Storage-induced morphological changes in erythrocytes

Raúl Carrillo-Esper,* Carlos Alberto Carrillo-Córdova,**
Jorge Raúl Carrillo-Córdova,** Luis Daniel Carrillo-Córdova**

RESUMEN

Introducción. Los avances en las técnicas de almacenamiento eritrocitario permiten utilizar concentrados eritrocitarios que presentan importantes anormalidades morfológicas asociadas a mayor lesión en la microcirculación, disfunción orgánica múltiple e incremento en la morbimortalidad. Objetivo. Evaluar las modificaciones de la morfología eritrocitaria relacionadas con el tiempo de almacenamiento. **Material y métodos.** Se analizaron los cambios morfológicos eritrocitarios en relación con el tiempo de almacenamiento de los concentrados eritrocitarios transfundidos en la Unidad de Terapia Intensiva de la Fundación Clínica Médica Sur mediante la evaluación del frotis de una muestra obtenida de los concentrados eritrocitarios obtenidos. Cada uno de los frotis se tiñó con tinción de Wright; dos observadores independientes evaluaron el porcentaje de alteraciones morfológicas eritrocitarias. El tiempo de almacenamiento se definió como el lapso entre la extracción de la sangre y la transfusión del concentrado eritrocitario. **Resultados.** Se recolectaron 67 muestras. El promedio de anormalidades eritrocitarias fue de 40%. La media del tiempo de almacenamiento fue de 12.25 ± 6.9 días. El coeficiente de correlación de Pearson demostró correlación entre el porcentaje de anormalidades eritrocitarias y los días de almacenamiento. Después de siete días de almacenamiento 26.57% de los eritrocitos de los concentrados eritrocitarios tuvieron anormalidades morfológicas eritrocitarias que se incrementaron significativamente en los siguientes días. **Conclusión.** Los eritrocitos que rebasan siete días de almacenamiento presentan alteraciones eritrocitarias significativas.

Palabras clave. Transfusión sanguínea. Anormalidades eritrocitarias.

ABSTRACT

Introduction. Advances in storage techniques of erythrocyte concentrates have contributed to develop significant erythrocyte morphologic abnormalities which have been associated with greater damage in the microcirculation, multiple organ dysfunction and increased morbidity and mortality. Objective. To evaluate the changes in erythrocyte morphology related to storage time. **Material and methods**. We analyzed the morphologic changes in erythrocyte in relation to the storage time of the globular packages that were transfused. The blood smear was wisdom with Wright's technique and was evaluated by two observers who evaluated the percentage of morphologic alterations. Results. 67 samples were collected. The average of abnormalities in the erythrocyte package was 40%. The storage time was of 12.25 ± 6.9 days. The Pearson correlation coefficient demonstrated correlation between the percentages of erythrocyte abnormalities and storage time. After seven days of storage 26.57% of the erythrocytes of the packages had morphological abnormalities which increased significantly in the following days. **Conclusions.** Red blood cells with more than seven days of storage, present significant morphologic alterations, erythrocyte abnormalities.

Key words. Blood transfusion. Abnormal erythrocytes.

INTRODUCTION

Recent investigations in the process of blood transfusion have focused on studying the morphologic changes secondary to conservation and storage in erythrocytes, these morphologic changes are named "storage lesions" and are related with erythrocyte damage and decrease of the survival after the transfusion. The efforts and investigations made during the last 40 years to maintain the corpuscular integrity and the viability and function after the transfusion have increased the storage time to up to 42 days, this was achieved thanks to the phosphate, ade-

* Unidad de Terapia Intensiva, Fundación Clínica Médica Sur. ** Facultad de Medicina, UNAM.

Correspondencia:
Dr. Raúl Carrillo-Esper
Unidad de Terapia Intensiva, Fundación Clínica Médica Sur. Puente de Piedra, Núm. 150. Col. Toriello Guerra, C.P. 14050.
Tel.: 5424-7200. Correo electrónico: rcarrillo@medicasur.org.mx

nine and glucose added help with the preservation of the blood. These advances are counterproductive, because now-a-days the transfusion of morphologically-altered erythrocyte concentrates is more common, this is related with the lesion and dysfunction of the microcirculation which is an independent risk factor in the critically ill patient with organic dysfunctions and increased morbidity.[1-3]

OBJECTIVE

The aim of this report is to correlate the morphologic alterations of erythrocytes with the storage time of the transfused erythrocyte concentrates in the ICU of the Medica Sur Foundation.

MATERIAL AND METHODS

A longitudinal, observational and prospective study was realized in the ICU of the Medica Sur hospital, blood smears of every erythrocyte concentrate were taken; the samples were taken after the transfusion, to ensure the quality and the safety of the procedure. Every smear was dyed with Wright stain and was evaluated to determine the percentage of erythrocyte abnormalities by the investigator and a citotechnician, whom didn't know the time of storage of the transfused erythrocyte concentrates. Both observers evaluated the percentage of abnormal erythrocytes, calling abnormal every erythrocyte with a different morphology than the one of a mature erythrocyte (crenovyte, schistocyte, fragmented erythrocyte). An arithmetic average was realized of both stimations. The time of storage, defined as the time between the extraction of the blood from the donator to the transfusion, was registered.

The statistic analysis was realized with the median and the SD. To evaluate the correlation of the variables, time of storage and morphologic alterations we used the Pearson correlation coefficient.

RESULTS

67 consecutive samples were analyzed, the mean days of storage were 12.25 ± 6.9. Samples were classified in accordance with the storage time in three groups:

- Group I from 0 to 10 days.
- Group II from 11 to 20 days, and
- Group III from 21 or more days.

Group 1 included 30 samples, group II 29 samples and group III 8 samples. The mean of morphologic abnormalities in the red blood cells (RBC) from the transfused concentrates was 40.4 ± 12, with a median of 40 and a mode of 50, percentage of alterations went from 10% to a maximum of 90%.

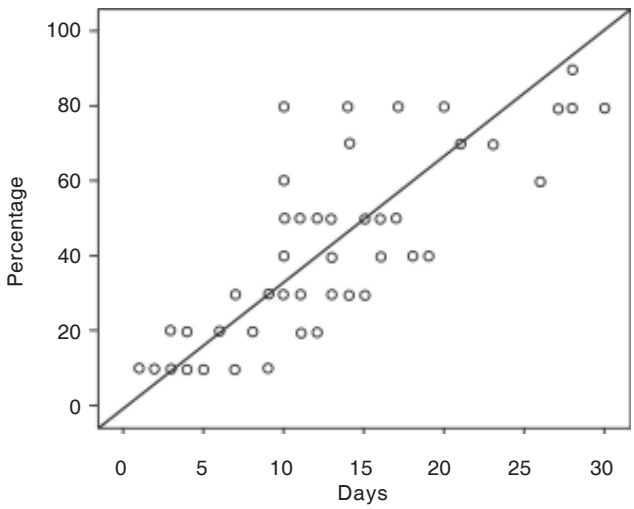

Figure 1. Correlation between the time of storage and the erythrocyte changes. With a longer time of storage the morphologic changes are found in a larger percentage of the transfused erythrocytes.

In each group Pearson correlation coefficient was used finding association between the abnormalities percentage and the storage time, r = 0.8251, this result show relation with the 2 variables (Figure 1). Simple determination coefficient was r = 0.689, this shows a variability percentage of 68.09%. The inclination slope angle was 71 degrees 48 min. This indicates the relation of the morphologic abnormalities and the storage time.

Based in this and according to linear correlation, after seven days of storage, 26.7% of transfused red blood cells have some morphology abnormalities, which represents one third of the transfused red blood cells. By the seventh day this number raise to 75%.

DISCUSSION

First autotranfusion was done in 1914, with this, the clinical limitation that vein to vein transfusion was eliminated, but it was during the Second World War when Dr. Oswald Robertson used the solution Rous-Turner, a combination of citrate and dextrose, to practice the first autotransfusion of storaged blood, and this established the first principles of preservation and storaging of blood.

Like an answer to the increasing transfusional needs during Second World War, Luoitt and Mollison presented

a new solution for preservation of RBC, this is the today well known ACD solution (Acid-citrate-dextrose). ACD solution simplified the sterilization procedure, decreased the volumen of the conservative solution and increased viability of RBC up to 21 days. On the other side, addition of phosphate and 2,3 diphosphoglycerate allow to increase the storage amount of days up to 42 and also increased the viability.[4-6]

The main result of this investigation is to demonstrate that there is a direct relation between storage time and percentage of abnormalities in the RBC. There is enough scientific evidence that storage time from the transfused RBC during transoperatory or during their stay in the intensive care unit is associated with an increased risk of death, more complications and more hospital stay.[7-9]

It is known that transfusion of RBC with a long storage time, which have energetic depletion, membrane alterations and distortion, amplify the inflammatory response when they damage and obstruct the vascular endothelium, this induce more microcirculation alteration and organic dysfunction[10,11] (Figure 2).

Red blood cell characteristics and changes during storage are characterized by (Table 1):

- **Morphologic changes.** From their normal biconcave disc form, RBC deform during the pass of the storage days. The most important form are the crenocytes which are spheric erythrocytes with spicules, this spicules correspond to lipidic vesicles that protrude from the membrane. This transformation is associated with a loss of the surface/volume of the RBC, this increases the mean corpuscular heamoglobin concentration and osmotic fragility, this leads to a decrease in the capacity of the RBC to deform. There is some evidence that all this morphologic changes correlates with the ATP depletion of the RBC.[12] Nakao[13] reproduced these

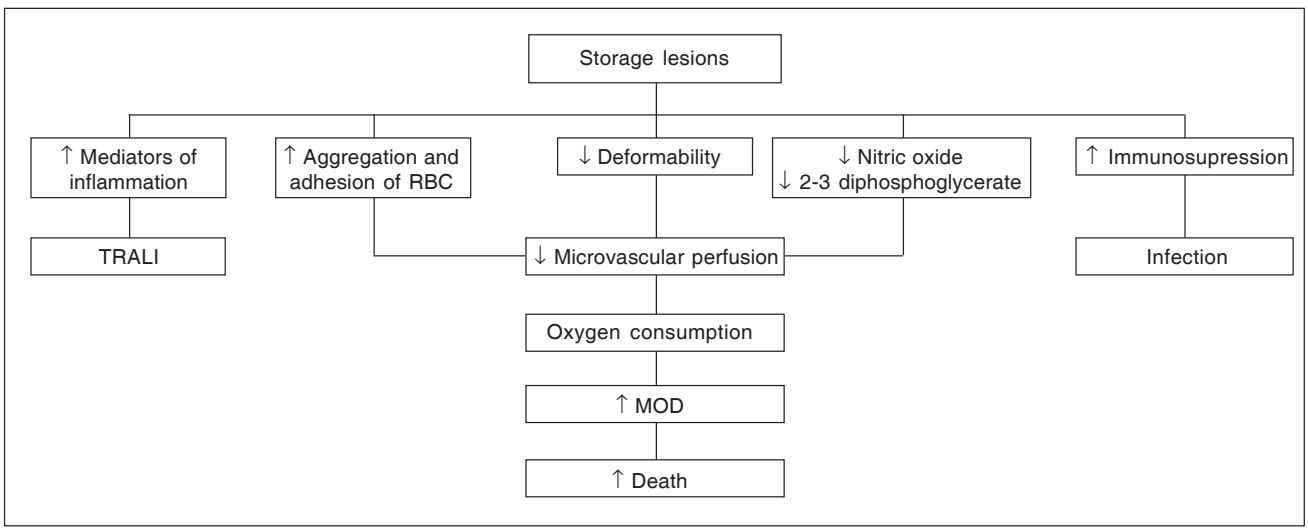

Figure 2. Effects of the red blood cell storage. TRALI: transfusion related acute lung injury. MOD: multiple organ dysfunctions.

Table 1. Changes during storage.

Effects during the storage	Consequences
Decrease 2,3 diphosphoglycerate	Increase of the affinity to oxygen and decrease of tissular contribution.
Depletion of ATP	Changes on the erythrocytes morphology. Increase of the osmotic fragility. Decrease of the deformability.
Microvesiculation	Decrease of the erythrocytes viability.
Lipidic peroxidation	Celular damage or death.

morphologic changes secondary to prolonged storage. He also showed that reestablishing the ATP levels with adenosine made the RBC to obtain their normal biconcave structure after being crenocytes.

- ATP levels. It is considered that biomechanical surface changes of RBC and cytoplasmatic viscosity occurs after the decrease of ATP. There is a low correlation between RBC ATP levels and surviving of RBC, except when ATP levels decrease > 50%. This inconsistency emphasizes in studies that very high levels of ATP have no impact in RBC surviving post-transfusion. Even though ATP depletion does not explain by itself the membrane damage, it has been thought that depletion of ATP increases the levels of secondary mediators like intracellular calcium, protein phosphorilation and membrane kinases which maintain the integrity of cell membrane. The phospholipids analysis shows a change in the phosphorylation/dephosphorilation balance in the RBC during the second and third storage week. The increase in the phosphorilated form of the phosphoinositol-4-phosphate, is accompanied with an appearance of crenocytes, this suggests a direct relation between dephosphorilation and change in the morphology of RBC.[14,15]
- Biomechanical changes. There is a complex interaction between the membrane phospholipids, transmembrane proteins and cytoplasmic components which has an impact in the morphology and deformability of the RBC during storage. This modifications decrease the RBC half life post-transfusion. RBC biomechanical changes are the result of the alterations of the lipidic bilayer, proteins and cytoskeleton. The interactions between these 3 components favor the formation of 20 to 80 nm vesicles, which could be detected by the second storage week. Oxidative damage is another important mechanism of RBC damage, this impacts the biomechanical by damaging the phospholipids of the membrane and the spectrine, this contributes to the formation of squistocytes and spherocytes, also contributes to the osmotic fragility.[16-18]

Changes in the RBC during the storage produce RBC with low efficacy and efectivity to improve oxygenation in tissues, this can be related with a depletion of the 2-3 diphosphoglycerate (DPG). This 2-3 DPG depletion is bigger within the seventh day of storage, this implies a shift to the left in the haemoglobin curve with a decrease of the p50.

The clinical impact of the RBC changes induced with the storage reverberates in the poor effectiveness of these to improve tissue oxygenation this is related with de decrease of 2-3 diphosphoglycerate. This depletion is the mayor alosteric modificator over the affinity to haemoglobin. The deformability reduction of the RBC during storage has been explained like of the physiopathological mechanisms in the development of tissue lesion induced by the storaged RBC.[19,20]

The main objective of RBC transfusions in the critically ill patient is to improve oxygen disponibility in tissues and increase survival and organic function. All this lead to the wrong idea of tranfusing all the critically ill patients and maintain haemoglobin values > 13 g/dL, which is wrong. Different studies has demonstrated that this is not truth and using transfusions in a bad way or abusing of them increases in a significative way complications, specially infections, and morbimortality, specially when the transfused packages has more that 7-10 days of storage.

Based in this, current concepts of RBC transfusions indications is to limit the number of transfusions, follow rigid transfusion politics, set transfusion cutting points in haemoglobin 7-10 g/dL, try to use new RBC, utilize leucoreduced packages in patients with sepsis and when there is evidence of microcirculation damage and trying to avoid the transfusions of RBC with more than 7 days of storage.[21-24]

CONCLUSIONS

The morphologic, biological and molecular changes induced by the storage of erythrocytes are subjects of basic and clinical investigation because of their effects in transfused patients. The scientific evidence shows that the erythocytes with a storage time grater than 14 days induce lesions on the microcirculation and don't increase the consumption of oxygen of cells, which favours the development of multiple organ dysfunctions. Because of that is necessary to develop a specific politic about the transfusions in the ICU's where the use of erythrocyte concentrates with shorter storage life is prioritary, especially with patients with sepsis and organ or microcirculatory dysfunction.

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