Usefulness of Delta Value of Platelet Parameters on ADVIA 120 for the Functional Reactivity of Stored Platelets

Il Joong Park, Young Ae Lim,* and Sung Ran Cho

Department of Laboratory Medicine, Ajou University School of Medicine, Suwon, South Korea

An agonist-induced expression of CD62P by flow cytometry analysis for evaluating platelet functional reactivity has some disadvantages. We investigated the usefulness of platelet parameters by ADVIA 120 to predict an agonist-induced expression of CD62P in stored platelets. The CD62P expression by flow cytometry and the platelet parameters by ADVIA 120 were studied in samples from 27 platelet pheresis products. Delta (Δ) values were calculated as the degree of change of the platelet parameters studied with or without adenosine 5'-diphosphate sodium (ADP) stimulation. The CD62P expression of the

ADP-activated platelets were correlated with the Δ platelet count ($r = 0.517$) in the short-term storage group (within 10 hr from preparation), with the platelet component distribution width (PCDW) without ADP ($r = -0.744$) and the Δ PCDW ($r = -0.755$) in the long-term storage group (after 10 hr from preparation). Therefore, the delta values of platelet parameters on ADVIA 120 analysis in platelets between with and without ADP stimulation could be useful as a simple predictor for the functional reactivity of stored platelets. J. Clin. Lab. Anal. 24:38–43, 2010.

© 2010 Wiley-Liss, Inc.

Key words: platelet function; platelet pheresis; ADVIA120; ADP

INTRODUCTION

Platelets become activated during the preparation process and prolonged storage (1). P-selectin (CD62P) is released from only activated platelet granules, and is expressed on the plasma membrane after activation (2). The activation-dependent increase of P-selectin on the platelet surface is not reversible under standard blood banking conditions. Therefore, the platelet CD62P expression, without agonist stimulation, measured by flow cytometry, has been traditionally used as a quality control tool for the evaluation of platelet activation during management, processing, storage, and manipulation of platelet products (3–6).

Flow cytometry can determine both the activation state of platelets and the reactivity of platelets to stimulation with agonists (7). Currently, agonist-induced expression of CD62P, or other markers, has been investigated in several studies to determine platelet function in platelet products (8–15), because the functional reactivity of platelets could be used to predict platelet transfusion efficacy. However, agonist-induced expression of CD62P by flow cytometry analysis has some disadvantages, including the expense of the equipment and reagents, and the difficulty of the associated procedures.

The recently developed ADVIA 120 hematology analyzer (Bayer, Tarrytown, NY) evaluates the platelet activation state by calculating the mean platelet component (MPC) parameter (16). MPC is calculated from the platelet component histogram and reflects platelet density. Our laboratory has found that the platelet component width distribution (PCDW) by ADVIA 120 analysis was correlated with CD62P expression of the platelets, without agonist stimulation, during the storage (17). However, there is paucity of studies on the use of hematology analyzer for a simple indicator of a functional reactivity of platelet products.

The goals of this study were to investigate the possible use of the ADVIA 120 for the detections of changes in the *in vitro* adenosine 5'-diphosphate sodium (ADP)-activated platelets, in stored products to be used for

*Correspondence to: Young Ae Lim, Department of Laboratory Medicine, Ajou University School of Medicine, San-5, Woncheon-Dong, Yeongtong-Gu, Suwon, 443-721, South Korea. E-mail: limyoung@ajou.ac.kr

Received 18 February 2009; Accepted 3 December 2009

DOI 10.1002/jcla.20364

Published online in Wiley InterScience (www.interscience.wiley.com).

transfusion, and the possible use of these changes for predictions of a functional reactivity of stored platelets.

MATERIALS AND METHODS

Collection of Platelets

Twenty-seven batches of single donor apheresis platelets (SDP) using acid-citrate-dextrose (ACD) as an anticoagulant were prepared from healthy volunteer blood donors with no recent history of aspirin intake. Seventeen were prepared at the Gyeonggi Korean Red Cross and ten at the Ajou University Hospital. Both organizations supply all platelets for patients at the Ajou University Hospital who need a transfusion; directed donations were performed only at Ajou University Hospital. The samples were stored at 22°C in a platelet incubator with continuous gentle agitation until release for transfusion. Before the platelets were released, 1 ml from each sample was collected from the line of bags, for analysis of the platelets using the ADVIA 120 and CD62P labeling.

The storage time was defined as the interval between complete preparation of the platelet pheresis product and dilution of the platelets with phosphate-buffered saline (PBS; pH 7.4, Sigma, St Louis, MO) for CD62P labeling. According to the storage time, we divided the SDP into two arbitrary groups, 10 hr or less was defined as the short-term group, and 10 hr or more as the long-term group.

Platelet Parameters by the ADVIA 120

An equal volume of PBS was added to the platelet suspension, which was already diluted 1:10 with PBS. The platelet parameters were directly determined using the ADVIA 120 for a diluted platelet suspension without ADP, and defined as parameter_{NS}. The platelet parameters were also estimated using the ADVIA 120 for activated platelets after incubation with ADP for 20 min, which was defined as parameter_{ADP}. The absolute value of the difference for each platelet parameter was defined as the Δ parameter (with or without ADP).

The parameters included platelet count (PLT), mean platelet volume (MPV), platelet volume distribution width (PDW), MPC, and PCDW. Using normal and abnormal, 1 and 2 controls, (ADVIA 120), the platelet parameters on the ADVIA showed reasonable coefficients of day-to-day variation (CVs less than 10%).

CD62P Labeling, ADP Activation and Flow Cytometry Analysis of Platelets

The platelets from the 27 SDPs were diluted 1:10 with PBS. For the evaluation of the functional reactivity to ADP of stored platelets, the platelet suspensions were

labeled with CD62P antibody. We used the method which minimizes artificial platelet activation, and simultaneously performs the labeling of monoclonal antibodies and agonists stimulation of the platelets suspension (18). CD62P labeling and ADP stimulation were performed simultaneously. One aliquot containing 20 μ l of diluted platelet suspension, 5 μ l of phycoerythrin (PE)-conjugated mouse antihuman monoclonal CD62P antibody (CD62P antibody, BD Biosciences, BD Pharmingen, San Diego, CA), or PE-conjugated mouse antihuman monoclonal IgG₁ antibody (BD Biosciences, BD Pharmingen) for isotype control was added simultaneously with a 20 μ M final concentration of ADP (Sigma) to evaluate the functional reactivity to the ADP of platelets. The mixtures were incubated for 20 min at room temperature, and in the dark. To evaluate the activated state, under the blood banking condition, of the platelets that were not stimulated with ADP, PBS was added to another aliquot containing 20 μ l of diluted platelet suspension to adjust for the total volume without the addition of ADP. The labeled platelet suspensions, with or without ADP, were fixed immediately with 1 ml of 1% (W/V) paraformaldehyde at room temperature for 30 min.

For each labeled platelet sample, 10,000 platelet events were evaluated by the FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA), equipped with CELLQuest software. The flow FACScan was optimized for the acquisition of platelets by logarithmic signal amplification for the forward and side scatter, and fluorescence channels FL2; the threshold for the forward scatter was set at 200. For the analysis, the gates were set around single intact platelet populations, as defined by the forward and side scatter characteristics. The platelet gating was confirmed by staining with FITC-CD61P antibody (17).

The results are presented as the percent of the total number of platelets that expressed CD62P. The CD62P expression of the platelets without ADP stimulation, activated state under the blood-banking condition, was defined as CD62P_{NS}, and the CD62P expression of ADP-activated platelets, functional reactivity to ADP, as CD62P_{ADP}.

Statistics

The acquired data were statistically analyzed using SPSS 12.0 for Windows (SPSS, Chicago, IL) and Microsoft Office Excel 2003 (Microsoft, Redmond, WA). The results of time and the platelet parameters and the percent CD62P expression are reported as the median (interquartile range), and the correlation coefficients were determined by Spearman's correlation coefficient. The Mann-Whitney *U* test was used to

determine the differences in the value of the stored products between the short- and long-term storage groups. Analysis of the differences of the mean CD62P expression or the parameters of the platelets analyzed by the ADVIA120 were compared between the ADP stimulated and nonstimulated platelets by Wilcoxon signed ranks test. *P* values <0.05 (two-sided) for multiple tests were considered significant.

RESULTS

Characteristics of Platelet Products

The time interval was 4.3 hr (1.5~23.2 hr) between the preparation of the platelets at the blood center and release from the blood bank. Fifteen SDP were released

TABLE 1. The Median (interquartile range) of CD62P Expression (%) in Platelets without ADP (CD62P_{NS}) and with ADP Activation (CD62P_{ADP}) or Between Short- and Long-Term Storage Groups

	Total (n = 27)	Short (n = 15)	Long (n = 12)
CD62P _{NS}	11.1 (5.8~16.6)	7.6 (4.2~14.3)*	15.2 (9.8~19.8)
CD62P _{ADP}	46.0 (31.4~53.7) [†]	52.3 (32.5~61.5)*, [†]	45.3 (26.7~47.2) [†]

Short, short-term storage group was 15 SDP released within 6 hr from preparation; Long, long-term storage group was 12 SDP released after 28 hr from preparation. **P* value<0.0001 vs. unstimulated platelets with ADP group using Wilcoxon signed ranks test. [†]*P* value<0.05 vs. long-term group using Mann-Whitney *U* test.

for transfusion within less than 10 hr from the short storage group (1.5 hr, 1.0~2.8 hr), and 12 SDP were released after 10 hr of storage from the long storage group (23.2 hr, 18.7~43.8 hr).

The percent of CD62P_{NS} was 11.1% (5.5%~16.6%), and CD62P_{ADP} was 46.0% (31.4~53.7%); the CD62P expression was significantly increased after ADP stimulation (*P*<0.0001) (Table 1).

Change of Platelet Parameters on the ADVIA 120 on Platelet Suspensions after ADP Stimulation (Table 2)

There were significant changes in the median values of MPV, MPC, and PCDW in the platelet suspension on the ADVIA 120 following activation with ADP. [7.6 fl to 6.3 fl for the MPV (*P*<0.0001), 25.0 g/dl to 28.0 g/dl for the MPC (*P*<0.0001), and 7.7 g/dl to 5.6 g/dl for the PCDW (*P*<0.0001)]. The change of the MPV and PCDW, and the MPC after activation with ADP was observed on the platelet suspensions from both the short- and long-term storage groups. However, the PLT was significantly decreased after activation with ADP only in the short-term storage group but not the long-term storage group.

The MPC were higher without ADP (*P* = 0.0012) and with ADP (*P* = 0.025) in the short-term storage group compared with the long-term storage group. However, the PLT, MPV, PDW, and PCDW were not significantly different between short- and long-term storage group.

TABLE 2. The Median (Interquartile Range) of Platelet Parameters on the ADVIA 120: without ADP (Parameter_{NS}) and with ADP Activation (Parameter_{ADP}) for 20 min According to the Storage Group

	Total (n = 27)		Short (n = 15)		Long (n = 12)	
PLT _{NS} (μl)	53.0	(46.0~61.0)	54.0	(49.0~61.0)	49.5	(43.5~62.5)
PLT _{ADP} (μl)	52.0	(43.0~58.0)	52.0	(42.0~58.0) [‡]	51.5	(43.5~61.8)
ΔPLT (μl)	5.0	(3.0~8.0)	6.0	(3.0~13.0)	4.0	(2.3~6.0)
MPV _{NS} (fl)	7.6	(7.1~8.0)	7.5	(6.8~8.0)	7.9	(7.5~8.3)
MPV _{ADP} (fl)	6.3	(5.8~6.9)*	6.2	(5.8~7.5) [†]	6.4	(5.9~6.8) [†]
ΔMPV (fl)	1.2	(0.8~1.7)	1.1	(0.5~1.5)	1.6	(1.2~1.8)
PDW _{NS} (%)	52.9	(46.7~57.2)	51.3	(46.0~60.1)	53.5	(49.9~57.0)
PDW _{ADP} (%)	51.3	(47.4~64.1)	59.2	(49.4~71.2)	48.9	(42.7~52.8)
ΔPDW (%)	6.2	(2.4~14.2)	6.2	(2.4~17.0)	6.0	(2.4~11.0)
MPC _{NS} (g/dl)	25.0	(23.6~26.0)	25.5	(24.6~26.4)	23.7	(21.9~24.8) [§]
MPC _{ADP} (g/dl)	28.0	(27.1~29.5)*	28.8	(27.8~30.3) [†]	27.8	(26.5~28.7) ^{†,}
ΔMPC (g/dl)	3.7	(2.7~4.8)	3.3	(1.5~4.7)	4.4	(3.6~5.7)
PCDW _{NS} (g/dl)	7.7	(7.4~7.9)	7.7	(7.5~8.1)	7.6	(7.3~7.9)
PCDW _{ADP} (g/dl)	5.6	(5.1~6.2)*	5.5	(5.0~5.8) [†]	6.1	(5.1~6.2) [†]
ΔPCDW (g/dl)	2.0	(1.3~2.7)	2.1	(1.8~2.7)	1.6	(0.7~2.6)

The data were obtained from final 1:20 diluted platelet suspension with PBS-. Short, short-term storage group was 15 SDP released within 6 hr from preparation; Long, long-term storage group was 12 SDP released after at least 17 hr from preparation. Delta (Δ) of the platelet parameters on ADVIA120 was defined as the value of the absolute difference for each platelet parameter with and without the addition of ADP. PLT, platelet count; MPV, mean platelet volume; PDW, platelet volume distribution width; MPC, mean platelet component; PCDW, platelet component width distribution. *P* value*for<0.0001, [†]for<0.01, [‡]for<0.05 vs. parameters on platelets without ADP using Wilcoxon signed ranks test. *P* value [§]for<0.01, ^{||}for<0.05 vs. parameters of platelets from short storage group using Mann-Whitney *U* test.

Correlation between CD62P Expression and Platelet Parameters on the ADVIA 120 (Fig. 1)

For the short-term storage group, the CD62P_{NS}, as an activated state under the blood-banking condition, was negatively correlated with the MPC_{NS} ($r = -0.524$, $P = 0.045$), and the CD62P_{ADP}, as a functional reactivity of platelets, was correlated with only the PLT count ($r = 0.517$, $P = 0.048$).

For the long-term storage group, the CD62P_{NS} was negatively correlated with the Δ MPV ($r = -0.670$, $P = 0.017$) and the MPC_{NS} ($r = -0.701$, $P = 0.011$); the CD62P_{ADP}, as a functional reactivity of platelets, was correlated with the PCDW_{NS} ($r = -0.744$, $P = 0.006$) and the Δ PCDW ($r = -0.755$, $P = 0.005$).

DISCUSSION

The platelet expression of CD62P without agonist has been traditionally used as a quality control method using flow cytometry for the detection of platelet activation under the blood-banking condition. Several studies have showed that high levels of platelet activation may be related to a low survival potential for transfused platelets (3,4,19,20). However, Cardigan et al. (21) reported that the reactivity of platelets to stimuli such as physiological agonists, rather than the glycoprotein expression without agonists, might be a better predictor of in vivo platelet transfusion efficacy. Recently, several studies have reported on CD62P expression in agonist-stimulated platelets for the evaluation of platelet function in platelet products (8–15). However, agonist-induced expression of CD62P by flow cytometry analysis is not easy to use routinely.

The MPV (22), PCDW (23), and MPC (24) platelet parameters, determined by a hematology analyzer, have been reported to reflect the storage lesion of the platelets to be used for transfusions. We evaluated whether ADP stimulation of the platelets could induce changes in the volume, granulation, and shape distribution of the stored platelets, and the degree of such changes might be associated with the functional reactivity of stored platelets to ADP. We had used only a final concentration of 20 μ M/l of ADP as an agonist because a high concentration of an agonist would be easy to use due to one concentration and enough to induce the morphologic changes of platelets.

The results of our study showed that the changes of the activated platelets, after stimulation with ADP, could be detected by the ADVIA 120 and the degree of change correlated with the CD62P_{ADP} functional reactivity of the platelets to ADP. In addition, the patterns and degree of change were different according to the storage time period.

In this study, the MPV, MPC, and PCDW significantly changed after 20 min of ADP stimulation in both the short- and long-term storage groups; however, the PLT significantly decreased only in short-term storage groups. Giacomini et al. (24) also reported a significant decrease in the PLT, MPV, and PCDW, and an increased MPC in platelet rich plasma, from fresh citrated treated whole blood, after 10 min of stimulation with a final concentration of 2 μ M/l of ADP. However, they evaluated platelet activation only in platelet rich plasma obtained from fresh whole blood treated with

Fig. 1. The correlation between platelet parameters on the ADVIA 120 and the expression of CD62P in platelets. The CD62P expression of the platelets without ADP stimulation, activated state under the blood banking condition, was defined as CD62P_{NS}, and the CD62P expression of ADP activated platelets, functional reactivity to ADP, as CD62P_{ADP} in the short-term storage groups (A) and long-term storage groups (B). Platelet parameters included the platelet count (PLT), mean platelet volume (MPV), mean platelet component (MPC), and platelet component width distribution (PCDW). Delta (Δ) of the platelet parameters on ADVIA120 was defined as the value of the absolute difference for each platelet parameter with and without the addition of ADP.

sodium citrate, not from stored platelet products using ACD; in addition, they did not evaluate the delta values (Δ) of platelet parameters in platelets between with and without ADP stimulation. To our knowledge, this is the first study on the use of hematology analyzer as a simple indicator of a functional reactivity to agonist in platelet products.

In this study, we found a finding that the delta values (Δ) of platelet parameters in platelets between with and without ADP stimulation were more useful to predict functional reactivity in stored platelets than values of platelet parameters in platelets with ADP stimulation. For example, and the $CD62P_{ADP}$ was correlated with only the ΔPLT count ($r = 0.517$, $P < 0.05$), not with the PLT , after ADP. The change in PLT in response to ADP, however, was observed only in the short-term storage group. This finding suggests that platelets from the short-term storage group were more active and more easily formed aggregates than those from the long-term storage group. The decreasing PLT may be explained by ADP-induced platelet aggregation. This possibility is supported by the finding that the greater the decrease in the PLT , after activation with ADP, the higher the $CD62P_{ADP}$ in the short-term storage group. This finding can help predict the reactivity of platelets from short-term storage. In this study, however, all of the short-term storage platelets were stored for up to 6 hr after preparation, and the long-term storage platelets were stored for at least 17 hr (data not shown). We do not know whether the stored platelets during the interval from 6 to 17 hr would demonstrate decreasing $PLTs$ and this requires further study.

In this study, the MPC is considered to be a better indicator for platelet activation for long-term storage of platelets, under the blood-banking condition, than the MPC_{NS} . The more changes are in the values of the MPC or MPV , after ADP activation, the lower the expressions of $CD62P_{NS}$ are among the long-term storage platelets. This finding suggests that platelets already activated with high expression levels of $CD62P_{NS}$, before ADP activation, have less potential for change of the MPV or MPC after ADP activation compared with platelets with low expression of $CD62P_{NS}$. This is because platelet activation induces changes in platelet volume and granule content. Therefore, the ΔMPC or ΔMPV are considered to be more useful for the prediction of $CD62P$ expression of activated platelets than the MPC_{NS} or MPV_{NS} among long-term stored platelet products.

The $PCDW$, which is calculated from “ $(MPC/\text{standard deviation of } MPC) \times 100$,” has been reported to gradually decrease over five days of storage (17). The reactivity to ADP, for the long-term stored platelet products, showed that the lower the values for the

$PCDW_{NS}$ or $\Delta PCDW$, the greater the expression of $CD62P_{ADP}$. Therefore, the $PCDW$, from both ADP-stimulated and -unstimulated platelets, was a useful indicator of the functional reactivity of platelets in the long-term storage group.

In summary, the ADVIA 120 was used to detect changes in platelet parameters of *in vitro* activated platelets, with ADP, from stored products planned to be used for transfusions. Some of the observed changes were correlated with the activated state and the functional reactivity of the stored platelets. Therefore, the delta values of platelet parameters on ADVIA 120 analysis in platelets between with and without ADP stimulation could be useful as a simple predictor for the functional reactivity of stored platelets.

REFERENCES

1. Metcalfe P, Williamson LM, Reutelingsperger CP, et al. Activation during preparation of therapeutic platelets affects deterioration during storage: a comparative flow cytometric study of different production methods. *Br J Haematol* 1997;98:86–95.
2. Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. *J Cell Biol* 1985;101:880–886.
3. Holme S, Sweeney JD, Sawyer S, Elfath MD. The expression of p-selectin during collection, processing, and storage of platelet concentrates: relationship to loss of *in vivo* viability. *Transfusion* 1997;37:12–17.
4. Rinder HM, Murphy M, Mitchell JG, Stocks J, Ault KA, Hillman RS. Progressive platelet activation with storage: evidence for shortened survival of activated platelets after transfusion. *Transfusion* 1991;31:409–414.
5. Triulzi DJ, Kickler TS, Braine HG. Detection and significance of alpha granule membrane protein 140 expression on platelets collected by apheresis. *Transfusion* 1992;32:529–533.
6. Schmitz G, Rothe G, Ruf A, et al. European Working Group on Clinical Cell Analysis: consensus protocol for the flow cytometric characterisation of platelet function. *Thromb Haemost* 1998;79:885–896.
7. Michelson AD, Barnard MR, Krueger LA, Frelinger III AL, Furman MI. Flow cytometry. In: Michelson AD, editors. *Platelets*. London: Academic Press, Inc. 2002. p 297–315.
8. Jilma-Stohlawetz P, Horvath M, Eichelberger B, Koren D, Jilma B, Panzer S. Platelet function under high-shear conditions from platelet concentrates. *Transfusion* 2008;48:129–135.
9. Wang C, Mody M, Herst R, Sher G, Freedman J. Flow cytometric analysis of platelet function in stored platelet concentrates. *Transfus Sci* 1999;20:129–139.
10. Mondoro TH, Shafer BC, Vostal JG. Restoration of *in vitro* responses in platelets stored in plasma. *Am J Clin Pathol* 1999;111:693–699.
11. Hagberg IA, Akkoc CA, Lyberg T, Kjeldsen-Kragh J. Apheresis-induced platelet activation: comparison of three types of cell separators. *Transfusion* 2000;40:182–192.
12. Rinder HM, Snyder EL, Tracey J, et al. Reversibility of severe metabolic stress in stored platelets after *in vitro* plasma rescue or *in vivo* transfusion: restoration of secretory function and maintenance of platelet survival. *Transfusion* 2003;43:1230–1237.

13. Curvers J, van Pampus EC, Feijge MA, Rombout-Sestrienkova E, Giesen PL, Heemskerk JW. Decreased responsiveness and development of activation markers of PLTs stored in plasma. *Transfusion* 2004;44:49–58.
14. Vetlesen A, Mirlashari MR, Torsheim IA, Kjeldsen-Kragh J. Platelet activation and residual activation potential during storage of hyperconcentrated platelet products in two different platelet additive solutions. *Transfusion* 2005;45:1349–1355.
15. Apelseth TO, Bruserud O, Wentzel-Larsen T, Bakken AM, Bjorsvik S, Hervig T. In vitro evaluation of metabolic changes and residual platelet responsiveness in photochemical treated and gamma-irradiated single-donor platelet concentrates during long term storage. *Transfusion* 2007;47:653–665.
16. Macey MG, Carty E, Webb L, et al. Use of mean platelet component to measure platelet activation on the ADVIA 120 haematology system. *Cytometry* 1999;38:250–255.
17. Lim YA, Hyun BH. Evaluation of platelet parameters on the ADVIA 120 as the quality indicator for stored platelets. *Clin Lab Haematol* 2002;24:377–384.
18. Lim YA, Cho SR, Lee WG, Park JS, Kim SW. Change of platelet activation markers using flow cytometry in patients with hematology/oncology disorders after transfusion. *Platelets* 2008;19:387–393.
19. Leytin V, Allen DJ, Gwozdz A, Garvey B, Freedman J. Role of platelet surface glycoprotein Ibalph and P-selectin in the clearance of transfused platelet concentrates. *Transfusion* 2004;44:1487–1495.
20. Dumont LJ, AuBuchon JP, Whitley P, et al. Seven-day storage of single-donor platelets: recovery and survival in an autologous transfusion study. *Transfusion* 2002;42:847–854.
21. Cardigan R, Turner C, Harrison P. Current methods of assessing platelet function: relevance to transfusion medicine. *Vox Sang* 2005;88:153–163.
22. Fijnheer R, Pietersz RN, de Korte D, Roos D. Monitoring of platelet morphology during storage of platelet concentrates. *Transfusion* 1989;29:36–40.
23. Rock G, Freedman M, Hamilton C, Bormanis J. New methods showing in vitro changes of platelets. *Transfus Apheresis Sci* 2006;35:145–149.
24. Giacomini A, Legovini P, Antico F, et al. Assessment of in vitro platelet activation by Advia 120 platelet parameters. *Lab Hematol* 2003;9:132–137.