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# Determination of the optimum platelet-rich plasma method for the essential amount of platelets

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## Abstract

**Aim:** Platelet-rich plasma (PRP) is the cellular plasma component containing a higher platelet concentration obtained by centrifugation of autologous peripheral blood. PRP has an auxiliary role in regenerative medicine treatment. Current treatment protocols for tissue repair strategies may be globally insufficient. PRP treatment via Platelet-released growth factors (GF) supports inflammation, proliferation, and wound healing. PRP studies have progressed and have proposed different formulations in recent years. Although various methods and devices are used, there are few and different standardized protocols to obtain PRP with the highest platelet concentration. Although anticoagulants containing citrate are primarily preferred in PRP production To provide effective platelet concentration in PRP, we noticed differences in the literature regarding PRP tube volumes, anticoagulant-citrate, and blood amounts. We aimed to obtain the most appropriate platelet concentration in PRP treatment by using different concentration ratios of Acid Citrate dextrose (ACD) solution A and different blood amounts and introducing a new treatment method to the literature.

**Materials and Methods:** The blood of 30 individuals aged between 30 and 75 was taken into 10cc laboratory tubes, and 15cc and 18cc hourglass-shaped PRP tubes containing 8% ACD-solution A. Blood fractions were separated by centrifugation, buffy coat area was collected, and count platelet amount by Hematology Analyzer.

**Results:** The platelet amount per µl was statistically significantly higher in 18cc blood compared to 15cc and 10cc. Platelet amounts obtained from 15 and 18cc peripheral blood with 8% ACD-solution A were above the effective treatment amount recommended in the literature.

**Conclusion:** It was observed that the amount of used blood volume was correlated with the amount of obtained platelets.



## Introduction

Platelets are predominantly 1-4 µm sized, anucleate, and disc-shaped blood cells with protruding, formed by budding from Megakaryocytes (MKs), which have a size of 50-100 mm and differentiate from pluripotent hematopoietic cells in the bone marrow [1, 2]. Platelets circulate 5-7 days after formation and primarily function as regulators of hemostasis and thrombosis. Platelets regulate vascular hemostasis, innate immunity, vascular tumor growth, and extravasation. They also affect inflammation, angiogenesis, regeneration, stem cell migration, and cell proliferation with the GF and cytokines they secrete. The abundance and easy availability of GFs have made them preferable in the treatment [3-6].

PRP is a plasma component obtained by centrifugation of venous blood and contains a higher concentration of platelets than venous blood. Blood cellular component includes 95% erythrocytes, 5% platelets, less than 1% leukocytes, and many fibrin; on the other hand, PRP consists of 4% erythrocytes, 95% platelets, and 1% leukocytes [7-10]. Citrated blood prevents coagulation during centrifugation to obtain PRP and plasma-rich GF (PRGF) [11]. Platelet-rich fibrin (PRF), a second-generation platelet aggregate, is widely used to accelerate soft and hard tissue healing due to various GFs. In vitro studies have shown that PRF induces strong and highly significant osteoblast differentiation and sustained stimulation and proliferation of all cell types [12]. For PRF, blood is collected without anticoagulant and immediately centrifuged during the natural coagulation [11].

In the 1970s, the term PRP was first coined by Hematol-

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ogists, using platelet-derived from transfused peripheral blood in treating patients with thrombocytopenia [13]. In the same year, an experimental study was conducted to heal skin wounds using fibrin glues in a rat model. Platelets in the fibrin gel also showed excellent preliminary results in ophthalmology, neurosurgery, and general surgery. In the following years, successful results were obtained in treating skin ulcers with platelet-derived wound healing factors (PDWHF) [9]. In a study, the therapeutic effects of PRP on cartilage, tendon, and muscle damage were demonstrated; however, there is no consensus on appropriate patient selection and the number and frequency of injections [14].

There are differences of opinion in the literature about obtaining the highest platelet with PRP. For this purpose, various methods have been used to prepare PRP [15]. There is no standardized PRP preparation technique. Citrate-containing anticoagulants used in producing PRP are easy to obtain and provide a high amount of platelet. Until now, it has always been discussed which anticoagulant might be the most effective for PRP preparation in regenerative therapy. It is stated that the most effective PRP may be related to the release and quality of GFs and the platelets they contain [16]. In a study, as a result of the use of anticoagulant sodium citrate (NC), ethylenediaminetetraacetic acid (EDTA), and ACD-solution A in the production of PRP, the highest platelet amount was obtained with NC. The researchers noted that with the use of ACD-solution A, they obtained even more platelets by dividing them into smaller tubes without the use of additional anticoagulants. It is stated that platelet recovery depends not only on the type of anticoagulant but also on the tube format and may even have a unique effect than serum centrifugation on whole blood/plasma, given the high viscosity of the tube format [17]. It is suggested that ACD-solution A is a much better anticoagulant than NC to preserve platelets' structural and physiological properties during the PRP preparation phase, where the processing of blood samples may take two or more hours [18].

The critical thing in PRP preparation is to choose an anticoagulant that can best preserve the functionality and morphology of platelets. Our study aims to determine the number of platelets in PRPs obtained with different concentrations of ACD-solution A and different amounts of venous blood and to introduce a new treatment method to the literature.

## Materials and Methods

### *Study design*

Akdeniz University Human Ethics Committee approved this study with protocol number 05.05.2021/KAEK-316. The research was carried out jointly with Çallı Meydan Medical Center Orthopedics and Traumatology Clinic and Akdeniz University Medical Faculty Histology and Embryology Department. Patients were collected from a single center. Patients aged from 30 to 75 years who applied to the Çallı Meydan Medical Center Orthopedics and Traumatology outpatient clinic were included in the study. Written and verbal informed consent were obtained from 30 randomly selected patients, and blood samples were included in the study, considering the following criteria. Since our study was experimental, not a therapeutic one, the sample size was determined as 30 patients by examining the literature, and considering that there may be variability due to individual differences, each patient's blood was used for each group of tubes. It was taken into account that the blood pressure values of each patient were within the normal range before blood was drawn. After the blood pressure measurements were made again 30 minutes after the blood collection, the blood pressure values were checked before and after the blood collection, and the patients were sent to their homes. While selecting the patients, a random distribution was made, with male and female populations in each group. Since the patients were selected from the regular patients who came to the outpatient clinic, the necessary follow-ups were evaluated by A.Y. Severe trauma, active infection, diabetes, serious cardiovascular disease, coagulation disorder, autoimmune disease, and cancer history, taking immunosuppressive therapy, hemoglobin value $< 11$ g/dl or platelet value $> 150{,}000$, using the anticoagulant or antiplatelet medication, pregnant or breast-feeding individuals were excluded from the study. The blood taken from each individual in the study was transferred to 10cc laboratory tubes, 15cc and 18cc hourglass-shaped PRP tubes. PRP obtaining and measurements were made at Çallı Meydan Medical Center.

When the results were first transferred to the researcher (E.K.) performing the analysis, the researcher was blind to the data. The researcher made and evaluated the statistics of the first groups as groups 1, 2, and 3 (Group1-10cc laboratory tube, Group2-15cc hourglass-shaped PRP tube, Group3-18cc hourglass-shaped PRP tube).

### *Obtaining PRP*

Whole venous blood was collected under solid aseptic conditions from each patient using a 22G needle tip into syringes containing 4% and 8% ACD-solution A. Hemogram values were determined by counting some of the blood with Medonic M16M Hematology Analyzer (Boule Medical AB SE-12613 Stockholm, Box 42056 SWEDEN). The other part of the blood was transferred to a 10cc laboratory tube, 15cc, and 18cc hourglass-shaped PRP tubes and centrifuged at 3000 rpm for 10 minutes (INODT5-6B centrifuge, Inovia technology). After centrifugation, the three regions of the erythrocyte, buffy coat, and plasma were separated in the tube. The Buffy coat region containing leukocytes and platelets was collected into the injector with a 22G needle. The number of platelets in it was measured with the help of Medonic M16M Hematology Analyzer. In using 4% ACD-solution A, a high rate of PRF occurred due to early coagulation, and a healthy platelet count could not be performed. For this reason, the study was continued with 8% ACD-solution A, and the platelet numbers were determined from tubes with different volumes and shapes.

### *Statistical analysis*

The results obtained from all groups were evaluated with the One-way ANOVA Bonferroni's Multiple Comparison Test using the GraphPad Prisms10.0.2(232) version (BOSTON, USA) statistical analysis program. P values

<0.01 were considered significant. ±SEM values were taken in the evaluation.

## Results

Our study compared ACD-solution A percentage, tube type, and blood amounts to determine the PRP containing the most appropriate platelet amount and bring a new treatment method to the literature. It was determined that 4% ACD-solution A is unsuitable for obtaining PRP, and the minimum platelet amount required for treatment can be obtained with 8% ACD-solution A. The platelet amount obtained from the blood taken into 10cc, 15cc, and 18cc PRP tubes containing 8% ACD-solution A used to prevent clotting was compared by analyzing them with the GraphPad statistical program. We observed that 15cc and 18cc hourglass-shaped PRP tubes have higher platelet capture efficiency than 10cc laboratory tubes. Although there was significant interindividual variation in platelet yield from the buffy coat collected using both types of tubes, the amount of platelets obtained with the hourglass-shaped PRP tubes was statistically higher than that obtained with the laboratory tube (Figure 1).

As a result of our study, it was found that the platelet amounts obtained with 18cc were statistically higher than 15cc and 10cc, and that obtained with 15cc was statistically higher than 10cc. Platelet amounts for 15 and 18cc were obtained as equal and higher than the minimum platelet amounts [19-21] recommended for treatment, respectively.

## Discussion

PRP applications are among the auxiliary regenerative medicine treatment options. There is a global need to treat patients with osteoarthritis (OA) and spine disorders. OA, in particular, is a worldwide disease that increases in prevalence with age, affecting many joints and causing disability in load-bearing joints [22, 23]. There are several commercially marketed PRP systems. These systems work with the principle of preparing platelet-rich suspensions ready for application by collecting and concentrating platelets and leukocytes with different centrifugation methods and times of a small amount of blood, such as 20-60 ml on average. The different concentrations

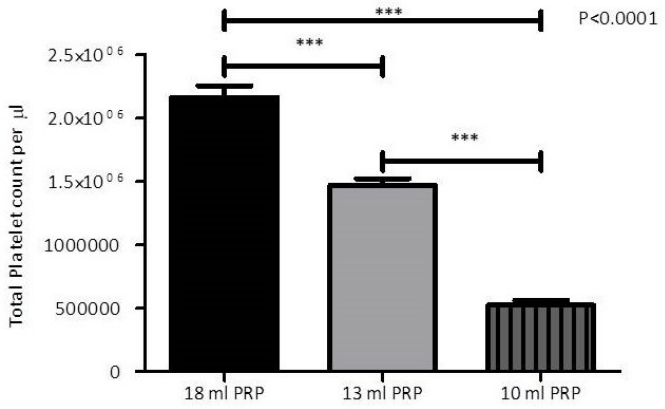


**Figure 1.** Platelet quantities obtained from 18cc, 15cc and 10cc tubes containing 8% ACD-solution A. P<0.0001.

of growth factors in the PRP content are related to the differences in the platelet and WCB concentrations. PRP platelet concentrations obtained by commercial systems have significant differences within themselves. Low (2.5-3 times the initial concentration) and high (5-9 times the initial concentration) efficiency systems are used to obtain PRP. High-efficiency devices include Biomet GPS II and III (platelet count 3-8 times), platelet SmartPRep 2 APC+ (4-6 times), and ArterioCyte-Medtronic Magellan (3-7 times); among the systems with low concentration is Arthrex ACP (2-3 times), Cascade PPR treatment (1-1.5 times) and PRGF (2-3 times) from Biotech Institute Vitoria, Spain [19].

Plasma-based PRP systems such as ACP, Cascade, Endoret, and RegenPrep, defined as low-efficiency devices, often contain a platelet concentration between baseline and 3x baseline. On the other hand, buffy-coat-based systems from high-efficiency PRP-producing devices such as GPS III, SmartPrep, and Magellan give platelet concentrations ranging from 4x to 6x (average 750 × $10^3$ platelets/µL to 1800 × $10^3$ platelet/µL). Successful results have been obtained from the results of many in vitro, in vivo, and clinical studies performed with medium (2x and 3x) and high platelet concentration (4x to 6x) PRP preparations. In an in vitro study, it was pointed out that the best angiogenic effect in PRP treatment was obtained with 1500 × $10^3$ platelets/µL, and increased angiogenesis contributed to the healing process [24].

In their study, Marx et al. showed that they increased bone and soft tissue healing with a minimum 1× $10^6$/µL platelet count [22,25]. It was observed that the platelet cell counts we obtained as a result of our study with two separate volumes of PRP tubes in the shape of an hourglass were correlated with the number of platelet cells (1×$10^6$ / µL) suggested by Marx et al. in the literature results for bone and soft tissue healing.

A study made with novel generation platelet concentrated growth factors (CGFs) has shown that the dose of PRP also affects the magnitude of the treatment outcome [26]. Giusti et al. demonstrated that a dose of 1.5 × $10^9$ platelets/mL is required for tissue repair by inducing a functional angiogenic response by endothelial cell activity [27]. However, an excessive amount of platelets causes a paradoxical inhibitory effect through cellular apoptosis, downregulation and desensitization of growth factor receptors [20]. Apart from being dose-dependent, the effects of PRP on cell activity appear to be time-dependent. Soffer et al. reported that short-term exposure to human platelet lysate stimulates bone cell proliferation, and chemotaxis, while long-term exposure to PRP results in low alkaline phosphatase levels and mineral formation [28].

In our study, using an hourglass-shaped tube and 8% ACD-solution A, we provided the platelet amount required for effective bone and soft tissue treatment recommended in the literature with less peripheral blood (15cc).

## Conclusion

Our study did not achieve the necessary cell levels for treatment with 10 cc tubes with 8% ACD-solution A. We have always determined the number of cells required for

treatment in hourglass-looking tubes in 15 and 18-cc tubes. Our results showed that the number of platelets obtained with similar tubes in our study correlated with the initial blood volume ratio.

*Conflict(s) of interest/ disclosure(s)*

The authors declare no conflict of interest

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*Ethical approval*

Akdeniz University Human Ethics Committee approved this study with protocol number 05.05.2021/KAEK-316.

*Authors contributions*

Esma Kirimlioglu (E.K.) performed the data analysis, drafted the manuscript, and assisted this project's hypothesis. Ahmet Yapıcı (A.Y.) provided the funding and consent from patients, collected all patients' blood and obtained their PRP, counted their platelets, and assisted in drafting the manuscript.

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