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Development of classification and criteria of quality and safety of autologous platelet concentrates for regenerative injection therapy in traumatology and orthopedics



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ABSTRACT

Regenerative injection therapy (also known as orthobiology or prolotherapy) is a burgeoning field in orthopedics that explores the use of cell technologies and new materials to promote reparative or physiological regeneration in various musculoskeletal diseases and injuries. The substantiation of the therapeutic effectiveness of autologous platelet concentrates, based on their manufacturing characteristics and features, remains an ongoing challenge.

THE PURPOSE of the study was to develop the classification, quality and safety criteria of autologous platelet concentrates for regenerative injection therapy in traumatology and orthopedics.

MATERIALS AND METHODS. A comparative study was conducted on 778 autologous platelet concentrates of various types, derived from the blood of patients with osteoarthritis, aseptic necrosis of the hip and knee joints due to ligament and tendon damage, and osteochondrosis of the spine. In biotechnological products, the content of platelets and leukocytes was determined, the ratio of platelets and leukocytes among themselves and relative to their content in peripheral blood was calculated, respectively. During the production of autologous platelet concentrates, the visual characteristics of cell sediment and plasma were evaluated. Products of platelet cryolysates were subject to microbiological testing.

RESULTS. A classification of autologous platelet concentrates based on platelet and leukocyte concentrations was developed, along with the determination of quality and comprehensive safety criteria for cellular forms of autologous platelet concentrates: platelet index, leukocyte index, and leukocyte-platelet index. Visual characteristics of platelet concentrates were established: "ideal", "classic", "erythrocytic", "low cell" and "alimentary" phenotype. Based on the results of a microbiological study, the safety of platelet concentrates for intra-articular administration in orthopedic and trauma patients was established.

CONCLUSION. The developed classification of biotechnological products derived from human peripheral blood, along with their quantitative and qualitative criteria, serves as the foundation for creating differentiated and personalized approaches to their use in musculoskeletal diseases and injuries.

KEY WORDS: regenerative injection therapy; autologous platelet concentrates; leukocyte rich platelet rich plasma; leukocyte poor platelet rich plasma; platelet cryolysate

Regenerative injection therapy (also known as orthobiology, prolotherapy) is considered the newest direction of orthopedics, which studies the use of cell technologies and new materials to ensure reparative or physiological regeneration in various diseases and injuries of the musculoskeletal system [1].

The clinical effectiveness of the means of regenerative medicine and wide possibilities of application arouse the interest of practicing doctors, contributing to its active implementation in everyday practice. At the same time, the need for fundamental knowledge and understanding of key processes in medicine, physiology and regeneration processes, the insufficient number of studies, including by domestic authors, make regenerative techniques vulnerable to compliance with the principles of evidence based medicine and often compromise their application.

It should be noted that although regenerative orthopedics is considered a fairly "young" and innovative field, its history dates back to the beginning of the last century. Thus, in 1905, the German surgeon of the well-known hospital "Charite" (Berlin, Germany) August Beer, who was one of the founders of German surgery, first described the successful use of autohemotherapy in a patient with a fracture that did not heal [2]. This method can rightfully be considered the "ancestor" and prototype of modern regenerative technologies.

The next evolutionary step in the introduction of regenerative injection therapy should be considered the technique of prolotherapy (synonyms of sclerotherapy, tissue therapy). For the first time, this method using a dextrose solution was applied by the American surgeon and osteopath Earl Gedney in 1923 for the treatment of abdominal hernias, and in 1930 it was recognized by the American Association of Osteopaths and Herniologists as official for the treatment of this pathology. The next step was a method for non-surgical treatment of joint, ligament and tendon pathologies [3].

In 1933, V. P. Filatov, who founded and was the first director of the Institute of Eye Diseases and Tissue Therapy (Odesa), first used tissue therapy in ophthalmology, and in 1936 his monograph "Tissue Therapy" was published. Subsequently, he began to apply the techniques of tissue therapy in other specialties, including for wound healing and fracture fusion. In 1940, V. P. Filatov began to use herbal preparations (aloe extract, FIBS) for tissue therapy. According to his assumption, under the influence of these products, substances with pronounced biological activity are formed. At the suggestion of the author, these substances were called "resistance factors" or "biogenic stimulants" [4].

The founders of modern prolotherapy in orthopedics and traumatology with the use of dextrose and other stimulating solutions are the American orthopedic traumatologists George Hacket and Gustav Hemwall, who worked in the period from 1940 to 1950. They developed the first regional prolotherapy protocols for musculoskeletal pathology [5]. In 1986, the Italian neurobiologist Rita Levi Montalcini discovered "growth factors", for which she was awarded the Nobel Prize [6]. This momentous event marked the beginning of the modern era of regenerative injection therapy as a treatment method. In 1987, the first publication by Italian authors was published on the use of platelet rich plasma (PRP) in cardiac surgery [7]. In 1990, the successful use of platelet rich plasma for the treatment of pathology of the musculoskeletal system, namely tendinitis and tendinopathies, was first mentioned in the literature [8].

Currently, PRP has become the most famous biotechnological product and used by doctors in many fields, including dentistry, maxillofacial surgery, vertebrology, orthopedics and traumatology, in general surgery for the treatment of chronic wounds, etc.

Platelet rich plasma today refers to the patient's autologous plasma, in which the concentration of platelets has been increased several times compared to the values of peripheral blood by stepwise centrifugation. If the concentration of platelets is paid enough attention by doctors, then the role of other cells, in particular leukocytes, as a constituent component of PRP, in many cases remains overlooked [9 10].

Therefore, a separate product leukocyte rich platelet rich plasma (LR-PRP) – has recently been released. This product means autologous plasma with an increased, compared to the initial values, concentration

of platelets (more than 1 million in 1 μ L) and leukocytes. Conversely, leukocyte poor platelet rich plasma (LP-PRP) is platelet rich plasma with a minimal content of leukocytes [11 13]. The specified variety of variants of platelet rich plasma indicates that the latter is not a single product. Platelet rich plasma is a group of biotechnological products with a different ratio of cell composition, where platelets and leukocytes play a key role, since they are its "active substances", if parallels are drawn with pharmaceuticals. The clinical effect and differentiated application of the specified variants of biotechnological products in any pathology of the musculoskeletal system will depend on the concentration of these cells, as well as their ratio.

THE PURPOSE of the study was to develop a classification of autologous platelet concentrates for the use in patients with pathology of the musculoskeletal system and to develop criteria for the quality and microbiological safety of these minimally manipulated biotechnological products. The developed classification will contribute to the further development of a differentiated approach to the use of various variants of platelet concentrates depending on the pathology of the musculoskeletal system.

MATERIALS AND METHODS

The material for the production of biotechnological products from autologous blood was the peripheral venous blood of 778 patients (338 men and 440 women) aged 22 to 83 years (average age 52.5 years) with osteoarthritis and aseptic necrosis of the hip and knee joints, ligament and tendon damage, spinal osteochondrosis.

The following variants of biotechnological products from autologous blood were produced for the mentioned patients:

- leukocyte rich platelet rich plasma – 98 patients,
- leukocyte poor platelet rich plasma – 143 patients,
- platelet poor plasma – 74 patients,
- high density platelet rich plasma – 112 patients,
- platelet cryolysate – 351 patients.

Blood sampling procedure. The blood collection procedure plays a significant role in the subsequent quality and safety of autologous biotechnological products based on platelets and other peripheral blood cells. All patients received informed consent for the course of treatment and research.

At the first stage, Complete Blood Count was performed. If the indicators deviated from the norm, blood sampling for the production of platelet concentrates was not performed for the patient. In the absence of abnormalities, patients were recommended to stop taking non-steroidal anti-inflammatory drugs within 72 hours, prescribed a special diet (no fatty, fried, salty and spicy food, up to 3 liters of fluid per day, no coffee) and no smoking and drinking alcohol. Immediately before blood sampling, a rapid immunochromatographic combined test "Profitest" (*InTec Products, Inc.*, China) was performed to detect antibodies to human immunodeficiency virus types 1 and 2 (HIV 1/2), surface antigen of hepatitis B virus (HBsAg), antibodies to hepatitis B (HBcAb) and hepatitis C viruses (HCV), antibodies to the *Treponema pallidum*.

During the sampling, the patient was in a supine position to avoid orthostatic reactions. The patient's condition was assessed prior to venipuncture to select an appropriate vein. In some cases, the median ulnar vein was used for blood sampling. Selecting a large and stable vein is essential for successful blood collection. In some cases, with the difficulty of visual identification of the vein for blood sampling, ultrasound navigation was used. Before venipuncture, the node of the needle for blood sampling was treated with an anticoagulant dextrose citrate to prevent blood clotting. Blood was collected in test tubes containing anticoagulant dextrose citrate, then it best supports the metabolic needs of platelets and their release without destruction and best supports the vital activity of cells [14].

Aspiration was performed slowly in a controlled manner at a rate of approximately 1 mL/s to prevent the vein from collapsing and creating too much negative pressure that could cause cell damage. During aspiration, the blood was mixed twice in the tube with the anticoagulant to ensure proper mixing, as whole blood is denser than the anticoagulant and will not mix properly on its own during collection, which may cause it to clot. After filling, the tube was inverted five times to better mix the anticoagulant with blood.

Processing of blood for biotechnological products. To prepare platelet poor plasma, 18 mL of venous blood was collected using 9 mL vacuum tubes containing dextrose citrate anticoagulant (*BD Vacutainer*, USA). It was centrifuged at 250 ×g for 10 min and the blood plasma was collected into new tubes with a volume of 10 mL without mixing. After that, the selected plasma was centrifuged again at 2300 ×g for 5 minutes. The plasma was aspirated in the required volume, the rest was disposed.

To prepare leukocyte rich platelet rich plasma, 54 mL of venous blood was collected using 9 mL Vacutainer vacuum tubes containing dextrose citrate anticoagulant (*Becton Dickinson*, USA). The collection of such a volume of blood is due to the fact that to obtain the biotechnological product we used a centrifuge with 6 cassettes for 9 mL tubes. This is the optimal volume of blood to achieve a platelet concentration of more than 1 million cells per 1 µL. After collection, venous blood was centrifuged at 250 ×g for 10 min to separate plasma and blood cells using a CM 3 centrifuge (*MICROMed*, China). After centrifugation, cells were lifted into the plasma from the upper layer of the gradient (buffy coat), which contains leukocytes and platelets, using a Pasteur pipette. After that, 30 mL of blood plasma with a buffy coat was transferred to new 9 mL round bottom tubes and centrifuged at 2300 ×g for 5 min. Exactly three 9 mL tubes were used, as centrifugation was performed in a centrifuge. Furthermore, in round bottom tubes, the sediment of leukocytes and platelets, along with residual erythrocytes, is more visible compared to a 50 mL conical bottom tube. All plasma was collected using a Pasteur pipette with a graduated scale, 3 mL of which was used to resuspend the cell pellet. The 3 mL product volume is optimal for injection into the patient's joint to prevent injection pain from excess fluid, and was therefore chosen for the study. Most (approximately 90 %) of the platelet poor plasma was disposed.

To prepare leukocyte poor platelet rich plasma, 54 mL of venous blood was collected using 9 mL vacuum tubes containing dextrose citrate anticoagulant (*BD Vacutainer*, USA). After the first centrifugation at 250 ×g for 10 min, the blood plasma was collected into new 10 mL tubes without mixing to reduce the leukocyte content in it. After that, the collected plasma was centrifuged at 2300 ×g for 5 min. The resulting precipitate containing platelets was also resuspended in 3 mL of platelet poor plasma, and the remaining plasma was disposed.

To prepare high density platelet rich plasma (HD-PRP), 54 mL of peripheral blood was collected using a sterile system into vacuum tubes containing dextrose citrate as an anticoagulant (*Vacumed*, Italy). Whole blood was centrifuged to separate plasma and cells at 250 ×g for 10 min using a CM 3 centrifuge. Plasma (upper fraction) was carefully collected, transferred to 9 mL round bottom tubes and centrifuged at 2300 ×g for 5 min. The supernatant from platelet poor plasma was collected, 1.2 mL of which was used to resuspend platelets.

To prepare autologous platelet cryolysate, 120 mL of peripheral blood was collected using a sterile 10 mL syringe with a 22G needle into sterile 50 mL conical tubes containing dextrose citrate as an anticoagulant (up to 10 % of the total volume). Whole blood was centrifuged to separate plasma and cells at 250 ×g for 10 min. in a CM 3 centrifuge. Plasma was transferred to sterile tubes (*Vacumed*, Italy) and centrifuged at 2300 ×g for 5 min. The supernatant was collected, the pellet with platelet concentrate was transferred in a 50 mL tube, where 18 mL of plasma was added using a graduated Pasteur pipette.

The resulting suspension was divided into 3 mL aliquots in cryovials and stored in a cryochamber at a temperature of 20 °C. Before use,

the cryolysate was thawed in a water bath VB 4 (*MICROMed*, Ukraine) at a temperature of 37 °C for 10 min. The suspension was centrifuged at 150 ×g for 5 min. The supernatant was collected with a sterile syringe.

All manipulations with blood samples were performed under aseptic conditions in a DFZ M680 biosafety cabinet (Ukraine) to protect the product from contamination.

Quality criteria were assessed by counting cells in biotechnological products and assessing their ratio. For cell counting, an automatic hematology analyzer HumaCount 5D (Human, Germany) was used.

Microbiological study of platelet concentrates. The microbiological safety of the product was assessed, namely the determination of the presence of aerobic, anaerobic, and fungal flora in the biotechnological product. Microbiological study was carried out in the microbiology laboratory of the State Institution "Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine". The obtained platelet concentrates were tested for sterility. 0.5 mL of the obtained platelet concentrate was collected and added to a bottle with a nutrient medium for aerobic, anaerobic and fungal flora, which was incubated in a BACT/Alert analyzer (*Biomeriaux*, USA). Exposure took place for 7 days, the growth of aerobic, anaerobic flora and fungal flora was determined. The specified sterility testing method is a mandatory requirement and is also validated.

RESULTS

Depending on the application of the biotechnology product in patients with different diagnoses, different variants of biotechnology products were purposefully produced from autologous peripheral blood, which were classified based on the number of platelets, leukocytes, and their ratio. To achieve the required cell content, the initial levels of platelets and leukocytes in the blood sample were taken into account. If necessary, the volume of blood collected or the volume of plasma used for resuspending the resulting precipitate was adjusted to obtain the desired product type. Based on the results of manufacturing biotechnological products from autologous platelets, the following classification was developed.

Classification by platelet concentration:

- Platelet Poor Plasma (single platelets per product volume unit);
- Autologous Concentrated Plasma (the number of platelets in the biotechnological product is greater than the initial concentration in the blood, but less than 1 million per 1 µL);
- "Classic" Platelet Rich Plasma (the number of platelets in the biotechnological product is 1.3 million per 1 µL);
- Concentrated Platelet Rich Plasma (the number of platelets in the biotechnological product is 3.10 million per 1 µL);
- High Density Platelet Rich Plasma (the number of platelets in the biotechnological product is more than 10 million per 1 µL).

Thus, platelet poor plasma has anti-inflammatory properties, so we recommend using it for paraarticular administration, in particular combined with other variants of thromboconcentrates. We recommend using the "classic" product for intraarticular administration, in particular in the early stages of osteoarthritis. The high concentration of cells is recommended for intraosseous administration. In particular, the concentrated option is for subchondral administration in osteoarthritis, and the high density option is for aseptic necrosis of various localizations.

Platelet rich plasma can be classified

By leukocyte concentration:

- leukocyte rich platelet rich plasma (leukocyte concentration in platelet rich plasma ≥ 2 thousand per 1 µL);
- leukocyte poor platelet rich plasma (leukocyte concentration in platelet rich plasma is less than 2 thousand per 1 µL);

Accordingly, it is possible to produce different cryolysate variants:

- platelet cryolysate based on leukocyte rich platelet rich plasma;
- platelet cryolysate based on leukocyte poor platelet rich plasma.

We recommend using leukocyte poor products in the early stages of the disease (particularly in osteoarthritis) in order to avoid the possible chondrotoxic effect of leukocytes, and leukocyte rich variants in the later stages, since for these patients the effect of leukocytes, particularly taking into account the role of macrophages, is important for eliminating the inflammatory component in the joint and paraarticular tissues.

Based on the results of analyses of manufactured biotechnological products, the following quality criteria for cellular forms of autologous platelet concentrates were established:

Platelet index of autologous platelet concentrate (PIAPC) – the ratio of the platelet concentration in the biotechnological product to the initial platelet concentration in the patient's blood. The following criteria for evaluating this indicator have been identified:

- PIAPC < 4 – low index;
- PIAPC within 4 6 – average index;
- PIAPC > 6 – high index.

This indicator is a criterion of product quality, as it allows to characterize the level of platelets in the biotechnological product compared to their level in the patient's native blood and allows to determine the indications for its use (paraarticular, intraarticular or intraosseous administration). We can adjust it depending on the need for the use of the biotechnological product based on the localization and volume of tissue damage. The greater the volume of tissue damage is, the greater the concentration of platelets should be compared to the concentration in whole blood. Regarding the application depending on the localization of the pathological process, with a low index value, the product will be indicated for paraarticular administration, with an average index value – for intraarticular administration, and with a high index value – for intraosseous injection.

Leukocyte index of autologous platelet concentrate (LIAPC) – the ratio of the initial leukocyte concentration in the patient's initial blood test and the leukocyte concentration in the biotechnological product:

- LIAPC < 2 – low index;
- LIAPC within 2 3 – average index;
- LIAPC > 3 – high index.

This indicator is a criterion for the safety of the product, as it allows predicting the likelihood of adverse reactions upon its administration caused by the presence of leukocytes. Thus, the lower the index is, the greater the likelihood of adverse reactions upon administration of the product is due to the content of leukocytes in it.

Platelet leukocyte index of autologous platelet concentrate (PLIAPC) – the ratio of the concentration of leukocytes and platelets in a biotechnological product:

- PLIAPC < 500 – low index;
- PLIAPC within 500 1000 – average index;
- PLIAPC > 1500 – high index.

This criterion makes it possible to determine the indications for the use of the product depending on the stage of the disease. Thus, a biotechnological product with a low and medium index is advisable to use in patients with the initial stages of osteoarthritis, a product with a high index in the late stages of osteoarthritis due to the need to influence the leukocyte component, namely the influence of macrophages on the affected tissues.

The proposed indices were determined in the obtained cellular biotechnological products, namely in leukocyte, leukocyte poor and high density platelet rich plasma (Tables 1-3).

Table 1. Platelet index of autologous platelet concentrate in manufactured biotechnological products.

Biotechnological product	Number	PIAPC <4	PIAPC 4 6	PIAPC >6
Leukocyte rich platelet rich plasma	98	10	46	42
Leukocyte poor platelet rich plasma	143	34	91	18
High density platelet rich plasma	112	4	21	87

Table 2. Leukocyte index of autologous platelet concentrate in manufactured biotechnological products.

Biotechnological product	Number	LIAPC <2	LIAPC 2 3	LIAPC >3
Leukocyte rich platelet rich plasma	98	64	34	0
Leukocyte poor platelet rich plasma	143	0	64	79
High density platelet rich plasma	112	48	47	17

Table 3. Leukocyte platelet index of autologous platelet concentrate in manufactured biotechnological products.

Biotechnological product	Number	PLIAPC <500	PLIAPC 500 1000	PLIAPC >1500
Leukocyte rich platelet rich plasma	98	32	76	0
Leukocyte poor platelet rich plasma	143	0	114	29
High density platelet rich plasma	112	0	15	97

Leukocyte rich platelet rich plasma is characterized by an average or high platelet index and a low or average leukocyte and leukocyte platelet index. For leukocyte poor platelet rich plasma, the average platelet index, the average or high leukocyte index and the average leukocyte platelet ratio index are characteristic. High density platelet rich plasma is characterized by the high platelet and leukocyte platelet index as well as the low or average leukocyte index. These indicators mainly depend on the initial concentration of leukocytes and platelets in the patient's native blood, but the operator, if necessary, can adjust them when manufacturing the biotechnological product.

A visual quality criteria for autologous platelet concentrates.

In fact, the visual characteristics of autologous platelet concentrates reflect their morphological composition, as well as various variants of violations of the technology of their manufacturing process starting from improper preparation of the patient for the blood collection procedure and ending with errors in the cell isolation technology.

According to visual characteristics, we divided all autologous platelet concentrates into the following phenotypes:

- "ideal" phenotype;
- "classic" phenotype;
- "erythrocytic" phenotype;
- "low cell" phenotype;
- "alimentary" phenotype.

Fig. 1 shows a variant of the ideal phenotype of platelet-rich plasma. It can be described as follows: the plasma in the supernatant is clear, the cell ring is well-defined, and the erythrocyte sediment is barely visible (Fig. 1A). The final product is amber in color and opaque compared to plasma (Fig. 1B).



Fig. 1. Photos of platelet rich plasma with the "ideal" phenotype. A – visual characteristics of the cell pellet, B – visual characteristics of the final product (left) compared to plasma (right).

Fig. 2 shows the “classic” phenotype of platelet rich plasma. It differs from the “ideal” one in that it has a more pronounced erythrocyte sedimentation rate.

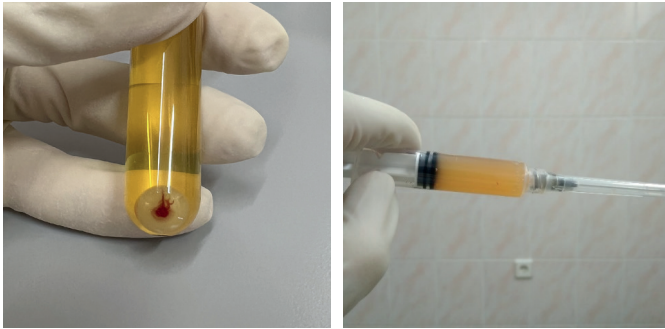


Fig. 2. Photos of platelet rich plasma with the “classic” phenotype.

Figures 3-5 present variants of “defective” phenotypes of autologous platelet concentrates. In most cases, they are associated with violation of the rules of patient preparation for blood sampling and violation of the sampling technology itself.

The “erythrocytic” phenotype is characteristic of patients who did not adhere to the fluid intake regimen before blood sampling for the manufacture of a biotechnological product. The recommended fluid intake is 2-3 liters per day for 2 days prior to the planned sampling of biological material (Fig. 3).



Fig. 3. Photograph of the autologous platelet concentrate with the “erythrocytic” phenotype: significant erythrocyte sedimentation is observed after the second centrifugation due to hypohydration.

The “low cell” variant of the phenotype is characteristic of patients who violated their dietary regimen before blood sampling (insufficient food and fluid intake, insufficient protein intake, veganism, smoking electronic cigarettes) (Fig. 4).



Fig. 4. Photo of autologous platelet concentrates at the platelet rich plasma stage with the “low cell” phenotype (a cell ring is visualized).

The “alimentary” phenotype is characteristic of patients who, during preparation for the blood sampling procedure, violate their diet (eating fatty, smoked, salty and fried foods, drinking sweet carbonated drinks). This phenotype variant is characterized by the absence of clear layers during centrifugation, premature activation of platelets (Fig. 5).

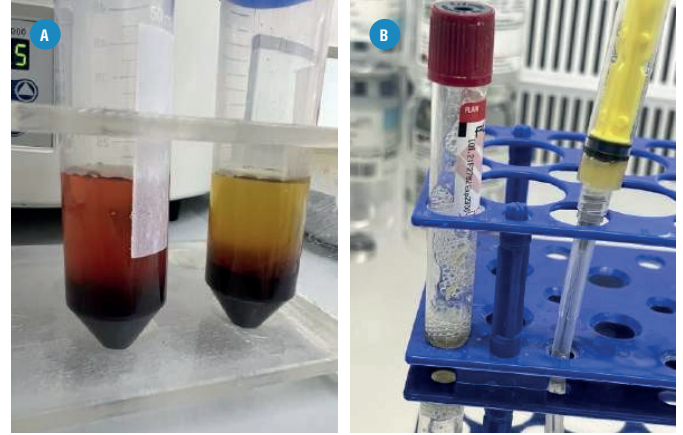


Fig. 5. Photos of autologous thromboconcentrates with the “alimentary” phenotype. The layers appear indistinct during the centrifugation stages (A), and premature platelet activation is observed in the final product (B).

Microbiological safety of autologous platelet concentrate products.

For microbiological testing, platelet cryolysate samples were used, since this product is not used immediately after manufacture, but is in quarantine storage in a cryostorage until test results are received. Microbiological testing results showed no detectable flora in any sample, indicating proper adherence to aseptic conditions throughout all stages of autologous platelet concentrate manufacturing. In case of detection of microbial contamination, the product should be immediately disposed of with subsequent verification of the proper quality of reagents, consumables and compliance with standard operating procedures by personnel.

DISCUSSION

Platelet rich plasma has been used in traumatology and orthopedics for over 30 years, as a stand-alone product or in the complex treatment of musculoskeletal diseases and injuries, but there are still very few publications that study the cellular content of the drugs and their additional characteristics [15]. It should be noted that the concept of platelet rich plasma is quite general and characterizes a whole group of biotechnological products based on the patient’s peripheral blood. At the same time, there are studies that indicate the clinical significance of cell concentration and their ratio in the treatment of a particular musculoskeletal injury, as well as differences in clinical effect depending on the patient’s personal characteristics [16, 17]. If the concentration of platelets is given sufficient attention by practicing physicians, the role of other cells, in particular leukocytes, as a component of the PRP in many cases remains unnoticed.

At the same time, it has been proven that leukocytes are able to secrete a large number of pro inflammatory cytokines, enzymes (interleukin IL-1β, metalloproteinase MMP9, tumor necrosis factor TNF-α, etc.), which can cause increased catabolism of the extracellular matrix and inhibition of regeneration processes [18, 19]. In particular, regarding the use of platelet rich plasma in osteoarthritis, modern literature mainly notes the role of growth factors contained in α granules of platelets as the main therapeutic “tool”, but insufficient attention is paid to the influence of macrophages contained in the product on modeling the course of the pathological process and their influence on regenerative processes in the joint as well, depending on the stage of the disease [20, 21].

The most well-known classification of platelet rich plasma in the literature is the Dohan Ehrenfest classification, which is based on the technique of isolating a particular product [22]. Other classifications have subsequently appeared [23], but the need for more detailed product characterization has not disappeared, in particular due to conflicting data on the role of leukocytes in the product and the maximum number of platelets. Some studies indicate the chondrotoxic effect of leukocytes and advise to completely remove them from platelet rich plasma [24, 25], while others, on the contrary, claim their positive effect, especially in the late stages of osteoarthritis [26, 28].

Therefore, it is precisely because of the clinical significance of the cellular composition and cell ratio in a particular variant of platelet rich plasma that our study is relevant, and the developed classification and numerical criteria (indices) allow us to more clearly characterize the biotechnological product from human peripheral blood and develop a differentiated and personalized approach to their use in a particular pathology of the musculoskeletal system.

CONCLUSION

- 1. A classification of autologous thromboconcentrates based on platelet and leukocyte content has been developed, with quality and comprehensive safety criteria determined by the cell ratio and visual characteristics.**
- 2. Compliance with aseptic conditions is a mandatory safety criterion in the manufacture of autologous thromboconcentrates, and platelet cryolysate products must undergo preliminary microbiological testing before use.**
- 3. The proposed classification of biotechnological products derived from human peripheral blood, along with their quantitative and qualitative characteristics, forms the basis for developing differentiated and personalized approaches to their use in musculoskeletal diseases and injuries.**

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Розробка класифікації та критеріїв якості і безпеки аутологічних тромбоконтратів для регенеративної ін'єкційної терапії в травматології та ортопедії



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РЕЗЮМЕ

Регенеративна ін'єкційна терапія (синоніми – ортобіологія, пролотерапія) вважається новітнім напрямком ортопедії, який вивчає використання клітинних технологій та нових матеріалів для забезпечення репаративної або фізіологічної регенерації при різних захворюваннях та пошкодженнях опорно-рухової системи. Актуальною проблемою залишається обґрунтування терапевтичної ефективності аутологічних тромбоконтратів, залежно від особливостей виготовлення та характеристик цих біотехнологічних продуктів.

МЕТОЮ ДОСЛІДЖЕННЯ стала розробка класифікації та критеріїв якості і безпеки аутологічних тромбоконтратів для регенеративної ін'єкційної терапії в травматології та ортопедії.

МАТЕРІАЛ І МЕТОДИ. Проведено порівняльне дослідження 778 аутологічних тромбоконтратів різного типу, виготовлених з крові пацієнтів з остеоартрозом та асептичним некрозом кульшового та колінного суглобів, пошкодженням зв'язок та сухожилків, остеохондрозом хребта. У біотехнологічних продуктах визначали вміст тромбоцитів, лейкоцитів, розраховували співвідношення тромбоцитів і лейкоцитів між собою та відносно їх вмісту в периферичній крові, відповідно. Під час виготовлення аутологічних тромбоконтратів оцінювали візуальні характеристики клітинного осаду та плазми. Препарати кріолізатів тромбоцитів підлягали мікробіологічному тестуванню.

РЕЗУЛЬТАТИ. Розроблено класифікацію аутологічних тромбоконтратів за концентрацією тромбоцитів та лейкоцитів, а також визначено критерії якості та комплексної безпеки клітинних форм аутологічних тромбоконтратів: тромбоцитарний індекс аутологічного тромбоконтрату, лейкоцитарний індекс аутологічного тромбоконтрату, лейкоцитарно-тромбоцитарний індекс аутологічного тромбоконтрату. Встановили візуальні характеристики тромбоконтратів: "ідеальний", "класичний", "еритроцитарний", "малоклітинний" та "аліментарний" фенотип. За результатами мікробіологічного дослідження встановлено безпеку тромбоконтратів для внутрішньосуглобового введення пацієнтам ортопедо-травматологічного профілю.

ВИСНОВОК. Запропонована класифікація біотехнологічних продуктів з периферичної крові людини, а також їхні кількісні та якісні критерії є основою для впровадження диференційованого та персоналізованого підходів до їх застосування при захворюваннях та травмах опорно-рухового апарату.

КЛЮЧОВІ СЛОВА: регенеративна ін'єкційна терапія; аутологічні тромбоконтрати; лейкоцитарна збагачена тромбоцитами плазма; малолейкоцитарна збагачена тромбоцитами плазма; кріолізат тромбоцитів