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Review article

Recent advances and clinical applications of red blood cell lifespan measurement

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ABSTRACT

The red blood cell (RBC) lifespan is a crucial indicator used in clinical diagnostics, treatment, and disease monitoring. This biomarker quantifies the duration that red blood cells (RBCs) circulate within the bloodstream after being released from the bone marrow, serving as a sensitive and direct indicator of red blood cell turnover. Conventional techniques for RBC lifespan measurement, including differential agglutination, ^{51}Cr labeling, and ^{15}N glycine labeling, each present their own set of challenges, such as complexity, radioactive exposure, and potential allergic reaction. The carbon monoxide (CO) breath test has emerged as an advanced and non-invasive alternative, indirectly assessing RBC lifespan through hemoglobin (Hb) renewal rates. This method is convenient, rapid, and lacks the drawbacks of traditional approaches. The CO breath test for RBC lifespan is widely utilized in benign anemia, malignant hematological disorders, neonatal hyperbilirubinemia, and diabetes mellitus, offering valuable insights into disease mechanisms, progression, and treatment outcomes.

1. Background

The red blood cell (RBC) lifespan refers to the duration that RBC circulate in the peripheral blood post their release from the bone marrow. In healthy adults, the RBC lifespan averages around 120 days, with a range of 70–140 days [1]. However, this equilibrium can be disrupt under various pathological conditions, leading to increased destruction of RBCs and a shortened RBC lifespan. The measurement of RBC lifespan is valuable for understanding the maturation, survival, and clearance of RBCs in circulation. It provides insights into the mechanisms underlying anemia and helps evaluate the efficacy of anemia treatments [2].

Several methods are commonly used to detect RBC lifespan, including the radioisotope assay (^{51}Cr labeling method) [3], stable isotope method (^{15}N-glycine labeling method) [4], biotin method [5], RBC population dynamics [6,7] and carbon monoxide (CO) breath test method [8]. The radioisotope labeling method, mainly the ^{51}Cr labeling method, has disadvantages, including the

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requirement for multiple blood collections, cumbersome operation, extended measurement cycles, radioactive hazards, and high cost associated with radioactive waste disposal. The stable isotope labeling method primarily involves ^{15}N-glycine labeling. However, it has the disadvantage of labeling both Hb and other proteins, which can be reused by new RBCs. The biotin labeling method utilizes biotin as a marker, but it is hindered by being time-consuming, complicated and prone to allergies. In contrast, the CO breath test is an important approach to measuring the RBC lifespan. It is characterized by its simplicity, rapidity, non-invasiveness, repeatability, as well as its high accuracy and sensitivity. Furthermore, it can accurately reflect the severity of diseases.

Research and applications related to RBC lifespan primarily focus on treatment of various disease including benign anemia, malignant hematologic diseases, neonatal hyperbilirubinemia, and diabetes mellitus. For example, treatment with ribavirin for chronic hepatitis C has been associated with a reducted RBC lifespan and hemolytic anemia [9]. Additionally, immunotherapy has shown promise in improving RBC lifespan in patients with severe aplastic anemia [10]. Patients with thalassemia experience fluctuations in RBC lifespan following blood transfusions [11]. Chronic kidney disease (CKD) also influence RBC lifespan [12]. The extent of RBC lifespan shortening is positively correlated with the severity of CKD [13]. In microcytic hypochromic anemia, variations in RBC lifespan aid in distinguishing between iron deficiency anemia (IDA) and thalassemia(Thal) [14]. Iron removal therapy can improve RBC lifespan in patients with myelodysplastic syndrome [15]. Acute leukemia patients exhibit different RBC lifespan patterns based on subtype and disease status [16]. Additionally, RBC lifespan plays a role in neonatal care, influencing decisions regarding discharge and phototherapy intervals [17]. In diabetes, RBC lifespan reductions contribute to underestimating of glycated Hb levels [18].

Overall, RBCS measurement serves as a valuable tool across various medical contexts, offering insights into disease progression, treatment efficacy, and patient management.

2. Detection methods of RBC lifespan

2.1. Direct methods

The radioisotope method primarily utilizes ^{51}Cr labeling [3]. This ^{51}Cr marker labels RBCs at all stage, from neonatal to senescent, during the mixing of blood with radioactive sodium chromate in vitro. Hexavalent chromium ions penetrate the RBC membrane, enter the RBCs, and are reduced to trivalent chromium ions, and attached to the globin of hemoglobin molecules. After RBCs destruction, the trivalent chromium ions released into the bloodstream and cannot be reused, thus avoiding the impact on the test results. This method offers advantages such as avoiding repeated labeling and demonstrating good reproducibility. However, it entails multiple blood collections, intricate procedures, lengthy determination cycles, and concerns regarding radioactive hazards and disposal costs.

The stable isotope labeling method predominantly employs ^{15}N-glycine labeling [4]. The ^{15}N-glycine marker label the same stage RBCs. Since the four nitrogen atoms of Hb are derived from glycine, ^{15}N-glycine is incorporated into in Hb synthesis throughout the RBCs life cycle as a substrate, thus labeling the RBCs. The labeled RBCs develop into reticulocytes and are released into the peripheral circulation, where they subsequently differentiate into mature RBCs. Consequently, the intracellular levels of ^{15}N-glycine in RBCs gradually increase as they mature. Studies have shown that ^{15}N-glycine in RBCs reaches 50 % of the total marker within 2.8–6.3 days after labeling and peaks at 20–25 days post-labeling. This method presents the benefit of non-radioactivity and oral administration for RBC labeling. Nevertheless, it also labels other proteins besides Hb, affecting test outcomes and requiring analysis by a mass spectrometry analyzer.

The biotin labeling method utilizes biotin as a marker [5]. Biotin labels RBCs in vitro by covalently binding to the RBC membrane proteins. After transfusion of the biotinylated RBCs (BioRBCs) into the test subject, peripheral blood is collected at different time. Using the strong biotin-avidin affinity, BioRBCs specifically are bound to fluorescein-containing avidin or streptavidin, and then the number of BioRBCs with fluorescein is measured by flow cytometry (FCM). The percentage of BioRBCs relative to the total RBCs was calculated at different time. By comparing the percentage of BioRBCs in the initial sample (1–2 h post-infusion) with subsequent samples, the RBCs survival rate at each time point can be determined. This approach provides stable isotope labeling that can be covalently bind to membrane proteins with minimal detachment from RBCs. It facilitates the labeling of RBCs populations of different ages using various concentrations or types of biotin, enabling simultaneous detection of diverse RBC populations. However, this method is time-consuming, involves complex perform, and may provoke allergies reactions. Table 1 presents a comparison of the advantages and disadvantages of these detection methods.

Table 1
Methodological comparison of direct method.

	Radioisotope method	Stable isotope method	Biotin labeling
Accuracy	Accurate	Accurate	Accurate
Steady-state Detection	Applicable	Applicable	Applicable
Dynamic Detection	Not applicable	Not applicable	Not applicable
Multiple blood collections	Yes	Yes	Yes
Allergy risk	No	No	Yes
Radiation injury	Yes	No	No
Analysis time	About four weeks	About four weeks	About four weeks
References	[3]	[4]	[5]

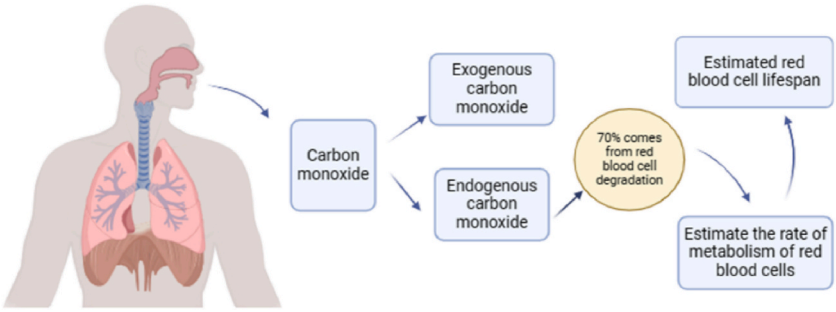

Fig. 1. How the carbon monoxide breath test works.

Table 2
Methodological comparison of indirect method.

	CO breath method	RBC population dynamics
Accuracy	Accurate	Accurate
Steady-state Detection	Applicable	Applicable
Dynamic Detection	Applicable	Not applicable
Multiple blood collections	No	Yes
Allergy risk	No	No
Radiation injury	No	No
Analysis time	No more than 5 minutes	Long time for digital modeling
References	[8]	[6,7]

2.2. Indirect methods

There are three sources of CO in the exhaled breath of the human body: endogenous heme degradation and non-heme metabolism, and exogenous inhalation. 86 % of endogenous CO comes from heme degradation, and no more than 14 % of CO is produced by non-heme metabolism. 85 % of the heme that produces CO comes from the degradation of hemoglobin in RBCs, and 15 % comes from non-RBC heme. Therefore, about 70 % of endogenous CO comes from the degradation of RBCs, and the rate of CO excretion in the lungs can be deduced from the rate of destruction of RBCs under the premise of excluding exogenous interference, and RBC lifespan can be extrapolated at this rate. It is calculated as follows: RBC lifespan (days) = hemoglobin concentration (unit: g/L) × 1.38/exhaled endogenous CO concentration (ppm). The CO breath test as a prominent indirect method for measuring RBC lifespan [8], based on Hb renewal rates. Endogenous CO in human exhaled gas, comprising about 70 % from RBCs degradation, serves as a key component of this method. When Hb breaks down into bilirubin, the α-methylene carbon in Hb produces CO, with one molecule of Hb produceing one molecule of CO. By assessing the concentration of endogenous CO in exhaled gas, independent of exogenous CO influences, the metabolism rate of RBCs can be inferred, allowing for the derivation of RBC lifespan (as illustrated in Fig. 1). This method offers several advantages, including simplicity, rapidity, noninvasiveness, ease of repetition, high accuracy, sensitivity, and the ability to accurately reflect disease severity. However, factors such as smoking, vigorous exercise, and impaired lung function can influence results, rendering the method inapplicable to such individuals. RBC population dynamics, linked to variations RBC distribution width (RDW), is an indirect method [7]. Increased RDW can result from one of the following four possibilities: 1) decreased mean corpuscular volume (MCV), 2) increased variance in reticulocyte volume, 3) increased heterogeneity in the rate of RBC volume reduction in the peripheral circulation, and 4) delayed RBC clearance. Recent studies have demonstrated that an increased RDW is associated with a slight shortening of RBC lifespan [6]. Table 2 provides a detailed overview of the specific advantages and disadvantages of those approach.

2.3. Other methods

It is well established that RBCs production is controlled by erythropoietin (EPO), low oxygen levels increase the concentration of EPO in the blood, which accelerates the proliferation and differentiation of erythroid precursors in the bone marrow, boosting the number of RBCs. The rise in the concentration of EPO in response to low oxygen levels would lengthen RBC lifespan, reducing the rate of RBCs destruction. An excess of oxygen inhibits EPO production, which delays the recruitment of new RBCs into the blood. Low EPO levels would shorten RBC lifespan, increasing the destruction of RBCs in the liver and the spleen. The effect of EPO on CD47 and SIRP-α would also lead to changes in the autoimmunity threshold. High EPO levels might upregulate CD47 in newly formed RBCs and SIRP-α in macrophages, thus lengthening the lifespan of circulating RBCs, while hyperoxia would have the opposite effect. However, the deeper mechanisms of immunity between EPO and CD47, SIRP-α are not clearly. Recent studies have demonstrated that using FCM to detect markers related to the RBC lifespan can effectively reflect RBC lifespan. One study showed that EPO could influence RBC lifespan by regulating the expression of CD47 in newly formed RBCs and SIRP-α in sinusoidal macrophages. EPO can also modulate the onset and intensity of anti-RBC autoimmune responses, thereby shortening the RBC lifespan [19]. Previous research have shown that CD47 regulates the phagocytosis of macrophages, affects RBC lifespan, and modulats immune cell activation through interactions with

SIRPα, TSP-1 and integrins [20]. These interactions are crucial for maintaining RBC homeostasis and preventing premature destruction. Additionally, another study found that increased anti-band-3 antibodies in serum lead to increased binding of RBC membranes to IgG resulting in their premature clearance from circulation [21,22]. As researchers continue to explore the roles of EPO, CD47,anti-band-3 in RBCs, these new markers can help us better understand the role of RBC lifespan in clinical practice.

3. Application of RBC lifespan in different diseases

3.1. Effect of therapeutic drugs and treatment modalities on RBC lifespan

Virtue et al. divided chronic hepatitis C patients into two groups: those undergoing ribavirin treatment and those without ribavirin treatment [9]. Their results indicated a significant reduction in RBC lifespan among patients receiving ribavirin, leading to the emergence of hemolytic anemia. This suggests that ribavirin antiviral treatment for chronic hepatitis C may shorten RBC lifespan. Ye et al. utilized the CO breath test to assess RBC lifespan in patients with severe/very severe aplastic anemia (SAA/VSAA). They observed significantly shorter RBC lifespan in untreated SAA/VSAA patients compared to healthy individuals. However, immunosuppressant therapy improved RBC lifespan duration, with some patients returning to normal levels. This indicates that immunosuppressant treatment can enhance RBC lifespan and serve as an efficacy indicator during SAA/VSAA treatment. Li et al. studied RBC lifespan changes during blood transfusion therapy in Thal patients, noting a significant increase in RBC lifespan and reduced end-expiratory CO concentration in the 1st to 2nd week post-transfusion. However, RBC lifespan gradually shortened after the 3rd to 4th weeks. This highlights RBC lifespan as an evaluation marker for transfusion therapy in Thal patients. Li. et al. also observed gradual RBC lifespan shortening in CKD patients across stages 1–5 [12], with better nutritional status correlating to longer RBC lifespan. Hemodialysis (HD) patients with CKD stages 1–4 and improved nutritional status exhibited extended RBC lifespan. Additionally, roxadustat was found to enhance Hb levels in HD patients [13,23] with the roxadustat-treated group showing longer RBC lifespan compared to the rhuEPO-treated group [24]. Jiang et al. demonstrated that a single hemodialysis filtration (HDF) treatment reduced plasma concentrations of indole sulphate phenol (IS), leading to prolonged RBC lifespan in end-stage renal disease (ESRD) patients on HD [25]. Moreover, Zhao et al. identified diabetes mellitus with nephropathy as an independent risk factor for RBC lifespan shortening in continuous peritoneal dialysis (PD) patients, emphasizing the importance of regular RBC lifespan monitoring and a structured PD regimen for ESRD patients [26].

3.2. Effect of mechanical injury on RBC lifespan

The mechanical hemolysis observed in non-physiological flow conditions has been attributed to the periodic deformation of RBCs as they traverse confined spaces. This deformation triggers the release of adenosine triphosphate (ATP) from the cells. A subsequent decline in cellular ATP levels results in RBC fatigue, morphological alterations, and a diminished capacity for deformation, ultimately culminating in hemolysis [27,28]. Mitlyng et al. measured RBC lifespan in patients with implanted mechanical valves and prosthetic bioprosthetic valves using the CO breath test. They found that RBC lifespan in these patients was significantly shorter compared to healthy individuals. Despite normal Hb levels, both types of valves were associated with hemolysis [29]. Luo et al. investigated the impact of different dialysis membranes on RBC lifespan in CKD patients. Their study using the CO breath test showed that polysulfone dialysis membranes did not affect RBC lifespan in end-stage renal disease patients before and after dialysis [30].

3.3. Effect of increased erythropoiesis on RBC lifespan

Phubu et al. studied RBC lifespan in high-altitude polycythemia (HAPC) patients, finding significantly shorter RBC lifespan in Tibetan HAPC patients compared to healthy individuals, while Han Chinese patients showed RBC lifespan levels similar to healthy individuals [31]. They also noted a negative correlation between RBC lifespan and serum VitB12 levels. RBC mono-collecting treatment prolonged RBC lifespan in Tibetan HAPC patients, making RBC lifespan a useful assessment index for HAPC treatment. Zhang et al. observed significantly shortened RBC lifespan in patients with polycythemia vera (PV) compared to healthy controls [32]. The increased peripheral blood Hb in PV patients primarily resulted from enhanced erythropoiesis rather than prolonged RBC lifespan, suggesting a compensatory organism response. Hereditary spherocytosis (HS) is a common hereditary hemolytic anemia. Shi et al. found significantly shortened RBC lifespan in HS patients compared to healthy individuals, highlighting RBC lifespan as a direct indicator of hemolysis severity in HS diagnosis and disease assessment [33].

3.4. Effect of chronic infection on RBC lifespan

Infections can destroy RBCs through various mechanisms. For example, infected microorganisms can cause hemolysis either by directly destroyin or by triggering immune responses [34,35]. Mitlyng et al. conducted a CO breath test to evaluate RBC lifespan in patients with rheumatoid arthritis (RA), osteoarthritis (OA), and anemia of chronic disease. They found that RBC lifespan was significantly shorter in the RA group and the anemia of chronic disease group compared to healthy controls and OA patients. However, RBC lifespan in OA patients was not shorter than in healthy controls, indicating that OA does not induce hemolysis [36].

3.5. Effect of insufficient hematopoietic raw materials and hereditary diseases on RBC lifespan

IDA and Thal are both microcytic hypochromic anemia (MHA), making RBCs differentiation challenges. Iron deficiency reduces catalase and glutathione peroxidase activities, causing oxidative damage to cell membranes, poor deformability of RBCs, and a shortened lifespan. Thal, caused by a single nucleotide mutation, results in decreased production of globin chain and adult hemoglobin (HbA), leading to excess unstable globin tetramers in erythroid cells. These unstable globin generates cytotoxic reactive oxygen species and cell precipitates, impairing maturation and viability of RBC precursor cells, resulting in ineffective erythropoiesis and premature destruction of RBCs in the circulatory system. Lei et al. conducted an RBC lifespan study in patients with these conditions and found that MHAs with normal RBC lifespan levels were indicative of IDA, whereas significant RBC lifespan shortening without chronic gastrointestinal bleeding indicated Thal [37]. RBC lifespan plays a crucial role in distinguishing between IDA and Thal. James et al. compared end-expiratory CO concentrations in patients with Thal, sickle cell anemia (SCA), and healthy individuals. They observed higher CO concentrations in SCA patients, followed by Thal patients, and the lowest levels in healthy individuals [38,39]. This study suggests varying extent of RBC lifespan shortening among different types of anemia, consistent with previous research by Lal [40], Sylvester [41], and Suriyun et al. [42]. Kang et al. discovered that patients with Gilbert's syndrome (GS) exhibited significantly smaller mean RBC lifespan compared to normal reference values, with lower end-expiratory CO concentrations. CO concentration inversely correlated with RBC lifespan and positively correlated with anemia severity [43,44]. Wang et al. investigated RBC lifespan in patients with hemophagocytic syndrome, finding a 65 % shorter mean RBC lifespan than healthy individuals, with Hb levels positively linked to RBC lifespan and CO concentration inversely correlated with RBC lifespan. Increased RBC destruction and shortened lifespan are critical mechanisms contributing to anemia development in hemophagocytic syndromes [45].

3.6. Application of RBC lifespan in malignant hematologic diseases

The presence of malignant tumors within the hematological system often leads to alterations in RBC lifespan. Pei et al. investigated RBC lifespan in patients with malignant lymphoma and anemia, noting a 46.7 % incidence of anemia with 40.0 % of patients experiencing shortened RBC lifespan [46]. Splenomegaly and elevated lactate dehydrogenase (LDH) levels were significantly associated with decreased RBC lifespan, exacerbated by intense chemotherapy regimens that further reduced RBC lifespan and aggravated anemia. Wang et al. studied RBC lifespan in patients with multiple myeloma (MM), finding significantly shorter RBC lifespan compared to healthy individuals, with negative correlations observed between RBC lifespan and reticulocyte levels, EPO, clonal plasma cells, β2-microglobulin (MG), and creatinine (Cr) levels [47]. Zhang et al. explored the impact of iron overload (IO) on RBC lifespan in patients with myelodysplastic syndromes (MDS), revealing shortened RBC lifespan with IO, which improved with iron chelation therapy (ICT), leading to reduced inflammatory cytokine expression and prolonged RBC lifespan [15]. Yuan et al. assessed RBC lifespan in acute leukemia (AL) patients [16], finding shorter RBC lifespan in males compared to females, with varying RBC lifespan among different AL types and minimal residual disease (MRD) status. Additionally, studies have shown that patients with acute myelogenous leukemia (AML), MM, MDS, and primary myelofibrosis (PMF) exhibit shorter RBC lifespan compared to control group, and PMF patients experiencing the most pronounced RBC lifespan shortening [48,49].

3.7. RBC lifespan in neonatal hyperbilirubinemia

In the relatively hypoxic environment of the womb, the fetus produces extra RBCs to compensate. After birth, as the baby begins breathing oxygen, the excess RBCs are rapidly destroyed, leading to an overload of bilirubin. The newborn's liver cannot process all this bilirubin quickly, causing jaundice and a shortened RBC lifespan. The CO breath test is currently recommended by the American Academy of Pediatrics for diagnosing neonates with or without clinical hemolysis and hyperbilirubinemia. According to the September 2022 Guidelines of the American Academy of Pediatrics Hyperbilirubinemia Group and the Expert Consensus on the Diagnosis and Treatment of Neonatal Hyperbilirubinemia [50], RBC lifespan serves as a crucial indicator for neonatal hospital discharge decisions. Neonates with low-risk RBC lifespan measurements can be discharged, while those with moderate to high-risk measurements require hospital observation. RBC lifespan also aids in diagnosing rebound bilirubin concentrations and determining appropriate phototherapy intervals [17]. Bhutani et al. demonstrated that hemolyzed neonates and children had significantly higher end-expiratory CO concentrations and lower RBC lifespan compared to healthy children of the same age [51], improving the detection rate of neonatal hyperbilirubinemia through RBC lifespan testing. Ni et al. observed similar findings, highlighting RBC lifespan as a valuable diagnostic tool for neonatal hyperbilirubinemia [52].

3.8. Application of RBC lifespan in diabetes mellitus

Long-term exposure to high glucose levels induce oxidative stress, damaging RBC membranes and shortening their lifespan. Because of this, RBC lifespan has become a valuable biomarker for assessing glycemic control. Hyperglycemia significantly impacts RBC lifespan in diabetes mellitus patients. Studies by Huang and others have shown a notable reduction in RBC lifespan among diabetic individuals [18], with fasting glucose levels exerting the most significant influence. Poor glycemic control exacerbates RBC lifespan shortening, as observed by Zhang et al., who found a negative correlation between RBC lifespan and fasting glucose levels, particularly affecting women's RBC lifespan [53]. This shortened RBC lifespan can lead to underestimation of glycated hemoglobin (HbA1c) levels, impacting accurate diagnosis and treatment. One study on HbA1c and RBC lifespan in type II diabetic patients corroborates these findings, indicating that hyperglycemia causes RBC lifespan shortening and distorts HbA1c values [54]. Chu et al. further confirmed

Table 3
The relationship between diseases and RBC lifespan in comparison to healthy individuals.

Diseases	RBC lifespan	Results	Hemolysis causes	References
Hepatitis C	Short	Hemolysis	Increased inflammatory cytokines	[9]
Chronic kidney disease	Short	Hemolysis	Increased inflammatory cytokines	[12]
Implantation of valves	Short	Hemolysis	Activated coagulation	[29]
High-altitude polycythemia (Tibetan)	Short	Hemolysis	Intracellular hypoxia	[31]
High-altitude polycythemia (Han Chinese)	Normal	Normal	Intracellular hypoxia	[31]
Polycythemia vera	Short	Hemolysis	Hypersplenism	[32]
Hereditary spherocytosis	Short	Hemolysis	Abnormalities in RBC membranes	[33]
Osteoarthritis	Short	Hemolysis	Increased inflammatory cytokines	[36]
Rheumatoid arthritis	Short	Hemolysis	Increased inflammatory cytokines	[36]
Iron deficiency anemia	Short	Hemolysis	Intracellular iron deficiency	[37]
Tthalassemia	Short	Hemolysis	Impaired globin production	[37]
Sickle cell anemia	Short	Hemolysis	Abnormal amino acid structure	[38,39]
Gilbert's syndrome	Short	Hemolysis	UTG1A1 mutation	[43,44]
Hemophagocytic syndrome	Short	Hemolysis	Enhanced CTL and NK cell-mediated killing	[45]
Acute myeloid leukemia (AML)	Short	Hemolysis	Morbid hematopoiesis	[35]
Multiple myeloma (MM)	Short	Hemolysis	Morbid hematopoiesis	[35]
Myelodysplastic syndrome (MDS)	Short	Hemolysis	Morbid hematopoiesis	[35]
Primary myelofibrosis (PMF)	Short	Hemolysis	Morbid hematopoiesis	[35]
Neonatal Hyperbilirubinemia	Short	Hemolysis	Oxidative stress	[50–52]
Diabetes Mellitus	Short	Hemolysis	Oxidative stress	[56]

the relationship between RBC lifespan and HbA1c through CO breath testing, showing a positive correlation between HbA1c levels and RBC lifespan, independent of gender or age [55]. Mao et al. explored RBC lifespan, HbA1c, and pancreatic β-cell function in various glucose-tolerant populations and diabetes mellitus patients. They found significantly lower RBC lifespan in impaired glucose regulation (IGR) and type II diabetes mellitus patients compared to normal individuals, with RBC lifespan further decreasing with diabetes progression [56]. The relationship between diseases and RBC lifespan is shown in Table 3.

4. Summary and prospects

4.1. Summary

RBC lifespan can be assessed through various methods including the radioisotope method, stable isotope method, biotin method, RBC population dynamics [6,7] and CO breath test. Various studies have shown that BioRBCs has significant advantages over radioisotope method(^{51}Cr)in terms of method, analysis and safety [57–59]. All kinds of labeling methods mentioned in this paper take about a month to get results, and the labeling method is only suitable for the period when the body's erythropoiesis and destruction rate is stable, and is not applicable when there is a dynamic change [60]. Advancements in detection technology have reduced the drawbacks of older methods, such as cumbersome operations, long cycle times, radiation hazards, and allergy risks. The emerging CO breath test requires very simple equipment and only necessitates the collection of exhaled air from patients. Multiple acquisitions are possible, there is no harm to the patients, and it does not take more than 5 minutes from blowing to getting the result. This indirect detection method offers simplicity, speed, non-invasiveness, repeatability, high accuracy, and sensitivity, effectively reflecting disease severity. In addition, as newly discovered markers of RBC lifespan, the advantage and disadvantage of EPO, CD74, and anti-band-3, need to be summarized by a large studies. RBC lifespan testing holds significant clinical value, aiding in the diagnosis of anemia, enhancing the accuracy of HbA1c measurements in diabetic patients, and gauging anemia severity in malignant hematological diseases and neonatal hyperbilirubinemia. In summary, the advancements in RBC lifespan assessment methods, especially the popular of the CO breath test, have provided an important approache for monitoring treatment efficacy in various diseases. This method offer enhanced efficiency, accuracy, and safety, contributing significantly to the management and treatment of various blood-related disorders.

4.2. Challenges and prospects

Certain factors influencing RBC lifespan remain unclear, leading to divergent research results among scholars. For example, some experts believe that the size of RBC become smaller as the aging, and a slight reduction in the rate of RBC turnover allows smaller cells to continue circulating, thereby increasing RBC lifespan, but others take the opposite attitude. Over the past few decades, most doctors considered that RBC, Hb, Mean corpsular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) are better than RBC lifespan at diagnosing hemolysis and anemia. Some medical professionals still have reservations about RBC lifespan and its detection methods. However, as testing technology continues to advance, more and more doctors are recognizing the importance of RBC lifespan. Future research could explore additional factors such as climate, mood, basal metabolic rate, and intestinal microenvironment to comprehensively understand RBC lifespan. Publicizing and explaining existing research outcomes could aid in enhancing acceptance. Additionally, accurate RBC lifespan detection relies on a comprehensive and robust quality control system. Establishing standardized operating procedures (SOPs), strictly implementing SOPs, and refining quality control systems will elevate the accuracy

of test result. This will provide clearer clinical guidance for diagnosis and treatment.

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Ethics statement

Not applicable.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] H. Shijin, Y. Jie, Y. Yongbin, L. Jie, Detection of red blood cell lifespan and its clinical application, journal of Central South University(Medical Science) 49 (3) (2024) 476–481.
[2] Q.Y. Xuepeng Zhang, Analysis of influencing factors of erythrocyte lifespan in patients with acute leukemia, Chongqing Medicine 53 (11) (2024) 1684–1689.
[3] A.W. Hutcheon, P.W. Horton, J.S. Orr, J.H. Dagg, The assessment of red cell survival in normal subjects and in patients with haemolytic disorders and ineffective erythropoiesis using the radioiron occupancy method, Br. J. Haematol. 37 (2) (1977) 195–205, https://doi.org/10.1111/j.1365-2141.1977.tb06835.x.
[4] P.K. Khera, E.P. Smith, C.J. Lindsell, et al., Use of an oral stable isotope label to confirm variation in red blood cell mean age that influences hba1c interpretation, Am. J. Hematol. 90 (1) (2015) 50–55, https://doi.org/10.1002/ajh.23866.
[5] R.P. Shrestha, J. Horowitz, C.V. Hollot, et al., Models for the red blood cell lifespan, J. Pharmacokinet. Pharmacodyn. 43 (3) (2016) 259–274, https://doi.org/10.1007/s10928-016-9470-4.
[6] H.H. Patel, H.R. Patel, J.M. Higgins, Modulation of red blood cell population dynamics is a fundamental homeostatic response to disease, Am. J. Hematol. 90 (5) (2015) 422–428, https://doi.org/10.1002/ajh.23982.
[7] J.M. Higgins, Red blood cell population dynamics, Clin. Lab. Med. 35 (1) (2015) 43–57, https://doi.org/10.1016/j.cll.2014.10.002.
[8] S.M. Krishnan, N.M. Dixit, Estimation of red blood cell lifespan from alveolar carbon monoxide measurements, Transl. Res. 154 (1) (2009) 15–17, https://doi.org/10.1016/j.trsl.2009.04.002.
[9] M.A. Virtue, J.K. Furne, S.B. Ho, M.D. Levitt, Use of alveolar carbon monoxide to measure the effect of ribavirin on red blood cell survival, Am. J. Hematol. 76 (2) (2004) 107–113, https://doi.org/10.1002/ajh.20069.
[10] L. Ye, L. Jing, J. Guo, et al., Red blood cell lifespan is reduced in severe aplastic anemia and improves with response to immunosuppressive treatment, Am. J. Hematol. 96 (11) (2021) E441–E443, https://doi.org/10.1002/ajh.26344.
[11] Y.C.Z.T. Jing Li, Research progress of eastern mediterranean combined with auto or allogeneic antibodies, Chinese and Foreign Medical Research 22 (2) (2024) 181–184.
[12] J.H. Li, J.F. Luo, Y. Jiang, et al., Red blood cell lifespan shortening in patients with early-stage chronic kidney disease, Kidney Blood Pressure Res 44 (5) (2019) 1158–1165, https://doi.org/10.1159/000502525.
[13] B. Zhao, X. Yang, W. Li, et al., Effect of roxadustat on red blood cell lifespan in patients with long-term haemodialysis: a single-centre, prospective, single-arm study, Clin. Kidney J. 16 (9) (2023) 1500–1507, https://doi.org/10.1093/ckj/sfad080.
[14] D.P. Wu, J. Bai, S.L. Chu, et al., Clinical Value of Erythrocyte Lifespan in Common Erythrocyte Diseases and its Relationship with Related Indicators, Lanzhou University, Lanzhou, 2022, p. 64.
[15] Y. Zhang, Y. Qian, L.X. Song, C. Xiao, C.K. Chang, The use of carbon monoxide breath test to detect the effect of iron overload on erythrocyte lifespan in mds, Front. Oncol. 122022) 1058482, https://doi.org/10.3389/fonc.2022.1058482.
[16] Y.Q.Y. Qian, A Study of the Lifespan of Red Blood Cells in Patients with Acute Leukemia, Lanzhou University., Lanzhou, 2023, p. 46.
[17] S.S. Coquery, A. Georges, A. Cortey, et al., Discharge of newborns with risk factors of severe hyperbilirubinemia: description of a hospital at home-based care monitoring and phototherapy, Eur. J. Pediatr. 181 (8) (2022) 3075–3084, https://doi.org/10.1007/s00431-022-04461-4.
[18] Z. Huang, Y. Liu, Y. Mao, W. Chen, Z. Xiao, Y. Yu, 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 (2) (2018) 26004, https://doi.org/10.1088/1752-7163/aa9081.
[19] C.F. Arias, N. Valente-Leal, F. Bertocchini, S. Marques, F.J. Acosta, C. Fernandez-Arias, A new role for erythropoietin in the homeostasis of red blood cells, Commun, Biol. 7 (1) (2024) 58, https://doi.org/10.1038/s42003-023-05758-2.
[20] A.C. Gomes, A.C. Moreira, T. Silva, et al., Ifn-gamma-dependent reduction of erythrocyte life span leads to anemia during mycobacterial infection, J. Immunol. 203 (9) (2019) 2485–2496, https://doi.org/10.4049/jimmunol.1900382.
[21] S. Kawamoto, [Identification of the target molecule of autoantibodies in acquired autoimmune hemolytic anemia], Rinsho Ketsueki 61 (5) (2020) 491–501, https://doi.org/10.11406/rinketsu.61.491.
[22] A. Kitao, S. Kawamoto, K. Kurata, et al., Band 3 ectopic expression in colorectal cancer induces an increase in erythrocyte membrane-bound igg and may cause immune-related anemia, Int. J. Hematol. 111 (5) (2020) 657–666, https://doi.org/10.1007/s12185-020-02831-y.
[23] J. Barratt, F. Dellanna, J. Portoles, et al., Safety of roxadustat versus erythropoiesis-stimulating agents in patients with anemia of non-dialysis-dependent or incident-to-dialysis chronic kidney disease: pooled analysis of four phase 3 studies, Adv. Ther. 40 (4) (2023) 1546–1559, https://doi.org/10.1007/s12325-023-02433-0.
[24] X. Yang, B. Zhao, J. Wang, et al., Red blood cell lifespan in long-term hemodialysis patients treated with roxadustat or recombinant human erythropoietin, Ren. Fail. 43 (1) (2021) 1428–1436, https://doi.org/10.1080/0886022X.2021.1988968.

[25] Y. Jiang, J.H. Li, J.F. Luo, et al., Hemodiafiltration improves red blood cell lifespan in patients with end-stage renal disease, Semin. Dial. 35 (3) (2022) 215–221, https://doi.org/10.1111/sdi.13037.

[26] H.Z.B.W. Yuchao Zhao, Analysis of erythrocyte lifespan and its influencing factors in patients undergoing maintenance peritoneal dialysis, Chin J Blood Purification 21 (5) (2022) 322–325.

[27] X. Zhang, F. Florini, J.E. Visone, et al., A coordinated transcriptional switching network mediates antigenic variation of human malaria parasites, Elife 11 (14) (2022) e83840, https://doi.org/10.7554/eLife.83840.

[28] B.S. Ferguson, L.E. Neidert, M.J. Rogatzki, K.R. Lohse, L.B. Gladden, H.A. Kluess, Red blood cell atp release correlates with red blood cell hemolysis, Am. J. Physiol. Cell Physiol. 321 (5) (2021) C761–C769, https://doi.org/10.1152/ajpcell.00510.2020.

[29] B.L. Mitlyng, Y. Chandrashekhar, J.K. Furne, M.D. Levitt, Use of breath carbon monoxide to measure the influence of prosthetic heart valves on erythrocyte survival, Am. J. Cardiol. 97 (9) (2006) 1374–1376, https://doi.org/10.1016/j.amjcard.2005.11.074.

[30] J.F. Luo, J.H. Li, J.J. Nie, P.P. Li, H.D. Zhang, Y.J. Ma, Effect of hemodialysis on the red blood cell life span in patients with end-stage kidney disease, Ther. Apher. Dial. 23 (4) (2019) 336–340, https://doi.org/10.1111/1744-9987.12775.

[31] F.J.L.B. Pu Buwangdui, Detection of red blood cell longevity in patients with high-altitude erythrocytosis by endogenous carbon monoxide breath test, Shanghai Medical Journal 44 (4) (2021) 227–230.

[32] H.D. Zhang, Y.J. Ma, Q.F. Liu, et al., Human erythrocyte lifespan measured by levitt's co breath test with newly developed automatic instrument, J. Breath Res. 12 (3) (2018) 36003, https://doi.org/10.1088/1752-7163/aaacf1.

[33] Y. Shi, Y. Li, X. Yang, et al., Genotype-degree of hemolysis correlation in hereditary spherocytosis, BMC Genom. 24 (1) (2023) 304, https://doi.org/10.1186/s12864-023-09364-8.

[34] M. Kosecka-Strojek, J. Trzeciak, J. Homa, et al., Effect of staphylococcus aureus infection on the heat stress protein 70 (hsp70) level in chicken embryo tissues, Poult. Sci. 100 (6) (2021) 101119, https://doi.org/10.1016/j.psj.2021.101119.

[35] Y. Pan, Y. Li, Y. Li, J. Li, H. Chen, Fatigue of red blood cells under periodic squeezes in ecmo, Proc. Natl. Acad. Sci. U. S. A. 119 (49) (2022) e2084148177, https://doi.org/10.1073/pnas.2210819119.

[36] B.L. Mitlyng, J.A. Singh, J.K. Furne, J. Ruddy, M.D. Levitt, Use of breath carbon monoxide measurements to assess erythrocyte survival in subjects with chronic diseases, Am. J. Hematol. 81 (6) (2006) 432–438, https://doi.org/10.1002/ajh.20644.

[37] M.Q. Lei, L.F. Sun, X.S. Luo, et al., Distinguishing iron deficiency anemia from thalassemia by the red blood cell lifespan with a simple co breath test: a pilot study, J. Breath Res. 13 (2) (2019) 26007, https://doi.org/10.1088/1752-7163/aafe4f.

[38] D.P. Wu, J. Bai, S.L. Chu, et al., Changes and clinical significance of erythrocyte lifespan in megaloblastic anemia], Zhonghua Nei Ke Za Zhi 62 (6) (2023) 688–692, https://doi.org/10.3760/cma.j.cn112138-20221025-00788.

[39] E.B. James, H.J. Vreman, R.J. Wong, et al., Elevated exhaled carbon monoxide concentration in hemoglobinopathies and its relation to red blood cell transfusion therapy, Pediatr. Hematol. Oncol. 27 (2) (2010) 112–121, https://doi.org/10.3109/08880010903536227.

[40] A. Lal, L. Patterson, A. Goldrich, A. Marsh, Point-of-care end-tidal carbon monoxide reflects severity of hemolysis in sickle cell anemia, Pediatr. Blood Cancer 62 (5) (2015) 912–914, https://doi.org/10.1002/pbc.25447.

[41] K.P. Sylvester, R.A. Patey, G.F. Rafferty, D. Rees, S.L. Thein, A. Greenough, Exhaled carbon monoxide levels in children with sickle cell disease, Eur. J. Pediatr. 164 (3) (2005) 162–165, https://doi.org/10.1007/s00431-004-1605-8.

[42] T. Suriyun, W. Kaewsakulthong, P. Khamphikham, et al., Association of the degree of erythroid expansion and maturation arrest with the clinical severity of beta0-thalassemia/hemoglobin e patients, Acta Haematol. 144 (6) (2021) 660–671, https://doi.org/10.1159/000518310.

[43] L.L. Kang, Z.L. Liu, H.D. Zhang, Gilbert's syndrome coexisting with hereditary spherocytosis might not be rare: six case reports, World J. Clin. Cases 8 (10) (2020) 2001–2008, https://doi.org/10.12998/wjcc.v8.i10.2001.

[44] L.L. Kang, Z.L. Liu, H.D. Zhang, Gilbert's syndrome coexisting with hereditary spherocytosis might not be rare: six case reports, World J. Clin. Cases 8 (10) (2020) 2001–2008, https://doi.org/10.12998/wjcc.v8.i10.2001.

[45] J.W.Y.W. Xinkai Wang, Detection of erythrocyte lifespan in patients with hemophagocytic syndrome and its effect on anemia, Chin J Experimental Hematol 28 (2) (2020) 652–656.

[46] S.H.Z.W. Pei Qiang, Preliminary discussion on the significance of erythrocyte lifespan determination in patients with malignant lymphoma and anemia, Chin J Experimental Hematol 27 (1) (2019) 80–85.

[47] Y. Wang, Z. Zhang, Y. Liu, H. Jing, X. Ke, F. Dong, Use of carbon monoxide breath test to assess red blood cell lifespan in newly diagnosed multiple myeloma patients, J. Breath Res. 13 (4) (2019) 46008, https://doi.org/10.1088/1752-7163/ab2c12.

[48] Z.L. Liu, H.D. Zhang, Abnormally short erythrocyte lifespan in three patients with primary myelofibrosis despite successful control of splenomegaly, Am. J. Med. Sci. 361 (2) (2021) 274–277, https://doi.org/10.1016/j.amjms.2020.09.002.

[49] L.L.I.J. Limj, Analysis and detection significance of rbc lifespan length in four common malignant hematological diseases detected by co breath test modality, Int. J. 42 (5) (2021) 574–578.

[50] L. Daggle, N. Sharma, I. Setiady, K. Leonard, Management of neonatal hyperbilirubinemia: shedding light on the american academy of pediatrics 2022 clinical practice guideline revision, Pediatr, Ann 53 (6) (2024) e208–e216, https://doi.org/10.3928/19382359-20240407-02.

[51] V.K. Bhutani, M.J. Maisels, D.L. Schutzman, et al., Identification of risk for neonatal haemolysis, Acta Paediatr. 107 (8) (2018) 1350–1356, https://doi.org/10.1111/apa.14316.

[52] Z.H. Niby, Clinical significance of rbc lifespan testing in neonatal hyperbilirubinemia, Heilongjiang Medicine 36 (3) (2023) 548–550.

[53] Z.G. Zhangzw, Research progress on the effects of hyperglycemia on the physicochemical properties and immune function of rbcs, Clinical Blood 23 (5) (2021) 677–680.

[54] S. Zhou, R. Dong, J. Wang, et al., Red blood cell lifespan < 74 days can clinically reduce hb1ac levels in type 2 diabetes, J. Pers. Med. 12 (10) (2022) 1738, https://doi.org/10.3390/jpm12101738.

[55] H.W. Chu, Y.J. Ma, Z.H. Huang, A pilot study: effect of erythrocyte lifespan determined by a modified carbon monoxide breath test on glycosylated hemoglobin interpretation, J. Breath Res. 14 (2) (2020) 27101, https://doi.org/10.1088/1752-7163/ab75f8.

[56] H.S. Maoyf, Study on the relationship between red blood cell lifespan and glycosylated hemoglobin and pancreatic islet β cell function in people with different glucose tolerances and diabetes mellitus, Practical Chinese and Western Medicine Clinic 21 (12) (2021) 20–21, https://doi.org/10.13638/j.issn.1671-4040.2021.12.009.

[57] D.M. Mock, G.L. Lankford, N.I. Matthews, et al., Accelerated removal of antibody-coated red blood cells from the circulation is accurately tracked by a biotin label, Transfusion 52 (5) (2012) 1097–1105, https://doi.org/10.1111/j.1537-2995.2011.03397.x.

[58] C.R. Valeri, H. Macgregor, A. Giorgio, R. Srey, G. Ragno, Comparison of radioisotope methods and a nonradioisotope method to measure the rbc volume and rbc survival in the baboon, Transfusion 43 (10) (2003) 1366–1373, https://doi.org/10.1046/j.1537-2995.2003.00528.x.

[59] D.M. Mock, D. Nalbant, S.V. Kyosseva, et al., Development, validation, and potential applications of biotinylated red blood cells for posttransfusion kinetics and other physiological studies: evidenced-based analysis and recommendations, Transfusion 58 (8) (2018) 2068–2081, https://doi.org/10.1111/trf.14647.

[60] D.M. Mock, N.I. Matthews, S. Zhu, et al., Red blood cell (rbc) survival determined in humans using rbcs labeled at multiple biotin densities, Transfusion 51 (5) (2011) 1047–1057, https://doi.org/10.1111/j.1537-2995.2010.02926.x.