# A New Economic Method for Preparing Platelet-rich Plasma

Mototsugu Fukaya, MD
Asako Ito, MD

**Background:** Although platelet-rich plasma (PRP) is nowadays a common method in various medical fields, including cosmetic surgery or dermatology, the expensiveness of the kit for processing is still a hurdle.
**Methods:** A new unique economic method for preparing PRP was reported. The method consists in a simple modification of a disposable 5-mL syringe that allows insertion into a common centrifuge and positioning of the syringe on the centrifuge so the PRP separates next to the tip of the syringe. Platelet-derived growth factor BB in PRP was measured under anticoagulant dextrose solution A (ACD-A) or heparin as anticoagulant and with or without prostaglandin E1 (PGE1) as a platelet aggregation suppressant.
**Results:** The new method successfully created PRP with high platelet-derived growth factor BB in all conditions, and the highest value was obtained by using ACD-A and PGE1.
**Conclusions:** The new method is useful, and the use of ACD-A and PGE1 is the most recommended. *(Plast Reconstr Surg Glob Open 2014;2:e162; doi: 10.1097/GOX.0000000000000109; Published online 3 June 2014.)*

**P**latelet-rich plasma (PRP) is a common treatment nowadays in the cosmetic dermatological field.[1,2] It stimulates fibroblasts within the skin and makes them produce collagen fibers. As PRP is made from one's self blood, the treatment is regarded as very safe. The problem exists in rather a high cost of the PRP processing kit considering its mild efficacy. If there were a more economical way to process PRP, this treatment would become more popular and common. Moreover, although the treatment is empirically regarded as useful, there are still few scientific evidences.[3] If more economical method were offered, researchers could access to various

experiments much easier. From that point of view, the authors invented a unique method to prepare PRP using only medical disposables and a common laboratory centrifuge.

Most of commercially available kits adopt anticoagulant dextrose solution A (ACD-A) as an anticoagulant even though there are others such as heparin or EDTA (ethylenediaminetetraacetic acid). Moreover, no kits take the platelet aggregation inhibitor into consideration although coagulation and platelet aggregation are very different and anticoagulants never suppress platelet aggregation. In the course of creating the new method, anticoagulants of ACD-A and heparin were compared in the final concentration of platelet-derived growth factor BB (PDGF-BB) in PRP and utility of prostaglandin E1 (PGE1), a platelet aggregation inhibitor, was also studied. EDTA was not adopted in the study because there is no clinically available medication of EDTA in the authors' country. As the study aims to construct a practical way of preparing PRP, using a nonmedical reagent was considered unethical and should be excluded. PGE1 was adopted in the study as it is

the only one platelet aggregation inhibitor available from the same viewpoint.

## METHODS

### Subjects

Two healthy female volunteers at the age of 49 (subject A) and 50 (subject B) were enrolled under a written consent.

### Preparation of PRP

The whole blood of 60 mL was taken from each healthy volunteer. Thirty milliliters was under existence of ACD-A solution, and the rest 30 mL was under heparin. The mixing rate was 9:1 in volume.

Sixteen pieces of disposable 5-mL syringes were prepared for the first centrifugation with their finger-holders cut by scissors (Fig. 1). Four milliliters of the whole blood was put into each syringe and centrifuged as it stands (Fig. 2). The rotation speed and time was 3000 rpm × 3 minutes, which was the minimum for separating red blood cells (RBCs) from plasma empirically by KOKUSAN H-19α centrifuge with RF-109 rotor and MA-109K buckets. The centrifugal force was calculated with the corrected real



**Fig. 1.** Disposable 5-mL syringe with the part of finger-holder cut by scissors.

radius (Fig. 3) to be 704$g$. The syringes were then taken out from the centrifuge and arranged on a special handmade holder (Fig. 4). A 3-way cock and an extension tube (both are common medical disposables) were connected, and the other end of the extension tube was attached to the syringe after centrifugation. Eight syringes for the second centrifugation were prepared as the same way as the syringes used for the first centrifugation. One microgram of PGE1 diluted in 0.05 mL of saline was added to each of 4 syringes beforehand. The syringe was connected to the 3-way cock and the plasma was aspirated (Fig. 5). The second centrifugation was performed at 4000 rpm (1252$g$) for 15 minutes, which is the fastest speed of the machine and considered to be the realistic time as a daily practice. The supernatant was discarded leaving 0.65 mL in each group (namely, ACD-A PGE1+, ACD-A PGE1−, Heparin PGE1+, and Heparin PGE1−), and the sediment was mixed with it using a vortex mixer (Vortex V-1 plus, BIOSAN). Finally, 0.65 mL of PRP solution was prepared from 16 mL of the whole blood in each group.

### Activation of PRP and Platelet Count

PRP in each group was divided into 3 pieces of 0.2 mL. Twenty microliters of 0.5% Triton X in saline was added to 1 of the 3 and 20 μL of 8.5% calcium glucuronate to another. Triton-X is a kind of detergent that destroys cell membrane and let α-granules discharge nonspecifically. Calcium glucuronate neutralizes the anticoagulant effect of ACD-A while it has no effect to heparin. Platelet count of the 4 groups was performed by using disposable counting chambers.

### Measurement of PDGF-BB

The PRP solutions were stored at 22–29°C[4] overnight for the delivery to a laboratory (Institute of Applied Technology for Innate Immunity, Kagawa, Japan). PDGF-BB was measured there using Human PDGF-BB immunoassay microplate (R&D systems). Ten microliters of the specimen was taken from each whole blood or PRP solution after mingling, diluted to 190 mL buffer and measured. The rest 190 μL of the samples were centrifuged
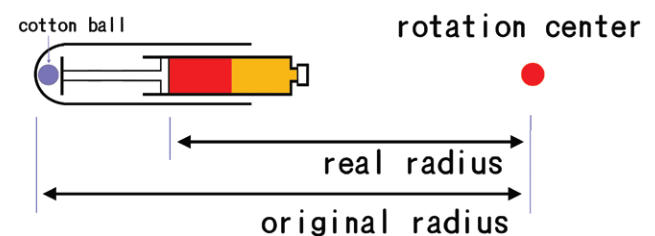


**Fig. 2.** As the radius becomes shortened by the method, the centrifugal force must be calculated by the real radius.

**Fig. 3.** The appearance after the first centrifugation.

at 10,000 rpm for 10 minutes, and the supernatants were also measured.

## RESULTS

**PDGF-BB**

All data are shown in Table 1, and an illustration of the procedure for better understanding is shown in Figure 6. PDGF-BB in the whole blood was 867.7 pg/mL in subject A and 477.8 pg/mL in subject B. The supernatant after the first centrifugation (plasma) contained 1066.6 (ACD-A) and 889.7 (heparin) pg/mL in subject A and 627.9 (ACD-A) and 671.2 (heparin) pg/mL in subject B. The finally processed

PRP solutions in subject A contained 30109.4 pg/mL (ACD-A, PGE1+), 12281.5 pg/mL (ACD-A, PGE1−), 9232.1 pg/mL (heparin, PGE1+), and 7341.9 pg/mL (heparin, PGE1−), whereas 21094.9 pg/mL (ACD-A, PGE1+), 7987.2 pg/mL (ACD-A, PGE1−), 6530.8 pg/mL (heparin, PGE1+), and 5104.6 pg/mL (heparin, PGE1−) in subject B. The PRP solutions added by Triton-X in subject A showed the values of 41517.0 pg/mL (ACD-A, PGE1+), 41599.3 pg/mL (ACD-A, PGE1−), 8920.6 pg/mL (heparin, PGE1+), and 7819.8 pg/mL (heparin, PGE1−), whereas 27041.8 pg/mL (ACD-A, PGE1+), 26493.3 pg/mL (ACD-A, PGE1−), 6816.9 pg/mL (heparin, PGE1+), and 5959.4 pg/mL (heparin, PGE1−) in subject B. The PRP solutions added by calcium glucuronate in sub-
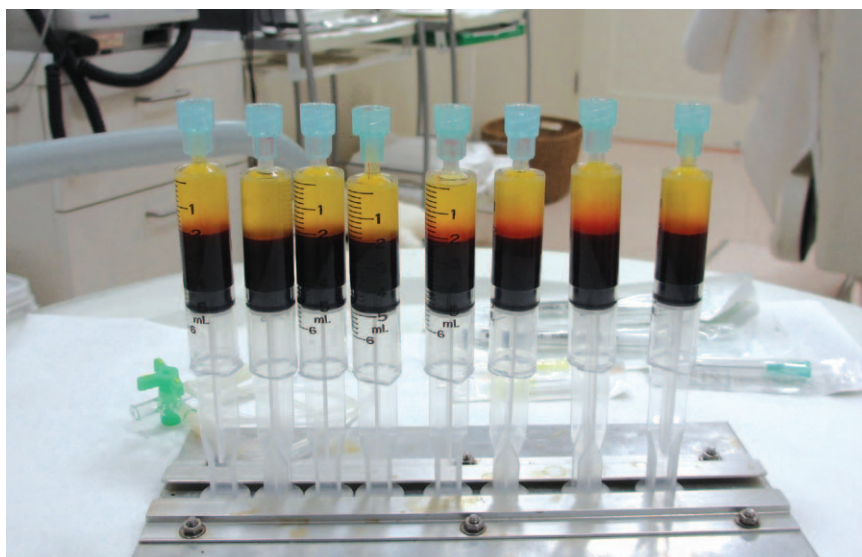


**Fig. 4.** The handmade syringe holder plate.

**Fig. 5.** Extraction of the plasma from syringes after centrifugation.

ject A showed the values of 38535.1 pg/mL (ACD-A, PGE1+), 20972.8 pg/mL (ACD-A, PGE1−), 8681.2 pg/mL (heparin, PGE1+), and 6578.4 pg/mL (heparin, PGE1−), whereas 34732.2 pg/mL (ACD-A, PGE1+), 21437.2 pg/mL (ACD-A, PGE1−), 4702.2 pg/mL (heparin, PGE1+), and 3290.8 pg/mL (heparin, PGE1−) in subject B. The values increased by addition of Triton-X or calcium glucuronate in PRP solutions processed by ACD-A, whereas the values did not increase in heparin-treated ones (Fig. 7).

In comparison of raw PRP solutions and centrifuged supernatants, the values decreased after centrifuge in all ACD-A–treated PRP solutions, whereas the values increased in all heparin-treated ones (Fig. 8). The largest value in nonactivated PRP solutions was obtained in the ACD-A, PGE1+ in both subjects, and the largest value in calcium glucuronate–activated PRP solutions was also in the ACD-A, PGE1+.

**Platelet Count**

Platelet count by counting chambers resulted in $6.4 \times 10^6/\mu L$ in ACD-A, PGE1+, $6.4 \times 10^6/\mu L$ in ACD-A, PGE1−, $6.4 \times 10^6/\mu L$ in heparin, PGE1+, and $6.4 \times 10^6/\mu L$ in heparin, PGE1− in subject A, where-

as $4.0 \times 10^6/\mu L$ in ACD-A, PGE1+, $4.2 \times 10^6/\mu L$ in ACD-A, PGE1−, $5.2 \times 10^6/\mu L$ in heparin, PGE1+, and $6.0 \times 10^6/\mu L$ in heparin, PGE1− in subject B.

## DISCUSSION

**Our New Method of Preparing PRP Solution**

Our new method of preparing PRP solution has 2 advantages. One is economy, and the other is safety. Preparation of PRP for 1 client for cosmetic purpose usually requires 12 disposable 5-mL syringes (8 for the whole blood and 4 for the second centrifuge), caps, one 3-way cock, and 1 extension tube, which costs about 7 USD totally. They are all medical disposables, and the blood or plasma never goes out of them. So the method is safe and ethical.

**Selection of the Anticoagulant**

From the data of PDGF-BB, it is obvious that ACD-A is more appropriate than heparin as an anticoagulant for PRP preparation. However, platelet count revealed that the numbers of platelets are almost the same between ACD-A-treated ones and heparin-treated ones. How should this controversial result be interpreted? The authors consider that heparin-treated platelets more easily discharge α-granules, which contains PDGF than ACD-A–treated ones. The data of the change before and after centrifuge of the last processed PRP (raw PRP and supernatant) show that the concentration of PDGF decreases in ACD-A while increases in heparin. It is the clear evidence that platelets are less instable in heparinized plasma against centrifugation.

In fact, the authors had considered that heparin is more proper than ACD-A. Because the latter has low pH (acidic) that causes less painful sensation when injected to the skin. As the low pH can be neutralized by sodium bicarbonate just before injection, the authors admit that ACD-A is superior to heparin as an anticoagulant for preparation of PRP. Moreover, Araki et al[5] reported that the plasma finally added to the pellet can be replaced to normal saline and the concentration of PDGF increased 3 times more than original PRP. From the viewpoint of measures to the pain, it is a good idea.

Araki et al[5] also reported that PRP processed by EDTA showed higher value of PDGF-BB than by ACD-A although the platelet counts were almost similar. So it is better to adopt EDTA in the laboratory experiments than ACD-A, whereas ACD-A is still appropriate in daily practice in the country where no clinical medication of EDTA is available. It is because there is an ethical problem to use EDTA under such a circumstance.

**Table 1. PDGF-BB Concentrations of the Whole Blood, Plasma, and PRP of Subjects A and B Under Different Conditions of Anticoagulant and Platelet Aggregation Suppressant**

| Subject | Whole Blood/Plasma/PRP | Anticoagulant | Platelet Aggregation Suppressant | Activation | PDGF-BB Concentration (pg/mL) | |
|---|---|---|---|---|---|---|
| | | | | | Raw | Supernatant after Centrifuge |
| A | Whole blood | ACD-A | None | None | 867.7 | 520.4 |
| | | Heparin | None | None | 867.7 | 714.6 |
| | Plasma | ACD-A | None | None | 1066.6 | 955.9 |
| | | Heparin | None | None | 889.7 | 933.8 |
| | PRP | ACD-A | PGE1 | None | 30109.4 | 7700.3 |
| | | | | Triton-X | 41517.0 | 13460.9 |
| | | | | $CaCl_2$ | 38535.1 | 22171.7 |
| | | | None | None | 12281.5 | 4230.0 |
| | | | | Triton-X | 41599.3 | 3947.4 |
| | | | | $CaCl_2$ | 20972.8 | 16695.5 |
| | | Heparin | PGE1 | None | 9232.1 | 14352.6 |
| | | | | Triton-X | 8920.6 | 14063.3 |
| | | | | $CaCl_2$ | 8681.2 | 12113.1 |
| | | | None | None | 7341.9 | 8513.6 |
| | | | | Triton-X | 7819.8 | 9951.4 |
| | | | | $CaCl_2$ | 6578.4 | 6840.7 |
| B | Whole blood | ACD-A | | None | 477.8 | 736.4 |
| | | Heparin | | None | 477.8 | 671.2 |
| | Plasma | ACD-A | | None | 627.9 | 823.8 |
| | | Heparin | | None | 671.2 | 563.3 |
| | PRP | ACD-A | PGE1 | None | 21094.9 | 6006.9 |
| | | | | Triton-X | 27041.8 | 12233.4 |
| | | | | $CaCl_2$ | 34732.2 | 24188.2 |
| | | | None | None | 7987.2 | 2430.5 |
| | | | | Triton-X | 26493.3 | 2361.3 |
| | | | | $CaCl_2$ | 21437.2 | 16792.3 |
| | | Heparin | PGE1 | None | 6530.8 | 12041.0 |
| | | | | Triton-X | 6816.9 | 11103.7 |
| | | | | $CaCl_2$ | 4702.2 | 9495.8 |
| | | | None | None | 5104.6 | 7556.9 |
| | | | | Triton-X | 5959.4 | 8058.9 |
| | | | | $CaCl_2$ | 3290.8 | 4182.9 |

The background of the values of (ACD-A, PGE1+), (ACD-A, PGE1−), (heparin, PGE1+), and (heparin, PGE1−) is colored light blue for easy understanding.

There are few reports of measurement of growth factors in PRP.[6,7] As the concentration of growth factor in PRP solution might not necessarily correlate with the platelet count, the efficacy of PRP treatment is not also ensured by platelet count.

**Utility of Platelet Aggregation Inhibitor**

Anticoagulants such as ACD-A or heparin never interrupt aggregation of platelets. This point of view has been overlooked in preparing PRP. Aggregated platelets stick to the wall of syringes and
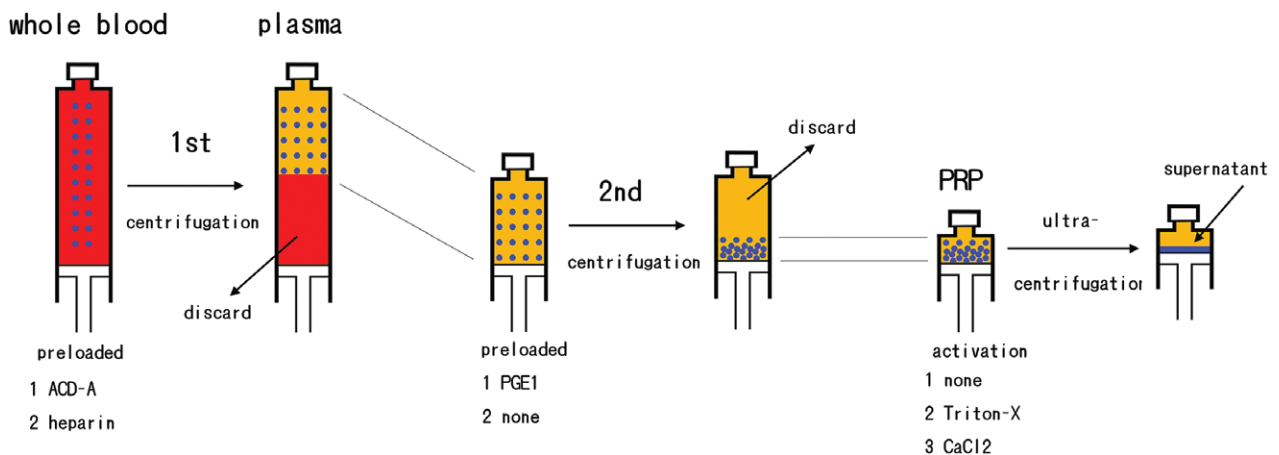


**Fig. 6.** Illustration for understanding the procedure of the study. Blue rounds indicate platelets.
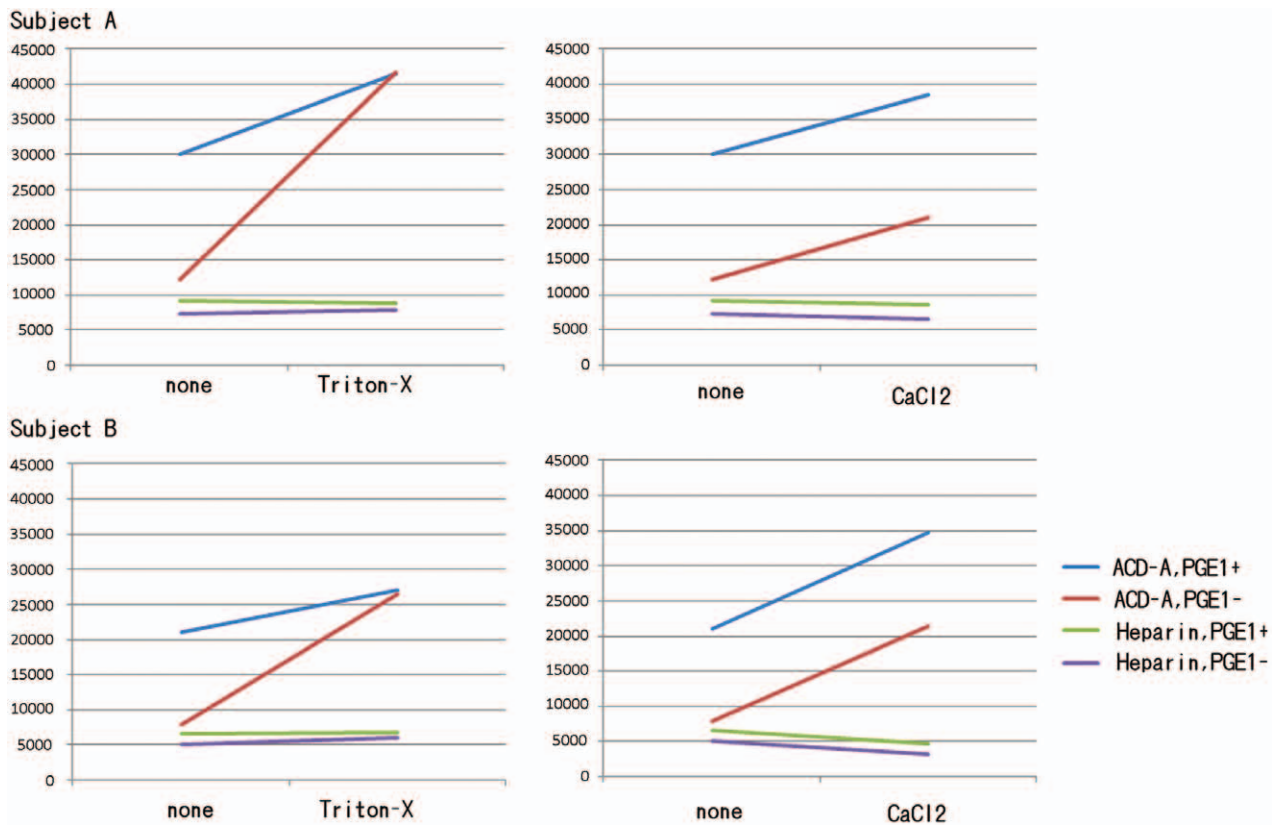
**Fig. 7.** PDGF-BB values (pg/mL) in the vertical axis before and after addition of Triton-X or $CaCl_2$ in PRPs.

do not easily detach from them. As the primary aggregation of platelets is reversible, the platelets come off from the wall and float in the plasma again after several hours. But we cannot wait until then in the daily practice. So the authors suggest using platelet aggregation inhibitor (PGE1). The data also ensure it.

**Rotation Speed and Time of Centrifugation**
The first centrifugation is aimed to separate RBC from other segments of the whole blood. From this point of view, the rotation speed and time was thought to be easily selected. As RBC is red, visible, and heavy in specific gravity, the best condition must be the lowest and shortest speed and time at which RBC and plasma can be clearly separated visibly. Too much speed and time will let platelets also precipitate and should be avoided. There is a report about the efficiency of platelet yield at various speed of centrifugation.[5] 230–270$g$ for 10 minutes is reported to be the most efficient for the first centrifugation. Our experiment adopted 704$g$ for 3 minutes from the reason that the shorter the better at daily clinical practice.

From the viewpoint that the second centrifugation is aimed to separate platelets from plasma, the speed and time should be as high and as long as possible. The higher the speed and the longer the time is, the more platelets will precipitate. However, as the centrifuge is revealed to be stimulus for platelets to discharge PDGF, the best speed and time should be studied by measuring PDGF in plasma discharged from platelets. It is a future-related issue.

**The Difference among Individuals of PDGF-BB Was Not Clarified in the Study**
Further investigation in many individuals is expected. Various commercial kits for preparing PRP are available nowadays, and their main target seems to be cosmetic or rejuvenating field although there are still only a few reports[3] about the efficacy for antiaging. On the other hand, there are also many exciting reports[8] that PRP accelerates wound healing process. Using the method in the study, researchers could try much more experiments without consuming expensive commercial kits.

### CONCLUSIONS
Our new method is useful and ethical in clinical practice. The use of ACD-A and PGE1 is recommended for preparing PRP with dense PDGF-BB.
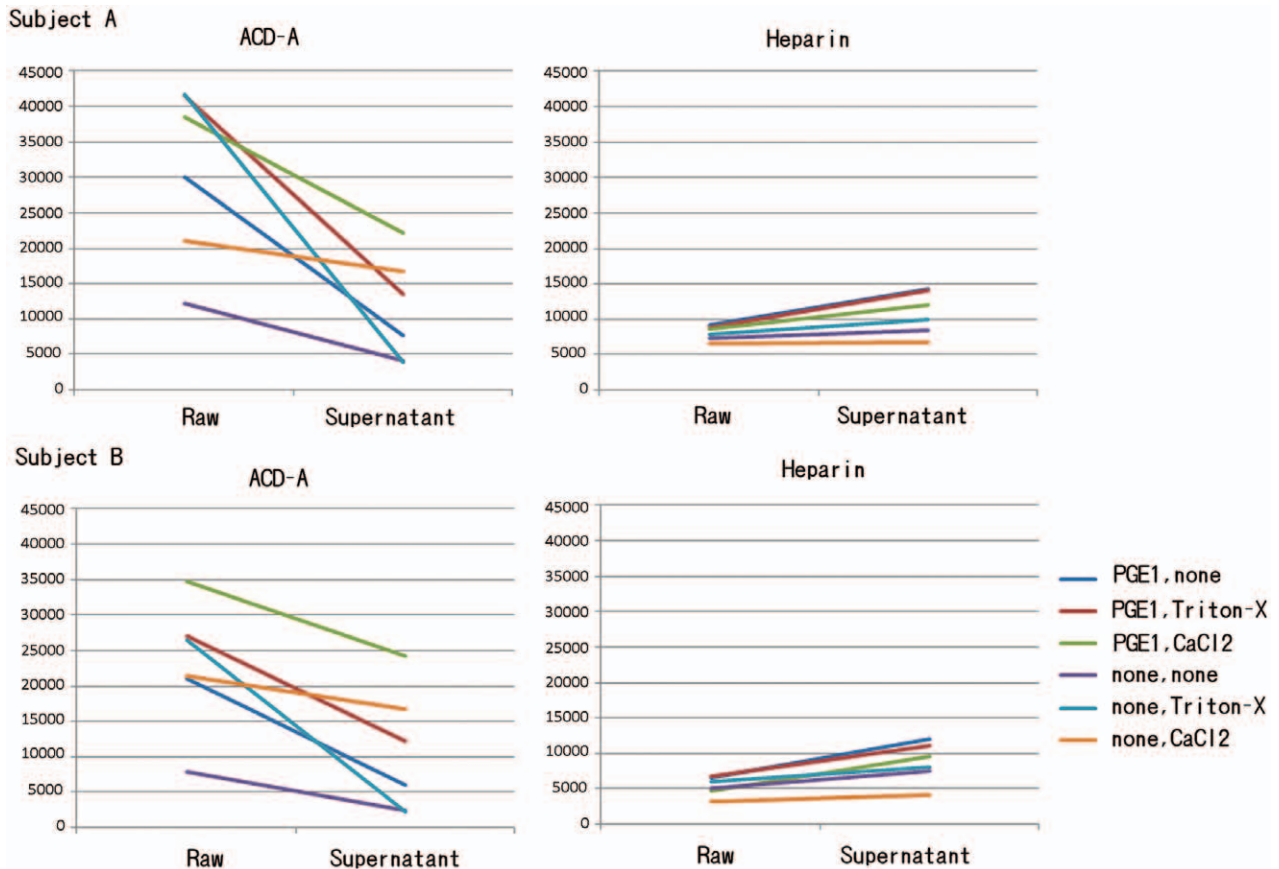
**Fig. 8.** PDGF-BB values (pg/mL) in the vertical axis before and after the high-speed centrifugation of PRPs.

*Mototsugu Fukaya, MD*
Tsurumai Kouen Clinic, 5-20-6 Chiyoda
Nakaku, Nagoya 460-0012, Japan
E-mail: moto@earth.ocn.ne.jp

**REFERENCES**

1. Redaelli A, Romano D, Marcianó A. Face and neck revitalization with platelet-rich plasma (PRP): clinical outcome in a series of 23 consecutively treated patients. *J Drugs Dermatol.* 2010;9:466–472.
2. Kim DH, Je YJ, Kim CD, et al. Can platelet-rich plasma be used for skin rejuvenation? Evaluation of effects of platelet-rich plasma on human dermal fibroblast. *Ann Dermatol.* 2011;23:424–431.
3. Cho JM, Lee YH, Baek RM, et al. Effect of platelet-rich plasma on ultraviolet b-induced skin wrinkles in nude mice. *J Plast Reconstr Aesthet Surg.* 2011;64:e31–e39.
4. Pietersz RN, Loos JA, Reesink HW. Survival in vivo of platelets stored for 48 hours in the buffycoat at 4 degrees C compared to platelet rich plasma stored at 22 degrees C. *Blut* 1987;54:201–206.
5. Araki J, Jona M, Eto H, et al. Optimized preparation method of platelet-concentrated plasma and noncoagulating platelet-derived factor concentrates: maximization of platelet concentration and removal of fibrinogen. *Tissue Eng Part C Methods* 2012;18:176–185.
6. Lee JW, Kwon OH, Kim TK, et al. Platelet-rich plasma: quantitative assessment of growth factor levels and comparative analysis of activated and inactivated groups. *Arch Plast Surg.* 2013;40:530–535.
7. Giraldo CE, López C, Álvarez ME, et al. Effects of the breed, sex and age on cellular content and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel. *BMC Vet Res.* 2013;9:29.
8. Marck RE, Middelkoop E, Breederveld RS. Considerations on the use of platelet-rich plasma, specifically for burn treatment. J Burn Care Res. 2014;35:219–227.