Original Paper

Erythrocyte Sodium Sensitivity and Eryptosis in Chronic Hemodialysis Patients

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Key Words

End stage renal disease • Eryptosis • Glycocalyx • Oxidative stress • Reactive oxygen species • Salt blood test • Sodium buffer capacity • Erythrocyte death

Abstract

Background/Aims: In hemodialysis (HD) patients the endothelial and erythrocyte glycocalyx is impaired which in turn correlates with elevated erythrocyte sodium sensitivity (ESS). Additionally, apoptotic erythrocyte death (eryptosis), characterized by phosphatidylserine (PS) exposure on the cell surface, is increased in this population. We aimed to explore the relationship of ESS and eryptosis. **Methods:** Blood samples were collected from 11 healthy controls and 20 chronic HD patients before and after midweek HD. ESS was quantified by the salt blood test. PS-exposure, intracellular reactive oxygen species (ROS) of erythrocytes and reticulocytes were assessed by flow cytometry. **Results:** Compared to controls ESS was significantly higher in HD patients preHD and did not change during treatment. The percentage of eryptotic cells did not differ between controls and patients preHD. However, eryptosis decreased during HD. ESS and eryptosis were uncorrelated, while eryptosis was positively correlated with intracellular ROS and percent reticulocytes. **Conclusions:** Higher ESS levels in HD patients indicate a pathologic glycocalyx. ESS and eryptosis were not correlated. The decreased eryptosis postHD may possibly be related to dialytic uremic toxin removal, but is likely multifactorial. The relationship between eryptosis and intracellular ROS warrants further research.

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Introduction

The most prevalent comorbidity in patients with chronic kidney disease is hypertension [1]. Although dietary salt restriction and removal of sodium and water during hemodialysis (HD) lower blood pressure, patients with end-stage renal disease (ESRD) undergoing HD rarely have normal blood pressure [2, 3]. Furthermore, high salt intake is associated with higher mortality in HD patients [4, 5].

Sodium overload is known to damage the endothelial glycocalyx [6], a gel-like carbohydrate-rich biopolymer covering the luminal surface of blood vessels. This negatively charged surface layer is hundreds of nanometers thick and plays an important role in maintaining vascular homeostasis. It modulates interactions between blood cells and vessel wall, regulates the redox state, serves as a sodium buffer by selectively binding sodium ions, and is involved in mediating shear induced release of nitric oxide [7, 8]. Erythrocytes are likewise covered with a glycocalyx, preventing direct contact between the red blood cells (RBC) themselves and the vascular endothelium. The glycocalyx of erythrocytes “mirrors” specific properties of the endothelial glycocalyx. Thus, an impaired endothelial glycocalyx is reflected by an impaired erythrocyte glycocalyx and vice versa [9]. Recently, a biochemical assay, the salt blood test, was developed to quantitate the sodium buffering capacity of the glycocalyx of RBCs and thus indirectly the degree of glycocalyx integrity [10]. Because of an association between the capacity of the glycocalyx to buffer sodium and sodium sensitivity of blood pressure the read-out of the test was called erythrocyte sodium sensitivity (ESS) [11]. Of note, an impaired glycocalyx and shedding of major components of the glycocalyx have been observed in patients with chronic kidney failure in previous studies [12-14].

Another major complication in HD patients is anemia [1, 15]. The main reasons are limited erythropoiesis caused by insufficient renal production of erythropoietin (EPO), functional iron deficiency, an impaired bone marrow response to EPO, and a shortened RBC life span [16-18]. The decreased survival of RBCs may partially be explained by elevated premature apoptotic erythrocyte death, called eryptosis, in HD patients [19]. Hallmarks of eryptosis are cell shrinkage, cell membrane blebbing, cell membrane scrambling, and exposure of the aminophospholipid phosphatidylserine (PS) on the outer surface of the cell. Similar to apoptosis, eryptosis may be induced by activation of Ca^{2+} -permeable cation channels leading to elevated cytosolic calcium. Increase of intracellular Ca^{2+} leads to cell membrane scrambling, which is further enhanced by ceramide [20]. Signaling molecules that may be involved in the eryptosis pathway include the G protein subunit Gai2 [21], the mitogen- and stress-activated kinase MSK1/2 [22], and the cyclin-dependent kinase 4 (CDK4) [23]. PS-exposing RBCs are prone to bind to the surface of endothelial cells and macrophages and are thereafter rapidly cleared from circulation [24-26]. Accelerated eryptosis is observed in several clinical pathologies including diabetes, metabolic syndrome, sepsis, malignancy, hepatic and renal insufficiency [25, 27-29]. Additionally, it may be induced by various xenobiotics and endogenous substances [30-42]. Triggers of eryptosis that are particularly relevant in the HD population include uremic toxins, elevated phosphate concentrations, and oxidative stress [20, 28, 43]. In circulation RBCs are constantly undergoing normoxic and hypoxic cycling and continuously exposed to endogenous and exogenous sources of reactive oxygen species (ROS). Therefore, erythrocytes have an extensive antioxidant system rapidly neutralizing ROS to minimize their effect and resultant oxidative stress [44].

Nothing is currently known about the relationship between glycocalyx integrity and erythrocyte survival. Since both ESS and eryptosis relate to RBC surface properties we aimed to explore their relationship. Secondary, the acute impact of HD on ESS and eryptosis was further explored in this research.

Materials and Methods

Population and study design

We studied 20 chronic HD patients from two facilities of the Renal Research Institute (RRI) located in Manhattan, New York between December 2015 and February 2016. Eleven healthy individuals served as control group. All HD patients were on thrice weekly single pass HD using high-flux dialyzer and had arterio-venous fistulae as dialysis access. Bicarbonate buffered dialysate containing 137 mmol/L sodium and polysulfone membranes were used as standard of care. At a midweek treatment 6 mL of blood were drawn immediately before and after HD using heparinized blood collection tubes (BD Life Science, New Jersey).

Patients were excluded if they used systemic antibiotics in the 60 days preceding enrollment, systemic immunosuppressive medication within 3 preceding months, or received a blood transfusion within the preceding 4 months. Patients were further excluded if they had a systemic autoimmune or connective tissue disease, a hemoglobinopathy, or a history of solid organ or hematologic malignancy.

The study was approved by the New England Institutional Review Board and the Mount Sinai Beth Israel Institutional Review Board, and all procedures were performed in accordance with the declaration of Helsinki. All subjects provided written informed consent before participating in this study.

Salt blood test

The salt blood test was performed as described by Oberleithner and Wilhelmi [10]. In short, 4 mL of blood were transferred into plastic vials and centrifuged. Plasma and buffy coat were removed. Erythrocytes were washed twice in HEPES-buffered (4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid) saline containing 1% bovine serum albumin. Measurements were performed in triplicates. For a single measurement 75 μ L of 150 mmol/L sodium or 125 mmol/L sodium solution were mixed with 50 μ L of erythrocytes each to ensure a fixed hematocrit of 40%. The sodium solutions contained 3% dextran to trigger RBC aggregation and 150 mmol/L sodium or 125 mmol/L and 50 mmol/L sucrose to maintain constant osmolality. Hematocrit capillary tubes (SAFE-TEC Clinical Products, Pennsylvania) were filled with the suspensions and put on stands in upright position. Supernatant length was measured after 60 minutes. Smaller numbers of free negative charges around the erythrocyte lead to more formation of cell aggregates and a larger sedimentation velocity. Performing this sedimentation procedure the dimensionless ESS can be obtained by calculating the ratio of the supernatant lengths at 150 mmol/L and 125 mmol/L sodium solution ($ESS = L_{150}/L_{125}$).

Flow cytometry

The percentage of PS-exposing RBCs, as a measure of eryptosis, has been assessed using Annexin-V-Fluos (ROCHE, Germany). Annexin V binding is well defined and used to study apoptotic events in different cells, including erythrocytes. In brief, freshly isolated RBCs were washed with Ringer solution and incubated with fluorescence labeled annexin V. Reticulocyte count was determined using RETIC-Count™ (BD Biosciences, USA). Presence of intracellular ROS (iROS) was evaluated using Total ROS/Superoxide detection Kit (Enzo Life Sciences Inc., USA), which contains two dyes. The non-fluorescent, cell-permeable Oxidative Stress Detection Reagent reacts directly with a wide range of ROS, such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^\cdot$), and peroxynitrite ($ONOO^-$), yielding a fluorescent product indicative of ROS and reactive nitrogen species. The Superoxide Detection Reagent reacts specifically with superoxide ($O_2^{\cdot-}$). The result is the percentage (%) of cells containing ROS, which is indicative of oxidative stress. See Fig. 1 for more details. All measurements were performed in duplicates using flow cytometry with FACS Calibur™ (BD Biosciences, USA) within two hours after blood collection. Instrument settings were kept the same for all experiments.

Clinical and Laboratory Data

Laboratory measurements (Spectra Laboratories, New Jersey) were downloaded to the RRI data warehouse and extracted to the study database. Diabetes and congestive heart failure were defined using International Classification of Diseases codes, Ninth Revision (ICD-9). In HD patients the body mass index (BMI) was calculated using postHD weight.

Fig. 1. Representative plots of (A) Erythrocyte gating on FSC/SSC plot to exclude debris and white blood cells; (B) positioning of untreated Erythrocyte on FL1/FL2 plot; (C) Erythrocytes pre-treated with reactive oxygen species (ROS) inducing agent (Pyocyanin) and stained with cell-permeable ROS detection dye; the shift to the right along FL1 channel reflects cells with intracellular hydrogen peroxide, peroxynitrite, hydroxyl radicals; (D) Erythrocytes pretreated with Pyocyanin and stained with cell-permeable superoxide detection dye; shift up along FL2 reflects cells with intracellular superoxide; (E) Erythrocytes pretreated with Pyocyanin and stained with both dyes; the shift to the right and top reflects total intracellular ROS; (F) fresh Erythrocytes from healthy subject stained with both dyes; (G) fresh RBCs from HD subject stained with both dyes; plots (F) and (G) represent cells with naturally occurring intracellular ROS reported in the study as % of cells with elevated fluorescent signal compared to unstained cells.

Statistical analysis

Descriptive statistics comprised means ($\pm$ standard deviation) for continuous variables and percentages for categorical variables. Differences were analyzed by Student's *t* test, Mann-Whitney U test, or Wilcoxon's signed rank test as appropriate. Linear correlation between variables was assessed by calculating Pearson or Spearman correlation coefficient as appropriate. Results were considered significant if $p < 0.05$. All statistical analysis was performed using R 3.0.2 (Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

We examined 20 HD patients (age 28 - 76 years, 11 males, 11 white, 10 diabetic) and 11 healthy subjects (age 30 - 71 years, 5 males, 9 white). Detailed demographic and clinical characteristics of the 31 subjects included in this analysis are presented in Table 1. None of the control subjects and 9 HD patients were anemic defined as hemoglobin level below 11 g/dL. Compared to healthy subjects the dialysis patients had significantly lower hematocrit, serum sodium and calcium concentrations. Serum potassium and systolic blood pressure before as well as after the hemodialysis treatment were significantly higher. Diabetic HD patients were on average 12.6 years older than patients without diabetes (49.5 ± 10.0 years vs. 62.1 ± 8.2 years, $p = 0.01$).

Table 1. Demographic and clinical characteristics of the study population

	Healthy control (n=11)	Hemodialysis Patients (n=20)	p-value
Demographics			
Males (%)	5 (45.5%)	9 (45.0%)	
Age (years)	47.2 ± 11.6	55.8 ± 11.1	0.070
Whites (%)	9 (72.7%)	11 (55.0%)	
BMI (kg/m ²)	24.7 ± 4.1	30.7 ± 6.6	0.014
Height (cm ²)	169.1 ± 8.6	166.8 ± 9.2	0.509
Weight (kg)	72.1 ± 16.1	85.4 ± 20.1	0.060
Vintage (years)	n.a.	6.80 ± 5.86	
Comorbidities (%)			
Diabetes mellitus	0	50	
Congestive heart failure	0	10	
Laboratory parameters			
Hemoglobin (g/dL)	14.0 ± 1.6	11.3 ± 1.1	<0.001
Red blood cells (x10 ⁶ /μL)	4.82 ± 0.38	3.74 ± 0.48	<0.001
Hematocrit (%)	42.0 ± 4.0	34.7 ± 3.8	<0.001
Serum sodium (mmol/L)	141.0 ± 1.6	137.7 ± 2.4	<0.001
Serum calcium (mmol/L)	9.31 ± 0.40	8.46 ± 0.62	<0.001
Serum potassium (mmol/L)	0.33	5.06 ± 0.71	0.002
Creatinine (mg/dL)	0.82 ± 0.13	10.20 ± 2.48	<0.001
Systolic blood pressure (mmHg)	117 ± 10	preHD: 153 ± 22 postHD: 135 ± 19	
Diastolic blood pressure (mmHg)	76 ± 7	preHD: 84 ± 14 postHD: 72 ± 12	
Erythropoiesis stimulating agents	None	Mircera (n=11), Epogen (n=5), none (n=4)	
Antihypertensive Medication	None	Beta blocker (n=8), Calcium channel blocker (n=7), ACE inhibitor (n=4), Angiotensin II receptor blocker (n=3), none (n=5)	

Blood flow was 427 ± 37 mL/min, dialysate flow 669 ± 71 mL/min, treatment time 231 ± 25 min and equilibrated Kt/V 1.45 ± 0.17. Ultrafiltration volume was 2.24 ± 0.8 L and ultrafiltration rate 6.9 ± 2.0 mL/h/kg, with an interdialytic weight gain of 2.13 ± 0.91 kg.

Erythrocyte Sodium Sensitivity (ESS)

Four HD patients and no control subject showed an ESS value above 4.3, the reference for the healthy population (Fig. 2). Compared to controls ESS was significantly higher in HD patients before treatment (2.0 ± 0.5 vs. 3.9 ± 1.5, p < 0.001). Compared to preHD levels, postHD ESS was not materially different (3.7 ± 1.5, p = 0.18) (Fig. 3). Within groups, ESS was not correlated with any of the demographic or clinical parameters, with the exception of a negative correlation with BMI in dialysis patients (preHD: ρ = -0.52, p = 0.02; postHD: ρ = -0.56, p = 0.01). Upon analysis of this correlation in HD patients with and without diabetes separately, ESS correlated with BMI in non-diabetics only (diabetic: ρ = -0.39, p = 0.16; non-diabetic: ρ = -0.91, p = <0.001).

Measurements of Red Blood Cell Eryptosis, Reticulocytes and iROS

Levels of eryptosis were quantified by determining the percentage of annexin V binding erythrocytes, which was not significantly different between controls and HD patients preHD (0.48 ± 0.26% vs. 0.60 ± 0.42%, p = 0.36). Compared to preHD, postHD eryptosis decreased significantly (0.34 ± 0.13%, p = 0.004) (Fig. 4 A). Univariate correlation analysis revealed a positive association between PS-exposure before HD treatment and serum phosphate concentration (ρ = 0.37, p = 0.10). PostHD PS-exposure was negatively correlated with

Fig. 2. Erythrocyte sodium sensitivity (ESS) by subject. The horizontal line indicates the reference value determined by Oberleithner and Wilhemi [10]. Black bars mark individuals with ESS exceeding this reference value. C: control; HD: hemodialysis patients.

Fig. 3. (A) Erythrocyte sodium sensitivity (ESS) by group and (B) linear regression of preHD ESS with body mass index. *** ($p < 0.001$) indicates significant difference from control (Wilcoxon's signed rank test).

Fig. 4. (A) Eryptosis levels and (B) percentage of reticulocytes by group. (C) Linear regression between eryptosis levels and reticulocytes before HD treatment. * ($p < 0.05$) indicates significant difference from control (Mann-Whitney U test).

serum iron ($\rho = -0.48$, $p = 0.03$). An association between eryptosis levels and ESS could not be confirmed (preHD: $\rho = 0.10$, $p = 0.67$; postHD: $\rho = -0.03$, $p = 0.91$) (Fig. 5).

To examine if erythrocyte turnover was altered we assessed the percentage of reticulocytes, which was not significantly different between controls and HD patients before as well as after the HD treatment ($1.47 \pm 0.33\%$, $1.34 \pm 0.5\%$ and $1.29 \pm 0.54\%$, respectively) (Fig. 4 B). In HD patients the percentage of reticulocyte correlated positively with the % of PS-exposing RBCs before HD ($r = 0.43$, $p = 0.05$) (Fig. 4 C).

In addition, we assessed the role of oxidative stress by measuring the percentage of erythrocytes with iROS. There was no significant difference between controls and HD patients before as well as after the HD treatment ($42.6 \pm 13.5\%$, $36.5 \pm 11.0\%$, and $38.9 \pm 14.7\%$) (Fig. 6). Measurement examples are shown in Fig. 1. The % of RBCs with iROS was positively correlated with % of PS-exposing RBCs before dialysis ($r = 0.45$, $p = 0.05$) and % reticulocytes ($\rho = 0.47$, $p = 0.04$). ESS and iROS were not correlated ($\rho = 0.14$, $p = 0.53$).

Discussion

The present study revealed increased erythrocyte sodium sensitivity in chronic dialysis patients. However, a correlation between glycocalyx integrity measured by the salt blood test and red blood cell eryptosis was not found. Interestingly, a decrease of eryptotic RBCs during the dialysis treatment was noted, while ESS, the percentage of reticulocytes, and erythrocytes with iROS were not appreciably influenced by HD.

During senescence the glycocalyx of vascular endothelium and erythrocytes is gradually compromised [45, 46]. In dialysis patients this process is supposedly accelerated. An elevated ESS, which indicates a disturbed vascular glycocalyx, in the dialysis population was expected, given the fact that HD patients frequently suffer from vascular damage. Kliche *et al.* found that the RBC cell surface properties could be partially restored through the dialysis treatment, especially in those individuals with high serum sodium concentrations before the treatment [47]. It has been shown *in vitro* that sodium concentrations higher than 140 mmol/L impair the glycocalyx and increase adherence of erythrocytes to endothelial cells [48]. In our

Fig. 5. Correlation of annexin V binding red blood cells with clinical parameters.

Fig. 6. (A) Red blood cells (RBCs) containing intracellular reactive oxygen species (iROS) by group and linear regression of % of RBCs containing iROS with (B) % of cells binding Annexin V and (C) % reticulocytes before hemodialysis (HD) treatment.

population the ESS was not significantly altered through the HD treatment itself, possibly due to preHD serum sodium concentrations (137.7 ± 2.4 mmol/L) close to the prescribed dialysate sodium of 137 mmol/L. Of note, our patients were treated with conventional single pass HD. We hypothesize that the fact that Kliche et al. used post-dilutional hemodiafiltration (HDF) is relevant in that respect. Middle molecules, such as beta-2 microglobulin, and protein-bound uremic toxins, such as indoxyl sulphate, p-cresyl sulphate, and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), are poorly removed by conventional HD. HDF enhances their convective removal and is superior to HD in eliminating several small and larger uremic toxins [49, 50]. Of note, a complex pattern of >250 proteins is removed by HDF [51]. It is conceivable that some of these toxins may interact with the RBC glycocalyx and that their inferior removal may affect ESS. In addition, Kliche et al. recruited patients that were admitted to the hospital which points to an in general sicker population.

We found no significant association between ESS and blood pressure which is not surprising because anti-hypertensive treatment may mask blood pressure changes. Interestingly, a negative correlation between ESS and BMI was noted, meaning that patients with a low BMI were more likely to have an impaired glycocalyx. This finding is at variance with previous reports from healthy volunteers [52, 53]. However, it is tempting to speculate that this could be related to the paradoxical finding of improved survival of obese patients on dialysis [54, 55]. Inflammation is thought to be one of the potential causes of the positive relationship between BMI and survival in HD patients; a comparable relationship has also been observed in type II diabetes, congestive heart failure, coronary heart disease, stroke, rheumatoid arthritis, cancer, and dementia [56]. Of note, rapid glycocalyx degradation has been demonstrated under various systemic and local inflammatory conditions such as diabetes, atherosclerosis, and surgical ischemia/reperfusion injury and sepsis [12, 57]. It is intriguing to speculate that more pronounced inflammatory processes increased the ESS in patients with low BMI.

In the early days of HD mechanical destruction of erythrocytes was thought to contribute to anemia in these patients. Technical improvements of the dialysis equipment reduced the mechanical stress of the process itself on erythrocytes and erythrocyte lifetime increased [58]. In our population HD patients did not have a significantly higher percentage of eryptotic RBCs as determined by annexin V binding to PS-exposing erythrocytes. This is contrary to the recent findings of Abed *et al.* and Bonan *et al.* [19, 59]. The low level of eryptosis in our population is paralleled by higher levels of hemoglobin and a smaller age difference between controls and HD patients compared to the previous studies. Of note, eryptosis levels increase with age [60], possibly confounding previous findings. Nitric oxide (NO) is a potent inhibitor of eryptosis [61]. Peridialytic blood pressure drops are associated with high plasma concentrations of NO [62]. Noteworthy, in our study the systolic blood pressure decreased on average by 18 mmHg, hence an elevated production of NO in our population may contribute to the low level of PS-exposure. Uremic serum of ESRD patients promotes a pro-inflammatory CD14++/CD16+ monocyte phenotype with increased erythrophagocytosis, promoting an accelerated clearance of PS-exposing RBCs and thus suggesting an additional pathway for renal anemia, without necessarily increasing the number of eryptotic RBCs [59].

While Abed *et al.* found an increase of eryptotic RBCs after HD, in our study the percentage of phosphatidylserine exposing RBCs decreased during the dialysis treatment. It has been shown that a reduction of endotoxin levels in the dialysate can improve anemia by extending erythrocyte life span and promoting erythropoiesis [63]. In RRI facilities the use of endotoxin filters in the water rooms and an additional filter at each machine is standard of care, leading to endotoxin levels that are usually less than 0.3 EU/mL which is well below the 1 EU/mL standard of the Association for the Advancement of Medical Instrumentation (AAMI). This may be one explanation of these divergent findings, but the causes are likely multifactorial. In addition, we excluded patients with catheter as dialysis access. Patients with catheters are more prone to inflammatory processes, which in turn correlate positively with eryptosis [64, 65].

An intriguing question is what happens to the PS-exposing RBCs during the HD treatment. One possibility is an inhibition of PS-exposure. The application of erythropoietin is able to facilitate a reduction of annexin V binding both *in-vivo* and *in-vitro* [66]. Although there was no association between ESA treatment and reduction of eryptosis in our population, this indicates that a reversion may in principle be possible. One may also speculate that the removal of uremic toxins that are known to increase eryptosis such as vanadate [67], acrolein [68], indoxyl sulfate [69], and methylglyoxal [70] may reduce the number of eryptotic RBCs. Hyperphosphatemia is frequent in ESRD patients and it has been shown that high phosphorus levels induce eryptosis as well [28]. Correcting hyperphosphatemia during HD may thus reduce eryptosis. Of note, serum phosphate and annexin V binding were correlated before dialysis, although not significantly, probably due to the small sample size. Another explanation would be an enhanced clearance of PS-exposing cells during the HD treatment. It is known that chronic kidney disease leads to an accelerated clearance of circulating erythrocytes [18], with increased spleen size in dialysis patients compared to patients on peritoneal dialysis, indicating that the dialysis treatment itself plays an instrumental role [71]. Medina *et al.* have shown that the dialysis procedure led to a 20% increase in RBC destruction, measured by an increase in carbon monoxide concentration in alveolar air during treatment [72]. Fluid removal during the HD treatment leads to diminished flow in the microcirculation. Lower flow rates increase the adherence of erythrocytes to the endothelium, possibly leading to increased removal of annexin V binding RBCs from the central circulation during the treatment [73]. The increased adherence in turn could interfere with blood flow leading to a subsequent impairment of microcirculation. We are currently not in a position to test these hypotheses, and careful designed future experiments will be required to shed light on the underlying mechanisms.

The percentage of eryptotic RBCs was positively correlated with the percentage of RBC with iROS. Oxidative stress is a well-known stimulator of eryptosis [25]. Of note, oxidative

stress may result in reduced cell deformability and thus contribute to retention of eryptotic RBC in the microvascular bed [74, 75].

Previous studies have shown that diabetes influences the glycocalyx as well as the erythrocyte membrane properties [20, 76]. Analyzing diabetic and non-diabetic HD patients separately did not show a significant difference between groups with respect to ESS, eryptosis or oxidative stress. However, the negative correlation between ESS and BMI was much stronger in non-diabetic patients, indicating that diabetes might confound this relation.

Unexpectedly, glycocalyx integrity estimated by the salt blood test was not correlated with levels of eryptosis, reticulocytes, or the percentage of erythrocytes with iROS. Additionally, a comparison between of HD patients with ESS ≤ 3.42 and ESS > 3.42 , the median ESS value, failed to show a significant difference between groups in any of the analyzed variables, possibly due to the small sample size. Of note, the salt blood test covers only one aspect of glycocalyx functionality, the sodium buffering capacity. Eryptosis and oxidative stress could well be correlated with other glycocalyx properties.

The main limitation of the study is the small sample size, hence the results are only exploratory in nature. In addition, there may be an inherent self-selection bias. Although participants were not informed about their results, patients who decided to volunteer in this observational research study may have been more health conscious than patients that were not interested in taking part.

Conclusion

Although no connection between glycocalyx integrity and premature erythrocyte death could be established here, future carefully designed experiments are warranted to investigate other aspects of the multiple properties of the glycocalyx.

Disclosure Statement

PK holds stock options in Fresenius Medical Care North America (Waltham, MA). The remaining authors declared no competing interest.

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