Polish Journal of Medical Physics and Engineering
The Journal of Polish Society of Medical Physics
ISSN 1898-0309, doi: 10.2478/pjmpe-2023-0004

March 2023
Vol 29, Issue 1

Scientific Paper

The Changes of Elongation Index of erythrocytes caused by storage of blood at low temperature

Iwona DYLONG [1,DEF], Paweł DOLIBOG [1,ABF], Tomasz MULARZ [2,B], Jacek MŁYNARSKI [1,BE], Sławomir Grzegorczyn [1,ACDEF]

[1]*Department of Biophysics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Poland*
[2]*Regional Blood Center and Blood Treatment in Katowice, Poland*
[*]*Corresponding author: Dr. Jacek Młynarski; jmlynarski@sum.edu.pl*

(received 23 September 2022; revised 6 December 2022 and 12 January 2023; accepted 17 January 2023)

Abstract

Introduction: An important parameter characterizing the ability of erythrocytes to deform depending on the blood flow conditions is the Elongation Index (EI), and it is a parameter defined by the shape of the erythrocyte obtained as a diffraction pattern of erythrocytes at different values of shear stresses.

Material and methods: EI measurements at different shear stress were performed by Laser-assisted Optical Rotational Cell Analyzer (LORRCA) for erythrocytes derived from Tissue Bank in Katowice. Measurements were performed immediately after receiving them from Tissue Bank and after 2, 9, and 28 days of storage of samples at the temperature of 4°C in solution with the anticoagulant.

Results: An increase in the erythrocytes Elongation Index in the first 9 days of storing samples at low temperatures was observed in the entire range of applied shear stresses. This indicates an increase in the elasticity of erythrocytes during short-term storage at 4°C. In turn, on the 28th day of erythrocyte storage, a significant decrease in the Elongation Index for shear stresses greater than 1 Pa was observed, which indicates the stiffening of the erythrocyte membrane structure, reducing their elasticity. The relative decrease in the Elongation Index of erythrocytes stored for 28 days compared to erythrocytes measured at the beginning was similar and slightly greater than 30% for shear stresses greater than 3 Pa. For shear stresses lower than 3 Pa, the relative change in elongation index was smaller than for shear stresses greater than 3 Pa and increased with the increase in shear stress.

Conclusions: The elongation index of erythrocytes stored in the anticoagulant solution at 4°C, initially increases in the entire range of applied shear stresses in the first few days from the moment of blood collection and preparation at the Tissue Bank, and then decreases, but on the ninth day of storage the elongation index is still higher than for blood immediately after collection.

Keywords: shear stress; erythrocyte; elongation index; storage of blood; LORRCA.

Introduction

Blood is a biological material used to replenish blood deficiencies in the body in emergency and planned life-saving interventions. It is a complex material, the main components of which are erythrocytes, used in the human body to carry oxygen and carbon dioxide. Therefore, the mechanical properties of erythrocytes play an important role in the functions of blood. Important properties of erythrocytes include their ability to deform, thanks to which they can move through blood vessels with a diameter smaller than the size of the erythrocyte itself[1,2] as well as the ability of erythrocytes to aggregate, which affects the conditions of blood flow in blood vessels.[2] These properties of erythrocytes have been intensively studied for their potential change due to various health conditions.[3,4]

The collection of blood by Tissue Banks and its further use in medicine has been constantly improved for many years.[1] The developed technologies of blood processing allow for its storage for a long period of time[1] and its subsequent use. Therefore, the question arises whether the process of collecting and processing blood in the Tissue Bank and then its storage at a sufficiently low temperature affects the properties of erythrocytes. Erythrocytes stored in blood banks are subject to faster ageing processes than in their natural environment. 120 days is the average lifetime of red blood cells in the body.[5] During storage, erythrocytes undergo many metabolic, biochemical and

© 2023 Authors
This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Authors' contribution:
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article.

molecular processes.[6,7] One of the most important parameters influencing the quality of erythrocytes is the concentration of ATP. During the storage of erythrocytes, it decreases over time, which leads to changes in the shape of the erythrocytes, loss of membrane lipids and a reduction in cell deformability.[8,9] In the Blood Banks erythrocytes are stored under refrigeration (at temperatures between 2 and 6ºC) for a maximum of 42 days as cellular components of red blood cells concentrate.[1,5] For this, the donor's blood is collected into a container with a suitable preservative and the additive solution. Then it is subjected to centrifugation and the buffy coat and plasma are separated. The storage time of blood products depends on the composition of the fluid containing nutrients and prevents blood clots. Up to the 14th day after blood collection and its proper preservation, erythrocytes can be treated as 'fresh',[10-13] while after the 15th day after blood collection, erythrocytes should be treated as 'old'.[13-15] What is mainly changed in erythrocytes during their storage are changes in erythrocytes membrane causing changes in the mechanical properties of erythrocytes,[1] which is important for the erythrocyte flow through the small vessels of the blood system.

An important parameter characterizing the ability of erythrocytes to deform depending on the blood flow conditions is the Elongation Index. It is a parameter defined by the erythrocyte shape, i.e. the radii of the long and short axes of the ellipse obtained as a diffraction pattern of erythrocytes at different values of shear stresses. One of the instruments used in this type of research is the Laser-assisted Optical Rotational Red Cell Analyser LORRCA.[3,16,17] It is an instrument that uses laser light with a wavelength of 655 nm, illuminating erythrocytes suspended in a buffer solution and rotating in an appropriate measuring cup with determined shear stresses.[18] The changes that occur in erythrocytes during their storage can be determined using rheological parameters. One of them is the elongation index (EI), which allows the determination of erythrocyte deformability. The curves of the dependence of the Elongation Index on applied shear stresses allow to obtain a dynamic image characteristic for changes in the size of erythrocytes, caused by external conditions. Studies on erythrocytes from individuals of various pathological conditions have shown changes in the Elongation Index in comparison with the elongation index of the erythrocytes from healthy people.[2,3]

Whole blood viscosity changes nonlinearly with the change of the flow velocity – its value decreases with the increase in velocity and vice-versa.[19-21] The changes in blood viscosity observed at high velocities flow depend on the elongation of erythrocytes.[4] Erythrocyte deformability disorders include such factors as: sodium and calcium concentration, oxidation, pH, osmolarity or temperature.[4,21] The shape of erythrocytes changes during their storage, which causes rheological changes, an increase in blood viscosity as well as a slower flow through the capillaries.[5] The correct shape and function of the erythrocytes can be restored using the rejuvenation procedure used in transfusion medicine. Due to the price and short shelf life, this procedure is rarely used.[9]

The question arises whether the storage of blood samples after their preparation in the Tissue Bank at low temperatures (4°C) has an effect on the deformability of erythrocytes. For this reason, blood from the Tissue Bank in Katowice, obtained from young and healthy people, was measured. Measurements of the Elongation Index using the LORRCA instrument under various dynamic conditions allow to answer this question.

Materials and methods

Blood samples from the Tissue Bank in Katowice (Regional Blood Center and Blood Treatment in Katowice) were used to measure the erythrocyte elongation index at different shear stresses. The Elongation Index (EI) is calculated as[18]

$$EI = \frac{A-B}{A+B} \qquad \text{Eq. 1}$$

where A and B are respectively the major and minor radius of the semi-axis of the ellipse, obtained from the diffraction spectrum of erythrocytes. For EI measurements, a LORRCA laser-optical rotating cell analyser was used, allowing the measurement of erythrocyte elongation indices in the shear stresses range from 0.3 to 100 Pa. Due to the fact that above 30 Pa the erythrocytes slightly change shape with increasing shear stress, the upper range of the shear stress was limited to 30 Pa.

Measurements were performed on 10 randomly selected blood samples of young and healthy people. The study group consisted of only men, with the mean age being 35.5 ± 4.5 years (range: 31-42). Among the samples, 5 were blood group A, 3 were blood group 0, and 2 were blood group B. The mean haematocrit was 45.0 ± 1.9% by volume (range: 42.9-48.2%). Blood samples were protected against permanent changes in blood with an anticoagulant (sodium edetate, K_2EDTA) and stored at 4°C.

For the Elongation Index measurements, 25µl of blood dissolved in polyvinylpyrrolidone buffer solution was used. The measurement temperature was stabilized and equal to 37.0 ± 0.2°C. The unused portion of the blood sample was stored at a temperature of 4°C. The measurement procedure was repeated on the same blood after 2, 9 and 28 days from the moment of blood collection. The stored blood, for a short time of collecting a small volume needed to measure the erythrocyte elongation index, was removed from the refrigerator, and then the unused part of the blood was placed again at 4°C. The time of taking the sample from the refrigerator was so short that it did not significantly change the temperature of the stored blood.

Table 1. Changes of the Elongation Index on the selected shear stress (0.3; 3; 30 Pa) for blood samples on days 0, 2, 9 and 28.

Shear Stress [Pa]	Elongation Index (mean value ± SD)				p ANOVA analysis of variance **, and * Bonferroni test
	Day 0	Day 2	Day 9	Day 28	
0.3	0.0581 ±0.0195	0.0620 ±0.0138	0.0844 ±0.0158	0.0608 ±0.0125	p(Main Effect)** = 0.0295 p(0 – 2)* = 1 p(0 – 9)* = 0.0159 p(0 – 28)* = 1 p(2 – 9)* = 0.0424 p(2 – 28)* = 1 p(9 – 28)* = 0.0206
3	0.3383 ±0.0355	0.3759 ±0.0098	0.3875 ±0.0243	0.2350 ±0.0149	p(Main Effect)** < 0.0001 p(0 – 2)* = 0.0504 p(0 – 9)* = 0.0040 p(0 – 28)* = < 0.0001 p(2 – 9)* = 1 p(2 – 28)* < 0.0001 p(9 – 28)* < 0.0001
30	0.5293 ±0.0633	0.5997 ±0.0225	0.5623 ±0.0405	0.3609 ±0.0265	p(Main Effect)** < 0.0001 p(0 – 2)* = 0.0229 p(0 – 9)* = 0.0229 p(0 – 28)* = < 0.0001 p(2 – 9)* = 0.5290 p(2 – 28)* < 0.0001 p(9 – 28)* < 0.0001

Statistical analysis

Statistical analysis was performed using the Statistica 13 program (TIBCO, Inc., USA). Quantitative variables were analyzed with the Shapiro–Wilk test to examine the normality of distribution. Within-group comparisons for measurements 0–28 days were performed by Annova's analysis of variance and a post-hoc Bonferoni test. The comparisons between pairs of variables 0-9 and 0-28 were performed with the t-Student test and Wilcoxon test. The values for $p < 0.05$ were taken as statistically significant.

Results and discussion

Tests carried out on blood from the Tissue Bank in Katowice allowed to determine erythrocyte elongation indices for various shear stresses. Different values of shear stresses are related to different flow conditions in the circulatory system. The increase in shear stresses leads to an increase in the erythrocyte elongation index, due to the greater deformation of erythrocytes, taking on a more elliptical shape. **Table 1** shows changes of the EI on the 0.3, 3 and 30 Pa shear stress for blood samples on days 0, 2, 9 and 28. The ANOVA analysis of variance showed that changes of EI index were statistically significant (main effect) for each the shear stress. **Figure 1** shows the dependence of the erythrocyte elongation index on the shear stress determined with the LORRCA device. The curves and measurement points in **Figure 1** show the dependence of the elongation index as a function of shear stress for the mean values of the same 10 blood samples, but at different storage times at a temperature of 4°C. The number 0 corresponds to the Elongation Index measured immediately after receiving the blood from the Tissue Bank, and the following numbers represent the number of days the samples were stored at 4°C.

As can be seen from **Figure 1**, the dependence of the Elongation Index on the shear stress is a non-linear one, with the greatest changes observed in the range of changes in small values of shear stresses. With an increase in shear stress, the erythrocytes change shape and become more elliptical (an increase of Elongation Index). As can be seen from the curves presented in **Figure 1**, in the first two days of blood storage, an increase in their elasticity was observed (an increase in the elongation index in the entire range of tested shear stresses by approximately 15%). Measurement on blood storage day 9 shows a decrease in the Elongation Index, especially in the range of higher shear stress values, greater than 3 Pa.

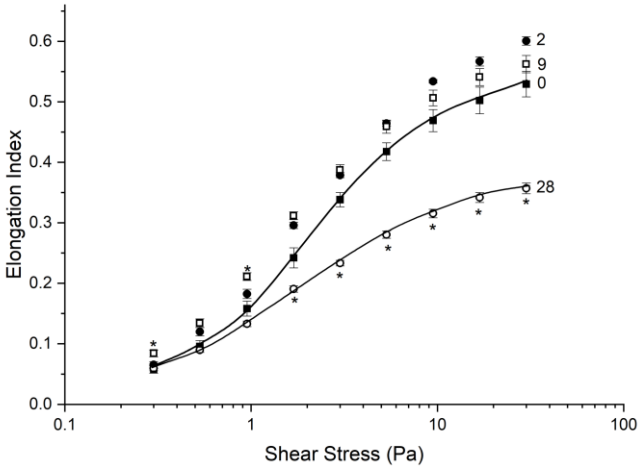


Figure 1. Dependence of the Elongation Index (Average ± Standard Error) on the shear stress for blood samples immediately after receiving the samples (0) and for samples at different storage times at 4°C, on days 2, 9 and 28, respectively, after receiving blood from the Tissue Bank. (Asterisk near point is for the case of statistical difference of this point from all others for this same Shear Stress, Repeated measures ANOVA, post hoc Bonferroni test, $p < 0.05$).

On the other hand, the measurement performed after 28 days of blood storage shows a significant reduction in the Elongation Index, the greater for greater the shear stresses determined during the measurement. **Figure 2** shows the dependence of the Elongation Index derivative after shear stress as a function of the shear stress calculated from the data presented in **Figure 2**.

The graphs in **Figure 2** relate to changes in the Elongation Index caused by changes in shear stress. Similarly to the Elongation Index itself, the changes in the Elongation Index with shear stresses (described by the derivative of the Elongation Index after shear stress) compared to the curve measured immediately after receiving the blood for low blood storage times take higher values, while after 28 days of storing blood at 4°C the derivative of the Index Elongation after shear stress is smaller than at the beginning (index 0). The greatest reduction in the derivative of the Elongation Index after shear stress is observed for small values of shear stresses. Increasing the shear stress causes that the difference between the initial curve (measurement start) and the end curve (measurement after 28 days of storage) is smaller and smaller. In order to capture changes in the Elongation Index related to the storage time of blood samples, in **Figure 3** the dependence of the relative change in the Elongation Index (compared to the values of the Elongation Index obtained on day zero) as a function of the shear stress is shown.

As results from **Figure 3**, on the second day of blood storage at 4°C, the elongation index increases by about 15% in the entire range of shear stresses, which may indicate an increase in the deformability of erythrocytes. After nine days of storing blood at 4°C, in the range of low shear stresses, less than 3 Pa, we observe a further increase in the relative change in the Elongation Index to about 30-40%. For shear stresses greater than 3 Pa, a decrease in the relative change in the Elongation Index is observed to values less than 10%. On the other hand, after 28 days of storing blood at 4°C, the relative changes in the Elongation Index are negative, which is related to much lower elongation indices for blood after long-term storage. As can be seen from the graph for 28 days of storage, presented in **Figure 3**, the greater the shear stress, the greater the relative change in the elongation index. For shear stresses greater than 3 Pa, the relative change in elongation index is approximately –30%. A significant decrease in the elongation index after 28 days of storing blood at 4°C indicates "stiffening" of the erythrocytes, which causes them to significantly lose their ability to deform. This can make it difficult for erythrocytes to pass through the capillaries after they are introduced into the body. The reason for such "stiffening" of erythrocytes may be changed in the erythrocyte membrane.

Loss of the rheological properties of erythrocytes during their storage results from a decrease in the concentration of ATP, 2,3-DPG (2,3-bisphosphoglyceric acid) and NO.[1] During the storage of red blood cell preparations, selected biochemical changes occur that affect erythrocytes and the environment in which they are located. For erythrocytes, these are: a decrease in the concentration of ATP and 2,3-DPG energy compounds, an increase in the affinity of haemoglobin for oxygen, oxidation of proteins related to the cell membrane, an increase in osmotic instability and haemolysis, the formation and removal of microbubbles and a decrease in the ability of erythrocytes to deform.[1,22] The appropriate concentration of ATP allows, among other things, to maintain the characteristic discoidal, biconcave shape of the erythrocyte and its deformability, which allows for the maintenance of viability and transport functions.[23]

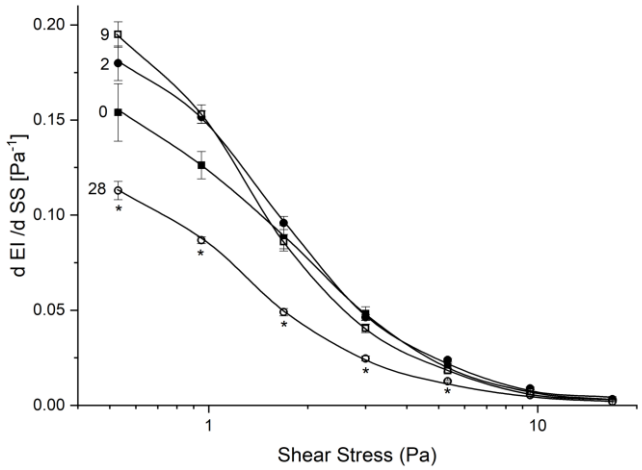


Figure 2. Dependence of the Elongation Index derivative after shear stress (dEI/dSS) as a function of shear stress, for blood samples immediately after receiving the samples from the Tissue Bank (0) and samples at different storage times at 2, 9 and 28 days after receiving the samples, respectively. (Asterisk near point is for the case of statistical difference of this point from all others for this same Shear Stress, Repeated measures ANOVA, post hoc Bonferroni test, $p < 0.05$).

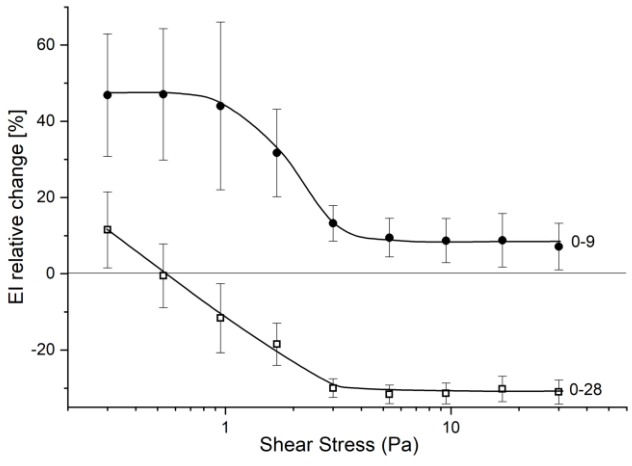


Figure 3. Relative change in Elongation Index as a function of shear stress for different storage times of blood samples at temperature 4°C. (In all cases of direct comparisons for each Shear Stress, the mean differences are $p < 0.01$ – t-Student test and Wilcoxon test).

The excess of the membrane, resulting from the biconcave shape of the erythrocyte, makes it possible for erythrocytes to deform and pass through the appropriate vessels of organs with a diameter often smaller than their own. As the erythrocytes mature and age, the loss of the cell membrane can be observed, which is also associated with the change in their shape during storage.[1] Erythrocytes do not have the mechanism of renewing the lost cell membrane, and lose less than 1/3 of the membrane due to storage.[24] The decrease in the deformation ability of erythrocytes may also result from defects of the cell membrane, which include membranopathies.[25] The high intracellular concentration of Ca2+ also reduces the deformability of the membrane.[26] Protein 4.2 is one of the main erythrocyte membrane proteins, and its role in erythrocyte deformability is to stabilize the ankyrin/AE1 complex and interaction with CD47 and membrane lipids.[25]

The graphs of the dependence of the Elongation index (**Figure 1**) and the derivative of the elongation index with respect to shear stress (**Figure 2**) as functions of shear stress for different storage times of blood samples show significant differences due to the shear stress in the range of medium and high shear stresses (**Figure 1**) and small and medium shear stresses (**Figure 2**), which can be treated as complementary information about the characteristics of the Elongation Index as a function of shear stress. To get a better picture of these types of characteristics, **Figure 4** shows the relationship between the product of the elongation index and its derivative with respect to shear stress (IE*dEI/dSS) as a function of the shear stress.

As shown in **Figure 4**, the dependence of the IE*dEI/dSS as a function of the shear stress shows a maximum, the height and position of which (coordinates of maximum) depend on the storage time of the samples. It follows that this type of plot could complement the most frequently used one, i.e. the elongation index as a function of shear stress, which would allow for a more detailed description of the processes accompanying the deformation of erythrocytes caused by shear stress. A characteristic feature of the graphs presented in **Figure 4** is the maximum, whose coordinates depend on the storage time of the blood samples. For this reason, **Figure 5** shows the relationship of the maximum coordinates, i.e. its amplitude (MIE * dIE/dSS) and the shear stress at which the maximum is observed (Mshear stress) as a function of the number of days of storing blood samples.

As it can be seen from **Figure 5**, the shear stress at which the maximum of the product of the elongation index and its derivative on shear stress decreases with the passage of storage time by about 40% on the 28th day of storage. The greatest changes in the position of this maximum are observed for small amounts of storage days. In turn, the amplitude of this maximum in the first days of storage increases, reaching a maximum at about day 6 of blood sample storage, and then decreases. The maximum amplitude after 28 days of blood storage is approximately 45% below the baseline value.

As we believe, this type of interpretation of the results of the Elongation Index as a function of shear stress may allow for a more precise differentiation and characterization of elongation indices for various shear stresses and processes accompanying erythrocyte deformation under various flow conditions, not only when examining the effect of blood storage but also in various pathological states.

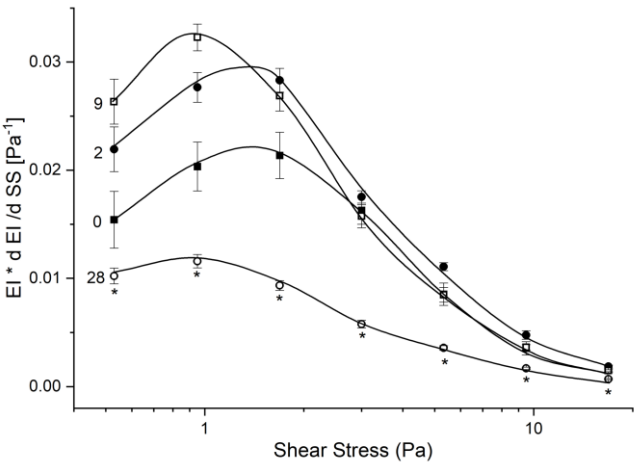


Figure 4. The dependence of product of elongation index and its derivative on shear stress as a function of shear stress for different days of storage of erythrocytes in 4°C. (Asterisk near point is for the case of statistical difference of this point from all others for this same Shear Stress, Repeated measures ANOVA, post hoc Bonferroni test, $p < 0.05$).

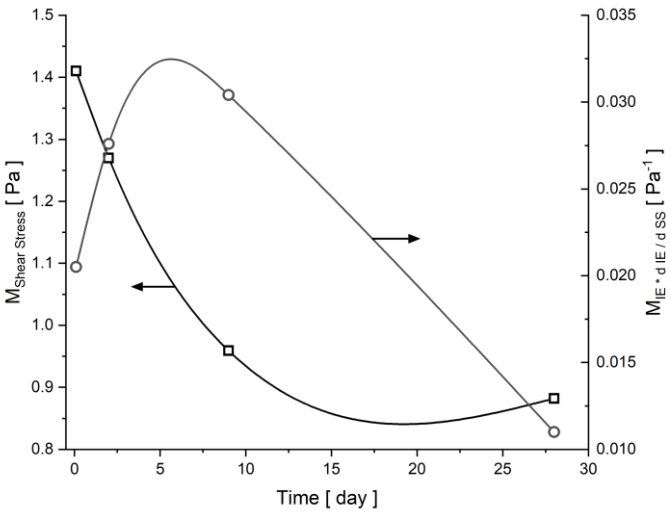


Figure 5. The shear stress at maximum (left axis) and product of elongation index and its derivative at maximum (right axis) as functions of days of storage of erythrocytes in 4°C.

Conclusions

1. The elongation index of erythrocytes stored in the anticoagulant solution at 4°C, initially increases in the entire range of applied shear stresses in the first few days from the moment of blood collection and preparation at the Tissue Bank, and then decreases, but on the ninth day of storage the elongation index is still higher than for blood immediately after collection.
2. On the 28th day of storing of erythrocytes in the anticoagulant solution at 4°C, significantly lower values of Erythrocyte Elongation Indexes were observed compared to the Erythrocyte Elongation Index at the initial time (day 0). For small shear stresses (less than 1 Pa), the differences are insignificant, while with the increase of the shear stress, the changes in the elongation index are increasing, and for shear stresses greater than 3 Pa, they are set at about 34%.
3. The derivatives of the elongation index with respect to shear stress show that it is lower after 28 days of storage compared to those measured immediately after blood collection. However, in contrast to the elongation index itself, the differences in the derivatives are greater the lower the shear stress. For shear stresses greater than 15 Pa, the differences between the derivatives elongation index on the shear for erythrocytes immediately after collection and after 28 days are statistically insignificant.
4. The introduction of the shear stress dependence of the elongation index multiplied by the derivative of the elongation index on shear stress allows to obtain additional information, allowing for better differentiation of the studied values in relation to the determined parameter.

Funding

This work was supported by the project of MUS (Medical University of Silesia): PCN-1-201/N/1/I.

References

1. Gmerek K, Fabijańska-Mitek J. Zmiany zachodzące w krwinkach czerwonych przechowywanych w bankach krwi. Postępy Nauk Med. 2016;XXIX(2):119-125. http://www.pnmedycznych.pl/wp-content/uploads/2016/03/pnm_2016_119-125.pdf
2. Kępińska-Szyszkowska M, Szyguła Z, Dąbrowski Z, Szarek M. Czynniki wpływające na zmiany właściwości reologicznych krwi - przegląd piśmiennictwa. J Lab Diagnostics. 2017;53(4):247-250. https://doi.org/10.5604/01.3001.0013.7992
3. Słowińska L, Monkos K. Kliniczne zastosowania laserowo-optycznego rotacyjnego analizatora krwinek czerwonych Clinical applications of the Laser-assisted Optical Rotational Cell Analyser LORCA. Ann Acad Medicae Silesiensis. 2010;64(3-4):42-47. https://annales.sum.edu.pl/Kliniczne-zastosowania-laserowo-optycznego-rotacyjnego-analizatora-krwinek-czerwonych,114862,0,2.html
4. Libionka A, Figiel W, Maga P, et al. Lepkość krwi w chorobach układu krążenia ze szczególnym uwzględnieniem kardiologicznego zespołu X Blood viscosity in cardiac syndrome X and other cardiovascular disorders. Cardiol J. 2005;12(7):465-470. https://journals.viamedica.pl/cardiology_journal/article/view/21881
5. Antosik A, Żbikowska HM. Koncentraty krwinek czerwonych w transfuzjologii. Kosmos. 2014;63(1(302)):13-23. http://kosmos.icm.edu.pl/PDF/2014/13.pdf
6. Scott KL, Lecak J, Acker JP. Biopreservation of red blood cells: past, present, and future. Transfus Med Rev. 2005;19(2):127-142. https://doi.org/10.1016/j.tmrv.2004.11.004
7. Łęjowska M, Zupańska B. Current opinions on some transfusion reactions. Acta Haematol Pol. 2009;40(2):407-423.

8. Hogman CF, Meryman HT. Storage parameters affecting red blood cell survival and function after transfusion. Transfus Med Rev. 1999;13(4):275-296. https://doi.org/10.1016/S0887-7963(99)80058-3
9. Almac E, Ince C. The impact of storage on red cell function in blood transfusion. Best Pract Res Clin Anaesthesiol. 2007;21(2):195-208. https://10.1016/j.bpa.2007.01.004
10. Steiner ME, Assmann SF, Levy JH, et al. Addressing the question of the effect of RBC storage on clinical outcomes: The Red Cell Storage Duration Study (RECESS) (Section 7). Transfus Apher Sci. 2010;43(1):107-116. https://doi.org/10.1016/j.transci.2010.05.014
11. Benjamin RJ. Evidence-based transfusion: young versus old blood as a case study. ISBT Sci Ser. 2009;4:323-328. https://doi.org/10.1111/j.1751-2824.2009.01262.x
12. Hébert PC, Chin-Yee I, Fergusson D, et al. A pilot trial evaluating the clinical effects of prolonged storage of red cells. Anesth Analg. 2005;100(5):1433-1438. https://doi.org/10.1213/01.ANE.0000148690.48803.27
13. Kiraly LN, Underwood S, Differding JA, Schreiber MA. Transfusion of aged packed red blood cells results in decreased tissue oxygenation in critically injured trauma patients. J Trauma - Inj Infect Crit Care. 2009;67(1):29-32. https://doi.org/10.1097/TA.0b013e3181af6a8c
14. Corwin HL. Blood transfusion: First, do no harm! Chest. 1999;116(5):1149-1150. https://doi.org/10.1378/chest.116.5.1149
15. Robinson SD, Janssen C, Fretz EB, et al. Red blood cell storage duration and mortality in patients undergoing percutaneous coronary intervention. Am Heart J. 2010;159(5):876-881. https://doi.org/10.1016/j.ahj.2010.02.018
16. Hardeman MR, Dobbe JG, Ince C. The Laser-assisted Optical Rotational Cell Analyzer (LORCA) as red blood cell aggregometer. Clin Hemorheol Microcirc. 2001;25(1):1—11. http://europepmc.org/abstract/MED/11790865
17. Hardeman MR, Goedhart PT, Dobbe JGG, Lettinga KP. Laser-assisted optical rotational cell analyser (L.O.R.C.A.); I. A new instrument for measurement of various structural hemorheological parameters. Clin Hemorheol Microcirc. 1994;14(4):605-618. https://doi.org/10.3233/CH-1994-14416
18. RR Mechatronics. User Manual Lorrca Laser-Assisted Optical Rotational Cell Analyzer Version 5.07 MRN-231-EN. https://manuals.rrmechatronics.com/Lorrca/PDF/LorrcaMaxsis_User_Manual.pdf
19. Dąbrowski Z. Fizjologia Krwi - Wybrane Zagadnienia. Wydawnictwo Naukowe PWN; 1998.
20. Traczyk WZ, Trzebski A. Fizjologia Człowieka z Elementami Fizjologii Stosowanej i Klinicznej. Wydawnictwo Lekarskie PZWL; 2004.
21. Başkurt OK. Pathophysiological Significance of Blood Rheology. Turkish J Med Sci. 2003;33(6):347-355. https://journals.tubitak.gov.tr/medical/vol33/iss6/2
22. Aubron C, Nichol A, Cooper DJ, Bellomo R. Age of red blood cells and transfusion in critically ill patients. Ann Intensive Care. 2013;3(2):1-11. https://doi.org/10.1186/2110-5820-3-2
23. Karger R, Lukow C, Kretschmer V. Deformability of red blood cells and correlation with atp content during storage as leukocyte-depleted whole blood. Transfus Med Hemotherapy. 2012;39(4):277-282. https://doi.org/10.1159/000339809
24. Hess JR. Measures of stored red blood cell quality. Vox Sang. 2014;107(1):1-9. https://doi.org/10.1111/vox.12130
25. Spychalska J. Membranopatie krwinek czerwonych – patogeneza, obraz kliniczny i diagnostyka. Hematologia. 2012;3(2):81-119. https://journals.viamedica.pl/hematology_in_clinical_practice/article/viewFile/19213/15117
26. Pasini EM, Lutz HU, Mann M, Thomas AW. Red blood cell (RBC) membrane proteomics - Part I: Proteomics and RBC physiology. J Proteomics. 2010;73(3):403-420. https://doi.org/10.1016/j.jprot.2009.06.005