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FEATURES OF THE COURSE OF INFECTIOUS DISEASES OF VIRAL ETIOLOGY WITH NEUTROPENIA

DOI 10.25789/YMJ.2022.77.05

УДК 616.155.394.5:616.9

The aim of the study is to establish the features of the course of infection of viral etiology with neutropenia. Study included analysis of immunological parameters of 628 people with a viral infection and 359 practically healthy people aged 31-51 years living in Arkhangelsk. It was found that the course of infection of viral etiology against the background of neutropenia is accompanied by a more significant decrease in the percentage of active phagocytes and an increase in the concentration of circulating immune complexes, which occurs against the background of a low content of properdin, which is a risk of a chronic course of the disease. The deficiency of the phagocytic activity of neutrophils in neutropenia is due to the insufficiency of the main chemotaxis factor C5a, which is a product of the enzymatic cleavage of C5. An increase in the content of autoantibodies, which is characteristic of viral pathology, speaks in favor of a more significant level of cytolytic and secretory function of neutrophils in neutropenia.

Keywords: neutropenia, viral infection, phagocytosis, circulating immune complexes, autoantibodies.

Introduction. Neutropenia is not a mandatory hematological manifestation of most diseases. In infectious diseases that do not directly affect the organs of hematopoiesis, neutropenia is not associated with a violation of the processes of maturation and differentiation of neutrophils. In infections, neutropenia is temporary and mostly disappears during recovery. But the presence of neutropenia does not have a positive effect on the course of any inflammatory process, including viral etiology. The questions of neutropenia attract little attention of researchers, but this does not make them less relevant. In the North, where neutropenia is quite widespread and depends on the climatic and geographical conditions of life, the issues of neutropenia become very topical. Neutropenia is often combined with a deficiency of active phagocytes. Neutropenia, in combination with a deficiency of phagocytic protection, creates serious defects of natural and acquired resistance to various factors that adversely affect a person. The frequency of registration of phagocytic defense deficiency in practically healthy children, aged 1-1.5 years on average is 5.56±0.24%, in children 10-12 years, the level of registration of this immunodeficiency increases to 8.39±0.31%, and at the age of 16-19 years, the frequency of phagocytic activity deficiency of neutrophils is already 29.76±0.84% [6]. Based on the data of Almazov V.A. (1981), Dale D.C. (1995) and Lichtman M.A. (1995), the frequency of registration of so-called "spontaneous" neutropenia is quite significant [1, 14, 19]. Neutropenia is registered in the first hours after the introduction of vaccines, when infected with respiratory viruses, with some bacterial infections [2, 4, 5, 10].

The aim of the study is to establish the features of the course of infection of viral etiology with neutropenia.

Materials and methods. The immunological results of the preanalytical and analytical stages of the examination of 628 people with a viral infection aged 31-51 years living in Arkhangelsk, who applied to the professional diagnostics center "Biolam" after a previous illness with a previously established diagnosis, including 126 people with enterovirus infection, 164 – herpes infection, 223 – Acute respiratory viral infections (ARVI), 115 – Grippe, were analyzed. As a comparison group, 359 practically healthy people of the specified age living in Arkhangelsk were examined. The survey was carried out with the written consent of the respondents in compliance with the basic norms of biomedical ethics in accordance with the document "Ethical principles for medical research involving human subjects" (WMA Declaration of Helsinki 1964, amended in 2013).

For the study, peripheral venous blood was taken from the ulnar vein in the morning on an empty stomach. The number of leukogram cells and neutrophil phagocytosis were calculated in blood smears stained by the Romanovsky-Giemsa method; the calculation was performed at the rate of 100 cells. The content of complement system factor P (properdin) (Cloud-Clone Corp., CCC, Wuhan), C4, C3, C5-components of the complement system (AssayPro, USA) was determined in blood serum by enzyme immunoassay (ELISA) with appropriate reagents. Anti-double stranded DNA (Anti-dsDNA) antibodies, Anti-RNP antibodies, and autoleucoagglutinins were determined. Autoantibodies were detected using ELISA using the ENA Profile kits from Bio-Rad (USA) and ORGenTec Diagnostika (Germany). The results were recorded using a Multiskan series spectrophotometer (Finland) and an automatic enzyme immunoassay analyzer "Evolis" from Bio-RAD (Germany). Serum autoantibodies to leukocytes were determined in leukoagglutination reactions, the results of which were taken into account in a "thick drop" type preparation. Leucoagglutinins were determined in dilutions 1/5, 1/20, 1/40, 1/80, 1/60, etc., the titers of which were recorded in log2. The concentration of circulating immune complexes (CIC IgG) was determined by the standard precipitation method using 7.5 % PEG-6000 in blood serum. The reaction was evaluated on an automatic enzyme immunoassay analyzer "Evolis" of the company "Bio-RAD" (Germany). A deficiency in the content of neutrophil granulocytes (neutropenia) was established at a content of < 2.0×109 cells/l. Statistical processing of the obtained data was carried out using the Statistica 10.0 software package (StatSoft, USA). The critical level of significance (p) in the work was taken equal to 0.05.

Results and discussion. The frequency of neutropenia registration in viral infections varies within 12.56-26.96% (19.46±1.23%), it is most often detected with influenza and enterovirus infection. Deficiency of active phagocytes and neutropenia coincide in 78-93% in viral infections (table 1).

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Table 1

Frequency of registration of immune defense defects in viral infections in adults (number / %)

Indicator	Enterovirus infection, n=126	Herpes infection, n=164	ARVI, n=223	Grippe, n=115
Neutropenia	26 / 20.63	29 / 17.68	28 / 12.56	31 / 26.96
Deficiency of active phagocytes	23 / 18.25	25 / 15.24	24 / 10.76	26 / 22.61
Neutropenia and deficiency of active phagocytes	21 / 80.76	24 / 82.76	22 / 78.57	29 / 93.55

Table 2

Immunological parameters of peripheral blood in patients and practically healthy people with neutropenia and normal neutrophil granulocytes (M±m)

Studied parameters	Viral infection		Practically healthy persons	
	Neutropenia, n=114	Normal neutrophil content, n=125	Neutropenia, n=134	Normal neutrophil content, n=225
	1	2	3	4
Neutrophils, 109 cells/l	1.62±0.25	2.29±0.19	1.83±0.17	2.68±0.22
% of active phagocytes	35.32±1.32 ***1-3	49.28±1.05 ***2-4	41.34±1.93	54.27±1.26
Properdin, g/l	0.33±0.03**1-3	0.27±0.04 ***2-4	0.67±0.05	0.89±0.07
C4, g/l	0.52±0.01	0.51±0.06	0.62±0.09	0.59±0.05
C3, g/l	1.61±0.08**1-3	1.54±0.07**2-4	0.62±0.09	0.53±0.09
C5, g/l	0.32±0.04 ***1-2	0.68±0.05	0.28±0.08**3-4	0.49±0.06
CIC IgG, g/l	3.89±0.09**1-3	2.36±0.07	2.83±0.09	2.23±0.12
Autoleucoagglutinins, $\log_2$	2.32±0.02*1-3	2.86±0.08*2-4	1.49±0.07	1.36±0.09
Anti-dsDNA, IU/ml	49.60±1.26 **1-2	38.67±1.35	39.71±1.11	35.32±1.03
Anti-RNP, IU/ml	0.79±0.04	0.69±0.06	0.57±0.08	0.89±0.13

* p<0.05, ** p<0.01, *** p<0.001

The infection is accompanied by an increase in the CIC content in the blood; statistically significant differences were found relative to the CIC IgG, whose concentrations are higher in patients with neutropenia (table 2).

A decrease in the percentage of phagocytic neutrophils in viral infections, regardless of the etiology of the infectious disease, occurs against the background of a low content of properdin, an activator of the alternative pathway of the complement system, as well as an increase in C3 concentrations. Without going into the details of the functional activity of the complement system, we will mention only the basic concepts related to the analyzed results. The classical complement activation pathway begins with the binding of C1q to the Fc fragment of complement-binding immunoglobulins (IgM, IgG1-3) and the formation of either their aggregates or complexes with an antigen. After C1, C4 and C2 are activated, and only after that C3 convertase is formed. An alternative or properdin activation pathway immediately begins with the formation of active C3a and C3b without the participation of C1, C4 and C2 [17, 28]. Low concentrations of C4 and slight fluctuations in its concentrations indicate the predominance of activation of the complement system by an alternative route. Properdin (from the Latin perdition – destruction, factor P) was discovered by L. Pillemer in 1954, electrophoretically – it is a gamma-globulin of blood serum. A long-term low content of properdin is a poor prognostic sign, as well as the risk of a prolonged or chronic course of the disease [3, 9]. Non-complement-binding IgA and Fab fragments of all classes of immunoglobulins, which can aggregate not only antigens, are also able to immediately activate C3

The deficiency of the content of phagocytic granulocytes is combined with neutropenia not only in viral infections, but also in practically healthy individuals; in patients with neutropenia, the content of phagocytic neutrophils is noticeably lower (p<0.01). The reason for the decrease in phagocytic activity in neutropenia, in our opinion, is the predominant exocytic activity of neutrophils. Immediately after contact with the object, first part of the granules merges with the outer surface of the cell membrane, then breaks or is thrown out of the cell, and phagocytosis occurs much later. The release of pyrogen is the earliest reaction of activated neutrophils in tissues and entails the aggregation of granules, the release of cationic proteins from lysosomes, the marginal standing of cationic proteins under the cell membrane and their secretion into the intercellular medium [11]. The decrease in the level of active phagocytes during the activation of the secretory function of neutrophils is confirmed by a sufficiently large number of convincing facts [7, 8, 12, 18, 20, 22, 25, 35].

Both in patients and in practically healthy individuals, neutropenia is combined with a low content of C5 (p<0.001), which is quite understandable since the chemotaxis factor C5a is formed mainly by an enzyme in the lysosomal granules of polymorphonuclear leukocytes. Activation of the serum complement leads to the enzymatic cleavage of C3 and C5 into fragments of C3a and C5a, which contribute to the release of histamine, but only C5a is a true chemotactic factor for granulocytes [15, 21].

If there is a sufficient concentration gradient of the chemoattractant, the location

of the receptors on the surface of the cell membrane becomes asymmetric, concentrates on one of the poles in the form of a cap (capping) and determines the direction of its movement [13]. Well-studied chemotactic agents are components of activated complement or short peptides with N-terminal formyl-methyl residues. Activation of the complement system can be induced by bacterial endotoxins, bacterial cell walls (zymosan), various proteases. Bacterial growth products stimulate chemotaxis because, unlike eukaryotic cells, bacteria initiate the synthesis of products with an N-terminal formyl-methionyl group [26]. The number of such newly discovered chemotactic peptides in recent years has numbered several hundred and their number continues to increase, but, most likely, all these substances react with the same, common neutrophil membrane receptor. At elevated concentrations of chemoattractant, the activity of chemotaxis may even decrease. At high concentrations of various chemotaxis factors, there is a decrease in the activity of signal transmission and the mechanism of receptor concentration on pseudopods with a parallel increase in the secretion of hydrolases, metalloproteases and free radical production. At the same time, the activity of chemokinesis does not decrease, which is manifested by the absence or decrease in the severity of the pointed front of cell movement in agarose [24, 36]. The speed and number of moving neutrophils significantly decreases by 10 and even 100 times at low concentrations of granulocytes [23].

So, with neutropenia, the content of C5 in the blood is lower, which is formed mainly by the enzyme of lysosomal granules of polymorphonuclear leukocytes, which leads to a low formation of the product of its enzymatic cleavage of C5a – the main factor of granulocyte chemotaxis. The content of C5 in practically healthy and sick people with neutropenia is noticeably lower in these cases, the differences in their concentrations in the blood of patients and healthy people are actually not significant. In addition to reducing the content of the main chemotaxis factor in neutropenia, their chemokinesis is also lower.

The adhesion of the object to the phagocytosis wall (opsonization) is provided by the treatment of the object with the complement system enzyme C3b and immunoglobulins. Opsonins have been known since 1903 [34]. They act as ligands between the object and the phagocyte [32]. The adhesion of the object to the phagocyte wall causes changes in the cell membrane, leading to the organization of contractile proteins and the movements of the membrane around the phagocytosis object [29, 30].

The possibility of an insufficient effect of opsonins on the deficiency of phagocytic protection in neutropenia is hardly real. Opsonins act on the object of phagocytosis as ligands. The most important opsonins are C3 (C3b), activated both by the classical and alternative pathways, and immunoglobulins [16, 27, 30-33]. Complement-binding immunoglobulins, as well as non - complement-binding IgA, IgE and Fab fragments of immunoglobulins of any classes capable of aggregating antigens, play only a predominantly initiating role. Opsonization, in all probability, does not suffer from neutropenia, since the serum content of C3, the source of C3b, does not actually change with neutropenia (1.61±0.08 and 1.54±0.07 g/l). It should be noted that the activity of autoantibody formation in northerners is significantly higher than in people living in favorable climatic conditions and an excessive increase in autoantibodies can cause low concentrations of neutrophils. Thus, in neutropenia, the content of autoleucoagglutinins in persons with a viral infection is higher than in practically healthy people (2.32±0.02 and 1.49±0.07 log2). The concentrations of Anti-dsDNA in viral infections on the background of neutropenia are noticeably higher than their level in patients with normal neutrophil content in peripheral venous blood. In itself, the fact of an increase in the content of Anti-dsDNA in a viral infection is not surprising and, most likely, is the result of the cytopathic effect of viral infection of the cell. An increase in the content of Anti-dsDNA against the background of neutropenia may be the result of the predominance of cytolytic and secretory activity of neutrophils in the conditions of neutropenia. Cytolysis and phagocytosis are a mechanism for protecting organs and tissues from the damaging influence of factors and actions that activate the complement system.

Conclusion. The course of infection of viral etiology against the background of neutropenia is accompanied by a more significant decrease in the % of active phagocytes and an increase in the concentration of CEC. The deficiency of the phagocytic activity of neutrophils in neutropenia is due to the insufficiency of the main chemotaxis factor C5a, which is a product of the enzymatic cleavage of C5 formed by enzymes of lysosomal neutrophil granules, as well as a decrease in the activity of their chemokinesis. An increase in the content of autoantibodies, which is characteristic of viral pathology, speaks in favor of a more significant level of cytolytic and secretory function of neutrophils in neutropenia.

The study was performed under the program of fundamental scientific research, № 122011800217-9.

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