Chapter 5

Red Blood Cell Transfusion and Functional Dose

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Additional information is available at the end of the chapter

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Abstract

Objective: The objective is to study the relationship between cell age and function in the process of the red blood cell (RBC) normal metabolism and investigate the functional changes of red blood cells in the preservation process.

Methods: The methods are (1) the use of discontinuous density gradient separation to divide the whole blood into different fractions; (2) the use of spectrophotometry and flow cytometry determinated red blood age; (3) the use of flow cytometry, erythrocyte rosette test, and spectrophotometry detected red blood cell function; (4) exploration of the condition of in vitro RBC oxygen-carrying assay system and analysis of the change of RBC during preservation; and (5) in vivo, the changes of hemoglobin concentration after RBCs stored for variable time were transfused to β-thalassemia major (TM) patients have been studied.

Results: PS expression increased gradually with the increase of cell age and PK expression reversed. The positive rate of erythrocyte CR1 receptor expression and the number of CD35+ reduced with the cell age. *Q* value, P50, 2,3-DPG, and Na^+-K^+-ATPase gradually declined with the preservation time.

Conclusion: There is a close correlation between the red blood cell density and the age.

Keywords: red blood cell, transfusion, cell age, storage, function, dose

1. Introduction

Transfusion is important in clinical treatment, while red blood cell (RBC) is one of the most widely used components in transfusion medicine, but the function of RBC changed during

their preservation as RBC storage lesion. Therefore, calculating the function dose of different storage time RBCs can achieve quantitative RBC transfusion, and improve the therapeutic effect of RBC transfusion. Here is the brief introduction to RBC transfusion and function dose as follows.

2. Focus of red blood cell transfusion research

2.1. Constant updating of red blood cell transfusion guidelines

A scientific reasonable blood transfusion can cure diseases and save lives, but as a coin has two sides, blood transfusion may bring transfusion risks. Blood transfusion cannot only transmit infectious diseases but also may give rise to hemolytic transfusion risk, as well as "Class III risk" [1]—anaphylactic reaction, transfusion-associated graft versus host disease (TA-GvHD), transfusion-related acute lung injury [2] (TRALI), transfusion-related immune modulation (TRIM), etc.

As an effective therapeutic measure for improving oxygen-carrying capacity and tissue hypoxia of anemia patients during clinical treatment, red blood cell (RBC) transfusion is being extensively applied clinically. There are various blood group antigens on RBC surface; thus transfused RBCs may bring hemolytic transfusion risks. According to statistics, the amount of transfusion is about 85 × 106U RBCs around the world every year [3]; however, RBC transfusion criteria vary with countries in RBC transfusion practice; one of the primary reasons may be the lack of high-quality evidence of advantages and disadvantages arising from RBC transfusion, so it is difficult to reach a consensus about RBC transfusion criteria. With deeper understanding of transfusion-related adverse reactions in clinical practice and increasingly significant blood supply versus demand contradiction, efficacy and safety of transfusion of stored RBCs become a problem to be addressed urgently in clinical transfusion practice.

"Red Blood Cell Transfusion Guidelines" [4] issued by American Association of Blood Banks (AABB) in 2012 proposed the recommendations of restrictive blood transfusion strategies and RBC transfusion criteria for adults and children with stable hemodynamics, in which RBC transfusion should be decided depending on patient symptoms and hemoglobin (Hb) level together. During the systematic retrospective analysis, no clinical study on transfusion threshold assessment for patients with acute coronary syndrome was found; therefore no recommendation can be given due to lack of clinical randomized controlled trial (RCT).

In October 2016, the latest version of "Red Blood Cell Transfusion Guidelines" was issued by AABB, aimed at establishing criteria for blood storage and transfusion behavior. Comparative study of restricted transfusion threshold and free transfusion threshold on 31 clinical trials from clinical randomized controlled trials with quantitative study hemoglobin threshold for RBC transfusion from 1950 to May 2016 revealed that the proportion of poor prognosis was

not increased. Meanwhile 13 RCTs of 5515 patients subjected to random transfusion of fresh or stored RBC suspension from 1948 to May 2016 were compared, and it was found that fresh RBCs could not improve clinical prognosis. Furthermore, evidence-based advice for transfusion safety in terms of Hb level and RBC preservation time during RBC transfusion for most hemodynamically stable hospitalized adult patients was raised through Grading of Recommendations Assessment, Development and Evaluation (GRDE) study. Therefore, two Hb levels were set as transfusion thresholds in the recommendations: the restrictive RBC transfusion threshold for adult patients with stable hemodynamics, including severe patients, is 7 g/dL; for patients with past cardiovascular disease or patients subjected to cardiac or orthopedic surgery, the restrictive RBC transfusion threshold is 8 g/dL. The guidelines also demonstrate equal safety for most hospitalized patients in stable condition (even including neonates) to transfuse both stored RBCs within the validity period and stored blood within 10 days.

As can be seen from updates and advances of blood transfusion guidelines in recent years, RBC transfusion criteria have been changed from open strategy to restrictive strategy, and it has to further evolve to individualized transfusion strategy. In other words, RBC transfusion decision should not only rely on Hb level of a patient but also consider other factors, such as oxygen supply-associated symptoms and vital sign of patient individual and application of alternative blood transfusion protocol [5].

Although the Blood Transfusion Guidelines, issued by AABB in 2016, validate current restrictive transfusion strategy based on more randomized controlled studies of RBC transfusion and recommend two restrictive transfusion thresholds applicable to most patients in most cases so that blood transfusion decision becomes more individualized [6], the guidelines are still primarily limited in that setting blood transfusion threshold based on Hb level fails to consider fully other factors of oxygen supply balance and neither quantitates or semi-quantitates medical condition of a patient nor sets out a target Hb level of RBC transfusion; therefore, transfusion time and amount still rely more on clinical experience. And the new guidelines are applicable to most patients in perioperative period, critically ill patients with normal volume anemia, and internal medicine and geriatric patients. However, acute hemorrhage patients, patients with unstable hemodynamics, or selective operation patients with very low hemoglobin level due to acute massive hemorrhage are excluded. For patients with acute coronary syndrome, serious thrombocytopenia, and transfusion-dependent chronic anemia, no recommendation was made because of insufficient evidence.

2.2. Fresh RBCs and stored RBCs transfusion

RBC preservation time is a hot issue debated a lot in blood transfusion field; there are more than 50 observational studies reported about it [7, 8]. Most clinical transfusion researches discuss about effects of transfusion of “fresh” and “stored” RBCs on clinical prognosis of blood recipients. As the most common blood component, RBCs are one of the most widely used ingredients with the largest amount in transfusion therapy. The amount of blood collected worldwide each year exceeds 1 × 108 U. For preservative solutions ranging from sodium

citrate, ACD, and CPD to CPDA and SAGM in popular application, RBC preservation time extends from the initial 5 to 42 days; nonetheless, a preservative solution can only delay aging of ex vivo RBCs, but cannot prevent its own aging process; therefore, as preservation time proceeds and metabolite accumulates [9], RBCs will change in shape and aggregation property, reduced oxygen-carrying capacity, and increased immunogenicity; changes of RBCs in oxygen-carrying capacity and immune functions are called "storage injuries" [10]. Current FDA standard allows frozen RBCs to be stored for up to 42 days prior to blood transfusion and stipulates that stored RBCs can be transfused back only after meeting two criteria as follows: (1) hemolytic rate of RBCs is less than 1% and (2) within 24 h after infusion, more than 75% of the red blood cells can survive in the body [11]. But more and more evidences demonstrate that, even if the above criteria are met, transfusion of RBCs which stored for a long time may still increase patient's risk of transfusion-associated adverse complications.

A large number of retrospective clinical studies show that transfusion of stored RBCs is associated with increased risk of cardiovascular events and higher mortality of clinically critical patients [11–13]; it is found in a study of cardiac surgery patients that, compared with patients who receive transfusion of stored RBCs alone or both fresh and stored RBCs, the fresh RBC transfusion group had a significantly decreased postoperative length of stay (PLOS). Among patients who receive transfusion of 1 URBCs, PLOS of patients receiving stored blood transfusion was 3.8 times more than that of patients receiving transfusion of fresh blood alone [14]. Nevertheless, this finding is still controversial. Lelubre and Vincent [15] conducted a systematic evaluation of such papers, which searched from MEDLINE covering a period from 1983 to December 2012, and studied the relevance of RBC preservation time to mortality or morbidity of adult patients. And they did not find any explicit argument to support superiority of fresh RBC transfusion (4 days) to stored RBC transfusion (26.5 days) in this systematic evaluation. Through the investigation of 1153 cardiac surgery patients with perioperative blood transfusion, McKenny et al. [16] also found that postoperative mortality, pulmonary infectious complication, ICU admission time, and postoperative ventilation duration were relevant to transfusion volume but irrelevant to blood preservation time. In 2015, the New England Journal of Medicine (NEJM) published a multicenter randomized clinical trial with important reference value [17], showing that, with 90-day mortality as primary outcome measure, disease outcomes were not statistically different between critically ill patients who received transfusion of fresh RBCs stored for up to 10 days (6.1 ± 4.9 days) and critically ill patients who received transfusion of stored RBC (storage time, 22 ± 8.4 days) in line with "first-in-first-out" principle, and between-group comparisons in secondary outcome measures (main complications, respiration, hemodynamics, renal support treatment duration, length of stay, and transfusion reaction incidence) and comparisons between subgroups (age, APACHE score, number of transfused RBC units, disease type such as medicine, surgery, and injury) show no statistical difference. In the same year, NEJM reported that Steiner et al. performed a prospective multicenter randomized study of cardiac surgery patients [18], finding that even if storage time was longer than 21 days, prognosis of transfusion recipients would not be affected. It is found that comparison between transfusion of fresh RBCs (<10 days) and transfusion of RBCs having a storage period >21 days show no significant difference in adverse events, except high probability of hyperbilirubinemia occurring in the long-term storage group. In

a trial of 377 extremely low birth weight neonates and preterm infants who received randomly fresh RBCs (<8 days) or stored RBCs [19], adverse outcome incidence had no difference between groups. Heddle's observational study [20] also suggested that, in general, nosocomial mortality of hospitalized patients was irrelevant to storage time of transfused RBCs, but this study failed to observe transfusion of old RBCs having a storage time of 35–42 days [21], so it is biased. Dhabangi and Fergusson et al. [22, 23] did not find any difference in clinical prognosis between transfusion of RBCs having a storage time of 21–28 days and transfusion of RBCs having a storage time <7 days. The Blood Transfusion Guidelines issued by AABB in 2016 strongly recommended that RBCs at any time within storage period can be transfused into hospitalized patients including neonates, based on evidence that fresh RBCs (stored for <10 days) and RBCs within standard storage period are transfused in most RCT samples, whereas only a very few samples receive transfusion of old RBCs having a storage time of 36–42 days. The National Blood Collection and Utilization Survey Report issued by the US Department of Health and Human Services (US DHHS) pointed out that RBCs transfused in the USA had mean storage duration of 17.9 days, so the AABB guidelines did not assess RBCs stored for more than 35 days, either. Unexpectedly, The Lancet published a retrospective cohort study in 2016 [24], in which the investigators divided 91,065 transfusion events (all RBCs were leukofiltered) occurring in 23,634 adult patients during 2008–2014 into fresh RBC group (1–7 days), mid-term RBC group (3–5 days), and long-term RBC group (36–42 days) by RBC storage time, assessed effects of blood storage time on a 90-day mortality of transfusion recipients and concluded that overall mortality risk of fresh RBC group was higher than that of long-term RBC group and a 90-day mortality of patients receiving transfusion of fresh RBCs was higher than those of patients receiving transfusion of stored RBCs; the research team said that its causes had to be further studied.

We can find from a number of observational (retrospective and prospective) study reports that transfusion of RBCs having average blood storage times differing by more than 10 days was mostly studied, though the dividing time point between fresh RBCs and stored RBCs has not yet been uniformly defined; meanwhile, due to scarcity of blood resource, patients seldom receive transfusion of old RBCs (>35 days) in clinical practice, which enables the presence of multiple independent confounding variables that may influence outcome measures in RCT studies and clinical trials, such as site (treatment difference), disease severity, transfusion occurring after a clinical event, etc., all of which will cause biases. Therefore, research that lacks multicenter and large-sample randomized controlled data cannot help one assess correctly benefits and hazards of intervention measures [24]. Importantly, none of the studies clarified that transfusion of stored RBCs would cause any harm in clinical transfusion [7, 25].

2.3. Open transfusion strategy and restrictive transfusion strategy

RBC transfusion plays an irreplaceable important role in correcting anemia and surgical and trauma ischemia rescue. Upon anemia, Hb level falls, blood viscosity declines, blood flow volume increases, and 2,3-DPG activity is stronger, so that tissue blood flow volume and oxygen release increase [26], not only lowering tissue (including myocardium) oxygen supply but also needing higher cardiac output to maintain sufficient systemic oxygen supply so as

to meet myocardial oxygen demand; therefore RBC transfusion becomes an effective treatment mode for full restoration of tissue oxygen supply in the event of insufficient oxygen supply [27]. Given that anemia is closely related to preoperative anemia or poor prognosis of cardiovascular disease [28], most clinicians select free transfusion strategy based on clinical experience; however, when most patients are hemodynamically stable, hemoglobin threshold of ordinary critically care patients [29] is 7 g/dL; patients having Hb level of 7–9 g/dL did not need blood transfusion, unless there is any particular comorbidity or acute disease-related factor changed clinical decision. Initially, RBC transfusion used to adopt open transfusion strategy or use higher Hb level to trigger blood transfusion. Adams and Lundy proposed initially "10/30 criterion" in 1942 [30], that is, blood is transfused when Hb level declines below 10 g/dL or hematocrit (Hct) falls below 30%, which has served as a trigger of RBC transfusion in decades though there is a paucity of clinical evidence [27]. From updates and advances of blood transfusion guidelines and blood transfusion indications in the past years, we can see that RBC transfusion indications have gradually transformed from open transfusion strategy to restrictive transfusion strategy in clinical transfusion practice; the Blood Transfusion Guidelines updated by AABB in 2016 also support restrictive transfusion strategy with large-scale multicenter RCT evidence without increasing incidence rate of adverse clinical outcome.

In recent years, a large amount of data of many RCTs studying clinical influences of transfusion strategies support more and more vigorous RBC transfusion strategy. In terms of clinical therapeutic effect, restrictive RBC transfusion strategy is at least not inferior to or even superior to open RBC transfusion strategy. For people receiving surgery or critical care, the restrictive transfusion strategy has been proven to be safe and even safer than free transfusion strategy in some cases. In a prospective RCT conducted by Hajjar et al. [31], during hospitalization of adult patients receiving cardiac surgery with extracorporeal circulation, differences of 30-day mortality and severe morbidity (cardiogenic shock, acute respiratory distress syndrome, or acute kidney injury necessitating renal dialysis or hemodialysis) between free transfusion strategy group and restricted transfusion strategy group were not statistically significant. In 2011, the New England Journal of Medicine reported that [32], for high cardiovascular-risk patients aged above 50 under hip surgery, starting blood transfusion at a Hb level of 100 g/L did not yield a lower mortality or hospitalized morbidity than starting blood transfusion at 80 g/L. For adult acute leukemia patients under chemotherapy [33], restrictive transfusion strategy had no impact on 30–100-day mortality, hemorrhage, and length of stay. To determine hemoglobin threshold of patients with acute gastroenteric hemorrhage, investigators recruited 921 patients with acute upper gastrointestinal hemorrhage, assigned transfusion strategy at random, and stratified the subjects depending on whether or not a subject contracts liver cirrhosis, finding that restrictive transfusion strategy improved significantly prognosis of patients with acute upper gastrointestinal hemorrhage, as compared with free transfusion strategy [34], which agrees with the finding of previous observational study and RCT that restrictive transfusion strategy did not increase [32] but even decreased [35] mortality. As shown in a study [36], the use of restrictive hemoglobin threshold enabled RBC transfusion rate to fall by 43%, and compared with free transfusion strategy, no evidence indicated that the restrictive transfusion strategy affected a 30-day mortality; the researchers further assessed other adverse clinical prognoses, including infection (pneumonia, wound infection, and sepsis),

heart disease, apoplexy, and thrombosis and did not find any difference between both transfusion strategies, either. Therefore, we can know that, for hemodynamically stable hospitalized patients, restrictive transfusion strategy (7–8 g/dL) is at least effective like free transfusion strategy (hemoglobin threshold, 7–10 g/dL) and does not produce any result adverse to clinical prognosis, including a 30-day mortality, cardiac morbidity, and infection. In other words, no RCT data can verify that higher hemoglobin threshold (9–10 g/dL) will benefit clinical prognosis. In addition, a single-center RCT conducted by Shehata et al. [37] also demonstrated that, for high-risk heart disease patients who adopted randomly restricted transfusion strategy or open transfusion strategy, between-group comparison shows no difference in individual adverse prognosis. Therefore, triggering blood transfusion in strict accordance with restrictive hemoglobin threshold in clinical transfusion practice would decrease greatly transfusion volume of a patient and could lower unnecessary blood transfusion risk of the patient. Rohde et al. [38] made a systematic review of clinical RCT data of hospitalized patients involving RBC transfusion threshold and ran meta-analysis of relevance of restrictive and free RBC transfusion strategies to medical infection, and the results indicate that restrictive transfusion strategy is irrelevant to holistic medicine-associated infection reduction, but restrictive RBC transfusion strategy is relevant to reduction in serious infection risk.

The transfusion should be started with one unit of RBCs rather than two in clinical transfusion practice when following the restrictive transfusion strategy; this may have important impact on blood transfusion behavior. Moreover, development of blood protection measures also enriches connotation of the restrictive transfusion strategy, so as to attain no or less blood transfusion. Blood protection measures include certainty of blood transfusion indications, treating anemia with iron supplement, minimizing the use of ischemia drugs, using autologous blood transfusion to patients with large blood loss, etc.

3. Functional dose of RBC

3.1. Concept of RBC "functional dose"

The main functions of RBCs consist of oxygen-carrying function and immune functions. Oxygen-carrying function of mature RBCs is evaluated worldwide by using many methods: P50, 2,3-DPG, effective oxygen-carrying capacity (Q), and Na^+-K^+-ATPase. The most primary immune function of RBCs is to remove circulating immune complexes (CICs); the complement C3b receptor (CR1) on membrane surface is able to adhere to and bind CICs in blood, bring them to mononuclear phagocyte systems of the liver and spleen, and then dissociate and remove them, so as to reduce CIC deposition in tissues. Therefore, quantitative assay of CR1 molecules in RBCs and quantitative evaluation of their bioactivity can help assess immune function state of RBCs at different days of age.

So far the quality standards of suspended RBC mainly focused on RBC count and hemolytic change during the preservation; however, the stored RBC in the preservation period could experience the aging process of RBC itself on one hand; on the other hand, RBC oxygen-carrying

function and immune function will change due to damage of RBC in preservation. Following the prolonging of preservation time of RBC, aging RBC will increase concurrently, while the cell volume will decrease, hemoglobin content will decrease, cell density will increase, the activity of pyruvate kinase (PK) will decrease, P50 will decrease significantly, RBC oxygen affinity will increase [39], and RBC oxygen-binding ability gets stronger, which is not conducive to oxygen release. At the same time, the level of C3b is decreased, which gives rise to the apparent weakening of its immune adherence function as well as the capacity to clear the pathological circulating immune complex. Therefore, the amount of RBC used in the present situation as well as oxygen-carrying function cannot accurately reflect the true situation of RBC. However, the function of storing RBCs and fresh RBCs was regarded as the same in clinical at present. It is reported that storage damages of red blood cells in vitro and at low temperature can reduce the deformability of red blood cells, 2,3-DPG, and oxygen-carrying capacity which lead to reduction of infusion efficiency [40]. In order to accurately reflect the functional status of RBC at different preservation time, we proposed that the dose unit of RBC should use the "functional dose" unit. "Functional dose" of 1 RBC refers to the function of the RBC contained in 200 mL whole blood under physiological conditions (oxygen-carrying capacity and immunity).

3.2. Study on the days of age and function of mature RBC under physiological conditions

The average life expectancy of mature RBC in healthy adults is about 115–120 days [41]; RBCs are constantly emerging and damaged, which maintain a dynamic balance. Adults need to update 200 billion (2 × 1012) RBC per day to maintain the total amount of 2–3 × 1013 RBC in the body [42]. Therefore, the RBC in the body is a heterogeneous group of RBC with different days of age.

Based on the change of red blood cell density, Wu Zhou et al. from our laboratory [43] divided the whole blood RBCs into six different density gradients by discontinuous density gradient separation method at low temperature and low centrifugal force within a short time and then detected the ratio of pyruvate kinase of each layer of RBC and pyruvate kinase activity in RBC of whole blood, days of age of RBC were calculated according to Bracey on the RBC pyruvate kinase ratio and the average age of the RBC data, different density of RBC represent RBC of different days of age, and the correlation between RBC density and its days of age was revealed according to the expression positive age of phosphatidylserine on the surface layers of RBC membrane; the quantitative measurement of CRI molecules and the quantitative evaluation of its bioactivity were conducted to evaluate the immune function of RBC of different days of age, the natural immune adhesion tumor cell rosette test was used to detect immune activity of RBC immune activity, and the method of measuring the oxygen-carrying capacity of RBC was discussed.

3.2.1. Isolating RBC populations with variable days of age by discontinuous density gradient method.

As shown in **Figure 1** courtesy of *Wu Zhou*, centrifuging RBCs at 3500 g and 10 °C for 20 min yielded six clearly stratified density layers, and their average RBC days of age (in the ascending order of density) could be obtained from ratio values and the calculation formula for average RBC days age: 11.5, 46, 63.8, 74.6, 80.5, and 102.2.

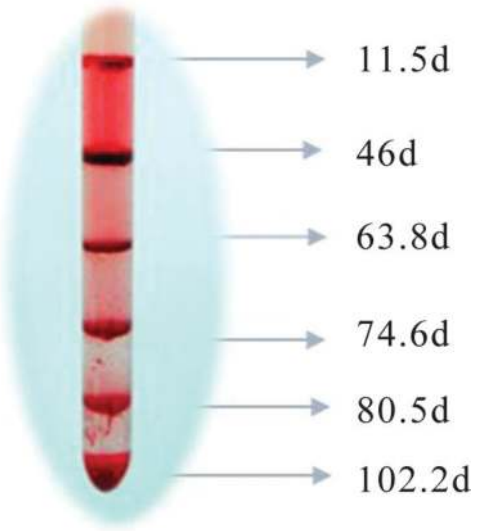

Figure 1. Different ages of red blood cell separation.

3.2.2. Assay of pyruvate kinase activity

Table 1 lists ratios of pyruvate kinase (PK) activity of stratified RBCs at different density to that of the whole blood, from low-density portion (layer 1) to high-density portion (layer 6); PK activity weakened gradually and phosphatidylserine (PS) expression increased gradually. According to ratio formula, ratio = PK activity of stratified RBCs with variable density/PK activity of non-isolated RBCs (whole blood), days of age of RBCs with variable density in **Figure 1** were calculated.

Cell stratification	PK (U/gHb)	PK (Ratio)	PS^{+} (%)
Whole blood	5.62 ± 1.15		0.73 ± 0.46
1	8.11 ± 1.70	1.49 ± 0.44	0.36 ± 0.16
2	6.21 ± 1.24*	1.14 ± 0.32	0.53 ± 0.24
3	5.16 ± 1.23*	0.96 ± 0.31	0.59 ± 0.12
4	4.53 ± 1.06*, $^{\sharp}$	0.85 ± 0.26	0.67 ± 0.21
5	4.37 ± 0.68*, $^{\sharp}$	0.79 ± 0.13	0.61 ± 0.19
6	3.20 ± 0.74*, $^{\sharp}$, $^{\triangle}$	0.57 ± 0.11	1.03 ± 0.88*

*Compare with the first layer, $P < 0.05$.
$^{\sharp}$Compare with the second layer, $P < 0.05$.
$^{\triangle}$Compare with the third layer, $P < 0.05 (n = 10)$.

Table 1. Erythrocyte PK activity and the ratio of PK activity to the whole blood.

3.2.3. Study on days of age versus function for mature RBCs under physiological condition

Difference in number of CD35 (CR1 receptor) molecules shown between RBC populations at different days of age is just one manifestation of immune function difference shown in process of RBC aging, while innate immune adherence rosette test is just one aspect of RBC receptor immunoactivity. As shown in **Figure 2**, the higher the RBC density, the fewer the RBCs with positively expressed CD35 molecules, and the lower the immune adherence rosette rate of different stratified cells, and the rosette rate declined more significantly than CD35 RBC percentage.

Mature RBC age in physiological state and oxygen-carrying function is shown in **Figure 3**. As shown in **Figure 3**, RBCs at different days of age differed significantly in oxygen-carrying capacity, and RBC function was inversely correlated with RBC days of age.

3.3. Regulated changes of RBC function under storage condition

It is well known that RBCs will change in morphology and function during storage: RBC volume is getting smaller, and RBC density is increasing, relating to RBC aging associated with cell membrane vesiculation [44], resulting in loss of cell membrane and some intracellular hemoglobin and weakened regulating capacity of supramembrane Na^+-K^+ ion pump. RBC transfusion in stored state cannot improve an organism's capability of taking in oxygen, which is associated with reduction in oxygen-carrying capacity of stored RBCs [45]. Such reduction is even earlier than reduction in 2,3-DPG [45, 46]. Therefore, measured *Q* value, 2,3-DPG, P50, and Na^+-K^+-ATPase of stored blood can be used to evaluate effects of blood stored for different times on oxygen-carrying function of RBCs [46]. Then, how will oxygen-carrying capacity of RBCs change with variable storage time? Is transfusion of stored RBCs further capable of supplying oxygen to organism tissues very efficiently? How is RBC transfusion volume determined to

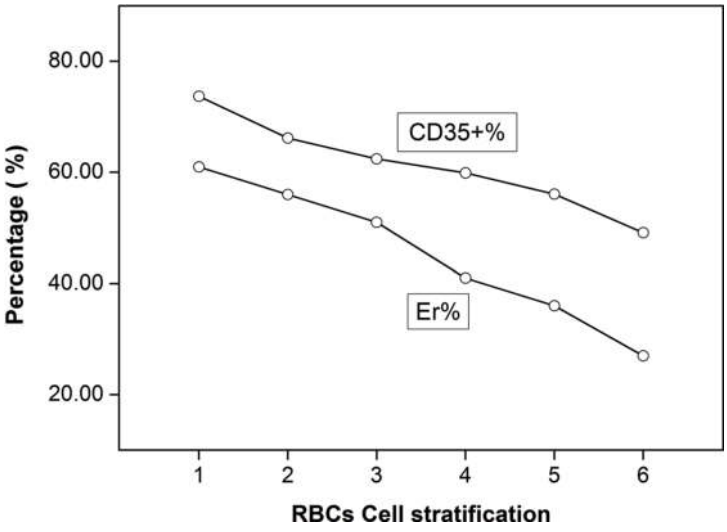

Figure 2. Changes in immune functions of various ages of RBC under physiological conditions.

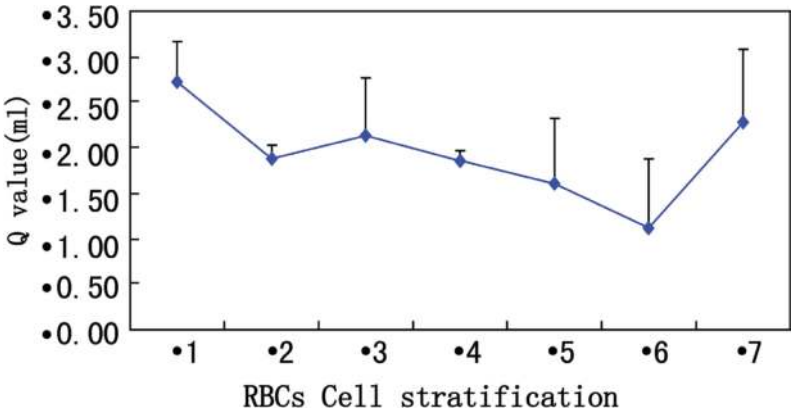

Figure 3. Different ages of RBCs and *Q* changes under physiological status. Note: 7 is not separated before the whole blood specimens.

achieve the same treatment effect? Can we quantitate oxygen-carrying capacity of stored RBCs to provide transfusion dose to clinical transfusion more accurately?

In 2013, Ting et al. [47] conducted a study on oxygen-carrying capacity of RBC in different storage time and obtained the change law of oxygen-carrying capacity of RBC suspension at different ages. As shown in **Figures 4–7**, the values of *Q* and P50 were decreased with the increase of the number of days during storage, among which the faster decrease appeared in the first 14 days. With the concentration of 2,3-DPG was decreased gradually, it was difficult to release O_2, ATPase decreased with the increase of storage time, and the most severe decrease appeared in the first 7 days. It can be seen that in a full linear correlation between *Q* value and P50, the oxygen-carrying capacity of RBCs depends on their own aging variation. The authors established a mathematical multivariate linear model, with effective oxygen-carrying volume as the dependent variable and 2,3-DPG, Na^+-K^+-ATPase, and storage time as independent variables: the multivariate linear model was $Q = 5.457 - 0.925 \times$ 2,3-DPG $+ 0.142 \times Na^+$-K^+-ATPase $-0.076 \times T$ (Storage days), and we obtained functional doses of RBCs stored in vitro for different days. Physical packaging dose unit fails to effectively reflect functions of RBCs transfused at various time points during storage period; therefore establishing a conversion formula for determining oxygen-carrying capacity coefficient of unit RBC stored for different days can enable realistic quantitative transfusion, guide clinical blood use with scientific functional dose, and improve RBC treatment effect.

3.4. Functional dose of RBCs for clinical trial

Based on the above in vitro RBC tests, we found that oxygen-carrying capacity of RBCs declined progressively with increasing storage time. In order to explore clinical transfusion efficacy of RBCs versus storage time and provide experimental data for quantitative transfusion of RBCs, Yunayuan et al. [48] observed changes of hemoglobin concentration after RBCs stored for variable time were transfused to β-thalassemia major (TM) patients. The authors collected 52 (persons) parts (400 mL/part) of blood that were leukofiltered within 6–8 h, had

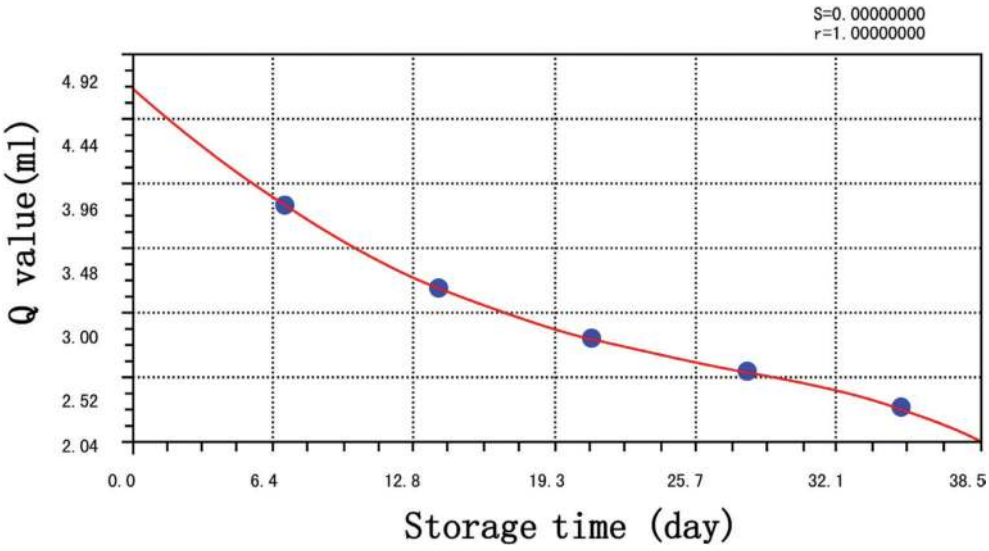

Figure 4. Changes of *Q* value of red blood cells in stock of different ages.

a RBC Hct of 45–47% and passed routine inspection, then divided each part into two aliquots of 1 U (200 mL/U) leukoreduced RBCs for later use; leukoreduced RBCs that were collected from one same blood donor and stored until Day 3 (fresh blood, 1U) and Day 17 (old blood, 1U) were transfused into 52 TM patients enrolled as per study criteria; blood routine, blood gas analysis, and 2,3-DGP concentration were assayed 24 h prior to and 24 h after transfusion

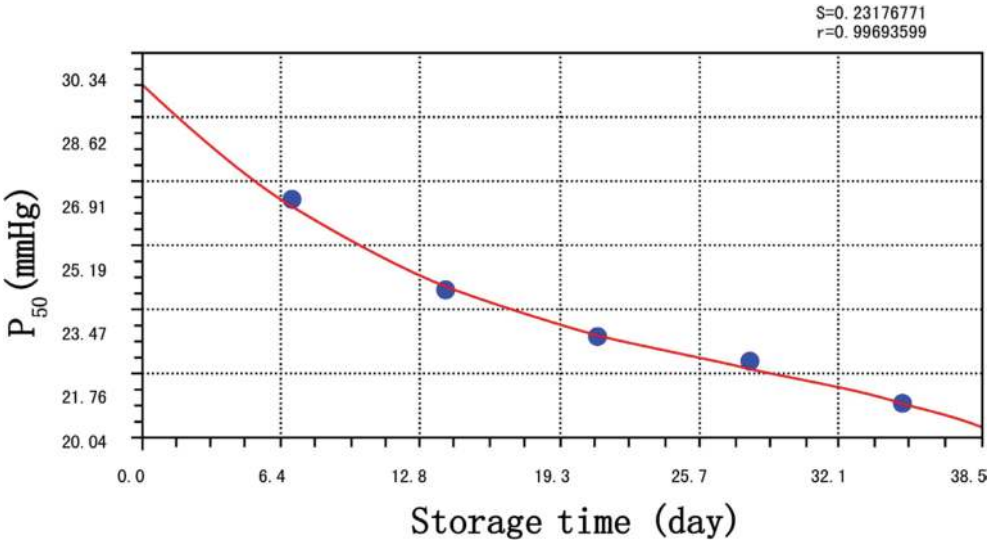

Figure 5. Changes of P50 of red blood cells in stock of different ages.

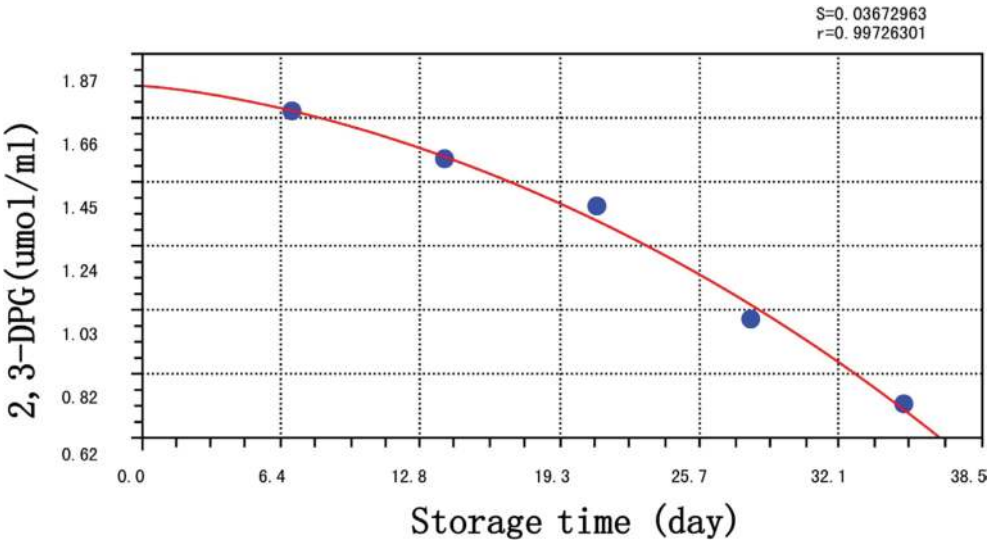

Figure 6. Changes of 2,3-DPG of red blood cells in stock of different ages.

and on day 14 after transfusion, respectively. Variations per 10 kg of body weight 24 h and 14 days after every transfusion were compared. In the trial, there are 10 dropout cases and 42 completed cases. The results are shown in **Figures 8** and **9**: Hb and 2,3-DGP of patients receiving transfusion of blood stored for 3 days were obviously better than those of patients receiving transfusion of blood stored for 17 days.

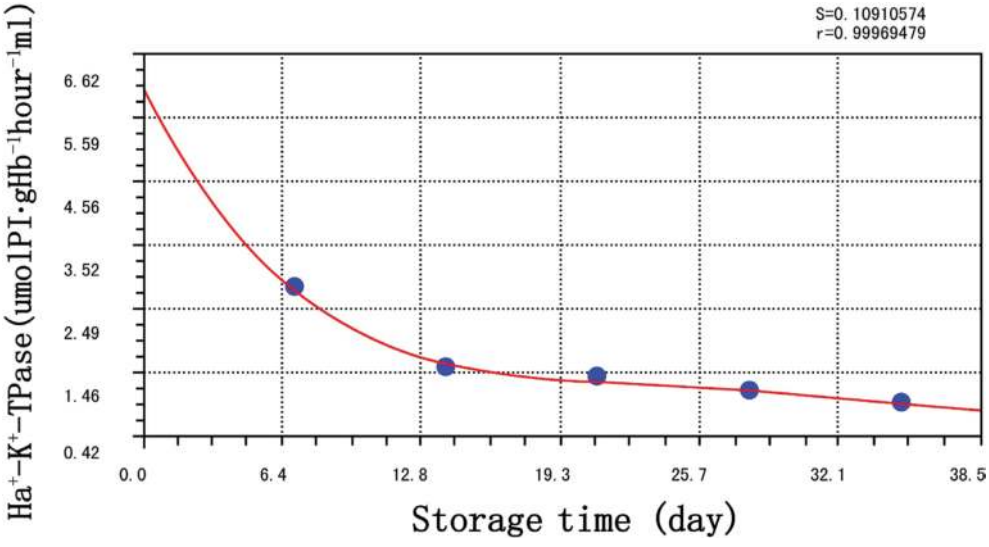

Figure 7. Changes of Na^+-K^+-ATPase of red blood cells in stock of different ages.

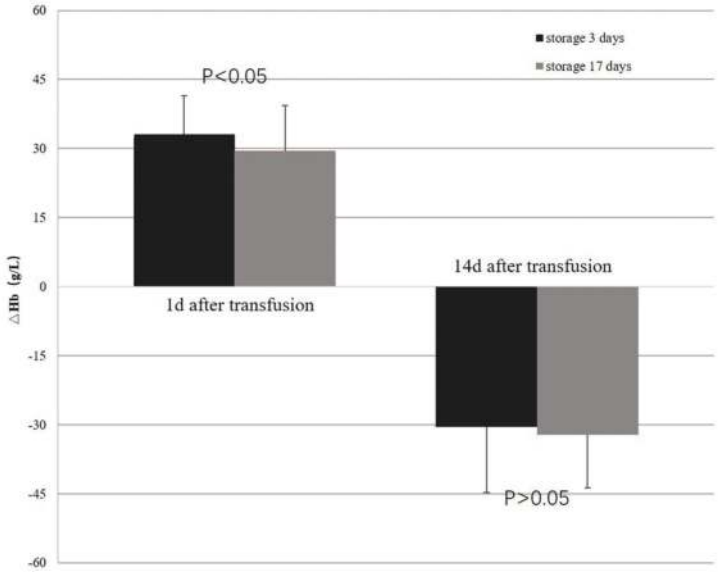

Figure 8. Different storage times of RBC transfusion in patients with Hb changes. $n = 42$.

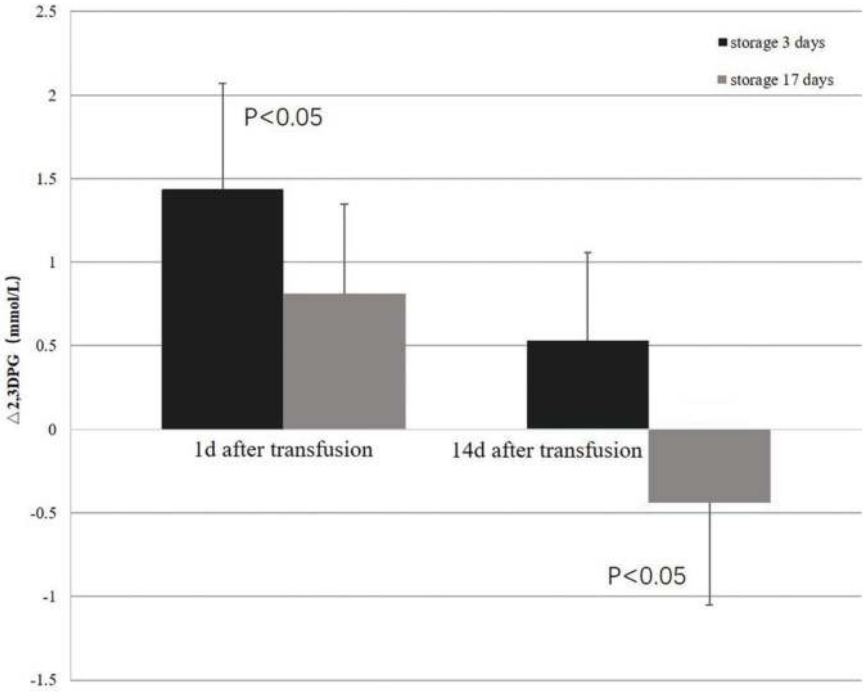

Figure 9. Different storage times of RBC transfusion in patients with 2,3-DGP changes. $n = 42$.

4. Conclusion

Blood transfusion has an irreplaceable position and significance in rescuing patients, and the blood belongs to the scarce resource because of the long-time shortage worldwide. In light of this situation, it is most necessary to have a good command of transfusion indications in clinical transfusion practice, determine the optimum therapeutic dose, and supply safe and effective blood within storage period for rescue treatment of patients. After functional doses of RBCs with different storage times are determined, quantitative RBC transfusion can be achieved to minimize transfusion risk and improve RBC transfusion efficacy, which is the ultimate objective of our study.

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