

Antioxidant Capacity of Plasma may Predict the Wounds Healing Process

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ABSTRACT We associated conventional other new parameters, to show the ratio between the endogenous (exogenous) oxidants and antioxidants and the influence exercised by this ratio upon the sensibility of organism to the standard therapy applied. In the same time, we sustain that a good and frequently clinic and paraclinic observations of evolution assure the possibility to select the opportunity for applying the most adequate schema of treatment, to correct the functional and humourale disequilibrium. The capacity of tissues to be very sensible to the applied therapy could be evaluated by means of the new parameters proposed. Understanding the basic mechanisms underlying the organ failure, suggests that efficient therapies have to be developed, and applied early, not as a very known classical schema, but as a pathophysiological one, before the irreversible alterations to appear, and so to reduce death rates.

KEY WORDS oxidants, antioxidants, healing process

Introduction

A completely healed wound in postoperative period, reflects tissular potential to return to normal anatomical structure, function and appearance, within a reasonable period of time, and attained complete skin closure, without drainage or dressing requirements. In postanesthetic-surgical period, individual reactivity of organism contributes significantly to the wound healing evolution, marked by its great individual variability of responses to surgical injury, because of genomic variations. Genes encoding proteins, involved in signal transduction and in inflammatory process (Toll receptors), are implicated in individual's response to injury, and also, in surgical wound healing. Clinical significance of genomic markers remains to be tested, for understanding their mechanism of action, and how could they be used to select the therapeutic management, into the future. Several authors(1) consider that individual genotype is implicated and is influenced by the ratio between seric oxidants (O)/ antioxidants (Ao), the maintenance of this one, having a crucial role upon the cellular cycle and cellular homeostasis (cells production: proliferation - apoptosis) which can influencing the wound healing. Some authors (2) appreciated that modulation of the oxidative stress and the antioxidant response of serum against free radicals reactions could represent an important therapeutic strategy in obtaining a rapid and efficient wound healing. Oxidants/antioxidants systems interaction, so the potent free radical reactions, starting with OH_·, do

not end in only a one-step reaction; generally, they continue, even forming a free radical chain reaction, and antioxidants prevent the prolongation of this kind of reactions. The relative antioxidant responses of the antioxidant components of serum, against the ascendant potent free radical reactions, represents, the way of oxidants/antioxidants modulation, and may be one of the major pathophysiological objective for surgical wound therapy. Oxidants interfere with the gene expression for adhesion molecules, and reactive oxygen species (ROS) and activate the mechanism of signal transduction, inducing an increasing of cellular metabolism (3) and immune response. Takahashi (4) demonstrated that ROS also activates the signaling intracellular pathways for cellular mitogenesis. It was reported data underlining that the Oxidants could induce the overexpression of adhesion molecules: IL-8 and ICAM, increasing their special reception by the tissues as an important characteristic of endothelial cells activation. It was reported that IL-8 expression for epithelial cells and IL-8 mRNA are dependent of the ROS concentration (5). As a biochemical signal, oxidants are the stimuli for cell growth and for overexpression of the gene c-fos, because of a paracrine mechanism of stimulation (6), belonging to the endothelium itself. It is possible to induce the endothelin 1 (ET1) release, local substance, known to act as a mitogenetic factor.

Material and Methods

Clinical study included: 60 patients, age: 40-60 years, submitted to a mild anesthetic surgical stress for autologous skin grafting.

We divided patients into four groups:

- group A: 10 cases (17%), control, healthy volunteers;
- group B: 28 cases (48%): with localized infection, or patients, at risk;
- group C: 12 cases (20%): several with multi-organic-dysfunctions (MODS) and failure (MOF);
- group D: 10 cases (17%), with SIRS (6 cases), septic shock (2 cases) and death (2 cases).

We calculated the scores: SOFA and APACHE, and from each group we have determined, from blood (serum) the values of circulating proinflammatory cytokines (IL1, TNF alpha), C reactive protein (CRP), procalcitonin (PCT), coagulability status of plasma, leukocytes number, thrombocytes count, and total antioxidant response (TAR) at three different moments: T1 (4-12 hours postoperative), T2 (7-10 days postoperative), T3 (21 days postoperative).

The study was approved by Ethical Committee of University of Medicine and Pharmacy and Clinical Emergency County Hospital of Craiova. Also, the patients were informed and approved their participation in this kind of clinical research.

We determined the biological values at Clinical Emergency County Hospital Laboratory. We used Ozcan Erel (7) technique for obtaining values for oxidative stress parameters.

Results

Morpho-clinical aspects of surgical wounds highlighted the three kinds of healing:

- efficient healing : 90% after 7 days and 100% after 14 days, from the skin grafts application.**
- inefficient healing after 6 weeks associated with reintervention for restoration of skin grafting.**
- no healing and bad evolution after 2 months.**

We evaluate the evolution of skin grafts, not only the local aspects of wounds, but verifying clinical status, presence of organic dysfunctions (calculating SOFA scores), level of hematocrit, and hemoglobin.

Cellular oedema was the most important clinical sign, which firstly appeared; it was correlated with TAR decreasing. We observed the organism's resistance to antibio-or/and corticoid

therapy, in T2 period for the cases from C, D groups, who didn't receive antioxidant therapy.

D. TAR values

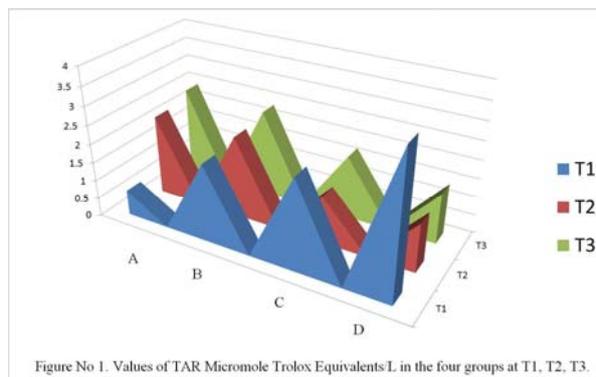


Figure No 1. Values of TAR Micromole Trolox Equivalents/L in the four groups at T1, T2, T3.

TAR values were differentiated in two groups:

- A-B, who received “per os” fruits and vegetables, rich in antioxidant polyphenolic compounds. To these ones, total antioxidant capacity was increased.
- C-D groups, increased lipid peroxidation and a great potent free radical reactions were dominant, inducing an important oxidant damage, expressed by a very low serum TAR.

E. PCT values rise earlier, before CRP and CK, and have a high specificity for patients with multiorgan dysfunctions and failure.

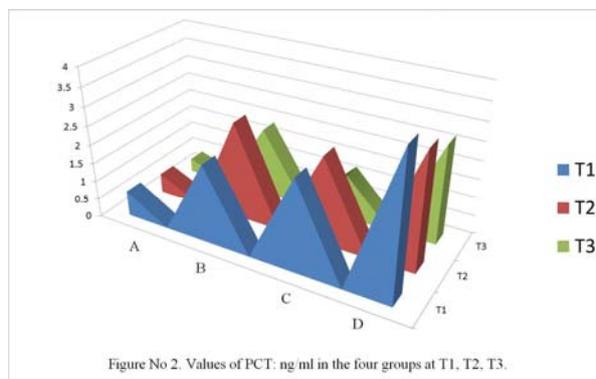


Figure No 2. Values of PCT: ng/ml in the four groups at T1, T2, T3.

For our cases the discrete elevated PCT values in patients after major surgery, was indicator for the risk of developing of any complications, namely: multiple organ failure and sepsis. PCT values are predictive for the initiation of infection (T1) its generalization (T2) and give the information about a bad evolution of wound healing.

Several authors (8) sustain predictive power of PCT is almost equal to that of fine needle biopsy- as the gold standard.

F. CRP is elevated during postoperative states, and its increasing is observed after 24 hours, at T2 simultaneously to cytokines' level increasing.

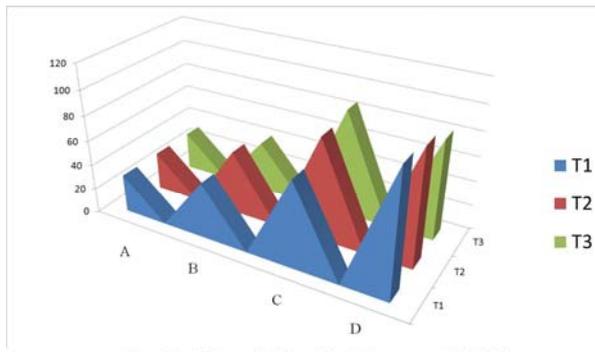
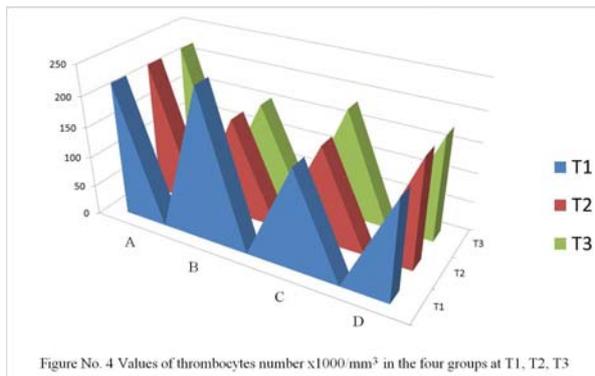


Figure No. 3 Values of PCT: mg/l in the four groups at T1, T2, T3

G. Trombocytes count:

Figure No. 4 Values of thrombocytes number $\times 1000 \text{ mm}^3$ in the four groups at T1, T2, T3

We recognized the hypercoagulability in all postoperative status, because of autonomic vegetative system stimulated by presence of pain. The decreasing of inhibitors of the coagulation system may be initiators of SIRS or sepsis appearance, level of antithrombin III (AT III), activated factor VII, prothrombin fragments (1+2) and D-dimers reflect the hypercoagulant state. Fluctuations of trombocytes count, especially their lower level, could be explained by dilutional thrombocytopenia or by direct effect of free radicals species upon the cells from vascular structure and from blood.

Discussion

Reactive oxygen species (ROS) are generated at sites of inflammation and injury, and at low levels, ROS can function as signaling molecules participating as signaling intermediates in regulation of fundamental cell activities such as cell growth and cell adaptation responses, whereas at higher concentrations, ROS can cause cellular injury and death. The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissues, is a major target of oxidant stress, playing a critical role in the pathophysiology of several vascular diseases and disorders. Specifically, oxidant stress increases vascular endothelial permeability and promotes leukocyte adhesion, which is coupled

with alterations in endothelial signal transduction and redox-regulated transcription factors such as activator protein-1 and nuclear factor-B. This review discusses recent findings on the cellular and molecular mechanisms by which ROS signal events leading to impairment of endothelial barrier function and promotion of leukocyte adhesion. Particular emphasis is placed on the regulation of cell-cell and cell-surface adhesion molecules, the actin cytoskeleton, key protein kinases, and signal transduction events.

Several aspects of oxidant stress-mediated endothelial dysfunction should be emphasized for future investigation. For example, what are the molecular mechanisms by which ROS disassemble/assemble the tight and adherens junctions?

Furthermore, oxidant-mediated remodeling of the actin cytoskeleton is implicated in the loss of junctional organization and stability, but this hypothesis has not been vigorously tested. Several protein kinases have been identified to regulate barrier function; yet specific roles and targets of these kinases (and phosphatases) remain unresolved.

An exciting area of study is defining the molecular communication or interactions between the endothelial cell and the transmigrating leukocyte under oxidant stress conditions. This is particularly important in light of the fact that mechanisms governing leukocyte extravasation are highly stimulus, and likely tissue, specific. It has been shown that the adherent or transmigrating leukocyte can induce endothelial cell activation responses, including remodeling of endothelial junctions and the actin cytoskeleton. The functional significance and the regulatory mechanisms engaged by these interactions during oxidant stress have yet to be determined.

A redox imbalance in endothelial cells results in both a transcription-independent and -dependent surface expression of different endothelial cell adhesion molecules, suggesting that oxidant stress induces acute and chronic phases of leukocyte adhesion to the endothelium. The control of these different facets of leukocyte adhesion likely engages different regulatory mechanisms. Future studies are needed to identify and define the molecular events by which the endothelium regulates leukocyte adhesion and barrier function in the context of acute (minutes) vs. chronic (more than several hours) oxidant stress. Selenium can enhance the ability of cells to scavenge ROS. Moreover, it is an essential integral component of glutathione peroxidase, which catalyzes the reduction of organic

hydroperoxides and hydrogen peroxide, so that sodium selenite enhances the ability of ER-1 cells to scavenge ROS.

Conclusions

-Early diagnosis in postoperative, burn patients is important to prevent the complications of microbial infection, so to eliminate the disturbances in wound healing process.

-Routine laboratory parameters, frequently suffice to establish the diagnosis of sepsis, but for prevention of a bad evolution to unapparent multiorgan failure, or may be death, the patients could be observed not only clinically, but using very sensible parameters, such as: TAR, PCT, CK (IL1, TNF alpha) and coagulability index.

-The disequilibrium Ox/Ao, as a determinant factor in wound healing alteration could be observed, early postoperative, by means of clinically aspects (discrete interstitial oedema, at site of incision) and seric values of TAR.

-There are two periods of endogenous antioxidants level increasing (at 4 and 24 hours postoperative), produced as an adaptive mechanism of defence, or may be because of the antioxidant administered immediately postoperative.

-The final conclusion is, to use the combinative therapies, according with the pathophysiological mechanisms, expressed by the presented parameters.

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