

Potassium in Red Blood Cells - A New Biomarker of Oxidative Stress by Sepsis

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Abstract: There is increasing evidence that oxidants and antioxidants play a key role in the pathogenesis of sepsis. Sepsis is also characterized by excessive production of oxidants. Although many biomarkers for oxidative stress have been developed, the most biomarkers are technically very complex and they are not suitable for clinical routine. We discuss the possibility of using as a biomarker for oxidative stress by sepsis a new parameter - the concentration of potassium in red blood cells (RBC). The method for measuring potassium in erythrocytes, as well as the explanation for the increased potassium in the RBC, as a result of eryptosis (absorption of released potassium), was described by us earlier. Oxidative stress is known to be a major trigger for eryptosis - as a consequence, the increased potassium concentration in RBC directly reflects the intensity of oxidative stress. We also detect a tight buffer-like interaction between potassium in RBC and chlorine in plasma. Based on results from our study, we designed a nomogram for acid-base status of RBC. Method: In 66 patients (meeting criteria "Sepsis-3"), measurements of potassium in RBC were performed on the 1st, 3rd and 5th day stay at intensive care unit (ICU). The results: all patients had increased potassium in RBC on the 1st day. In the RBC nomogram, all measurements were in the range of metabolic acidosis. Over time, potassium in RBC normalized in surviving patients (n=42). In deceased patients (n=24), potassium in RBC fell with transition to metabolic alkalosis. A clear relationship was also noted between the concentration of potassium in the RBC and SOFA scale. The transition from metabolic acidosis to the metabolic alkalosis was accompanied by increased mortality. So, ROC - analysis showed high sensitivity and specificity of RBC acid-base status in predicting in-hospital mortality (AUROC = 0,78). Conclusion: The preliminary diagnostic model created on the basis of the nomogram allows assess the relationship of this parameter with the clinical course of sepsis. Basically, three approaches are conceivable for clinical practice: 1. Estimation of oxidative stress; 2. Estimation of RBC insufficiency or potassium deficiency; 3. Monitoring of antioxidant therapy. For the introduction into clinical routine, the automation of the method by Medical Industry is essential.

Keywords: Potassium in Red Blood Cells, Ion-Selective Electrodes, Biological Marker, Oxidative Stress, Sepsis, Multiorgan-Failure

1. Introduction

Sepsis is the leading cause of mortality in the intensive care units. Despite the research, sepsis pathogenesis is not completely understood. In the past, the widely accepted theory reported that sepsis was an uncontrolled inflammatory response to a pathogen. The failure of numerous studies using anti-inflammatory agents questioned the hypothesis of hyperinflammation [1-4]. Definition of oxidative Stress

(2007): "An imbalance between oxidants and anti-oxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage". [5]

There is increasing evidence that oxidants and antioxidants play a key role in the pathogenesis of sepsis [6]. Sepsis is also characterized by excessive production of oxidants. Therefore, they may represent a generator for various organ dysfunctions that lead to increased mortality. The clinical significance of oxidative stress in sepsis is demonstrated by several studies [6-8]. Despite the increasing evidence that oxidative stress is a

cornerstone on sepsis pathogenesis, the role of oxidative stress in sepsis may be underestimated [6]. In this context, the monitoring of redox homeostasis in clinical routine may play a key role - and consequently, therapies targeted to redox abnormalities may be useful for better management of septic patients. An increased understanding of the biology behind diseases and redox biology has led to more specific and sensitive tools to measure oxidative stress markers [9]. But most biomarkers for oxidative stress are sophisticated and technically very complex (flow cytometry, mass spectrometry etc.) and none of them are suitable for clinical routine.

2. Red Blood Cells as Organ

For decades, erythrocytes have been used as a cellular model for studying cell membranes. The development of highly technological methods in molecular biology (including proteomics) in the last 15–20 years has led to the point of view that erythrocytes are an organ which is involved in many “non-canonical” functions (beyond gas transport) which impact systemic metabolic homeostasis [10].

We emphasize that this point of view on erythrocytes, as an independent organ, is decisive for the interpretation of intracellular potassium homeostasis data, as well as for the consistency of further logical reasoning. We accept this paradigm shift and consider the red blood cells as follows in our discussion:

- 1) RBC's are an independent liquid cellular organ, dynamically distributed between all organ systems of the body and takes an active part in the overall homeostasis of the body.
- 2) As an organ, it has its own anatomical and functional sovereignty. RBC's are strictly separated from the extracellular fluid by colloid osmotic plasma gradient that corresponds to the "capsule" of the organ.
- 3) All electrolyte shifts between erythrocytes and plasma occur initially only in plasma - that is, within one organ. Plasma is the intercellular fluid of erythrocytes.
- 4) RBC's have their own autonomous system of homeostasis and maintenance of integrity.
- 5) The metabolic status of erythrocytes is dynamically equilibrated with other cells of the body and reflects the overall intracellular state of other organs.
- 6) Measurement of potassium in erythrocytes is their functional diagnostics.

2.1. Oxidative Stress and Red Blood Cells

Erythrocytes are rather poorly adaptable for survival. Exposure to high concentrations of oxygen radicals, the lack of nucleus and mitochondria, inability to synthesise new protein and degradation of detoxifying enzymes makes red blood cells (RBCs) uniquely vulnerable to oxidative stress [11]. RBC's are unable to generate ATP using molecular oxygen because of lack of mitochondria. Glycolysis is the only source of ATP generation in mature RBCs – they tend to faster energy depletion (RBC-Hypoxia). Although their primary function is transportation of the respiratory gases, O₂

and CO₂, between lungs and tissues, these circulatory cells are equipped with effective anti-oxidative systems that make them mobile free radical scavengers, providing antioxidant protection not only to themselves but also to other tissues and organs in the body (non-canonical functions of RBC's). So, they are among the first cells to be affected by alterations in the redox status of the body and can be explored for the early detection of pathophysiological alterations of the body in early stages [11].

2.2. Oxidative Stress and Eryptosis

Human RBCs have an average life span of 120 days. During their lifespan, RBCs are exposed to a large number of stressful situations. On average RBCs pass once a minute through the lungs where it is exposed to oxidative stress. More than once an hour it travels through the kidney medulla where it faces osmotic shock [7]. Prior to senescence, erythrocytes may, however, experience injury which compromises their integrity and thus triggers suicidal erythrocyte death or eryptosis. An important physiological function of eryptosis is to prevent the occurrence of haemolysis and the resulting complications. Numerous triggers for eryptosis have been identified, including hyperosmotic shock, energy depletion, oxidative stress, inflammatory mediators, oxygen free radicals, hepatic failure, heart failure, sepsis, fever, dehydration, and diverse xenobiotics [12-14]. Excessive eryptosis is observed by sepsis and hyperthermia [12]. The process of eryptosis has been well researched and the intracellular machinery always follows the same sequence, regardless of the origin of the trigger. Ultimately, oxidative stress is the main trigger of eryptosis. Here is a very simplified process. Oxidative stress activates Ca²⁺ - permeable nonselective cation channels in the cell membrane, thus stimulating Ca²⁺ - entry and subsequent cell membrane scrambling, resulting in activation of Ca²⁺ -sensitive K⁺ channels, leading to K⁺ exit, hyperpolarisation, Cl⁻ exit and ultimately cell shrinkage due to loss of KCl and osmotically driven water. The damaged RBC are eliminated from the circulation.

3. Potassium in RBC – A New Biological Marker

This parameter is largely unknown in clinical medicine - due to the lack of a practicable method of measurement. We developed a very simple method for measuring the potassium concentration in RBC's with ion-selective electrodes, i.e. with any blood analyzer in a few minutes [15]. The results of the first exploratory study, as well as the model for clinical interpretation we published earlier [16]. Normal values of potassium concentration in the RBC, measured by flame photometry and our method is 90-110 mmol / l. We noted both low (<70 mmol/l) and high (>120 mmol/l) values in patients in intensive care units. All experimental and few clinical studies of this parameter date back to the second half of the 20th century. At present, this parameter is practically not studied, and the reasons for the decrease in potassium in erythrocytes have not been discussed anywhere. Based on the results of

experimental studies, we suggest three possible pathophysiological mechanisms for decrease the concentration of potassium in the RBC: a) loss of potassium and its intracellular deficiency; b) inhibition of the function of the potassium-sodium pump; c) osmotic displacement of plasma by sodium (sodium-attack). We described clinical cases corresponding to these reasons in our previous publication. And the increased concentration of potassium is not mentioned anywhere in the literature. We noticed that increased potassium content in RBC was especially often observed in patients in inflammatory conditions. We hypothesized a relationship between elevated RBC potassium levels and eryptosis, which has been proven to be elevated in the presence of oxidative stress in patients with sepsis.

3.1. Potassium in RBC and Oxidative STRESS

When trying to explain the increased content of potassium in the body, we must return to the last phase eryptosis. In the final phase of eryptosis, KCl and water are fully excreted from erythrocytes to plasma. The damaged erythrocytes are eliminated from the blood circulation, while released KCl remains definitive in plasma. RBC's represent a potassium concentrate, its concentration in erythrocytes is 25 times higher than in plasma. It's easy to calculate - if anaemia associated with eryptosis leads to the decrease of the Hb level from 13 to 12 g/L, then the released potassium must increase the potassium concentration in plasma from 4.5 to fatal 9.5mmol/L, but it does not occur. We hypothesize, that released KCl is immediately absorbed by healthy erythrocytes. This process is a physiologically logical continuation of the protective function of eryptosis - first against haemolysis and then against hyperkalaemia. The uptake of released potassium consequently leads to an increase in its concentration. And this process occurs within one organ - erythrocytes. This phenomenon is indirectly confirmed by the

results of our exploratory study [16]. In the group of measurements with a potassium concentration of more than 120 mmol/l, a significant decrease haematocrit and plasma chloride was noted. The uptake of Cl anions inevitably leads to acidosis, and vice versa - loss of potassium and chlorine inevitably leads to alkalosis. So, we are allowed to discuss here from the theoretical intracellular acidosis or alkalosis. This constellation is also known to us from similar relationships in plasma - acidosis with hyperkalaemia, alkalosis with hypokalaemia.

3.2. Acid-Base Status of RBC's – The Model for Clinical Interpretation

By statistical analysis of our data from our exploratory study we did not find any correlations to any parameters in plasma, but very close and buffer-like ratios to chlorine in plasma – analogy to Acid-Base-Status. Following this logic, we calculated three derived parameters - all related to chlorine in plasma and potassium concentration in red blood cells. In order to interpret these parameters, it is necessary to imagine a mental equivalent in acid-base status. To make the interpretation more conventional, we have swapped cations and anions.

Three new Parameter – the Legend:

KaEry – (Potassium concentration in RBC, mmol/l) - this is equivalent for PCO₂. Normal Values 90-110 mmol/l.

KaEryExc - Potassium Excess, in relation to chlorine in plasma, mmol/l. $KaEryExc = Cl - KaEry$. Similar to BE (Basic Excess). Can take positive and negative values. Normal Values $\pm 5-6$ mmol/l.

pRCB = $Cl / KaEry$ Ratio, is similar to pH in acid-base balance ($pH = pCO_2 / HCO_3$). Since the calculated values are too small (norm ≈ 1.0), they are multiplied by 7 for convenience. Normal Values 7.0 ± 0.5 .

This creates a mental chain: pCO_2 - BE- $pH \approx KaEry - KaEryExc - pRCB$.

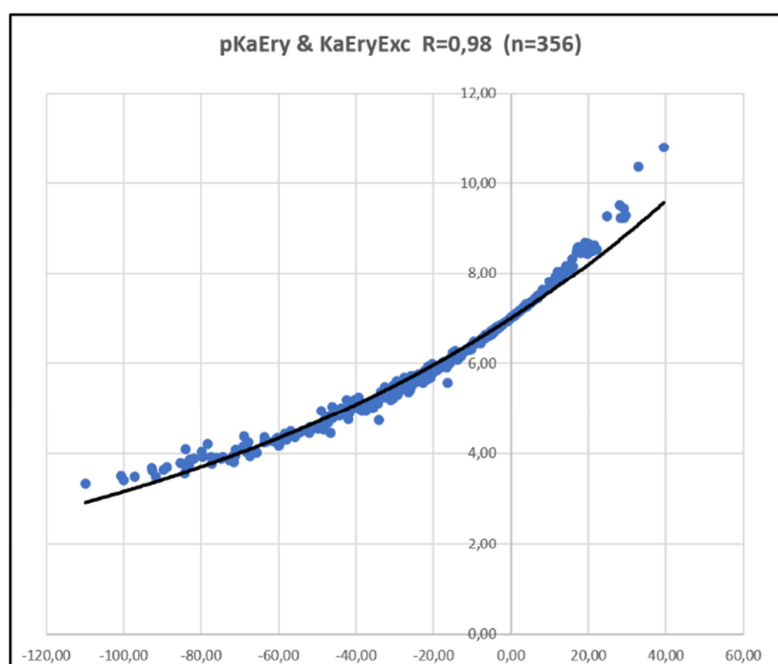


Figure 1. Point scatter diagram *KaEryExc* & *pRCB*.

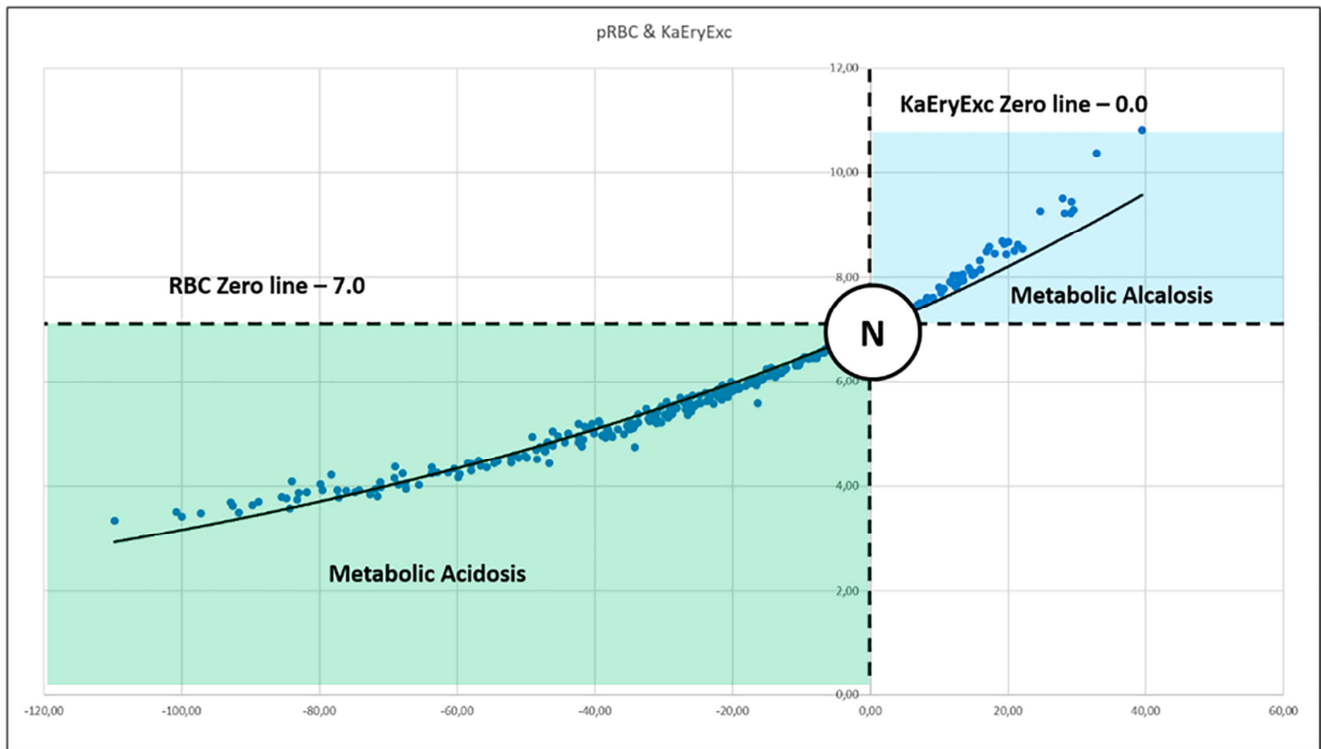


Figure 2. RBC Acid-Base Nomogram. The vertical dotted line - zero line of KaEryExc (0,0). The horizontal dotted line - zero line of pRBC (7,0). Explanations in text.

The correlation between these parameters showed very high linear ratios. This confirms our hypothesis about a close buffer connection of the potassium concentration in erythrocytes (the main cellular cation) with the concentration of chlorine in blood plasma (the main extracellular anion). We present only one plot of linear correlation between KaEryExc and pRBC, which turned out to be the most informative. This graph is the basis of our interpretation model (Figure 1).

Based on graph (Figure 1) we designed a nomogram for the acid-base status of RBC's (Figure 2).

The vertical line is a zero line from KaEryExc and divides the nomogram into two areas. On the left is the zone of metabolic acidosis - KaEryExc < 0 (think Basic Excess). To the right of the line is the zone of metabolic alkalosis. Above the pRBC zero line is the area of decompensated metabolic alkalosis (pRBC > 7.0), below - of decompensated metabolic acidosis (pRBC < 7.0, think pH). Any value of potassium concentration in RBC's will always lie exactly on this line and is unambiguously interpretable. However, the causes of this dynamic are still completely in the dark.

4. Prospective Diagnostic Study in Patients with Sepsis

The aim of the study was to confirm the following hypotheses: 1. Acid-base status of RBC's changes according to the severity of the clinical course of the sepsis; 2. Acid-base Status of RBC's have prognostic value. 3. Changes in Acid-Base-Status of RBC occur before clinical manifestations.

5. Material and Method

We studied 66 patients (37 males, 29 females) with sepsis who met "Sepsis-3" criteria. Exclusion criteria: ASA Classification > III, severe comorbidities. The Study and was approved by the Ethics Committee of the South Ural State Medical University (Chelyabinsk, Russia). Written informed consent was obtained from all patients. Period of study - October 2021 to August 2022.

Measurement of potassium in RBC's and calculation of derived parameters was performed on 1th, 3th and 5th day of ICU stay. The potassium content in erythrocytes was measured using analyzer COBASb -221 (Roche Diagnostics GmbH, Germany) in parallel with routine analyses. All parameters and the corresponding diagnostics of RBC acid-base status were automatically generated in our specially developed database. At the same time as the measurement, clinical data were documented according to the SOFA scale. Statistical analysis was performed with the statistical software R, version 4.2.1.

6. Results and Discussion

We emphasize again, that for the assessment of the acid-base status of RBC's we will mention only one parameter - KaEryExc, the deviation of chlorine in plasma (Cl-KaEry). This parameter corresponds logically to the Basic Excess in plasma and presents the metabolic component of acid-base status of RBC's. For a better

understanding, we deliberately avoid a large number of tables, especially with parameters that are still unfamiliar. The main results of our studies will be presented in the form of graphs, which, in our opinion, is much clearer. We only mention that all optical differences in the graphs are confirmed as well as statistical significant.

6.1. Statistic

Survived patients - 41, deceased – 24. The values of all measurements are presented in Table 1.

Table 1. Descriptive Statistic new Parameters.

	N	Min	Max	Mean	SD
KaEry	194,0	61,3	241,0	126,0	32,4
KaEryExc	194,0	-144,2	60,0	-20,9	36,0
pRBC	194,0	2,8	13,4	6,3	1,9

Table 2. T-Test KaEryExc between Survivors and Non-Survivors on 1-3-5th Days.

1. Day				
Exitus		N	Mean	Sign.
KaEryExc	Surv.	41	-38,7	p < 0,001
	Non Surv.	24	-10,5	
3. Day				
Exitus		N	Mean	Sign.
KaEryExc	Surv.	41	-29,9	p < 0,01
	Non Surv.	24	-3,7	
5. Day				
Exitus		N	Mean	Sign.
KaEryExc	Surv.	41	-27,5	p < 0,001
	Non Surv.	24	11,9	

Noteworthy is the large scatter of parameters, which indicates about the very high dynamics and sensitivity of this parameter. When comparing KaEryExc values between surviving and deceased patients (t-Test), highly significant differences were found (Table 2).

On day 1st, the values in both groups were in the range of metabolic acidosis (KaEryExc < 0). In the further course in the group of deceased patients there is a shift to metabolic alkalosis, which reached its maximum on the 5th day (KaEryExc + 11.9). This trend can also be seen on the corresponding boxplot chart (Figures 3 and 4).

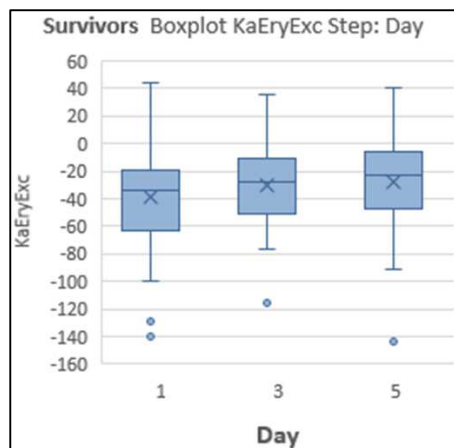


Figure 3. Boxplots KaEryExc by Survivors on 1-3-5th Days.

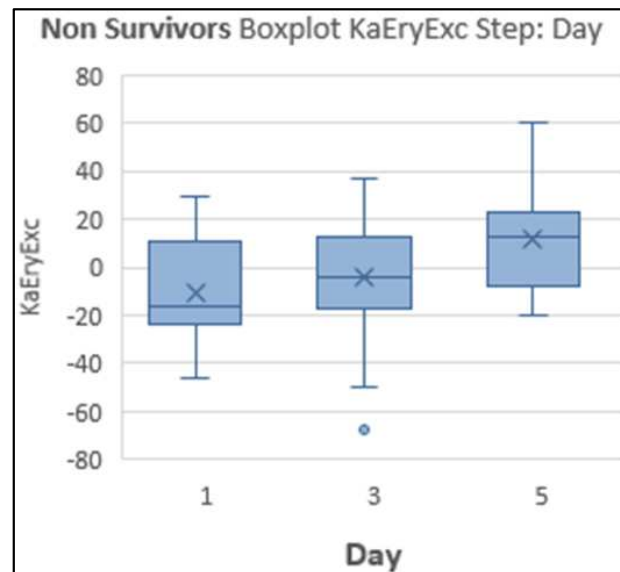


Figure 4. Boxplots KaEryExc by Non-Survivors on 1-3-5th Days.

Now we present the same data as an area diagram, projected on our RBC acid-base nomogram (Figures 5 and 6).

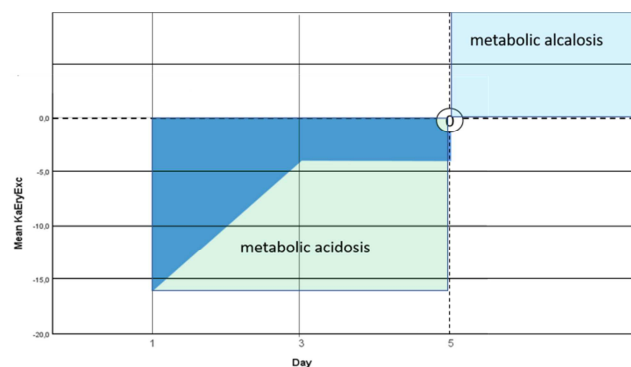


Figure 5. Mean Values of KaEryExc by Survivors, projected on RBC-Nomogram.

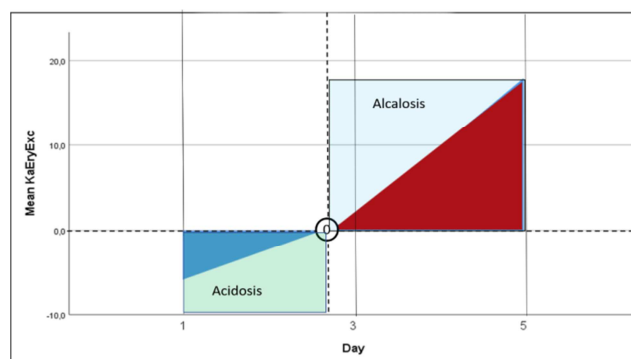


Figure 6. Mean Values of KaEryExc by Non-Survivors, projected on RBC-Nomogram.

The differences in the distribution of the values between both groups are obvious. In patients who survived, all values are in the range of metabolic acidosis. In the case of non-survivors, a clear shift into the zone of alkalosis can be seen - this trend is obviously associated with the poorer prognosis.

6.2. Correlations with Clinical Course

The following graphics show the same data from KaEryExc - together with the simultaneously recorded clinical data (SOFA scale).

Figure 7 clearly shows the correlation between KaEryExc and the SOFA scale by Survivors. With the clinical improvement, KaEryExc values are approaching the norm,

but remain in the zone of mild acidosis.

We see a completely different dynamic in Figure 8. With the deterioration of the condition, the KaEryExc values shift in the direction of metabolic alkalosis. It is noticeable, that the shift to alkalosis occurs earlier than the SOFA scale changes (Day 3). Now the same data in linear representation, along with SOFA-Scale and Basic Excess in plasma.

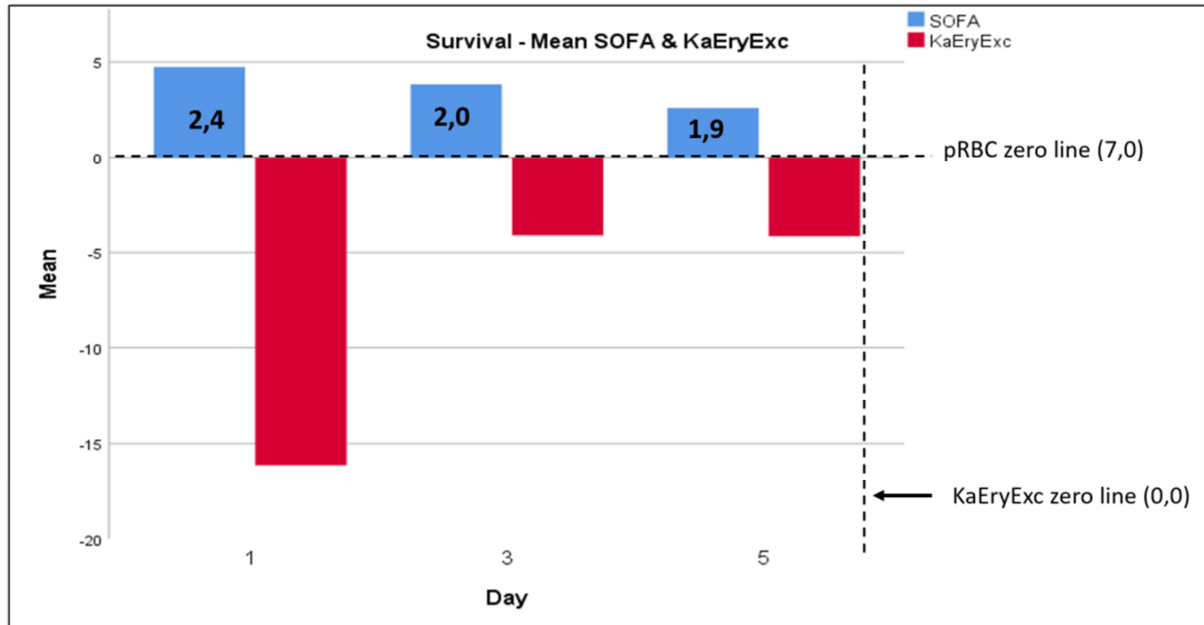


Figure 7. Mean Values of KaEryExc by Survivors, together with SOFA-Scale. The vertical dotted line - zero line of KaEryExc (0,0). The horizontal dotted line - zero line of pRCB (7,0).

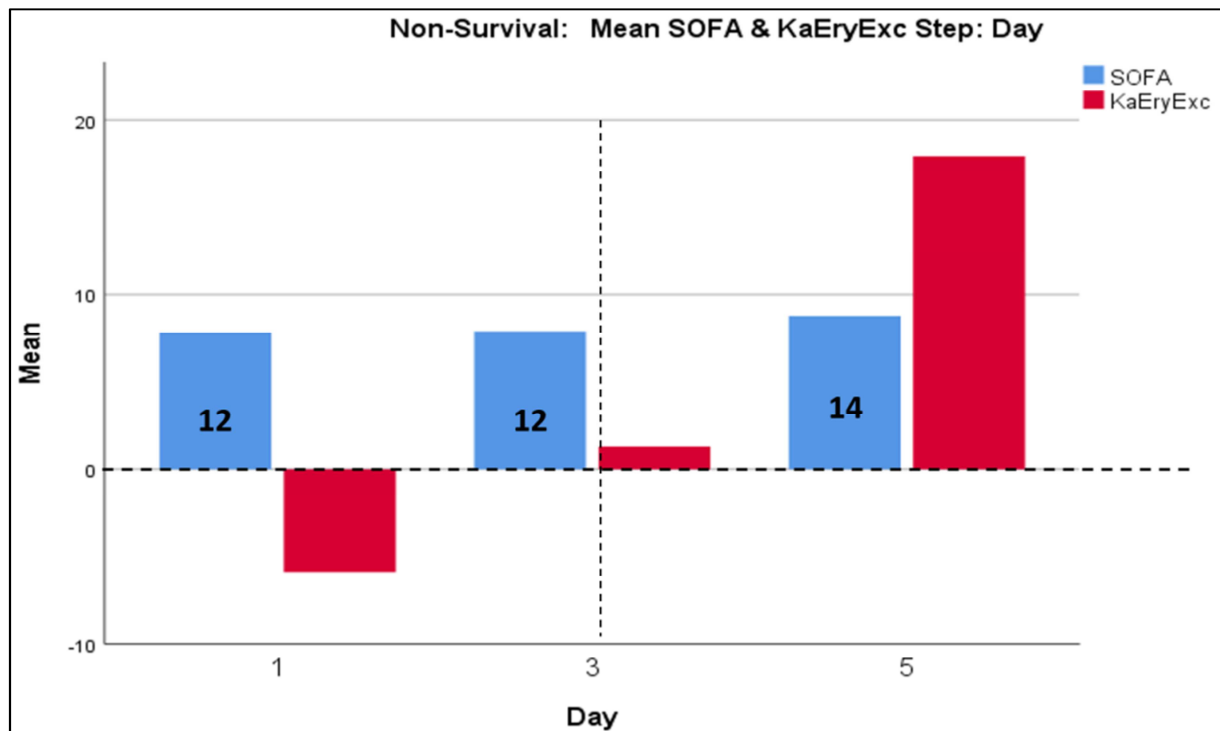


Figure 8. Mean Values of KaEryExc by Non-Survivors, together with SOFA-Scale. The vertical dotted line - zero line of KaEryExc (0,0). The horizontal dotted line - zero line of pRCB (7,0).

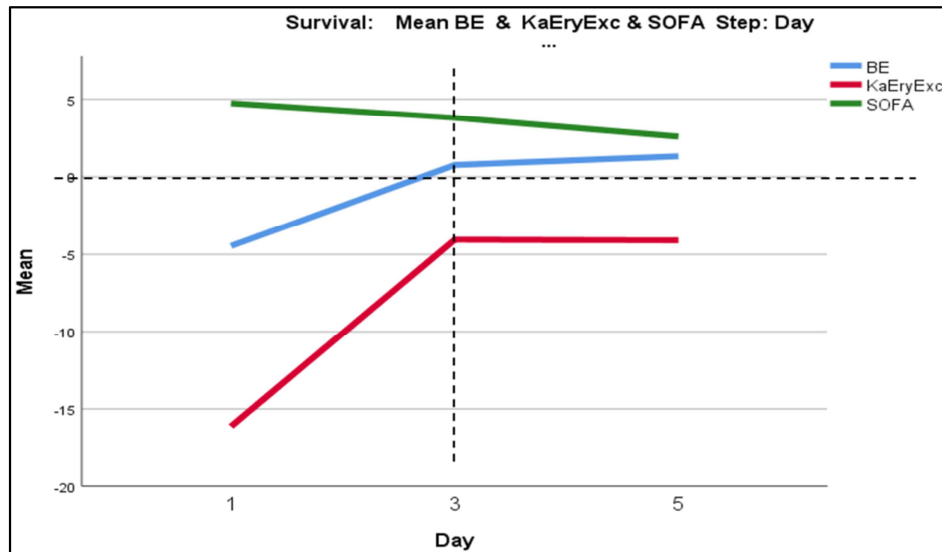


Figure 9. Survivors - linear representation KaEryExc, SOFA and BE in Plasma. Dotted lines - see above.

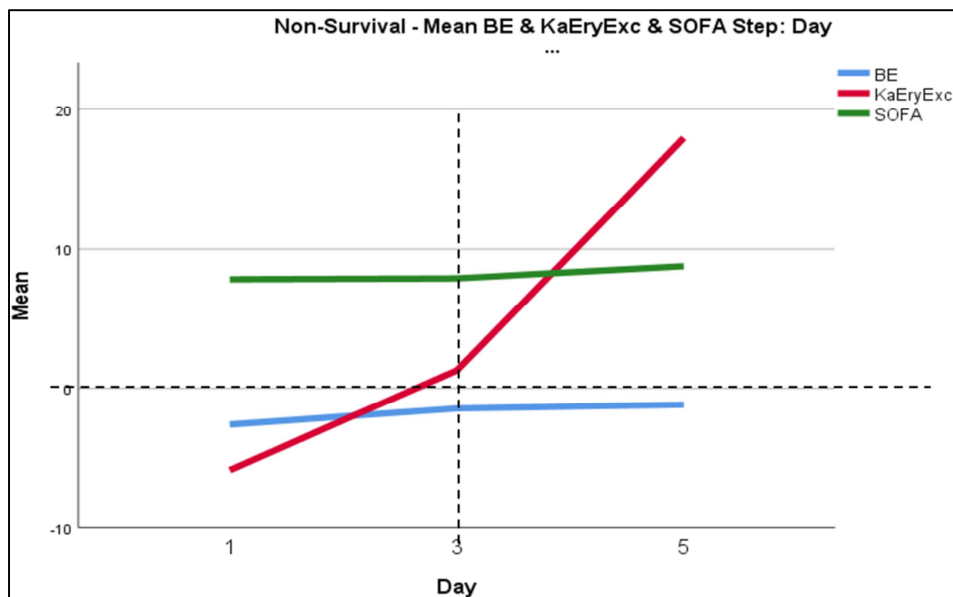


Figure 10. Non-Survivors - linear representation KaEryExc, SOFA and BE in Plasma. Dotted lines - see above.

Figure 9 probably shows a "normal" course of KaEryExc with a favourable prognosis. The response to oxidative stress manifests with extracellular and intracellular acidosis. With clinical improvement (see SOFA dynamics), both parameters normalize. With an unfavourable prognosis (Figure 10) we see a rather sharp transition to intracellular metabolic alkalosis (kink on the KaEryExc line). However, this phenomenon is easy to understand and has a consistent pathophysiological logic. Naturally, confrontation with metabolic acidosis is much more common, and we are not evolutionarily equipped to compensate for metabolic alkalosis. This also explains the difficult to treat metabolic alkalosis, which is well known in intensive care medicine.

6.3. The Prognostic Value of RBC Status

The following pie chart shows the mortality associated with the KaEryExc values - measured on the 5th day of stay.

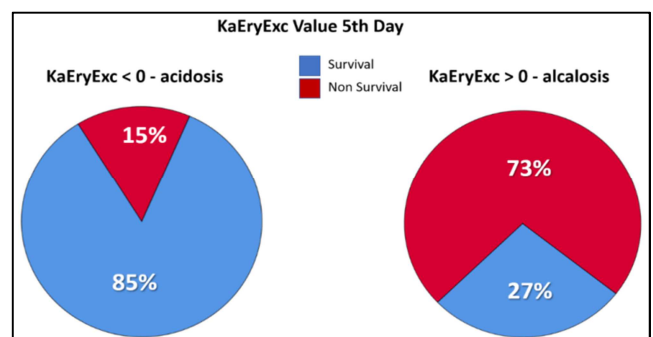


Figure 11. Mortality rate (red in%) associated with the RBC acid base status. Left - metabolic acidosis, right - metabolic alkalosis.

To check sensitivity and specificity for mortality we performed ROC analysis with KaEryExc value. These are the results.

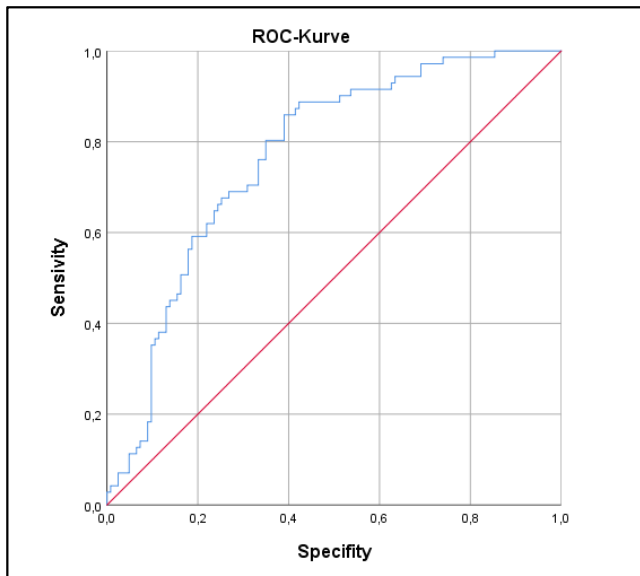


Figure 12. Predictive validity for in-hospital mortality. Area Under Curve (AUC) = 0,78; 95% CI; 0,75-0,84; $p < 0,01$.

These data clearly demonstrate a high prognostic value of RBC acid-base status. This also very evident that the tendency to intracellular metabolic alkalosis is associated with a poor prognosis.

6.4. Red Blood Cells - The First Organ in the Cascade of Multi-Organ Failure

A plausible and pathophysiologically understandable explanation for the described phenomena would be the following. Increased concentration of potassium in RBC's with metabolic acidosis is a normal response to oxidative stress und eryptosis - uptake of potassium in RBC's. If the course of the sepsis is positive, the intensity of oxidative stress also falls, the RBC' release the absorbed potassium again - all values normalize. With clinical deterioration and pathophysiological escalation RBC's suffer from stress, energy loss and depression of potassium-sodium pump follows. The RBC's lose their ability to absorb released potassium. Potassium loss follows - with metabolic alkalosis, sodium entry, and edema. This development can be seen as an RBC failure. Since RBCs are the most sensitive and vulnerable cells, it can be assumed that they are the first organ in the cascade of multiple organ failure. This moment is manifest with the transition to metabolic alkalosis (kink in KaEryExc values in the graph).

RBC-Failure – a Clinical Case

This clinical case empirically supports our discussion of RBC failure. A 58-year-old patient S. was admitted to the ICU with extensive facial phlegmon. The measurements on 1.3. and 5th day are shown on Figure 13. The first measurement showed a metabolic acidosis (oxidative stress). The measurement on the 3rd day showed an increase in acidosis, which can be interpreted as an increasing intensity of oxidative stress. On the 4th day of the stay, the patient's condition had deteriorated drastically. The phlegmon expanded, further surgical intervention was required. The measurement on day 5 showed a huge drop in potassium in RBC (from 130 to 65 mmol/l) and

shift from acidosis (KaEryExc -20 mmol/l to alkalosis (KaEryExc + 60 mmol/l) within 24 hours. The patient died a day later in septic shock and fulminant multiorgan failure. The moment of transition to metabolic alkalosis (3) is the beginning decompensation of RBC's.

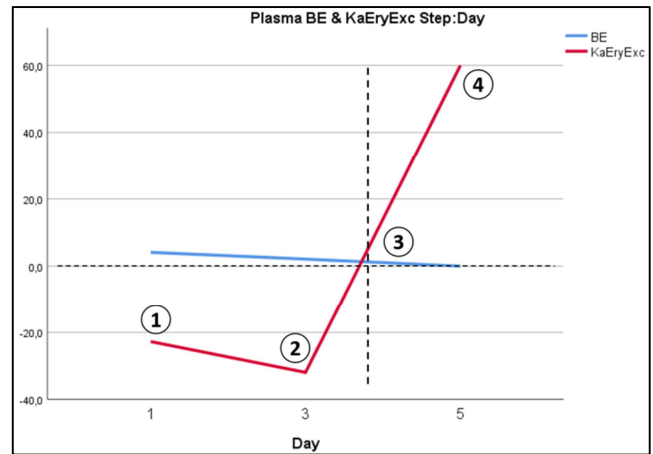


Figure 13. Patient S., KaEry and BE values (Explanations in Text) 1. Oxidative stress; Increased oxidative stress; 3. RBC – Decompensation; 4. RBC-Failure.

Summing up this discussion, we can state that all three hypotheses formulated by us are fully proven:

1. Acid-base status of RBC's changes according to the severity of the clinical course of the sepsis;
2. Acid-base status of RBC's have prognostic value.
3. Changes in Acid-Base-Status of RBC occur before clinical manifestations. Furthermore, our concept for RBC failure is based on a consistent pathophysiological logic and is corroborated with clinical observations.

7. Potassium in Red Blood Cells as Biomarker for Oxidative Stress

The World Health Organization has defined a biomarker as any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease [5].

Markers of oxidative stress often fulfil the first part of the criteria (i.e., they can be measured). But to be a clinically relevant biomarker, must be able to meet one of the following criteria: (1) show specificity for a certain disease (diagnostic), (2) have prognostic value, and (3) correlate with disease activity. This then allows treatment efficacy to be assessed. To be clinically useful, a biomarker must also be reasonably stable, present in an easily accessible tissue, and cost-effective to measure reproducibly on a large scale [5]. Looking back at the presented data, we can assume that Potassium in RBC meets all of these criteria.

Of course, the evidence for this assumption must be provided by further large-scale studies.

The demonstrated representative of oxidative stress, KaEryExc, is often in the negative range (acidosis) and is therefore not convenient for interpretation. When inverting the values, the

parameter is easier to interpret - an increase means the intensity of stress, a trend towards negative values means a deterioration in the RBC status. RBC Stress Factor – can be a new Name.

8. Potassium in Red Blood Cells – Business Case for Medical Industry

Manual execution of the method is largely acceptable for scientific purposes. But for the introduction into clinical practice, the automation of the method is essential. That wouldn't be a problem for the medical industry - provided it recognizes a business case. That only happens when the large studies with convincing data are published - and the method gets broad acceptance in the clinical world. We also designed a disposable device in which all sample preparation steps take place in a closed container - without contact with reagents. This device can be easily manufactured and would greatly simplify the measurement. And even more - our experiments have shown that it is also possible to measure calcium and magnesium in RBCs using the same principle (haemolysis of erythrocytes via ultrasound). The complete measurement of intra- and extracellular electrolytes means a revolutionary development in laboratory monitoring with great scientific and diagnostic potential.

9. Conclusion

The discovered close buffer-like relationship between potassium in RBC and chlorine in plasma made it possible to design a nomogram for theoretical RBC acid-base status. The preliminary diagnostic model created on the basis of the nomogram allows assess the relationship of this parameter with the clinical course of sepsis. The first results of the study of this parameter showed its prognostic significance for the outcome of the disease, as well as for assessing the degree of oxidative stress of RBC's. In addition, this parameter may indicate organ failure of red blood cells and suggest incipient multiple organ failure. Basically, three approaches are conceivable for clinical practice:

- 1) Estimation of oxidative stress.
- 2) Estimation of RBC insufficiency or potassium deficiency (not discussed here).
- 3) Monitoring of antioxidant therapy.

The study of this parameter in fields related to medicine (experimental medicine, pathophysiology, etc.) would allow a more complete study of this potentially informative marker. For the introduction into clinical routine, the automation of the method by Medical Industry is essential.

Hans Selye, the father of oxidative stress, once said [17]: “If only stress could be seen, isolated and measured, I'm sure we could enormously lengthen the average human lifespan”. Perhaps potassium in RBC can become this tool.

The method described step by step and the special database can be downloaded here:

<https://1drv.ms/u/s!AtLL0yThFD4kxX4UYiHxyk5oK8Eq?e=I2okqz>

Conflict of Interest

All the authors do not have any possible conflicts of interest.

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