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Rheological parameters as prognostic factors in blood loss

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Abstract

The aim of the work was to study the rheological status in experimental blood loss of varying severity. The experiments were carried out on out bred male rats. Depending on the severity of blood loss, the animals were divided into subgroups. In each subgroup, the erythrocyte aggregation index, erythrocyte membrane deformability index, and blood plasma viscosity were measured. As a result of statistical data processing, it was found that in case of blood loss of any severity, erythrocyte aggregation plays a leading role, followed by the blood viscosity parameter, and changes in membrane deformability are significant only in the decompensatory phase. Thus, it is once again emphasized that with blood loss, the presence of a persistent, starting from the first stage, changes in the rheological status, which mainly depends on the profile of erythrocyte aggregation. These experimental data, coupled with changes in the viscosity and membrane deformability of erythrocytes in the later stages of blood loss, lead to the idea of a careful use of the mass of erythrocytes for the treatment of critical conditions caused by blood loss. Continuation of experimental studies and translation of their findings into clinical medicine will most likely improve treatment tactics and increase its effectiveness in the treatment of patients with hemorrhagic shock.

KEYWORDS: COVID-19; shock; erythrocyte aggregation; erythrocyte deformability; plasma viscosity

Introduction

In recent years, the death rate of the world's population has exceeded the birth rate. The increase in the mortality rate of the population is primarily due to the pandemic caused by the new corona virus. Pneumonia, heart attacks, strokes, diabetic commas, tumor processes against the background of corona virus caused even more deaths than in previous years. In the leading role of the list hemorrhagic shock. Hemorrhagic shock against the background of urbanization, man-made disasters caused by the increased armament of the planet, wars, natural disasters is the cornerstone of resuscitation and critical care medicine. Despite the well-known algorithm of replacement infusion therapy, the rate of disability and mortality in this pathology remains high. This is a complex pathophysiological process with an unexplored mechanism. Against the background of microcirculatory and metabolic disorders with blood loss, hypovolemic shock is formed. In some cases, treatment with blood-substituting solutions, donor blood has a positive effect, but often gives complications associated with the irrational use and incorrect tactics of treatment with these substances.

Blood loss and massive blood transfusion pose a serious threat to the life of patients from multiple disorders caused by transfused blood components and pathophysiological damage in response to critical hypovolemia. Mortality remains extremely high. In modern therapy, acute blood loss or massive blood loss is usually defined as the loss of one volume of blood within a day, the normal blood volume is approximately 7% of ideal body weight in adults and 8-9% in children. Alternative definitions include a 50% blood volume loss within 3 hours, or a loss rate of 150 ml/min. Such definitions highlight the importance of early detection of blood loss and the need for intensive care to prevent shock and its consequences.

Blood loss is a multifactorial diagnosis that requires special attention from various areas of biomedicine. The relationship between clinicians of different specialties, diagnostic laboratories, blood banks, local blood transfusion centers against the backdrop of basic and applied research in the field of physiology and pathophysiology gives hope for a detailed study of blood loss in order to discover the mechanisms for the development of a critical condition in order to improve new treatment tactics that will provide better treatment , and as a result, will increase the survival rate and reduce the percentage of disability in the population as a whole.

Severe hypovolemic/hemorrhagic shock is associated with high mortality due to organ dysfunction and disseminated intravascular coagulation. Restoration of blood volume is initially achieved by rapid administration of saline or colloid. There are confirmed factors that the quality and composition of the infused solution, compared with how quickly its introduction began, does not make sense [1]. The use of protein and non-protein colloids instead of crystalloids for bulk replacement has been a matter of debate until recently. A meta-study on colloid use, in the American College of Surgeons Advanced Trauma Life Support Guidelines, found that the “European” and “American” models of hydration therapy remain equally valid ie. the initial infusion can begin with both crystalloid and colloid solutions.

Blood loss leads to the failure of many body systems. The fluidity of the blood, which is associated with the rheological status, with the coagulation system and with the vascular factor, is disturbed. The bulk of the literature is devoted to coagulation/anticoagulation and vascular reactions in blood loss [2,3,4]. There are no specific manuals and recommendations for streamlining the hemorheological properties of blood in terms of improving the clinical course of the disease. Blood rheology, studies in this direction until recently were not recognized as central in terms of the study of blood loss as a pathophysiological process. There is no way to calculate the probability of transition from the compensatory phase of hemorrhagic shock to non-compensatory, there is no optimized management of hemorrhagic shock, which would allow to predict various situational changes in the body systems. All this is undoubtedly important from the point of view of increasing the effectiveness of the treatment of hemorrhagic shock. With such data, the prognosis of the treatment of patients with blood loss would be better. Recent works by Georgian and foreign authors on rheological predictors of the staging of the process forced us to look at the problems differently [5,6].

The aim of the work was to study the rheological status in experimental blood loss of varying severity. The rheological status is a combination of factors that ensure the fluidity of blood – the aggregability of the erythrocyte membrane, and the deformability of the erythrocyte membrane and the viscosity of the plasma.

The authors hope that this work will initiate a new era of hemorheological research in the field of shock, where the analysis of small series of experimental data will provide biomedical specialists with meaningful and relevant conclusions.

Materials and methods

The experiments were carried out on white out bred male rats (n=12) with an average weight of 240±13 g. The study was carried out in accordance with the 3R principle [7]. For persistent anesthesia, urethane (Purum, Switzerland) was used (1 ml of 20% urethane solution per 100 g of body weight). Modeling of hemorrhagic shock of different stages was carried out by the standard method [5,8]. Animals were divided into subgroups depending on the severity of blood loss. Subgroup I included individuals with blood loss less than 8% of the animal's weight – stage I of shock was observed; subgroup II included individuals with blood loss of 1-15% of the animal's weight – stage II of shock was observed; subgroup III included individuals with blood loss of more than 15% of the animal's weight. Each subgroup contained four individuals. The control group consisted of 8 male animals. To determine an additional criterion for shock (in addition to the severity of blood loss), the animals measured blood pressure in the tail artery using an MPX5050D manometer (Motorola). According to the manufacturer's recommendation, in the case of out bred rats, the average diastolic and systolic pressure was measured. No pharmacological preparations were administered to the experimental animals.

The utility model was reproduced using an experimental setup designed to simulate hemorrhagic hypotension. The objective of the model is to exclude hemolysis of erythrocytes in experimental hemorrhagic hypotension. The problem was solved in the following way. In a device consisting of a blood collection column connected to a syringe and a manometer, an air valve was installed after the column. Laboratory and experimental study of erythrocyte aggregation was carried out 15 minutes after bloodletting. The group of false-negative animals was not included in the experiment. In subgroups, the coefficient of erythrocyte aggregability was measured by texture analysis using a TAS-plus computer unit (manufactured by Lietz, Germany), the coefficient of erythrocyte membrane deformability by the Reid principle, and blood viscosity. To achieve the main goal of the work, the Kruskal-Wallis criterion was defined. Statistical analysis of the data was carried out using the standard routines of Origin 4.1. (Microsoft Software Inc) and Microsoft Excel. The conditions of work on animals corresponded to the European Convention for the Protection of Experimental Animals, adopted in 1986 in Strasbourg [9], the consent of the Ethics Committee of the organizations on the basis of which the work was performed was obtained for the experiment.

Results and discussion

In control experiments, the average erythrocyte aggregation index was 20.5%, which was much higher than the average erythrocyte aggregation index already in the first subgroup and increased as the blood loss of the animal increased. Depending on the amount of blood lost in animals, the average values of the erythrocyte aggregation index differed significantly from each other. Changes in the viscosity and membrane deformability of erythrocytes occurred in the second and third subgroups of animals, i.e. with large blood loss. The index of erythrocyte aggregation, the index of membrane deformability and viscosity of the blood plasma of animals are shown in the table.

Table. *Erythrocyte aggregation index, erythrocyte deformability index, blood plasma viscosity, arithmetical mean of systolic and diastolic pressure of animals with experimental hemorrhagic shock depending on the volume of blood lost and in the control group. (M ± m)*

Parameters	Subgroup I	Subgroup II	Subgroup III	Control
Volume of blood, ml	2.5	3.5	4.5	4.5
Erythrocyte aggregation index, %	18.5±2.38	23.5±2.38	30.5±2.38	30.5±2.38
Erythrocyte deformation index, %	2.22±0.05	2.23±0.05	2.31±0.05	2.20±0.05
Blood plasma viscosity, sP	1.1±0.05	1.2±0.05	1.5±0.05	1.1±0.05
Arithmetical mean of systolic and diastolic pressure, mmHg	75±4	45±4	23±18	23±18

In the early works of the authors, it was described that it is the increase in erythrocyte aggregability that causes impaired blood flow in hemorrhagic shock and is responsible for the development of critical situations [5,10].

The mean systolic and diastolic pressure in the control group was 85±3 mm Hg. Art. As blood loss increased, a logical change in pressure occurred. Pressure monitoring emphasized the dependence of the degree of circulatory compensation on the severity of hemorrhagic shock. With small blood loss, blood pressure was close to normal, and then decreased as the volume of lost blood increased.

The crisis of macro – and microcirculation with blood loss has a special specific character. The number of aggregates in the blood plays a certain role in the main arteries and a special dominant role in the microvasculature. The blood circulating in the vessels is inhomogeneous. The parabolic blood profile changes as the caliber of the vessels decreases [11,12]. Even in healthy mammals and humans, erythrocytes in the blood circulating in the body stick together more or less randomly, attaching disparate erythrocytes, which, connecting with their surfaces, form coin columns – aggregates (not conglomerates!). Aggregates account for 15-30% of the total area of erythrocytes [6,12,13]. Analyzing the data obtained, it was found that even relatively small volumes of bloodletting increased erythrocyte aggregation. Apparently, in blood loss, a change in aggregability is associated with the inclusion of compensatory reactions of the body, and/or enhanced aggregation is the basis for the activation of this mechanism. With an increase in blood volume during bloodletting, there was a further increase in intravascular aggregation of erythrocytes. This fact causes a decrease in blood flow velocity in microvessels, up to the development of complete blood stasis, which ends with blockage of all open capillaries and the disappearance of blind capillaries, sometimes against the background of unchanged perfusion pressure [5,12,13].

If we consider in detail the moment of treatment, which is associated with erythrocyte transfusion, then one of the most important factors for starting treatment is the loss of 30-40% of blood volume, with losses of more than 40% of blood volume, five emergency transfusions of erythrocytes and other components are necessary, and with the presence of the examined donor – whole donor blood of short shelf life. These recommendations are based on empirical knowledge, analysis of an incredible number of records. If we compare the data of the so-called. evidence-based medicine (evidence medicine) with our experimental data, it is easy to explain how important the transfusion of erythrocytes is for large blood losses and how prognostically it is not unambiguous for small losses. At the first stage, when the viscosity and membrane deformability are not particularly changed, but the number of aggregates in the blood is already increased, an additional number of erythrocytes will lead to an even greater increase in aggregation, which will adversely affect the hemodynamic parameters of the blood. Hemodynamic measurements, such as heart rate, blood pressure, pulmonary capillary wedge pressure, and cardiac function, can help guide the decision, but it should be emphasized that occult blood loss can occur in the presence of stable vital signs.

From a practical point of view, our experiments have an undeniable contribution to the estimation of the amount of blood loss, which is very often underestimated and is one of the confounding factors. Usually, the values of hemoglobin and hematocrit are not markers and assessors of the condition, so they do not change within a few hours after acute bleeding, but change after 48-72 hours, while the erythrocyte aggregability index changes immediately and therefore may well be a predictor of the condition and an estimate of the stage patient bleeding.

As the volume of blood lost increases, the violation of erythrocyte aggregation increases with high reliability, and always in the case of an increase in blood loss, an increase in erythrocyte aggregability necessarily occurs. From the point of view of the effectiveness of treatment, it is undoubtedly important to control the aggregation of erythrocytes in case of blood loss of any volume. The high accuracy of calculations and the reliability of measurements made it possible to adapt the experimental data.

Reference

1. Kirov M Yu, Gorobets ES, Bobovnik SV et al. Principles of perioperative infusion therapy for adult patients. Anesthesiology and resuscitation. 2018; (6):82-103. https://doi.org/10.17116/anaesthesiology201806182
2. Rasmussen KC, Pedersen T, Secher NH [Perioperative fluid therapy influences coagulation, blood loss and post-operative complications]. Ugeskr Laeger. 2018; 180(21):V06170498. Danish. PMID: 29804566
3. Brake MA, Ivanciu L, Maroney SA, Martinez ND, Mast AE, Westrick RJ. Assessing Blood Clotting and Coagulation Factors in Mice. Curr Protoc Mouse Biol. 2019; 9(2):e61. DOI: 10.1002/cpmo.61. Epub 2019 Mar: 15 PMID: 30875463; PMCID: PMC6771260
4. Jang MS, Han JH, Lee S, Kim SE. Postoperative Blood Loss and Coagulation Changes After Balanced 6% Hydroxyethyl Starch 130/0.4 Administration During Spine Surgery: A Retrospective Study. Clin Spine Surg. 2019; 32(2):E65-E70. DOI: 10.1097/BSD.0000000000000727. PMID: 30334822
5. Mantskava M, Momtselidze N, Davlianidze L, Mitagvaria N. Hemorrhagic shock and stress. Cause and concequence of hemorheology disturbances on the example of the changes in ertythrocyte

aggregation. Journal of Stress Physiology and Biochemistry. 2014; 10(2):238-246

6. Baskurt O, Neu B, Meiselman H. Red blood cell aggregation. Clinical research. 2012; 1:1-26
7. Bie P, Debrabant B. Gonadal sex and animal experimentation: Perfection vs. 3R principle? Basic Clin Pharmacol Toxicol. 2020; 127(2):111-119.
   DOI: 10.1111/bcpt.13411. Epub 2020 Apr 21. PMID: 32267064
8. Zyblev SL, Dundarov ZA, Zybleva SV. Modeling of hemorrhagic shock // Problems of health and ecology. 2017; 1(51).
   https://cyberleninka.ru/article/n/modelirovanie-gemorragichesko-go-shoka (date of access: 08/26/2021)
9. European convention for the protection of vertebrate animals used for experimentation and other scientific purposes, no. 123 of 18 March; 1986 [ER].
   http://www.worldlii.org/int/other/COETSER/1986/1.html (Accessed: 01/15/2019)
10. Mantskava M, Momtselidze N., Davlianidze L. Blood rheological properties in blood loss (an experimental study). General Resuscitation. 2014; 10 (5):27-32
    https://doi.org/10.15360/1813-9779-2014-5-27-32
11. Bishop JJ, Nance PR, Popel AS, Intaglietta M, Johnson PC. Effect of erythrocyte aggregation on velocity profiles in venules. American Journal of Physiology. 2001; 280(1):222-236
12. Mchedlishvili G. Dynamic structure of blood flow in microvessels. Microcirc Endothelium Lymphatics. 1991; 7(1-3):3-49. PMID: 1762608
13. Mchedlishvili G.I. Pathogenesis of angiosperms [Pathogenesis of an angiospasm]. Patol Fiziol Eksp Ter. 1974; 10(2):6-15. Russian. PMID: 4600285