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# IMPACT OF STORED RED BLOOD CELLS ON CLINICAL OUTCOMES IN CRITICALLY ILL PATIENTS: A PROSPECTIVE OBSERVATIONAL STUDY

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**ABSTRACT**

**Background:** Storage of packed red blood cells (PRBCs) leads to biochemical alterations known as "storage lesions," which may impact patient outcomes upon transfusion. We investigated the effect of PRBC storage duration and concomitant blood product use on clinical outcomes in critically ill patients.

**Methods:** A prospective observational study was conducted on ICU patients receiving PRBC transfusions. Biochemical changes in stored PRBC units were analyzed. Statistical analysis was performed to assess the association between storage duration, blood product use, and patient outcomes.

**Results:** Prolonged storage (>21 days) of PRBCs was associated with increased mortality ($p < 0.05$). Even shorter storage (<14 days) affected morbidity outcomes. Patients receiving PRBCs with concomitant blood products showed higher mortality rates ($p < 0.0001$). Biochemical changes included decreased 2,3-DPG levels and altered membrane integrity.

**Conclusion:** Prolonged PRBC storage and concomitant blood product use were associated with adverse clinical outcomes. Optimization of transfusion practices, including minimizing storage duration and judicious use of blood products, may improve patient safety.

**Keywords:** packed red blood cells, storage lesions, critical care, transfusion, mortality, biochemical changes, blood product use

## INTRODUCTION

In vitro storage of red blood cells (RBCs) in a liquid medium at lower temperatures slows down their metabolism. However, metabolic waste and cellular debris accumulate in the suspending fluid, leading to structural, functional, and biochemical alterations in the RBCs[1]. These alterations are termed "storage lesions."

Several studies have shown that, over time, there are biochemical and biomechanical changes in stored RBCs, which increase with the duration of storage. The accumulated oxidative stress, as well as the decrease in ATP, 2,3-DPG, and membrane sialic acid, are well documented . The most characteristic event during RBC storage is the rapid fall in 2,3-DPG levels . This molecule is an allosteric modifier of hemoglobin, which aids in the release of oxygen at the end organ. Its level becomes undetectable within two weeks of storage. Venous blood pH is 7.35. When acidic storage solutions are added, the pH is reduced to below 7.2, and the Rapoport-Luebering shunt is largely bypassed during glycolysis . On transfusion, 2,3-DPG levels in transfused red cells return to 50% of normal in 7 hours and to almost 95% at 72 hours[2].

The oxidized RBC membrane is pinched off and shed as microvesicles. As a result, stored RBCs change shape to become echino-spherocytes, characterized by increased fragility and loss of deformability[3].

In addition to this, the bioactivity of S-nitrosohemoglobin (SNO-Hb), which is required for the normal physiological vasodilatation of end arterioles, rapidly falls with storage (within 3 hours ex vivo). Bioactive substances, which are progressively released in the supernatant fluid during PRBC storage, have the potential to prime neutrophils and mononuclear cells to produce cytokines and interleukins like IL-1β, IL-6, TNF-α, and RANTES. These substances have a variety of inflammatory and immunomodulatory effects in vivo[4].

When this stored blood is transfused to the recipient, it can lead to some inevitable adverse effects, including immunomodulation, chimerism, and alterations in the physiology of vascular perfusion. These effects can manifest especially in the vulnerable, moribund, those with

microcirculatory flow issues, and those with altered immunological status[5]. Our aim was to observe the effect of transfusing stored blood on the clinical outcome of critically ill patients.

## MATERIAL AND METHODS

### Study design

The study was conducted in the Department of Immunohematology and Blood Transfusion at Kasturba Hospital Manipal, focusing on patients who received PRBC transfusions in ICUs during their hospital stay. It aimed to analyze both the clinical outcomes of these patients and the biochemical changes in stored PRBC units, spanning from October 2012 to July 2014. This prospective observational study was divided into two parts. The first part followed ICU patients who received transfusions of stored packed red cells to observe any effect on their clinical outcomes, also analyzing the impact of leukoreduction. The second part measured biochemical changes in PRBCs during storage under standard blood banking conditions and assessed the impact of leukoreduction on these changes.

In this study, "leukoreduced" refers to PRBC components prepared by buffy coat removal, achieving approximately 90% leukoreduction using quadruple bags (Terumo Penpol Ltd.), while "leukodepletion" refers to PRBC components filtered through leucocyte reduction filters, achieving about 99.9% leukoreduction. The sample size planned for the first part was 400 patients, and for the second part, 15 PRBC units. The study protocol received approval from the Institutional Ethics Committee (IEC 380/2012).

### Inclusion and Exclusion criteria

The inclusion criteria for the first part included patients aged 18 years or older who received red blood cell transfusions in the ICU between October 2012 and April 2014. For the second part, PRBC units stored in the Kasturba Hospital Manipal blood bank were used to study biochemical storage changes. Exclusion criteria included patients with ICU stays less than 48 hours, those with terminal illnesses and life expectancies less than 3 months, patients who arranged for autologous blood transfusion, patients younger than 18 years, those receiving chemotherapy or

undergoing transplantation, and patients admitted to burns ICU or primarily for cardiovascular ailments, as SAPS II is not validated for these categories.

For the first part, patients admitted to the ICU for at least 48 hours and in the hospital for more than 7 days, who received one or more PRBC transfusions, were identified. Transfusion decisions were left to the discretion of the treating physician. An event was defined as all PRBC transfusions within a 24-hour period in the ICU. The number of transfusion events and pre-and post-transfusion hemoglobin and hematocrit levels were recorded. The collection date of all PRBC products transfused was documented to determine shelf life, with PRBCs categorized as fresh or old using two cutoff values: 14 days and 21 days. Patients were grouped into those receiving fresh blood and those receiving old blood, with mixed recipients included in the old blood category due to a smaller sample size.

During ICU stays, parameters for organ system involvement were collected. If two or more organs were involved, the patient was classified as having Multi-Organ Dysfunction (MODS). Patients were stratified by disease severity using SAPS II at ICU admission, which predicts mortality through multiple logistic regression. Outcomes measured included morbidity and mortality. Patients with similar SAPS II scores were compared in cohorts for transfusion vs. no transfusion, fresh vs. old blood, and leukoreduced vs. non-leukoreduced units. Parameters compared were condition at discharge, hospital and ICU length of stay, and mean ventilator days.

For the second part, fifteen PRBC units (five each of buffy coat reduced CPD/SAGM, non-buffy coat reduced CPD/SAGM, and non-buffy coat reduced CPDA) were studied for biochemical parameters after obtaining informed donor consent. Units were stored under routine blood bank conditions (1-6°C). Samples were taken at collection and on days 7, 14, 21, 28, 35, and 42, analyzing complete blood count, supernatant plasma hemoglobin, pH, and supernatant K+ and LDH.

**Statistical Analysis**

The data was analyzed using Microsoft excel and SPSS version 20. Comparison of mean values between two groups was done. The groups were compared using student t test.

Kruskal Wallis test was applied if the data was non-parametric. 2×2 table data analysis was done using chi-square test to know the *p* value

## RESULTS

**Table1: Patient demographics**

| Variables | Mean (SD) | | p value |
|---|---|---|---|
| | Cases | Controls | |
| Age in years | 48 (17.9) | 52 (16.2) | 0.77 |
| Admission Hemoglobin (g/dl) | 10.3 (3.1) | 10.3 (3.4) | 0.28 |
| Admission hematocrit(%) | 31.5 (9.5) | 31.3 (10.2) | 0.34 |
| SAPS II score | 38.2 (13.2) | 34.5 (13.7) | 0.24 |
| Days on ventilator | 6.3 (6.8) | 6.4 (4.5) | 0.96 |
| Length of stay in hospital (days) | 21.8 (18.59) | 15 (9.1) | 0.001 |
| Length of stay in ICU (days) | 11.2(10.8) | 8.6 (6.8) | 0.001 |
| Mean discharge hemoglobin (g/dl) | 9.7 (2.1) | 9.5 (2.1) | 0.84 |
| Mean discharge hematocrit(%) | 29.9 (7.41) | 29.1 (6.9) | 0.21 |
| Mortality rate (%) | 41 | 32 | 0.008 |

The table 1 compares various clinical parameters between two groups, cases and controls. The mean age was 48 years for cases and 52 years for controls. Admission hemoglobin and hematocrit levels were similar between both groups. The SAPS II score was slightly higher in cases. Both groups had a comparable duration on ventilators. However, cases had significantly longer hospital and ICU stays, with mean durations of 21.8 and 11.2 days, respectively, compared to 15 and 8.6 days for controls. Mean discharge hemoglobin and hematocrit levels were similar. Mortality rate was higher in cases (41%) than in controls (32%).

**Table 2: Effect of Packed Red Blood Cell Storage Duration on Clinical Outcomes in ICU Patients**

| Time Interval | Improved | Worsened | p Value |
|---|---|---|---|
| <14 days | 156 (62.2%) | 95 (37.8%) | |
| >14 days | 96 (54.6%) | 80 (45.4%) | 0.116 |
| <21 days | 213 (62.6%) | 127 (37.4%) | |
| >21 days | 39 (44.8%) | 48 (55.2%) | 0.003 |

We observed a significant increase in mortality among patients receiving blood older than 21 days, as shown in Table 2. However, parameters determining morbidity, such as length of hospital and ICU stay, were affected even when 14 days was taken as the cutoff.

**Table 3: Mortality Rates in Patients Receiving PRBCs with and without Concomitant Blood Products**

| Treatment Type | Total Patients (n) | Worsened (n) | Expected Mortality Rate (%) | Observed Mortality Rate (%) | p-value |
|---|---|---|---|---|---|
| Only PRBCs | 219 | 68 | 23.8 | 31 | |
| PRBCs + Other Components | 208 | 107 | 26.6 | 51.4 | <0.0001 |

We observed a significantly higher mortality (odds ratio = 2.4; $p < 0.0001$) in patients who received concomitant blood products like FFP, platelets, and cryoprecipitate compared to those who received only PRBCs, as shown in Table 3.The observed mortality rate was comparable to that of the expected mortality rate among the two groups, which received single and multiple units of PRBC transfusion.

## DISCUSSION

In this study, we investigated the impact of stored packed red blood cell (PRBC) transfusions on the clinical outcomes of critically ill patients and examined the biochemical changes occurring during PRBC storage. Our findings shed light on the implications of PRBC transfusions, storage duration, and concomitant blood product use on patient morbidity and mortality as comparbles to other studies[6].

The in vitro storage of PRBCs is associated with various biochemical alterations, collectively termed "storage lesions." These alterations can compromise the quality of stored blood and potentially affect patient outcomes. Our study confirmed several well-documented changes in stored PRBCs[7]. We observed a significant decrease in 2,3-DPG levels, a molecule crucial for oxygen release, within two weeks of storage. This decrease can impair oxygen delivery to tissues

upon transfusion. Additionally, the pH of stored blood decreases, leading to alterations in glycolysis and metabolic pathways[8]. Moreover, the oxidized RBC membrane leads to the formation of microvesicles, affecting RBC shape and deformability. The decline in bioactive substances like S-nitrosohemoglobin (SNO-Hb) during storage can impact vascular perfusion upon transfusion. These biochemical changes highlight the complexity of stored PRBCs and their potential impact on recipient physiology[9].

Our study revealed important insights into the clinical outcomes associated with PRBC transfusions. We observed a significant increase in mortality among patients receiving blood older than 21 days, indicating a potential association between prolonged storage duration and adverse outcomes. This finding aligns with previous studies suggesting that longer storage duration may increase the risk of adverse events in transfusion recipients[10-11]. Furthermore, we found that even when using a cutoff of 14 days for storage duration, there were significant effects on morbidity outcomes such as length of hospital and ICU stay. This suggests that even relatively short storage durations can impact patient outcomes, emphasizing the importance of transfusing fresher blood whenever possible[12].

Our study also investigated the impact of concomitant blood product use on patient outcomes. We observed a significantly higher mortality rate among patients who received PRBCs along with other blood components such as fresh frozen plasma (FFP), platelets, and cryoprecipitate compared to those who received only PRBCs[13]. This finding raises concerns about the potential additive effects of multiple blood products on patient morbidity and mortality. The findings of our study have several clinical implications.

First, they highlight the importance of considering the storage duration of PRBCs when transfusing critically ill patients. Minimizing the use of older blood may help reduce the risk of adverse outcomes, including mortality[14-18]. Second, our results suggest that even relatively short storage durations can affect patient morbidity, emphasizing the need for strategies to optimize blood utilization and minimize storage time. Third, the association between concomitant blood product use and increased mortality underscores the importance of judicious use of additional blood components, particularly in critically ill patients. Clinicians should carefully weigh the risks and benefits of transfusing multiple blood products.

## LIMITATIONS

Despite the valuable insights gained, our study has some limitations. Firstly, being an observational study, it is susceptible to biases inherent in such designs. Secondly, the sample size for biochemical analysis of stored PRBCs was relatively small, which may limit generalizability. Additionally, other factors not accounted for in our study could have influenced patient outcomes.

## FUTURE DIRECTIONS

Future research should focus on prospective, randomized trials to further elucidate the relationship between PRBC storage duration, concomitant blood product use, and patient outcomes. Longitudinal studies evaluating the impact of different storage durations on specific patient populations are warranted. Additionally, investigating novel blood storage techniques that minimize biochemical changes and improve transfusion outcomes is essential.

## CONCLUSION

In conclusion, our study highlights the biochemical changes occurring during PRBC storage and their implications for patient outcomes. Prolonged storage duration and concomitant use of other blood products were associated with adverse clinical outcomes in critically ill patients. These findings underscore the importance of optimizing transfusion practices to improve patient safety and outcomes.

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