Intravascular Hemolysis as a Biomarker of Acute Postoperative Kidney Injury

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Abstract

Acute postoperative renal damage (ATP) is accompanied by at least 1/3 of patients whose operations are accompanied by II-III-IV blood loss (ATLS), and require the introduction of donor plasma erythrocytes and auto-erythrocyte reinfusion.

The development of this complication, as an integral part of polyorganism disorders in the first phase of postoperative disease, significantly increases the lethality.

Intravascular hemolysis, which determines hemosiderin - ferritin, tubulo-obturation and hypoxic - ischemic damage of the pores, has been chosen as a marker for the formation of OPPT.

The dependence of the degree of hemolysis severity on the severity of hemorrhage was established: its critical values for class IV, and also for the volume of introduced donor red blood cells, reaching 7 doses from different donors and reinfusion 8-9 ml / kg. The correlation dependence of the increase in hemolysis level with a decrease in the clearance of creatinine and urea was deter-
mined. Pathognomonic morphological signs of acute postoperative renal damage, including focal hemosiderosis, erythrocytes in the lumen of the tubules and necrosis of their epithelium, were established.

**Keywords:** Intravascular hemolysis; Postoperative kidney injury

**Introduction**

Postoperative Acute Kidney Injury (Postoperative AKI) occurs in 30% of patients [1, 9, 15] after abdominal surgery, in peritonitis, pancreatitis and thrombosis of mesenteric vessels. About 50 – 52% of such incidents had been noted in cardiovascular surgery, 47% - in thoracic surgery [3, 10, 13].

At the same time the risk of developing AKI increases three times and accompanied by high mortality after surgery due to massive hemorrhage [1, 5, 14] with the subsequent emergency allotransfusion [4, 6].

The real possibilities of modern diagnostics of kidney injury include studies of troponins, apoptosis, inflammatory factors, nephrotoxins and factors of endothelial injury [1, 9, 15]. A list of biomarkers of Postoperative AKI comprises a few hundred of items [11, 12, 15]. The tools of modern science allow reflecting on the mechanisms and importance of biomarkers in kidney injury in postoperative period [7, 8, 13].

One of such markers, surely, remains the intravascular hemolysis associated with hemorrhage that is compensated for allotransfusion in surgical treatment. The physiological destruction of red blood cells (RBCs) due to their natural aging is determined by their life span that is on average, from 100 to 130 days. During the whole period of life, RBC "covers" the distance of 150-200 km, about one-half are so-called narrow areas. Within one minute the RBC passes twice through the capillaries of smaller diameter (2-4 µm) than its own diameter (7.5 µm). The normal RBC passes through the spleen sinuses due to its ability of changing shape. The spleen sinuses are the center of concentration of specialized filter system and removal of aged erythrocytes. The RBCs can linger in the spleen sinuses for a little while [1, 11, 14]. Intracellular hemolysis is a physiological fragmentation (erythrorhexis) of erythrocytes with a subsequent lysis and erythrophagocytosis in organs of the reticuloendothelial system (RES) with the concentration mainly in the spleen, partly in the liver.

About 90% of the erythrocytes don’t last long there, without being filtrated. 10% of RBCs get into the system of vascular sinuses, being filtrated through the pores (fenestrae) which size is smaller (0,5-0,7 µm) than the diameter of RBC. The "old" RBCs tend to change the membrane rigidity; they are fixed in the sinusoids. Due to the reduced pH and glucose concentration in the sinuses of the spleen, red blood cells are exposed to metabolic exhaustion. Macrophages, located on both sides of the sinuses of the spleen eliminate "old" RBCs. The red blood cells are destroyed by macrophages of the RES - intracellular hemolysis. Nearly 90% of red blood cells are broken down [3, 15].
Intravascular hemolysis is a physiological rupture of red blood cells directly into the bloodstream. The destroying red blood cells release from 1 to 4 mg of free Hb (ferrohematogen with Fe$^{2+}$) in 100 ml of blood plasma. Released into the bloodstream Hb is bounded with plasma protein by haptoglobin, which belongs to α2-globulins. The created hemoglobin-haptoglobin complex weighs of from 140 to 320 kDa, while the filter of the kidney glomeruli «lets through» molecules less than 70 kDa. Thus, the ability of haptoglobin of binding hemoglobin prevents its extrarenal elimination with the subsequent absorption of complex by RES and destroying cells.

The pathologic intravascular hemolysis is characterized by three main factors of AKI formation.

**The first factor is hemosiderin – ferritin**

Hemoglobin binding capacity of haptoglobin makes up 100 mg per 100 ml of blood. The excess reserve hemoglobin binding capacity of haptoglobin in the blood is accompanied by the release of hemoglobin through the kidneys with urine. This process occurs during massive intravascular hemolysis. Although the molecular weight of hemoglobin is significant (64 600 D), it is still able to penetrate through the glomerular barrier. Passing through the renal tubules, hemoglobin is adsorbed by the renal epithelial cells. Subsequently, the degradation of hemoglobin in the tubules is accompanied by the release of free iron, which catalyzes the formation of radicals. The influence of activated xantinoxidase leads to the formation of highly toxic superoxide radical [2, 8]. This radical is able to damage biomolecules both directly and through its derivatives, influenced the carbonyl group of phospholipids membranes. This process causes lipid peroxidation and reduction of trivalent iron (Fe III) in the active centers of hemin enzymes to divalent (Fe II) [1, 3, 13]. Further, influenced by reduced iron occurs the formation of hydroxyl radical and hydroxyl anion, which is accompanied by powerful oxidizing action, causing damage not only lipids of cellular membranes, but also ion channels proteins, structural proteins of cells and enzymes. It activates thereby necrosis of epithelial cells of the tubules and formation of AKI [1, 15].

**The second is tubular – obstructive factor**

During massive hemolysis and inflow of a considerable amount of erythrocytic and proteins decay products into the blood, they are precipitated with the formation of cylinders, obstructed the renal tubules – tubular obstruction [2, 14]. Obstruction of the tubules may lead to increased tubular pressure, decreasing of the filtration pressure and GFR decline and promotes the development of inflammation of the renal parenchyma, activation of the immune component of inflammation and sensitization and production of autoantibodies against renal tissue. It is resulting from a loss of villi brush borders of renal tubules cells, exfoliation of epithelial cells from the basal membrane, which leads, ultimately, to the formation of an acute nephrotoxic tubular necrosis [4, 6].
The third factor is hypoxic – ischemic

The kidneys take the first place among other organs in the intensity of blood supply. The total blood flow makes 20 — 25 % of the stroke volume of the heart. The tone of the arterioles is regulated by hormones and vasoactive substances, most of which are formed directly in the kidney. Anatomical and functional unit of the kidney is nephron, including Schumlanisky-Bowman's capsule that contains a ball of capillaries called renal or Malpighi body. The diameter of the afferent arterioles is twice higher than the diameter of the efferent one. Over leaving the glomerulus, the efferent arteriole branches into capillaries, creating a dense network around the proximal and distal convoluted tubules.

The significant feature of the juxtamedullary circulation is that the efferent arteriole doesn't break with the formation of pericanalicular capillary network but forms the «straight vessels», located in the kidney medulla. By decreasing of systemic pressure less 70 mmHg the autocontrolling mechanisms of renal blood circulation are incapable resulted in a «blood reset» through the juxtamedullary "straight vessels" Trueta renal vascular shunts. This led to formation of ischemia of the renal cortex and the development of an acute nephrotoxic tubular necrosis [1, 5, 13]. Beyond that, renal ischemia reduces the discharge of vasodilator prostaglandin in the renal cortex, which may increasingly reduce renal circulation.

Thus, the study of the mechanisms of an acute postoperative renal damage in pathological intravascular hemolysis remains relevant.

The purpose of the work

To establish the role of the intraoperative hemolysis as a biomarker of AKI.

Methods and materials

The retrospective non-randomized, observational, cohort study was conducted to verify the hypothesis of the effect of intravascular hemolysis on indices of renal function in 407 patients after abdominal aorta surgery and in 107 cardio surgical patients operated with artificial circulation. The volume of hemorrhage was defined according to the ATLS Protocol (2013). Hemolysis was determined by hemiglobincyanide method [1, 2, 3].

Postoperative acute kidney injury was diagnosed according to the RIFLE scale.

Histological study of the renal autopsy material was carried out at the Department of Pathological Anatomy of Donetsk National Medical M. Gorky University, (Doctor of Science, PhD, Professor Vasilenko I. V.) and comprised light microscopy in 46 persons.
Discussion and results

It was founded an acute postoperative renal injury in 118 patients out of all 514 patients in total. In 70 persons was detected stage "I", and 40 patients were in stage "F".

The blood loss class II (ATLS) 1127 – 1449 ml was observed in 40 (40%) patients, class III 1932 – 2208 ml was identified in 42 (36%) patients, blood loss of class IV 2989 – 6588 ml was observed in 28 (24%) patients.

To achieve the target hemoglobin value of 90 g/l (blood loss severity Class II) it was required the introduction of allogeneic Packed red cells 1 – 2 doses and performing of non-hardware reinfusion 5 ml/kg; in case of severity Class III were required 3 to 4 doses of allogeneic Packed red blood cells and non-hardware reinfusion of 6 ml/kg; in severity of blood loss Class IV were required 7 doses of allogeneic Packed red blood cells with reinfusion of 8 – 9 ml/kg.

It became evident that depending on the severity of hemorrhage the patient was given packed red blood cells not from one donor but from 2-6 persons. The factor of developing of intravascular hemolysis was confirmed by available information about the existence of about 300 erythrocyte antigens that determine a huge number of blood groups [2].

It is established that in blood loss class II the intravascular hemolysis, as a rule, made 0.4±0.01 g/l (for control of 0.3±0.001 g/l), in class III that amounted to 0.67±0.01 g/l, at the fourth class was of 0.9±0.001 g/l, sometimes having reached critical value up to 4 – 6 g/l.

In addition, in all patients have been determined the criteria of postoperative acute kidney injury in the early postoperative period according to the KDIGO recommendations, 2012. That was confirmed by a decrease in glomerular filtration rate 35±0, 08 ml/min, increase (p<0.001) of creatinine indicators 0.3±0.01 mg/DL, urea raise for more than 1.5 times from baseline, and a reduction of 45% clearance-creatinine.

The analysis of the obtained results revealed significant (p <0.001) moderate negative correlation between the level of clearance creatinine and hemolysis amount (Figure 1). It was noted the following dependence, the more is the index value of hemolysis, the less is the creatinine clearance index value.
Fig. 1. Dependence of clearance on the hemolysis indicators.

Fig. 2. Dependence of creatinine value on hemolysis indicators.

At the same time, it was established a statistically significant ($p < 0.001$) strong positive correlation between the amount of hemolysis and creatinine level (Figure 2.): The higher is the hemolysis rate, the higher is the value of creatinine.
Intravascular hemolysis as a biomarker…

Fig. 3. Dependence of plasma urea values on hemolysis indicators.

In addition, it was revealed that in all patients of cardio surgical and angiosurgical profile the value of plasma urea (Figure 3.) \( p < 0.001 \) is in direct proportion to the value of hemolysis: the higher the index of hemolysis is, the greater is the value of plasma urea.

The obtained data were reported in histological-morphological study of the kidneys of deceased persons. The typical changes are reported in the following observation. Case History № 9259 aged 64, Diagnosis: rupture of the infrarenal division of abdominal aorta in the retroperitoneal space, extensive retroperitoneal hematoma. Patient is in condition after resection of the aneurysm. Aortal-femoral bifurcation prosthesis was performed. Intraoperative blood loss was 8500, 0 ml (Class IV according to ATLS). The blood reinfusion was carried out in the amount of 3600,0 ml, allogeneic blood transfusion of packed red cells was 1540,0 ml (6 doses). The hemolysis indicator after performed transfusion was 2,42g / l.

Death occurred on the 5th day. The light microscopic examination of the autopsy material of the patient's kidney reveals that morphological substrate of kidney injure were severe dystrophic changes and necrosis of the convoluted tubule epithelium, obstruction of the lumen by hyaline casts and stagnant plethora of the kidney glomeruli.
Hyaline cylinders in the tubular lumen (1). Necrosis of the tubular epithelium (2). Evident congestive hyperemia of the glomeruli (3).

Staining with hematoxylin and eosin, ×100

The light microscopic examination of the autopsy material of the patient's kidney reveals that morphological substrate of kidney injure were severe degenerative changes in the epithelium of the convoluted tubules, stagnant plethora of the kidney glomeruli and renal capillaries, tubule lumen occlusion and focal hemosiderosis.

Conclusions

The evidence of hemolysis significantly (p< 0.001) depends on the class of blood loss severity (ATLS) and methods of blood replacement. The maximum index value is determined in class of blood loss III and IV according to ATLS.
The severity of hemolysis correlates with the violation of the indices of renal function: decreased creatinine clearance and increased urea level in plasma. Intravascular hemolysis is considered to be not only a marker but also pathogenetically conditional factor of the formation of postoperative AKI. This is confirmed by the presence of hemosiderosis, tubular obstruction and hypoxic-ischemic injury of the renal structures with the development of atrophy and necrosis of the tubular epithelium, which were detected by light and of the kidneys in patients with intravascular hemolysis.

References


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