

Deformability of Red Blood Cells and Correlation with ATP Content during Storage as Leukocyte-Depleted Whole Blood

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Keywords

ATP content · Deformability · Quality ·
Red cell storage · Storage lesion · In vivo survival ·
Whole blood

Summary

Background: Storage duration of red cells has been associated with increased morbidity and mortality following transfusion. This association has been attributed to the loss of deformability of stored red cells leading to deterioration of microvascular perfusion. ATP content is considered a critical determinant of the deformability of stored red cells. **Methods:** ATP content and deformability were determined after storage for up to 49 days in 40 leukocyte-depleted whole blood units. Red cell deformability was determined using a laser-assisted optical rotational cell analyzer (LORCA®) employing shear stress (SS) ranging from 0.3 to 30 Pa. Deformability was expressed as the elongation index (EI). EI was correlated with ATP content. **Results:** ATP content decreased from 3.5 to 1.7 $\mu\text{mol/g}$ hemoglobin. EI increased from 0.03 to 0.05 at an SS of 0.3 Pa, and decreased from 0.62 to 0.59 at an SS of 30 Pa. Correlation coefficient (r) of ATP vs. EI at 0.3 Pa ranged from -0.17 to $+0.15$ during storage. At 30 Pa, r ranged from -0.03 to $+0.45$. Correlation increased with storage irrespective of SS, and increased with SS irrespective of storage. **Conclusions:** ATP content is not a valid surrogate marker for red cell deformability and may not reflect in vivo survival of stored red cells.

Schlüsselwörter

ATP-Gehalt · Verformbarkeit · Qualität ·
Erythrozytenlagerung · Lagerungsschaden In-vivo-
Überleben · Vollblut

Zusammenfassung

Hintergrund: Die Hinweise mehrten sich, dass die Lagerungsdauer von Erythrozyten mit einer erhöhten Morbidität und Mortalität nach einer Transfusion assoziiert ist. Für diesen Zusammenhang werden die verminderte Flexibilität gelagerter Erythrozyten und eine damit einhergehende Verschlechterung der mikrovaskulären Perfusion verantwortlich gemacht. Der ATP-Gehalt wird als kritische Determinante der Verformbarkeit gelagerter Erythrozyten angesehen. **Methoden:** ATP-Gehalt und Erythrozytenverformbarkeit wurden über einen Lagerungszeitraum von 49 Tagen an 40 leukozytendepletierten Vollblutkonserven untersucht. Die Erythrozytenverformbarkeit wurde mit einem «laser-assisted-optical-rotational-cell-analyzer» (LORCA®) über Scherstress (SS) von 0,3 bis 30 Pa als sogenannter Elongationsindex (EI) gemessen. Der EI wurde mit dem ATP-Gehalt der Erythrozyten korreliert. **Ergebnisse:** Der ATP-Gehalt nahm von 3,5 $\mu\text{mol/g}$ Hämoglobin auf 1,7 $\mu\text{mol/g}$ Hämoglobin ab. Der EI stieg bei einem SS von 0,3 Pa von 0,03 auf 0,05, und nahm bei 30 Pa von 0,62 auf 0,59 ab. Der Korrelationskoeffizient r (ATP vs. EI) lag bei einem SS von 0,3 Pa zwischen $-0,17$ und $+0,15$ und bei 30 Pa zwischen $-0,03$ und $+0,45$. Die Korrelation nahm mit der Lagerungsdauer unabhängig vom SS und mit dem SS unabhängig von der Lagerungsdauer zu. **Schlussfolgerungen:** Der ATP-Gehalt ist kein valider Surrogatmarker für die Erythrozytenverformbarkeit und dürfte somit nicht das In-vivo-Überleben gelagerter Erythrozyten widerspiegeln.

Introduction

The measurement of the ATP content of red cells during storage has become a routine approach for evaluating the storage lesions in red cells. The main concern, however, is not the storage lesion per se, but the integrity and viability of the red cells in the recipient's circulation. Storage lesions, basically characterized by a couple of biochemical markers, including the ATP content of the red cells, represent only a surrogate marker for the red cell's ability to survive in the recipient's circulation. As early as 1947, Rapoport had shown that there was a close association between the ATP content of stored red cells and their viability [1]. A fall in ATP content is usually associated with a shape change from discs to spheres, a loss of membrane lipid, and an increase in cellular rigidity. That the loss in ATP is actually the cause for the increased rigidity of the red cells has been inferred from the observation that restoring the ATP content of the red cells before transfusion restores their normal flexibility, and greatly enhances viability. Maintaining red cell integrity and flexibility is an energy-consuming process [2, 3]. It is presumed that when ATP content is below a certain critical level, red cells lose their capacity to phosphorylate glucose and, therefore, to derive energy from it. However, the association between ATP content of the red cell and viability is only modest, suggesting that other processes independent of ATP content, like loss of membrane lipid, have a significant influence on red cell survival. Thus, the association between ATP content and red cell flexibility might not be as causal as sometimes presumed.

Several unfavorable effects of transfusion may be associated with storage lesions of the red cells in blood products [4]. There is increasing evidence of an association between the duration of storage and clinical outcome [5, 6], although a causal relationship has not yet been established [7–9]. Possible mechanisms explaining this association are the 2,3-diphosphoglycerate (2,3-DPG) deficiency of stored red cells, which results in a reduced capacity of the red cells to release oxygen to the tissues, and an increased rigidity of the red cells. This increased rigidity results in elevated adherence to endothelial cells [10], precluding them from readily passing small capillaries [11], and leading to increased vascular resistance [12]. Several randomized clinical trials are under way to clarify this issue [7]. Since red cell flexibility or deformability are major determinants of red cell viability [13], the aim of this study was to investigate, in addition to established biochemical markers, rheological properties of red cells at different shear rates in leukocyte-depleted whole blood over a 7-week storage period. This study is a post hoc analysis of a randomized experiment comparing different blood collection methods [14].

Material and Methods

Blood Collection

In this study, 2×20 leukocyte-depleted whole blood (LD-WB) units were investigated in a randomized controlled fashion [14]. In 1 group, an anti-coagulant (citrate phosphate dextrose adenine 1; CPDA-1) was continuously added to 500 ml donated whole blood via a roller pump attached to scales, using a new blood drawing device, the 'Automat for Blood Collection' (MacoPharma ABC[®], MacoPharma, Langen, Germany). In the comparison group blood was drawn using a conventional drawing device (Compomixer M2[®], Fresenius HemoCare, Bad Homburg, Germany); for this the collection bag was prefilled with 70 ml CPDA-1, and mixing was accomplished by automated tilting of the scales. All whole blood units were put on 4 °C cooling plates immediately after collection (Compo-cool[®], Fresenius HemoCare) and were leukocyte-depleted 2 h post donation at room temperature using an in-line LST2 filter (MacoPharma). The study was approved by our institutional review board. The data from the 2 groups were pooled for this post hoc analysis.

Laboratory Investigations

Red cell quality parameters, i.e. ATP, 2,3-DPG, free hemoglobin, potassium, glucose, lactate, pH, and parameters of plasma coagulation (prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen, coagulation factors V and VIII (FV and FVIII), antithrombin (AT), D-dimer, thrombin-AT complexes (TAT)) were determined on days 1, 7, 21, 35, 42, and 49 of storage. Samples were taken from all whole blood units after thorough but gentle mixing of the units. Except for ATP and 2,3-DPG measurements, parameters were determined in plasma/CPDA-1 supernatants after centrifugation of the samples. The ATP concentration was measured enzymatically using a commercial test kit (Rolf Greiner BioChemica, Flacht, Germany). Details of the different methods are reported elsewhere [14].

Red cell deformability was analyzed using a laser-assisted optical rotational cell analyzer (LORCA[®]) [15–17]. In brief, samples of the whole blood units were diluted 1:200 in polyvinylpyrrolidone to generate a Newtonian fluid. The suspensions were then subjected to shear stress (SS), which was increased in 9 logarithmic steps from 0.3 to 30 Pa, representing shear rates of 10–1,000/s. This led to a change of the typical biconcave to an ellipsoid shape of the red cells, which could be visualized and quantified by means of the laser diffraction pattern. The elongation index (EI) was calculated as $EI = (A - B) / (A + B)$, where A represents the longer and B the shorter axis of the ellipsoid red blood cell. The EI is thought to be proportional to the deformability of the cells. To measure the aggregability, undiluted original samples were preincubated at 37 °C with air to obtain a hemoglobin (Hb)-O₂ saturation of 99–100%. A shear rate of 500/s was then applied to the samples to achieve a complete disaggregation of red cells. Subsequently, shear force was stopped and the extent and kinetics of the spontaneous rouleaux formation of the red cells were measured by detecting the backscattered laser light. An aggregation index was calculated that describes the overall aggregation behavior of the cells. A higher aggregation index indicates higher aggregability, whereas $t_{1/2}$ (the time elapsing until peak intensity of the light signal is reduced by half) represents the velocity of the aggregation. The amplitude between maximum and minimum light intensity reflects the extent of the aggregation quantified in arbitrary units (au) [15, 16].

Statistical Analysis

Pearson's correlation coefficient r was calculated for the correlation analyses [18]. Regression analyses were also performed to study the influence of covariates other than ATP content on deformability. All statistical tests were 2-sided. p values < 0.05 were considered statistically significant.

Results

Donors and LD-WB Units

Donor characteristics and baseline blood product data are depicted in table 1. All tested units contained less than 1×10^6 leukocytes per unit. Red cell and plasma quality parameters during storage are listed in table 2. Mean ATP content decreased from 3.5 $\mu\text{mol/g}$ Hb on day 1 to 1.7 $\mu\text{mol/g}$ Hb on day 49. A pronounced fall in ATP content was only observed from day 21 onwards. 2,3-DPG decreased during the first 2 weeks of storage to trough levels. These changes, as well as

Table 1. Donor demographics and blood product data (n = 40)

Donors	
Sex, male/female	20/20
Blood group, A/O	20/20
Mean predonation Hb, mg/l (SD)	149 (12.0)
Median PT, % (range)	100 (91–100)
Mean aPTT, s (SD)	30.8 (3.4)
Mean fibrinogen, g/l (SD)	2.9 (0.4)
LD-WB units	
Mean net volume, ml (SD)	525 (8.1)
Mean Hb content, g/unit (SD)	66.7 (6.6)
Median leukocytes, 10^5 /unit (range)	0.66 (0.00–1.61)

Hb = Hemoglobin, PT = prothrombin time, aPTT = activated partial thromboplastin time, LD-WB = leukocyte-depleted whole blood.

hemolysis rate, potassium leak, glucose consumption and lactate production were similar to the changes usually observed during red cell storage. Except for FVIII activity, which fell to nearly a third of its initial value, the coagulation profile of the plasma supernatant showed only slight changes.

Red Blood Cell Deformability and Aggregation (Table 3)

Mean EI at an SS of 0.3 Pa increased from 0.03 on day 1 to 0.06 on day 49 ($p < 0.0001$). At higher SSs, EI consistently decreased during storage, e.g. at an SS of 30 Pa from 0.62 on day 1 to 0.59 on day 49 ($p < 0.0001$). The magnitude of this decrease (ΔEI) was comparable for an SS of 1.7 Pa and above. A trend towards an increase of deformability during storage was only seen at the low SS of 0.3 Pa. All aggregation parameters consistently showed a continuous decrease in aggregability, i.e. a tendency to rouleaux formation.

Correlation Analyses (Table 4)

Pearson's correlation coefficients (r) of ATP vs. EI at 0.3 Pa SS ranged from -0.17 to $+0.15$ during storage. At 30 Pa SS, they ranged from -0.03 to $+0.45$. In general, r increased with storage irrespective of shear rates, and with higher shear rates irrespective of storage. When the analysis was not stratified by day of storage, r was -0.26 at 0.3 Pa SS and $+0.75$ at 30 Pa SS. Thus, time course had a significant influence of the relationship between ATP content and deformability, particularly at high SS, suggesting a typical example of a spurious correla-

Table 2. LD-WB quality parameters^a

	Days of storage					
	1	7	21	35	42	49
<i>Red cell storage parameters</i>						
ATP, $\mu\text{mol/g}$ Hb	3.5 (0.4)	3.8 (0.4)	3.5 (0.4)	2.7 (0.4)	2.3 (0.4)	1.7 (0.4)
2,3-DPG, $\mu\text{mol/g}$ Hb	16.3 (17.8)	8.0 (2.8)	2.2 (1.3)	1.3 (0.9)	1.4 (1.0)	0.9 (0.6)
K^+ , mmol/unit	1.4 (0.11)	3.8 (0.38)	6.5 (0.59)	8.5 (0.59)	9.2 (0.72)	10.0 (0.74)
Free hemoglobin, g/unit	0.14 (0.08)	0.18 (0.08)	0.28 (0.11)	0.35 (0.11)	0.39 (0.12)	0.48 (0.15)
Hemolysis rate, %	0.13 (0.07)	0.18 (0.08)	0.27 (0.10)	0.33 (0.11)	0.37 (0.11)	0.46 (0.14)
pH (22 °C)	7.3 (0.11)	7.2 (0.08)	7.0 (0.08)	6.8 (0.06)	6.8 (0.06)	6.7 (0.05)
Glucose, mg/dl	429 (20.1)	386 (19.1)	307 (31.7)	262 (30.0)	237 (31.2)	217 (34.5)
Lactate, mmol/l	2.9 (0.75)	7.3 (0.79)	15.3 (1.63)	20.1 (2.13)	22.2 (2.26)	23.6 (2.78)
<i>Plasma coagulation parameters</i>						
PT, %	90 (8.1)	89 (10.6)	81 (8.7)	71 (10.7)	68 (12.6)	66 (14.2)
aPTT, s	36.4 (4.1)	40.2 (4.9)	40.9 (5.7)	41.7 (6.2)	41.6 (6.5)	41.8 (7.1)
TT, s	21.1 (1.47)	21.4 (1.44)	21.6 (2.20)	21.8 (4.26)	22.3 (4.44)	22.8 (5.93)
Fibrinogen, g/l	2.4 (0.36)	2.4 (0.36)	2.4 (0.39)	2.4 (0.42)	2.4 (0.42)	2.5 (0.44)
FV, %	80 (9.6)	80 (9.2)	77 (10.4)	70 (8.6)	72 (8.6)	75 (8.6)
FVIII, %	89 (17.9)	56 (14.1)	51 (13.7)	47 (14.3)	46 (14.7)	37 (13.8)
AT, %	92 (10.1)	94 (10.0)	90 (9.6)	91 (10.3)	90 (9.5)	87 (9.8)
D-dimer, $\mu\text{g/l}$	206 (102)	213 (101)	199 (99)	229 (100)	237 (97)	239 (95)
TAT, $\mu\text{g/l}$	2.2 (< 2–7.1)	2.2 (< 2–8.9)	< 2.0 (< 2–6.9)	2.1 (< 2–7.9)	2.3 (< 2–11.0)	2.1 (< 2–10.2)

ATP = Adenine triphosphate, 2,3-DPG = 2,3-diphosphoglycerate, TT = thrombin time, FV and FVIII = coagulation factors V and VIII, AT = antithrombin, TAT = thrombin-AT complexes.

^aData given as mean (SD) except for TAT where median (minimum-maximum) are reported.

Table 3. LD-WB rheological properties^a

	Red blood cell deformability and aggregation						
	1	7	21	35	42	49	ΔEI*
EI at SS, Pa							
0.3	0.032 (0.016)	0.035 (0.012)	0.043 (0.011)	0.047 (0.019)	0.042 (0.016)	0.055 (0.031)	+0.023
0.5	0.064 (0.015)	0.063 (0.012)	0.063 (0.012)	0.056 (0.012)	0.053 (0.011)	0.053 (0.011)	-0.011
1.0	0.141 (0.021)	0.138 (0.017)	0.137 (0.018)	0.126 (0.019)	0.120 (0.017)	0.119 (0.016)	-0.022
1.7	0.247 (0.022)	0.242 (0.017)	0.241 (0.017)	0.227 (0.020)	0.221 (0.018)	0.217 (0.017)	-0.030
3.0	0.361 (0.019)	0.354 (0.014)	0.351 (0.015)	0.337 (0.017)	0.331 (0.015)	0.325 (0.016)	-0.036
5.3	0.458 (0.014)	0.452 (0.011)	0.448 (0.011)	0.436 (0.013)	0.429 (0.012)	0.421 (0.013)	-0.037
9.5	0.528 (0.011)	0.524 (0.009)	0.521 (0.008)	0.511 (0.009)	0.505 (0.009)	0.497 (0.010)	-0.031
16.9	0.582 (0.010)	0.578 (0.009)	0.574 (0.008)	0.563 (0.008)	0.560 (0.008)	0.552 (0.009)	-0.030
30.0	0.621 (0.008)	0.618 (0.008)	0.613 (0.007)	0.606 (0.007)	0.599 (0.008)	0.591 (0.008)	-0.030
Aggregation index	38.9 (10.4)	36.7 (8.2)	35.9 (7.0)	34.9 (6.7)	34.5 (6.5)	33.3 (6.6)	./.
Amplitude, au	17.0 (2.2)	16.3 (2.1)	13.6 (1.7)	12.7 (2.0)	13.0 (2.0)	12.6 (2.2)	./.
t _{1/2} , s	6.9 (3.9)	7.3 (3.4)	7.6 (2.9)	8.2 (3.6)	8.3 (3.2)	8.9 (3.4)	./.

EI = Elongation index, SS = shear stress.
^aData given as mean (SD).
* EI on day 1 minus EI on day 49.

Table 4. Correlations (Pearson's *r*) between ATP content and deformability (EI) at different SS

Days of storage	All days combined***	1	7	21	35	42	49
EI at SS, Pa							
0.3	-0.262	0.068	-0.016	-0.171	0.015	0.149	0.098
0.5	0.348	0.109	0.166	0.030	0.067	0.098	0.065
1.0	0.435	0.099	0.221	0.116	0.107	0.113	0.112
1.7	0.505	0.044	0.256	0.157	0.154	0.165	0.140
3.0	0.589	0.011	0.264	0.185	0.198	0.204	0.210
5.3	0.690	0.002	0.315	0.205	0.232	0.309	0.319**
9.5	0.734	-0.000	0.258	0.202	0.170	0.404*	0.419*
16.9	0.725	-0.007	0.213	0.116	0.097	0.407*	0.434*
30.0	0.753	-0.029	0.216	0.103	0.126	0.397**	0.454*

*p < 0.01, **p < 0.05, ***p < 0.0001 for all coefficients.

tion [18]. However, when we included the day of analysis as a variable into a regression model together with other covariates (study group, ATP content, 2,3-DPG, hemolysis rate, pH, glucose, PT, FVIII activity, and aggregation index), after backward stepwise elimination of covariates, ATP content was the only covariate apart from day of analysis that contributed significantly to the variability of the EI (data not shown), particularly at high SS (5.3 Pa and above). The percentage of variability of EI explained by ATP content was still rather low, e.g. only 20% at 30 Pa SS on day 49 ($r^2 = 0.206$).

Discussion

This is, to our knowledge, the first study formally analyzing the relationship between ATP content and deformability of stored red cells using state-of-the-art methodology. Our study demonstrates that a significant association between ATP con-

tent and deformability only becomes apparent at high SS and when ATP content has decreased to less than 2.5 $\mu\text{mol/g}$ Hb. However, most red cell concentrates nowadays have, at the end of their approved shelf life, ATP contents well above this threshold. Thus, there only seems to be an independent effect of ATP content on deformability at high SS, which is, however, most prominent only after 6 weeks of storage and only explains approximately 20% of the variability of deformability, at best. This statistical association is, therefore, of little clinical value. ATP content only slightly decreases during storage, and is too insensitive to indicate potentially relevant changes of red cell deformability. The ATP content of the red cells does not, therefore, reliably reflect red cell deformability at approved storage times. Similar results have been reported by others for LD red cell concentrates resuspended in saline adenine glucose mannitol (SAGM) [19].

The results of our study are in accordance with previous studies investigating deformability during storage. That red

cells lose their deformability during storage has been consistently shown by indirect methods using filtration techniques [20] or the LORCA system [21]. Only the time course and the magnitude of the loss of deformability have differed between the studies, most likely for methodological reasons. The tendency of red cells to aggregate, i.e. their tendency for rouleaux formation by responding to rouleaugenic proteins, like fibrinogen, has been shown to increase in vivo with ageing of the red cells [22] and in vitro with storage time [23]. This is in contrast to the findings of our study where red cells exhibited a decrease in aggregability with storage time, most probably because the loss in deformability prevented rouleaux formation of the red cells. Alternatively, a growing number of non-biconcave-shaped red cells during storage could also explain the decrease in aggregability [24]. The discrepancy of our results to the former in vivo study might be easily explained by the fact that the mechanisms of aging in vivo appear to be quite different from the changes in stored red cells in vitro. In the latter in vitro study, increased aggregability with storage was only observed when red cells were resuspended in a dextran/buffer solution or fresh allogeneic plasma, but not in autologous plasma. The authors concluded that the loss of fibrinogen of autologous plasma might have compensated the increase in aggregability, resulting in no change of aggregability in autologous plasma. Thus, according to the reasoning of the authors of this study, we should have observed an increase in aggregability since fibrinogen did not decrease during storage in our LD-WB units. In our view, this reasoning is very speculative and unsatisfying. We believe that methodological differences may explain the discrepant results of the 2 studies. Whereas in our study original whole blood samples were used, which were only re-oxygenated (see Material and Methods), in the other study, before investigating aggregability with an in-house method, the red cells were subjected to centrifugation and washing steps, which might have caused a number of artifacts.

Henkelman et al. [19] also used LORCA to study the aggregability and deformability in LD red cell concentrates in SAGM over a storage period of 7 weeks. In contrast to our study, aggregability did not drop steadily during storage, probably due to the low number of units investigated ($n = 10$), resulting in unstable estimates of the parameter values. Another explanation might be the difference in storage medium and hematocrit in comparison to our LD-WB units. Other studies have shown that the storage medium may have an influence on aggregability [25]. Furthermore, in addition to containing red cell membrane-stabilizing compounds, e.g. sorbitol or mannitol [26, 27], red cell concentrates lack a significant amount of plasma, which might also explain the observed differences.

In our study a consistent decrease in deformability during storage was found above an SS of 0.5 Pa, and most prominently at SSs found in the microcirculation, i.e. around 3–5 Pa [28]. In contrast, the data of Henkelman et al. [19] show an

inconsistent course of deformability at 3.9 Pa, and a steady decrease only at 50 Pa. This adds to the conflicting results observed by others [25, 29, 30]. Given that ATP only plays a small role in explaining the loss in deformability (see above), its decrease during storage might be attributed to the loss of Hb-bound nitric oxide, a variable that participates in controlling red cell deformation [31]. The discrepancies between the different studies might also be explained by the different storage media used for the red cells.

The results of our study are also in line with accumulating evidence that several changes other than a decrease in ATP content might play a bigger role in the deterioration of red cell function during storage. For example, the hemolysis occurring during storage has recently been proposed as a mechanism for endothelial injury and impaired vascular function [32]. Our data might also explain why studies investigating the association of ATP content with red cell viability have been conflicting [13].

Consequently, for state-of-the-art blood component preparations, ATP content measurement might imply an unsubstantiated sense of safety, resembling the fallible conclusions drawn from the investigations of surrogates or biomarkers in clinical research [33]. More sophisticated approaches, in particular including techniques that determine red cell deformability, are required to determine the in vivo quality of stored red cells since it is known that red cell deformability, i.e. the flexibility of the red cell membrane, is strongly related to normal red cell function and viability [13]. These methods, however, may need to be validated against the gold standard of red cell viability determination, i.e. radioactive ^{51}Cr labeling [1].

There is still the need to prove that the loss of red cell function and viability is of such an importance that it causes unfavorable clinical outcomes. Observational studies showing an association between red cell storage and clinical outcome are usually biased [8] and are, therefore, unsuitable for establishing a causal relationship. An animal model of hemorrhagic shock could not provide proof of a causal role of prolonged storage for survival [34]. Such studies can only forego clinical trials in humans. Several randomized clinical trials are currently under way to clarify this issue, and their results are eagerly awaited [7].

A limitation of the present study might be that LD-WB units preserved in CPDA-1 were investigated. In most developed countries, LD red cell concentrates resuspended in additive solutions are the state-of-the-art red cell products for allogeneic transfusion. However, the results of this study are still valuable for autologous transfusion and the many clinical settings, particularly in developing countries [35] where allogeneic whole blood is still mainly employed. Furthermore, the use of whole blood is gaining renewed interest in developed countries [36–38] because it has been shown that leukocyte depletion results in a considerably improved plasma quality in whole blood [39].

Conclusion

Red cell ATP content is not a valid surrogate marker for red cell deformability, irrespective of the SS to which the red cells are subjected. This holds true even though ATP content and deformability of red cells do change significantly during storage. Deformability increases significantly at the SS found in the microcirculation. However, because this change is not likely to be biologically significant its clinical relevance is questionable.

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Disclosure Statement

The authors declare that they have no conflicts of interest relevant to this manuscript.

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