ROLE OF THE HEPCIDIN IN THE DEVELOPMENT OF ANEMIA IN PATIENTS WITH JUVENILE RHEUMATHOID ARTHRITIS

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Abstract. Overproduction of hepcidin in the liver is considered to be due to the effect of high levels of proinflammatory cytokines. Interleukin-6 is known to play a key role in the development of anemia in patients with juvenile rheumatoid arthritis. During the study of 35 patients with juvenile rheumatoid arthritis treated with biological disease-modifying drugs (tocilizumab and golimumab), direct relations between the level of hemoglobin and hepcidin were not found. Most probable, anemia in rheumatoid arthritis is multifactorial and involves violation of iron metabolism in the organism and the negative effects of proinflammatory cytokines on erythropoiesis. Severe anemia can be associated with iron deficiency, or with the macrophage activation syndrome in children with juvenile rheumatoid arthritis.

Keywords: hepcidin, anemia, juvenile rheumatoid arthritis.

Introduction. Anemia of chronic disease, the anemia that is the second most prevalent after anemia caused by iron deficiency, occurs in patients with acute or chronic immune activation [15]. It is believed that the anemia in patients with chronic illness can significantly worsen the prognosis and quality of life of these patients.

For the first time this condition was described by Cartwright G.E, Wintrobe M.M. in 1952 [5]. However, the key pathogenic moment became clear after the opening of the peptide hepcidin in 2001 [8]. Name of the new peptide was formed by abbreviation Latin word **hep**ar (lat. - liver - the site of synthesis of peptide) and **cidin** (lat. - destroy - emphasizing antibacterial properties of peptide). It was shown that hepcidin synthesis occurs in renal tubules. Also, low levels of expression noted in other cells, tissues and organs, including macrophages, adipocytes, and brain cells, which may indicate the important role of hepcidin in the autocrine and paracrine control of iron metabolism at the local level [13]. Hepcidin is encoded as an 84-amino acid prepropeptid. The active form of the hormone circulates in plasma and it is binding to α_2-macroglobulin [9]. The main

route of elimination of hepcidin – renal clearance.

Currently, hepcidin is the main regulator of systemic iron metabolism in the organism [7]. Hepcidin acts by modulating the cellular export of iron in the plasma and extracellular fluid through ferroportin. Ferroportin - is a receptor of hepcidin and is the only known cellular iron exporter in vertebrates [21]. Hepcidin inhibits iron absorption in the intestine and the presentation of iron from macrophages, dispose of old red blood cells. Hepcidin injection into mice resulted in a significant reduction in serum iron within just 1 hour. Even though hepcidin is rapidly cleared from the plasma, the effect of a single dose was apparent for up to 72 hours. Probably, this time required for the re-synthesis of sufficient amounts of the hepcidin receptor - ferroportin [20]. Hepcidin synthesis rapidly increases in infection and inflammation. IL-6 is a major inductor hepcidin and acts through STAT3-dependent transcriptional mechanism. In volunteers who was introduced the IL-6 urinary hepcidin excretion increased several times and serum iron levels decreased in 2 hours after infusion [10].

Thus, IL-6 is a leading point of contact in the pathogenesis of juvenile rheumatoid arthritis and anemia. Production of IL-6 was significantly increased in patients with juvenile rheumatoid arthritis and correlated with the degree of disease activity [2] and to the development of anemia. In patients with rheumatoid arthritis indicated a significantly higher level of serum pro-hepcidin compared to patients with systemic lupus erythematosus and healthy control group [19]. In addition, serum pro-hepcidin was significantly lower in patients with iron deficiency anemia than in patients with rheumatoid arthritis and anemia without iron deficiency and a control group of healthy volunteers [12].

Anemia in rheumatoid arthritis is a typical example of the anemia of chronic disease [14]. The main mechanism of anemia – a disturbance of iron utilization in the bone marrow by the action of hepcidin, whose expression is increased due to overproduction of IL-6 [16]. Anemia is highly prevalent among patients with rheumatoid arthritis in the absence of effective treatment of the underlying disease. According to studies published from 1966 to 2003, the prevalence of anemia ranged from 33.3 to 59.1% [17]. According to a multicenter study of patients with rheumatoid arthritis, the prevalence of anemia in the first year after onset of the disease was 5%, 3 y - 11%, 5 - 13%, 7 y - 16%, 10 y - 7% [23]. In another study, the prevalence of anemia was 16.7% and was associated with the severity of rheumatoid arthritis [18]. In a placebo-controlled trial of infliximab anemia was observed in 39% of patients, 39% of women and 32% men [3].

Treatment of anemia in rheumatoid arthritis requires from rheumatologist understanding of

the pathogenic mechanisms underlying in the development of anemia. The best means of correction of anemia in rheumatoid arthritis is a systemic disease control by administrating synthetic and biological disease-modifying drugs: methotrexate [4], antagonists of TNF-α, rituximab, abatacept, tocilizumab [6]. In studies of combination therapy with infliximab-methotrexate noted an increase of hemoglobin and its normalization in 43% of anemic patients with rheumatoid arthritis [22]. Tocilizumab - recombinant humanized monoclonal antibody to human IL-6 receptor (IL-6). Tocilizumab selectively binds to and inhibits both soluble and membrane IL-6 receptor (sIL-6R and mIL-6R). In clinical trials of tocilizumab the hemoglobin level in patients with rheumatoid arthritis and anemia increased by 17 g/L at 2 weeks after the start of treatment [11]. Thus, with the using drugs acting on the overall important pathogenic link of rheumatoid arthritis and rheumatoid anemia is possible to implement a system control of the underlying disease and the effective correction of anemia.

The aim of our study was to determine the prevalence of anemia in patients with juvenile rheumatoid arthritis receiving treatment by the biological disease-modifying drugs and estimate the dependence of the level of hemoglobin in this group of patients from the level of hepcidin and other parameters of iron metabolism.

Materials and methods. The study included 35 children (12 boys and 23 girls) suffering from juvenile rheumatoid arthritis. At 19 children diagnosed polyarticular juvenile rheumatoid arthritis (pJRA), 16 - system onset JRA (sJRA).

All patients were treated by biologic disease-modifying drugs. Of these, 25 patients received tocilizumab - recombinant humanized IgG1 class monoclonal antibodies to human IL-6 receptor, 10 patients receiving golimumab - recombinant humanized monoclonal antibody IgG1 class, forming high-affinity stable complexes "antigen-antibody" with soluble and transmembrane bioactive forms with tumor necrosis factor alpha (TNF-α), preventing binding of TNF-α to its receptors. The study was approved by the Ethics Committee, prior to study the patients and their parents signed informed consent.

Estimate the dynamics of the following laboratory parameters: hemoglobin (n = 554), red blood cells count (n = 554), and reticulocytes (n = 135), mean corpuscular volume (n = 236), mean corpuscular hemoglobin (n = 241), mean corpuscular hemoglobin concentration (n = 191), white blood cells count (n = 554), and platelets (n = 543), absolute neutrophil count (n = 192), eosinophils (n = 191), monocytes (n = 192), basophils (n = 192), lymphocytes (n = 192), erythrocyte sedimentation rate (n = 554), the level of C-reactive protein (n = 383), soluble transferrin receptor

(n = 35), total serum iron (n = 76) and ferritin (n = 154), total iron binding capacity of serum (n = 60), the level of serum hepcidin (n = 25). All studies were conducted in certified laboratories on standardized methods, the results of the study are presented in SI units.

In addition, clinical signs of activity of juvenile rheumatoid arthritis were evaluated: joint swelling, pain and articular syndrome restriction of joint movement. Also evaluated the response to treatment according to the guidelines of the American College of Rheumatology (ACR score).

Anemia was diagnosed based on the WHO criteria for the different age groups.

To evaluate the results were used methods of the statistical description, statistical hypothesis testing and modeling.

According to the results of the study the prevalence of anemia in patients with juvenile rheumatoid arthritis receiving treatment by biological disease-modifying drugs was 60.0%. At the same time, the prevalence of anemia in patients with polyarticular JRA was 42.1%, and in patients with systemic JRA - 81.3% (Table 1). At 71.4% was found mild anemia, in 23.8% - moderate anemia and 4.8% - severe anemia (Figure 1).

Based on these data was performed statistical analysis and constructed multivariate models describing the dependence the level of hemoglobin from hepcidin and other parameters of iron metabolism.

Thus, the low level of hemoglobin is noted for the high-level of serum hepcidin and low-level of serum ferritin, which can probably be attributed to associated iron deficiency in patients with juvenile rheumatoid arthritis. In addition, the low level of hemoglobin is observed at combination of high serum ferritin levels and low levels of serum hepcidin, which in its turn, may be due to the development of macrophage activation syndrome at these patients, however, the nature of this phenomenon is not fully understood and requires in-depth study in larger number of patients (Figure 2).

The decrease hemoglobin levels is observed at combination of high level of serum hepcidin and high total iron binding capacity (TIBC), which may also be due to associated iron deficiency. Moderate decrease hemoglobin levels is observed at the mean values of serum hepcidin, and total iron binding capacity, which is characteristic of anemia of chronic disease (Figure 3).

And moderately low hemoglobin level celebrated with a combination of high serum hepcidin level and low level of soluble transferrin receptor (sTFR), which is characteristic of

anemia of chronic disease, due to inhibition of sTFR expression by proinflammatory cytokines (Figure 4).

Conclusions. Anemia widely distributed in patients with juvenile rheumatoid arthritis receiving treatment by the biological disease-modifying drugs. It is caused by the fact that this group includes patients with severe pJRA and children with sJRA. However, the prevalence of anemia in this group higher than that for patients with pJRA and sJRA receiving standard treatment of juvenile rheumatoid arthritis [1].

Anemia in juvenile rheumatoid arthritis is multifactorial and develops as a result of hepcidin overproduction and disorders of iron reutilization accompanied iron restricted erythropoiesis. However, severe cases of anemia, probably association with the iron deficiency, or with the development of macrophage activation syndrome.

To estimate the iron metabolism in patients with juvenile rheumatoid arthritis in addition to standard indicators such as the level of serum iron, TIBC, transferrin saturation and ferritin levels, is necessary to determine the level of soluble transferrin receptor.

Greatest interest is the reduction of hemoglobin accompanied by increased ferritin levels with low serum hepcidin. This condition is probably be associated with the development of macrophage activation syndrome in patients with juvenile rheumatoid arthritis and requires further study.

Table 1.

Prevalence of anemia in JRA patients.

	Anemia	No anemia	Total
pJRA (prevalence, %)	8 (42,1%)	11	19
sJRA (prevalence, %)	13 (81,3%)	3	16
Total (prevalence, %)	21 (60,0%)	14	35

Figure 1.

Frequency of various degrees of anemia in JRA patients.

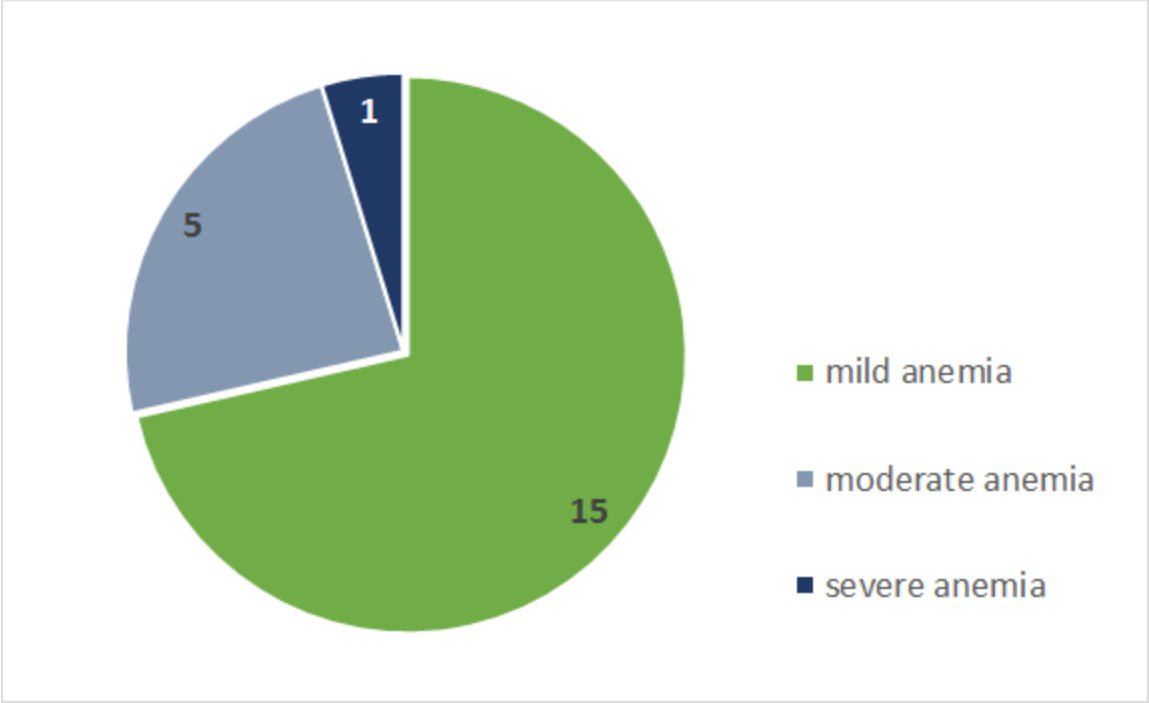
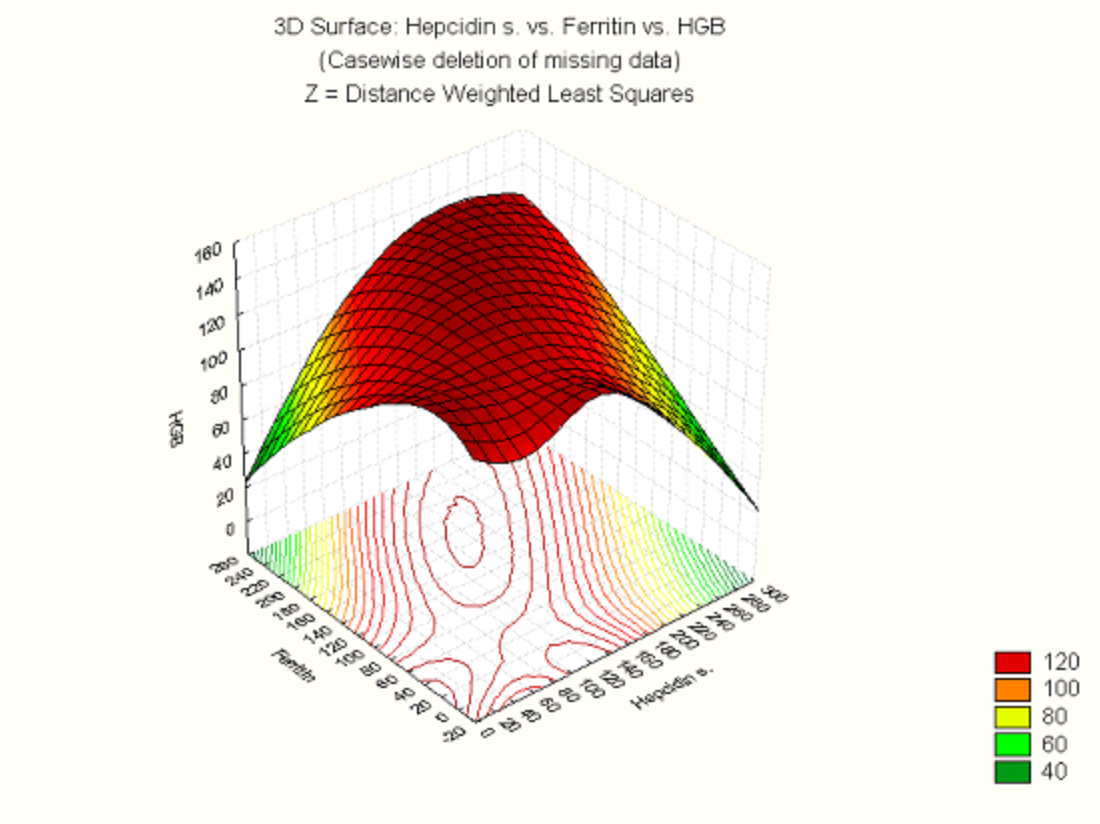
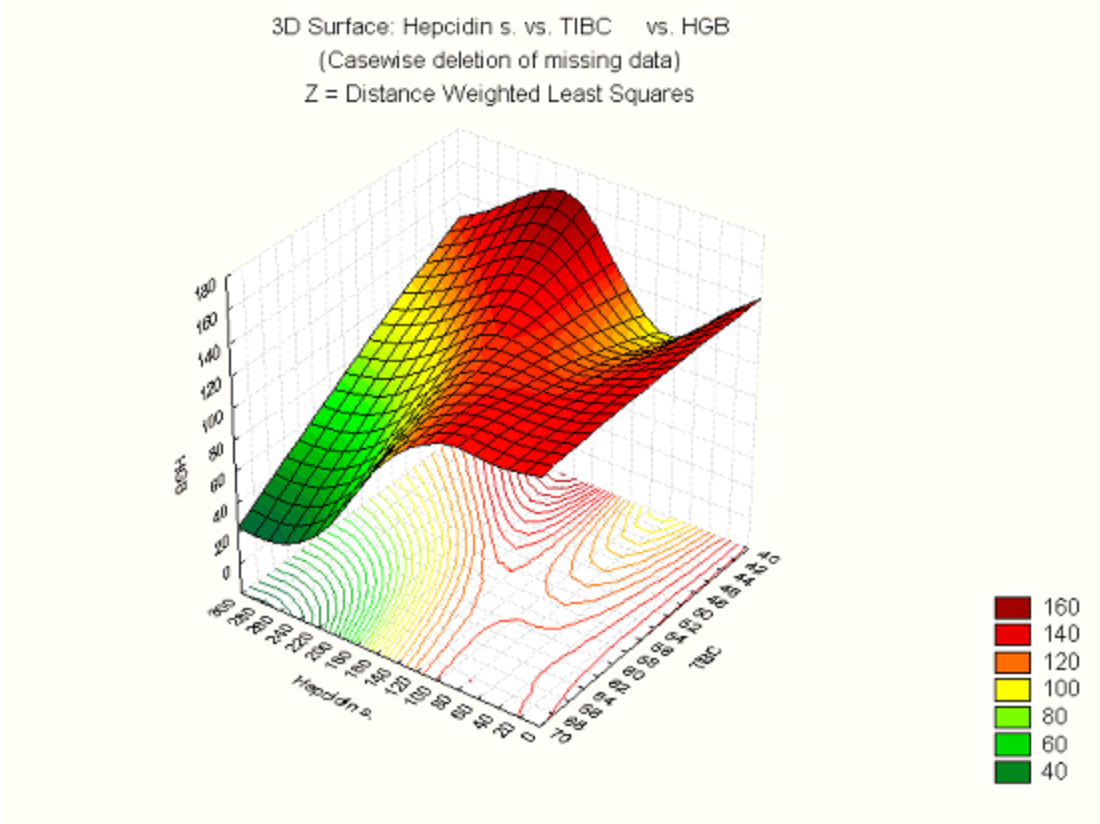


Figure 2.



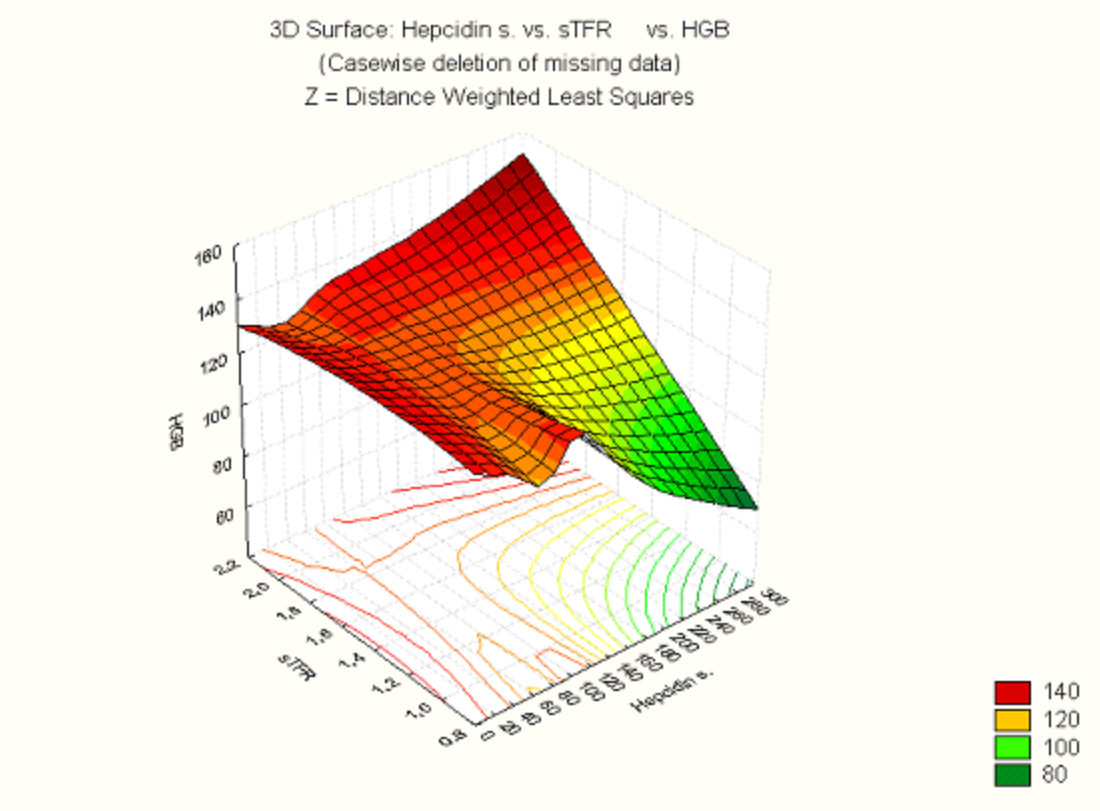
Dependence of hemoglobin level from hepcidin and ferroportin levels.

Figure 3.



Dependence of hemoglobin level from hepcidin and TIBC levels.

Figure 4.



Dependence of hemoglobin level from hepcidin and sTFR levels.

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