

Review

New Clues to Cardiovascular Disease: Erythrocyte Lifespan

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[Received January 1, 2023; Revised May 5, 2023; Accepted May 6, 2023]

ABSTRACT: Determination of erythrocyte lifespan is an important part of the diagnosis of hemolytic diseases. Recent studies have revealed alterations in erythrocyte lifespan among patients with various cardiovascular diseases, including atherosclerotic coronary heart disease, hypertension, and heart failure. This review summarizes the progress of research on erythrocyte lifespan in cardiovascular diseases.

Key words: erythrocyte lifespan, heart failure, atherosclerosis, hypertension, statins, heart chamber assist device

Introduction

The lifespan of erythrocytes is the duration they remain in the bloodstream. In healthy individuals, erythrocytes usually survive for an average of 120 days [1]. However, previous research has demonstrated that several conditions, including hereditary xerocytosis (HX), severe aplastic anemia (SAA), and sickle cell anemia (SCA), may result in a shortened lifespan of erythrocytes, leading to anemia [2-7]. Patients with renal insufficiency may experience varying degrees of anemia [8, 9] that could be linked to reduced production of endogenous erythropoietin (EPO) and iron deficiency [10, 11]. Furthermore, shortened erythrocyte lifespan has been observed in patients on dialysis with renal insufficiency and proteinuria [12-18], as well as in those with diabetes [19-25].

Anemia, which can result from a shortened lifespan of erythrocytes, is closely associated with cardiovascular diseases (CVD) [26] and increases the risk of developing CVD [27]. Recently, more attention has been given to the alterations in erythrocytes' lifespan associated with cardiovascular diseases such as heart failure, hypertension, and atherosclerosis (AS). Additionally,

statins, commonly used to treat CVD, have been linked to a shortened lifespan of erythrocytes as a side effect.

This review aims to summarize the changes in erythrocyte lifespan in CVD patients and suggest future research directions. Our goal is to highlight the importance of the erythrocyte lifespan for researchers.

Role of Erythrocyte lifespan in cardiovascular diseases

Erythrocytes are unique in lacking a nucleus and having a double concave disc shape. They possess several physiological characteristics, including plasticity, suspension stability, and osmotic fragility. Erythrocytes are primarily responsible for transporting oxygen and carbon dioxide [28] and play a crucial role in buffering acid-base substances in the blood [29], clearing immune complexes [30], metabolizing systemic nitric oxide, regulating redox reactions, and modulating blood viscosity [31]. Erythrocyte production is mainly influenced by EPO and sex hormones. Besides, thyroid, adrenocortical, and growth hormones can indirectly impact erythropoiesis.

As the most abundant cell type in circulation, alterations in erythrocyte lifespan can contribute to the

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development of cardiovascular diseases such as acute heart failure, hypertension, and AS.

Erythrocyte lifespan and acute heart failure

Patients with heart failure have a higher risk of developing anemia [32, 33] due to excessive eryptosis, a process characterized by erythrocyte contraction, phosphatidylserine (PS) exposure, and clustering of band-3 on eryptotic erythrocytes, along with a decrease in CD47 levels [34-36]. Mahmud *et al.* [37] found that erythrocyte PS exposure was more prominent in rats with acute heart failure, leading to increased phagocytosis by macrophages and a shortened erythrocyte lifespan. The authors analyzed erythrocytes from five patients with acute heart failure and found a significant increase in PS binding to annexin V, indicating erythrocyte senescence [38]. These findings suggest that the erythrocyte death rate is accelerated, and the erythrocyte lifespan is shortened in rodents and human patients with heart failure. Oxidative stress is another factor that affects erythrocyte lifespan [39], and Mahmud *et al.* [37] suggested that sustained oxidative stress in patients with acute heart failure contributes to accelerated eryptosis.

Attanasio *et al.* [40] conducted a study on 22 acute heart failure patients and 10 healthy individuals as the control group, all with the same number of erythrocytes. The rate of eryptosis was measured using annexin V, and they found that the binding rate of PS in erythrocytes to annexin V was significantly higher in the patient group than in the control group. The study results indicate that the rate of eryptosis increased in patients with acute heart failure, leading to anemia and a shortened erythrocyte lifespan. Furthermore, the findings suggest that oxidative stress can accelerate the rate of eryptosis [40].

Erythrocytes lifespan and hypertension

Hypertension is China's most prevalent chronic non-communicable disease, with 244.5 million patients in 2015 [41]. Hyperlipidemia often accompanies hypertension in diabetic patients [42]. Pinzón-Díaz *et al.* [43] investigated erythrocyte lifespan in patients with hypertension and hyperlipidemia. They classified 81 patients as healthy (NT), hypertensive (HT), hyperlipidemic (ND), and hypertensive and hyperlipidemic (HTD). Patients with HTD had the highest calcium ion levels in erythrocytes, followed by those with HT. Regardless of hyperlipidemia, calcium ions in erythrocytes were elevated in patients with hypertension, leading to PS exposure. All three groups (HT, ND, and HTD) had lower glutathione (GSH) concentrations than the NT group. Both HT and HTD groups had a higher degree of lipid peroxidation. Pinzón-Díaz *et al.* [43]

suggested that increased intracellular calcium concentration in erythrocytes in patients with hypertension can lead to PS exposure, but it is not entirely proportional. Because an increase in blood cholesterol causes an increase in cholesterol on the cell membrane [44], the enzymes required for PS inversion could weaken the sensitivity to calcium ions, decreasing the activity of inverting enzymes. At the same time, the decrease of GSH on the erythrocyte membrane could also weaken the antioxidant capacity of erythrocytes and shorten their lifespan.

Pinzón-Díaz *et al.* observed that hypertension increased eryptosis due to oxidative stress, although other molecular mechanisms may be at play. Huang *et al.* [45] created a hypertension mice model and injected angiotensin II (Ang II) in the experimental group and saline in the control group. Their study showed that Ang II decreased the expression of CD47 on erythrocyte surfaces and increased the binding of Annexin V to PS. However, losartan, an angiotensin 1 receptor antagonist, reversed these effects. Angiotensin II also reduced antioxidant enzymes, and the addition of antioxidant N-acetylcysteine (NAC) produced similar effects to losartan and maintained a redox balance. The authors suggested that angiotensin II induces erythrocyte redox imbalance through the angiotensin II type 1 receptor. Pinzón-Díaz *et al.* also proposed that angiotensin II may promote the formation of NADPH oxidase and reactive oxygen species (ROS), which generate superoxide ions in the cells. Interestingly, Guimarães-Nobre *et al.* [46] demonstrated that in sickle cell anemia patients, moderate concentrations of angiotensin II solution reduced the binding of PS to Annexin V through angiotensin 1 receptor (ATR1), possibly by reducing PS valguus. However, high and low concentrations of Ang II could not reduce PS valguus, suggesting that angiotensin II and ATR1 have different effects on erythrocytes in different diseases.

Erythrocyte lifespan and AS

Recent studies have indicated that erythrocyte lifespan may play a crucial role in the progression of AS [47]. Individuals with AS were reported to have erythrocytes with a reduced lifespan, making them more susceptible to spleen-mediated removal from circulation [47]. Delbosc *et al.* [48] conducted a study on rabbits and induced AS through a high-fat diet. The study found that hypercholesterolemic rabbits had erythrocytes with accelerated senescence and shortened lifespan. During the early stages of AS, erythrocytes were detected in the vessel wall of arteries and were phagocytosed by vascular smooth muscle cells. Both humans and rabbits with early-stage AS exhibited iron and Hb deposits in their arteries,

which promoted the progression of AS. The hemolysis of erythrocytes in AS was caused by oxidized low-density lipoprotein (ox-LDL) and lipids, resulting in a shorter erythrocyte lifespan and increased iron and heme production, which further promoted the oxidation of lipids and the production of atheromatous substances and endothelial stress [49]. Hemeoxygenase-1 (HO-1) has been shown to have anti-AS effects [50], and its production is increased in AS. Additionally, Sánchez *et al.* [51] observed increased erythropoiesis in mice with AS.

Wang *et al.* [52] demonstrated that AS was associated with Jak2 gene mutation. In their study, they transplanted bone marrow cells from Jak2V617F, a prevalent mutation that causes myeloproliferative disorders [53], and wild-type (WT) mice into low-density lipoprotein receptor knockout mice and fed them a high-fat diet. Their results demonstrated that the Jak2V617F mutation promoted the occurrence and development of AS. Individuals carrying the Jak2V617F mutation had increased erythrocyte numbers and decreased CD47 expression on erythrocytes, which promoted erythrocyte phagocytosis and shortened the lifespan of erythrocytes. The decrease in CD47 on the surface of erythrocytes led to erythrocyte phagocytosis, which shortened the lifespan of erythrocytes. Jak2V617F mice had reduced levels of MerTK, a receptor expressed on macrophages that promotes phagocytosis, while erythrocyte phagocytosis inhibited efferocytosis. Efferocytosis can prevent secondary necrosis of near-death cells, thereby preventing the release of inflammatory substances. The Jak2V617F mutation can promote thrombocytosis, enhance platelet activity, and further promote the occurrence of AS.

Erythrocyte lifespan and thrombus

Red blood cells play a role in the formation of thrombi, which can lead to heart attacks. Patients with Sickle Cell Anemia (SCA) are at a significantly higher risk of pulmonary embolism than healthy individuals [54-58]. These emboli form within the lungs rather than from lower limb deep veins [55, 56]. Patients with hereditary spherocytosis (HS) also have an increased risk of arterial thrombosis after splenectomy [59]. Deformed erythrocytes can increase blood viscosity [60] and promote thrombosis in patients with SCA and HS [61]. Damaged erythrocytes can release Hemoglobin (Hb) and Adenosine Diphosphate (ADP), which can promote platelet aggregation and activation [62-64]. PS exposure in erythrocytes drives thrombin production [65-69]. However, it did not predict thrombotic risk in HS mice [70]. It was reported that PS exposure increases in erythrocytes [70-72], and erythrocyte lifespan is shortened in patients with SCA. Elevated prothrombin

fragment 1.2 has been observed in patients with SCA, and it was associated with PS in erythrocytes [73]. Furthermore, erythrocytes may increase thrombin production and inhibit thrombolysis [61].

In a study by Schleicher *et al.* [74], platelets were found to express FasL, a ligand for cell death receptors, which induced apoptosis. Similarly, erythrocytes were also found to express FasR, a cell death receptor [75]. Klatt *et al.* [76] discovered that erythrocytes can expose FasL on platelets, leading to FasR activation in erythrocytes. This binding induced PS exposure on erythrocytes and promoted platelet activation and thrombosis. Additionally, erythrocytes can contribute to thrombosis by increasing blood viscosity [77, 78]. Dayal *et al.* [79] demonstrated that mice deficient in superoxide dismutase-1 had a shorter erythrocyte lifespan, exhibited eryptosis, and experienced faster thrombosis, indicating that superoxide dismutase is crucial for erythrocyte lifespan and thrombosis [80].

Mechanisms linking erythrocyte lifespan and cardiovascular diseases

Oxidative stress and inflammation

Patients with heart failure experience accelerated eryptosis, the process of programmed cell death in erythrocytes. This is caused by oxidative stress or activation of calcium-sensitive potassium channels by calcium ions, leading to erythrocyte shrinkage and increased exposure of PS on erythrocytes. As a result, the lifespan of erythrocytes is shortened in these patients [81]. As shown in Figure 1, oxidative stress induces PS exposure through several mechanisms. Firstly, it activates caspase, an apoptosis-related enzyme expressed in erythrocytes, leading to PS exposure on the erythrocyte membrane [37, 82]. Secondly, oxidative stress activates cation channels on the erythrocyte surface, resulting in calcium influx into erythrocytes and increased calcium activity. This high calcium concentration in erythrocytes stimulates scramblase, which transfers PS from inside to outside erythrocytes [37, 81, 82]. Additionally, the activation of Gardos channels, which are calcium-sensitive potassium channels, promotes PS exposure through increased potassium ion outflow, membrane hyperpolarization, and anion outflow through chloride channels. These processes lead to the loss of intracellular potassium chloride, decreased intracellular osmotic pressure, and erythrocyte contraction [81]. Intracellular calcium ions could also activate proteolytic enzymes, causing the degradation of the erythrocyte cytoskeleton [37, 82]. Energy depletion during heart failure damages the supply of GSH and weakens the antioxidant barrier of erythrocytes, leading to eryptosis [37, 82]. This process

can be activated through the hyperosmotic shock pathway, where cationic channels are stimulated by the release of prostaglandin E2 [83]. Furthermore, sepsis caused by excessive inflammation and tissue damage can

promote eryptosis by increasing the production of ceramide, which facilitates the entry of calcium ions into erythrocytes [84].

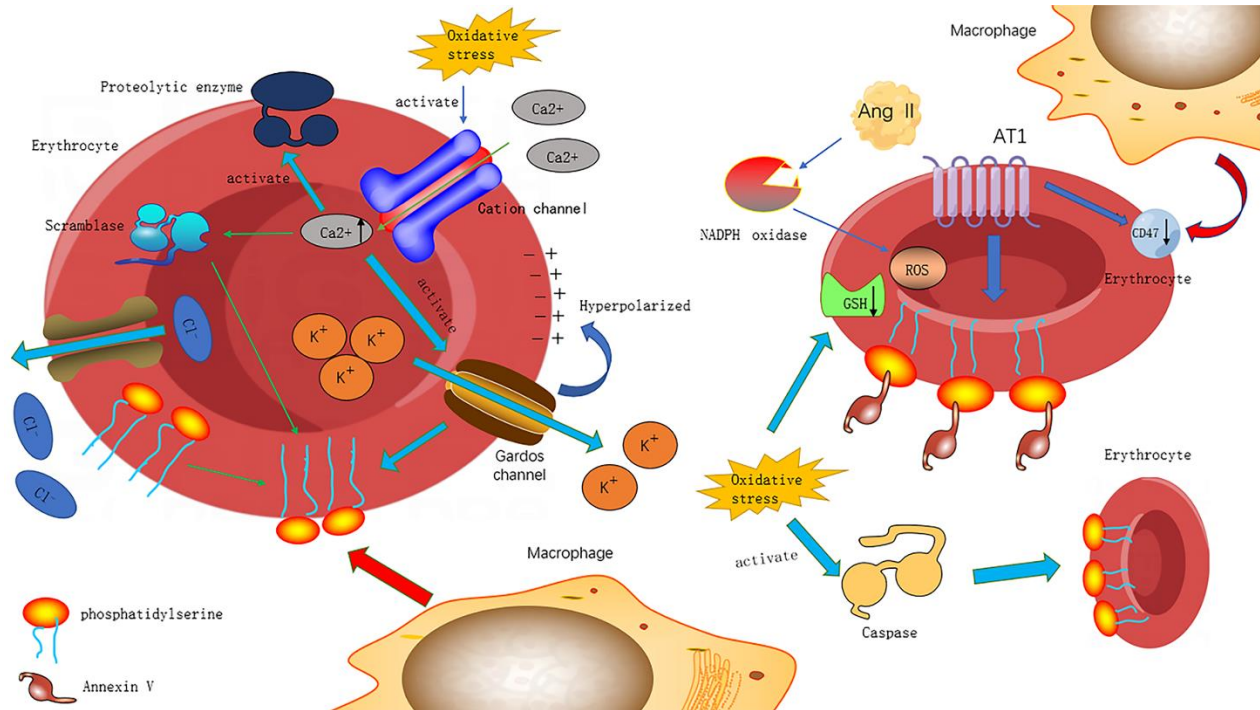


Figure 1. The potential mechanism of oxidative stress leading to shortened lifespan of erythrocyte. Oxidative stress triggers the activation of caspase, an enzyme that stimulates the exposure of phosphatidylserine (PS) on the erythrocyte membrane. Additionally, oxidative stress activates cation channels on the erythrocyte surface, leading to an influx of calcium ions into the cell, which enhances calcium activity. The increased concentration and activity of calcium ions in erythrocytes stimulates scramblase, which transfers PS from the inside to the outside of the erythrocyte. Intracellular calcium also activates calcium-sensitive potassium channels, known as Gardos channels. This leads to an increase in potassium ion outflow and membrane hyperpolarization, driving anion outflow through chloride channels. As a result, there is a loss of intracellular potassium chloride, a decrease in intracellular osmotic pressure, and erythrocyte contraction. The activation of Gardos channels also promotes PS exposure. Intracellular calcium ions can activate proteolytic enzymes, leading to the degradation of erythrocyte cytoskeleton. Moreover, angiotensin II reduces the expression of CD47 on the surface of erythrocytes but increases the binding of Annexin V to PS. It promotes the formation of NADPH oxidase and reactive oxygen species (ROS), leading to the oxidation of cells. During oxidative stress, the level of glutathione (GSH) on erythrocytes decreases, and the antioxidant capacity of erythrocytes decreases.

Inflammation can have a negative impact on erythrocyte deformability. Normally, erythrocytes can undergo plastic deformation and regain their biconcave disc shape after passing through capillaries. However, erythrocytes lose this ability in the presence of inflammation and cannot maintain their biconcave disc shape after deformation [85]. Hyperactivated platelets and increased adhesion to erythrocytes may also impair erythrocyte deformability [85]. Furthermore, when erythrocytes interact with inflammatory factors, it can lead to pathological deformation and, ultimately, eryptosis [85].

Anemia and reduced oxygen delivery

Anemia triggers the sympathetic nerve and activates RAAS, causing an increase in oxidative stress [31]. Oxidative stress can damage the erythrocyte membrane, reducing erythrocyte lifespan [86]. Additionally, anemia could exacerbate coronary heart disease by causing tissue hypoxia, increasing anemic patients' heart rate. This acceleration, coupled with increased preload, could lead to left ventricular hypertrophy and cardiac cavity enlargement [87], ultimately resulting in heart failure. Anemia is also closely associated with hypertension, arrhythmia, and cerebral stroke [86].

Iron and pro-inflammatory molecule release

Approximately two-thirds of the body's iron is found in erythrocytes [88], which is vital to their lifespan [89]. Iron deficiency can lead to increased programmed cell death, shortened erythrocyte lifespan, and anemia [90]. A shortage of iron results in more calcium ions entering the red blood cells, which is the leading cause of their shortened lifespan [90]. In conditions like thalassemia, iron stored outside red blood cells leads to non-transferrin-plasma iron (NTPI) accumulation, which is toxic and can cause lipid peroxidation on cell membranes, damaging cells and causing hemosiderin cardiomyopathy [91].

Ferroptosis is a type of non-apoptotic cell death caused by the RAS-selective lethal small molecule erastin and is different from other forms of iron metabolism. During ferroptosis, the cystine/glutamate antiporter is inhibited [92], leading to a depletion of GSH, an increase in oxidative stress, and the inhibition of glutathione peroxidase 4 (GPX4) [93]. These effects increase the peroxidation of lipids and can destroy cell membranes, including those of red blood cells [94].

The destruction of erythrocytes leads to the release of various components, which induces aseptic inflammation via Toll-like receptors (TLR) and NOD-like receptors (NLR) [95]. Heme can cause oxidation and inflammation [96] and activate TLR4 to induce macrophages to secrete TNF- α [97]. Additionally, heme can activate neutrophils and enhance the production of ROS and interleukin-8 (IL-8) [98]. The prolonged state of heme overload can result in heart damage [99]. Studies have shown that methemoglobin can cause extensive neuroinflammation through TLR4 after subarachnoid hemorrhage [100]. Upon stimulation, activated NOD-like receptors (NLR) can promote the activation of caspase-1, which converts the IL-1 family of cytokines into their active forms, IL-1 β and IL-18, leading to cell death [101].

Cardiovascular diseases, including acute heart failure, hypertension, thrombosis, and AS, have been linked to the shortened lifespan of erythrocytes. However, the exact nature of the relationship between erythrocyte lifespan and cardiovascular disease is still being explored.

Factors affecting the lifespan of erythrocytes

The lifespan of erythrocytes is influenced by various factors, including genetics, cardiovascular diseases, and environmental factors. HX, an inherited congenital hemolytic disease, is one example of how genetics can play a crucial role. HX pathogenesis is believed to be induced mainly by a PIEZO1 mutation, which leads to calcium influx, Gardos channel activation, and potassium and water outflow [2, 4]. As a result, an imbalance of

sodium and potassium ions in erythrocytes occurs, leading to erythrocyte dehydration, decreased deformability, and shortened lifespan [2, 4]. Smoking is another factor that significantly affects erythrocyte lifespan. Studies have shown that smoking reduces CD47 expression on erythrocyte surfaces, leading to shortened erythrocyte lifespan [102]. Moreover, diabetes, associated with widespread oxidative stress and inflammation, has been linked to shortened erythrocyte lifespan in several studies [19-25]. Patients with renal insufficiency and proteinuria undergoing dialysis have also shown shortened erythrocyte lifespan [12-18]. Finally, some iatrogenic factors also affect the lifespan of erythrocytes.

Statins

Due to their plaque-stabilizing and lipid-lowering effects, statins were a significant breakthrough in the treatment of various diseases, including hyperlipidemia [103], hypercholesterolemia [104], AS, and leukemia [52, 105]. Biswas *et al.* [106, 107] found that arsenic reduced erythrocyte lifespan, which was restored after treatment with atorvastatin and N-acetylcysteine (NAC). Studies have shown that atorvastatin can reverse the altered properties of erythrocyte membranes in patients with hypercholesterolemia [108]. Similarly, simvastatin was reported to decrease lipid peroxidation of erythrocyte membranes [109]. Lipid-lowering therapy with atorvastatin combined with ezetimibe or atorvastatin alone has also been shown to improve erythrocyte membrane parameters in patients with coronary artery disease [110].

However, there are conflicting findings about the effects of statins on erythrocyte lifespan. *In vitro* experiments by Rana *et al.* [80] showed that atorvastatin administration at different concentrations (1-10 μ M) induced oxidative stress in erythrocytes, resulting in decreased activities of GSH peroxidase, superoxide dismutase, and catalase, and shortened erythrocyte lifespan. Al Mamun Bhuyan *et al.* [111] showed that erythrocytes exposed to a 1 μ g/ml solution of simvastatin experienced eryptosis, characterized by an increase in the percentage of PS exposure and induction of calcium ions into the erythrocytes, causing oxidative stress and eryptosis.

Therefore, more studies are necessary to fully understand the effect of statins on erythrocyte lifespan, given the current ambiguity on this matter.

The cardiac chamber assists devices

Advancements in science and technology have led to the development of intra-cardiac assist devices, which can now be used to treat end-stage heart failure and valvular

heart disease. The left ventricular assist device (LVAD) is one such device that is used to slow down the pressure of ventricular pumping by introducing blood from the ventricle into the device, which is then pumped into the aorta. This device is commonly used in patients with end-stage heart failure.

Taimeh *et al.* [112] investigated the relationship between erythrocyte lifespan and the constant-flow left ventricular assist device (CF-LVAD). The study showed that erythrocytes lifespan in patients with CF-LVAD was shorter than that of normal subjects. Additionally, patients with thrombus formation had a shorter erythrocyte lifespan than those without thrombus, and both groups experienced anemia. The study suggested that mechanical damage to erythrocytes by the pump is the primary mechanism of anemia [112]. Although CF-LVAD can improve survival and alleviate symptoms in patients with end-stage heart failure, it comes with complications, such as anemia and infections [113].

Vrtovec *et al.* [114] found that non-anemic patients had twice the survival rate of anemic patients after 6 months of LVAD application. Infection is also a common complication in patients with LVAD [115], and Hernandez *et al.* [116] suggested that readmission rates could be reduced by anticoagulation and treatment of complications. Therefore, it is essential to give adequate attention to the potential complications of CF-LVAD, including the shortened lifespan of erythrocytes, in the treatment of patients with end-stage heart failure.

Erythrocyte lifespan assays

Discriminative agglutination method

In 1919, Ashby devised the discriminative agglutination method to determine erythrocyte lifespan. This method involved injecting type O blood erythrocytes into individuals with type A blood, followed by mixing blood samples with anti-A serum after transfusion to produce an agglutination reaction. The survival time for type O erythrocytes could be tracked by collecting blood regularly for agglutination reaction [117-121]. Nonetheless, implementing this method in a clinical setting proved challenging.

Labeling method

The ^{51}Cr and ^{15}N glycine labeling methods were developed later for measuring erythrocyte lifespan. ^{51}Cr can label erythrocytes of all ages, and when hexavalent chromium ions enter the erythrocytes, they are reduced to trivalent chromium ions and remain in the erythrocyte until the cell dies. This method involves injecting the labeled erythrocytes into subjects and drawing blood

regularly to measure the radiation intensity of ^{51}Cr . As time passes, the labeled erythrocytes gradually die out, and the radioactivity of ^{51}Cr decreases. The survival curve of erythrocytes can be drawn based on the decreasing rate of radiation intensity, and the lifespan of erythrocytes can be determined. However, this method is time-consuming, and the results are not the exact value of erythrocyte life [122]. Correction is also required using a coefficient table. Additionally, this method is not suitable for children or pregnant women due to the use of radioactive elements.

Creatine method

Kameyama *et al.* [123] proposed a method for determining erythrocyte lifespan based on the logarithm of creatine through a linear model. The formula is $\log \text{EC} = -0.04379 \text{MRBC} + 2.882$, and the transformed formula is $\text{MRBC} = -22.84 \log \text{EC} + 65.83$, where EC represents erythrocyte creatine ($\mu\text{mol/g Hb}$), and MRBC represents mean erythrocyte age (days). However, this method has a complex formula and is not straightforward to obtain results. Moreover, it is significantly influenced by renal function.

CO expiratory method

The carbon monoxide (CO) breath test is a commonly used method to measure erythrocyte lifespan. When eryptosis occurs, endogenous CO is produced by heme oxidation, which accounts for approximately 70% of the total endogenous CO produced by the body. These CO are eliminated through the lungs [124, 125]. By measuring the concentration of CO in the environment, the concentration of endogenous CO can be determined, and the metabolic rate of erythrocytes can be calculated. When the rate of hemoglobin synthesis equals the rate of decomposition, a dynamic balance is achieved, and the average lifespan of erythrocytes on that day is equal to the total amount of hemoglobin/hemoglobin decomposition. One millimole of hemoglobin contains four millimoles of heme, and one millimole of heme oxidizes to produce one millimole of CO. Thus, the above formula can be modified to the total amount of heme-derived CO/CO lung output on the same day, and the formula is erythrocyte lifespan = $(4 \times \text{Hb} \times 22,400) / (0.7 \times \text{endPCO} \times 64,400 \times 1,440) \times (\text{Vb}/\text{Vt})$ [Hb: hemoglobin (g/L); 22,400: standard state gas molecular volume (mL); 4: 1 mmol hemoglobin releases 4 mmol CO; 0.7: ratio of CO produced by heme to endogenous CO; 64,400: blood red egg white fraction; 1,440: total minutes in a day (min); endPCO: endogenous CO partial pressure (ppm); Vb: blood volume (mL); Vt: resting alveolar ventilation (mL/min)]. The values of Vb and Vt are approximately the same. Thus, erythrocyte lifespan =

$(\text{Hb} \times \text{K})/\text{endPCO}$ [K: 1,380, unit: mL/(dg)] [124-127]. Hemoglobin concentration can be determined through a routine blood test, while the endogenous CO concentration during expiration can be measured using specific instruments. As a result, the CO breath test is relatively simple and can be conducted in a clinical setting.

However, due to the requirement for a greater amount of sample gas than that exhaled by a healthy individual, at least two collections are necessary to obtain the required amount of gas, which may result in errors. In the case of patients with chronic obstructive pulmonary disease (COPD), exhalation is difficult, and the error may be magnified. Therefore, research on how to reduce this error is needed.

Clinical significance.

The potentials of erythrocyte lifespan measurement in clinical diagnosis and prognosis

The measurement of erythrocyte lifespan plays a crucial role in clinical practice, serving as the "gold standard" for the diagnosis of hemolytic diseases and aiding in the investigation of therapeutic effects and mechanisms of such diseases. For example, the erythrocyte lifespan measurement can detect hemolysis and guide compensatory bone marrow hematopoiesis in patients with heart valve implantation. Additionally, it can reveal the effects of exercise on erythrocytes and the causes of secondary polycythemia at high altitudes [128].

Traditionally, a shortened erythrocyte lifespan was associated with hemolytic diseases in the blood system, but now it also suggests the possibility of other system diseases, including cardiovascular, endocrine, urinary, and others.

Large-scale population studies are necessary to confirm the diagnostic value of erythrocyte lifespan in cardiovascular diseases, particularly in risk stratification and prognosis evaluation.

Potential therapeutic strategies targeting erythrocyte lifespan and future research directions

One potential therapeutic strategy for addressing shortened erythrocyte lifespan is to target risk factors such as high blood pressure, blood sugar, and lipids. Additionally, treatments like enhanced external counterpulsation (EECP) and cardiac rehabilitation may help improve erythrocyte lifespan. Hyperbaric oxygen is also being studied for its potential benefits on erythrocyte lifespan.

Dapagliflozin is a drug that can promote erythropoietin (EPO) production [129], which has been

shown to protect erythrocytes from oxidative stress [130, 131]. EPO has been found to reduce the area of myocardial infarction in animal experiments during myocardial ischemia-reperfusion [132]. However, according to Gao *et al.* [133], EPO does not improve cardiac function in patients with acute myocardial infarction. Nevertheless, EPO certainly benefits erythrocytes by preventing oxidative stress, though further studies are needed to determine if it affects erythrocyte lifespan.

Nitric oxide (NO) is a signal molecule that can enter erythrocytes and inhibit oxidative stress [5, 89]. Erythrocytes also contain nuclear factor (NFκB), which prevents eryptosis [134] and may be related to erythrocyte aging [135]. NFκB is almost absent in old erythrocytes, which could be a potential future research direction for erythrocyte lifespan.

Other areas of research that merit investigation include the effect of chronic heart failure on erythrocyte lifespan, the use of statins in patients with hyperlipidemia and anemia, and ways to improve erythrocyte lifespan in patients with CF-LVAD and artificial valves. Chinese herbal medicines are also being used to treat heart failure in China, and it remains to be seen whether they can prolong erythrocyte lifespan when combined with current treatment protocols.

Conclusion

This paper aimed to shed light on the relationship between erythrocyte lifespan and cardiovascular diseases. The shortening of erythrocyte lifespan in patients with cardiovascular diseases is linked to oxidative stress, increased calcium ion concentration, decreased activity of GSH peroxidase and Superoxide dismutase (SOD), and increased phosphatidylserine exposure. However, this study has limitations due to the lack of research on erythrocyte lifespan. Future research can focus on 1) investigating the effect of other diseases on erythrocyte lifespan, 2) exploring the impact of different drugs on erythrocyte lifespan, 3) identifying additional molecular mechanisms that contribute to the shortening of erythrocyte lifespan, and 4) investigating the relationship between erythrocyte lifespan and aging to determine whether prolonging erythrocyte lifespan can delay aging.

Conflicts of Interest

The authors declare no conflict of interest.

References

- [1] Corrons JLV, Casafont LB, Frasnado EF (2021). Concise review: how do red blood cells born, live, and

- die? *Ann Hematol*, 100:2425-2433.
- [2] Jankovsky N, Caulier A, Demagny J, Guitton C, Djordjevic S, Lebon D, et al. (2021). Recent advances in the pathophysiology of PIEZO1-related hereditary xerocytosis. *Am J Hematol*, 96:1017-1026.
 - [3] Kim J, Usmani A, De Simone N, Sarode R (2018). Mathematical calculation of lifespan of transfused RBCs in sickle cell disease patients. *Transfus Apher Sci*, 57:46-49.
 - [4] Mohandas N (2018). Inherited hemolytic anemia: a possessive beginner's guide. *Hematology Am Soc Hematol Educ Program*, 2018:377-381.
 - [5] Nader E, Romana M, Guillot N, Fort R, Stauffer E, Lemonne N, et al. (2020). Association Between Nitric Oxide, Oxidative Stress, Eryptosis, Red Blood Cell Microparticles, and Vascular Function in Sickle Cell Anemia. *Front Immunol*, 11:551441.
 - [6] Ye L, Guo J, Jing LP, Peng GX, Zhou K, Li Y, et al. (2018). The life span of red blood cell in patients with severe/very severe aplastic anemia. *Zhonghua Xue Ye Xue Za Zhi*, 39:137-142.
 - [7] Ye L, Jing L, Guo J, Zhao X, Peng G, Li Y, et al. (2021). Red blood cell lifespan is reduced in severe aplastic anemia and improves with response to immunosuppressive treatment. *Am J Hematol*, 96:E441-e443.
 - [8] Gafter-Gvili A, Schechter A, Rozen-Zvi B (2019). Iron Deficiency Anemia in Chronic Kidney Disease. *Acta Haematol*, 142:44-50.
 - [9] Ma J, Dou Y, Zhang H, Thijssen S, Williams S, Kuntsevich V, et al. (2017). Correlation between Inflammatory Biomarkers and Red Blood Cell Life Span in Chronic Hemodialysis Patients. *Blood Purif*, 43:200-205.
 - [10] Batchelor EK, Kapitsinou P, Pergola PE, Kovesdy CP, Jalal DI (2020). Iron Deficiency in Chronic Kidney Disease: Updates on Pathophysiology, Diagnosis, and Treatment. *J Am Soc Nephrol*, 31:456-468.
 - [11] Portolés J, Martín L, Broseta JJ, Cases A (2021). Anemia in Chronic Kidney Disease: From Pathophysiology and Current Treatments, to Future Agents. *Front Med (Lausanne)*, 8:642296.
 - [12] Bissinger R, Nemkov T, D'Alessandro A, Grau M, Dietz T, Bohnert BN, et al. (2021). Proteinuric chronic kidney disease is associated with altered red blood cell lifespan, deformability and metabolism. *Kidney Int*, 100:1227-1239.
 - [13] Bomholt T, Oturai P, Rix M, Almdal T, Knop FK, Rosthøj S, et al. (2021). Reduced erythrocyte lifespan measured by chromium-51 in patients with type 2 diabetes undergoing long-term hemodialysis. *Hemodial Int*, 25:198-204.
 - [14] Daenen K, Andries A, Mekahli D, Van Schepdael A, Joutet F, Bammens B (2019). Oxidative stress in chronic kidney disease. *Pediatr Nephrol*, 34:975-991.
 - [15] Daimon S (2020). Shortened red cell life span as a factor of anemia of mild inflammation in hemodialysis patients. *Ther Apher Dial*, 24:742-744.
 - [16] Fishbane S, Pollock CA, El-Shahawy M, Escudero ET, Rastogi A, Van BP, et al. (2022). Roxadustat Versus Epoetin Alfa for Treating Anemia in Patients with Chronic Kidney Disease on Dialysis: Results from the Randomized Phase 3 ROCKIES Study. *J Am Soc Nephrol*, 33:850-866.
 - [17] Matsumura K, Okumiya T, Sugiura T, Takahashi N, Yamamoto Y, Kikuchi S, et al. (2020). Shortened red blood cell age in patients with end-stage renal disease who were receiving haemodialysis: a cross-sectional study. *BMC Nephrol*, 21:418.
 - [18] Yang X, Zhao B, Wang J, Wang L, Tao M, Lu J, et al. (2021). Red blood cell lifespan in long-term hemodialysis patients treated with roxadustat or recombinant human erythropoietin. *Ren Fail*, 43:1428-1436.
 - [19] Arita T, Maruyama T, Yokoyama T, Hieda M, Fukata M, Fujino T, et al. (2020). Impaired deformability and association with density distribution of erythrocytes in patients with type 2 diabetes mellitus under treatment. *Clin Hemorheol Microcirc*, 76:73-83.
 - [20] Chu HW, Ma YJ, Huang ZH (2020). A pilot study: effect of erythrocyte lifespan determined by a modified carbon monoxide breath test on glycosylated hemoglobin interpretation. *J Breath Res*, 14:027101.
 - [21] Huang Z, Liu Y, Mao Y, Chen W, Xiao Z, Yu Y (2018). Relationship between glycated haemoglobin concentration and erythrocyte survival in type 2 diabetes mellitus determined by a modified carbon monoxide breath test. *J Breath Res*, 12:026004.
 - [22] Jagadish S, Hemshekhar M, NaveenKumar SK, Sharath Kumar KS, Sundaram MS, Basappa, et al. (2017). Novel oxolane derivative DMTD mitigates high glucose-induced erythrocyte apoptosis by regulating oxidative stress. *Toxicol Appl Pharmacol*, 334:167-179.
 - [23] Kameyama M, Takeuchi S, Ishii S (2018). Steady-state relationship between average glucose, HbA1c and RBC lifespan. *J Theor Biol*, 447:111-117.
 - [24] Turpin C, Catan A, Guerin-Dubourg A, Debussche X, Bravo SB, Álvarez E, et al. (2020). Enhanced oxidative stress and damage in glycated erythrocytes. *PLoS One*, 15:e0235335.
 - [25] Zhou S, Dong R, Wang J, Zhang L, Yu B, Shao X, et al. (2022). Red Blood Cell Lifespan < 74 Days Can Clinically Reduce Hb1Ac Levels in Type 2 Diabetes. *J Pers Med*, 12.
 - [26] Estcourt LJ, Fortin PM, Hopewell S, Trivella M, Wang WC (2017). Blood transfusion for preventing primary and secondary stroke in people with sickle cell disease. *Cochrane Database Syst Rev*, 1:Cd003146.
 - [27] Kang SH, Moon JY, Kim SH, Sung JH, Kim IJ, Lim SW, et al. (2022). Association of hemoglobin levels with clinical outcomes in acute coronary syndromes in Koreans. *Medicine (Baltimore)*, 101:e32579.
 - [28] Pernow J, Mahdi A, Yang J, Zhou Z (2019). Red blood cell dysfunction: a new player in cardiovascular disease. *Cardiovasc Res*, 115:1596-1605.
 - [29] Rees SE, Klastrup E, Handy J, Andreassen S, Kristensen SR (2010). Mathematical modelling of the acid-base chemistry and oxygenation of blood: a mass balance, mass action approach including plasma and red blood cells. *Eur J Appl Physiol*, 108:483-494.

- [30] Pasqualetti G, Brooks DJ, Edison P (2015). The role of neuroinflammation in dementias. *Curr Neurol Neurosci Rep*, 15:17.
- [31] Kuhn V, Diederich L, Keller TCSt, Kramer CM, Lückstädt W, Panknin C, et al. (2017). Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. *Antioxid Redox Signal*, 26:718-742.
- [32] Ezekowitz JA, Zheng Y, Cohen-Solal A, Melenovský V, Escobedo J, Butler J, et al. (2021). Hemoglobin and Clinical Outcomes in the Vericiguat Global Study in Patients With Heart Failure and Reduced Ejection Fraction (VICTORIA). *Circulation*, 144:1489-1499.
- [33] Siddiqui SW, Ashok T, Patni N, Fatima M, Lamis A, Anne KK (2022). Anemia and Heart Failure: A Narrative Review. *Cureus*, 14:e27167.
- [34] Burger P, Hilarius-Stokman P, de Korte D, van den Berg TK, van Bruggen R (2012). CD47 functions as a molecular switch for erythrocyte phagocytosis. *Blood*, 119:5512-5521.
- [35] Repsold L, Joubert AM (2018). Eryptosis: An Erythrocyte's Suicidal Type of Cell Death. *Biomed Res Int*, 2018:9405617.
- [36] Xu W, Peng F, Deng Y, Fan X, Li N (2019). The emerging roles of eryptosis in liver diseases. *Transfus Clin Biol*, 26:336-340.
- [37] Mahmud H, Ruifrok WP, Westenbrink BD, Cannon MV, Vreeswijk-Baudoin I, van Gilst WH, et al. (2013). Suicidal erythrocyte death, eryptosis, as a novel mechanism in heart failure-associated anaemia. *Cardiovasc Res*, 98:37-46.
- [38] Crowley LC, Marfell BJ, Scott AP, Waterhouse NJ (2016). Quantitation of Apoptosis and Necrosis by Annexin V Binding, Propidium Iodide Uptake, and Flow Cytometry. *Cold Spring Harb Protoc*, 2016.
- [39] Hannemann A, Rees DC, Brewin JN, Noe A, Low B, Gibson JS (2018). Oxidative stress and phosphatidylserine exposure in red cells from patients with sickle cell anaemia. *Br J Haematol*, 182:567-578.
- [40] Attanasio P, Bissinger R, Haverkamp W, Pieske B, Wutzler A, Lang F (2015). Enhanced suicidal erythrocyte death in acute cardiac failure. *Eur J Clin Invest*, 45:1316-1324.
- [41] Wang Z, Chen Z, Zhang L, Wang X, Hao G, Zhang Z, et al. (2018). Status of Hypertension in China: Results From the China Hypertension Survey, 2012-2015. *Circulation*, 137:2344-2356.
- [42] Tojo A, Asaba K, Onozato ML (2007). Suppressing renal NADPH oxidase to treat diabetic nephropathy. *Expert Opin Ther Targets*, 11:1011-1018.
- [43] Pinzón-Díaz CE, Calderón-Salinas JV, Rosas-Flores MM, Hernández G, López-Betancourt A, Quintanar-Escorza MA (2018). Eryptosis and oxidative damage in hypertensive and dyslipidemic patients. *Mol Cell Biochem*, 440:105-113.
- [44] Gottlieb MH (1980). Rates of cholesterol exchange between human erythrocytes and plasma lipoproteins. *Biochim Biophys Acta*, 600:530-541.
- [45] Huang C, Gao J, Wei T, Shen W (2022). Angiotensin II-Induced Erythrocyte Senescence Contributes to Oxidative Stress. *Rejuvenation Res*, 25:30-38.
- [46] Guimarães-Nobre CC, Mendonça-Reis E, Teixeira-Alves LR, Miranda-Alves L, Berto-Junior C (2022). ATR1 Angiotensin II Receptor Reduces Hemoglobin S Polymerization, Phosphatidylserine Exposure, and Increases Deformability of Sick Cell Disease Erythrocytes. *Cell Biochem Biophys*, 80:711-721.
- [47] Turpin C, Catan A, Meilhac O, Bourdon E, Canonne-Hergaux F, Rondeau P (2021). Erythrocytes: Central Actors in Multiple Scenes of Atherosclerosis. *Int J Mol Sci*, 22.
- [48] Delbosc S, Bayles RG, Laschet J, Ollivier V, Ho-Tin-Noé B, Touat Z, et al. (2017). Erythrocyte Efferocytosis by the Arterial Wall Promotes Oxidation in Early-Stage Atheroma in Humans. *Front Cardiovasc Med*, 4:43.
- [49] Nagy E, Eaton JW, Jeney V, Soares MP, Varga Z, Galajda Z, et al. (2010). Red cells, hemoglobin, heme, iron, and atherogenesis. *Arterioscler Thromb Vasc Biol*, 30:1347-1353.
- [50] Soares MP, Bach FH (2009). Heme oxygenase-1: from biology to therapeutic potential. *Trends Mol Med*, 15:50-58.
- [51] Sánchez Á, Orizaola MC, Rodríguez-Muñoz D, Aranda A, Castrillo A, Alemany S (2020). Stress erythropoiesis in atherogenic mice. *Sci Rep*, 10:18469.
- [52] Wang W, Liu W, Fidler T, Wang Y, Tang Y, Woods B, et al. (2018). Macrophage Inflammation, Erythrophagocytosis, and Accelerated Atherosclerosis in Jak2 (V617F) Mice. *Circ Res*, 123:e35-e47.
- [53] Liu W, Östberg N, Yalcinkaya M, Dou H, Endo-Umeda K, Tang Y, et al. (2022). Erythroid lineage Jak2V617F expression promotes atherosclerosis through erythrophagocytosis and macrophage ferroptosis. *J Clin Invest*, 132.
- [54] Bates DDB, Liu Z, Gibbons J, LeBedis CA, Holalkere NS (2019). Sick cell disease and venous thromboembolism: A retrospective comparison of the rate of positive CT pulmonary angiography in the emergency department. *Eur J Radiol*, 110:256-259.
- [55] El-Amin N, Lauzon SD, Nietert PJ, Kanter J (2021). Which adults with sickle cell disease need an evaluation for pulmonary embolism? *Br J Haematol*, 195:447-455.
- [56] Khan MI, Patel N, Meda RT, Nuguru SP, Rachakonda S, Sripathi S (2022). Sick Cell Disease and Its Respiratory Complications. *Cureus*, 14:e28528.
- [57] Pervaiz A, El-Baba F, Dhillon K, Daoud A, Soubani A (2021). Pulmonary complications of sickle cell disease: a narrative clinical review. *Adv Respir Med*, 89:173-187.
- [58] Sange I, Cherukuri PB, Parchuri V, Srinivas N, Ramanan SP, Sange AH, et al. (2021). Sick Cell Disease and the Respiratory System: A Tangential Perspective to the Hematopulmonological Dilemma. *Cureus*, 13:e15562.
- [59] Schilling RF, Gangnon RE, Traver MI (2008). Delayed adverse vascular events after splenectomy in hereditary spherocytosis. *J Thromb Haemost*, 6:1289-1295.
- [60] Piety NZ, Reinhart WH, Pourreau PH, Abidi R, Shevkoplyas SS (2016). Shape matters: the effect of red blood cell shape on perfusion of an artificial

- microvascular network. *Transfusion*, 56:844-851.
- [61] Byrnes JR, Wolberg AS (2017). Red blood cells in thrombosis. *Blood*, 130:1795-1799.
- [62] Reimers RC, Sutra SP, Joist JH (1984). Potentiation by red blood cells of shear-induced platelet aggregation: relative importance of chemical and physical mechanisms. *Blood*, 64:1200-1206.
- [63] Rother RP, Bell L, Hillmen P, Gladwin MT (2005). The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *Jama*, 293:1653-1662.
- [64] Santos MT, Valles J, Marcus AJ, Safier LB, Broekman MJ, Islam N, et al. (1991). Enhancement of platelet reactivity and modulation of eicosanoid production by intact erythrocytes. A new approach to platelet activation and recruitment. *J Clin Invest*, 87:571-580.
- [65] Peyrou V, Lormeau JC, Hérault JP, Gaich C, Pflieger AM, Herbert JM (1999). Contribution of erythrocytes to thrombin generation in whole blood. *Thromb Haemost*, 81:400-406.
- [66] Kuypers FA, Lewis RA, Hua M, Schott MA, Discher D, Ernst JD, et al. (1996). Detection of altered membrane phospholipid asymmetry in subpopulations of human red blood cells using fluorescently labeled annexin V. *Blood*, 87:1179-1187.
- [67] Reddel CJ, Tan CW, Chen VM (2019). Thrombin Generation and Cancer: Contributors and Consequences. *Cancers (Basel)*, 11:100.
- [68] Tripisciano C, Weiss R, Eichhorn T, Spittler A, Heuser T, Fischer MB, et al. (2017). Different Potential of Extracellular Vesicles to Support Thrombin Generation: Contributions of Phosphatidylserine, Tissue Factor, and Cellular Origin. *Sci Rep*, 7:6522.
- [69] van Beers EJ, Schaap MC, Berckmans RJ, Nieuwland R, Sturk A, van Doormaal FF, et al. (2009). Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. *Haematologica*, 94:1513-1519.
- [70] Wandersee NJ, Tait JF, Barker JE (2000). Erythroid phosphatidyl serine exposure is not predictive of thrombotic risk in mice with hemolytic anemia. *Blood Cells Mol Dis*, 26:75-83.
- [71] Lombardi E, Matte A, Risitano AM, Ricklin D, Lambris JD, De Zanet D, et al. (2019). Factor H interferes with the adhesion of sickle red cells to vascular endothelium: a novel disease-modulating molecule. *Haematologica*, 104:919-928.
- [72] Faes C, Sparkenbaugh EM, Pawlinski R (2018). Hypercoagulable state in sickle cell disease. *Clin Hemorheol Microcirc*, 68:301-318.
- [73] Setty BN, Rao AK, Stuart MJ (2001). Thrombophilia in sickle cell disease: the red cell connection. *Blood*, 98:3228-3233.
- [74] Schleicher RI, Reichenbach F, Kraft P, Kumar A, Lescan M, Todt F, et al. (2015). Platelets induce apoptosis via membrane-bound FasL. *Blood*, 126:1483-1493.
- [75] Mandal D, Mazumder A, Das P, Kundu M, Basu J (2005). Fas-, caspase 8-, and caspase 3-dependent signaling regulates the activity of the aminophospholipid translocase and phosphatidylserine externalization in human erythrocytes. *J Biol Chem*, 280:39460-39467.
- [76] Klatt C, Krüger I, Zey S, Krott KJ, Spelleken M, Gowert NS, et al. (2018). Platelet-RBC interaction mediated by FasL/FasR induces procoagulant activity important for thrombosis. *J Clin Invest*, 128:3906-3925.
- [77] Bettiol A, Galora S, Argento FR, Fini E, Emmi G, Mattioli I, et al. (2022). Erythrocyte oxidative stress and thrombosis. *Expert Rev Mol Med*, 24:e31.
- [78] Çınar T, Hayıroğlu M, Selçuk M, Çiçek V, Doğan S, Kılıç Ş, et al. (2022). Association of whole blood viscosity with thrombus presence in patients undergoing transoesophageal echocardiography. *Int J Cardiovasc Imaging*, 38:601-607.
- [79] Dayal S, Gu SX, Hutchins RD, Wilson KM, Wang Y, Fu X, et al. (2015). Deficiency of superoxide dismutase impairs protein C activation and enhances susceptibility to experimental thrombosis. *Arterioscler Thromb Vasc Biol*, 35:1798-1804.
- [80] Rana RB, Jilani K, Shahid M, Riaz M, Ranjha MH, Bibi I, et al. (2019). Atorvastatin Induced Erythrocytes Membrane Blebbing. Dose Response, 17:1559325819869076.
- [81] Föller M, Lang F (2020). Ion Transport in Eryptosis, the Suicidal Death of Erythrocytes. *Front Cell Dev Biol*, 8:597.
- [82] Lang F, Lang KS, Lang PA, Huber SM, Wieder T (2006). Mechanisms and significance of eryptosis. *Antioxid Redox Signal*, 8:1183-1192.
- [83] Lang F, Gulbins E, Lang PA, Zappulla D, Föller M (2010). Ceramide in suicidal death of erythrocytes. *Cell Physiol Biochem*, 26:21-28.
- [84] Luo MC, Zhou SY, Feng DY, Xiao J, Li WY, Xu CD, et al. (2016). Runt-related Transcription Factor 1 (RUNX1) Binds to p50 in Macrophages and Enhances TLR4-triggered Inflammation and Septic Shock. *J Biol Chem*, 291:22011-22020.
- [85] Pretorius E (2018). Erythrocyte deformability and eryptosis during inflammation, and impaired blood rheology. *Clin Hemorheol Microcirc*, 69:545-550.
- [86] Mozos I (2015). Mechanisms linking red blood cell disorders and cardiovascular diseases. *Biomed Res Int*, 2015:682054.
- [87] Metivier F, Marchais SJ, Guerin AP, Pannier B, London GM (2000). Pathophysiology of anaemia: focus on the heart and blood vessels. *Nephrol Dial Transplant*, 15 Suppl 3:14-18.
- [88] Lasocki S, Longrois D, Montravers P, Beaumont C (2011). Hepcidin and anemia of the critically ill patient: bench to bedside. *Anesthesiology*, 114:688-694.
- [89] Dreischer P, Duszenko M, Stein J, Wieder T (2022). Eryptosis: Programmed Death of Nucleus-Free, Iron-Filled Blood Cells. *Cells*, 11.
- [90] Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, et al. (2006). Enhanced programmed cell death of iron-deficient erythrocytes. *Faseb j*, 20:368-370.
- [91] Hershko C, Link G, Cabantchik I (1998). Pathophysiology of iron overload. *Ann N Y Acad Sci*,

- 850:191-201.
- [92] Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*, 149:1060-1072.
- [93] Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell*, 156:317-331.
- [94] Yang WS, Stockwell BR (2016). Ferroptosis: Death by Lipid Peroxidation. *Trends Cell Biol*, 26:165-176.
- [95] Jeney V (2018). Pro-Inflammatory Actions of Red Blood Cell-Derived DAMPs. *Exp Suppl*, 108:211-233.
- [96] Immenschuh S, Vijayan V, Janciauskiene S, Gueler F (2017). Heme as a Target for Therapeutic Interventions. *Front Pharmacol*, 8:146.
- [97] Lin S, Yin Q, Zhong Q, Lv FL, Zhou Y, Li JQ, et al. (2012). Heme activates TLR4-mediated inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage. *J Neuroinflammation*, 9:46.
- [98] Graça-Souza AV, Arruda MA, de Freitas MS, Barja-Fidalgo C, Oliveira PL (2002). Neutrophil activation by heme: implications for inflammatory processes. *Blood*, 99:4160-4165.
- [99] Sawicki KT, Shang M, Wu R, Chang HC, Khechaduri A, Sato T, et al. (2015). Increased Heme Levels in the Heart Lead to Exacerbated Ischemic Injury. *J Am Heart Assoc*, 4:e002272.
- [100] Kwon MS, Woo SK, Kurland DB, Yoon SH, Palmer AF, Banerjee U, et al. (2015). Methemoglobin is an endogenous toll-like receptor 4 ligand-relevance to subarachnoid hemorrhage. *Int J Mol Sci*, 16:5028-5046.
- [101] Guo H, Callaway JB, Ting JP (2015). Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med*, 21:677-687.
- [102] Eltayeb MM, Waggiallah HA, Hakami NY, Elmosaad YM (2023). Correlation between smoking and downregulation of red cell CD47 as eryptosis marker. *Eur Rev Med Pharmacol Sci*, 27:476-482.
- [103] Tarantino N, Santoro F, De Gennaro L, Correale M, Guastafierro F, Gaglione A, et al. (2017). Fenofibrate/simvastatin fixed-dose combination in the treatment of mixed dyslipidemia: safety, efficacy, and place in therapy. *Vasc Health Risk Manag*, 13:29-41.
- [104] Alonso R, Cuevas A, Cafferata A (2019). Diagnosis and Management of Statin Intolerance. *J Atheroscler Thromb*, 26:207-215.
- [105] Jang HJ, Woo YM, Naka K, Park JH, Han HJ, Kim HJ, et al. (2021). Statins Enhance the Molecular Response in Chronic Myeloid Leukemia when Combined with Tyrosine Kinase Inhibitors. *Cancers (Basel)*, 13.
- [106] Biswas D, Sen G, Biswas T (2010). Reduced cellular redox status induces 4-hydroxynonenal-mediated caspase 3 activation leading to erythrocyte death during chronic arsenic exposure in rats. *Toxicol Appl Pharmacol*, 244:315-327.
- [107] Biswas D, Sen G, Sarkar A, Biswas T (2011). Atorvastatin acts synergistically with N-acetyl cysteine to provide therapeutic advantage against Fas-activated erythrocyte apoptosis during chronic arsenic exposure in rats. *Toxicol Appl Pharmacol*, 250:39-53.
- [108] Koter M, Broncel M, Chojnowska-Jezierska J, Klikczynska K, Franiak I (2002). The effect of atorvastatin on erythrocyte membranes and serum lipids in patients with type-2 hypercholesterolemia. *Eur J Clin Pharmacol*, 58:501-506.
- [109] Coccia R, Spadaccio C, Foppoli C, Perluigi M, Covino E, Lusini M, et al. (2007). The effect of simvastatin on erythrocyte membrane fluidity during oxidative stress induced by cardiopulmonary bypass: a randomized controlled study. *Clin Ther*, 29:1706-1717.
- [110] Jackowska P, Pytel E, Koter-Michalak M, Olszewska-Banaszczyk M, Legęza A, Broncel M (2016). The Effect of Combined Ezetimibe/Atorvastatin Therapy vs. Atorvastatin Monotherapy on the Erythrocyte Membrane Structure in Patients with Coronary Artery Disease: A Pilot Study. *Adv Clin Exp Med*, 25:433-439.
- [111] Al Mamun Bhuyan A, Nüßle S, Cao H, Zhang S, Lang F (2017). Simvastatin, a Novel Stimulator of Eryptosis, the Suicidal Erythrocyte Death. *Cell Physiol Biochem*, 43:492-506.
- [112] Taimeh Z, Koene RJ, Furne J, Singal A, Eckman PM, Levitt MD, et al. (2017). Erythrocyte aging as a mechanism of anemia and a biomarker of device thrombosis in continuous-flow left ventricular assist devices. *J Heart Lung Transplant*, 36:625-632.
- [113] Smedira NG, Hoercher KJ, Lima B, Mountis MM, Starling RC, Thuita L, et al. (2013). Unplanned hospital readmissions after HeartMate II implantation: frequency, risk factors, and impact on resource use and survival. *JACC Heart Fail*, 1:31-39.
- [114] Vrtovec B, Radovancevic R, Delgado RM, Radovancevic B, Bracey AW, Gregoric ID, et al. (2009). Significance of anaemia in patients with advanced heart failure receiving long-term mechanical circulatory support. *Eur J Heart Fail*, 11:1000-1004.
- [115] Cikirikcioglu M, Ponchant K, Murith N, Meyer P, Yilmaz N, Huber C (2021). Treatment of HeartMate III-LVAD driveline infection by negative pressure wound therapy: Result of our case series. *Int J Artif Organs*, 44:912-916.
- [116] Hernandez RE, Singh SK, Hoang DT, Ali SW, Elayda MA, Mallidi HR, et al. (2015). Present-Day Hospital Readmissions after Left Ventricular Assist Device Implantation: A Large Single-Center Study. *Tex Heart Inst J*, 42:419-429.
- [117] Ashby W (1919). The determination of the length of life of transfused blood corpuscles in man. *J Exp Med*, 29:267-281.
- [118] Ashby W (1921). Study of transfused blood : i. the periodicity in eliminative activity shown by the organism. *J Exp Med*, 34:127-146.
- [119] Ashby W (1948). The span of life of the red blood cell; a resume. *Blood*, 3:486-500.
- [120] Zhang HD, Ma YJ, Liu QF, Ye TZ, Meng FY, Zhou YW, et al. (2018). Human erythrocyte lifespan measured by Levitt's CO breath test with newly developed automatic instrument. *J Breath Res*, 12:036003.
- [121] Callender ST, Powell EO, Witts LJ (1947). Normal red-cell survival in men and women. *J Pathol Bacteriol*,

- 59:519-532.
- [122] Mock DM, Matthews NI, Zhu S, Strauss RG, Schmidt RL, Nalbant D, et al. (2011). Red blood cell (RBC) survival determined in humans using RBCs labeled at multiple biotin densities. *Transfusion*, 51:1047-1057.
- [123] Kameyama M, Koga M, Okumiya T (2020). A novel method for calculating mean erythrocyte age using erythrocyte creatine. *Aging (Albany NY)*, 12:8702-8709.
- [124] Peng YF, Zhang ZX, Cao W, Meng CR, Xu SS, Zhang Q (2015). The association between red blood cell distribution width and acute pancreatitis associated lung injury in patients with acute pancreatitis. *Open Med (Wars)*, 10:176-179.
- [125] Yasin Z, Witting S, Palascak MB, Joiner CH, Rucknagel DL, Franco RS (2003). Phosphatidylserine externalization in sickle red blood cells: associations with cell age, density, and hemoglobin F. *Blood*, 102:365-370.
- [126] Strauss RG, Mock DM, Widness JA, Johnson K, Cress G, Schmidt RL (2004). Posttransfusion 24-hour recovery and subsequent survival of allogeneic red blood cells in the bloodstream of newborn infants. *Transfusion*, 44:871-876.
- [127] Ye L, Ji Y, Zhou C, Luo J, Zhang L, Jing L, et al. (2021). Comparison of Levitt's CO breath test and the (15) N-glycine labeling technique for measuring the lifespan of human red blood cells. *Am J Hematol*, 96:1232-1240.
- [128] Gao QY, Ye L, Zhang FK (2019). [Clinical application and significance of the technique detecting the lifespan of red blood cells]. *Zhonghua Xue Ye Xue Za Zhi*, 40:447-448.
- [129] Lopaschuk GD, Verma S (2020). Mechanisms of Cardiovascular Benefits of Sodium Glucose Co-Transporter 2 (SGLT2) Inhibitors: A State-of-the-Art Review. *JACC Basic Transl Sci*, 5:632-644.
- [130] Vota DM, Crisp RL, Nesse AB, Vittori DC (2012). Oxidative stress due to aluminum exposure induces eryptosis which is prevented by erythropoietin. *J Cell Biochem*, 113:1581-1589.
- [131] Sun Y, Liu G, Jiang Y, Wang H, Xiao H, Guan G (2018). Erythropoietin Protects Erythrocytes Against Oxidative Stress-Induced Eryptosis In Vitro. *Clin Lab*, 64:365-369.
- [132] Sato T, Tanno M, Miki T, Yano T, Sato T, Shimamoto K, et al. (2010). Erythropoietin (EPO) affords more potent cardioprotection by activation of distinct signaling to mitochondrial kinases compared with carbamylated EPO. *Cardiovasc Drugs Ther*, 24:401-408.
- [133] Gao D, Ning N, Niu X, Dang Y, Dong X, Wei J, et al. (2012). Erythropoietin treatment in patients with acute myocardial infarction: a meta-analysis of randomized controlled trials. *Am Heart J*, 164:715-727.e711.
- [134] Ghashghaeinia M, Toulany M, Saki M, Bobbala D, Fehrenbacher B, Rupec R, et al. (2011). The NFκB pathway inhibitors Bay 11-7082 and parthenolide induce programmed cell death in anucleated Erythrocytes. *Cell Physiol Biochem*, 27:45-54.
- [135] Ghashghaeinia M, Cluitmans JC, Toulany M, Saki M, Köberle M, Lang E, et al. (2013). Age sensitivity of NFκB abundance and programmed cell death in erythrocytes induced by NFκB inhibitors. *Cell Physiol Biochem*, 32:801-813.