Biochemical and functional quality assessment of platelet concentrates
Biohemijska i funkcionalna ispitivanja kvaliteta koncentrata trombocita

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Abstract

Background/Aim. Preparation of platelet concentrate (PC) from a unit of whole blood is a method dependent on a number of factors, which alone or together, can have a significant impact on the quality of the final product. Quality of PCs is determined with criteria defined in the Guide to the preparation, use and quality assurance of the Council of European Committee for Blood Transfusion. The aim of the study was to analyze the quality of PCs prepared from buffy coat (BC) and to allocate the factors of improvement and standardization of the quality of PCs. Methods. The study included a total of 80 PCs prepared from BC according to the standard procedure in the Blood Transfusion Institute Niš. The quality of PCs was determined according to the product volume and laboratory testing on the first (PC1), the third (PC3) and the fifth day (PC5) of the PC storage. The following parameters were determined: platelets, residual erythrocytes and leukocytes count, gas analysis [partial pressure of oxygen, carbon dioxide (pO2, pCO2)], pH, sterility and platelet aggregation triggered by using 3.2, 6.4 and 9.6 μg collagen/mL (impedance aggregometry). Results. There were significantly lower platelet count, pO2, pCO2 and pH in the PC3 and PC5 samples (p < 0.001). Except for the fulfillment of the criteria for platelet count, all the other quality parameters were in accordance with recommended criteria. Platelet aggregation for all the concentrations of collagen showed a decrease during the storage period, with statistically significant differences for PC3 and PC5 as compared to PC1 (p < 0.01). There was statistically significant decrease in activity of PCs triggered with higher concentrations of collagen (6.4 and 9.6 μg collagen/mL) in comparison with lower concentration of collagen (3.2 μg collagen/mL). Conclusion. Platelet count, evaluated biochemical parameters and the platelet function were significantly changed during the storage period. In order to improve the quality of PCs it is important to store the products under proper conditions, change the type of plastic bag for PC storage and use platelet additive solutions (PAS) instead of plasma.

Key words: blood platelets; reference standards; blood chemical analysis; platelet aggregation; platelet count.

Apstrakt

Uvod/Cilj. Priprema koncentrata trombocita (KT) iz jedinice celokrvne krv je metoda osnovljena velikim brojem faktora koji pojedinačno ili udruženo mogu imati značajan uticaj na kvalitet končanog proizvoda. Kvalitet koncentrata trombocita određen je kriterijumima definisanim u Vodiču za pripremu, korišćenje i obezbedenje kvaliteta Evropskog komiteta za transfuziju krvi Saveta Evrope. Cilj istraživanja bio je da se ispita kvalitet KT dobijenim izdvajanjem iz "buffy coat"-a (BC) i izdvojiti faktore koji doprinose poboljšanju kvaliteta koncentrata trombocita. Metode. Ispitivanje je obuhvatio ukupno 80 KT izdvojenih iz BC prema standardnoj proce- duri pripreme u Zavodu za transfuziju krvi u Nišu. Kvalitet KT određivan je na osnovu zapremine produkta i laboratorijskih testiranja prvog (KT1), trećeg (KT3) i petog dana skladištenja (KT5). Određivan je broj trombocita, zaostalih eritrocita i leucocita, parcijalni pritisak kisine i ugljen-dioksida (pO2, pCO2), pH, sterilnost i agregacija trombocita izazvana dodavanjem 3,2; 6,4 i 9,6 μg kolagena/mL (impedance aggregometrija). Rezultati. Nađen je statistički značajan manji broj trombocita, pO2, pCO2 i pH u uzorcima KT1, KT3 i KT5 (p < 0,001). Osim isp.ujenosti kriterijuma za broj trombocita, svi ostali parametri bili su u saglasnosti sa preporukama Vodiča. Agregacija trombocita za sve koncentracije kolagena pokazala je pad tokom skladištenja, sa statistički značajnom razlikom za uzorke KT5 i KT1 u odnosu na uzorak KT1 (p < 0,01). Postojala je statistički značajna razlika u smanjenju aktivnosti uzoraka KT aktiviranih pri- menom visokih koncentracija kolegena (6,4 i 9,6 μg kolagena/mL) u odnosu na nižu koncentraciju kolagena (3,2 μg kolagena/mL). Zaključak. Broj trombocita, posmatrani bi...
The transfusion of platelet concentrates (PC) is used in the prevention and treatment of bleeding in patients with thrombocytopenia or thrombocytopenia. Other most common indications are hematological diseases, transplantation of bone marrow and other organs, treatment of cardiac surgery patients and the gastrointestinal bleeding. Applications of aggressive medical treatments including chemotherapy increase the rate of applied transfusions of random donor platelets. Transfusion of PCs is intended to bring a sufficient number of platelets in the patient’s circulation in order to increase their number and allow normal hemostasis, achieve prevention and treatment of bleeding, and, on the other side, reduce side effects, infections and alloimmunization to the lowest level. Contemporary data show that each year about 1.5 million of PCs in the United States and about 2.9 million of PCs in Europe are transfused.

PCs are the blood products obtained from whole blood units [prepared from platelet rich plasma (PRP) or "buffy coat" (BC – a layer of white blood cell and platelets occurred after red blood cells sedimentation in an unit of whole blood)] or apheresis of platelets obtained from a single procedure of thrombocytapheresis, using automated blood cell separator. Preparation of PCs from an unit of whole blood is a method dependent on a number of factors, which alone or together, can have a significant impact on the quality of the final product. Standardization of PCs is very difficult to execute. The most important factors influencing the quality of PC are the type and quality of blood bags, the characteristics of centrifugation, separation method and storage conditions of the prepared PCs. Today, in all developed countries, including our, most of PCs are prepared by removing the BC layer and their quality is improved especially if blood is collected in "top & bottom” blood bags. Platelets are stored in special plastic bags for storage that allow the transport of oxygen, at a temperature of 22 ± 2 °C, with continuous agitation on a horizontal agitator (approximately 70 cycles per minute) to prevent platelet aggregation and accelerate the transfer of oxygen for up to 5 days. Application of additive solution for platelets (PAS) extends the storage life of concentrate to 7 days, and generally speaking has numerous advantages over the plasma which is normally used as a medium for storage of platelets, both in terms of improving the quality of PCs and their efficiency in patients.

The standard therapeutic dose of platelets for adults is prepared from 4–6 units of blood (dose: 1 concentrate/10 kg body weight – BW), and some number of PCs can be merged into one bag by pooling. Pooled platelet concentrate is now commonly prepared by pooling several BC units of the same ABO blood group before centrifugation. Dilution of prepared pool can be performed with plasma or PAS.

Quality of PCs is determined with criteria defined in the Guide to the preparation, use and quality assurance of the Council of European Committee for Blood Transfusion. Mandatory requirements of quality control of PCs prepared from BC are volume of 50–75 mL which contains at least 60 ×10⁹ platelets, less than 0.05 ×10⁹ leukocytes, the number of erythrocytes 0.2–1 ×10⁹ and pH greater than 6.4. In addition, it is recommended to test PC for the presence of bacteria till the end of storage as well as to test platelet function in PCs, which usually means the examination of platelet aggregation by adding the appropriate agonist. This is essential to assess the in vitro function of platelets in respect to different activation pathways.

The study aimed to analyze the quality of PCs prepared from BC by determination platelet count, gas analysis, biochemical parameters in vitro, platelet aggregation and sterility during the whole storage period. On the basis of the results we evaluated if random donor PCs were prepared in accordance with the criteria of the Guide of Council of Europe, and whether we can allocate the factors of improvement and standardization of the quality of PCs.

Methods

The study included a total of 80 PCs prepared from BC according to the standard procedure in the Blood Transfusion Institute Niš. Whole blood from voluntary blood donors of both sexes, non-reactive on the markers of transmissible diseases, with normal clinical and laboratory parameters, who were not taking antiplatelet drugs for last 7 days, was collected in a system of quadruple plastic bags containing 63 mL of CPD anticoagulant solution and 100 mL of additive solution of saline, adenine, glucose and mannitol (SAGM) for storage of red blood cells (JiaxingTianhe Pharmaceutical, China). All units of blood in the assay were stored at room temperature (20–24°C) and within a period of 6 hours of collection centrifuged for 15 minutes at 3200 rpm and a temperature of 22°C (centrifuge with a cooling for blood, Heraus, Cryoefuga 8500). After that, automatic separation of whole blood units was done on the T-ACE II (Terumo) device, and from each blood unit concentrate of erythrocytes, the plasma unit and platelet-leukocyte layer – “buffy coat” (BC), which was left to stand at room temperature for two hours, were prepared. After that, BCs were centrifuged for 8 minutes at 1100 rpm and a temperature of 22°C, and PCs were transferred in bags that allow storage of platelets for five days. PCs were left a short time on a flat surface (in order to disaggregate platelets), labeled (ISBT 128) and stored.

horizontally on the agitator (Helmer PC4200i) until the expiration of the storage.

The quality of the PCs was determined according to the volume of the product and laboratory testing was performed on the first (PC1), the third (PC3) and the fifth day (PC5) of the storage. PCs with a volume less than 20% of the standard volume of the product, chylous, hemolyzed or in any way contaminated were excluded from this investigation. In order to obtain aliquots from samples of PCs, a sterile connection (TSCD Terumo) was used which ensured the integrity of the environment. From each PC sample, the following parameters were determined: hematological analysis – count of platelets, residual erythrocytes and leukocytes on a Abbott Cell-Dyn Rubyanalyser (Abbott Laboratories); gas analysis – pO2, pCO2 on the AVL Compact 3 Blood Gas Analyzer (Roche Diagnostics); pH – on the CyberScan pH510 device (Eutech Instruments); sterility – on the Bact / ALERT 3Ddevice (Biomerieux, France): the tested samples were plated in one vial for the presence of aerobic (BPN) and one for anaerobic bacteria (BPA). The bottles were incubated for 7 days at 37°C. The sensor system detects a change in the color of the surface, sensitive to the change of carbon dioxide concentration. If the bacteria grow, concentration of carbon dioxide is increasing, which leads to a color change on the base of the bottle. Platelet aggregation was determined by impedance aggregometry method (Multiplate Platelet Function Analyzer, Roche). The method measures platelet aggregation that is ex vivo stimulated by application of various platelet agonist (eg. adenosine diphosphate, arachidonic acid, collagen). Multiplate test cells have two independent measuring units, each of them is composed of two copper, silver-coated electrodes 3.2 mm high and 0.3 mm in diameter. The procedure involves mixing of 150 μL PC with 450 μL of buffer (0.81% NaCl, 0.0067 M PO4, pH 7.2) in a particular test cell. After incubation at 37°C for a period of three minutes 20 μL of the selected agonist is added, e.g. collagen in concentrations of 3.2, 6.4 and 9.6 μg/mL. A blood sample containing added agonist is automatically stirred (800 U/min) using a magnetic stirrer coated with poly-tetra-fluoro-ethylene (PTFE). Activated platelets adhere to the electrodes and increase the electrical impedance between them, which is registered within 6 minutes, and the increase in impedance is converted into arbitrary units of aggregation [aggregation arbitrary units (AU)]. The most important parameters monitored are: area under the aggregation curve (AUC), which is directly dependent on the height of the curve, and shows the overall activity of platelets, the height of the aggregation curve, which shows the degree of platelet aggregation and the maximum slope of the aggregation curve, which indicates the rate of platelet aggregation.

Statistical analysis was performed using Statistical Package for Social Science (SPSS Software GmbH, Germany), version 15.0. The results are presented in tables and figures, using the mean values, standard deviations (SD) and medians (Me). Qualitative characteristics of the investigated variables are given as frequency (n) and the percentage (%). Normality of the distribution of continuous variables according to the size of the sample was examined by Kolmogorov-Smirnov or Shapiro-Wilk test. Statistical significance of the experimental data during the storage period was analyzed using the Wilcoxon Signed Ranks Test and the Paired Samples t-test. Statistical significance of the differences between the absolute frequencies of samples was analyzed by the Pearson’s χ² test or Fisher's exact test. The effect of different concentrations of collagen on the monitored variables changes over time was determined by analysis of variance for repeated measures (RM ANOVA).

Results

The average volume of investigated PCs was 58.75 ± 3.92 mL. The results of hematological analysis are shown in Table 1. Comparing values of the investigated hematological parameters of PCs during storage, it was found significantly lower platelet count in PCs on the Days 3 (PC3) and 5 (PC5) in relation to that on the the Day 1 (PC1).

Conformity of PCs quality with the standard quality criteria for blood products during the storage period is shown in Table 2.

Except for the fulfillment of the criteria for platelet count, all the other hematological parameters were in accordance with recommended criteria. On the first day of storage 91.25% of PCs had the required platelet count, on the third day the percentage was reduced to 83.75%, while on the fifth day only 66.25% of the PCs had more than 60 × 10^9/L of platelets.

Values of laboratory parameters and gas analysis during the study period are shown in Table 3. There were significantly lower values of pO2, pCO2 and pH on the third and fifth day of storage. pH, as a required criterion for testing the quality of PCs met the recommended criterion for all the concentrates (80/80, 100%) during the whole period of storage till the fifth day.

### Table 1

<table>
<thead>
<tr>
<th>Blood components</th>
<th>PC1 mean ± SD (median)</th>
<th>PC3 mean ± SD (median)</th>
<th>PC5 mean ± SD (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (x10^12)</td>
<td>0.005 ± 0.010 (0.000)</td>
<td>0.005 ± 0.011 (0.000)</td>
<td>0.004 ± 0.015 (0.000)</td>
</tr>
<tr>
<td>Platelet (x10^9)</td>
<td>62.166 ± 2.416 (62.190)</td>
<td>60.915 ± 2.337*** (61.190)</td>
<td>59.642 ± 2.478*** (60.315)</td>
</tr>
<tr>
<td>Leukocytes (x10^9)</td>
<td>0.007 ± 0.004 (0.006)</td>
<td>0.008 ± 0.004 (0.007)</td>
<td>0.007 ± 0.004 (0.006)</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>58.753 ± 3.919 (58.870)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PC1 – PC on the first day; PC3 – PC on the third day; PC5 – PC on the fifth day; SD – standard deviation; ***p < 0.001 (Wilcoxon Signed Ranks Test).

Table 2
Conformity of platelet concentrates (PC) quality with standard quality criteria during storage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PC1, n (%)</th>
<th>PC3, n (%)</th>
<th>PC5, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes &lt; 0.2–1 × 10^9</td>
<td>80 (100)</td>
<td>80 (100)</td>
<td>80 (100)</td>
</tr>
<tr>
<td>Platelets &gt; 60 × 10^9</td>
<td>73 (91.25)</td>
<td>67 (83.75)</td>
<td>53 (66.25)**</td>
</tr>
<tr>
<td>Leucocytes &lt; 0.05 × 10^9</td>
<td>80 (100)</td>
<td>80 (100)</td>
<td>78 (97.50)</td>
</tr>
<tr>
<td>Volume &gt; 40mL</td>
<td>80 (100)</td>
<td>80 (100)</td>
<td>80 (100)</td>
</tr>
</tbody>
</table>

PC1 – PC on the first day; PC3 – PC on the third day; PC5 – PC on the fifth day; *** − p < 0.001 (χ²-test).

Table 3
Laboratory parameters and gas analysis of platelet concentrates (PC) during storage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PC1</th>
<th>PC3</th>
<th>PC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.191±0.067 (7.178)</td>
<td>7.181±0.063*** (7.171)</td>
<td>7.170±0.062*** (7.161)</td>
</tr>
<tr>
<td>pO₂</td>
<td>137.056 ± 7.888 (137.50)</td>
<td>130.87 ± 7.593*** (130.75)</td>
<td>123.70 ± 6.544*** (122.95)</td>
</tr>
<tr>
<td>pCO₂</td>
<td>49.259 ± 2.604 (48.950)</td>
<td>42.339 ± 2.739*** (42.30)</td>
<td>23.854 ± 3.012*** (23.650)</td>
</tr>
<tr>
<td>pH &gt; 6.4, n (%)</td>
<td>80 (100)</td>
<td>80 (100)</td>
<td>80 (100)</td>
</tr>
</tbody>
</table>

PC1 – PC on the first day; PC3 – PC on the third day; PC5 – PC on the fifth day; SD – standard deviation; *** − p < 0.001 (Wilcoxon Signed Ranks Test, Paired Samples t test).

Table 4
Platelet aggregation induced by collagen during storage

<table>
<thead>
<tr>
<th>Collagen concentration (μg/mL)</th>
<th>PC1 mean ± SD (median)</th>
<th>PC3 mean ± SD (median)</th>
<th>PC5 mean ± SD (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>1057.29 ± 98.07 (1056.0)</td>
<td>590.14 ± 80.15*** (591.50)</td>
<td>252.46 ± 56.75*** (250.00)</td>
</tr>
<tr>
<td>6.4</td>
<td>1111.18 ± 111.3 (1133.5)</td>
<td>966.90 ± 127.04*** (985.00)</td>
<td>734.18 ± 120.12*** (741.00)</td>
</tr>
<tr>
<td>9.6</td>
<td>1181.65 ± 119.3 (1215.0)</td>
<td>1094.49 ± 121.89*** (1119.0)</td>
<td>956.15 ± 120.16*** (986.00)</td>
</tr>
</tbody>
</table>

PC1 – Platelet concentrates on the first day; PC3 – Platelet concentrates on the third day; PC5 – Platelet concentrates on the fifth day; SD – standard deviation; *** − p < 0.001 (Wilcoxon Signed Ranks Test, Paired Samples t-test).

Table 5
The effects of different collagen concentrations on platelet aggregation after 1st, 3rd, and 5th storage day – the results of generalized linear method for repeated measurements

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All concentrations</th>
<th>3.2 μg/mL</th>
<th>6.4 μg/mL</th>
<th>9.6 μg/mL</th>
<th>The effect of different concentration</th>
<th>Interaction concentration× time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet aggregation</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Size effect</td>
<td>0.9695</td>
<td>0.9826</td>
<td>0.9636</td>
<td>0.9327</td>
<td>0.7669</td>
<td>0.9013</td>
</tr>
</tbody>
</table>

*Partial eta squared (ηp²).

Platelet aggregation of PC1, PC3 and PC5 triggered with different concentration of collagen is shown in Table 4. It is observed that for all the concentrations of collagen there was a decrease in the aggregation during the storage period, with statistically significant differences for PC3 and PC5 as compared to aggregation for PC1 (p < 0.01).

Based on the data in Table 4, as well as a generalized linear model for repeated measurements (Table 5), taking into account the different concentrations of collagen, it was confirmed that during the storage there was a significant decrease in capability of platelets to aggregate regardless of collagen concentrations used (p < 0.001).

Also, it was found that these changes were statistically significantly different among the different collagen concentrations (the smallest decrease of aggregation was observed for collagen at the highest concentration, and the highest decrease of platelet activity was at the lowest concentration of collagen, p < 0.001).

All PCs had negative results for microbiological control till the end of the storage period.

Discussion
Considering that the main objective of the transfusion of blood products is to achieve the highest therapeutic effect...
with high safety of the transfusion, it is clear that the evaluation of the quality of prepared blood products is of great importance for efficient transfusion. History of platelet transfusion begins in 1960s with the detection of association between hemorrhagic syndrome and decreased number of platelets in the circulation of patients. Since then there have been many changes in the way of preparation and storage of PCs, with the aim of improving the quality of the product. On the other hand, the quality of PC is not just an accordance with the criteria of quality system, but also the ability of transfused PC to provide in vivo hemostatic support. Many studies confirmed that only 66% of transfused platelets circulate freely, and many factors can affect their function, such as infection, the use of antibiotics and anti-inflammatory drugs, as well as previous separation and storage lesions in PCs. According to Gulliksson there are the three most important points in the process of preparation and storage of PCs that are essential for maintaining good quality of the products. First, it is important to prevent or reduce the platelet activation during the blood collection, preparation and storage of PCs. Second, the level of glycolytic activity, anaerobic glucose consumption and lactate production should be maintained at the lowest level. Third, it is important that a certain amount of glucose must be present in tissue during the whole period of storage.

Numerous studies have shown that there are significant changes in PCs during the storage period, both in number and platelet function, and biochemical alterations with consequent changes of intracellular metabolism. During storage period platelet count in PCs progressively decreases comparing to their numbers immediately after preparation. There are changes of the morphological distribution and morphological score of platelets, wherein the number of the discoid and spherical shaped platelets decreases, while dendritic and ballooned cells, which have functionally lesser value, are increasing. This investigation showed that the average number of platelets on the first and third day of the storage met the required recommendations (on the first day of storage 91.25% of investigated PCs had a platelet count greater than $6 \times 10^9$, and on the third day 83.75% of PCs had the required number of platelets). During storage till the fifth day, platelet count was statistically significantly decreased, while the average number of platelets was below the required value, and only 66.25% of the PCs had a number of platelets greater than $6 \times 10^9$.

In the analysis of the leukocyte content in PCs, we obtained less than $0.05 \times 10^9$ leukocytes, which indicated normality. This is very important because leukocytes are responsible for a variety of acute and delayed transfusion reactions, primarily an immune-mediated reactions, transmission of the viruses and graft-versus-host disease, but also in vitro production of inflammatory cytokines and the development of febrile non-haemolytic transfusion reactions. All the investigated samples of PCs remained sterile for aerobic and anaerobic microorganisms until the end of the storage. This is also of the great importance as the presence of bacteria in PC can lead to sepsis and other transfusion reactions. Data from the literature presents the incidence of bacterial contamination of 1 in 2,000 PCs.

PH is an important marker of the quality of PCs in vitro since at values below 6.8 platelets become spherical and this change in shape becomes irreversible when PH drops below 6.2. This investigation showed statistically significant decrease in pH, which appears to be a consequence of a greater permeability of the plastic bag for PC storage to gases, particularly CO$_2$. These changes in gas concentrations lead to changes in the concentrations of bicarbonate, with a resulting buffering of the system and the change of pH.

Although gas analysis do not belong to the group of mandatorv parameters for quality testing, recommendations imply that the level of blood gases should stay at a constant level, ideally as on the first day. A number of studies show that this does not occur in the practice, mostly depending on the kind of the used bag and a temperature of 22°C. Obtained results showed that pO$_2$ and pCO$_2$ significantly decreased on the third and the fifth storage day. It is known that during storage of PCs extracellular alterations can cause cellular lesions, implying metabolism variations and function decrease.

The focus of this study was functional testing of platelets in PCs. Collagen is known as a very potent platelet agonist which activates several intracellular metabolic systems with different receptors on platelet membranes. Thus, it binds to von Willebrand factor (vWF), creating an adherence bridge between collagen and the platelet glycoprotein Ib receptor. Granule content secretion is triggered by platelet surface receptor agonists. In our study, the results of platelet aggregation showed that after 5 days of storage a reproducible aggregation response could be determined, but there was statistically significant decrease in platelet aggregation on the third and fifth day of storage compared to the first day. Also, there was statistically significant decrease in activity loss between PCs triggered with higher concentration of collagen (6.4 and 9.6 μg collagen/mL) and with smallest concentration of collagen used (3.2 μg/mL). A possible explanation for these changes might be the changes of pH, increase of lactate concentration as well as the variation in the composition of the platelet membrane.

Considering the changes that have been proven in the PC quality during storage it is important to identify factors that may be important to reduce the level of these changes and to improve the quality of PCs, especially from the third to the fifth day of storage. A large number of investigations on this subject concluded that there are many factors that can be singled out as determinants of functional and biochemical changes in PCs. The most important of them are temperature, volume, agitation and the kind of plastic bag used for conservation of PCs. As all the investigated parameters of PC1 showed a high level of quality, we can conclude that the blood collection process did not significantly affect the platelet quality. Our results lead us to the conclusion that in the conditions under which PCs were prepared and stored, there were the two main factors affecting the quality of PCs. These were the kind of bag for storage of PCs and plasma, as a medium for storage of platelets. The observed gas exchanges are capable of causing platelet lesions, altering their metabolism, which, on the other hand, lead to significant platelet activation and reduction of functional capacity.
to choose some other type of plastic bags for PC storage which can provide an environment that results in an improved product quality and will permit 5-day storage of PCs with preserved quality. On the other hand, it is known that platelets derive energy from glucose oxidation through glycolysis and β-oxidation of long-chain fatty acids, and during the storage of PC there is an increase of glucose metabolism by the glycolitic process, lactate production is also increased while pH and production of bicarbonate are decreased. Various authors have suggested that instead of plasma should be used platelet additive solutions (PAS) for PCs storage in order to improve their quality. PAS contains acetate as a nutrient medium for platelets, which is a basic substrate for normal platelet metabolism, it reduces the production of lactate and increases the production of hydrogen carbonate, which finally leads to pH stabilization. Additionally, acetate acts as a buffer. The other ingredient of PAS is phosphate, which has a double effect on the metabolism of platelets. In addition to its action as a buffer, a phosphate also stimulates glycolysis, which can lead to a significant drop in pH, but also the production of higher levels of ATP, which allow greater platelet viability. Magnesium and potassium in PAS reduce the activation and aggregation of platelets, maintain the morphological score and decrease in lactate production. A number of previous studies have shown that in vitro quality of platelets stored in the PAS is a statistically significantly improved compared to platelets that are stored in the plasma, and bearing in mind the other advantages of PAS (reduction of the incidence of allergic and febrile transfusion reactions, ABO-incompatible transfusion of platelets, pathogen inactivation, increased amount of plasma available for fractionation, storage of PCs for 7 days), it is necessary to include PAS in routine transfusion practice for PC preparation and storage.

**Conclusion**

Platelet count, pO2, pCO2, pH and the platelet aggregability were statistically significantly changed during the storage period. In order to improve the quality of PCs it is important to store the products under proper conditions, change the type of plastic bag for PC storage and the use PAS instead plasma.

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