The effect of different methods of leucoreduction on plasma coagulation factors

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Removal of leucocytes from blood products, namely leucoreduction, improves the safety of blood transfusion by reducing adverse events associated with the incidental transfusion of leucocytes. Coagulation factors might be compromised during leucoreduction because of exposure of plasma to a variety of filter materials. The aim of the current study was to assess the effect of different methods of prestorage leucofiltration (apheresis and whole blood filters) on prothrombin time, international normalized ratio, partial thromboplastin time and factors V and VIII. There was a significant prolongation of prothrombin time as well as elevation of international normalized ratio in plasma after leucoreduction (14.5 ± 0.7 s vs. 13.9 ± 0.7 s, $P = 0.008$ and 1.14 ± 0.07 vs. 1.09 ± 0.07, $P = 0.005$, respectively). Also, there was a statistically significant prolongation of activated partial thromboplastin time in nonleucoreduced plasma (55.6 ± 9.9 s vs. 43.2 ± 12.8 s, $P = 0.001$). There was no significant filtration effect on factors V and VIII levels. Furthermore, there was no significant difference in factors V and VIII levels between plasma filtered by inline whole blood filters and apheresis machine. Leucodepleted plasma originating from both inline whole blood filter and apheresis machine maintained satisfactory levels of factors V and VIII.

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Introduction

Leucocyte reduction of cellular blood components to less than 5×10^6/unit has been repeatedly studied as a way to prevent different blood transfusion complications, such as immune platelet refractoriness, febrile transfusion reactions (FTRs) and transmission of human cytomegalovirus [1]. In addition, leucoreduced red cells may play a role in reducing postoperative infections [2].

Leucoreduction has been widely used at the bedside, but many studies had reported its lack of efficacy in avoiding clinical complications, particularly FTR to platelet concentrates [3]. Inflammatory cytokines released from contaminating leucocytes during platelet storage have been implicated in FTR to platelets; this has raised the attention to prestorage leucodepletion [4]. Elevated cytokine levels in stored red cells are minimized by prestorage leucodepletion [5]. In addition, several factors associated with bedside leucodepletion and difficulty in quality control [6] make prestorage leucodepletion of red cells a preferred solution [7].

Leucoreduction is usually achieved by filtration of whole blood or blood components, or by certain apheresis-associated filtration techniques, and most filters provide a 3- to 4-log removal of leucocytes [8]. Guidelines for assessing the processes of leucodepletion before their routine use have been adopted [9]. Previous studies have investigated the effect of leucofiltration on residual clotting factors using different types of filter [10–12]. In this study, we aimed to evaluate the quality of plasma in terms of prothrombin time, international normalized ratio (INR), partial thromboplastin time and factors V and VIII after leucoreduction by apheresis and whole blood filters.

Materials and methods

Fifty samples of fresh platelet poor plasma (PPP) products were collected randomly from volunteer donors in Blood Bank Unit of Kasr-Al Aini Hospitals-Cairo University, who met all American Association of Blood Banks and US Food and Drug Administration criteria, into 500-ml packs using citrate phosphate dextrose adenine (CPDA)-1 : 70 ml as anticoagulant. Apheresis plasma was collected in packs containing acid citrate dextrose (ACD). The research protocol was approved by the Research Ethics Committee of the Clinical Pathology Department of Cairo University. A written informed consent was obtained from donors.

Samples were divided into two major groups:

Nonleucoreduced group

Twenty samples of fresh plasma were prepared from citrate phosphate dextrose adenine-whole blood units by centrifugation of the whole blood within 8 h at 5000*g* for 6 min using a centrifuge (MSE CELLSEP

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6/720r Centrifuge, UK) to produce PPP and a red blood cell (RBC) concentrate.

Leucoreduced group

Thirty samples of fresh plasma were collected as follows:

Whole blood filter subgroup: 15 samples of fresh plasma were prepared by centrifugation of the whole blood within 8 h at 5000*g* for 6 min using a centrifuge (MSE CELLSEP 6/720r Centrifuge) to produce PPP and an RBC concentrate. Plasma was then immediately filtered using Leucotrap system integral whole blood filters with uncharged polyester fibres in polyvinyl chloride (Dockable Filtration System; Haemonetics Corporation, USA).

Apheresis subgroup: 15 samples of fresh plasma were immediately filtered from donors using the apheresis machine (TrimaAccel Collection System, USA) into acid citrate dextrose packs.

Plasma samples from both groups were collected and aliquoted into 2 ml portions and stored at −20°C for 21 days until testing. All units of filtered plasma had the quality to yield a residual leucocyte count 1×10^6 per unit or less as counted by automated cell counter before [mean ± standard deviation (± SD): 4.875 ± 1.136, range: $4–6 \times 10^6$/unit] and after [mean ± SD: 0.906 ± 0.0365, range: $0.8–0.99 \times 10^6$/unit] leucoreduction (Cell Dyn 3500, Abbott, California, USA).

Coagulation assays

Plasma samples were tested for prothrombin time, activated partial thromboplastin time (APTT), factors V and VIII. These tests were performed on STA Coagulometer (Diagnostica Stago, Asnieres, France) according to the manufacturer's recommendations.

Statistical analysis

Data were statistically described in terms of mean ± SD or median and range. Comparison between the study groups was done using Student *t* test for independent samples in comparing two groups of normally distributed data and Mann–Whitney test when comparing two groups of non-normal data. Comparison of numerical data between more than two groups was done using one-way analysis of variance with Bonferroni post hoc test. *P* value less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, Illinois, USA) version 15 for Microsoft Windows.

Table 1 Coagulation assay results in nonleucoreduced and leucoreduced groups

Parameter	Nonleucoreduced ($n=20$)	Leucoreduced ($n=30$)	*P* value
Prothrombin time (s)	13.9 ± 0.7	14.5 ± 0.7	0.008
INR	1.09 ± 0.07	1.14 ± 0.07	0.005
APTT (26–34s)	55.6 ± 9.9	43.2 ± 12.8	0.001
Factor V (71–95%)	78.4 ± 11.6	74.7 ± 15.3	0.370
Factor VIII (82–116%)	79.5 (31–100)	52.5 (30–89)	0.086

APTT, activated partial thromboplastin time; INR, international normalized ratio. Data presented as mean ± SD were analysed by Student *t* test, whereas data presented as median (range) were analysed by Mann–Whitney test.

Results

In this study, leucoreduced samples showed a significantly prolonged prothrombin time as compared with nonleucoreduced plasma (14.51 ± 0.66 s vs. 13.96 ± 0.73 s, $P=0.008$) as well as a *higher INR level* compared with nonleucoreduced samples (1.14 ± 0.07 vs. 1.09 ± 0.07, $P=0.005$). There was a significant prolongation of the APTT in nonleucoreduced plasma compared with the leucoreduced one (55.60 ± 9.98% vs. 43.17 ± 12.84%, $P=0.001$). There was no significant difference in factors V and VIII levels postfiltration. Factor VIII was not significantly lower in the leucoreduced group, yet there was a trend (52.5% vs. 79.5%, $P=0.086$) (Table 1). One-way analysis of variance between the three studied groups revealed a statistical difference in the prothrombin time, INR and PTT ($P=0.017$, 0.019 and 0.001, respectively). Post hoc Bonferroni test revealed a significantly prolonged prothrombin time and higher INR in apheresis plasma as compared with nonleucoreduced plasma (14.6 ± 0.7 s vs. 13.9 ± 0.7 s, $P=0.016$ and 1.16 ± 0.07 vs. 1.09 ± 0.07, $P=0.017$, respectively). APTT was more prolonged in the nonleucoreduced group as compared with the whole blood filters group (55.6 ± 9.9 s vs. 39.5 ± 2.9 s, $P=0.001$). There was no statistically significant filtration effect on factors V and VIII levels. FVIII level was lower in the plasma filtered using whole blood filters than the nonleucoreduced plasma (47.4 ± 13.1% vs. 68.0 ± 35.3%), and the difference was nonsignificant ($P=0.076$), yet there was a trend. There was no significant difference between both methods of leucoreduction regarding prothrombin time, INR, PTT, factors V and VIII levels (Table 2).

Table 2 Coagulation assay results in nonleucoreduced, whole blood filter and apheresis groups

Parameter	Nonleucoreduced ($n=20$)	Whole blood filter ($n=15$)	Apheresis ($n=15$)
Prothrombin time (s)	13.9 ± 0.7[a]	14.4 ± 0.6	14.6 ± 0.7[a]
INR	1.09 ± 0.07[b]	1.12 ± 0.06	1.16 ± 0.07[b]
APTT (26–34s)	55.6 ± 9.9[c]	39.5 ± 2.9[c]	46.5 ± 17.4
Factor V (71–95%)	78.3 ± 11.5	72.2 ± 12.7	77.1 ± 17.6
Factor VIII (82–116%)	68.0 ± 35.2	47.4 ± 13.0	60.9 ± 20.9

INR, international normalized ratio; APTT, activated partial thromboplastin time. Data are presented as mean ± SD and analysed by one-way analysis of variance and post hoc Benferroni test. [a] $P=0.016$ nonleucoreduced vs. apheresis. [b] $P=0.017$ nonleucoreduced vs. apheresis. [c] $P=0.001$ nonleucoreduced vs. filters.

Discussion

Prestorage leucodepletion is now commonly practiced on different blood components including whole blood, intermediate products as platelet rich plasma or final blood products [13–15]. Standards regarding residual leucocytes after filtration exist though it differs slightly between the United States and Europe. However, no specifications exist concerning the residual clotting factor levels for the two licensed plasma products prepared from whole blood in the United States: fresh frozen plasma and 24-h frozen plasma, leucoreduced or not. This study investigated the effect of two different methods of leucofiltration (filtration by inline whole blood polyester filters vs. automated apheresis machine) on the extrinsic and intrinsic pathway clotting factors in terms of prothrombin time, INR and PTT as well as on clotting factors V and VIII. There was a significantly prolonged prothrombin time in leucodepleted plasma samples, yet in our opinion, this might not be clinically significant as the absolute clotting time difference was 0.5 s. Contrary to our results, Kretzschmar *et al.* [16] documented shorter prothrombin time postfiltration. They attributed this decrease to the release of factor V from platelets during storage or filtration process. Also, platelet microparticles that may have factor VII-activating potential could be released during platelet removal by filter sheets. Our results showed a prolonged APTT in nonleucoreduced plasma as compared with the leucoreduced one. No clear explanation could be found for prolongation of the APTT. This could be a storage effect during freezing at −20°C on APTT results. This prolongation was not evident to the same extent in the leucoreduced samples. This was in agreement with Gosselin and Dwyre (2015) [17], who studied the effect of freezing on coagulation testing and found significant differences between fresh and frozen tested plasma for prothrombin time, APTT, factors V and VIII and ATIII. Contrary to our results, Kretzschmar *et al.* [16], reported prolongation in APTT postfiltration with a polyurethane whole blood filter as well as a decrease in factor VIII:C; however, this was only significant after plasma storage at room temperature for 18 h preprocessing.

The study showed no significant decline in factors V and VIII postfiltration. Factor VIII was not significantly lower in the plasma filtered using whole blood filter ($47.4 \pm 13.1\%$ vs. $68.0 \pm 35.3\%$, $P = 0.076$), yet there was a trend. There were no significant differences between whole blood filter and apheresis regarding prothrombin time, INR, APTT and factors V and VIII. Many published European and American studies have investigated the effect of leucofiltration of either whole blood or plasma on residual clotting factors. Heiden *et al.* [18], used five different whole blood filters, four polyester and one polyurethane for leucofiltration. They found no statistically significant reductions in clotting factors postfiltration. Runkel *et al.* [19], assessed the effect of two different whole blood polyester filters, one positively charged and the other negatively charged. Postfiltration fibrinogen and factors V and VIII:C levels were not different from those of a control population. Alhumaidan *et al.* [20], observed a variable effect of platelet filters on the residual clotting factors of their plasma products, with significant reduction of factors VII, VIII and XI postfiltration. No significant changes were seen with fibrinogen and factors V and VIII. Plasma filtered using whole blood filter showed a significant decline in factor V, but a minimal decline in factor VIII. They attributed these changes to an unclear mechanism, and the effect of simple protein attachment to a foreign surface was unlikely. The coating of these polyester filter surfaces might have an influence as it might contribute to a degree of selectivity in clotting factor adherence. Shooshtari and Hosseini [21], in their study for plasma quality after filtration using an integral filter system, found that there was no measureable impairment of coagulation factors VIII, IX, V, XI, fibrinogen, antithrombin III, antiplasmin and antitrypsin postfiltration. They found a negligible change in factor VII that apparently had no effect on the therapeutic quality of whole blood-filtered fresh plasma for transfusion.

Several limitations were present in this study: first, only one type of whole blood filters from a single manufacturer was used along with a single apheresis machine. So, our observations in this study are unique to these manufacturers. Second, our coagulation assays were limited to prothrombin time, INR, APTT and factors V and VIII. Other clotting factors especially for extrinsic pathway and anticoagulant proteins need to be investigated. Third, prefiltration samples should have been considered for better assessment of the effect of filtration on plasma quality, and finally, there was a relatively low sample size in each of our study groups. Thus, further studies on a larger scale are needed to confirm our findings.

Conclusion

Leucofiltration had no significant effect on factors V and VIII levels. Storage conditions might affect the quality of plasma in terms of the intrinsic pathway clotting factors as indicated by prolonged APTT in nonleucoreduced samples. Further studies on a larger sample size using filters from different manufacturers with coagulations assays of other clotting factors are needed to establish these effects.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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