JOURNAL OF CLINICAL AND BIOMEDICAL SCIENCES

ORIGINAL ARTICLE

OPEN ACCESS

Received: 08-10-2025
Accepted: 16-12-2025
Published: 15-06-2026

Citation: Sarvek B, Ajay Kumar K, Sumit K, Karandeep S, Swaranjeet S. Impact of Prestorage Leukoreduction on Various Parameters of Blood Component Units Collected in a Tertiary Care Centre. 2026; 16(2):52-57. https://doi.org/10.58739/jcbs/v16i2.25.365

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Funding: None

Competing Interests: None

Published By Sri Devaraj Urs Academy of Higher Education, Kolar, Karnataka

ISSN
Print: 2231-4180
Electronic: 2319-2453

Impact of Prestorage Leukoreduction on Various Parameters of Blood Component Units Collected in a Tertiary Care Centre

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Abstract

Background: The availability of safe and high-quality blood products hinges on effective storage systems. However, extended storage of red blood cells (RBCs) leads to biochemical and hematological alterations known as storage lesions, which can compromise transfusion efficacy and patient safety. **Objectives:** To evaluate the impact of storage duration and blood bag type (single, double, triple, and leukofilter CPD-SAGM) on biochemical and hematological parameters of stored RBC units over 28 days. Methods: A prospective study was conducted on 150 blood units collected and stored under standard conditions. Parameters including plasma sodium, potassium, lactate dehydrogenase (LDH), hematocrit, and total leukocyte count (TLC) were measured at multiple intervals over a 28-day period using standardized biochemical and hematological analyzers. **Results:** Significant biochemical changes were observed across all bag types: plasma potassium and LDH levels increased, while sodium levels decreased during storage ($p < 0.001$). Hematocrit showed a significant decline in non-leukoreduced units, while leukofilter bags maintained relatively stable values. TLC dropped significantly in leukoreduced units after processing, confirming the effectiveness of leukoreduction. Leukofilter bags consistently demonstrated lower biochemical deterioration and hemolysis markers compared to non-leukoreduced counterparts. **Conclusion:** Red cell storage induces progressive changes that can impact transfusion safety. Leukoreduction significantly mitigates these effects, as evidenced by lower potassium and LDH levels and better preservation of hematocrit. The use of leukofilter CPD-SAGM bags is recommended to improve the biochemical and hematological quality of stored blood, particularly for high-risk patients.

Keywords: Red blood cells, Blood storage, Leukoreduction, Potassium, LDH, Hematocrit, storage lesions, Transfusion safety

1 Introduction

Availability of safe and quality blood and blood products for all, relies on development of blood storage systems to separate and store collected blood before transfusions. This has caused improving the quality and availability of blood products[1]. While storing blood for extended periods offers several benefits, it also presents challenges[2]. On the positive side, blood storage helps maintain inventory, allows for processing

and testing, and reduces the risk of transfusion-associated graft-versus-host disease. However, as our understanding of red blood cell (RBC) physiology and transfusion practices grows, we are becoming more aware of the storage-induced damage, often referred to as "storage lesions," that affect RBCs[3].

When refrigerated, RBCs undergo various physiological and biochemical changes, known as RBC storage lesions, which can impact their quality, function, and survival after transfusion[3, 4]. Hemolysis, or the breakdown of red blood cells, can occur at any stage—from collection and transportation to preservation and handling in the blood bank[5, 6]. In India, the conditions under which blood is collected and transported differ from those in Western countries, and there are no clear guidelines regarding acceptable levels of hemolysis for RBC units. In contrast, the U.S. FDA has set a limit of 1% hemolysis by the end of the storage period, while European guidelines are more stringent, allowing only 0.8% hemolysis for transfused RBCs. These measures of RBC quality are important for ensuring the safety of transfusion recipients[7].

White blood cells (WBCs), when exposed to the acidic storage conditions and refrigerated temperatures, become activated, producing cytokines before they die within 24 hours of donation. These activated WBCs contribute to transfusion-related adverse reactions in recipients[8].

Additionally, the storage process negatively affects RBCs' ability to deliver oxygen, and transfusing allogeneic RBCs can potentially harm certain recipients, especially critically ill patients or those undergoing cardiac surgery. Units with high potassium levels should be used with caution in cardiac patients, neonates, individuals with renal failure, and those undergoing massive transfusions, as hyperkalemia has been linked to cardiac deaths in these situations[9, 10].

Finally, the hypothermic storage of RBCs contributes to several complications, such as prolonged hospital stays, impaired tissue oxygenation, pro-inflammatory and immunomodulatory effects, increased infections, multiple organ system failure, and, in some cases, higher mortality[10].

2 Materials and Methods

A prospective study was conducted at the blood centre, Department of Pathology, and Department of Biochemistry, MAMC Agroha, Hisar. A total of 150 blood units were examined to assess various parameters in stored blood. 450 ml blood was collected in double, triple and leukofilter bags while 350 mL is drawn into single bags. The collection process followed the NACO guidelines and the prescribed departmental Standard Operating Procedures (SOPs). Data is collected from various biochemical and hematological parameters of blood units stored for 28 days, based on the following investigative reports:

1. **Complete Blood Count**: Measured using a fully automated cell counter (Sysmex XS1000).
2. **Electrolytes (Na+, K+)**: Measured using a NOVA Biomedical Electrolyte 1 Analyzer (Ion Selective Electrode).
3. **Serum LDH**: Measured using the Erba Mannheim CHEM-7 Analyzer (DGKC method, kinetic).

Blood collection is performed by phlebotomy. All blood units are tested for HIV, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), Venereal Disease Research Laboratory (VDRL), and malaria parasites. Blood processing is done using the principle of centrifugation. Samples for analysis are collected after gentle mixing by inversion, using a sterile sampling procedure within a laminar airflow cabinet. The blood bag tubing is carefully stripped and refilled to collect representative samples for various parameter analyses.

Inclusion Criteria:

- Blood donors who meet the standard operating procedures for blood donation at the MAIMRE Blood Bank, Agroha.
- Written informed consent.
- Age: 18–65 years.
- Weight: >45 kg.
- Hemoglobin (Hb) >12.5 g/dL.

Exclusion Criteria:

- Blood donors who are deferred from donation according to the blood donor selection SOPs of the MAIMRE Blood Bank, Agroha.

3 Results

Blood collection was carried out using four different types of blood bags. The types and number of stored blood bags included in the study are as follows:

- Single CPDA bags – 60 units
- Double and Triple CPDA bags – 60 units
- Leukofilter CPD-SAGM bags – 30 units

Donors ranged in age from 18 to 65 years and were screened according to DGHS (Directorate General of Health Services) guidelines. All blood collection and processing were conducted in the Blood Bank, Department of Pathology, Maharaja Agrasen Medical College (MAMC), Agroha, Hisar.

Stored red cell units were evaluated for various parameters. Representative samples from each bag type were collected as follows:

- For double, triple and leukofilter CPD-SAGM bags (processed to packed red cells): Samples were collected on Day 0 (pre-processing), Day 1 (post-processing), and subsequently on Days 7, 14, 21, and 28.
- For single bags (stored as whole blood without processing): Samples were collected on Day 0, and then on Days 7, 14, 21, and 28.

Samples were collected in:

- EDTA vials for hematological assessments
- Plain vials for biochemical testing

After collection, blood samples were centrifuged at 1000 rpm for 10 minutes. The supernatant plasma was then separated and used for testing.

Parameters assessed:

- Plasma Potassium (K^+) (Table. 1)
- Plasma Sodium (Na^+) (Table. 2)
- Lactate Dehydrogenase (LDH) (Table. 3)
- Hematocrit (Table. 4)
- Total Leukocyte Count (TLC) (Table. 5)

The findings are presented in Table. 1 - Table. 5.

Table 1: Potassium levels in plasma in different bags on various days

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	p Value
Single bags	4.975±1.12	_____	13.41±5.82	18.97±6.51	25.45±7.23	34.64±9.34	<0.001
Double and Triple bags	5.311±1.44	11.99±7.43	18.46±8.72	23.24±9.33	30.71±9.65	35.08±10.12	<0.001
Leukofilter bags	4.88±1.41	12.01±2.80	15.22±4.56	18.91±7.36	22.68±7.88	29.27±9.86	<0.001

Table 2: Sodium levels in plasma on various days

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	p Value
Single bags	144.80±2.81	_____	137.52±8.56	130.21±7.82	129.36±7.82	125.72±7.2	<0.001
Double and triple bags	143.24±5.63	144.34±9.82	138.61±6.42	129.50±7.92	114.58±9.44	116±10.34	<0.001
Leucofilter bags	145.02±2.82	147.25±6.12	142.58±7.21	131.22±6.94	130.66±8.73	125.78±10.35	<0.001

Table 3: LDH levels on different days

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	p Value
Single bags	1092.10±674	_____	1346±590.37	1898.92±651.53	1952.46±671.39	2460.70±710.84	<0.001
Double and Triple bags	924.23±610.52	1191.67±763.40	1467.34±591.11	1991.36±679.84	2304.75±662.47	2508.14±786.26	<0.001
Leukofilter bags	672.64±216.56	672.00±260.35	756.67±402.56	901.46±492.03	960.12±561.24	947.19±582.42	<0.001

Table 4: Hematocrit levels on various days of storage

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	p Value
Single bags	42.41±6.04	_____	38.20±11.15	34.16±12.68	32.70±11.84	31.04±9.86	<0.05
Double and Triple bags	38.22±6.22	48.87±8.91	45.73±1384	43.37±13.01	41.45±9.89	40.02±14.21	<0.05
Leukofilter bags	37.64±6.56	49.84±14.32	47.67±10.02	43.11±9.20	46.13±12.01	45.69±10.42	>0.05

Table 5: TLC values in different bags on various days

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	p Value
Single bags	6.42±1.84	_____	6.11±2.11	5.44±1.65	5.23±1.87	4.96±1.86	<0.001
Double and Triple bags	9.24±2.46	8.85±3.27	8.03±4.91	7.86±5.12	7.23±3.89	6.57±2.82	<0.001
Leukofilter bags	6.82±1.86	0.19±0.26	0.12±0.09	0.08±0.06	0.06±0.14	0.04±0.02	<0.001

Significant Biochemical and Hematological Changes noted in Stored Red Cell Units

1. Biochemical Changes: Plasma Potassium Levels

In this study, plasma potassium levels were observed to increase significantly over the 28-day storage period across all four types of blood bags ($p < 0.001$).

- **Leukofilter CPD-SAGM Bags (with leukoreduction):**
  - The highest mean potassium level was recorded on Day 28.
  - The most pronounced increase occurred between Day 21 and Day 28.
  - A sharp rise in potassium was noted immediately after processing (Day 0 to Day 1).
- **Double and Triple CPDA Bags:**
  - Also showed the highest potassium level on Day 28.
  - The maximum increase occurred between Day 14 and Day 21.
  - A significant rise followed processing (Day 0 to Day 1).
- **Single CPDA Bags (Whole Blood):**
  - The highest mean potassium was also found on Day 28.
  - The sharpest increase occurred between Days 21 and 28 ($p < 0.001$).

Statistically significant differences ($p < 0.001$) in potassium levels were noted between the three groups from Day 1 to Day 28, with single, double and triple bags showing higher mean potassium than leukofilter bags. This suggests that leukoreduction may mitigate potassium leakage to some extent.

2. Plasma sodium levels

All four types of bags showed a significant decline in sodium levels ($p<0.001$) over a period of 28 days.

3. LDH levels

LDH levels showed a significant rise ($p<0.001$) in all four types of bags over a period of 28 days.

4. Hematological Changes: Hematocrit Levels

- **Leukofilter CPD-SAGM Bags (with leukoreduction):**
  - There was an increase of about 8% in hematocrit over a period of 28 days which was not statistically significant ($p > 0.05$).
- **Single, double & Triple CPDA Bags:**
  - Demonstrated a significant decrease in hematocrit throughout the storage period ($p < 0.05$).

5. Total Leukocyte Count (TLC)

- **Lekofilter CPD-SAGM Bags (with leukoreduction):**
  - A marked drop was observed immediately after processing (Day 0 to Day 1), indicating effective leukoreduction.
- **Single, double and Triple CPDA Bags:**
  - No significant decline was seen in single, double and triple bags after processing (Day 0 to day 1)
- All the bags showed a decline in total leucocyte count over a period of 28 days.

These results emphasize the effectiveness of bags with in-line leukoreduction filters in removing leukocytes at the time of processing and minimizing their storage-related effects.

3 Discussion

Blood banking aims to deliver the life-saving benefits of transfusion by ensuring that blood components are available, safe, effective, and affordable. The primary goal is to efficiently match the right blood from suitable donors to the right patients at the right time. The most reliable way to ensure timely availability is to maintain an optimal blood inventory at all times.

However, a key challenge in red cell storage remains: the post-storage viability of red blood cells can vary significantly between donors. Identifying donors whose red cells demonstrate better storage characteristics could be especially beneficial for patients requiring frequent transfusions—such as children with thalassemia or sickle cell anemia—who are at risk of iron overload due to repeated transfusions.

Biochemical Changes During Storage: Plasma Potassium and Sodium

In this study, a significant increase in plasma potassium and a significant decline in sodium levels was observed throughout the 28-day storage period across all three types of blood bags. These changes can be attributed to sodium-potassium pumps in RBCs becoming inoperative at low storage temperatures (reduced metabolism) leading to potassium exiting the cells and sodium to enter via semi permeable membranes.

Rise in potassium noted in this study is consistent with previous research by Sawant *et al.* [7], who also reported a substantial rise in plasma potassium in stored red cell units over 28 days. Similarly, Michael *et al.* [11] studied plasma potassium concentrations in 20 units of whole blood, 27 units of packed red cells, and 20 units of packed cells derived from stored whole blood. Their results showed a consistent increase in potassium across all groups during the 21-day storage period.

In the current study, potassium levels began to rise within the first 7 days and continued to increase with ongoing storage. This aligns with the observations of Adias and Moore-Igwe [12],

who reported that potassium is the primary electrolyte affected during blood storage.

In clinical settings, this rise in potassium can be particularly dangerous for patients with severe renal impairment, where even small fluctuations in potassium levels can have serious consequences. In such cases, fresh or washed red cells are preferred. Conversely, plasma sodium levels decreased over the storage period, which can be attributed to stoppage of ion exchange pumps in RBCs at cold storage temperatures. suggesting that elevated sodium in stored blood may have undesirable effects post-transfusion.

Overall, increase in potassium levels was found to be less marked in leukofilter bags as compared to other types of bags supporting the preference for leukoreduced blood, which shows less biochemical deterioration in quality of stored blood over time in terms of potassium levels.

Hematological Changes: Hematocrit and Total Leukocyte Count (TLC)

A significant decline in hematocrit and total leukocyte count (TLC) was noted in single CPDA bags, consistent with findings by Adias and Moore-Igwe[12], who observed storage-related reductions in these hematological parameters. Our study also supports the results of Queen *et al.*[13], who reported a deterioration in white blood cell counts between Day 1 and Day 7, likely due to the degeneration of leukocytes during storage.

These degenerative changes are part of the broader set of alterations known as RBC storage lesions, which are associated with the release of bioactive substances from leukocytes, such as histamines, lipids, and cytokines. These substances can directly affect transfusion recipients. Additionally, as red cells age during storage, they undergo structural and biochemical changes such as:

- Membrane vesiculation
- Reduced cell size
- Increased cell density
- Cytoskeletal alterations
- Enzymatic desialylation
- Phosphatidylserine exposure

Despite these changes, other hematological parameters remained stable throughout the study period and may still be considered acceptable for transfusion purposes.

Leukoreduction and its Effects

Leukoreduction has a protective effect on stored blood. In this study, units stored in leukoreduced integral bags showed a relatively smaller increase in LDH levels compared to triple CPD-SAGM and single CPDA bags. This observation is in agreement with findings by Heaton *et al.*[14], who reported significantly lower levels of hemolysis and plasma potassium in leukoreduced units after 42 days of storage. In their study, leukoreduced units exhibited approximately one-third of the hemolysis observed in non-leukoreduced units.

Similarly, Gyongyossy-Issa *et al.*[15] found that RBCs filtered at 4°C and stored with leukoreduction were less prone to hemolysis than non-filtered units. These benefits are attributed to the absence of leukocyte-derived enzymes, cytokines, and free radicals, which are known to damage red cell membranes, accelerate potassium leakage, enhance glycolysis, and compromise ATP preservation.

The deleterious effects of leukocytes have led many blood banks to adopt leukoreduction as a standard practice. Its benefits include:

- Reduced incidence of febrile non-hemolytic transfusion reactions
- Lower risk of leucotropic virus transmission
- Prevention of platelet refractoriness

Although many developed countries like Canada, France, and the UK have adopted universal leukoreduction, its implementation remains challenging in resource-limited settings like India. In such contexts, selective leukoreduction for high-risk patients can still significantly enhance blood quality and transfusion safety.

While the core structure of blood banking and transfusion services is unlikely to change in the near future, continuous quality improvements—such as the adoption of selective leukoreduction—can help mitigate the adverse effects associated with red cell storage. There are currently no effective alternatives to red cell transfusions, making such enhancements essential for optimizing patient outcomes.

5 Conclusion

During storage of red cell units in the blood bank, inherent red cell storage lesions induce alterations in both the erythrocytes and the suspending medium. These changes progressively intensify with the duration of storage.

a) Prestorage leukoreduced red cell units demonstrate significantly lower plasma potassium concentrations compared to non-leukoreduced units. Therefore, the utilization of leukoreduced units is recommended to further enhance the quality of stored blood.

b) It may be concluded that the decline in hematocrit during storage is minimized when blood is preserved in CPD-SAGM bags incorporating integral leukoreduction filters.

c) Furthermore, it is concluded that the elevation of lactate dehydrogenase (LDH) levels is least pronounced in leukofilter

bags in comparison to single, double and triple bags. Given that LDH is abundant within erythrocytes, an increase in plasma LDH serves as an indicator of red cell hemolysis in stored blood.

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