

## Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count

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**SUMMARY.** Introduction: Platelet-rich plasma contains autologous thrombocyte growth factors and might be promising for acceleration of dentoalveolar bone regeneration. In this study, it was analysed for platelet counts and growth factor concentrations. Material and method: Platelet-rich plasma was isolated by discontinuous cell separation from 158 healthy men and 55 women aged 17–62 years. One hundred and fifteen specimens (stratified for age and gender of the donor) were analysed for growth factor concentrations and platelet count. Results: The platelet count in platelet-rich plasma ( $1,407,640 \pm 320,100/\mu\text{l}$ ) was 5 times higher than in donor blood ( $266,040 \pm 60,530/\mu\text{l}$ ). Platelet-derived growth factor AB ( $117 \pm 63 \text{ ng/ml}$ ), transforming growth factor (TGF)  $\beta$ -1 ( $169 \pm 84 \text{ ng/ml}$ ), and insulin-like growth factor (IGF) I ( $84 \pm 23 \text{ ng/ml}$ ) were found in large amounts, while platelet-derived growth factor (PDGF) BB ( $10 \pm 8 \text{ ng/ml}$ ) and transforming growth factor  $\beta$ -2 ( $0.4 \pm 0.3 \text{ ng/ml}$ ) were found in small amounts only. The growth factor content was not well correlated with the platelet count in whole blood nor with the platelet-rich plasma ( $r_p = 0.35$ ). No influence of gender or age on platelet count or growth factor concentrations was discovered (except IGF-I). Conclusions: While there was substantial variation in the growth factor content of platelet-rich plasma, the factors influencing this are still worthy of further investigation. Furthermore, a technique whereby the growth factor content could be rapidly assessed in platelet-rich plasma may be of therapeutic benefit. © 2002 European Association for Cranio-Maxillofacial Surgery. Published by Elsevier Science Ltd. All rights reserved.

### INTRODUCTION

Platelets contain a number of different growth factors, such as platelet-derived growth factor (PDGF), transforming growth factors  $\beta$ 1 and  $\beta$ 2 (TGF- $\beta$ 1 and TGF- $\beta$ 2), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial cell growth factor (ECGF), and a hepatocyte growth factor (HGF) (Kiuru et al., 1991). The effects of recombinant growth factors for dentoalveolar surgery have been much debated in recent years, particularly when used in combination with different bone regeneration materials for alveolar crest augmentation (Lynch et al., 1991a, b; Becker et al., 1992; Rutherford et al., 1992, 1993; Cho et al., 1995; Park et al., 1995; Anitua, 1999). Radiographically and histologically, Marx et al. (1998) have shown that the use of platelet-rich plasma (PRP) concentrate is a source of autologous growth factors and led to an increase in bone formation and density after autologous bone grafting in 44 patients. Treatment with PRP to support osseointegration of dental implants has also been described (Kim et al., 2002), suggesting that PRP may be useful in accelerating the osseointegration of titanium implants.

Some promising clinical cases in which PRP treatment was applied have already been reported (Carlson, 2000; Kassolis et al., 2000), but basic data and exhaustive studies on thrombocyte growth factor levels in PRP are still missing.

Platelet counts and PRP growth factor content are likely to depend on the particular technique used in obtaining the PRP. In addition, the donor's biological condition may be a determining factor in the composition of PRP and its observed biological effects. Since the possible influences of age, sex, and thrombocyte count of the donor have not been rigorously examined in a previous study, we analysed growth factor levels in PRP samples that were professionally produced by the Johannes Gutenberg University Transfusion Institute. This study provides data on platelet growth factor concentrations in standardized platelet concentrates and investigated the influence of the donor's age, sex, whole blood and PRP thrombocyte counts.

### MATERIAL AND METHODS

Blood samples were collected from 213 consecutive healthy donors (158 men, 55 women) aged 17–62 years (mean 36.4, median 34.0, SD 10.8, 25% percentile 28.0, 75% percentile 43.0) at the Johannes Gutenberg University Transfusion Institute between 20 and 28 December, 1999. Before platelet separation, a 50-ml bag of whole blood was collected for serologic analysis via a cannula already in place. Subsequently, approximately 300 ml of PRP concentrate was prepared from the remaining whole blood, using the discontinuous cell separation method

described below. All donors had thrombocyte counts >150,000/ $\mu$ l, as required by this institute's criteria for platelet donation.

The PRP samples were stored in Eppendorf tubes at  $-78^{\circ}\text{C}$ . The samples were thawed and centrifuged for 10 min at 10,000 rpm in a microcentrifuge immediately before assay at room temperature. From the 213 specimens, only 115 specimens could be analysed for growth factor content. To analyse a possible effect of age and gender, these 115 specimens were stratified for age and sex during the selection by the institute for medical statistics and documentation. The age and gender distributions in this stratified set are depicted in Figure 1. Commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Diagnostics, Wiesbaden, Germany) were used to quantify the concentrations of PDGF-AB, PDGF-BB, TGF- $\beta$ 1, TGF- $\beta$ 2, and IGF-I in these 115 specimens. ELISAs were performed according to the manufacturer's instructions below.

All quantitative measurements were described using summary statistics ( $n$ , mean, standard deviation, median, minimum, maximum, and other quantiles). Scatter plots and correlation coefficients were used to demonstrate the relationship of thrombocyte counts and growth factor content in whole blood and in PRP (Pearson's correlation coefficient  $r_P$ ; Spearman's correlation coefficient  $r_S$ ). An analysis of variance was performed to evaluate possible influences of gender and age.

**Production of platelet concentrates by discontinuous flow separation**

PRP was obtained by means of an Haemonetics gradient density cell separator (MCS 3p, Haemonetics, München, Germany) used at the transfusion institute. This cell separator withdrew 400–450 ml of

whole blood through a venous catheter, using an intermittent (discontinuous) flow. The donor blood was supplemented with an anticoagulant (1 ml of citrate phosphate dextrose per 5 ml of blood) and allowed to flow into a rotating centrifuge cup, which separated the cellular blood components into erythrocyte, buffy coat (mostly thrombocytes and some leukocytes), and plasma (containing relatively few cells) fractions. As the centrifuge cup was refilled, the individual fractions left the cup through automatic pressure valves and entered three separate bags. Some blood was allowed to recirculate into the centrifuge cup to increase the platelet output by keeping blood in the centrifuge chamber for a longer period. After the erythrocyte and plasma fractions were retransfused, the whole procedure was redone until a predefined volume of PRP is collected. The PRP and venous blood samples thus obtained were submitted for automated platelet counts.

**Measurement of PDGF-AB and PDGF-BB levels**

Samples were assayed for PDGF-AB and PDGF-BB using commercially available Quantikine ELISA kits #DHD00 and #DBB00, respectively, produced by R&D Systems. The minimum detectable doses were 8.4 and 15 pg/ml, respectively. The samples and standards were prepared in duplicate according to the manufacturer's protocol. The plates were incubated for 2 h, washed, and incubated with enzyme-conjugated antibodies to PDGF-AB or PDGF-BB, respectively, for an additional 2 h at room temperature.

The wells were then washed and substrate was added for 20–30 min, respectively, also at room temperature. Stop solution (50  $\mu$ l) was added to each

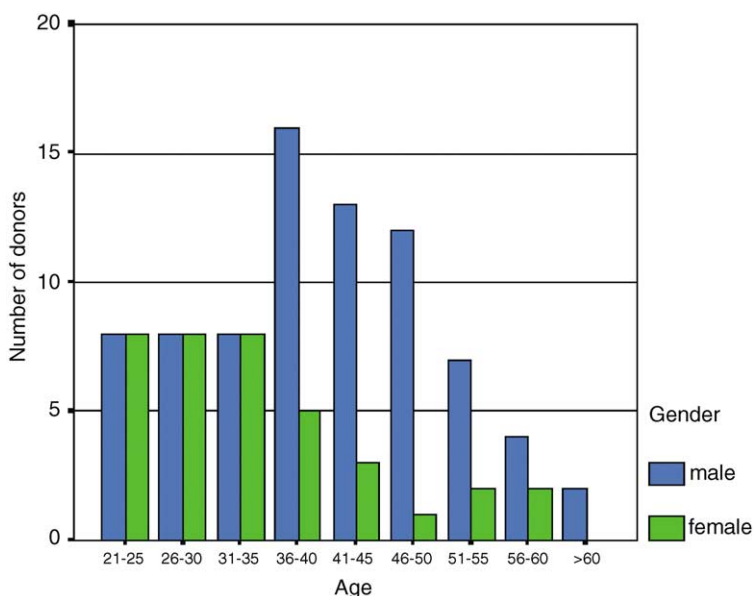


Fig. 1 – Distribution of gender and age ( $n = 115$ ).

well, and the absorptions at 450 nm were determined using a microtitre plate reader.

### Measurement of TGF- $\beta$ 1 levels

TGF- $\beta$ 1 was assayed using a commercially available Quantikine ELISA kit (#DB100, from R&D Systems). A dilution series of TGF- $\beta$ 1 standards (#890207) was prepared in 100- $\mu$ l volumes in 96-well microtiter plates coated with TGF- $\beta$ -receptor II. The minimum detectable dose of TGF- $\beta$ 1 was 7 pg/ml. Since a large proportion of TGF- $\beta$ 1 in biological samples is often present in a latent form (Roberts and Sporn, 1990), conversion of TGF- $\beta$ 1 to its active form was necessary to estimate total TGF- $\beta$ 1. For this purpose, 0.5 ml PRP samples were mixed with 0.1 ml of 1 N HCl, incubated at room temperature for 10 min, neutralized by an addition of 0.1 ml of 1.2 N NaOH/0.5 M HEPES (*N*-[2-hydroxyethyl] piperazine-*N'*-[2-ethanesulfonic acid]) from Sigma (#H-7523), and centrifuged. The supernatant fraction was then assayed for total TGF- $\beta$ 1 content. Aliquots (200  $\mu$ l) were applied in duplicate to the microtiter plate, which was then covered and incubated for 3 h at RT. The wells were then washed, enzyme-conjugated polyclonal antibody to TGF- $\beta$ 1 was added, and incubation continued for 1.5 h at room temperature. Measurements were completed as described above.

### Measurement of TGF- $\beta$ 2 levels

TGF- $\beta$ 2 was assayed essentially as described above, using a Quantikine ELISA kit (#DB250, from R&D Systems). A dilution series of TGF- $\beta$ 2 standards (#890267) was prepared in 100- $\mu$ l volumes in 96-well microtiter plates coated with a murine monoclonal antibody against TGF- $\beta$ 2. The MDD of TGF- $\beta$ 2 was 7 pg/ml. Primary incubation with duplicate 200- $\mu$ l PRP aliquots was for 2 h at RT. The wells were washed 3 times and then incubated with enzyme-conjugated polyclonal antibody to TGF- $\beta$ 2 for 2 h at RT. Measurements were completed as described above.

### Measurement of IGF-I levels

IGF-I levels were estimated using a Quantikine ELISA kit (#DG100, from R&D Systems). A dilution series of IGF standards (#890775) was prepared in 100- $\mu$ l volumes in 96-well microtiter plates coated with a monoclonal antibody specific for IGF-I. The minimum detectable dose of IGF-I ranged from 0.007 to 0.056 ng/ml and the mean MDD was 0.026 ng/ml, as reported by the manufacturer. Duplicate aliquots were applied to the microtiter plate, which was then covered and incubated for 2 h at 2–8°C. The wells were washed 3 times and then incubated with enzyme-conjugated IGF-I for 1 h at 2–8°C. Measurements were completed as described above.

## RESULTS

The donor platelet count from whole blood was  $266,040 \pm 60,530/\mu$ l (mean  $\pm$  SD). The platelet count in the PRP was  $1,407,640 \pm 320,100/\mu$ l. Three growth factors were principally found in the PRP: PDGF-AB ( $117.5 \pm 63.4$  ng/ml), TGF- $\beta$ 1 ( $169.4 \pm 84.5$  ng/ml) and IGF-I ( $84.2 \pm 23.6$  ng/ml). PDGF-BB ( $9.9 \pm 7.5$  ng/ml) and TGF- $\beta$ 2 ( $0.4 \pm 0.26$  ng/ml) were present only in small amounts (Table 1).

The discontinuous cell separation method for PRP production was useful in concentrating platelets from whole blood. This procedure yielded an approximately 5-fold increase in platelet concentration when compared with the initial platelet concentration in donor blood. Pearson's correlation coefficient was  $r_P = 0.77$  for platelets in PRP vs. whole blood.

Scatter plots of thrombocyte count and respective PRP growth factor levels revealed only slight correlations between these parameters (Fig. 2). The Pearson's correlation coefficients for whole blood platelets, PRP platelets, and growth factor contents demonstrated little relationship between these parameters (all  $r_P \leq 0.35$ ). On the other hand, levels of PDGF-AB, PDGF-BB, and TGF- $\beta$ 1 had relevant correlations. PDGF-AB was correlated with PDGF-BB ( $r_S = 0.79$ ) and TGF- $\beta$ 1 ( $r_S = 0.78$ ), and

**Table 1** – Descriptive statistical parameters

	Platelets blood	Platelets PRP	PDGF-AB	PDGF-BB	TGF- $\beta$ 1	TGF- $\beta$ 2	IGF-I
	(1000/ $\mu$ l)	(1000/ $\mu$ l)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
<i>n</i>	115	115	115	115	115	115	115
Mean	266	1408	117.5	9.9	169.4	0.4	84.2
95% CI	[255–277]	[1349–1467]	[105.8–129.2]	[8.5–11.7]	[153.8–185.0]	[0.4–0.5]	[79.8–88.5]
Median	258	1379	109.6	7.8	162.3	0.4	84.9
SD	61	320	63.4	7.5	84.5	0.3	23.6
Minimum	164	473	12.9	0.9	1.5	0.1	31.3
Maximum	464	2302	293.8	33.2	366.1	1.4	130.3
Percentile							
10	198	1036	48.5	3.0	63.1	0.7	51.1
25	220	1157	62.2	4.5	97.3	0.2	68.9
75	304	1620	165.4	14.3	238.1	0.6	101.0
90	347	1879	194.0	20.7	282.9	0.8	116.6

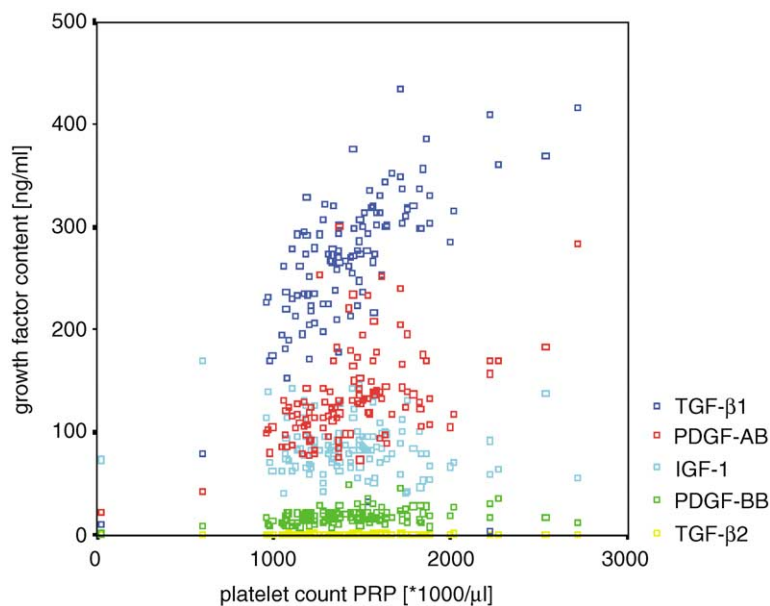


Fig. 2 – Scatter plots of thrombocyte count and PDGF-AB, PDGF-BB, TGF-β1, TGF-β2, and IGF-I levels in PRP samples.

Table 2 – Pearson's correlation coefficients for age, thrombocyte counts, and growth factor levels

	Age	Platelets blood	Platelets PRP	PDGF-AB	PDGF-BB	TGF-β1	TGF-β2
Platelets PRP	n.s. <sup>a</sup>	0.77 <sup>a</sup>					
PDGF-AB	0.28 <sup>a</sup>	n.s. <sup>a</sup>	0.20 <sup>a</sup>				
PDGF-BB	0.37 <sup>b</sup>	n.s. <sup>b</sup>	n.s. <sup>b</sup>	0.79 <sup>b</sup>			
TGF-β1	0.24 <sup>b</sup>	0.24 <sup>b</sup>	0.34 <sup>b</sup>	0.78 <sup>b</sup>	0.69 <sup>b</sup>		
TGF-β2	n.s. <sup>a</sup>	n.s. <sup>a</sup>	n.s. <sup>a</sup>	0.34 <sup>a</sup>	0.37 <sup>b</sup>	0.34 <sup>b</sup>	
IGF-I	0.56 <sup>a</sup>	0.24 <sup>a</sup>	-0.22 <sup>a</sup>	-0.19 <sup>a</sup>	-0.29 <sup>b</sup>	-0.21 <sup>b</sup>	n.s.

<sup>a</sup>Pearson's correlation coefficient,  $r_p$ .

<sup>b</sup>Spearman's correlation coefficient,  $r_s$ .

n.s. – not significant.

PDGF-BB was correlated with TGF-β1 ( $r_s = 0.69$ ) (Table 2).

The platelet concentrations in whole blood and PRP were slightly higher for women ( $n = 37$ ) than for men ( $n = 78$ ) (analysis of variance: sex  $p < 0.05$ , multiple correlation coefficients  $R^2 = 0.48$  and  $0.50$  for whole blood and PRP, respectively). Although age was included in this analysis of variance model, no significant influence on the outcome was found. Analysis of variance for PDGF-AB, PDGF-BB, TGF-β1, or TGF-β2 level also revealed no influence of gender or age. IGF-I levels, on the other hand, displayed a slight decrease in concentration with age (analysis of variance: age  $p < 0.01$ ,  $R^2 = 0.56$ ), but were not influenced by the gender of the donor.

## DISCUSSION

All measurements were performed in duplicate using validated commercially available ELISA kits, and no relevant scattering ( $< 10\%$ ) was observed. For all growth factor analyses specimens were used which were stored frozen. Deep freezing is a common

method for releasing intracellular thrombocyte growth factors (Pesonen et al., 1989; Ito et al., 1993). Sekido et al. (1987) have shown that freezing of specimens does not affect the levels of biologically active PDGF detected.

The whole blood platelet counts of our sample population were within the normal range. The platelet counts in the PRP were also in the expected range and corresponded to values reported in the literature (Marx et al., 1998).

The correlation ( $r_p = 0.77$ ) between the baseline (donor whole blood) and end (PRP) thrombocyte counts was lower than expected. This might be due to isolated incidents of inefficient concentration. Nevertheless, the level of correlation found between whole blood and PRP platelet counts may suggest that the whole blood platelet count can be used as rough estimate of the platelet count likely to be produced by discontinuous cell separation. From a clinical point of view, it is important that the thrombocyte concentration process is very reliable by using a certain PRP production method.

Theoretical levels of platelet-derived growth factors in PRP might be expected to depend on the

number of platelets involved. However, our data did not show a statistically significant correlation between platelet count and growth factor levels. This result might be explained by high individual variability in cellular production or storage of cytokines. For example, for a TGF- $\beta$  level of  $17.8 \pm 8.8$  ng/ $10^5$  platelets (Jiang et al., 1995), the platelet concentrate contained  $36.3 \pm 7.7$  pg/ml epidermal growth factor (after total thrombocyte lysis via 6 freeze/thaw cycles; Ito et al., 1993) and 38–505 pg/ml vascular endothelial growth factor (after thrombocyte lysis with CaCl<sub>2</sub> or thrombin; Banks et al., 1998). It seems that growth factor content in individual patients might be substantially influenced by additional, unknown biological factors. For the clinician using PRP, it is important to know the growth factor content to achieve predictable results in these patients. This could mean that different individuals need different thrombocyte concentrations to achieve a comparable biological effect.

Unfortunately, there is no simple procedure available for obtaining preoperative estimates of individual growth factor levels in a PRP sample. The data obtained in this study demonstrate that neither whole blood nor PRP platelet counts are predictive for the resulting growth factor levels in PRP.

Moderate, but significant, Spearman's correlation coefficients suggest that PDGF-AB levels may be used to estimate PDGF-BB ( $r_s = 0.79$ ) and TGF- $\beta$ 1 ( $r_s = 0.78$ ) levels. The low Pearson's correlation coefficient ( $r_p = 0.34$ ) obtained for PDGF-AB vs. TGF- $\beta$ 2 demonstrated little relationship between these parameters. No useful correlation was found for estimating the level of IGF-I; only donor age correlated slightly ( $r_p = 0.56$ ).

Knowledge (or at least reliable prediction) of growth factor levels in PRP samples is necessary to ensure reliable and reproducible use of PRP for clinical treatment, since the regenerative potency of PRP undoubtedly depends on its growth factor levels. Meanwhile, currently available case reports suggest that PRP treatment has a promising future in patient care. Further studies on this topic are awaiting.

## CONCLUSIONS

The discontinuous cell separation method for producing PRP is adequate for concentrating platelets from whole blood. From a clinical point of view, the platelet count of PRP produced by this method is highly predictable ( $r_s = 0.77$ ) from the platelet count of whole blood.

PRP contains many growth factors. Three of these (PDGF-AB, TGF- $\beta$ 1, and IGF-I) were found in high concentrations, and two (PDGF-BB and TGF- $\beta$ 2) were only in low concentrations (0.4–10 ng/ml). Major age- and gender-specific differences in individual growth factor levels were not found.

The data on thrombocyte and growth factor concentrations can be used for further studies concerning point of care production methods for

PRP. Although discontinuous cell separation is adequate for concentrating growth factors from whole blood, there is still considerable variability in the resulting concentrations of these growth factors. Prediction of resulting growth factor levels based on the thrombocyte counts of whole blood or PRP are not reliable. Predictive estimates for some growth factors are possible by analysing the PDGF-AB content, but are restricted as well. While there are limited correlations regarding growth factor levels, and while there is substantial variation in the growth factor content of individual platelet-rich plasma, the factors influencing this (e.g. an additional, unknown biological factor) are worthy of further investigation. From a scientific point of view, different dilutions of thrombocyte concentrates from one PRP specimen should be analysed for growth factor content, and the correlation between thrombocytes and growth factors should be evaluated. Furthermore, a technique whereby the growth factor content could be rapidly assessed in PRP may be of therapeutic benefit.

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