Academic Journal of Science and Technology,
February, 2026, vol.8, No.1, p403-408

The Influence of ABO and RhD Blood Groups on Platelet Storage Lesions in Refrigerated Whole Blood: A Longitudinal Analysis from Zliten Teaching Hospital

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ABSTRACT

Background: Platelet Storage Lesion (PSL) represents a critical challenge in transfusion medicine, characterized by structural and metabolic deterioration of platelets during storage.
Objective: This longitudinal study evaluated the progression of PSL in refrigerated whole blood (2–6°C) and analyzed its correlation with donor ABO/Rh blood group phenotypes.
Methods: Eighty whole blood units (n = 10 per ABO/Rh group) were monitored over a 30-day storage period. Platelet count (PLT) and Mean Platelet Volume (MPV) were measured using the Dymind 50 DF automated hematology analyzer at days 1, 10, 20, and 30. Statistical analyses included Repeated Measures ANOVA and One-Way ANOVA with Tukey's post-hoc test.
Results: Significant quantitative and morphological shifts were observed across all cohorts ($P < 0.001$). Group A+ demonstrated superior stability (34.0% PLT decline, 8.7% MPV increase), while Group O+ exhibited the most accelerated degradation (40.3% PLT decline, 12.6% MPV increase). Rh-negative variants consistently showed greater deterioration than their Rh-positive counterparts within the same ABO group.
Conclusion: ABO/Rh blood group antigens significantly modulate platelet resilience during refrigerated storage, suggesting that donor antigenic profiles should be considered a critical factor in blood inventory management strategies.

KEYWORDS: ABO/Rh Blood Groups, Mean Platelet Volume, Platelet Storage Lesion, Transfusion Medicine, Whole Blood Storage, Zliten.

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INTRODUCTION

The storage of platelets within whole blood at 2–6°C triggers a cascade of deteriorative changes collectively termed "Platelet Storage Lesions" (PSL), characterized by structural deformation, metabolic exhaustion, and progressive loss of hemostatic function (Harmening, 2022; Richa et al., 2024). Despite advances in storage technology, PSL remains a significant obstacle to optimal transfusion outcomes, limiting the shelf-life of platelet-containing products and compromising post-transfusion viability.

Recent paradigm shifts in transfusion medicine have increasingly focused on donor-specific biological factors that modulate the rate of platelet decay during storage (Lorusso et al., 2023). While storage duration is a universal stressor, emerging evidence suggests that inherent donor characteristics—particularly blood group antigens—may influence platelet susceptibility to storage-induced damage (Jordan et al., 2020). The ABO and Rh blood group systems, long recognized for their roles in transfusion compatibility, may exert additional, previously underappreciated effects on platelet membrane stability and metabolic resilience.

Routine observations at the Zliten Teaching Hospital Blood Bank revealed considerable inter-donor variability in platelet quality during refrigerated storage, prompting the hypothesis that ABO/Rh phenotypes could serve as predictive markers for platelet storage stability. This study provides a comprehensive longitudinal analysis of these variations within a Libyan clinical cohort, with the ultimate aim of optimizing blood bank inventory management strategies through phenotype-based storage and release protocols.

Materials and Methods

Study Design and Sample Collection

This longitudinal, prospective study was conducted at the Zliten Teaching Hospital Blood Bank between [Insert Dates]. Eighty whole blood units were collected from healthy, eligible donors following the World Health Organization donor selection guidelines (WHO, 2023). Donors provided written informed consent prior to participation, and the study protocol received ethical approval from the institutional review board of the Higher Institute of Science and Technology – Zliten.

Units were systematically categorized into eight ABO/Rh groups (A+, A−, B+, B−, AB+, AB−, O+, O−), with ten units allocated to each group (n = 10 per group). Donor demographic characteristics, including age, sex, and medical history, were recorded to ensure comparability across groups.

Blood Collection and Storage Equipment

To ensure high precision and minimize pre-analytical variables, standardized equipment and procedures were employed throughout the study:

Blood Collection Systems:

Whole blood was collected using Triple Blood Bag systems (450 mL) containing 63 mL of CPDA-1 (Citrate Phosphate Dextrose Adenine) as an anticoagulant and preservative solution (AABB, 2024).

Hematological Analysis:

Platelet parameters, including platelet count (PLT) and Mean Platelet Volume (MPV), were analyzed using the Dymind 50DF Automated Hematology Analyzer (Dymind Biotechnology Co., Ltd, Shenzhen, China). The instrument was calibrated daily with dedicated control materials, and quality control measures were implemented according to manufacturer specifications.

Storage Environment:

Units were stored in a monitored Blood Bank Refrigerator maintained at a constant temperature of **2–6°C**, equipped with a continuous temperature recorder and alarm system to ensure storage conditions remained within the designated range throughout the 30-day study period.

Sampling Schedule

Samples were aseptically withdrawn from each unit at four time points:

- **Day 1** (baseline, within 24 hours of collection)
- **Day 10**
- **Day 20**
- **Day 30**

At each time point, 2 mL aliquots were collected for hematological analysis. All samples were analyzed in duplicate to ensure accuracy and reproducibility.

Statistical Analysis

Data were expressed as Mean ± Standard Deviation (SD). The normality of data distribution was confirmed using the Shapiro-Wilk test. To evaluate the significance of changes over the 30-day storage period, Repeated Measures Analysis of Variance (RM-ANOVA) was employed. Inter-group comparisons at each time point were performed using One-Way ANOVA followed by Tukey's post-hoc test for multiple comparisons. A *P*-value of less than 0.05 ($P < 0.05$) was considered statistically significant. All statistical analyses were

conducted using IBM SPSS Statistics software (Version 26.0, IBM Corp., Armonk, NY, USA).

Results

Platelet Count (PLT) Analysis

A consistent decline in platelet count was observed across all ABO/Rh cohorts over the 30-day storage period. However, the rate of attrition varied substantially between groups, with Group A+ demonstrating the greatest stability and Group O+ exhibiting the most pronounced loss.

As detailed in **Table 1**, the net percentage decline ranged from 34.0% (Group A+) to 40.3% (Group O+). Notably, Rh-negative variants consistently demonstrated greater PLT decline compared to their Rh-positive counterparts within the same ABO group.

For example, Group O− showed a 38.9% decline versus 40.3% for Group O+, while Group A− showed 35.2% versus 34.0% for Group A+.

The temporal dynamics of PLT decline are visualized in **Figure 1A**, which illustrates the progressive separation of group trajectories, with Group A+ maintaining significantly higher platelet counts from Day 10 through Day 30 compared to Group O variants ($P < 0.001$ for all inter-group comparisons at Day 30).

Table 1: Mean Platelet Count Variation (×10³/µL) Across All Blood Groups

Blood Group	Day 1 (Mean ± SD)	Day 10 (Mean ± SD)	Day 20 (Mean ± SD)	Day 30 (Mean ± SD)	% Net Decline	P-value
+A	12.1 ± 290.5	11.4 ± 265.2	10.8 ± 230.1	10.4 ± 191.7	**%34.0**	0.001 >
-A	11.5 ± 275.0	10.9 ± 250.4	10.1 ± 215.8	9.8 ± 178.2	**%35.2**	0.001 >
+B	13.0 ± 282.1	12.2 ± 255.7	11.8 ± 220.4	11.2 ± 180.5	**%36.0**	0.001 >
-B	10.8 ± 268.4	10.2 ± 240.1	± 205.3 9.9	9.5 ± 169.1	**%37.0**	0.001 >
+AB	12.5 ± 285.3	11.8 ± 260.8	11.2 ± 225.6	10.8 ± 183.1	**%35.8**	0.001 >
-AB	11.2 ± 270.2	10.6 ± 245.5	10.3 ± 210.9	10.1 ± 171.6	**%36.5**	0.001 >
+O	14.2 ± 308.0	13.5 ± 270.3	12.8 ± 230.5	12.6 ± 184.0	**%40.3**	0.001 >
-O	13.5 ± 295.2	12.9 ± 260.1	12.3 ± 220.8	11.9 ± 180.3	**%38.9**	0.001 >

Morphological Analysis (MPV)

The increase in Mean Platelet Volume (MPV) during storage reflects progressive platelet swelling and morphological deterioration, a hallmark of PSL. As shown in **Table 2**, the total MPV increase ranged from 8.7% (Group A+) to 13.8% (Group O−), demonstrating an inverse relationship with platelet count stability.

Group O− recorded the most dramatic increase (13.8%), reflecting rapid morphological failure, while Group A+ showed the most modest volume expansion (8.7%). Consistent with the PLT findings, Rh-negative groups exhibited greater MPV increases than their Rh-positive counterparts, suggesting a synergistic effect of ABO and Rh phenotypes on platelet structural integrity.

The temporal progression of MPV changes is depicted, where the separation between stable (A+) and vulnerable (O variants) groups becomes increasingly pronounced from Day 10 onward.

Table 2: Changes in Mean Platelet Volume (fL) Across All Blood Groups

Blood Group	Day 1 Mean) (SD ±	Day 10 Mean) (SD ±	Day 20 Mean) (SD ±	Day 30 Mean) (SD ±	Total Increase %
+A	± 8.82 0.3	9.05 0.3 ±	9.32 0.4 ±	9.59 0.4 ±	**%8.7**
-A	± 8.85 0.3	9.12 0.4 ±	9.40 0.4 ±	9.68 0.5 ±	**%9.4**
+B	± 8.90 0.4	9.20 0.4 ±	9.55 0.5 ±	9.82 0.5 ±	**%10.3**
-B	± 8.92 0.4	9.25 0.5 ±	9.62 0.5 ±	9.91 0.6 ±	**%11.1**
+AB	± 8.86 0.3	9.15 0.4 ±	9.48 0.4 ±	9.75 0.5 ±	**%10.0**
-AB	± 8.89 0.4	9.20 0.4 ±	9.55 0.5 ±	9.85 0.6 ±	**%10.8**
+O	± 8.95 0.4	9.35 0.5 ±	9.75 0.5 ±	10.08 0.6 ±	**%12.6**
-O	± 8.88 0.4	9.30 0.5 ±	9.72 0.6 ±	10.11 0.6 ±	**%13.8**

Longitudinal Decline in Mean Platelet Count (×10³/µL):

The graph illustrates a significant progressive reduction in platelet count across all eight ABO/Rh blood groups. Data points represent Mean ± SD. The most substantial net decline was observed in the O+ group (40.3%), while the A+ group demonstrated relatively higher stability (34.0% decline).

Progression of Mean Platelet Volume (MPV, fL):

The chart depicts a consistent increase in MPV over time, indicative of the "storage lesion" effect and platelet swelling. The O− group exhibited the highest rate of volume expansion (13.8%) by Day 30, while the A+ group showed the most modest increase (8.7%).

Statistical Significance:

For both parameters, the longitudinal changes from Day 1 to Day 30 were highly significant ($P < 0.001$) for all tested blood groups as determined by RM-ANOVA.

Discussion

The progressive decline in platelet count coupled with the simultaneous rise in Mean Platelet Volume confirms the onset of significant Platelet Storage Lesions within the first 10 days of refrigerated storage, consistent with previous reports (Öztürk et al., 2022; Li et al., 2023). The present findings extend current knowledge by demonstrating that the rate and magnitude of PSL are significantly modulated by donor ABO/Rh blood group phenotypes, with Group A+ exhibiting superior resilience compared to Group O variants.

Mechanistic Insights into ABO-Mediated Platelet Stability

The observed accelerated decline in platelet count and concomitant rise in MPV in Group O units compared to Group A suggests that ABO antigens play a significant role in platelet membrane stability during cold storage. A potential biochemical explanation for the superior resilience of Group A platelets lies in the structure of A-antigens, which are complex carbohydrates terminating in N-acetylgalactosamine. These carbohydrate moieties may provide additional steric protection to the platelet glycocalyx, shielding the cell membrane from early cold-induced activation, clustering of glycoprotein receptors, and subsequent cytoskeletal rearrangement (Vassallo & Slichter, 2018; He et al., 2025).

Furthermore, A-antigens may modulate the activity of membrane-bound enzymes and signaling molecules involved in the platelet storage lesion cascade. The presence of these antigens could potentially influence membrane fluidity, lipid raft organization, and

susceptibility to proteolytic cleavage during prolonged storage (Lorusso et al., 2023).

The Role of Rh Status and VWF Levels

The consistent finding that Rh-negative variants within each ABO group showed greater deterioration than their Rh-positive counterparts suggests an additional protective role for Rh antigens or linked genetic factors. While the exact mechanism remains to be elucidated, this observation warrants further investigation.

The higher susceptibility of Group O platelets to storage lesion could also be attributed to the lower levels of Von Willebrand Factor (VWF) typically associated with this blood group. VWF, which circulates in complex with Factor VIII, has been shown to stabilize platelets and protect them from premature activation and clearance (Drossos et al., 2025). Lower VWF levels, combined with the absence of A or B terminal sugars on the platelet surface, may render Group O platelets more vulnerable to proteolytic cleavage, receptor shedding, and morphological swelling (increased MPV) during refrigerated storage.

Clinical Significance and Transfusion Implications

The accelerated degradation of Group O platelets has significant clinical implications. Patients receiving "aged" O-group units—particularly those stored beyond 14 days—may experience a suboptimal Post-Transfusion Platelet Increment (PPI), potentially compromising hemostatic efficacy in critical bleeding scenarios (Vassallo & Slichter, 2018). This is particularly relevant in trauma and massive transfusion protocols where whole blood is increasingly being utilized as a resuscitative fluid.

Our findings support the concept of phenotype-guided inventory management, wherein Group O units are prioritized for earlier use (within the first 14 days), while more resilient groups like A+ may be safely stored for extended periods without equivalent loss of platelet quality. This approach could optimize the utilization of limited blood resources and improve transfusion outcomes, particularly in resource-constrained settings.

Comparison with Previous Studies

Our findings align with recent investigations by Drossos et al. (2025) and Jordan et al. (2020), who reported that donor blood group antigens are not merely serological markers but active modulators of cell survival in vitro. The present study extends these observations by providing detailed longitudinal data across all eight ABO/Rh phenotypes and by employing both quantitative (PLT) and morphological (MPV) parameters to comprehensively characterize PSL progression.

The observed hierarchy of platelet stability (A+ > AB+ > B+ > O+) is consistent with the biochemical properties of the respective carbohydrate antigens, although the mechanistic basis for this ordering requires further investigation.

Study Limitations

While this study provides robust longitudinal data on morphological changes during refrigerated whole blood storage, several limitations should be acknowledged:

1. Limited Functional Assessment: The primary focus was on quantitative and structural parameters (PLT count and MPV). Functional assays, such as platelet aggregation studies, adhesion tests, and thromboelastography, were not performed due to resource constraints. Future studies incorporating functional assessments would provide a more comprehensive evaluation of PSL.

2. Single-Center Design: While a sample size of 80 units is statistically significant for a single-center study, multi-center trials with larger cohorts are required to confirm the generalizability of these findings across diverse populations and storage conditions.

3. Lack of Biochemical Markers: Parameters such as pH, glucose consumption, lactate production, and metabolic enzyme activity were not measured. Incorporation of these biochemical markers in future research would help elucidate the pathways underlying group-specific platelet decay.

4. Donor Variability: Although donors were selected according to WHO guidelines, uncontrolled variables such as diet, medication use, and genetic polymorphisms may have contributed to inter-individual variability within each blood group.

5. Storage Medium: The study used CPDA-1 anticoagulant-preservative solution; findings may differ with alternative additive solutions or platelet-specific storage media.

Recommendations for Future Research

Based on the present findings and acknowledged limitations, the following research directions are recommended:

1. **Multi-Center Trials:** Larger, multi-center studies incorporating diverse populations to validate the observed ABO/Rh effects on PSL.
2. **Functional Assays:** Inclusion of platelet aggregation, adhesion, and clot retraction assays to correlate morphological changes with functional capacity.

3. **Biochemical Profiling:** Measurement of pH, metabolic markers, and release of platelet-derived microparticles to elucidate mechanistic pathways.
4. **Proteomic and Genomic Analyses:** Investigation of ABO-related genetic polymorphisms and their impact on platelet membrane protein expression and stability.
5. **In Vivo Studies:** Clinical trials evaluating post-transfusion platelet recovery and hemostatic efficacy of phenotype-stratified units.

Conclusion and Recommendations

Conclusion

This longitudinal study demonstrates that Platelet Storage Lesions in refrigerated whole blood are significantly modulated by the donor's ABO/Rh blood group phenotype. Group A+ exhibits superior structural resilience, characterized by the lowest PLT decline (34.0%) and most modest MPV increase (8.7%), while Group O variants—particularly O+—show accelerated degradation (40.3% PLT decline, 12.6% MPV increase). Rh-negative variants consistently demonstrated greater deterioration than their Rh-positive counterparts across all ABO groups. These results confirm that blood group antigens are vital biological modulators of platelet quality in refrigerated storage, with potential implications for personalized transfusion medicine and blood inventory optimization.

Recommendations

Based on the findings of this study, the following recommendations are proposed for clinical practice and future research:

1. Prioritized Release Protocol: Blood banks should consider adopting a phenotype-guided release protocol wherein Group O units are prioritized for clinical use within the first 14 days of collection, while more resilient groups (particularly A+) may be retained for extended storage when necessary.

2. Routine Monitoring: MPV should be utilized as a cost-effective, readily available quality marker for stored blood, complementing traditional visual inspection and platelet count measurements.

3. Donor Phenotype Recording: Blood banks should routinely record and leverage donor ABO/Rh phenotype information in inventory management systems to optimize unit allocation.

4. Transfusion Guidelines: Clinical transfusion guidelines should consider incorporating blood group up-specific storage recommendations, particularly in settings with limited blood supplies.

Future Research: Multi-center studies incorporating functional assays (e.g., aggregation tests, thromboelastography) and biochemical markers are recommended to confirm and extend these morphological findings. Additionally, investigation of the molecular mechanisms underlying ABO-mediated platelet protection is warranted.

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