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RED BONE MARROW DAMAGE IN COVID-19 PATHOGENESIS CAUSED BY SARS-COV-2

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ABSTRACT — With the emerging of new strains of the SARS-CoV-2 coronavirus (such as B.1.1.529 for example), despite numerous studies to create effective vaccines, it becomes obvious that the relevance of studying the pathomorphology of tissue structures with damaged cellular targets has increased manifold. Most knowledge on genes of pathogenicity loses its importance for the development of antiviral agents since the reservoir for the virus is the cells, in which SARS-CoV-2 then persists. These data are more important for the development of vaccines, and the treatment strategy should be based on damaged cellular targets. The mechanisms of hypoxia in patients infected by SARS-CoV-2 with COVID-19 do not have an exhaustive explanation based only on the acute alveolar damage. Our investigation deals with the data on pathologic red bone marrow in patients with a fatal COVID-19 outcome against the background of various indicators of erythrocytes in clinical blood tests. We found in the structure of the red bone marrow that there is damage to the stroma and parenchyma as well as pathomorphological signs of damage to erythropoiesis in the patients of both groups. The data obtained on the cellular targets of SARS-CoV-2 can serve as a fundamental platform for the development of targeted conservative therapy in the treatment of patients infected with SARS-CoV-2, and should also be taken into account in severe COVID-19 cases with the risk of unfavorable prognosis.

KEYWORDS — COVID-19; SARS-CoV-2, cytokine storm; red bone marrow; erythrocyte; hematopoiesis; hemoglobin; hypoxia; hemophagocytosis; macrophages.

RELEVANCE

The damaging effect on cells when SARS-CoV-2 enters the body has not been fully studied and in some cases is hypothetical [1, 2]. New strains of SARS-CoV-2 turned out to be more aggressive and contagious than the first ones registered, while the strain of coronavirus B.1.1.529, omicron-VOC, contain an unusually high number of mutations in the S-protein gene, which helps it to avoid the protective effect of antibodies. Chisholm S.T., Coaker G., Day B., Staskawicz B.J. (2006), Megahed FAK, Zhou X., Sun P. (2020) argue that monitoring virus-host interactions is critical to understanding the pathogenesis of the disease [3, 4], especially given the global problem of SARS-CoV-2 and COVID-19. It is known that infection or vaccination induces a population of long-lived bone marrow plasma cells (BMPC), which are a persistent and important source of protective antibodies [5]. However, at the present stage, it is not known whether SARS-CoV-2 is capable of inducing this population in patients with developing severe acute respiratory syndrome (SARS-CoV-2). Recent reports suggest that convalescent SARS-CoV-2 patients experience rapid degradation of antigen-specific serum antibodies, raising concerns that humoral immunity against this virus may be short-lived [6, 7, 8]. This puts the study of the pathogenesis of COVID-19 at the forefront of medical problems in terms of relevance [9, 10, 11]. To develop a specific treatment for COVID-19, there is not enough large-scale work on sequencing the genomes of a new pathogen to monitor the genetic variability of the coronavirus, therefore, there is no pathogenetically justified treatment of infected patients with COVID-19 at the present stage, as well as there is no comprehensive information about the reasons for the development of a cascade of body reactions. leading to death [12, 13]. The mechanisms of hypoxia are not always associated with damage to alveolocytes, which requires a scientific search for the reasons for a decrease in oxygenation of blood and tissues [14, 15]. The least studied damage and the role of morphological changes in the red bone marrow in the pathogenesis of COVID-19, which determined the direction of our research.

Aim of the research:

to analyze the role of red bone marrow in the pathogenesis of hypoxia in COVID-19.

MATERIAL AND METHODS

The research was carried out following the fundamental ethical principles of the Declaration of Helsinki, GCP Rules (Good Clinical Practice), and approved by the ethical commission of the Ministry of Education and Science of the Russian Federation. The diagnosis in COVID-19 patients caused by SARS-CoV-2 was confirmed by PCR. The tissue of the red bone marrow was taken from the deceased no later than 24 hours from the onset of death. In total, 67 biopsies of the red bone marrow were studied in the work, of which in 2 patients a clinical blood test showed erythropenia, and in the rest the number of erythrocytes was within normal limits. After separating the soft tissues of the chest and iliac region, using a sectional saw, fragments of the rib and ilium with bone marrow with dimensions of 1 × 1 × 0.5 cm were taken. Biopsies were placed in buffered neutral 10% formalin from BIOVITRUM in a ratio of 1:20. The objects were fixed for 18-24 hours. After that, biopsies of red bone marrow were additionally placed in a decalcifying solution SoftDec (BIOVITRUM) in a ratio of 1:70. The material was kept for no more than 3 days. The decalcifying solution was replaced twice a day. During decalcification, bone fragments were checked with a medical alloy needle. Upon reaching the softness of the bone, it was removed from the decalcifying solution, placed in a container of 300–500 ml, fixed in a stationary state and continuously, with a weak flow of running water, washed for at least 2 hours. Then the material was carried out according to the standard protocol of embedding in paraffin and staining with hematoxylin and eosin. In each case, at least three preparations were observed with the analysis of at least 500 nuclear cells. Analysis of preparation and production of illustrations were carried out using an Olympus Bx52 microscope and a DP25 digital camera.

RESULTS OF RESEARCH AND DISCUSSION

A clinical analysis of the blood of deceased patients only in 2 out of 65 was characterized by erythropenia, the number of erythrocytes reached $1.4 \cdot 10^{12}$/l and $2 \cdot 10^{12}$/l. The rest of the patients had quantitative parameters of erythrocytes within the normal range. However, the pathomorphological analysis of red bone marrow (BMC) preparations of patients with fatal outcomes showed that BMC is one of the participants in the pathological events in COVID-19 caused by SARS- CoV-2, and plays an important role in the pathogenesis of the cascade of reactions leading to hypoxia and death. infected patients. The bone marrow preparations were characterized by a decrease in the number of stromal elements, as well as qualitative and quantitative changes in the structure of hematopoietic foci, not only erythrocyte germs but also granulocytic ones (Fig. 1).

For better clarity of the revealed pathomorphological features of the red bone marrow in patients who died as a result of the severe course of COVID-19 caused by SARS-CoV-2, the study results are presented in the form of illustrations performed with high magnification. The observed picture in patients with low erythrocyte counts in the blood was characterized by an almost complete absence of foci of erythropoiesis, in those who died from SARS-CoV-2 and a small number of macrophages containing granules of transferrin-bound iron (Fig. 2).

On preparations of the red bone marrow of this group of patients, 1–2 hypochromic, basophilic and oxyphilic erythrocytes are identified in the field of view, small or large, irregular in shape, as well as 1-2 macrophages in the field of view with iron granules and phagocytosed erythrocytes. Lymphocytes are characterized by brightly basophilic nuclei surrounded by a narrow rim of cytoplasm. Cells with fragmented or annular nuclei are also noted, indicating apoptosis. The blood vessels are identified, but the nuclei are hypertrophied and the cytoplasm is thinned.

In the material of patients who died from COVID-19 disease, against the background of low erythrocyte counts in the blood on the preparations of red bone marrow, there is a complete absence of stromal elements, blood vessels and reticular cells are not identified, macrophages are absent, oxyphilic erythroblasts with a homogeneously expanded cytoplasm are observed in a small amount. Hypochromic and polychromatophilic erythrocytes of irregular shape, eosinophilic granulocytes of varying degrees of maturity, mainly young and stab, are identified. In addition to eosinophils, stab neutrophils are also detected (Fig. 3).

Numerous pathological forms of hypochromic erythrocytes, lymphocytes, eosinophils, a small number of macrophages without granular inclusions in the cytoplasm are identified in the field of view. The presence of polychromatophilic non-nuclear erythrocytes indicates impaired differentiation and specialization of erythrocytes. The wall of the preserved sinusoidal capillaries is represented by the endothelium with intermittent thinned cytoplasm, with hypertrophied nuclei, in the lumen of which polychromatophilic erythrocytes are identified, thinned or destroyed sinusoidal capillaries and the reticular stroma of the CCM are also identified. Single megakaryocytes are found near the capillary wall. There are no normal erythrocytes, poikilo- and anisocytosis are noted, few spherocytes, hypochromic erythrocytes with Howell-Jolly bodies, Conde rings.

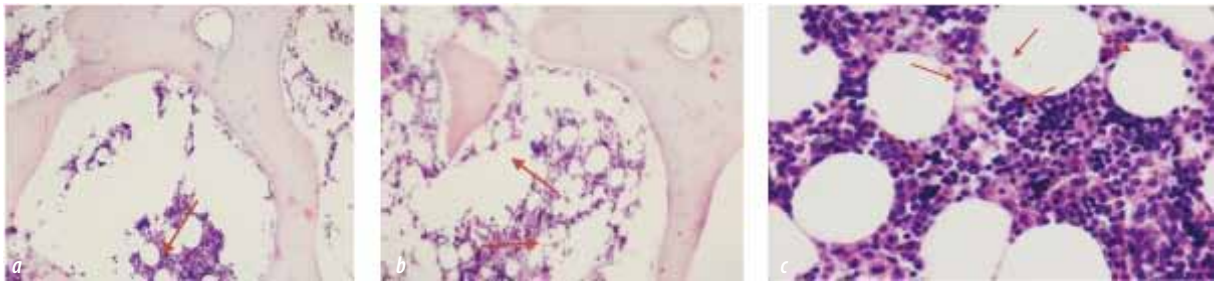
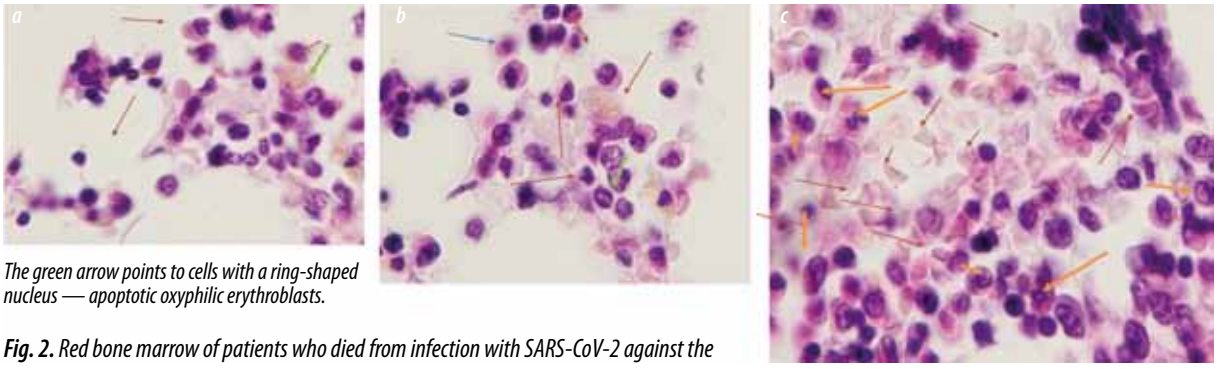


Fig. 1. *Red bone marrow of deceased patients infected with SARS-CoV-2. A, b) red bone marrow of the deceased against the background of low erythrocyte counts; c) red bone marrow of a patient who died from infection with SARS-CoV-2 against the background of erythrocyte counts within normal limits. Microphoto. Staining with hematoxylin and eosin. Magnification a, b) ×100; c) ×200*



The green arrow points to cells with a ring-shaped nucleus — apoptotic oxyphilic erythroblasts.

Fig. 2. *Red bone marrow of patients who died from infection with SARS-CoV-2 against the background of erythrocyte counts within normal limits. Staining with hematoxylin and eosin. Microphoto. Magnification a, b, c) ×400.*

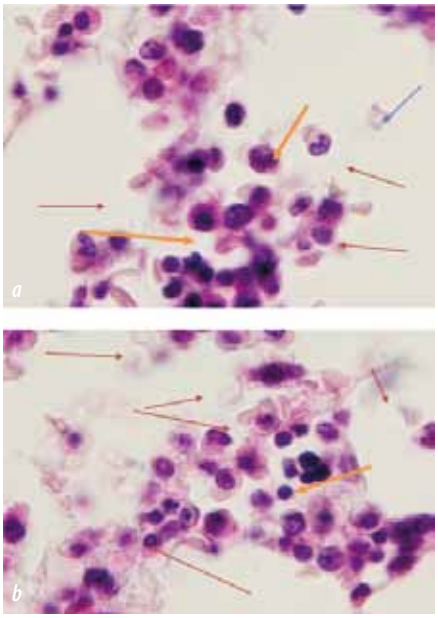


Fig. 3. *Red bone marrow of patients infected with SARS-CoV-2 who died against the background of low red blood cell counts in the clinical blood test. Staining with hematoxylin and eosin. Microphoto. Magnification a, b) ×400.*

Oxyphilic erythroblasts with annular nuclei, which are signs of apoptosis, surround macrophages.

Formerly H. Chandra, S. Chandra, Rm. Kaushik, Nk. Bhat, V. Shrivastava (2014) in bone marrow aspirates, the phenomena of hemophagocytosis, which is a life-threatening condition, were described based on the identification of macrophages with phagocytosed neutrophils and a large number of plasma cells in pancytopenia and erythroid hyperplasia [16]. Many coronaviruses cause autophagy. Monitoring and analysis of intercellular interactions under the influence of viral cellular behavior are critical for understanding the pathogenesis of the disease for the development of prevention and targeted treatment through the protection and regeneration of damaged targets. These studies are of particular importance because of the global problem of COVID-19 caused by the increasingly aggressive strains of SARS-CoV-2 [18].

CONCLUSION

The red bone marrow of COVID-19 patients who died of multiple organ failure, depending on the clinical parameters of the content of erythrocytes in the blood, morphologically differs from the red bone marrow of patients who died from complications of concomitant diseases, the absence of intact foci of erythropoiesis. With indicators of severe erythropenia in the blood of patients, disruption of erythropoiesis in the red bone marrow should be assumed, which should be taken into account when developing a

treatment strategy for this group of patients. Understanding of spectrum and frequency of histological findings in COVID-19 is essential for a better image of the pathogenetic aspects of the disease mechanisms and the prospects for predicting favorable outcomes. Hemophagocytosis, noted in the red bone marrow of dead patients, even in rare cases, must be taken into account in the study protocols since it is a marker of deep damage not only to the central organ of hematopoiesis, like red bone marrow but also to the systems involved in the regulation of hematopoiesis, leading to hypoxia and death.

Pancytopenia and cell hyperplasia of the erythroid lineage were frequent hematological manifestations. Moderate to severe hemophagocytosis was observed in the red bone marrow of the deceased. The study concluded that hemophagocytosis, even if observed in single cells, should always be documented in bone marrow reports. Bone marrow aspirates should be included in the differential diagnosis of COVID-19 caused by SARS-CoV-2. This may be the only sign of the development of COVID-19 infection caused by SARS-CoV-2, with damage to the red bone marrow and with the prediction of a severe course of COVID-19, as well as for the development of a pathogenetically based strategy. Thus, hypoxia in COVID-19 is associated not only with damage to the alveolar epithelium but also due to damage to the stroma and blood vessels of the BMC, abnormal activity of lymphocytes and macrophages in the tissue of the red bone marrow, as well as disturbances in the regulation system of hemoglobin synthesis and damage to erythrocyte diferon and erythropenia, which leads to impaired cellular respiration and severe damage to organs involved in the synthesis of erythropoietin.

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