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Research Article

Assessment of Blood Quality During Cold Storage: Correlating Electrical Impedance, Viscosity, and Cell Counts Using Impedance Spectroscopy

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ABSTRACT

Cold storage of whole blood is essential to maintain the availability and safety of transfusion products; however, prolonged storage induces progressive biochemical and biophysical changes that compromise cellular integrity and functionality. Electrical Impedance Spectroscopy (EIS) has emerged as a promising non-invasive technique for detecting early microstructural changes in biological systems, yet its relationship with viscosity and hematological parameters during extended blood storage remains insufficiently characterized. This study investigates time-dependent variations in electrical impedance, blood viscosity, and hematological indices in whole blood preserved with acidic citrate dextrose (ACD) for up to 35 days, to evaluate EIS as an early indicator of storage-related degradation. Twenty-five units of whole blood were stored at 1–6°C and analyzed on Days 0, 2, 7, 14, 21, 28, and 35. Electrical impedance over a frequency range of 100 Hz–100 kHz, hematological parameters, and blood viscosity were determined using a digital impedance analyzer, an automated hematology analyzer, and a digital rotational viscometer, respectively. The results revealed a gradual decline in total electrical impedance, with the most pronounced changes occurring within the first seven days, suggesting early membrane injury and ionic redistribution. Significant decreases were observed in leukocyte, platelet, and hemoglobin levels, while red blood cell count and hematocrit remained relatively stable throughout the storage period. Blood viscosity exhibited minimal variation during the first 30 days but showed a slight increase by Day 35, indicating delayed rheological alteration. Importantly, EIS demonstrated greater sensitivity for detecting early degradation than viscosity measurements and conventional hematological parameters. These findings point to the advantages of EIS as a rapid, sensitive monitoring tool for evaluating the quality and stability of stored blood in transfusion practice.

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1. INTRODUCTION

Maintaining the quality of whole blood during storage remains a major issue in transfusion medicine. During typical hypothermic storage (1-6°C), blood components undergo a variety of biochemical and biophysical modifications, collectively termed the red blood cell (RBC) storage lesion. Such modifications are triggered immediately after sampling and are accentuated by storage. The storage lesion is characterized by metabolic exhaustion, oxidative stress, membrane remodeling, ionic dysregulation, decreased ATP levels, and loss of erythrocyte deformability, all of which may reduce the efficacy of post-transfusion therapy and clinical outcomes. The PCDA-1 preservation solution can be stored for 28–35 days, but structural and metabolic damage can be detected 24-48 hours after collection (Tran et al., 2024; Tzounakas et al., 2021). These early changes show that traditional hematology parameters (simple cell counts that cannot capture subtle structural or dielectric modifications) are insufficient and that sensitive, non-invasive monitoring strategies are required.

Electrical Impedance Spectroscopy (EIS) is now considered a promising technique for investigating the electrical and structural characteristics of living cells. In contrast to typical laboratory assays, which may require labels or reagents and are time-consuming, EIS measurements are performed in a label-free, non-invasive manner across multiple frequencies. The method is responsive to variations in membrane capacitance, cytoplasmic resistivity, ionic redistribution, and cell geometry, all of which are directly influenced by storage-related membrane restructuring and metabolic degradation. Among other recent biomedical applications showing diagnostic capabilities with impedance-based techniques are the differentiation of RBC and platelet-rich thrombi in ischemic stroke (Sahin et al., 2025), dielectric monitoring of hemolysis in real time (Peng et al., 2025), and microfluidic impedance assays for the detection of biomechanical derangements in sickle cell disease (Patwardhan et al., 2024). Those studies indicated that the microstructural changes observed by dielectric measurements precede the onset of extensional morphological destruction. Widodo et al. (2024) report progressive changes toward low mean corpuscular hemoglobin concentration (MCHC) and membrane stability, as measured by impedance magnitude. In the same way, Herenda et al. (2020) found patterns of evolving impedance in whole blood samples stored under identical conditions. Collectively, these findings imply that impedance reflects erythrocyte membrane integrity and intracellular dielectric redistribution in stored cells. However, previous research has generally considered impedance or hematological measures in isolation, and storage duration has been limited to 28 days (Herenda et al., 2020; Widodo et al., 2024). There is therefore a lack of knowledge about how the dielectric, hematological, and rheological properties evolve in concert over long-term storage.

Rheological properties should therefore be a confounding factor: hemorheological properties (including blood viscosity) greatly influence TT results. Hct, erythrocyte deformability, plasma protein composition, and erythrocyte aggregation are the determinants of blood viscosity and aggregability of cells (Baskurt & Meiselman, 2003). Oxidative injury and membrane rigidification that occur during storage can significantly impair RBCs' ability to deform, potentially impairing microvascular flow. During the early stages of storage, the viscosity remains stable because the plasma's buffering action is active. Over time, however, as oxidative stress and membrane lipid peroxidation escalate, especially beyond the 28-day threshold, significant changes in viscosity tend to emerge (Kwag et al., 2023; Liao et al., 2019). During this storage period, dielectric measurements may offer early detection of ion and membrane alterations, potentially preceding noticeable changes in rheological properties. Although these are important observations, a full longitudinal study spanning the entire 35-day storage period using all three key approaches (dielectric, rheological, and hematological analyses) has not yet been conducted. Apart from a recently mentioned case in the experimental results, such integrative work is exceptionally rare. However, employing an integrated multimodal approach that combines these techniques has proven highly effective in elucidating the biophysical mechanisms associated with storage-induced degradation. Advanced calculations, such as impedance-based predictive modeling, are also expected to leverage large databases of dielectric characteristics and related physiological parameters, thereby enabling new discoveries. These integrative datasets will be critical for assessing whether impedance is merely a correlation of, or a biophysically meaningful indicator of, structural and functional decline during storage.

Thus, this study reports a detailed longitudinal study of electrical impedance spectra, hematological indices, and blood viscosity in CPDA-1 whole blood stored for 35 days. The present work is designed to investigate the early response of impedance and viscosity to cell death, the time-dependent evolution of dielectric, rheological, and hemorheological parameters over early, mid, and late storage, and the potential of impedance-related parameters to predict biochemical and rheological changes. By establishing a direct, consistent relationship among dielectric properties, cell counts, and viscosity for long-term storage, the present study aims to contribute to a more profound understanding

of the biophysical mechanisms of blood quality decay and to the possible role of impedance spectroscopy as an adjunct monitoring modality in transfusion medicine lacunas.

2. METHODOLOGY

2.1. Materials and Sample Preparation

Twenty-five units of whole blood were collected from volunteer donors at the Indonesian Red Cross (PMI) Malang Regency. Each unit consisted of packed red blood cells with CPDA-1 (Citrate-Phosphate-Dextrose-Adenine) as the anticoagulant and preservative. This additive solution is widely used in transfusion medicine to preserve erythrocyte quality, buffer pH, and extend storage to 28-35 days at refrigerated temperatures (1 to 6°C) (1). At the end of the collection, the bags were all inverted and, with gentle movements, rotated 10–15 times immediately to homogenize the samples and prevent clot formation. From each blood unit, 3-ml aliquots were collected into sterile blood collection tubes and stored in a laboratory-grade refrigerator at 1-6°C throughout the study. The samples were processed on schedule at Day 0 (baseline), 2, 7, 14, 21, 28, and 35.

2.2. Electrical Impedance Spectroscopy (EIS) Measurement

A signal generator, an IDE, and the acquisition software constituted the BIS apparatus for measuring electrical impedance. A sinusoidal current of 10 μA was forced at frequencies from 100 Hz to 100 kHz, as in Figure 1 (Specification and the design of IDE electrode). Sensitivity to the dielectric properties variation in the blood matrix was improved by designing the IDE on a glass substrate having 7 pairs of gold electrodes (width of 1 mm, inter-electrode gap of 0.5 mm, and diameter of 8 mm); refer to Figure 1. 100 μL of whole blood was pipetted onto the surface of the IDE for each assay, and the spectra of impedance, resistance (R), reactance (X), and magnitude of impedance (Z) were obtained.

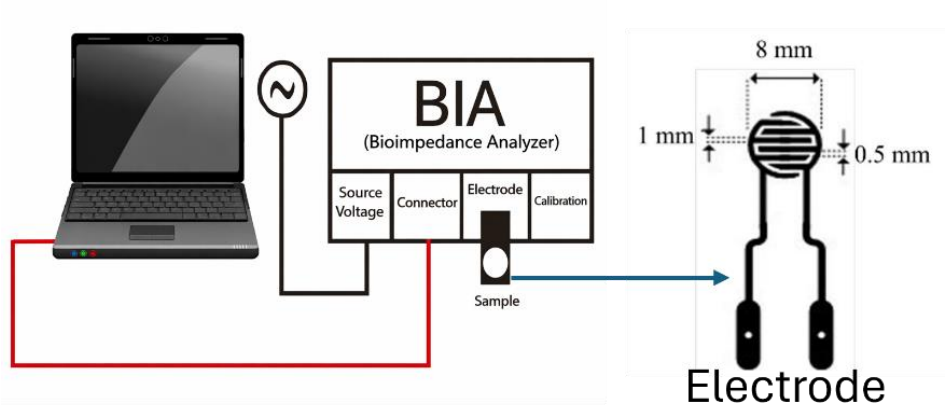

Figure 1. Schematic Diagram of a Bioimpedance Analyzer (BIA) Measurement System

2.3. Cell Count Analysis

The cell counts were analyzed using the Cobas hematology analyzer, an automated fluorescence-based system that counts red blood cells (RBCs), white blood cells (WBCs), and platelets. Hemoglobin concentration, hematocrit, and other hematological indices were also recorded. All cell count analyses were performed at Klinik Kawi Laboratory in Malang by trained technicians. Blood samples were tested at each time point in parallel with impedance measurements to ensure accurate temporal correlation.

2.4. Viscosity Measurement

Viscosity was assessed using a digital rotational viscometer at controlled room temperature (25 ± 1°C). Blood samples were equilibrated for 5 minutes before measurement to eliminate temperature-induced variability. Measurements were conducted at each storage interval using standard cylindrical spindles. Viscosity data were recorded in centipoise (cP) and used to assess the rheological behavior of stored blood over time.

3. RESULTS AND DISCUSSION

The 3C model predicts the hydration and cellular components of body composition from a body fluid estimate using a cancer patient-specific small panel of hematological and biochemical markers. The hemoglobin-impedance relationship was strongest (R^2 = 0.966, p<0.001), indicating that hemoglobin concentration explained 96.6% of the variability in impedance (Figure 2). The regression line (Z = 1319.40 Hb – 14867.18) was found to have a slope of 1319.40 (95% CI = 1032.99 to 1605.80), indicating a statistically significant increase in the inverse of impedance for every 1 g/dL increase in hemoglobin. Leukocyte counts were also strongly positively correlated with total impedance (R^2 = 0.892, p = 0.0014). The regression line (Z = 0.4025 Leukocyte + 426.95) is linear (slope 0.4025 [95% CI 0.2415 to 0.5635]) and indicates that leukocyte concentration was a positive determinant of this portion of the impedance. Furthermore, the thrombocyte count was significantly positively correlated with impedance (R^2 = 0.780, p = 0.0084), with a regression slope of 0.01308 (95% CI: 0.00510 to 0.02106), according to the model Z = 0.01308Thrombocyte − 65.15. The hematocrit was also highly correlated with total impedance (R^2 = 0.861, p = 0.0026). The regression (Z = 1912.48Hct − 67139.19) provided a slope of 1912.48 with a 95% CI of 1027.40 to 2797.55, indicating a significant increase in impedance with increasing % Hct. Significantly, all confidence intervals for the regression slopes excluded zero, further supporting the statistical significance and robustness of all identified correlations. In general, hemoglobin concentration had the strongest predictive value for total impedance, followed by zero, further count, hematocrit, and thrombocyte count. These findings indicate that the hematological makeup, particularly those related to cellular concentration and the oxygen-carrying capacity, has a significant influence on the bulk electrical impedance.

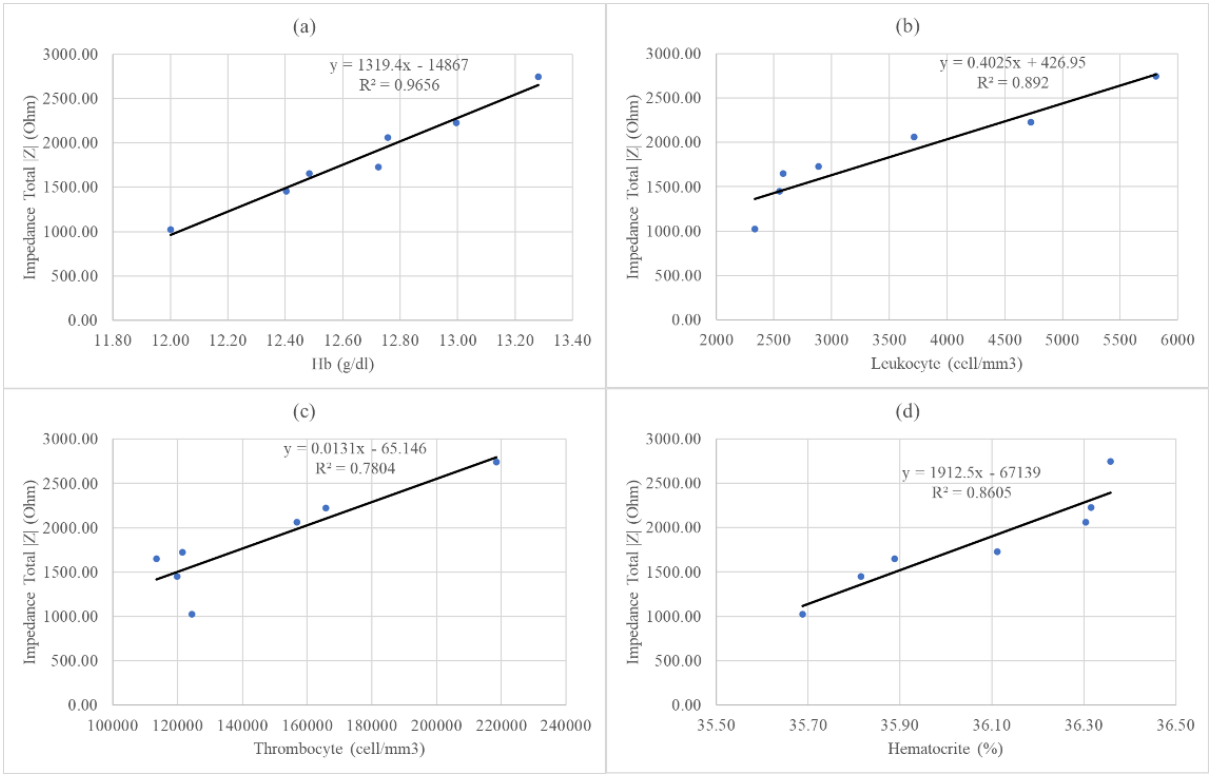

Figure 2. Linear regression analysis showing the correlations between total electrical impedance (Z) and hematological parameters, including (a) hemoglobin, (b) leukocyte count, (c) thrombocyte count, and (d) hematocrit. Regression equations and the coefficient of determination (R^2) are shown for each panel

These findings provide strong evidence that electrical impedance is a function of hematological parameters and viscosity during cold storage of whole blood. Electrical impedance monitored under laboratory conditions for 35 days showed a continuous decrease, while leukocyte and thrombocyte counts decreased significantly, with an increase in viscosity observed late in storage. These parallel behaviors suggest that electrical impedance spectroscopy (EIS) may be a sensitive indicator of early biochemical and structural alterations in stored blood. The cell counts demonstrated significant reductions in leukocyte, thrombocyte, and hemoglobin (Hb) concentrations during 35 d of storage. From Day 0 to Day 35, there was a 9.04% decrease in hemoglobin, from 13.2 g/dL at Day 0 to 12.0 g/dL at Day 35. Leukocyte counts decreased by more than 50%, from 5807.6 to 2334.6 cells/mm³. The

observed hematological decline is consistent with previous reports that storage induces oxidative damage, loss of cellular energy, and membrane disruption, leading to hemolysis and apoptosis (Tzounakas et al., 2021). The red blood cell count remained relatively stable at 4.5 million/mm³ throughout storage, suggesting that cellular lysis may not be preceded by morphological degradation. The hematocrit decreased slightly from 36.3% to 35.6%, indicating a slight hemoconcentration or less RBC deformability with increasing age. These findings reinforce the notion that biochemical lesions may occur even in the absence of significant numeric decline, underscoring the need for more sensitive monitoring tools.

The observed reduction in cell counts (hemoglobin, leukocytes, and thrombocytes) aligns with results from Tzounakas et al. (2021). The report on storage lesions in RBCs, including membrane rigidity and oxidative damage, was significant. Similarly, changes in hematological parameters were noted beyond 21 days of refrigerated storage, even with standard preservation protocols (Parimalbhai Lad et al., 2022). The electrical impedance response, as a marker of cellular integrity, depicted in Figure 3, shows temporal changes in electrical impedance. A significant reduction in the impedance magnitude was observed across all frequency bands, especially during the initial storage period (Days 0–7), after which it plateaued at Day 35. This phenomenon is consistent with the morphological changes that are supposed to occur at an early stage of erythrocyte and leukocyte, when membrane disruption and intracellular electrolyte leaking modify the dielectric properties of blood (Man et al., 2021).

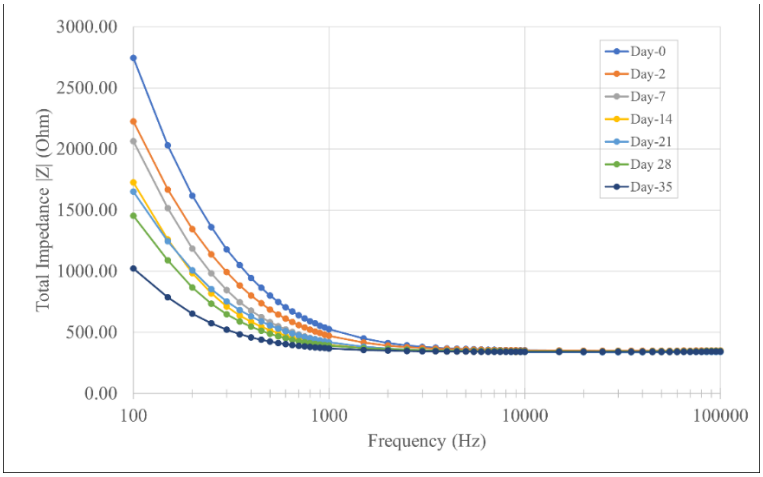

Figure 3. Total Electrical Impedance at Various Storage Durations

The slight decrease in impedance magnitude over time can be attributed to a combination of pore membrane degradation, ion leakage, and decreased cytoplasmic resistance due to energy source (ATP) depletion and oxidative stress in red blood cells (RBCs). These alterations have been termed the “storage lesion,” a collection of metabolic and mechanical changes that occur during cold storage. Previous work has shown that oxidative stress decreases deformability by cross-linking spectrin and actin filaments within the RBC cytoskeleton, a process that also modulates dielectric properties, as measured by impedance cytometry. With loss of membrane integrity, intracellular and extracellular ion concentrations are disturbed, leading to an increase in the resistive components of the impedance spectrum. The rightward and upward shift in Nyquist plots observed in this study further supports this phenomenon. This is consistent with the results of Mei et al. (2018), which indicated that comparable spectral shifts were associated with cell membrane breakage and electrolyte leakage in aged red blood cells.

The reduction in leukocytes and platelets suggests that erythrocytes are not the only cells to lose cell integrity. Leukocytes are known to be highly sensitive to cold storage, apoptosis, vacuolization, and lysis, with these events among the first to occur during preservation. These recent findings show that impedance was strongly associated with erythrocyte deformability and hemolysis in stored blood, confirming that electrical measurements reflect cumulative structural degradation rather than single-cell death events. Our finding of a relatively constant RBC count despite increasing impedance in our samples supports this interpretation. Structural damage can result without complete lysis, highlighting the fact that sublytic damage to cells can be quantified using EIS, which is invisible to traditional hematological assays. The Nyquist plot, as shown in Figure 4, showed a consistent rightward and upward shift in the impedance arcs over time, indicating increases in both the real and imaginary components. This shift points to increased intracellular resistance and altered membrane capacitance due to cell deterioration. These results agree with studies reporting higher impedance in aged or

damaged cells due to increased cytoplasmic resistance and altered cell morphology (Ivanov & Paarvanova, 2016; Li et al., 2016; Nguyen et al., 2017).

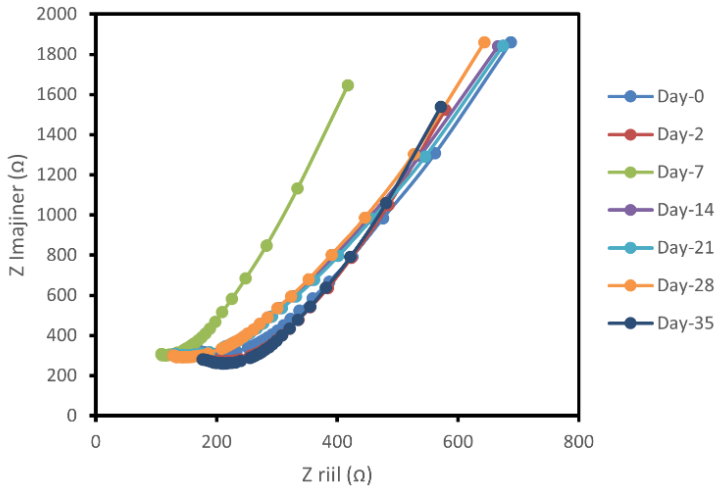

Figure 4. Nyquist Plot of Electrical Impedance at Various storage duration

The results of the investigation indicated a similar high decreasing trend in the electric impedance, which was more evident in the Nyquist plots. This result demonstrated that spectrin denaturation and membrane breakdown contribute to changes in dielectric properties measurable by impedance spectroscopy. Additionally, Nyquist plot shifts are indicative of morphological alterations and electrolyte imbalances in biological cells, further validating the findings (Mei et al., 2018). These findings will concern trends in blood viscosity and their hemorheological consequences. In contrast to impedance and cell count, blood viscosity (Figure 5) exhibited little change during the first 30 days of storage and increased slightly between Days 30 and 35. This trend suggests that blood rheology remains relatively stable under CPDA-preserved cold storage for approximately four weeks. The increase in viscosity at the end of the stage may be due to RBC aggregation, membrane stiffening, or microclot formation, and should thereby decrease flow resistance (Liao et al., 2019; Zhang et al., 2018).

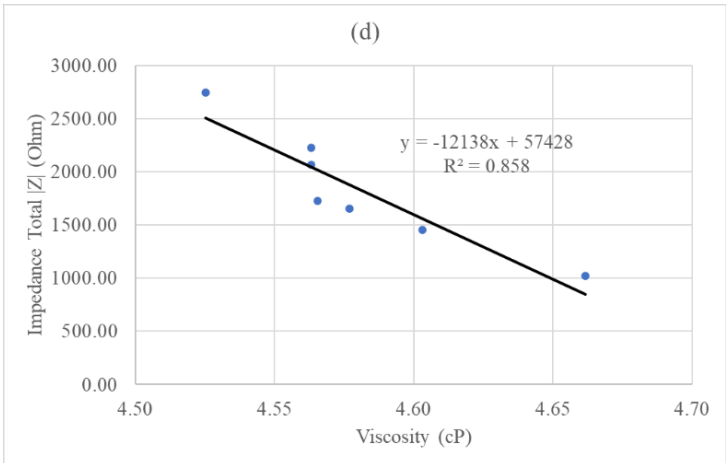

Figure 5. Whole blood viscosity with storage durations

Viscosity differences were minimal, but their effect on impedance implies a mild structural change in the components of the blood. This result reinforces the hypothesis that impedance spectroscopy is more sensitive to early-stage biophysical alterations than bulk viscosity measurements. Blood viscosity remained relatively stable for the first 30 days and increased only slightly towards the end of the storage period. This result aligns with the observations of Voiculescu et al. (2021), which indicated that viscosity is less susceptible to rapid changes unless there is significant protein aggregation or erythrocyte clumping. It is also established that mild alterations in the environment could have a retardant impact on viscosity, mainly because of modifications in the concentration of plasmatic proteins (Kwag et al., 2023).

Viscosity changes in a sluggish manner. Blood viscosity was nearly stable for the first 30 days, then slightly increased in the last week. This is the first study to demonstrate that the viscoelastic properties of blood stored under CPDA anticoagulant conditions, which initially preserve the rheological stability of stored blood, deteriorate after storage, in line with the findings of Voiculescu et al. (2021) and Kwag et al. (2023). The study revealed that plasma protein concentration and RBC aggregation cause late-stage increases in viscosity. The small rise after Day 30 is probably attributable to impaired deformability, microclotting, or membrane stiffening induced by protein oxidation and phospholipid rearrangement. These changes impair flow dynamics, leading to elevated resistance measurable by viscometry. Nevertheless, the subtle viscosity variation compared to the pronounced impedance shift highlights that EIS is more sensitive to early, microscopic alterations than bulk rheological measurements.

This is the first study to show that impedance is a reliable, non-invasive technique for detecting early declines in blood quality. Increased viscosity is preceded by impedance changes associated with internal damage, as apoptosis/hemolysis reduces cell populations. In addition to that, if one combines these three quantities, cell count, impedance, and viscosity, to obtain the full physiological picture of the blood state (Note Day 0-7:Quite a steep impedance drop with a slight viscosity increase and a sharp leukocyte drop). Day 14-28: Declining cell counts, stabilizing impedance, stable viscosity. D-30-35: Impedance plateau small, viscosity small decrease, nadir leukocytes, and platelets. These two trends indicate a two-phase degradation: a premature, rapid cell death and a late, slower rheological decay. These degradation curves also matter for transfusion and are discussed in Medicine: Clinical and Technological Implications. Although hematology analysis instruments quantify individual parameters (Hb or red blood cell count), impedance spectroscopy measures overall biophysical properties. Application of impedance-based techniques to blood storage monitoring could improve transfusion safety by identifying unsuitable units before morphological changes become evident. In addition, these results indicate that blood stored for up to 28 days in CPDA at 1-6°C remains physiologically viable, with only moderate disruption to viscosity. After Day 30, constant vigilance is recommended to prevent subclinical hemorheological alterations. When the triad of parameters is observed, it shows biphasic decay. Phase 1 (days 0-14) is an acute (biochemical) decline, with rapid declines in impedance and steep declines in leukocyte and platelet counts. Stage two (days 21-35) is characterized by stagnation in the impedance curve and a small increase in viscosity, suggesting a deceleration of morphologic and rheologic alterations. These two-phase processes link early oxidative stress to ATP loss, cellular mechanical fragility, and membrane lipid peroxidation. EIS, together with routine hematological parameters and viscosity, enables a comprehensive biochemical and mechanical evaluation of blood life.

From a clinical perspective, these results demonstrate the benefits of the EIS technique, based on real-time, non-contact monitoring of blood Re in blood transfusion services. Currently available conventional hematology analyzers primarily measure cellular features and cannot detect subtle subcellular or dielectric changes before over-hemolysis. Unlike standard UC, in which early phases of degradation cannot be detected, impedance analysis can correctly identify a compromised unit at an early stage of degradation, enabling timely discarding if necessary. The results also confirm that blood in CPDA remains stable when stored at a controlled temperature (1-6°C) for up to 28 days, and a degree of deterioration, such as this, suggests that Impedance Spectroscopy quality is monitored beyond the allowed window. Therefore, it is expected that the introduction of EIS into the routine screening of blood banks will provide further refinement in transfusion safety and cost reductions associated with extending storage beyond currently allowed periods, only when dielectric integrity indicates acceptable viability. Thus, the present study demonstrates, for the first time, that electrical impedance is a sensitive indicator of cellular and rheological changes associated with storage, even before significant changes in viscosity or severe hematological losses are observed. Its use in systems for blood quality management could enable early detection of degradation, support traditional tests, and add value to precision monitoring in blood transfusion.

4. CONCLUSION

The present study finds a strong correlation among electrical impedance, blood viscosity, and cellular degradation in whole blood stored for 35 days in a CPDA anticoagulant solution. Electrical impedance values gradually decline over time, indicating the biochemical and structural degradation of blood elements, especially erythrocytes, leukocytes, and platelets. By contrast, blood viscosity was almost unaffected for the first 30 days, with only a slight reduction at the end of the storage period, suggesting that the major rheological characteristics were well preserved under controlled conditions. The results indicate that EIS can be a potent, non-destructive method for evaluating the quality of stored

blood, in addition to conventional hematological analyses. The data also suggests that, even in the absence of discernible changes in viscosity, membrane integrity and intracellular content are considerably altered and can be observed in impedance spectra. These findings support the idea that blood can be held under controlled conditions for up to 28 days and still be deemed acceptable in physiological quality, and that monitoring after this point is required, even if it was not previously required. The addition of an impedance-based evaluation to the traditional protocols in a blood bank could enhance the safety and effectiveness of transfusion by enabling more timely detection of units approaching the limits of clinical use. A comparative study with other anticoagulants, a more detailed biochemical analysis of plasma constituents, and an in vivo investigation to confirm the presented results in a clinical setting are warranted and may be envisaged for future studies.

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CONFLICT OF INTEREST
Researchers confirm that they have nothing to declare regarding the paper's publication.

AUTHOR CONTRIBUTION
Viranita Qurotul Aini: Data collection, writing original draft. Chomsin Sulistya Widodo: Conceptualization, Methodology, Software, Writing, Reviewing, and Editing; Ekowati Retnaningtyas: Visualization, Investigation; Joel Rey U. Acob: Reviewing and Editing; Unggul Pundjung Juswono: Supervision, Validation.

DATA AVAILABILITY
The authors ensure the article contains data supporting their findings.

DECLARATION OF GENERATIVE AI
The authors declare that during the preparation of this work, the author(s) used Grammarly to proofread. After utilizing this software/service, the author(s) have reviewed and edited the material as they deemed necessary, and the author(s) are responsible for the content of the article.

ETHICS
Not applicable.

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