DYNAMICS OF SOME IMMUNOLOGICAL PARAMETERS IN CHILDREN WITH ASSOCIATED TRAUMA OF THE LOCOMOTOR SYSTEM DEPENDING ON THE PERIODS OF OSTEOSYNTHESIS

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During the period 2005-2009, there were operated 71 children with associated trauma of pelvis and extremities aged from 4 to 15 years. The patients were divided into 2 groups: early osteosynthesis (up to 3 days) was carried out in 52 (73,2%) and late osteosynthesis (from 3 to 7 days) in 19 (26,8%) suffered children. Immunological studies (determination of IL-1β, IL-2 and TNFα) were performed in both groups in the first hours after trauma and at the 1, 3 and 7 days. In trauma patients was observed a significant increase in the level of IL-1β, IL-2 and TNFα to control group. After late osteosynthesis, the levels of proinflammatory cytokines were elevated relative to the data in the first hours after injury, whereas in patients with early osteosynthesis their levels were significantly reduced on the 3rd day. Thus, early osteosynthesis showed positive dynamics of immune response mediators.

Keywords: associated trauma, pediatric polytrauma, osteosynthesis, immunity, cytokines

In recent years, morbidity and mortality rates in all developed countries has increased due to lethality from traumatism. In this case, there were changes in the character of trauma. Pozharisky (1989), Yermolov et al. (2003) noted the increased percentage of severe traumas, complicated by shock and hemorrhages, more often incidences of severe associated injuries or polytrauma, which is characterized by long-term rehabilitation and social adaptation, high disability and mortality (30% and 16-20%, accordingly) [11, 17].

Polytrauma is not just the sum of damages. Its treatment should include not only the control and correction of physiological damages, but the pathophysiological responses to them. Sokolov (2005), Yermolov et al. (2003) defined polytrauma as a systemic disease that causes the release of many inflammatory mediators. Systemic response to trauma can cause a lethal triad – hypothermia, acidosis, and coagulopathy, with particular acute course in children [14, 17].

All the body's response to trauma is difficult to explain only by shock. Therefore, it was introduced the concept of "»traumatic disease», which is a collection of local and general changes in the body due to severe mechanical trauma and determining its vitality and adaptive capabilities.

Traumatic disease occurs as a response to predominantly associated trauma, accompanied by acute disturbances of vital functions such as traumatic coma, spinal shock, traumatic shock or acute respiratory failure. Its pathogenesis was well studied by Klochkov, Yeryuhin et al. in 1989. According to authors, the first week after injury represents one of the key stages of traumatic disease when there is a gradual transition from the first phase with its mechanisms leading to the second one, which ensures the inclusion of long-term adaptation. If this stage is already completed (approximately by the end of 2-3 days), then any new extreme impact, including surgical intervention, causes failure, which is most often manifested in severe local and general surgical infections [7, 8, 18].

The main pathogenetic links of response to trauma defined by several studies are as follows: hemorrhagic shock, hemodynamic instability, respiratory distress syndrome, hypoproteinemia, disseminated intravascular coagulation (DIC) syndrome, low immunity, sepsis, multiorganic failure [2, 4, 7, 8, 12, 19].

Tcherne et al. (1995) distinguishes the following periods in the course of traumatic disease:

1) acute or resuscitation period (1-3 hours);
2) primary or stabilization period (1-72 hours);
3) secondary or regeneration period (3-8 days);
4) tertiary or recovery period (> 8 days) [15].

The risk of infection for any period of treatment of traumatic disease is of particular importance because every injury is accompanied by a depression of humoral and cellular immunity, the intensity of which increases sharply with polytrauma [9]. In addition, each surgical intervention causes a secondary immune deficiency, the severity of which depends on the volume and duration of operation [6]. Immunological parameters could serve as markers of prognosis of polytrauma severity and its outcome. In this occasion, Yagmur et al. (2005) have found a significant increase in the levels of interleukins (IL) 2, 6 and 8 in patients who died of polytrauma, whereas in the survivors with milder course of the disease, the concentration of these IL was significantly lower [16].

Peterson et al. (2004) considers that predictor of infectious complications in pediatric polytrauma is the initial shift of electrolyte balance in the direction of base deficit [10]. Ac-

cording to Galaktionov (2004), children with multiple traumas are also risk group for infections because of the limitations of protective immunological mechanisms, as maturation of the immune system is completed by age 14 [5].

In trauma, osteogenesis is regulated by a complex mix of factors, in which the immune system is also involved [1, 3]. Abakumov et al. (2001) observed irregularities in the synthesis of several cytokines after severe mechanical damage, both proinflammatory and anti-inflammatory [1].

According to Chechyotkin et al (2004), a number of cytokines such as TNFα, TNFβ, IL-6, and IL-17 stimulate the formation of osteoclasts and regulate their resorptive activity, resulting in the destruction of bone tissue. On the contrary, IL-4, IL-10, IL-13, IFNγ, and TGFβ inhibit both formation of new osteoclasts and activity of existing ones, stimulating bone formation [3].

Thus, there is no doubt that the study of cytokine levels in associated trauma in children will appreciate the role of immunological reactions in the body and evaluate their influence on the course of traumatic disease.

The purpose of this research was to study the levels of inflammatory cytokines in children with associated trauma of the locomotor system.

Materials and methods of research

Between 2005 and 2009, 71 children with associated trauma of pelvis and extremities were examined and operated in the department of pediatric traumatology of the Republican Scientific Centre of Emergency Medicine of the Republic of Uzbekistan.

On the periods of surgical treatment on the bones, the patients were divided into 2 groups: early osteosynthesis (up to 3 days) was carried out in 52 (73,2%) and late osteosynthesis (from 3 to 7 days) in 19 (26,8%) suffered children. To conduct immunological studies and for the objective quantitative evaluation of results, we selected 30 patients (each group consisted of 15 patients) with similar trauma severity. The patients' age ranged from 4 to 15 years.

Immunological studies were performed in both groups on the first hours after trauma and at the 1, 3 and 7 days. In blood serum we determined the concentrations of cytokines – IL-1β, IL-2 and TNFα (reactants of «Cytokine Ltd2, St. Petersburg) by immunoenzimatic method. The data obtained were processed using methods of variation statistics.

Results of research and their discussion

The periods and methods options for osteosynthesis at the stage of intensive care are an important task. Primarily, operations on bones must not to be accompanied by large blood loss. Additionally, we need to ensure sufficient stability of the fracture, which does not require plaster bandages, to facilitate care for severe sufferers. Where possible, osteosynthesis should be final and not to provide further reoperations. However, a number of specialists in children with severe associated traumas apply the concept of primary conservative and secondary surgical treatment, external or internal osteosynthesis.

The results of our studies on the concentration of proinflammatory cytokines in trauma patients demonstrated a significant increase in the levels of IL-1β, IL-2 and TNFα (P<0.001), compared with those of the control group (table 1).

Table 1

Levels of cytokines in blood serum of trauma patients, pg/ml

Cytokines	Control group	First hours after trauma	P
IL-1β	29,6 ± 2,3	178,7 ± 11,6	<0,001
IL-2	12,3 ± 1,6	49,1 ± 4,5	<0,001
TNFα	27,6 ± 2,5	68,9 ± 4,3	<0,001

Note: P – significant differences between the groups.

IL-1β is proved to be a cytokine of broad-spectrum, which is produced mainly by macrophages. It determines the starting immune response, plays a key role in the development of inflammation, is involved in regulation of hematopoiesis, and is a mediator of interactions between the immune and nervous systems [3].

The main function of IL-2 is to provide a cellular component of adaptive immunity. IL-2 is a factor in the growth and differentiation of T-lymphocytes and NK-cells. In addition to effects on proliferation and differentiation of these cells, IL-2 also participates in the regulation of the coordinated functioning of other factors and mechanisms of innate and acquired immunity.

Tumor necrosis factor (TNFα), produced by macrophages, lymphocytes and other cells, is an activator of endothelium and all types of white blood cells (primarily, their cytotoxic functions), and a stimulator of cell adhesion. It has pyrogenic effect and has been involved in the synthesis of acute phase proteins in liver [3].

The combination of these three cytokines provides a wide range of effects, ranging from the synthesis of acute phase proteins to enhance leukocyte migration and activation at the site of injury [3].

Thus, cytokine response, which starts immediately after injury, allows for a coordinated restructuring of metabolic and physiological functions of several organs and tissues. Excessive functioning of the cytokine response leads to shock-like states.

The results showed that in patients, who underwent conservative treatment and osteosynthesis on the 3rd day, the levels of studied cytokines increased and were significantly higher on the 7th day than on the 1st day (table 2). Table 2 shows that on the 1st day, the level of IL-1β was higher 6,5 times ($P < 0,001$), IL-2 – 3,9 times ($P < 0,001$), and TNFα – 2 times ($P < 0,01$), in comparison with the control group.

Table 2

Cytokine levels in blood serum of patients with late osteosynthesis, pg / ml

Cytokines	First hours after trauma	After first day	After 3 days	After 7 days
IL-1β	178,7 ± 11,6	193,4 ± 6,3	215,05 ± 11,9	273 ± 12,1**
IL-2	49,1 ± 4,5	45,7 ± 4,3	40,9 ± 3,3*	42,1 ± 2,9
TNFα	58,9 ± 4,3	59,9 ± 3,5	61,5 ± 2,9	74,7 ± 2,9**

Note: * – values are significant relative to the data in the first hours after trauma
** – values are significant relative to the data through the third day (P < 0,01 – 0,001)

At the 3rd day, the study revealed that there was a trend to increase in the level of IL-1β, while the concentration of IL-2 was significantly decreased ($P < 0,05$). The level of TNFα was steadily increased.

At the 7th day, there were some changes in the levels of studied cytokines. Thus, the levels of IL-1β and TNFα increased in 1,2 times ($P < 0,05$), in comparison with the data obtained on the 3rd day of investigation.

Thus, after late osteosynthesis the level of proinflammatory cytokines was elevated relative to the data in the first hours after trauma.

The results of monitoring the dynamics of post-traumatic period in patients with early osteosynthesis showed the significant reduction in the level of proinflammatory cytokines on the 3rd day (table 3).

At the 7th day, the study revealed positive dynamics of proinflammatory cytokines in patients with early osteosynthesis.

Thus, trauma belongs to the most common pathologic conditions encountered in humans. Regardless of the location and severity of injury, any trauma is accompanied by more or less deep stress, in which pathogenesis the essential role belongs to the immune system. According to some authors, the dynamics of indicators of immunity after injury correlates with the state of resistance in general adaptation syndrome and fit into the stages of traumatic disease [7, 8, 11].

Table 3

Cytokine levels in blood serum of patients with early osteosynthesis, pg/ml

Cytokines	First hours after trauma	After first day	After 3 days	After 7 days
IL-1β	178,7 ± 11,6	163,4 ± 5,3	151,2 ± 7,9*	98,7 ± 5,5**
IL-2	49,1 ± 4,5	37,2 ± 3,8	30,9 ± 4,1*	29,2 ± 2,5*
TNFα	58,9 ± 4,3	57,2 ± 2,8	51,9 ± 3,1*	49,2 ± 2,5*

Note: * – values are significant relative to the data in the first hours after trauma
** – values are significant relative to the data through the third day (P < 0,01 – 0,001)

In this regard, immunological monitoring, including the study of cytokine status in patients with injuries and diseases of bone tissue using the latest medical technology is relevant and possible to develop immunological criteria for predicting complications.

The most common reactions to traumatic exposure include changes in the system of nonspecific defense. Phagocytosis plays an important role in localization of damage, elimination of damaged tissue and destruction of contaminated flora. In parallel with the migration of neutrophils, macrophages and lymphocytes into the zone of damage, complement system is activated, particularly C3 component of complement, which together with transforming growth factor (TGF) induces production of IL-1 and TNFα. In turn, these cytokines promote the synthesis of IL-6. The combination of these three cytokines provides a wide range of effects, ranging from the synthesis of acute phase proteins to enhance leukocyte migration and activation at the site of injury. Systemic action of cytokines is manifested by stimulation of differentiation of bone

marrow precursors of immune cells that trigger the synthesis of acute phase proteins. Along with this, both IL-6 and IL-1 are pyrogenic, and induce febrile reaction [13].

It should be noted that the injury starts a cascade of cytokine responses aimed to limit the damaged area, to eliminate the necrotized tissue and contaminated flora, and, finally, to activate proliferating cells for reparations of damages. There are at least two types of interaction: a direct effect of the whole molecule of cytokines on immunocompetent cells or the effects of individual «key fragments», consisting of a limited number of amino acid residues [3]. Detection of violations in the functioning of individual components of the immune system can control the direction of bone regeneration and increases the clinical relevance of research.

Thus, the application of the concept of primary conservative and secondary surgical treatment did not contribute to changes in elevated levels of proinflammatory cytokines. It is known that in children due to the rapid course of reparative processes in the zone of fracture, immobilization of poor quality leads to fusion of bone fragments in the wrong position, or retards the process of consolidation. In this connection, associated trauma serves a problem in determining of site of operational stabilization of fractures, especially the volume and timing. We carried out early osteosynthesis showed positive dynamics of immune response mediators.

Summary

According to available literature data, every injury is accompanied by a depression of humoral and cellular immunity, the intensity of which increases sharply with severe associated trauma. In addition, each surgical intervention causes a secondary immune deficiency, the severity of which depends on the volume and duration of operation. Children with multiple injuries (polytrauma) are also risk group for infections because of the limitations of protective immunological mechanisms (as maturation of the immune system is completed by age 14). After severe mechanical injure, there are observed irregularities in the synthesis of several cytokines, both proinflammatory and anti-inflammatory.

The results of our studies on the concentration of proinflammatory cytokines in trauma patients demonstrated a significant increase in the levels of IL-1β, IL-2 and TNFα ($P < 0,001$), in comparison with the control group. After late osteosynthesis, the levels of proinflammatory cytokines were elevated relative to the data in the first hours after injury, whereas in patients with early osteosynthesis their levels were significantly reduced on the 3rd day.

Thus, we carried out early osteosynthesis showed positive dynamics of immune response mediators. Hence, early osteosynthesis is more effective in the treatment of associated and multiple traumas in children and should be started as soon as possible.

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