Correlation of Platelet Concentration in Platelet-rich Plasma to the Extraction Method, Age, Sex, and Platelet Count of the Donor

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An important reason to improve methods of isolating platelet-rich plasma (PRP) is the potential use of autologous platelet growth factors. In addition to discontinuous plasma separation, a second method for extraction of PRP has now become available, which can be performed directly by the surgeon. In this study, the suitability of the 2 methods of producing PRP was compared. Whole blood was drawn from 158 healthy donors (112 men, 46 women) aged 20 to 62 years (mean 34, SD 10). The PRP was separated by the discontinuous plasma separation method (by the blood bank) or by the so-called "buffy coat" method (the "self-concentration" method, analogous to the PRP Kit, Curasan, Kleinostheim, Germany). Platelet counts differed significantly according to donor blood (median men 237,500/μL, women 272,000/μL), blood bank PRP preparation (median men 1,302,000/μL, women 1,548,500/μL), and self-concentrated PRP (median men 944,000/μL, women 1,026,000/μL). The platelet concentration of the blood bank PRP correlated with the platelet count in the donor whole blood (Spearman's correlation coefficient $r_S = 0.73$). However, there was no significant correlation between the platelet count of self-concentrated PRP and donor whole blood ($r_S = 0.22$). Significant but irrelevant influences of sex on platelet concentration were found, but no influence of age was detected. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:693–699)

Key words: *growth factors, plasmapheresis, plateletpheresis, platelet count*

Platelets contain a number of different growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor beta 1 (TGF beta 1), transforming growth factor beta 2 (TGF beta 2), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial cell growth factor (ECGF), and a growth factor for hepatocytes.[1] Using platelet-rich plasma (PRP) concentrates as a source of autologous growth factors, Marx and coworkers[2] showed an increase in bone formation and bone density (radiographically and histologically) after autologous bone grafting in 44 patients. The use of recombinant growth factors in combination with different bone regeneration materials has been much debated in recent years.[3–10] Treatment with PRP to support osseointegration of endosseous dental implants has also been described.[4] To date, there have been some promising case reports, but no controlled studies.

The use of platelet concentrates obtained from blood banks (by the discontinuous plasmapheresis method) as a source of endogenous growth factors is limited because of known health risks[11] for the patient and high production costs (about DM 500).[12] However, a way to produce small amounts of PRP has recently become commercially available (PRP Kit, Curasan, Kleinostheim, Germany). This

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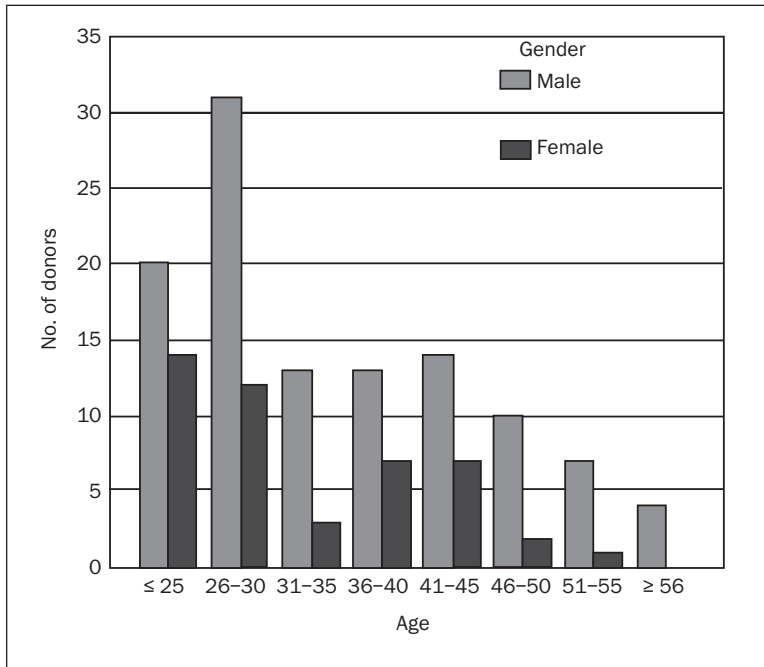
Fig 1 Distribution of age and gender of donors.

method is both more acceptable to the patient for clinical treatment and less expensive (about DM 20). However, it has not yet been proven whether this system is able to produce PRP with a platelet concentration equal to the PRP produced by the blood banks. The possible influence of patients' age, gender, and preoperative platelet count also remains unclear. Therefore, this study analyzed the influence of donor age, gender, and platelet count on the platelet concentration in the platelet concentrates produced by the 2 different methods.

MATERIALS AND METHODS

Between June 20 and 27, 2000, blood samples were collected from 158 healthy donors (112 males, 46 females) aged 20 to 67 years (mean 34, SD 10) at the Johannes Gutenberg University transfusion bank (Fig 1). The blood used for the presented analysis was residual from the routinely drawn blood (for serologic analysis, etc) via a cannula already in place for the plasmapheresis.

The blood was filled in an 8.5-mL tube containing 1 mL citrate, phosphate, dextrose, and adenosin (CPDA, 8.5-mL CPDA monovette, catalog no. 01.1610.001, Sarstedt, Nümbrecht, Germany) for "self-concentration" of 0.4 to 0.5 mL PRP by a method analogous to the PRP Kit (see below for method). Subsequently, the blood bank produced

approximately 300 mL of platelet concentrate using the discontinuous flow separation method (see below). The platelet counts of the whole blood, platelet concentrate, and self-concentrated PRP were determined automatically (Cell Dyn 3500, Abbott, Wiesbaden, Germany). All donors had platelet counts > 150,000/µL, as dictated by the blood bank's criteria for platelet donation.

To reduce the cost of materials, components of the PRP Kit were not ordered from the distributor (Curasan), but rather were purchased directly from the manufacturers (Sarstedt; Braun, Melsungen, Germany; and Kendro Laboratory Products, Osterrode, Germany). Replication of the commercial system was identical in every respect, except for the sterile filter; since the samples were not going to be used in patients, intake air cannulas without sterile filters were employed. Because there is no contact of the air intake cannulas with the PRP, no changes are to be expected to the resulting PRP.

Production of Platelet Concentrates by Discontinuous Flow Separation

The discontinuous flow separation procedure is characterized by an intermittent flow of donor blood that is supplemented with an anticoagulant and allowed to flow into a rotating centrifuge cup. The cellular blood components are then separated into erythrocytes, buffy coat (mostly platelets and some leukocytes), and plasma (containing relatively

Table 1 Descriptive Statistics of Platelet Counts of Donor Blood, PRP from Blood Bank, and Self-concentrated PRP

	Platelet count in whole blood (/µL)		Platelet count in blood bank PRP (/µL)		Platelet count in self-concentrated PRP (/µL)	
	M	F	M	F	M	F
n	112	46	112	46	112	46
Mean	244,723	280,609	1,334,387	1,606,587	892,830	979,587
Median	237,500	272,000	1,302,000	1,548,500	944,000	1,026,000
Standard deviation	46,241	61,369	301,807	341,061	459,771	532,299
Minimum	155,000	187,000	598,000*	1,076,000	41,000	56,000
Maximum	428,000	459,000	2,222,000	2,716,000	1,972,000	2,156,000
Percentile						
10	191,000	216,700	1,055,800	1,184,100	234,400	232,700
25	211,000	231,500	1,143,750	1,404,500	527,750	424,000
75	268,750	300,000	1,542,250	1,742,750	1,221,000	1,402,500
90	299,000	384,400	1,731,500	2,076,000	1,492,900	1,650,600
95% confidence interval	236,065–253,381	262,384–298,833	1,277,877–1,390,898	1,505,304–1,707,869	806,743–978,918	821,514–1,137,660

*There was one additional extreme value of 21,400/µL.

few cells) by a 2-step sedimentation procedure. As the centrifuge cup is refilled, the individual fractions exit the cup through automatic pressure valves and enter 3 separate bags. Some blood is allowed to recirculate into the centrifuge cup to increase the platelet output by keeping blood in the centrifuge chamber for a longer period. After retransfusion of erythrocytes and plasma, the separation steps are performed an additional 5 to 6 times until the predefined volume of PRP is attained.

Self-Concentration of Platelet-Rich Plasma
To produce PRP extracts, 8.5 mL of citrated blood (8.5 mL CPDA monovette, Sarstedt, catalog no. 01.1610.001) was centrifuged in a standard laboratory centrifuge (Heraeus Labofuge 300, Kendro Laboratory Products) for 10 minutes at 2,400 rpm. Subsequently, the yellow plasma (containing the platelets) was taken up into a neutral monovette with a long cannula, using an additional air-intake cannula. To combine the platelets into a single pellet, a second centrifugation step was performed with this second monovette for 15 minutes at 3,600 rpm. The plasma supernatant (containing relatively few cells) was then reduced to approximately 0.4 mL (by taking up with a second neutral monovette, a long cannula, and an air-intake cannula). The pellet of platelets was resuspended in the residual 0.4 mL of plasma using a conventional shaker (MS1 Min-

ishaker, IKA, Staufen, Germany) and transferred to an Eppendorf tube for later analysis.

Statistical Methods
All quantitative measurements are described using the summary statistics (n, mean, standard deviation [SD], and minimum and maximum). To account for non-normality, median, first and third quartiles, and other quartiles are reported additionally. The 3 platelet counts (donor whole blood, blood bank PRP, and self-concentrated PRP) were compared by Spearman rank correlation coefficients. Scatter plots were used to demonstrate the relationship between the whole blood and PRP platelet counts. For the analysis of difference of 2 dependent rank correlation coefficients, a test as proposed by Choi[13] was performed. In addition, Mann-Whitney U tests and Spearman rank correlation coefficients were calculated to evaluate possible influences of gender and age on the respective platelet counts.

The relationship between the 2 platelet concentration methods was depicted graphically in a plot representation proposed by Bland and Altman.[14] This plot shows the relationship between the mean and the difference of the 2 corresponding platelet measurements obtained by the 2 concentration methods. Spearman rank correlation coefficients (r_S) were calculated to show the relationship between the self-concentrated PRP platelet counts and the leukocyte and erythrocyte counts.
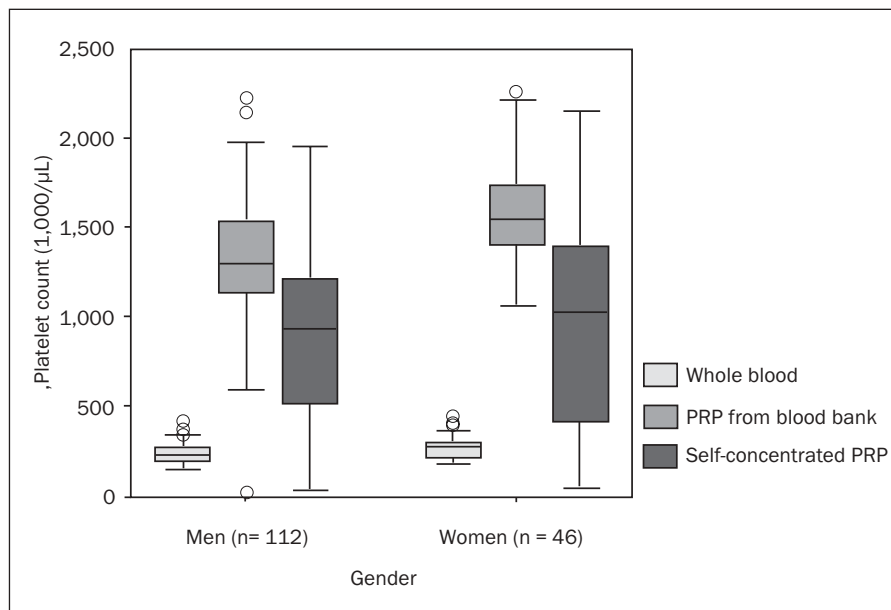
Fig 2 Platelet counts of the donor whole blood, the PRP of the Transfusion Center (blood bank), and the self-concentrated PRP.

RESULTS

Donor platelet counts from whole blood had a mean value of 255,170/μL (SD 53,470/μL) (Table 1). Platelet counts in blood bank–produced PRP had a mean value of 1,413,640/μL (SD 336,350/μL), including 1 extremely low value. The platelet counts in the self-concentrated PRP had a mean of 918,090/μL (SD 481,900/μL). For the latter method, 10% of the resulting platelet counts were lower than those of the donor whole blood. The summary statistics are shown in Table 1.

The platelet concentrations of whole blood (Fig 2) and blood bank–produced PRP were slightly higher for females (n = 46) than for males (n = 112) (mean gender-related difference, platelet count of whole blood 35,886/μL; mean gender-related difference, blood bank–produced PRP 272,200/μL; for both groups, Mann-Whitney U tests for differences in gender: $P < .001$ [$P = .0003$ for whole blood; $P = .04 \times 10^{-6}$ for blood bank PRP]). In the self-concentrated PRP, however, no statistically significant difference in gender of the donors was found (mean gender-related difference, platelet count of self-produced PRP 86,757/μL; Mann-Whitney U test for gender: $P = .286$).

Both analyzed methods of PRP production produced a statistically significant concentration of platelets versus whole blood (Spearman rank correlation coefficient, whole blood versus blood bank PRP, $r_S = 0.73$, $P < .001$; whole blood versus self-concentrated PRP, $r_S = 0.22$, $P = .006$) (Table 2). The platelet concentration in blood bank PRP differed significantly from that of self-concentrated PRP (test for difference of 2 dependent rank correlation coefficients as given by Choi[13] shows a significant difference: $P < .0001$). As shown in the Bland-Altman plot (Fig 3), the mean concentration of platelets/μL in the blood bank PRP was consistently higher, with an approximate difference of 500,000. The advantage of the blood bank method was even greater (an increase of approximately 800,000/μL) for donors with lower platelet counts in the PRP (600,000 to 1,200,000/μL). The discontinuous plasmapheresis method gave an increase in the platelet count of approximately 5 times the initial concentration in donor blood, whereas the self-concentrating PRP method resulted in a mean increase of approximately 3.5 times the initial concentration. The platelet counts of self-produced PRP (median 958.000/μL) showed a higher degree of data point scatter than the blood bank–produced PRP (median 1,366,000/μL) (Fig 4).

The platelet counts for PRP produced by the blood bank were correlated with those of the donor whole blood ($r_S = 0.73$), but no substantial relevant correlation was found between the platelet counts of the self-concentrated PRP and those of the donor whole blood ($r_S = 0.22$). No statistically significant influence of age on the respective platelet counts was seen (all correlations, $r_S < 0.08$; Table 2).

Table 2 Results of Spearman Rank Correlation (n=158)

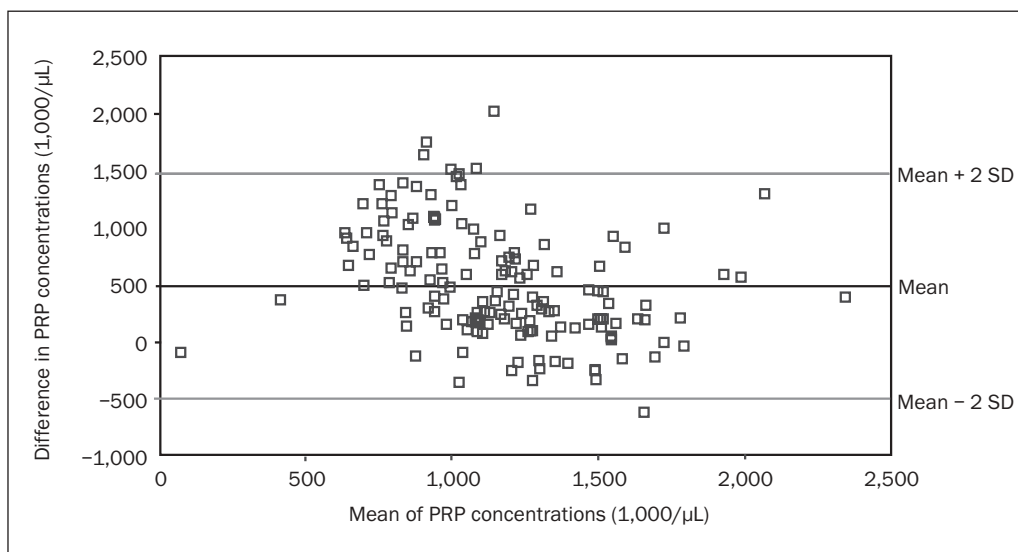|	Platelet count in whole blood	Platelet count in blood bank PRP	Platelet count in self-concentrated PRP
Platelet count in whole blood			
r_s	—	0.727	0.219
Significance 2-sided	—	$.1 \times 10^{-7}$	.006
White blood cells in self-concentrated PRP			
r_s	0.176	0.115	0.332
Significance 2-sided	.027	.151	$.02 \times 10^{-5}$
Red blood cells in self-concentrated PRP			
r_s	0.278	0.110	0.058
Significance 2-sided	$.04 \times 10^{-2}$	.170	.467
Age			
r_s	0.010	−0.062	−0.078
Significance 2-sided	.902	.438	.330

Fig 3   Bland-Altman plot. The mean difference between the platelet counts (PRP of the Transfusion Center minus self-concentrated PRP) was 496,000/µL. This demonstrates reliably higher concentration by the blood bank method. The distribution of the measurements is demonstrated by the mean and 2 standard deviations below (−504,000/µL) and above the mean (1,495,000/µL).
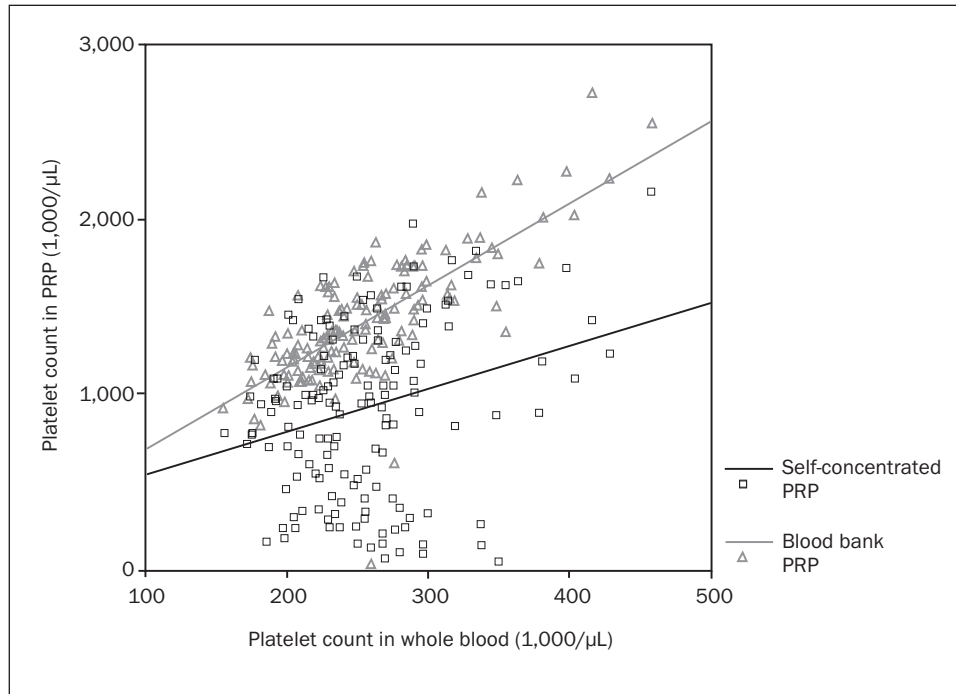
Fig 4 Scatter plot of the platelet counts of the different PRPs according to platelet counts of the donor blood.

Only small positive correlations were found between the platelet counts obtained from self-concentrated PRP and the corresponding leukocyte counts (mean 30,371/µL, SD 1,124/µL) (r_S = 0.33), but not for the erythrocyte counts (mean 3,976/µL, SD 135/µL). The PRP produced by the blood bank contained only small amounts of white leukocytes (mean 154/µL, SD 29/µL) and erythrocytes (mean 450/µL, SD 11/µL).

DISCUSSION

The whole blood platelet concentrations of this sample population were in the range of those expected for healthy individuals. Since no significant influence of age on the resulting platelet count in PRP had been found, the method would seem to be useful at all patient ages. Females have a slight advantage in PRP platelet counts (only slightly) because of their higher platelet counts in whole blood. Since the detected differences were small, a clinically relevant difference in the biologic effects of PRP is not expected.

The higher platelet counts obtained by the blood bank using discontinuous plasmapheresis might be attributed to the greater number of concentration steps used. The correlation between baseline (donor whole blood platelet counts) and the end concentration of PRP was slightly lower than expected (r_S = 0.73). However, the poor correlation between the platelet concentration from the self-concentrated PRP and the baseline whole blood platelet concentration is surprising (r_S = 0.22). This corresponds to the large data spread in the platelet concentration of self-concentrated PRP. The difference in the number of separation steps between the 2 methods may also have caused or contributed to this discrepancy. During discontinuous plasmapheresis, platelets are extracted in a number of successive steps. If one of these extraction steps is ineffective, the others can compensate for any loss. During self-concentration of PRP, there is only one separation step. If this concentration step is ineffective, the overall yield would also be reduced.

The correlation between platelet counts in whole blood and PRP samples is also reflective of the number of PRP samples that yielded platelet concentrations below the initial donor whole blood platelet count for each method. Only one PRP sample of 158 (< 1%) produced by discontinuous plasmapheresis was rejected for transfusion because of a low platelet count (21,400/µL), whereas 16 of 158 self-concentrated PRP samples (10%) had a

platelet count that was less than the platelet count in the donor's whole blood.

Marx and coworkers[2] showed an increase in platelet concentration from a whole blood platelet concentration of 232,000/µL (range 111,000 to 523,000/µL) to a final PRP platelet concentration of 785,000/µL (range 595,000 to 1,100,000/µL). Their results were obtained using 400 to 450 mL of whole blood (n = 44 patients) and a procedure similar to the blood bank method described here (Electromedics 500 cell separator, Medtronic, Minneapolis, MN).

The platelet counts for donor whole blood reported by Marx and coworkers were similar to those for the population described here.[2] However, their platelet counts were lower than those of the PRPs produced in the blood bank at the Johannes Gutenberg University (mean 785,000/µL versus 1,422,000/µL, respectively) using automated determination of platelet concentration. The mean PRP concentrations of Marx and coworkers were lower (mean 785,000/µL) than those of the present self-concentrated PRP (mean 923,000/µL). There is no information available regarding the degree of data scatter for their results.

At present, there is no simple procedure available to obtain a preoperative estimate of the content of individual growth factors in a PRP sample. However, the whole blood platelet count may be used to provide a sufficient estimate of the platelet count likely to be produced by the plasmapheresis method. Up to now, the empirical association between platelet count and growth factor content in PRP has not been proven. However, it is probable that such a correlation does exist.

CONCLUSIONS

Both of the methods tested herein for the production of PRP are useful, to very different degrees, for concentrating platelets from whole blood. Clinically meaningful age- and gender-related differences were not found. However, the PRP from the blood bank contained a significantly higher platelet concentration than the self-concentrated PRP. The platelet concentration of the PRP from discontinuous plasmapheresis is sufficiently predictable, but the platelet counts of self-concentrated PRP cannot be reliably estimated from the platelet counts of the whole blood. Both methods may be suitable for collecting autologous growth factors; however, no exact measurements of the growth factor content are available. Reliable predictions of growth factor content and the regenerative or inductive potency of platelet growth factors for the platelet concentra-tions shown here are necessary to ensure reliable use of PRP in the clinical treatment of patients. Further studies on this topic remain to be done.

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