ANAEMIA OR TRANSFUSION: A CLINICAL DILEMMA IN A PATIENT WITH CRANIOENCEPHALIC TRAUMA

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1. Summary

Anemia is a frequent complication in neurocritical care. Approximately 95% of patients admitted to the intensive care unit are anemic, presenting hemoglobin values below the normal range during the first 3 days of onset. Anemia compromises the blood's ability to transport O2 and is associated with increased morbidity and mortality among critically ill and traumatic brain injury (TBI) patients. To address this complication, some 45% of these patients receive at least 5 units of red blood cell (RBC) transfusion. However, recent studies have reported adverse effects in the transfusion of RBCs in TBI patients. Researchers and clinicians hypothesize that this is caused by storage lesion, the change of properties in stored red blood cells over time, which may lead to a diminished ability to transport oxygen in RBCs.

Main objective. The main objective of this study was to analyze the effect of blood storage and the encephalic and systemic response of patients with moderate or severe TBI who received transfusions.

Methods. This was a longitudinal study investigating changes in red blood cell properties after being stored in a blood bank using various markers and the repercussions of transfusion in anemic patients. A prospective study was also carried out using non-invasive methods (hybrid system of optical diffusion by infrared spectroscopy) to obtain indirect information on intracranial pressure, cerebral autoregulation, and cerebral hemodynamics in patients with moderate and severe TBI who required red blood cell transfusions.

Results. The study has allowed us to describe the effect of the storage and loss of properties of the blood that we administer to anemic patients. Our findings allow us to delve into how the storage of blood bags affects the quality of the red blood cells and the biochemical changes in the plasma, known as "storage lesions", responsible for the adverse clinical effects that they have following a blood transfusion. This will optimize the therapeutic measures that we apply to all neurocritical patients.

2. Results obtained

The most relevant results of the studies carried out within this project are:

- ***Lysis of erythrocytes releases free hemoglobin into the plasma***. The study carried out on 24 leukodepleted blood bags from day 3 to day 42 post-extraction (defined as the expiry date in Spain) showed a progressive increase in hemolysis. Specifically, the mean percent hemolysis on day 3 of blood storage is 0.00062%, while it is 0.00431% on day 42, an increase of 33% in hemolyzed red blood cells. Red blood cell hemolysis can occur during the processing, handling, transport and storage of blood bags, but it also depends on the characteristics of the blood donors. The transfusion of a blood bag with a high degree of hemolysis implies a lower availability of functional red blood cells, reducing the oxygen transport capacity to the tissues of the recipient patient. In addition, given the toxicity of free hemoglobin, neutralizing it reduces the bioavailability of the most important endogenous vasodilator, nitric oxide, thus reducing organ perfusion and the potential risk of organ injury. Therefore, hemolysis is a relevant quality indicator in storing red blood cells because it can be measured accurately. It is technically very easy to simply identify blood bags in poor condition to discard them and remove them from the active inventory.

- ***The levels of extracellular potassium [K+]e increase progressively***. The levels of [K+]e detected show values slightly higher than the physiological levels on the third day post-extraction; the third day values are close to the lower end of the physiological range seen in healthy persons in our center (3.5–5.1 mmol/L). However, the extracellular concentration of this ion increases progressively from day 3 to day 42 post-extraction, showing a linear increase in all blood bags and reaching values up to 10-times higher than the initial values. One of the possible risks of transfusing several bags of blood with high levels of [K+]e to the same patient is that it favors the appearance of arrhythmia due to induced hyperkalemia or even cardiac arrest if these ions have not been diluted in the total blood volume and reach toxic plasma levels. According to our findings, in the case of massive transfusions, it is recommended to monitor potassium levels in patients who already have hyperkalemia or kidney failure and who have problems eliminating excess potassium. To avoid problems with these patients, we recommend the transfusion of blood bags with 0–3 days of storage or potassium adsorption filters.

- ***Quantifying glucose consumption and pyruvate and lactate accumulation during storage of red blood cells***. Unlike other cell types, the metabolism of erythrocytes is limited by the absence of mitochondria, so the only way to obtain energy is by using glucose in glycolysis followed by lactic acid fermentation. Another characteristic of erythrocytes is that they do not have glycogen stores, so they depend solely on extracellular glucose, which is incorporated into the red blood cell by facilitated diffusion without energy expenditure and is metabolized to form lactate. In our studies, we observed that at the beginning of the study (on day 3 post-extraction), the mean extracellular concentration of glucose [Glu]e detected in the samples was 431.7 mg/dL, a very high value considering that in our center the normal values in healthy controls are 75 to 110 mg/dL. These higher values are because the preservative solution that is added to the blood in the bags, called SAGM, contains glucose in order to provide extra nutrients that ensure the viability of the red blood cells during the 42 days of storage. However, as storage days increase, glucose levels progressively and linearly decrease, indicating that the red blood cells use glucose from the medium to obtain energy. On the day that marks the expiration limit of the bags (day 42), an average concentration of 118.3 mg/dL was detected, which indicates that glucose consumption throughout the study was more than 70% higher than normal levels. These data demonstrate the importance of glucose supplementation through the preservative solution since the red blood cells would not have survived for 42 days with the donor's glucose levels
alone.

In glycolysis, the substrate is glucose, and the final product is two molecules of pyruvate. Our studies observed a tendency towards a progressive rise in pyruvate levels. Subsequently, a decrease in pyruvate levels was observed between days 10 and 17 of the study, which corresponded to an increase in lactate levels, so we can interpret that pyruvate levels decrease due to the activation of the last stage of the glycolytic pathway and the passage of pyruvate to lactate produced by LDH. The transfusion of blood with high levels of lactate poses a theoretical risk of facilitating the development of hyperlactatemia and lactic acidosis that increases with the number of concentrates administered, the duration of the transfusion and, as we have shown, with the time of administration and storage of administered erythrocytes.

Stored blood becomes acidic

This progressive acidification of the blood results from increased lactate levels (lactic acidosis) due to the glycolytic metabolism of glucose. In a normal biological system, the lactate generated is metabolized by the liver and kidneys and would not affect the system's pH. However, in the bags, not only is the blood exposed to an unavoidable build-up of lactate, but the added anticoagulant (CPD: citrate phosphate dextrose) is acidic (pH 5.6 at 37°C) and lowers the pH of the blood even further. Acidification of stored blood significantly affects the quality of stored red blood cells and cell survival after transfusion, especially in patients who need to receive numerous units of blood.

- ***The levels of 2,3-diphosphoglycerate (2,3-DPG) decrease rapidly and progressively.*** One of the most important objectives of blood transfusions is to ensure oxygen transport at the tissue level of patients and its correct transfer to the tissue. The hemoglobin dissociation curve conditions the release of oxygen from hemoglobin. One of the modulators of this curve is the levels of 2,3-DPG, a metabolite present in high concentrations in erythrocytes that, when bound to deoxyhemoglobin, facilitates the release of O2 from the erythrocyte to the tissues. 2,3-DPG depletion shifts the dissociation curve to the left and causes hemoglobin to have a high affinity for oxygen and is difficult to release into tissue. The results show that the levels of 2,3-DPG on the third day after blood extraction were lower than the physiological values described, from 10.5 to 16.2 μmol/g of hemoglobin. The levels of 2,3-DPG decreased rapidly in the first 2 weeks after blood extraction, and from day 17 of storage, the levels were so low that they became undetectable for the technique. Our study concludes that from day 17 of storage, the stored red blood cells are depleted of 2,3-DPG. The decrease in 2,3-DPG levels causes the stored erythrocytes to be transfused to have a lower capacity to release oxygen to the patient's tissues.

- ***Initial rise and subsequent progressive decline of energetic nucleotides***. The energy stored in ATP is essential to keep several processes going, such as glycolysis, electrolyte balance through the Na+/K+ pump, and regulation of the interaction between the spectrin network and the red blood cell membrane. The energy derived from these nucleotides is also necessary to maintain the shape and flexibility of red blood cells and transport phospholipids that prevent premature removal of the red blood cell by macrophages from the transfusion recipient's circulation. The decrease that we observed in ATP levels throughout the experiments showed that it is not a

linear decrease but that three stages can be distinguished: 1) an initial increase, which suggests a phase where ATP synthesis predominates, followed by 2) a plateau stage, where the synthesis and consumption of ATP are balanced, and 3) a final decline starting at three weeks, with linear characteristics that indicates a higher consumption of ATP. The presence of low levels of ATP compromises the survival of stored erythrocytes. Concerning other energetic nucleotides, such as ADP and AMP, the same downward trend was also observed after an initial rise, suggesting that during the first 10 days, the blood stored in the bags produces a synthesis of these compounds.

- ***Morphological changes of the erythrocytes and fragility in their cell membrane make them non-functional***. The morphology of the erythrocytes is critical for their function since its alteration weakens oxygen transport, decreases its deformability, worsens the rheological properties of the blood, and reduces the quality of the red blood cells. The results, analyzed by scanning electron microscopy, show that the most frequent form of red blood cells on the third day after blood extraction is the normal (biconcave) discocyte, followed to a lesser extent by the echinocyte (swollen cell with short, regular spicules) and the spherocyte (spherical shape). These last two are abnormal phenotypes. As the days of storage increase, the frequency of the dissociates decreases, while the abnormal erythrocyte forms become more evident. On day 42 of analysis, the echinocytes, the acanthocytes (with protoplasmic projections of different shapes and sizes), the crenocytes (smaller cells, wrinkled and with spicules), and the spherocytes together represent almost half of the morphology of the red blood cells. This means that on the last day of analysis, only half of the erythrocytes would have a normal phenotype and that in the case of a transfusion from a blood bag on day 42 of storage, only half of the cells would be functional for the patient. In addition, due to storage injuries, the erythrocyte membrane deteriorates, both morphologically and functionally, which influences and facilitates its hemolysis.

- ***Hemodynamic and intracranial pressure (ICP) changes were detected by non-invasive monitoring systems***. The non-invasive optical systems (hybrid diffuse optical monitor with time-resolved spectroscopy) used in this project have made it possible to fine-tune the system, reduce the dimensions of the devices, and improve the algorithms used. All this has made it possible to indirectly and non-invasively determine changes in ICP, study cerebral autoregulation, and objectify the

hemodynamic changes in the brain produced by transfusing red blood cell concentrates to the patients studied.

3. Relevance of the results with possible future implications

Anemia is a common adverse event in patients admitted to the intensive care unit (ICU). According to recent data, 37% of ICU patients receive at least one blood transfusion during their stay in this unit, and transfused patients receive a median of five units of packed red blood cells (RBC). Forty-six percent of patients with a TBI present anemia during the first week after admission and 76% are transfused. Anemia can affect cerebral oxygenation in neurocritical patients, and Siggaar-Andersen already described anemic hypoxia in 1995. The risk/benefit of transfusion when hemoglobin is less than 9 g/dL remains an unresolved and controversial issue. Blood transfusion can increase brain oxygen supply and reduce the risk of tissue hypoxia. However, some published works have reported that blood transfusion is associated in some cases with worse outcomes in patients with TBI, and some systematic reviews do not see a benefit from transfusion.

Blood transfusion in neurocritical patients is a frequent treatment that allows optimization of cerebral oxygenation in cases of anemia. Plasma-reduced and leukodepleted red blood cell concentrates are stored in blood banks for 42 days after collection from the volunteers. Extending the shelf life of erythrocytes is possible due to the combined application of suitable preparation methods and storage additives, such as saline-adenine-glucose-mannitol (SAGM) solution and storage in polyvinyl bags at low temperature. Despite these measures, stored erythrocytes are subject to metabolic, structural, biochemical, and molecular changes collectively referred to as "storage lesions". Storage injuries are characterized by ATP depletion, loss of 2,3-diphosphoglycerate (2,3-DPG), glutathione (GSH), and NADH/NADPH depletion. It has also been shown that stored red blood cells undergo reversible and/or irreversible morphological changes that make them more fragile.

Currently, transfusion practices in a moderate or severe TBI are highly variable, depending on the specialty of the treating physician and the type of patient. Permissive anemia is a recent strategy with great clinical impact. In our opinion, the debate on the

risk/benefit of transfusion has not been adequately focused and is largely biased by cost reduction in health systems.

From a physiological/pathophysiological point of view, as early as 1978 Miller described anemia as an avoidable secondary insult. The discrepancies between physiology and pathophysiology and the lack of positive results in randomized studies result from the design and biases. Our results show that a blood transfusion with less than 7 days of storage is not the same as with 22 days post-extraction since, in short, the blood bags that accumulate more days of storage present a predominantly abnormal morphology that influences the level of hemolysis and osmotic fragility, and also have a more acidic pH than physiological blood, which affects the functioning of multiple enzymes of the erythrocyte metabolism, compromising its viability. The results of the studies carried out in this project will allow a better understanding of the pathophysiology of blood transfusions and make recommendations on how they can be optimized. All this will have repercussions on adequate management of the neurocritical patient.

4. Generated scientific bibliography

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Federica Maruccia, "Lights and shadows of the benign external hydrocephalus syndrome. Investigating its neurological sequelae through the psychomotor assessment and non-invasive optical monitoring of a cohort of infants". Defense of the thesis for 2022 (Autonomous University of Barcelona, UAB)

Susana Tagliabue, Comprehensive monitoring of the injured brain by hybrid diffuse optics: towards brain-oriented theranostics. Defense date: April 29th, 2022

List of other articles in development to be published throughout 2022 and 2023:

Changes in the phenotype of long-stored red blood cell (RBC). The neglected side of storage lesion. Journal of Cerebral Blood Flow and Metabolism

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