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ORIGINAL ARTICLE

Evaluation of Coagulation Factors Activity in Different Types of Plasma Preparations

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Abstract Fresh frozen plasma (FFP) is a crucial substitute therapy in management of bleeding; producing plasma from whole blood stored within 24 h offers operational flexibility and leukocyte filtration significantly reduce transfusion reactions, it is necessary to consider the impact of these plasma preparations on clotting factors activity. Total of 75 plasma samples collected from 25 blood donors distributed as 3 groups; FFP (Group A), leukocyte filtrated FFP (Group B) and plasma frozen within 24 h i.e. PF24 (Group C), for all samples prothrombin time (PT), INR, (APTT), Factors V, VII, VIII, IX levels and Fibrinogen were done, also comparing coagulation factors levels in FFP in different blood groups. There were significant difference between three groups in (PT), INR and (APTT): ($P = 0.00$). Concerning Factor VII: significant difference ($P = 0.03$) between the three groups, FFP had a significantly higher level of FVII compared to filtrated FFP (98.92 vs. 82.52%; $P = 0.02$), while no significant difference between FFP and PF24 was detected ($P = 0.76$). Factor VIII: had significant difference ($P = 0.00$) between the three groups, FFP and Filtrated FFP had no significant difference regarding level of FVIII ($P = 0.72$), but FFP had significantly higher level of FVIII compared to PF24 ($P < 0.05$). Concerning Fibrinogen level: no significant difference between FFP and filtrated FFP ($P = 0.99$), while FFP had a higher level versus PF24 ($P < 0.05$). On the Contrary, no significant difference between three groups in Factor V: ($P = 0.22$) and Factor IX: ($P = 0.12$). ABO blood group effect on studied parameters in FFP: FVIII was statistically higher in Non-O blood group ($P = 0.03$), other factors had no statistical differences ($P > 0.05$). The leukocyte filtration of FFP did not affect the majority of coagulation factors activities, although FVII level was reduced, it stills enough for surgical hemostasis. The PF24 resulted in reduced FVIII and fibrinogen levels but no significant changes in FV, FVII or FIX, thus, can be used for FFP indications except that specifically requiring replacement of FVIII and/or fibrinogen as Hemophilia or DIC. No significant difference in coagulation factors of FFP between O and non-O blood groups except FVIII that was reduced in O blood group.

Keywords Leukocyte filtration · Fresh frozen plasma · Plasma frozen within 24 h · Coagulation factors

Introduction

Fresh frozen plasma (FFP) is the alternative therapy in coagulation factor deficiencies when suitable concentrate is not available [1]. The clinical application of FFP aim to supplementation of coagulation factors or correction of coagulation factor defects, so it is necessary to determine aspects affecting the level of coagulation factors of various conditions of plasma as fresh frozen plasma and 24-h frozen plasma, leucoreduced or not. The production of plasma from whole blood that has been stored within 24 h increases operational flexibility and availability to blood operators [2]. Studies have been performed to investigate the effect of extended storage of blood prior to FFP production [3–9]. Leukocytes are a non-therapeutic component of FFP, which increase the risk of adverse transfusion reactions [1]. pre-storage leukoreduction of plasma is the most broadly accepted approach, many advantages of pre-

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storage over post-storage leukoreduction that includes: elimination of inflammatory cytokine accumulation due to leukocytes during storage [10–12], also reduces HLA-alloimmunization risk in multitransfused cases [13, 14]. Pre-storage leukofiltration also reduce leukotropic virus transmission risk [15, 16].

The main technique used to clear the plasma of residual leukocytes is leukocyte filtration, removal of leukocytes by this method improves safety of transfusion by minimizing adverse effects associated with incidental transfusion of leucocytes [17, 18]. However, coagulation factors might be compromised during the leucoreduction process, it is necessary to consider whether leukocyte filtration would affect the coagulation factor activity of FFP or not and to what extent. No disclaimers exist to evaluate residual clotting factor activity for the licensed plasma products in the United States; fresh frozen plasma and 24-h frozen plasma either leucoreduced or not [19]. Aim of this study was to analyze and compare the coagulation profile in three types of plasma, the plasma prepared within 8 h after donation i.e. FFP, plasma with leukocyte filtration within 8 h of donation i.e. leukocyte filtrated FFP and plasma separated from blood stored at 4–6 °C after collection and separated and placed at − 20 °C within 24 h i.e. (PF24). Also to study effect of ABO blood group on studied parameters in FFP.

Materials and Methods

Total of 75 plasma samples were collected from twenty-five blood donors at Assiut, Egypt, in the period between January and July 2017 only donors who have met American Association of Blood Banks criteria were assigned to this study. The research protocol was approved by the Research Ethics Committee at Assiut University. Donors were informed that their plasma would be used for experimental investigations, efforts were made to achieve uniform representation of all blood groups (seven donors with blood group O, seven group A, seven group B and four group AB), plasma samples were separated into 3 groups: FFP (Group A), leukocyte filtrated FFP (Group B), PF24 (Group C). All samples were analyzed to evaluate their coagulation profile; prothrombin time (PT), INR, (APTT), Factors V, VII, VIII and IX activity levels and Fibrinogen were performed also comparing coagulation factors levels in FFP in different blood groups.

Blood Collection and Sample Processing (Fig. 1)

Twenty-five units of whole blood (WB) (about 500 ml each) were collected into JMS double blood bags (JMS Singapore Pte Ltd. ®) containing citrate phosphate dextrose adenine (CPDA-1) 70 ml as anticoagulant, under pressure of 50–60 mmHg to ensure similar collection conditions, collection times varied between 4 and 9 min. Because the level of FVIII is lower in group O donors, an equal number of group A and group O donations were selected to avoid biases in the results, following collection, two samples were withdrawn in Wassermann tubes (about 4.5 ml each); one of them was centrifuged within 8 h at 3800 RPM for 11 min at 4 °C. using (Hettich ROTOFIX 32A ®, Tuttlingen, Germany) to separate FFP (Group A) and the other sample was kept for 24 h at 4 °C, then centrifuged with the same technique to separate PF24 (Group C), the remaining part of each WB unit (About 480 ± 10 ml) was filtrated using disposable separate laboratory leukodepletion filters (BioR 02 plus BBS PF®, Fresenius Kabi AG, Germany) for red cell concentrates, then it was centrifuged within 8 h by hard spin (3880 RPM for 11 min. at 4 °C) using (KR4i centrifuge, Thermo Fisher Scientific ®, Germany), the separation of plasma was done according to standard practice to prepare FFP in our institute, and the red cells were separated from the plasma using a manual blood component extractor (JMS Singapore Pte Ltd. ®), There was no significant volume loss only about 10 ml blood that remain in the tubes connecting WB with filtrated blood bag, finally the third sample was ready as leukocyte filtrated FFP (Group B), residual leucocyte were measured in ten filtrated blood bags and it was less than 10^6 leukocytes. A total of 75 Plasma samples were collected representing the three groups, they were aliquoted into 2 ml portions and stored frozen at − 20 °C until testing within 2 months. Coagulation profile with clotting factor activity levels were determined using the automatic blood coagulation analyzer Sysmex CA-1500 System (Sysmex Corporation ®, Kobe, Japan). All assays were performed according to the manufacturer's instructions and the following assessments were done to all plasma samples: prothrombin time (PT), INR, Activated Partial Thromboplastin Time (APTT), Factors V, VII, VIII and IX activity levels and Fibrinogen.

Reagents and Principle

Thromborel S Reagent (for prothrombin time; solidification method), Pathromtin SL Reagent (for Activated partial thromboplastin time; solidification method), coagulation factors V, VII, VIII, IX deficient plasma (for FV:C, FVII:C, FVIII:C and FIX:C; solidification method), Dade Fibrinogen Determination Reagent (for fibrinogen; Clauss method). All reagents were supplied by (Siemens Healthcare Diagnostics Products ®, Marburg, Germany); samples testing were operated in accordance with the instructions of the instruments and reagents. Control plasma of known potency was used for each run to ensure reliability of the

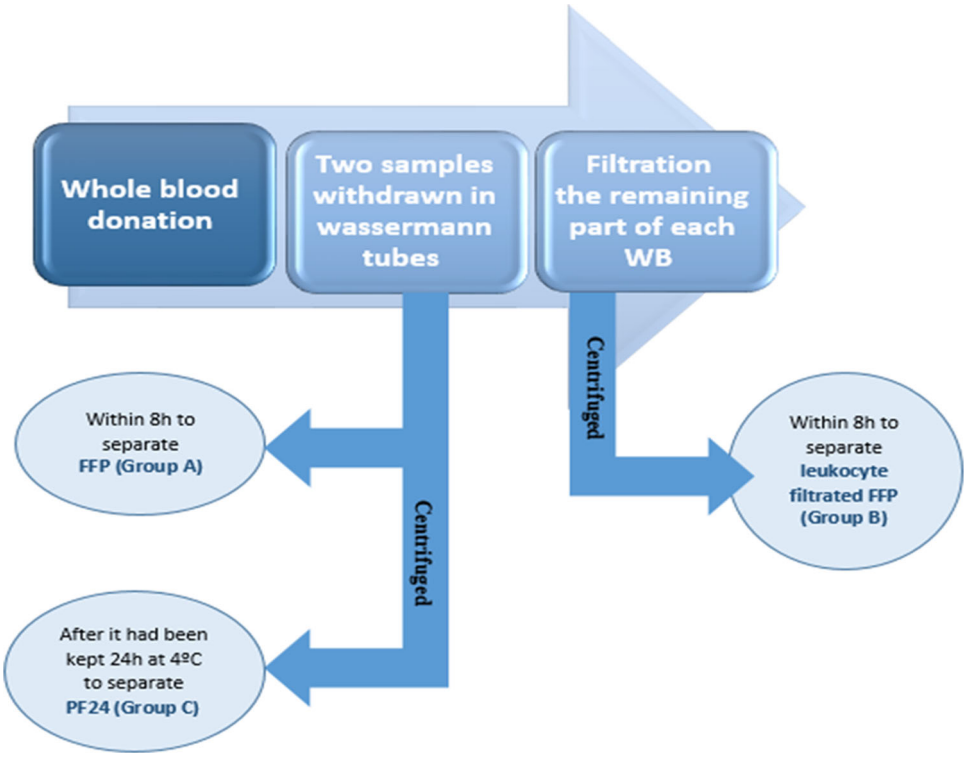

Fig. 1 Flow chart of blood collection and sample processing

tests; also, standard controls were run in all cases. Results were recorded for analysis.

Statistical Analysis

Data were normally distributed as tested by Shapiro test so; it was expressed in form of mean ± SD. ANOVA (ANalysis Of VAriance) was used to test differences between mean levels (± SD) of FV, FVII, FVIII, IX, fibrinogen, PT, INR and APTT in the three groups, post hoc Tukey test was used to compare each group to other. Student *t* test was used to analyze the effect of ABO blood group on all studied parameters. Analysis of data was done by IBM-SPSS version 20 and *P* value was considered significant if < 0.05.

Results

1-Coagulation Profile

FFP coagulation factors levels were considered as the baseline coagulation profile for the donors, as they were not affected by any delay in storage.

Table 1 shows the comparison between the three plasma products groups. Prothrombin time (PT): ANOVA test showed a significant difference in PT values between the three groups ($P = 0.00$), between FFP and filtrated FFP ($P = 0.03$), between FFP and PF24 ($P = 0.00$). International Normalized Ratio (INR): there was a significant difference between the three groups ($P = 0.00$), it was noticed that the INR value was statistically higher in PF24 > filtrated FFP > FFP with $P < 0.05$. Activated Partial Thromboplastin Time (APTT): showed a significant difference between the three groups ($P = 0.00$), PF24 had a significantly higher statistical difference of APTT in comparison to other groups ($P < 0.05$). Factor V: Although FFP had a higher level of FV in comparison to other groups, this difference was statistically insignificant ($P = 0.22$). Factor VII: there was a significant difference between the three groups with ($P = 0.03$). Post-hoc Tukey test showed FFP to have a significantly higher level of FVII compared to filtrated FFP (98.92 vs. 82.52%; $P = 0.02$), while no significant difference between FFP and PF24 ($P = 0.76$). Factor VIII: there was a significant difference between the three groups regarding the level of FVIII (105.24%, 110.64%, 62.76% respectively; $P = 0.00$). Post-hoc Tukey test showed that FFP and filtrated FFP had no significant difference regarding the level of FVIII ($P = 0.72$), but FFP had a significantly higher level of FVIII compared to PF24 ($P < 0.05$). Factor IX: there was no significant statistical difference ($P = 0.12$) between three groups, although FFP had a higher level (100.40%) compared to filtrated FFP and PF24 (95.96% and 87.12% respectively). Fibrinogen (FbgC) level by "Clauss method", for FFP, filtrated FFP and PF24, were

Table 1 Analysis of different coagulation factors in the in the three groups

Variables	Groups			
	Group A	Group B	Group C	*P* value
PT (11–14 s)	13.28 ± 0.63	13.98 ± 1.06	15.69 ± 1.16	0.00
INR	1.14 ± 0.05	1.23 ± 0.12	1.34 ± 0.13	0.00
APTT (28–40 s)	38.52 ± 1.66	50.01 ± 6.45	55.12 ± 6.04	0.00
Factor V (70–120%)	90.96 ± 20.43	89.04 ± 18.43	81.84 ± 18.93	0.22
Factor VII (70–120%)	98.92 ± 24.09	82.52 ± 18.28	94.60 ± 22.67	0.03
Factor VIII (70–150%)	105.24 ± 26.45	110.64 ± 22.72	62.76 ± 15.09	0.00
Factor IX (70–120%)	100.40 ± 19.95	95.96 ± 25.06	87.12 ± 23.12	0.12
Fibrinogen (1.8–3.5 g/l)	2.56 ± 0.91	2.55 ± 0.76	1.81 ± 0.76	0.00

PT prothrombin time, *INR* International Normalized Ratio, *aPPT* activated partial thromboplastin time, *Group A* FFP, *Group B* filtrated FFP, *Group C* PF24

2.56 ± 0.91, 2.55 ± 0.76 and 1.81 ± 0.76 g/l, respectively, ANOVA test showed a significant difference between the three groups ($P = 0.02$), it was noticed that no significant difference between FFP and filtrated FFP ($P = 0.99$) while FFP had a significantly higher level of fibrinogen compared to PF24 ($P < 0.05$).

2-Effect of ABO Blood Group

Table 2 samples distribution as 18 (72%)—non O blood group and 7 (28%) with O blood group. The current study showed that only FVIII was statistically higher in Non-O blood group with ($P = 0.03$), while other factors had no statistical differences between both ABO blood groups ($P > 0.05$).

Discussion

Current study investigated the effect of different plasma separation and storage techniques namely; leucocyte filtration of FFP plasma (Filtrated FFP/Group B) and holding of WB at 4 °C for 24 h before plasma separation (PF24/Group C), on the extrinsic and intrinsic pathway clotting factors in terms of PT, INR and APTT as well as on clotting factors V, VII, VIII, IX and fibrinogen and comparing them to plasma separated by centrifugation within 8 h of donation (FFP/Group A).

Our sample size was restricted to 75 plasma samples that were collected from twenty-five blood donors at Assiut, Egypt as we were limited by our financial fund. Similar to Aboul Enein et al. [12] who studied the effect of different methods of leucoreduction on plasma coagulation factors at Cairo University, Cairo, Egypt their total sample size were fifty plasma samples (Non leucoreduced group: Twenty samples, Leucoreduced group: Thirty samples.

In this study, there were significantly prolonged PT and INR in both filtrated FFP and PF24 when compared to FFP ($P = 0.03$, 0.00 respectively). Li et al. [1] and Acker et al. [2] have addressed changes in clotting factors after leucofiltration and came up with agreed results as ours. However, Weisert et al. [3] acknowledged a shorter PT post-filtration and Sohmer et al. [4] reported lack of the effect of holding blood at 4 °C overnight on the PT results.

The present study also showed significantly prolonged APTT in both filtrated FFP and PF24 ($P < 0.05$ in both) as compared with FFP. This was in agreement with [2–4]. Cardigan et al. [20] found a significant prolonged APTT in

Table 2 Effect of ABO blood group on all studied parameters in FFP

Variables	Blood groups		
	Non O blood group ($n = 18$)	O blood group ($n = 7$)	*P* value
PT (11–14 s)	13.26 ± 0.68	13.31 ± 0.56	0.13
INR	1.13 ± 0.06	1.15 ± 0.06	0.06
APTT (28–40 s)	38.14 ± 2.11	38.66 ± 1.51	0.26
Factor V (70–120%)	89.22 ± 14.82	89.22 ± 25.23	0.23
Factor VII (70–120%)	102.22 ± 22.76	89.85 ± 29.93	0.98
Factor VIII (70–150%)	107.78 ± 24.08	96.14 ± 22.08	0.03
Factor IX (70–120%)	101.11 ± 21.27	98.78 ± 17.45	0.11
Fibrinogen (1.8–3.5 g/l)	2.59 ± 0.91	2.47 ± 0.93	0.09

non-leucoreduced plasma as compared with the leucoreduced one, on the contrary, Nilsson et al. [5] reported that APTT was not significantly changed post filtration.

Regarding Factor V, there was no significant decrease of it neither in filtrated FFP nor in PF24 ($P = 0.22$), this matched with Li et al. [1] and Kakaiya et al. [6] who investigated the effect of leucofiltration on coagulation testing and others who studied the effect of holding of WB at 4 °C for approximately 24 h before plasma separation [7–9, 17, 18]. In contrast, Acker et al. [2] documented that there was a statistically significant loss in factor V in plasma prepared by using 2 of the 5 WB filters while one of the WB filters appeared to cause an increase in FV. Sohmer et al. [4] reported that Factor V significantly decreased by 15% after storage of WB at 4 °C for approximately 24 h before plasma separation.

Our results showed that Factor VII was significantly decreased in filtrated FFP compared with FFP (82.52% vs. 98.92%; $P = 0.02$), however, it was not significantly affected after storage of WB at 4 °C for approximately 24 h before plasma separation ($P = 0.76$), this was in agreement with [8, 9, 17, 18]. On the other hand, Kakaiya et al. [6] reported that there was a non-significant decrease in activity of FVII in filtrated plasma, furthermore, Nilsson et al. [5] reported that level of FVII was 3% higher post filtration as compared with FFP; however, it was not significant.

FVIII is recognized to be a labile coagulation factor in plasma. In Europe, its level is used as a quality control standard for FFP [19]. In our studied groups, FVIII level was not significantly affected by filtration ($P = 0.72$), this coincides with Nilsson et al. [5] and Kakaiya et al. [6] who investigated the effect of leucofiltration on coagulation factors. On the Contrary, Acker et al. [2] documented statistically significant losses in factor VIII with 2 of the 5 types of WB filters. Weisert et al. [3] also documented a decrease in FVIII post filtration, however, this was only significant after plasma storage at room temperature for 18 h pre-processing. Holding blood at 4 °C overnight resulted in 40% loss of FVIII. Its level in FFP and PF24 were 105.24%, 62.76% respectively ($P < 0.05$), these results are similar to that found by [7, 8, 17, 20]. Other studies reported that loss of FVIII in PF24 was 25% or less [4, 5, 9, 18].

In the present study, FIX was not significantly affected neither by filtration nor by holding blood at 4 °C overnight ($P = 0.12$), this was in agreement with [4, 6, 18].

In contrast, Acker et al. [2] documented statistically significant losses in FIX with 2 of the 5 types of WB filters, the decrease in FIX varies after consuming different plasma filters. In the present study fibrinogen (FbgC) was not significantly affected by filtration ($P = 0.99$), while FFP had a significantly higher level of fibrinogen compared to PF24 ($P < 0.05$), this was in agreement with Weisert et al. [3] and Kakaiya et al. [6] who found no significant effect of filtration on FbgC, while Cardigan et al. [20] reported that one of the WB filters seemed to result in an increased fibrinogen level; however, Li et al. [1] reported that FbgC significantly decreased by 14% post filtration. In the present study, FbgC decreased by 29% in PF24, this result was nearly in agreement with Cardigan et al. [21] who reported that FbgC decreased by 12% in PF24. Other studies found that FbgC was not affected after holding blood at 4 °C overnight [9, 17, 18], also, Weisert et al. [3] documented no significant effect on FbgC after holding blood up to 18 h at room temperature (20 ± 2 °C).

The previous studies assessing coagulation factors after filtration yielded mixed results, probably caused by different products being filtered (WB or plasma), types of filters, time and/or temperature of storage before processing and possible unidentified parameters related to filtration [21]. The present study investigated also the influences of ABO blood group on the extrinsic and intrinsic pathway clotting factors in terms of PT, INR and APTT as well as on clotting factors V, VII, VIII, IX and fibrinogen in FFP, the majority of the included samples 18 [72%] had Non-O blood group while only 7 [28%] had O blood group, our results showed that only FVIII that was statistically higher in Non-O blood group with (P value = 0.03) while other factors had no statistical differences between O and Non-O groups ($P > 0.05$), this coincides with previous studies which suggest that the diversities of FVIII was partially determined by ABO blood types [6, 7]. Naghadeh et al. [22] reported that ABO blood group had no noticeable influence on Fbg level, which is consistent with our results.

Conclusion

In conclusion, the leukocyte filtrated FFP retained majority of coagulation factors activities, it can be used as a safer alternative to FFP. The PF24 resulted in reduced FVIII and fibrinogen; it can be used for same indications as FFP except indications requiring FVIII and/or fibrinogen replacement. The FFP coagulation factors levels showed no difference between blood groups except FVIII was reduced in O blood group.

Author Contributions OA and MA designed the study, supervised the experiments and provided critical expertise, AA prepared the samples, measured the parameters, organized the resulting data, EN evaluated and interpreted the data and wrote the manuscript. All Authors read, edited, and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The Authors declare no conflicts of interest.

References

1. Li DY, Zhang HW, Feng QZ, Zhao H (2015) Impacts of leukocyte filtration and irradiation on coagulation factors in fresh frozen plasma. Exp Ther Med 9(2):598–602
2. Acker JP, Marks DC, Sheffield WP (2016) Quality assessment of established and emerging blood components for transfusion. J Blood Transfus 2016:4860284
3. Weisert O, Jeremic M (1973) Preservation of coagulation factors V and VIII during collection and subsequent storage of bank blood in ACD-A and CPD solutions. Vox Sang 24(2):126–133
4. Sohmer PR, Bolin RB, Scott RL, Smith DJ (1982) Effect of delayed refrigeration on plasma factors in whole blood collected in CPDA-2. Transfusion 22(6):488–490
5. Nilsson L, Hedner U, Nilsson IM, Robertson B (1983) Shelf-life of bank blood and stored plasma with special reference to coagulation factors. Transfusion 23(5):377–381
6. Kakaiya RM, Morse EE, Panek S (1984) Labile coagulation factors in thawed fresh frozen plasma prepared by two methods. Vox Sang 46(1):44–46
7. Hughes C, Thomas KB, Schiff P, Herrington RW, Polacsek EE, McGrath KM (1988) Effect of delayed blood processing on the yield of factor VIII in cryoprecipitate and factor VIII concentrate. Transfusion 28(6):566–570
8. O'Neill EM, Rowley J, Hansson-Wicher M, McCarter S, Ragno G, Valeri CR (1999) Effect of 24-hour whole-blood storage on plasma clotting factors. Transfusion 39(5):488–491
9. Smith JF, Ness PM, Moroff G, Luban NL (2000) Retention of coagulation factors in plasma frozen after extended holding at 1–6 degrees C. Vox Sang 78(1):28–30
10. Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD et al (2002) A randomized controlled trial comparing the frequency of acute reactions to plasma -removed platelets and Prestorage WBC- reduced platelets. Transfusion 42:556–566
11. Muylle L, Joos M, Wouters E, De Bock R, Peetermans ME (1993) Increased tumor necrosis factor alpha (TNF alpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNF alpha and IL-6 levels and febrile transfusion reactions. Transfusion 33:195–199
12. Rebulla P, Dzik WH (1994) Multicenter evaluation of methods for counting residual white cells in leukocyte-depleted red blood cells. Vox Sang 66:25–32
13. Williamson LM, Wimperis JZ, Williamson P, Copplestone JA, Gooi HC, Morgenstern GR et al (1994) Besides filtration of blood products in the prevention of HLA alloimmunization—a prospective randomized study. Blood 83:3028–3035
14. Sintnicolaas K, van Marwijk Kooij M, van Prooijen HC, van Dijk BA, van Putten WL, Claas FH et al (1995) Leukocyte depletion of random single- donor platelet transfusions does not prevent secondary human leukocyte antigen-alloimmunization and refractoriness: a randomized prospective study. Blood 85:824–828
15. Dzik WH, Anderson JK, O'Neill EM, Assmann SF, Kalish LA, Stowell CP (2002) A prospective, randomized clinical trial of universal WBC reduction. Transfusion 42:1114–1122
16. Paglino JC, Pomper GJ, Fisch GS, Champion MH, Snyder EL (2004) Reduction of febrile but not allergic reactions to red cells and platelets following conversion to universal Prestorage leukoreduction. Transfusion 44:16–24
17. Wieding JU, Vehmeyer K, Dittman J, Hiddemann W, Kohler M, Lanzer G (1994) Contamination of fresh-frozen plasma with viable white cells and proliferable stem cells. Transfusion 34(2):185–186
18. Yuan L, Sun L, Yang L, Jing Y (2014) Acute graft-versus-host disease in a nonhematopoietic stem cell transplantation candidate treated with decitabine followed by granulocyte colonystimulating factor-primed peripheral blood stem cells infusion: a special entity of the disease? Transfusion 54(1):190–193
19. Aboul Enein AA, Abdel Rahman HA, Abdel Maged MM, El Sissy MH (2017) The effect of different methods of leucoreduction on plasma coagulation factors. Blood Coagul Fibrinolysis Int J Haemost Thromb 28(2):117–120
20. Cardigan R, Sutherland J, Garwood M, Krailadsiri P, Seghatchian J, Beard M et al (2001) The effect of leucocyte depletion on the quality of fresh-frozen plasma. Br J Haematol 114(1):233–240
21. Cardigan R, Lawrie AS, Mackie IJ, Williamson LM (2005) The quality of fresh-frozen plasma produced from whole blood stored at 4 degrees C overnight. Transfusion 45(8):1342–1348
22. Naghadeh HT, Roudkenar MH (2009) A study of the quantity of some stable and labile coagulation factors in fresh-frozen plasma produced from whole blood stored for 24 hours inIran. Blood transfus = Trasfusione del sangue 7(1):39–42