DOCTORAL DISSERTATION

PRESERVED BLOOD ADMINISTRATION – DETERMINANT FACTOR ON CELLULAR STRESS IN SURGICAL PATHOLOGY

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CRAIOVA 2010
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Key words: burned patients, skin plasty, blood transfusions, oxidative stress

1. Introduction

The information offered by the specialty literature warns upon the risks that the patients who receive blood repeatedly expose to. We are given quotes regarding the biochemical and metabolic and functional perturbations of organisms in which organ dysfunctions, immunosuppressant situations (the increase of vulnerability to infection), malignancy and increase of mortality install.

The data regarding the increase of morbidity and mortality post-transfusion oriented the research on transfusions towards highlighting the risk factors and physiopathologic mechanisms involved in tripping these situations.

The impact of transfusion upon the clinic evolution in case of high blood loss (medical-surgical emergencies) compelled the initiation of researches regarding the finding of improved ways to check the quality of blood, of blood derivates and especially to achieve substitutes.

As far as the dilemmas of the effects of transfusions are concerned, we have proposed ourselves an enlarged retrospective and prospective study on patients who have the recommendation to receive blood.

In this context, our study intended to observe the reaction of organisms to which the volemic repletion is achieved, in order to highlight the physiopathologic mechanisms, cascade activated, progressively, after the administration of preserved blood. For this purpose we have used modern methods that can become usual methods for research labs, such as AFM (Atomic Force Microscopy) and determination of indicators of oxidative stress.

2. General Data

The blood is a living tissue, made of a variety of components that can be separated by complex procedures in order for its derivates to be obtained, that have specific indications and contraindications. Due to its proprieties and the importance of the effects produced after transfusions, the blood is considered to act as a transplant. The blood transfusions assume three important indications groups:
A. Restoration of a circulating blood volume in case of extended surgical interventions, traumas, bleedings of any types.

B. Rebuilding the numbers of red cells and, implicitly, of haemoglobin in case of acute and chronic anaemia.

C. Rebuilding some cellular component or excessively (consumed) lost plasmatic factors.

3. Specific Data

3.1. Motivation of the paper

The evaluation of blood transfusions effects on the recovery of burned patients subject to free split skin plasty surgical interventions and the highlighting of oxidative micro-injuries from the operated area and/or at distance, independently appeared or as a result of blood or blood products administration and which justifies the modification of post-transfusional morbidity and/or mortality.

3.2. Objectives

3.2.1. General Objectives

- Clinical-subclinical definition of post-transitional oxidative microinjury;
- Evaluation of the time period in which the extension of oxidative microinjury takes place and the dysfunction of one and/or more organs happens;
- Elaboration of the prediction on post-transitional oxidative microinjuries and establishment of the parameters of the oxidative risk induced by blood transfusion.

3.2.2. Specific Objectives

- Observance of the quality of skin graft healing in correlation with the number of the blood units received;
- Highlighting the inter-relations between the mentioned elements after the transfusion of two or more blood units, with the help of AFM (Atomic Force Microscopy) technique.
3.3. Material and methods

3.3.1. Material

Selection criteria regarding the group of patients included in the study

✓ Patient’s state:
  ● Burned surface (35±5%);
  ● Burnings degree (2nd/3rd degree).

✓ Age: 45±15 year old;

✓ Sex: men = 50% of the cases; women = 50% of the cases;

✓ Health state before the combustion injury happened:
  ● Apparent health - 50% of the cases;
  ● Comorbidities presence:
    ▪ Heart diseases under treatment - 20% of the cases;
    ▪ Hyperglycemia - 18% of the cases;
    ▪ Neurological diseases - 12% of the cases (epilepsy - 9%, cerebral vascular accident - 3%).

✓ Type of anaesthesia: same type of substances have been administrated for all interventions;

✓ Anaesthetic risk: ASA (American Society of Anaesthesiologists) 3rd degree for patients that needed more free split skin plasty surgical interventions;

✓ Type of surgical intervention:
  ● Wounds surgical toilet;
  ● Excision, necrotic tissues debridement and wounds degranulation;
  ● Free split skin plasty after degranulation in one or more sessions of tissue restoration.

3.3.2. Method

We proposed ourselves that the study takes place in two stages.

3.3.2.1. In the first stage we have achieved a retrospective, informational study out of which to extract the basic elements of the physiopathologic mechanisms activated after the blood transfusion.

The object of the study was to:

a) determine the usual parameters (clinical, functional and humoral) and to observe the post-transfusional modifications that appear at the level of the parameters.
b) **highlight the blood transfusion methods and techniques.**

The study was achieved on a group of 300 burned patients from the department of the Plastic and Reparatory Surgery of the County University Emergency Hospital of Craiova Municipality, hospitalized during 2005-2009, among whom, to a number of 200 cases the free split skin plasty was practiced, post-combustion, at the level of the injured areas. We also obtained the consent of the patients to cooperate and the Regional Blood Transfusion Centre of Craiova helped us with the study.

**Distribution of the study groups**

**Group 1:**
- 100 cases;
- surgical intervention – wound’s surgical toilet;
- transfusion not recommended, crystalloid and/or colloid solutions are administrated.

**Group 2:**
- 100 cases;
- surgical intervention - free split skin plasty;
- transfusion administrated immediately after intervention, being indicated by the values significantly modified of Hb, PaO2 and SaO2Hb.

**Group 3:**
- 100 cases;
- surgical intervention - free split skin plasty;
- transfusion indicated before and immediately after operation as a result of the important bleeding during operation. Severe acute anaemia installed, with values of haemoglobin ≤ 7 g%, associated with myocardium ischemia observable during EKG monitoring.

**The values have been noted during three evoluntional periods:**
- in 24 hours from the surgical intervention and the administration of crystalloid / colloid solutions or blood (P1);
- in 7 days from moment P1 (P2);
- at the discharge from the hospital (P3).

**3.3.2.2. In the second stage** we have achieved a prospective, conceptual study, oriented towards the highlighting the elements that participate in elaborating the prediction regarding the necessity of transfusion and evolution of the patient who received blood.
The leaving point for this study consisted in recent researches, orientated towards molecular and biochemical modifications which would determine tissue irreversible, death generators morpho-functional alterations, in which the essential factor is the intensity of the oxidative stress.

We have tried to observe these aspects throughout:

c) **AFM Technique (Atomic Force Microscopy).**

d) **Morpho-pathologic exam of tissues while recovering.**

e) **Determination of the balance between oxidants and antioxidants.**

We have selected a group of 100 burned patients during 2005-2009, hospitalized in the department of the Plastic and Reparatory Surgery of the County University Emergency Hospital of Craiova Municipality, the patients being subject to the reparatory therapy by free split skin plasty. The patients were selected by the criteria previously established and they were divided into 4 groups:

**Group A:**
- 25 cases;
- 1 free split skin plasty intervention;
- volemic repletion by crystalloid and colloid solutions.

**Group B:**
- 25 cases;
- 2 free split skin plasty interventions;
- volemic repletion by crystalloid + colloid solutions + 2 erythrocyte mass units.

**Group C:**
- 25 cases;
- 3 free split skin plasty interventions;
- volemic repletion by crystalloid + colloid solutions + 4 erythrocyte mass units.

**Group D:**
- 25 cases;
- 4 free split skin plasty interventions;
- volemic repletion by crystalloid + colloid solutions + 6 erythrocyte mass units.
We observed the fluctuations of the leucocytes’ and thrombocytes’ fluctuations, as well as the relation between the oxidants and the antioxidants in **three evolitional periods**:

- in 24 hours from the first surgical intervention and the administration of crystalloid/colloid solutions with or without erythrocyte mass transfusion (P1);
- in 10 days from moment P1 (P2);
- at the discharge from the hospital (P3).

We interpreted these values by their correlation with both the anatomic-pathologic aspects of the recovering tissues and the results obtained by using the AFM technique.

**3.4. Results**

a) In the case of the patients stable from the homodynamic point of view, to whom no active bleedings have been noticed, non-critic patients, with no heart and lungs dysfunctions, applying the erythrocyte mass transfusion for the Hb values around 8 g% proved to be beneficial.

In the case of patients in critical conditions and/or with heart and lungs problems, organic dysfunctions, noticed during the evolution within the hospital, the recommendation was imposed to administrate erythrocyte mass transfusion in order for the Hg values to be reestablished.

The transfusions before the operation preventively done in order to solve the already existent anaemia did not have the same effect as in case of blocking the mechanisms that generate the blood lost. Also, it is beneficial to use the pharmacological agents with the capacity to increase the haemoglobin level or to reduce the bleeding from the operated tissue mass: haemostatics, vasoconstrictors, gelaspon. Extremely valuable proved to be the macromolecular repletive solutions administrated before and after the operation, immediately and tardily.

In the case of patients that needed massive transfusions, multiple complications produced due to hypovolaemia, tissue ischemia and acid-basic perturbations. Some of these metabolic alterations, such as coagulation or respiratory alterations are amplified by preserved blood administration.
The results of this study sustain the fact that tissue alterations or hypo-perfusion secondary to an insufficient volemic recovery, amplify along with an insufficient release of O₂ towards mitochondria.

e) The AFM technique, applied for the peripheral blood samples from the four patients groups highlighted the fact that in case of group A the red-blood cells are exposed, at distance one from another, maintaining their shape, dimensions and lifetime. There are no signs of haemolysis. The morphological aspects (forms, dimensions, dispersion) are likely to the ones observed at the electronic microscope. Group A’s identical aspects have been noticed to group B, too.

On group C we noticed the presence of some red-blood cells agglomerations and increase of cellularization after the administration of blood, while on group D we noticed a triad red-blood cells disposal. The triads’ presence is associated with the apparition of necrosis and the lack of grafts attachment, possibly due to the increase of blood viscosity. Also, in the peripheral blood samples we noticed the establishment of leukocytosis and neutrophilia.

In case of the patients to whom we noticed the lack of healing, the AFM aspects had been suggestive, consisting in amorphous cells masses difficult to differentiate. Out of these cases we have noticed the patient’s decease.

The 3D images obtained with the AFM help allowed us to evaluate the intensity of the post-combustion defence reaction, noticing the redistribution of the elements at the level of the area next to the burned one.

The red-blood cells agglomerations and overlapping during the local defence process did not allow us to administrate blood, even when the haemoglobin values were at the inferior allowed limit. In such cases we practiced the restrictive transfusion.

These aspects have oriented us on the necessity of achieving an experimental model for the verification of the relations between the own elements and those brought by the preserved blood.

d) The presented morpho-pathological aspects are suggestive for the evolution of the post-combustion wounds. Their relation with the blood transfusion is only an ascertainment, to a reduced number of cases, which cannot be statistically argued. We
noticed that, on some patients with multiple blood transfusions and an increased number of grafts, the donated blood modified the viscosity of the receiver's blood, creating the disseminated coagulation state, a fact that supports also the cause of organ dysfunctions setting-up.

e) The tissues’ intense metabolic activity in hyper-catabolic state determines the apparition of O$_2$ radicals in blood at a larger scale compared to the preserved blood transfusion. There are no modifications of the antioxidant capacity of plasma after transfusion. An aspect to be observed is the increase of this parameter after the administration per bone of zinc sulfate. The fluctuations underline the local biochemical adaptation - defence capacity. The cellular antioxidizing capacity is maintained throughout the presence of the reduced glutathione, its values discretely modifying, within the normal limits.

3.5. Discussions

The blood transfusion achieves a balance between the loss and the restoration of the blood volume, being able to stimulate the restoration of all types of blood cells and proteins.

The researches on the evolution of the patients who received blood underlines the risk of producing infectious episodes and even death after a big number of blood transfusions, the need of blood being estimated according the death determinant factors: patients’ age, burned surface, the presence of the breathing injury, number of blood units received. Some authors appreciate that the mortality and the infectious complications are correlated to the number of blood units transfused. They recommend that the transfused blood volume be determined according to the haematocrit’s and haemoglobin’s values, usually low, under the limits accepted by other researchers as the new surgical techniques and those regarding the post-combustion wounds attendance allow their recovery without an increased transfusional volume.

Also, the human tissues are in constant flux between the anabolic, constructive and catabolic processes. The health state expresses the balance between the two processes. The oxidative injury has a major impact on the balance between anabolism and catabolism. The free oxygen radicals (RLO) are formed as a result of normal reactions of producing adenosine-triphosphate (ATP) and within the inflammatory processes. Antioxidants such
as vitamin C, vitamin E, selenium, glutathione, superoxid dismutase, are all substances that clean the area from the free oxygen radicals previously the establishment of the oxidative injury. In these circumstances the increased level of oxidants reflects the intensity of oxidative stress, their excess consuming the antioxidants.

The importance of the balance between oxidants and antioxidants at intercellular level, dependent on the genetic expression variability, is an essential element of therapeutic modulation of healing.

3.6. Conclusions

- The correct evaluation of the ratio between risk and benefit in case of blood transfusion allows the elaboration of the indication of transfusion which does not increase the death risk for the patients to whom the transfusions are restrictive (do not accumulate large blood volumes).
- The transfusion of fresh blood or blood stored for maximum 48-72 hours do not determine oxidative modifications in the receiver’s blood and tissues. The healing evolution of the grafted tissues is very good.
- The prediction on the post-transfusional evolutions of the burned patients who receive blood can be established by observing the values of erythrocytes and leucocytes, as well as by observing the values of the oxidant/antioxidants ratio.
- The contribution of additional antioxidants as nutrients assures the maintaining of the antioxidant capacity.
- The determination of the antioxidant capacity represents an original way of establishing the adequate therapies of preventing the installing of complications generated by the evolution not at all clinically visualized of the unbalance between oxidants and antioxidants.
- Our clinical-paraclinical observations do not support the hypothesis that preserved blood administration would have negative effect on tissues’ life, as the number cases studied is lower (trials would be necessary) and the administrated preserved blood did not have time (due to high solicitations) to become old.
- The correlation between the clinical parameters, the humoral values and the AFM results is weak. This aspect supports the conclusion that there are no statistic methods for the calibration the human model and neither there are successful standard therapies.


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