



ORIGINAL ARTICLE

Platelet Factor 3 Based-clotting Time Assay as a Quality Marker for Long-term Storage of Platelet Concentrates

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ABSTRACT

Background: Platelets rapidly lose their qualities usually after 5 day of storage. Different standard methods have been recommended to check the quality of platelets during storage which some of them show better correlation with other quality markers during storage. The purpose of this study was to demonstrate if platelet factor 3 (PF3) assay could be an indicator of storage lesion and provide a significant correlation with other quality markers during long-term storage of platelet concentrates (PC) up to 11 days.

Methods: Twelve random units of PC were placed in a standard platelet incubator under continuous agitation at 22-24°C for eleven days. Samples were taken on days 1, 3, 5, 8 and 11. Parameters such as pH, glucose, lactate dehydrogenase (LDH), platelet count of the bags, mean platelet volume (MPV) and platelet distribution width (PDW) and PF3 were measured. The correlation coefficient of PF3 and pH with the abovementioned parameters was evaluated.

Results: The mean percentage of changes for PF3, pH, glucose, LDH, platelet count, MPV and PDW on day 11 compared to the first day were found to be 61, 15, 52, 440, 19, 18 and 39%, respectively. After LDH, PF3 had the highest change relative to the other markers. PF3 demonstrated better correlation with glucose, platelet count, MPV and PDW compared with pH during long-term PC storage.

Conclusion: Platelet factor 3 based-clotting time assay could be a potential candidate for monitoring the quality of PC due to apparent trend of its changes during storage with better correlation between the quality markers.

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Introduction

Platelet storage lesions (PSL) are well-known events during preparation and storage of platelet concentrates (PC). Such changes are associated with decreased hemostatic function of platelets after transfusion and with poor post-transfusion recovery.¹ Various factors are responsible for the PSL and hence poor quality of PCs. There are numerous standards to check the quality of platelets during storage that have been published by various authorities, such as the European Council and American

Association of Blood Banks (AABB). Various tests are available varying from the simplest tests such as swirling to complex platelet function tests. Though swirling is a simple and non-invasive test, it is prone to observer bias.^{2,3} The swirling was observed in 94% of cases with a pH value in the range of 6.7-7.5.² This pH range is associated with adequate in vivo survival.^{4,5} Unfortunately, in practice there is no single laboratory test than can accurately predict the efficacy of a platelet transfusion and in other words its appropriate recovery following transfusion.⁶ Previous

studies have reported that pH, as a major quality marker, shows the highest correlations with recovery and survival of platelets in healthy subjects.^{7,8}

Platelet factor 3 (PF3), as a phospholipid lipoprotein blood coagulation factor derived from platelets acts with certain plasma thromboplastin factors to convert prothrombin to thrombin. The PF3 assay relies on the principle that incubation of platelet-rich plasma (PRP) with kaolin activates the procoagulant activity of platelets, resulting in a progressive shortening of both the recalcification time and Russell viper venom time and can be used specifically for assessing platelet procoagulant activity.^{9,10} This novel test may also be used to monitor procoagulant activity of platelet membrane in platelet substitutes such as lyophilized intact platelets¹¹ or lyophilized infusible platelet membranes¹²⁻¹⁶ in the future as well. We aimed to assess if PF3 assay indicates higher correlation compared with other quality markers such as pH during long-term storage of platelet concentrates up to 11 days and if it could be applied to monitor quality control of platelet concentrates in the future.

Materials and Methods

Study Design

In this study, 12 PCs were collected from healthy volunteers in Tehran Blood Transfusion Center as a routine procedure according to the platelet rich plasma method from whole blood with soft and hard spin centrifugation steps, respectively. After collection, standard PCs were kept undisturbed for one hour. The sterility test were performed to show no bacterial contamination of PCs and then they were placed in a standard platelet incubator with shaker (Danesh Pajoohesh Fajr Co, Iran) under continuous agitation at 22-24°C for eleven days.

Sampling

Sampling was done aseptically with a large bore needle.

Three milliliter samples of each PC unit were taken on 1, 3, 5, 8 and 11 days and the following tests were performed by biochemical and hematological analyzers.

Biochemical and Hematological Tests

Parameters such as pH, glucose as a main catabolic cell pathway (glycolysis), lactate dehydrogenase (LDH) as an enzyme marker of cell viability, pre-donation platelet count of donor, total platelet count of the bags, platelet yield extraction, mean platelet volume (MPV) and platelet distribution width (PDW) as morphostructural platelet indices were measured.

PF3 Assay

PF3 as a major platelet function based-clotting time assay was performed on the samples during storage of platelet concentrates according to the kaolin clotting time method.⁹ Fresh platelets and physiological normal saline were used as positive and negative controls, respectively. The clotting time was converted to percent of activity by use of a standard curve.

Statistical Analysis

True values was converted to mean percentage values with regard to initial value of 100 percent on day 1 and correlation coefficient (r) analysis was performed to compare the ability of PF3 and pH as quality markers during a 11-days storage period.

Results

The relevant data of twelve PCs in terms of quality control parameters of platelets such as pH, glucose, LDH, platelet count, MPV and PDW during eleven days storage are reported as mean percentage changes in table 1. Mean percentage platelet yield extraction of PCs during storage up to eleven days was shown in table 2. During storage, metabolic activity of platelets continued

Table 1: Mean percentage changes in the quality control markers of twelve platelet concentrates during standard storage up to eleven days

| Day of storage | PF3 | Glucose | pH | LDH | Platelet Count | MPV | PDW |
|----------------|------------|---------|---------|-----------|----------------|----------|----------|
| 1 | 100* (0)** | 100 (0) | 100 (0) | 100 (0) | 100 (0) | 100 (0) | 100 (0) |
| 3 | 90*** (10) | 90 (10) | 99 (1) | 171 (71) | 98 (2) | 108 (8) | 113 (13) |
| 5 | 77 (23) | 80 (20) | 98 (2) | 253 (153) | 92 (8) | 110 (10) | 115 (15) |
| 8 | 60 (40) | 65 (35) | 93 (7) | 377 (277) | 85 (15) | 114 (14) | 123 (23) |
| 11 | 39 (61) | 48 (52) | 85 (15) | 540 (440) | 81 (19) | 118 (18) | 139 (39) |

*Percent of initial value was assumed 100 on day 1 for all quality control markers; **Data shows mean percentage of increase or decrease relative to initial value of 100 percent; ***Mean Percentage: Each true value was converted into percent with regarding to initial value of 100 percent on day 1

Table 2: Mean percent of platelet yield extraction* of twelve platelet concentrates (PCs) during standard storage up to eleven days

| Day of storage | Total platelet count of PCs/bag ($\times 10^4$) | Platelet yield extraction/bag (%) | Platelet yield extraction/bag** (%) |
|----------------|---|-----------------------------------|-------------------------------------|
| 1 | 6862002 | 61 | 100 |
| 3 | 6711816 | 60 | 98 (2) |
| 5 | 6309816 | 56 | 92 (8) |
| 8 | 5803638 | 52 | 85 (15) |
| 11 | 5544546 | 49 | 81 (19) |

*Total pre donation platelet count/bag was calculated as 11149184×10^4 ; **Percent of initial value of platelet yield was assumed 100 on day 1 for PCs

leading to glucose consumption that led to a trend of decreasing pH due to glycolytic energy generation and lactate production. Better trend was observed in LDH production, PF3 activity decline, glucose consumption, increasing of PDW and MPV, platelet count depletion and decreasing pH, respectively (figure 1). Our results showed that platelet counts per bag do decrease slowly during storage, albeit with the acceptable platelet count ($>55 \times 10^9/\text{bag}$) according to the conventional standards even at the end of the storage (table 2).

The coefficient correlation of pH and PF3 versus other quality markers was calculated in table 3. Strong correlation was observed between pH and LDH, PF3, glucose, PDW, platelet count and MPV, respectively. High correlation was also observed between PF3 and glucose, platelet count, PDW, pH, MPV and LDH, respectively.

Discussion

We found that PF3 could be altered as a result of platelet storage and hence be associated with storage lesions during long-term storage of PCs similar to other quality parameters such as pH, glucose, LDH, platelet count,

MPV and PDW. Similar results have been reported by previous studies for pH,^{7,17-26} glucose,^{7,19,20,22-26} LDH,^{20,22,23,25} platelet count,^{7,20,21,23-26} MPV^{20,21,23} and PDW²¹ of PCs that confirm their roles in monitoring the quality of platelets during storage. Minor differences in these studies may be related to the preparation method of the platelets,^{27,28} the plastic material of the storage bag,⁴ the ability of bags to exchange gas across its surface,⁵ storage temperature,^{27,29} the type of anticoagulant used, the platelet concentration in the bag and the agitation.²⁷

On the other hand, PF3 which has phospholipid procoagulant activity of platelet membrane, as a clotting time assay, has not been used as a quality marker in the literature. Chao³⁰ used PF3 assay to show the functional properties of infusible platelet membrane product. Recently, studies have shown that flow cytometric analysis of CD41/CD61 and CD42b platelet receptors with PF3 based-clotting time assay may also show the status of platelet concentrates during storage.³¹

Accordingly, Bode³² analyzed platelet factor 3 in platelet concentrates during PC storage and demonstrated higher PF3 and LDH activity that were significantly correlated

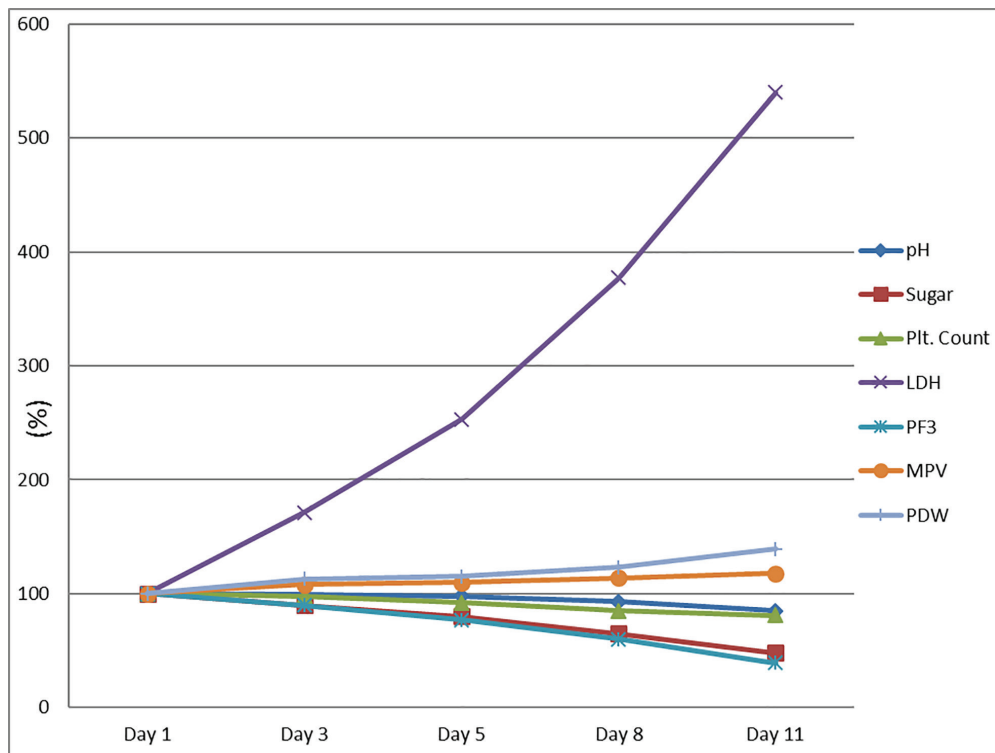


Figure 1: Trend in pH, glucose, platelet count, LDH, PF3, MPV and PDW. Each point represents the mean percentage value of twelve platelet concentrates stored in standard condition up to eleven days with regarding to initial value of 100 percent on day 1.

Table 3: Correlation coefficient values of pH and PF3 with other platelet quality control markers during 11 days storage of platelet concentrates

| Parameter | Correlation coefficient of pH (r) | Correlation coefficient of PF3 (r) |
|------------|-----------------------------------|------------------------------------|
| Glucose | 0.961 | -0.999 |
| LDH | -0.977 | 0.929 |
| Plt. Count | 0.934 | 0.982 |
| MPV | -0.868 | 0.958 |
| PDW | -0.959 | 0.978 |
| PF3 | -0.969 | 1.000 |
| pH | 1.000 | -0.969 |

together only in standard, manual PC. Measurement of cytoplasmic leakage of LDH may also be used as a quality parameter and reflects platelet membrane damage. LDH has been shown to correlate to platelet survival ($r=0.64$).³³ In our study, LDH and PF3 had trends showing storage lesions more relevant in comparison with other markers (Figure 1).

Our results demonstrated that pH declines steadily from day 1 (7.78) to day 5 (7.61) of storage, but sharper decline was observed between days 5 to 11 of storage with the mean pH of 6.61 at the end of storage which reflects better preservation of platelets during long-term storage with only 15 percent decrease in the pH. There was not an increase in pH between the first and third days of storage as reported by Dekkers³³ which may reflect the temporary changes in gas concentrations. The pH is an important marker for the quality of PCs *in vitro* since at values below 6.8, platelets become spherical; this change in shape becomes irreversible when the pH drops below 6.2. Platelet metabolism ceases completely when pH values drop below 6.0.³⁴

Conclusion

It may be concluded that platelet factor 3 based-clotting time assay can be a potential candidate for monitoring the quality of PCs due to the observed trend of its changes during storage. In comparison with pH, this test may also show better correlation with the other quality markers. However, further investigations are required to find the efficacy and precision of PF3 as quality marker and also look for other markers that help predict precisely status of PCs during storage.

Conflict of Interest: None declared.

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