.B., &Rochester, S.J. (2008). Blood sampling through intraosseous needles: time to stop? *Resuscitation*, 79(1), 168.

[7] Vos, G., Engel, M., Ramsay, G., &Van Waardenburg, D. (2006). Point-of-care blood analyzer during the interhospital transport of critically ill children. *European Journal of Emergency Medicine*, *13*(5), 304-307.

[8] Papadea, C., Foster, J., Grant, S., Ballard, S.A., Cate, J.C., Southgate, W.M., & Purohit, D.M. (2002). Evaluation of the i-STAT portable clinical analyzer for point-of-care blood testing in the intensive care units of a university children's hospital. *Annals of Clinical & Laboratory Science*, *32*(3), 231-243.

[9] Nolan, J.P., Soar, J., Zideman, D.A., Biarent, D., Bossaert, L.L., Deakin, C., & Böttiger, B. (2010). European resuscitation council guidelines for resuscitation 2010 section 1. Executive summary. *Resuscitation*, *81*(10), 1219-1276.

[10] Nikolaeva, L.P., Cherdashev, D.V., &Khvat, N.S. (2015). Features of tubular cells bone marrows myelogram. *Modern problems of Science and education*, *4*, 378.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 1775 - 1780
Received 20 January 2021; Accepted 08 February 2021.

[11] Nikolaeva, L.P. (2015). *Features of haemopoiesis in yellow bone marrow Modern problems of Science and Education*, *6*, 301.

[12] Onodera, R., Teramukai, S., Tanaka, S., Kojima, S., Horie, T., Matoba, S., & Fukushima, M. (2011). Bone marrow mononuclear cells versus G-CSF-mobilized peripheral blood mononuclear cells for treatment of lower limb ASO: pooled analysis for long-term prognosis. *Bone marrow transplantation*, *46*(2), 278-284. https://doi.org/10.1038/bmt.2010.110

[13] Kimura, F., Kanda, J., Ishiyama, K., Yabe, T., Yoshifuji, K., Fukuda, T., & Kanda, Y. (2019). ABO blood type incompatibility lost the unfavorable impact on outcome in unrelated bone marrow transplantation. *Bone marrow transplantation*, *54*(10), 1676-1685. https://doi.org/10.1038/s41409-019-0496-2

[14] Perseghin, P., Balduzzi, A., Galimberti, S., Dassi, M., Baldini, V., Valsecchi, M.G., & Uderzo, C. (2003). Red blood cell support and alloimmunization rate against erythrocyte antigens in patients undergoing hematopoietic stem cell transplantation. *Bone marrow transplantation*, *32*(2), 231-236. https://doi.org/10.1038/sj.bmt.1704114.