PATHOGENETIC CORRECTION OF ANEMIA WITH COMBINATION THERAPY IN PATIENTS WITH COVID-19

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ABSTRACT

The article presents experimental material on anemic syndrome (AS) pathogenesis in patients with coronavirus infection. Biomarkers of iron metabolic disorders and laboratory predictors of adverse outcomes of COVID-19 disease presented. The advantages and disadvantages of antianemic agents used to correct anemia of various origins in coronavirus infection are illustrated. We carried out the analysis of the effectiveness of the treatment of anemic syndrome using the combination therapy with erythropoiesis-stimulating, iron-containing drugs, and mineral complexes.

Keywords: COVID-19, coronavirus infection, SARS-CoV-2 virus, anemic syndrome, cytokines, ferritin, anemia treatment, erythropoietin, erythropoiesis-stimulating drugs, iron preparations, trace substance complexes, amino acids.

I. INTRODUCTION

To date, over 3 million cases of COVID-19 have already been identified worldwide. The new COVID-19 infection stimulates the destruction of red blood cells and contributes to the formation of the anemic syndrome. Conversely, anemia is one of the predictors of the severe course of COVID-19 since anemia, in particular iron deficiency anemia (IDA), is a factor in the activation and chronicity of inflammatory processes. [28].

Against the background of acute inflammation in COVID-19, destruction of erythrocytes occurs, which leads to a chaotic distribution of free iron in tissues, increases the loss of iron in the body, aggravates IDA and the severity of the course of coronavirus infection [10]. Accordingly, the combination of the anemic syndrome, especially of iron deficiency genesis, leads to a more severe course of coronavirus infection [18]. For example, during the epidemic of the MERS-CoV coronavirus infection, the need to connect to artificial lung ventilation in patients with IDA was more frequent than the average for the infected population [25]. When transferred to artificial lung ventilation, it was against the background of anemic syndrome that complications from vital organs most often occurred, and a higher mortality rate was noted [28].

This study presents the results of a systematic analysis of publications on disorders of erythropoiesis and iron metabolism in COVID-19, including the results of our original clinical studies of the use of combination therapy of antianemic agents. Hemabiological disorders against the background of coronavirus infection, the relationship of coronavirus infection with erythropoiesis dysfunction, iron deficiency, and anemia, as well as the importance of hyperferritinemia in COVID-19, are under review.

II. MATERIALS AND METHODS

Pathogenesis of anemia in coronavirus infection. Activation of the immune response against the causative agent of the SARS-CoV-2 virus can limit the availability of iron in the body during coronavirus infections, which is one of the mechanisms for the development of anemia [15,20]. Anemia, in turn, reduces the delivery of oxygen to the...
tissues. Thus, it turns out to be the cause of the development of multiple organ failure. Therefore, it is very important to consider the relationship between the violation of iron metabolism leading to anemia with the progression of COVID-19 and whether these associations have differences in age, gender, and the presence of chronic diseases.

Inflammatory processes significantly affect erythropoiesis through various mechanisms, in particular, disorders of iron metabolism, mediated by excessive production of interleukin (IL) -6, as well as through the overproduction of pro-inflammatory cytokines such as interferon-γ, IL-1, IL-3, and Tumor Necrosis Factor (TNF) -α [26]. The latter has an inhibitory effect on erythroid progenitor cells and can shorten the life span of erythrocytes [23-27]. These disorders often lead to the development of iron deficiency anemia (IDA), the second most common form of anemia in the world, as well as the most common among hospitalized patients in developed countries [16]. IDA is a disease characterized by a decrease in the filling of hemoglobin with iron, followed by a decrease in the content of hemoglobin in the erythrocyte with inhibition of erythropoiesis.

COVID-19 is characterized by a pronounced plethora of the capillaries of the interalveolar septa, as well as the branches of the pulmonary arteries and veins, with erythrocyte sludge, fresh fibrin thrombi; intrabronchial, intrabronchiolar, intralveolar, perivascular hemorrhages [4]. These hemorrhages, leading to cytolysis of erythrocytes, increase the uncontrolled loss of iron by the body and aggravate IDA in patients with coronavirus infection.

In general, there is very limited information on the disruption of iron content in COVID-19. For example, an analysis of patients with COVID-19 infection admitted to the intensive care unit (ICU) showed that they had a decrease in transferrin saturation by an average of 9% [1].

In a study of 50 PCR-confirmed COVID-19 patients, 45 had abnormally low serum iron concentrations (<7.8 μmol / L). Decreased serum iron levels have been associated with a higher risk of severe morbidity and mortality from COVID-19 [30].

Chinese researchers have studied how the SARS-CoV-2 virus infects red blood cells. It was found that the SARS-CoV-2 virus does not interact directly with red blood cells. Proteins are responsible for this, which are not part of the viral envelope but help the virus to gain a foothold in the host cell. So far, three such proteins have been identified - ORF1ab, ORF10, and ORF3a, which penetrate into erythrocytes and displace iron from hemoglobin. Hemoglobin, devoid of iron, is subsequently unable to perform its main function - to transport oxygen. These conclusions were made on the basis of computer simulation [15, 20].

By studying cellular blood counts of patients with COVID-19, the researchers found one interesting feature. Most patients have decreased hemoglobin levels and increased heme levels. An increased level of heme against the background of a decrease in hemoglobin suggests that hemoglobin is destroyed in the patient's body, which leads to the progress of an inflammatory process [15,26]. Recent case reports have described an association of COVID-19 with autoimmune hemolytic anemia (AHA), including one case of AHA cold agglutinin disease characterized by hyper inflammation, endothelial cell damage with systemic microcirculation and angiogenesis disorders, and acute respiratory distress syndrome (ARDS), which for most patients is the main cause of death [27].

A study of 1099 laboratory-confirmed cases of COVID-19 showed that severe patients had significantly lower hemoglobin levels than those diagnosed as mild cases. It should be noted that the decrease in hemoglobin was more pronounced in patients who achieved the combined endpoint of intensive care unit (ICU) or artificial lung ventilation (ALV), or death, indicating a low level of hemoglobin S may be associated with poor progression and prognosis. Therefore, anemia may be a risk factor for severe COVID-19 disease [28].

Anemia against the background of viral pneumonia can become chronic and even culminate into a phase of decompensation (due to increased oxygen demand, fever, hemoptysis, reduced tissue oxygen supply). Respiratory distress syndrome is characteristic of both the early symptoms of anemia decompensation and the severe clinical course of viral pneumonia: in both cases was reported rapid breathing with swelling of the nasal wings, intercostal and suprasternal skin retraction, feeling of lack of air, forced sitting position, reclining and pallescence. [11].

**Biomarkers of iron metabolic disorders and laboratory predictors of COVID-19 disease failures.** Patients with a severe course of COVID-19 have increased values of ferritin, a protein that stores iron in the body, associated with high mortality. Hyperferritinemia is a predictor of severe COVID-19 along with lymphopenia, hypoalbuminemia,
elevated levels of alanine aminotransferases (GPT), LDH, D-dimer, CRP, interleukin-1β, interleukin-2R, interleukin-6, interleukin-10, and tumor necrosis factor-alpha (TNF-α) [5]. Moreover, with a severe course of COVID-19, hyperferritinemia occurs in most patients. [17].

According to the Eurasian Association of Therapeutics (EAT, 2020), a high ferritin level is associated with an increased risk of complications such as bilateral pneumonia and acute respiratory distress syndrome (ARDS). However, EAT was unable to identify an accurate relationship between ferritin level and survival [6].

Another clinical study involving 5700 patients hospitalized with COVID-19 in district hospitals in New York also found that ferritin levels were significantly high in severe cases of coronavirus infection, which is in line with previous studies conducted in China [20,23]. An increased level of ferritin is associated with a pronounced inflammatory response in COVID-19 and is also associated with the penetration of the virus into the body with a subsequent effect on iron metabolism and the development of anemia [27]. It is obvious that anemia and hyperferritinemia, regardless of the underlying pathology, are priority predictors of mortality associated with coronavirus infection [24].

It is important to emphasize that hyperferritinemia progressively affects the integrity/permeability of alveolar-capillary/cell membranes. Respectively, inflammation, edema, and necrosis of lung cells ultimately lead to aggravation of pulmonary complications. The relative role of iron toxicity in the pathophysiology of COVID-19 is the putative hepcidin-mimetic effect of the SARS-CoV-2 virus, which promotes ferroportin activation, with subsequent progression of anemia and hyperferritinemia. Hepcidin promotes the entry of iron into cells, suppressing ferroportin, which is a key transporter of iron outside the cells; hepcidin is intended to enter the iron cell, its excess leads to ferroptosis. Physiologically, hepcidin increases or decreases, respectively, with a high or low concentration of serum iron [22].

Hyperferritinemia also induces ferroptosis, with high oxidative stress and lipid peroxidation, which ultimately increases mitophagy with accelerated apoptosis/cell necrosis. In fact, an overload of cellular iron is noted up to the hypoxic threshold (First phase of COVID-19). Increasing multi-organ oxidative stress associated with ferroptosis can trigger an excessive inflammatory/immune response (called a "cytokine storm") in the later, more critical stages of coronavirus infection. Sequestration of tissue iron leads to an increase in ferritin in the epithelium and immune cells of the lungs; this is probably due to the physiological need to protect lung cells from oxidative stress and hypoxia [16].

The fundamental units in the pathogenesis of COVID-19 include the interaction between the immune system and peripheral blood cells, which leads, in particular, to the activation of macrophages, inflammatory reactions, and increased release of TNF, interferon-γ, interleukin-1 [3]. They disrupt iron metabolism, reduce its release from macrophages, and incorporate it into erythrocytes. In coronavirus infection, an increase in ferritin production and impaired binding of transferrin to transferrin receptors were noted [19]. Cytokines inhibit the process of differentiation of erythroid progenitor cells and inhibit the production of endogenous erythropoietin (epoetin). Weak anti-A serum epoetin is consistently found in patients with inflammatory diseases. Epoetin reactive synthesis does not correspond to a decrease in hemoglobin (Hb) concentration. Loss of the relationship between epoetin and Hb levels is noted, which indicates a violation of the normal feedback mechanism in these diseases. Epoetin stimulates residual normal hematopoiesis, neutralizes the activity of cytokines, which have the ability to inhibit erythropoiesis, promotes the differentiation of erythroid progenitor cells of myelodysplastic hematopoiesis, and also inhibits the processes of apoptosis in the lung tissue [7].

A number of clinical studies have described specific diagnostic/therapeutic functions associated with myelodysplastic patterns in COVID-19, with improved hemobiological blood parameters after administration of recombinant human erythropoietin. [13]. This is due to the fact that erythropoietin, by stimulating the release of reticulocytes from the bone marrow, also helps to release leukocytes from there, which increases the body's immunity.

The most common formulations of recombinant human epoetin are considered to be epoetin alfa, epoetin beta, and long-acting epoetin alfa. In this regard, among many epoetin preparations, epoetin alfa "Epoxem" should be mentioned, the release forms of which (1000, 2000, 4000, 10,000 IU in syringes) allow the economical use of its various dosages, which makes its use convenient for the doctor and patient without loss of efficiency and additional costs.
According to the recommendations of the multicenter international study BRAVE (Breast Cancer-Anemia and the Value of Erythropoietin), the main criteria for starting therapy are the presence of symptoms of anemia and the level of Hb <100 g/l; the decision to use recombinant epoetin should be determined by the clinical situation; the recommended starting dose of epoetin is 150 units/kg, with the possibility of increasing the dose to 300 units/kg 3 times a week for the next four weeks. In our practice of administering patients with a moderate and severe form of COVID-19, we proposed recommendations for the use of epoetin alfa (Epoxem) in patients with anemic syndrome against the background of coronavirus infection, in the dosage regimen of injections of 4000 IU daily for 7-10 days, under the control of laboratory indicators and biomarkers of anemia.

According to international clinical practice guideline, indicating the possibility of achieving the target hemoglobin level and preventing adverse hemobiological consequences in patients with moderate and severe anemia associated with inflammatory diseases, when using a combination of epoetin with iron preparations, the advantage of intravenous forms of iron has been discovered in comparison with oral only preferably intravenous forms of iron. In particular, the combination of recombinant epoetin with parenteral iron preparations is effective in patients with chronic, oncological, lymphoproliferative, and autoimmune diseases accompanied by anemic syndromes [14], which, in turn, must be taken into account when administering patients with anemia against the background of viral infection in patients with comorbid conditions.

Recently, in clinical practice has appeared a new drug for intravenous iron administration - Sucrose complex of iron (III) hydroxide "Serofer", which has shown high efficiency, both in combination with epoetin and in mono-mode. Serofer does not require a test dose, has a minimal risk of adverse reactions, and is well tolerated.

In the case of IDA against the background of coronavirus infection, in addition to iron deficiency, there is an insufficient supply of micronutrients that inhibit the development of acute and chronic inflammation, prevent the formation of a cytokine storm [2], and aid support oxygen metabolism. For example, microcytosis of erythrocytes, which occurs against the background of a deficiency of minerals, aggravates the course of IDA, increases hemosiderosis of lung tissues due to the sweating of small erythrocytes through the capillaries and their subsequent cytolysis. Compensating micronutrient deficiencies is the most important preventive measure for the severe course of COVID-19. The results of an analysis of 20 000 publications on coronaviruses showed that an increase in the supply of zinc, magnesium, manganese, and vitamins contributes to an increase in the human body's resistance to coronavirus infection. [21].

The use of injectable forms of microelement complexes is a generally accepted and effective method of treating anemic syndrome in combination with the main antianemic agents. At the same time, when prescribing mineral preparations in the clinical practice of managing patients with coronavirus infection, preference is given to those trace element complexes or individual minerals, in particular zinc that plays a leading role in enhancing antiviral immunity, contribute to a decrease in viral load and the development of severe forms of COVID- 19. The injectable preparation of microelement complexes "Etaltis" is a unique preparation containing zinc, manganese, selenium, chromium, and copper, which are necessary for the elimination and prevention of anemia and viral diseases.

In the course of our own research in "Zangiota No.1- Specialized Hospital for the treatment of patients with coronavirus infection" from August to December 2020, 112 patients with COVID-19 were studied with laboratory confirmation with clinical stages of anemia (I -mild - the hemoglobin value is within 110 - 90 g/l; II-average - the hemoglobin content ranges from 90 to 70 g/l; III-severe - the hemoglobin level falls below 70 g/l) and concomitant diseases causing a number of complications.

The age of the patients ranged from 27 to 82 years, and the average age was 56.3 ± 6.99 years. The correlation of men and women was 1, 5/1. We included the research group of hospitalized patients with COVID-19 who underwent a course with a comprehensive treatment of various etiologies of anemia with recommendations. During hospitalization and during the observation period, anamnesis, clinical status (fever, shortness of breath, tachycardia, acrationosis, dizziness, weakness, fatigue, headaches, and loss of appetite), laboratory assessment, instrumental MSCT, and ultrasound data were studied. The level of hemoglobin, erythrocytes, hematocrits, average erythrocyte volume, the average hemoglobin content in erythrocytes, the number of reticulocytes, ESR, D dimer, CRP, GPT, SGOT, coagulation group, blood saturation, and external respiration were studied. In the course of the research, laboratory blood parameters were assessed in a comparative aspect before and after treatment.
Application in patients with anemic syndrome against the background of coronavirus infection:

- **Epoxem** in the injection dosage regimen of 4000 IU daily, subcutaneously for 7-10 days;
- **Serofer 5.0** per 100ml saline solution, intravenous drip, daily, for 7-10 days;
- **Etaltis 3.0** per 7 ml of saline solution, intravenous stream, every day, for 7-10 days;
- **Imun 50.0** intravenously and by drop infusion, every day, for 7-10 days;

The results were statistically processed on a computer using software Excel 2017. In order to compare the mean values, Student's t-test was used. Nonparametric features were compared by contingency tables using the χ2 test. p <0.05 was taken as the level of reliability of statistical indicators.

### III. RESULTS

"Zangiota No. 1 - Specialized Hospital for the treatment of patients with coronavirus infection" demonstrated that the inclusion of combination therapy with erythropoiesis-stimulating, iron-containing drugs, mineral complexes, and drugs for the synthesis of proteins, hormones in the treatment regimen for anemia in coronavirus patients could increase the effectiveness of antianemic therapy. Combined pharmacological correction of disorders characterizing anemia, complicating the course of COVID-19, at an earlier time normalizes the level of erythrocytes (increase by 1.6 times; p <0.001), hemoglobin (increase by 1.54 times; p <0.01), hematocrits (increase by 1.65 times; p <0.01), the average volume of erythrocytes (increase by 1.49 times; p <0.05), the moderate hemoglobin content in the erythrocyte (increase by 1.51 times; p <0.01) and ESR (decrease in 2.2 times; p <0.05). Treatment accompanied by an increase in the number of reticulocytes in the peripheral blood (increase by 1.3 times; p <0.05), indicating the activation of the erythroid germ of hematopoiesis to a greater extent with a complex effect.

The effect of combination therapy is more pronounced in the treatment of moderate anemia. Due to the fact that during the study period, patients did not show progression or regression of the underlying infectious disease, and there were no changes in the level of ferritin, a biomarker of the severity of COVID-19, the observed therapeutic effect was due only to the use of a combination of antianemic drugs. Erythropoietin alpha preparations in combination with iron and mineral preparations are quite effective. They can be recommended for the correction of anemia of any severity, including in the presence of comorbid conditions in COVID-19.

Thus, hematological disorders such as erythropoiesis abnormalities, iron metabolism disorders, anemia, transferrin dysfunction, hyperferritinemia are common features of the new coronavirus, especially in more severe cases. Accordingly, the literature provides some evidence of possible interaction of the virus with erythrocyte hemoglobin and with iron metabolism. Recombinant erythropoietin in combination with parenteral iron preparations and mineral complexes is probably a reasonable choice of rational pharmacotherapy for the anemic syndrome to mitigate the manifestations of COVID-19 in critically ill patients in order to improve their quality of life and the prognosis of the disease. However, to determine the optimal dose and duration of use of antianemic drugs, additional research is required.

### IV. DISCUSSION

Based on a systematic review and meta-analysis of the available results of a quantitative assessment of the average level of hemoglobin, ferritin, and other biomarkers of iron metabolism and erythrocyte indicators indices in patients with COVID-19, European researchers concluded that the concentration level of anemia biomarkers directly depends on age, gender, the presence of chronic conditions, and the severity of coronavirus infection, which determines their clinical and prognostic significance in COVID-19 [8, 20]. COVID-19 laboratory findings include hyperferritinemia, low hemoglobin, low serum iron, thrombocytopenia, and anisocytosis, with high RDW, elevated lactate, and LDH, which are reasonably compatible with erythrocyte/bone marrow dysmetabolism of the intended size and iron dysregulation. Some organs directly or indirectly target SARS-CoV-2 and slightly described pathomechanisms, both immune/inflammatory type and associated with hypoxia and ferroptosis; thromboembolism appears to play an essential role in the later stages [22].

### V. CONCLUSION

It should be emphasized that iron metabolism disorders in COVID-19, including IDA, should not be unambiguously associated with hyperferritinemia in COVID-19. Although ferritin is one of the proteins of iron homeostasis, at the same time, it is a protein of the acute phase of inflammation.
CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of apparent and potential conflicts of interest related to the publication of this article and report on each author’s contribution.

SOURCE OF FINANCING

No funding was required for this research.

REFERENCES

7. Эритропоэтин. Биологические свойства и клиническое применение / СА Гусева, ВГ Бебешко. Киев, 2005. 422 с