International Journal of Medical Laboratory 2022;9(1):47-54.

Original Article

A Root Cause Analysis of Haemolysis Encountered in Leuco-filtration of Stored Packed Red Cells Units

Amita R* MD., Arya AB PGDBBT., Sathyabhama S MBBS., Debasish Gupta MD.

Department of Transfusion Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, India

Article History
Received: 19 May 2021
Accepted: 12 Oct 2021
Available online: 16 Mar 2022

Keywords
Haemolysis
Leucofiltration
Packed red cells
Root cause analysis
Storage
Temperature

A B S T R A C T

Background and Aims: Leukoreduction of blood components has reduced the incidence of transfusion-associated adverse events. Leucofiltration is the most effective method of leukoreduction. We encountered haemolysis in a series of leucofiltered units. This stressed our precious inventory, added to financial loss, increased our turn-around time to issue leucofiltered blood units, and placed doubts on the safety of our leucofiltered products. A systematic root cause analysis was done to identify the reason for haemolysis.

Materials and Methods: A total of 69 units were leucofiltered during the study period, of which 13 units showed lysis following filtration.

Results: This study warranted a review of our existing leucofiltration standard operating procedures to keep strict adherence to manufacturer instructions to ensure quality in the end product.

Conclusions: Among the leucofiltered units showing hemolysis, 62% were due to higher temperature, 23% due to increased time for filtration, and 15% due to increased holding time of red cell units.

*Corresponding Author: Department of Transfusion Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, India. **Email:** amita.rnair@sctimst.ac.in

[DOI: 10.18502/ijml.v9i1.9086]

Introduction

Leucocytes in the blood product are considered the reason for a majority of the morbidity associated with transfusion of blood and blood components [1]. These include febrile non haemolytic transfusion reactions, Human leucocyte antigen (HLA) alloimmunization with subsequent refractoriness to platelet transfusions, graft-versus-host disease, transfusion-related acute lung injury, transfusion-related immune-modulation, and transfusion of leukotropic viruses, such as *cytomegalovirus*, *Epstein-Barr* virus, and human T-cell lymphotropic virus.

Leucoreduction of blood components is routinely practiced in blood centers to reduce the incidence of transfusion-associated morbidity. The American Association of Blood Banks (AABB) and Indian guidelines state that to qualify as leukoreduced, the blood components should contain less than 5 x 10^6 leukocytes per unit [2, 3].

Filtration is currently practiced as the standard technique for leukocyte reduction of packed red cells (PRC). High-efficiency leukocyte reduction filters have the capacity for approximately 4-5 log reductions in the leucocyte count. Filter performance depends on various factors, including the initial number of leukocytes, flow rate, pressure, priming, rinsing, temperature, blood viscosity, holding time between blood collection and filtration, erythrocyte and leukocyte deformability, and plasma content of the cell suspension [4]. Leukocyte reduction using filtration can be performed at three different time points, during processing, after processing (both in the laboratory), and at the patient bedside [5]. Laboratory leucofiltration is more preferred as it offers the evaluation of quality parameters.

We are a tertiary care super specialty hospital with a dedicated pediatric cardiothoracic surgery department. As a policy decision, pediatric patients undergoing cardiac surgery are given leucofiltered packed red cells. Of late, we encountered a series of units showing haemolysis, post leucofiltration. This stressed our precious inventory, added to financial loss, increased our turn-around time to issue leucofiltered blood units, and placed doubts on the safety of our leucofiltered products. Hence, we decided to undertake a root cause analysis to determine the reason for the haemolysis in leucofiltered units.

Materials and Methods

As per our existing standard operating procedure (SOP) for leucofiltration, packed red cells prepared from 350/450 ml whole blood collected in anticoagulant preservative solution (CPD or CPDA-1) with or without additive solution (SAGM) and relatively fresh stored at 2-6 ˚C are selected for leucofiltration. The selected units are kept outside the Blood bank refrigerator (BBR) at room temperature for 10-15 minutes with gentle mixing. The transfer bag with the leucofilter is connected to the packed red cell bag using a sterile connecting device and allowed to be filtered by gravity into the

[DOI: 10.18502/ijml.v9i1.9086]

transfer bag. The bag is visually checked for lysis, labeled, and issued per demand.

For undertaking the root cause analysis, the following additional data was collected: a) Type of packed red cells – 350/450 ml, with or without additive, b) Time kept outside BBR before filtration, c) Surface temperature of the blood bag on taking out of BBR and before filtration, d) Volume of packed red cell before filtration and after filtration, e) Haemoglobin, haematocrit, red cell count and a visual check for haemolysis after centrifugation from the proximal and distal segment of the leucofilter f) Duration required for complete filtration, g) Age of packed red cell units on the day of leucofiltration and h) post-filtration storage, if any.

We had leucofilter from two manufacturers, which was used alternately for the process, and it was also documented. All consecutive PRC subjected to leucofiltration were included in the study. PRC units, which showed visible haemolysis before leucofiltration or failed to filter due to any reason within 45 minutes altogether, were excluded from the analysis. The project was reviewed and approved by Institute Ethics Committee (SCT/IEC/1493/Nov-2019).

Results

A total of 69 packed red cell units were leucofiltered during the study period of 3 months. The age of PRC units on the day of leucofiltration varied from 2-13 days. The mean temperature of the blood bag taken out from BBR was 4.85 ˚C, and just before filtration was 6.38 ˚C. (Table 1). The time taken for complete filtration varied between 10-35 minutes for PRC with an additive solution and 20-45 minutes without an additive solution. There was no significant difference in the time for filtration between the different leucofilter used (Table 2). Percentage reduction in the volume of PRC units ranged from 10.58 to 14ml in cases of units with an additive solution and 14.6 to 19.23 ml in PRC units without an additive solution. There was no significant difference between the two types of leucofilter used (Table 3). The percent reduction in haemoglobin from 0.32 to 0.83% in PRC with the additive solution and 0- 0.98 % for PRC without an additive solution. Similarly, the change in haematocrit was between 1.1 to 2% and 0.79 to 5.05 %. There was no significant difference between the two types of leucofilter used (Table 4).

Table 1. The temperature of the blood units taken from BBR and before filtration

Red cell unit prepared from	Number of units	Temp on taking from BBR (in ˚C)	Mean Temp on taking from BBR (in ˚C)	Temp before filtration (in ˚C)	Mean temp before filtration (in ˚C)	Remarks
450 CPD with SAGM	26	3.5-6.4	5.04	3.6-15.7	8.308	3 units haemolysis in post filter sample
350 CPD with SAGM	19	3.5-6.2	5.03	4-14.6	7.3	3 units haemolysis in post filter sample
450 CPDA-1	5	3.8-6	4.7	4-6.2	4.92	No haemolysis
350 CPDA-1	19	3.5-6.3	4.65	3.9-9.6	5	2 units haemolysis in post filter sample

BBR=Blood bank refrigerator; CPD= Citrate phosphate dextrose; SAGM= Saline adenine glucose mannitol; CPDA-1= Citrate phosphate dextrose adenine -1

[DOI: 10.18502/ijml.v9i1.9086]

The red cell recovery following leucofiltration ranged between 93.96 to 98.33% (Table 5). This is in keeping with the manufacturer report of achieving more than 90% red cell recovery following leucofiltration [4].

Table 2. Time taken for complete filtration of packed red cells units

Red cell unit prepared from	Type of leucofilter	Number	Time taken (minutes)	Range (Minutes)	p-value	Remarks
450 CPD with SAGM	1	11	20.54	10 to 25	0.9	48 hours post storage, one unit showed lysis
	2	15	20.64	10 to 35		
350 CPD with SAGM	1	9	16.33	10 to 25	0.58	No haemolysis
	2	10	15	10 to 25		
450 CPDA-1	1	2	22.5	20 to 25	not calculated	24 hours post storage, one unit showed lysis
	2	3	33.33	25 to 40		
350 CPDA-1	1	10	25.5	15 to 30	0.25	one unit showed lysis
	2	9	33.33	15 to 45		

SAGM= Saline Adenine Glucose Mannitol; CPDA-1= Citrate Phosphate Dextrose Adenine -1

Table 3. Volume of packed red cells units after leucofiltration.

Red cell unit prepared from	Type of leucofilter	Number of units	Mean Vol pre (ml)	Mean Vol post (ml)	Mean reduction (ml)	% Reduction	p-value
450 CPD with SAGM	1	11	307	272	35	11	0.5
	2	15	307	274	32.5	10.58	
350 CPD with SAGM	1	9	210	183	26.88	12	0.07
	2	10	222	190	32	14	
450 CPDA-1	1	2	325	275	50	15.38	Not calculated
	2	3	331	283	48.33	14.6	
350 CPDA-1	1	10	233	185	37.7	16.18	0.42
	2	9	208.33	167.2	40	19.23	

SAGM= Saline Adenine Glucose Mannitol; CPDA-1= Citrate Phosphate Dextrose Adenine -1

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Table 4. Change in haemoglobin and haematocrit after leucofiltration.

Red cell unit prepared from	Type of leucofilter	Number	Mean pre haemoglobin (g/dl)	Mean post haemoglobin (g/dl)	% change in Haemoglobin	p-value	Mean Haematocrit pre (%)	Mean Haematocrit post(%)	% change in Haematocrit	p-value
450 CPD with SAGM	1	11	18.6	18.5	0.32	0.8	61.35	62.42	1.7	0.05
	2	15	19	18.88	0.63		57.63	58.27	1.1	
350 CPD with SAGM	1	9	18.44	18.34	0.5	0.21	53.56	54.94	2.5	0.45
	2	10	17.97	17.82	0.83		55.02	56.12	2	
450 CPDA-1	1	2	21.3	21.3	0	0.8	63.05	63.55	0.79	0.7
	2	3	20.23	20.03	0.98		59.23	59.83	1.01	
350 CPDA-1	1	10	17.21	17.08	0.75	0.8	56.33	58.6	3.9	0.6
	2	9	17.03	16.87	0.93		49.43	51.85	5.05	

CPD= Citrate Phosphate Dextrose; SAGM=Saline Adenine Glucose Mannitol; CPDA-1= Citrate Phosphate Dextrose Adenine -1

Table 5. Recovery of red cells following leucofiltration.

Red cell unit prepared from	Type of leucofilter	Number	RBC pre ($x10^9$/l)	RBC post ($x10^9$/l)	Difference	% Recovery	p-value
450 CPD with SAGM	1	11	7.2	7.08	0.18	98.33	0.8
	2	15	6.73	6.6	0.19	98.06	
350 CPD with SAGM	1	9	7.23	7.07	0.16	97.78	0.05
	2	10	6.79	6.38	0.41	93.96	
450 CPDA-1	1	2	7.44	7.2	0.19	96.77	0.83
	2	3	7.43	7.17	0.24	96.5	
350 CPDA-1	1	10	6.33	5.96	0.36	94.15	0.75
	2	9	5.88	5.77	0.32	98.12	

CPD= Citrate Phosphate Dextrose ; SAGM= Saline Adenine Glucose Mannitol ; CPDA-1= Citrate Phosphate Dextrose Adenine -1

Out of 69 units subjected for leucofiltration, samples from 17 units showed haemolysis on visual examination; of these four units had haemolysis in the pre-filter segment. Of the remaining 13 units that showed haemolysis after leucofiltration, two units were nine days and thirteen days old at the filtration time. Eight units had a temperature of more than

[DOI: 10.18502/ijml.v9i1.9086]

9 ˚C before filtration. Three units took more than 30 minutes for complete filtration, of which one unit showed haemolysis in the immediate post-filtration sample, and two units showed lysis upon storage for 24 hours and 48 hours, respectively (Table 6). All three units were PRC without additive solution, where optimum filter priming would not have occurred, causing plasma absorption by the leucofilter, resulting in high post-filtration hematocrit. Before and after filtration hematocrit were within quality control limits and all the units were less than five days.

Table 6. Details of PRC units taken more than half an hour for complete filtration.

Red cell unit prepared from	Unit no.	Days in storage	Volume of PRC unit		Temperature on taking out from storage	Temperature at the start of filtration	Type of leucofilter used	Duration taken for filtration	Hemoglobin		Hematocrit	
			pre	post					pre	post	pre	post
350 CPDA-1	182813	4	225	190	4.8	5	1	35	16.1	16	48.6	67.9
450 CPDA-1	182795	5	295	270	3.8	4	1	45	20.2	20.1	59.5	62
350CPDA-1	182821	4	200	180	3.9	4.2	1	40	17.8	17.7	53.2	57

CPDA-1= Citrate Phosphate Dextrose Adenine -1

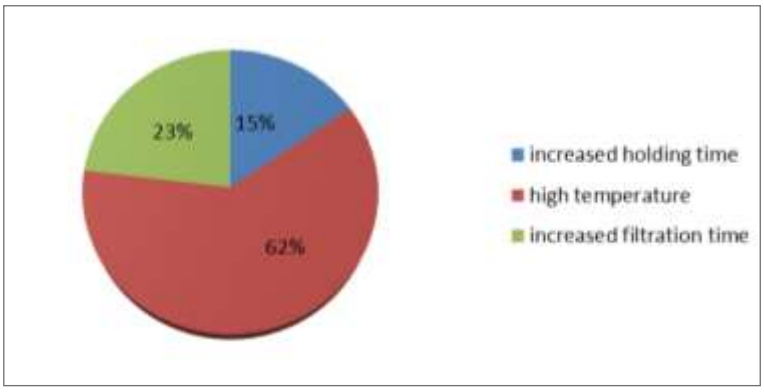

Fig.1. Postulated causes for haemolysis encountered during the process of leucofiltration

Discussion

Haemolysis post leucofiltration renders the unit unsuitable for transfusion. The study rationale was to analyze the causes of haemolysis encountered during the process of leucofiltration of units so that preventive/ corrective actions can be incorporated into our existing practice (Fig. 1).

All the units showed 4 log leuco-reduction and red cell recovery according to AABB and US FDA guidelines, which requires that the

[DOI: 10.18502/ijml.v9i1.9086]

filtration process produce no more than 15% loss of therapeutic cellular elements [6].

During the study period, we encountered 13 units with haemolysis post leucofiltration. Leucofilter manufacturer recommends that the red cell unit be filtration within 24 hours if stored at room temperature and within three days if stored at 4 ˚C [5]. We had two units nine and thirteen days old, which showed hemolysis post-filtration, possibly due to the increased holding time between blood collection and filtration, which was more than the manufacturer recommended.

As per our standard operating procedure for leucofiltration, units selected are kept outside the BBR for half an hour with gentle mixing. Eight units had a surface temperature of more than 9 ˚C at the time of leucofiltration, which showed haemolysis post filtration. The current practice was to keep the red cell units inside the platelet agitator cum incubator for half an hour before filtration for ease of work. The agitation and increased temperature likely contributed to hemolysis. This practice was discontinued, and instead, the blood units were gently mixed using the blood collection monitor for 15 minutes prior to filtration.

Time taken for filtration was found to be more than 30 minutes in three units, which showed post-filtration lysis, one immediately and two units after one and two days of storage, respectively. All three PRC units were without additive solutions. Inadequate priming of the leucofilter will result in increased air blood interface and interfere with filtration efficiency [7]. The blood bag supplied with the post-process leucofilter is meant for the collection of leucofiltered red cells and immediate issues. Post filtration storage is for a maximum of 24 hours only. (oral communication). Since slower filtration rates are associated with poorer efficiency of leukocyte reduction and increased plasma haemoglobin levels, it was decided that the red cell units taking more than 30 minutes to filter completely should not be issued for patient use, and all units leucofiltered should be issued immediately without storage.

Conclusion

This study helped in making the following changes in our SOP for leucofiltration

In keeping with the manufacturers instructions, packed red cell units selected for leucofiltration should be less than 3 days old. Units selected for leucofiltration should be placed on the blood collection monitor for gentle mixing for 15 minutes after taking out from BBR. Units taking more than 30 minutes for complete filtration should not be issued for patient use. Units should be leucofiltered on-demand and issued as soon as possible. PRC with an additive solution should be preferably selected for filtration to avoid filtration failure and optimize filter priming. Strict adherence to manufacturer instructions and periodic review of the existing operating practices are important to ensure quality in the end product.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgments

Not applicable

[DOI: 10.18502/ijml.v9i1.9086]

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[DOI: 10.18502/ijml.v9i1.9086]