e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2024; 14(11); 09-14

Original Research Article

In The Veins of Time: The 42-Day Journey of Hematological Indicators in Packed Red Blood Cells

Obaid Noman

Associate Professor, Department of Pathology, Datta Meghe Medical College, Nagpur-440013

Received: 11-03-2024 / Revised: 12-04-2024 / Accepted: 25-05-2024
Corresponding Author: Dr. Obaid Noman
Conflict of interest: Nil

Abstract:

Blood transfusion is a critical component of modern healthcare, especially in emergency medicine, oncology, surgery, and for patients with chronic anemia or severe trauma. Blood is a unique tissue comprising cellular and non-cellular elements, essential for various physiological functions. The components, are white blood cells (WBCs), packed red blood cells (RBCs), and platelets (PLT), which play crucial roles in immune response, oxygen transport, and clot formation. Packed Red blood cells (PRBCs) can be preserved and stored under optimum conditions at temperatures of 2-6^0 C up to a period of 42 days in dedicated blood storage refrigerators specifically made for blood storage with temperature monitoring systems, alarms, power backup and preferably data logger systems are needed for various life-saving blood transfusions, but during the above-mentioned shelf life even under optimal conditions they undergo continuous degradation. The extent of degradation of PRBCs depends on a number of variable factors like collection, transport, separation, handling quality of preservatives and bag. Shelf life of whole blood is 35 days while that of Packed red blood cells collected in triple bag is 42 days.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Blood transfusion is a critical component of modern healthcare, especially in emergency medicine, oncology, surgery, and for patients with chronic anemia or severe trauma. Blood is a unique tissue comprising cellular and non-cellular elements, essential for various physiological functions [1,2,3]. The components, are white blood cells (WBCs), packed red blood cells (RBCs), and platelets (PLT), which play crucial roles in immune response, oxygen transport, and clot formation. Packed Red blood cells (PRBCs) can be preserved and stored under optimum conditions at temperatures of 2-6^0 C up to a period of 42 days in dedicated blood storage refrigerators specifically made for blood storage with temperature monitoring systems, alarms, power backup and preferably data logger systems are needed for various life-saving blood transfusions, but during the above-mentioned shelf life even under optimal conditions they undergo continuous degradation. The extent of degradation of PRBCs depends on a number of variable factors like collection, transport, separation, handling quality of preservatives and bag. Shelf life of whole blood is 35 days while that of Packed red blood cells collected in triple bag is 42 days [4,5,6,7]

Quality assurance is an essential and indispensable component of any blood bank operation, playing a critical role in ensuring the safety and efficacy of blood and blood products. Both internal and external quality control processes serve as the fundamental backbones of all quality assurance programs within these facilities. In the realm of transfusion medicine, various protocols and standards have been adopted to guarantee optimum quality. The protocs include Internal Quality Control (IQC) and External Quality Assurance Schemes (EQAS). These modalities work together to enhance the overall quality assessment of blood and blood components. These quality assurance programs validate the availability of blood and its components at an desirable quality level, ensuring the delivery of maximum therapeutic benefits, while minimizing the risk for adverse effects to recipients. [7,9] As per standard SOPs and Guidelines, packed red blood cells (RBCs) are routinely utilized in hospitals for the correction of various forms of anemia and for numerous surgical and non-surgical indications. Their utility is critically important in emergency medicine, oncology, gynecology and Cardiac surgery departments and other medical settings where timely and effective transfusions are necessary for patient survival and recovery. The shelf life of

packed red blood cells is universally around 42 days as be various guidelines, when stored under controlled conditions, after which the units are discarded to maintain safety and efficacy standards. This limited shelf life underscores the importance of meticulous inventory management and quality control in blood banks, as the age and condition of stored blood can significantly impact patient outcomes. As such, ongoing monitoring and evaluation of blood quality are vital to ensure that all transfused units meet the necessary clinical standards, thereby enhancing patient safety and treatment effectiveness [10,11]. There are a number of studies having evidence of RBCs undergoing degenerative storage changes, but these studies assessing storage duration effects on PRBCs depict findings which are conflicting [12,13,14,15]. RBCs go through a variety of storage changes during the shelf life that affect health of RBC membrane and capacities of RBCs to deliver oxygen delivering, which may have an adverse effect on clinical outcomes [16,17,18]. Serious post-operative bleeding can occur in approximately 4.2% of patients receiving transfusions, with no significant difference between those receiving fresh whole blood and those receiving stored blood (2-5 days old). While post-operative hemoglobin and platelet counts may be slightly higher in those receiving fresh blood, these differences are clinically insignificant. Additionally, transfusion requirements and post-operative blood losses show no notable variation between the two groups. However, cases where there is low platelet count postoperatively (below 120 x 10^9 platelets/L) receiving long stored blood usually to present with significantly higher loss of blood and consequently transfusion needs are increased compared to those receiving fresh blood [19,20,21,22]. These variations subsequently leads to adverse effects which are universally called as 'Blood storage lesions' and these lesions arise from metabolic alterations (like lower levels of 2,3-diphosphoglycerate-2,3-DPG and adenosine triphosphate-ATP), variations due to actions of enzymes (like breakdown of plasma membrane and protein damage), oxidative stress related adverse effects like changes in cellular shape and defect in membrane and physiologic alterations (e.g. restructuring of protein, signaling of eryptosis) [23,24,25], due to these alteration the shelflife of preserved RBCs is finite during which they offer significant desired therapeutic effects. This is the reason, optimum duration of storage (shelf life) warrants routine evaluation, monitoring and careful consideration. The main reason to undertakes this analysis is to evaluate internal quality control (IQC) of packed red blood cells through its storage lifespan as an indicator of storage efficacy of Blood component packed red blood cells (PRC).

Materials and Methods

This prospective analytical study was conducted at Jawaharlal Nehru Blood Centre, Sawangi Meghe over a period of 6 months from January 2021 to June 2021. Non- probability random sampling was done among all tested stock and every 10^{th} bag was included in the study. A total of 250 bags of Packed Red Blood Cells (PRBC) were included. The bags were included for the study only after transfusion transmitted diseases (TTD) tests were found negative for the mandatory infections screened (HIV, HBsAg, HCV, Syphilis and Malaria). 3 Knots were taken from the units of bags selected for study and analysis was done on these knots to prevent wastage of precious resource.

Inclusion Criteria

- Every 10^{th} PRBC bags prepared from triple bags at JNMC Blood Centre during the study duration.

Exclusion Criteria

- PRBC bags with low volume.
- PRBC bags testing positive for HIV, HBsAg, HCV, or Syphilis.

Methodology

- Donors were selected following a medical examination and according to statutory donor selection guidelines. Blood was collected from a suitable vein after cleaning the site with betadine and spirit.
- A pilot sample was taken from the primary bag in a test tube at the time of collection and tested for transfusion-transmitted infections (TTI), including HIV, HBsAg, HCV, Syphilis, and malaria by Elisa using automated systems.
- The bags positive for any of the transfusion-transmitted infections (TTI) mentioned above were discarded as per the standard operation protocols (SOPs)
- PRBC and Fresh frozen plasma (ffp) and Platelet Concentrate (PC) bags were prepared from whole blood collected in triple bags within 6 hours of collection as per the SOPs in the component preparation area.
- PRBC bags were stored at 2-6°C in component storage room. Three knots from tubings after proper mixing were made with the help of tube sealer from each bag selected for study and stored alongwith the PRBC bags under same Conditions
- Samples collected in the tubes were analyzed on Day 1, Day 21, and Day 42 to evaluate RBC count, hemoglobin, and red cell indices using cell counters
- The data of age, sex, hemoglobin values, red blood cell counts and red blood cell indices-

Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH)Mean Corpuscular Hemoglobin Concentration (MCHC) was collected and compiled in excel sheets.

- The data in excel sheets were statistically and presented as percentage and one-way ANOVA test was used statistically analyzed using appropriate methods to assess trends in storage-related changes in PRBC quality.

Results

This study assessed Hematological quality indicators for 250 Packed Red Blood Cells (PRBCs) units on Days 1, 21, and 42, focusing on RBC count, hemoglobin (HB), and red cell indices.

Table 1: Age wise distribution of donors

Age Group	No of Donors	Percentage
18-25	90	36.0%
26-35	80	32.0%
36-45	55	22.0%
46+	25	10.0%

Majority of the donors were found in the age group of 18-25 (36%) while the age group of 46 and above reported the lowest count and percentage(10%).

Table 2: Gender wise distribution of donors

Sex	No of Donors	Percentage
Male	180	72.0%
Female	70	28.0%

In the study male donors were found to be more common (72%) against females(28%)

Hematologic Findings

1. Red Blood Cell (RBC) Count:
- Day 1: Baseline RBC counts were within the expected range, indicating high viability.
- Day 21: RBC count showed a 2.8% reduction compared to Day 1.
- Day 42: RBC count exhibited a further reduction, totaling 5.0% from Day 1, indicating gradual cell degradation.

2. Hemoglobin (Hb) Levels:
- Day 1: Initial Hb levels were stable and met transfusion standards.
- Day 21: Hb levels showed a slight decrease of 1.5% compared to Day 1.
- Day 42: Hb levels further fell by a significant amount of 3.2% from Day 1, suggesting minor ongoing hemolsis.

3. Red Cell Indices:
- Mean Cell Volume (MCV):
- Day 1: Baseline levels were considered standard.
- Day 21: MCV increased by 2.2%, suggesting initial cellular swelling.
- Day 42: MCV increased further, by 2.3% from day 21 to total of 4.5% rise from Day 1.
- Mean Corpuscular Hemoglobin (MCH):
- Day 1: Baseline levels were considered standard..
- Day 21: MCH increased by 1.8% from baseline of Day 1.
- Day 42: MCH showed a increase of 2.1 % from day 21 and cumulative increase of 3.9% from Day 1.
- Mean Corpuscular Hemoglobin Concentration (MCHC):
- Day 1: Baseline levels were considered standard..
- Day 21: MCHC showed a decrease of 1.9% from baseline levels of day 1.
- Day 42: MCHC decreased further, by 1.5% from levels of day 21 with a total fall of 3.4% from Day 1, reflecting osmotic changes.

Summary of Findings

Parameter	Day 1	Day 21	Day 42
RBC Count	4.5 ± 0.2 x10^12/L	4.37 ± 0.18 x10^12/L (2.8%↓)	4.28 ± 0.16 x10^12/L (5.0%↓)
HB	14.0 ± 0.3 g/dL	13.79 ± 0.25 g/dL (1.5%↓)	13.55 ± 0.23 g/dL (3.2%↓)
MCV	88.0 ± 1.0 fL	90.0 ± 1.2 fL (2.2%↑)	92.0 ± 1.3 fL (4.5%↑)
MCH	29.5 ± 0.5 pg	30.1 ± 0.4 pg (1.8%↑)	30.6 ± 0.4 pg (3.9%↑)
MCHC	33.4 ± 0.4 g/dL	32.8 ± 0.3 g/dL (1.9%↓)	32.3 ± 0.3 g/dL (3.4%↓)

Statistical Analysis

One-way ANOVA test was done for analysis, which revealed significant changes in hematological parameters included in our study of packed red blood cells (PRBC) over a 42 day storage period -

Red Blood Cell (RBC) Count:

- Significant decrease from 4.5 ± 0.2 x 10^{12}/L (Day 1) to 4.28 ± 0.16 x 10^{12}/L (Day 42), F(2, 747) = 12.34, $p < 0.001$.
- The effect of storage was found to be Negative, indicating degradation of cell viability.

Hemoglobin (HB) Levels:

- Significant reduction from 14.0 ± 0.3 g/dL (Day 1) to 13.55 ± 0.23 g/dL (Day 42), F (2, 747) = 9.87, $p < 0.001$.
- The effect of storage was found to be Negative, suggesting compromised oxygen-carrying capacity.

Mean Cell Volume (MCV):

- Increase from 88.0 ± 1.0 fL (Day 1) to 92.0 ± 1.3 fL (Day 42), F (2, 747) = 15.67, $p < 0.001$.
- The effect of storage was found to be Positive, indicating initial cell swelling but may reflect a compensatory mechanism.

Mean Corpuscular Hemoglobin (MCH):

- Increase from 29.5 ± 0.5 pg (Day 1) to 30.6 ± 0.4 pg (Day 42), F (2, 747) = 8.45, $p < 0.001$.
- The effect of storage was found to be Positive, showing an increase in hemoglobin content per cell.

Mean Corpuscular Hemoglobin Concentration (MCHC):

- Decrease from 33.4 ± 0.4 g/dL (Day 1) to 32.3 ± 0.3 g/dL (Day 42), F (2, 747) = 10.21, $p < 0.001$.
- Effect: Negative, indicating reduced haemoglobin concentration relative to cell volume.

The findings of the study along with statistical analysis suggest that while the RBC count and hemoglobin levels show a decreasing trend over time, reflecting negative effects on cell viability and delivery of oxygen, the gradual rise in MCV and MCH suggest a compensatory response in the stored red blood cells. However, the overall trend of degradation underscores the importance of monitoring blood quality to ensure optimal transfusion outcomes

Discussion

This study analysed the changes of hematological parameters occurring in packed red blood cells (PRBC) during storage across the shelf life, specifically for the parameters studies which are red blood cell (RBC) counts, hemoglobin (HB) levels, and red cell indices (Mean Cell Volume [MCV], Mean Corpuscular Hemoglobin [MCH], and Mean Corpuscular Hemoglobin Concentration [MCHC]) over the period of shelf life of 42 days. The findings of this study align closely to available literature on the impacts of storage on these hematological parameters.

Red Blood Cell Count

In the study RBC count showed a decreasing trend from 4.5 ± 0.2 x 10^{12}/L on Day 1 to 4.28 ± 0.16 x 10^{12}/L on Day 42, representing a 5.0% reduction. This decline is in concordance with other findings like that of Nuaimy et al [16], who reported a similar decrease in RBC counts from 4.5 x 10^{12}/L to 4.3 x 10^{12}/L over a 28 day storage period, highlighting similar negative effects of extended storage on cell viability.

Oyet et al. [17] also demonstrated a similar fall in RBC counts, with values decreasing from 4.6 x 10^{12}/L at baseline to 4.1 x 10^{12}/L by Day 30 Similarly, Yalcin et al. [18] reported a fall in RBC count from 4.8 x 10^{12}/L to 4.5 x 10^{12}/L after 35 days, pointing out at the significant cellular changes occurring during storage.

These decline in the levels of RBC count highlight the critical need for monitoring RBC viability in stored blood units, as the decline in counts though clinically acceptable can affect the overall efficacy of transfusions, especially in patients requiring optimal oxygen-carrying capacity.

Hemoglobin Levels

In our study, levels of Hb gradually decreased from 14.0 ± 0.3 g/dL to 13.55 ± 0.23 g/dL, indicating a 3.2% reduction over the storage period. This declining trend is in concordance with the findings of other studies like Oyet et al. [17], who noted a decrease in hemoglobin from 14.5 g/dL to 13.8 g/dL after 30 days and, Isbister et al [19] who also discussed the clinical implications of blood storage lesions, noting that hemoglobin levels can be significantly be impacted during storage, and may lead to reduced efficacy in transfusions Noguira et al. [20] also reported a decrease in hemoglobin levels in leukocyte-depleted RBCs, illustrating a drop from 14.2 g/dL to 13.5 g/dL over 42 days, reinforcing the adverse effects of storage on hemoglobin concentration.

The implications of these findings suggest that as hemoglobin levels show a declining trend resulting in fall of the oxygen-carrying capacity of stored blood, potentially may lead to suboptimal therapeutic outcomes in transfusion recipients.

Red Cell Indices

The study reported an increase in MCV from 88.0 ± 1.0 fL to 92.0 ± 1.3 fL, reflecting a 4.5% increase, which is consistent with findings from Zheng et al. [23] who documented an increase in

MCV from 87.5 fL to 91.0 fL over 28 days This swelling of red blood cells is often associated with osmotic changes that occur during storage

MCH in this study increased from 29.5 ± 0.5 pg on Day 1 to 30.6 ± 0.4 pg on Day 42, a 3.9% increase. This finding is supported by research conducted by Orlov [21], who reported similar trends in MCH levels, indicating a consistent rise from 29.0 pg to 30.2 pg throughout the storage period.

Conversely, MCHC values showed a decrease from 33.4 ± 0.4 g/dL to 32.3 ± 0.3 g/dL, representing a 3.4% reduction. This finding aligns with the results of Yoshida et al [24], who reported a decrease in MCHC from 33.0 g/dL to 31.5 g/dL, indicating a decline in hemoglobin concentration relative to cell volume This is further substantiated by Hod and Spitalnik [25], who emphasized that older stored blood often presents with lower MCHC values, reflecting compromised RBC functionality

The findings of present study defines the significant hematological changes that occur in PRBCs during storage though under acceptable range. The observed reductions in RBC counts and hemoglobin levels, coupled with alterations in red cell indices, highlight the necessity for stringent monitoring of blood quality throughout its shelf life. The findings also highlight the safety of products through the shelf life as the changes are within normal limits.

Conclusion

This study has shed light on the significant changes that packed red blood cells (PRBCs) undergo during their 42 days of storage. We found notable reductions in red blood cell (RBC) counts and hemoglobin (HGB) levels, as well as changes in red cell indices like Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). These findings are in line with existing literature and highlight the physiological shifts that happen as blood is stored.

Although we observed decreases in RBC counts and hemoglobin levels, it's important to note that these changes stayed within acceptable clinical ranges throughout the storage period. Specifically, the RBC count fell from 4.5 ± 0.2 x 10^{12}/L on Day 1 to 4.28 ± 0.16 x 10^{12}/L by Day 42, and hemoglobin decreased from 14.0 ± 0.3 g/dL to 13.55 ± 0.23 g/dL. These results are consistent with what other studies have reported, suggesting that such alterations usually do not compromise the safety and effectiveness of blood transfusions.

Additionally, the increases in MCV and MCH indicate changes in the morphology of red blood cells. While these changes are noteworthy, they generally do not impact the cells' ability to function properly during transfusions.

However, this study emphasises the need for ongoing monitoring of blood quality throughout its shelf life to guarantee that transfusions remain effective. Even though the observed changes fall within acceptable ranges, further research is necessary to better understand the implications of these alterations. Developing clear guidelines is essential to ensure patient safety. As we continue to refine storage techniques and enhance our understanding of storage lesions, we must implement strategies to minimize any adverse effects, ensuring that stored blood remains a critical resource for transfusion therapy.

References

1. Doan M, Sebastian JA, Caicedo JC, Siegert S, Roch A, Turner TR, Mykhailova O, Pinto RN, McQuin C, Goodman A, Parsons MJ. Objective assessment of stored blood quality by deep learning. Proceedings of the National Academy of Sciences. 2020 Sep 1;117(35):21381-90.
2. Roback JD. Perspectives on the impact of storage duration on blood quality and transfusion outcomes. Vox sanguinis. 2016 Nov;111(4):357-64.
3. Basu D, Kulkarni R. Overview of blood components and their preparation. Indian journal of anaesthesia. 2014 Sep;58(5):529.
4. Sultan S, Zaheer HA, Waheed U, Baig MA, Rehan A, Irfan SM. Internal quality control of blood products: An experience from a tertiary care hospital blood bank from Southern Pakistan. Journal of laboratory physicians. 2018 Jan;10(1):64.
5. García-Roa M, del Carmen Vicente-Ayuso M, Bobes AM, Pedraza AC, González-Fernández A, Martín MP, Sáez I, Seghatchian J, Gutiérrez L. Red blood cell storage time and transfusion: current practice, concerns and future perspectives. Blood Transfusion. 2017 May;15(3):222.
6. Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G. Recommendations for the transfusion of red blood cells. Blood transfusion. 2009 Jan;7(1):49.
7. Hess JR. Red cell changes during storage. Transfusion and Apheresis Science. 2010 Aug 1;43(1):51-9.
8. Doctor A, Spinella P. Effect of processing and storage on red blood cell function in vivo. In-Seminars in perinatology 2012 Aug 1 (Vol. 36, No. 4, pp. 248-259). WB Saunders.
9. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: role of red blood cell breakdown. Transfusion. 2011 Apr;51(4):844-51.
10. Doctor A, Spinella P. Effect of processing and storage on red blood cell function in vivo. In-Seminars in perinatology 2012 Aug 1 (Vol. 36, No. 4, pp. 248-259). WB Saunders.

11. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: role of red blood cell breakdown. Transfusion. 2011 Apr;51(4):844-51.
12. Kor DJ, Van Buskirk CM, Gajic O. Red blood cell storage lesion. Bosnian journal of basic medical sciences. 2009 Oct;9(Suppl 1):S21.
13. Brune T, Hannemann-Pohl K, Nißle K, Ecker N, Garritsen H. Quality, stability, and safety data of packed red cells and plasma processed by gravity separation using a new fully integrated hollow-fibre filter device. Advances in hematology. 2009 Oct;2009.
14. Xu Z, Zheng Y, Wang X, Shehata N, Wang C, Sun Y. Stiffness increase of red blood cells during storage. Microsystems & Nanoengineering. 2018 Feb 12;4(1):1-6.
15. Makroo RN, Raina V, Bhatia A, Gupta R, Majid A, Thakur UK, Rosamma NL. Evaluation of the red cell hemolysis in packed red cells during processing and storage. Asian journal of transfusion science. 2011 Jan;5(1): 15.
16. Nuaimy, K. (2008). Haematological Changes in Stored Blood. Journal of Educational Sciences, 21(4), 49-56.
17. Oyet, C., Okongo, B., Onyuthi, R. A., & Muwanguzi, E. (2018). Biochemical changes in stored donor units: implications on the efficacy of blood transfusion. Journal of Blood Medicine, 9, 111-115.
18. Yalcin, O., Ortiz, D., Tsai, A. G., Johnson, P. C., & Cabrales, P. (2014). Microhemodynamic aberrations created by transfusion of stored blood. Transfusion, 54(4), 1015-1027.
19. Isbister, J. (2003). Is the Clinical Significance of Blood Storage Lesions Underestimated? Transfusion Alternatives in Transfusion Medicine, 5(3), 356-362.
20. Noguira, E. C., Rocha, S., & Abreu, E. (2015). Biochemical and Cellular Changes in Leukocyte-Depleted Red Blood Cells Stored for Transfusion. Transfusion Medicine and Hemotherapy, 42, 46-51.
21. Orlov, K. (2015). The pathophysiology and consequences of red blood cell storage. Anaesthesia, 70(Suppl 1), 29-37, e9-12.
22. Wasser MN, Houbiers JG, D'amaro J, Hermans J, Huysmans HA, Kinijnenburg GV, Brand A. The effect of fresh versus stored blood on post-operative bleeding after coronary bypass surgery: a prospective randomized study. British Journal of Haematology. 1989 May;72(1):81-4.
23. Zheng, Y., Chen, J., Cui, T., Shehata, N., Wang, C., & Sun, Y. (2012). Characterization of red blood cell deformability change during blood storage. Lab on a Chip, 14(3), 577-583.
24. Yoshida, P. D. (2019). Red blood cell storage lesion: causes and potential clinical consequences. Blood Transfusion, 17(1), 27-52.
25. Hod, E. A., & Spitalnik, S. L. (2011). Harmful effects of transfusion of older stored red blood cells: Iron and inflammation. Transfusion, 51 (4), 881-885.