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S.V. Zakharov,
V.K. Zakharov

NONSPECIFIC PROTECTIVE FACTORS IN PATIENTS WITH EARLY LATENT SYPHILIS (report 1)

Dnipro State Medical University
V. Vernadsky str., 9, Dnipro, 49044, Ukraine
Дніпровський державний медичний університет
вул. В. Вернадського, 9, Дніпро, 49044, Україна
e-mail: dmu@dmu.edu.ua

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Ключові слова: *ранній прихований сифіліс, прозапальні та протизапальні інтерлейкіни, субпопуляції Т-лімфоцитів, тест відновлення нітросинього тетразолію, циркулюючі імунні комплекси, фагоцитоз*
Ключевые слова: *ранний скрытый сифилис, провоспалительные и противовоспалительные интерлейкины, субпопуляции Т-лимфоцитов, тест восстановления нитросинего тетразолия, циркулирующие иммунные комплексы, фагоцитоз*

Abstract. Nonspecific protective factors in patients with early latent syphilis (report 1). Zakharov S.V., Zakharov V.K. *The objective of this work is to study nonspecific protective factors in patients with latent early syphilis. The results of the study are based on the data of a comprehensive examination before treatment of 142 patients with early latent syphilis and 20 patients of the comparison group. Methods of investigation: serological methods for the diagnosis of syphilis complex of serological reactions, enzyme-linked immunosorbent assay (IgM, IgG), Indirect immunofluorescence reaction (RIF) RIF-200, RIF-abs; determination of cytokines IL-2, IL-6, IL-10, TNFα and INFγ in blood serum by enzyme-linked immunosorbent assay; determination of the phenotype of lymphocytes (CD-receptors), the concentration of circulating immune complexes, phagocytic number, phagocytic index, Nitroblue tetrazolium test. In the blood serum of patients the concentration of IL-2, IL-6, IL-10 and TNFα, INFγ was significantly increased. The concentration of IL-10 and IL-6 was also significantly increased in patients with more than 1 year of infection. The most significant disorders of the parameters of the metabolic activity of neutrophils were found in patients with an infection of more than 1 year; an imbalance in the circulating immune complexes concentration was also established. Under the increasing duration of the infection, the concentration of CD16+ in the blood serum progressively decreases.*

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The content of pro-inflammatory and anti-inflammatory cytokines in the blood serum of patients with early latent syphilis was significantly increased and depended on the timing of infection. This relationship was most pronounced for IL-6 and IL-10. In patients with early latent syphilis there is also an imbalance between CD25+ and late activation factor HLA-DR against the background of a progressive decrease in the number of CD16+ lymphocytes. A direct relationship was established between the time of infection and the number of NK-cells. The revealed disorders may be the consequence of significant disorders on the part of neutrophils and may be one of the factors of the latent course of syphilitic infection.

Реферат. Неспецифічні фактори захисту у хворих на ранній прихований сифіліс (повідомлення 1). Захаров С.В., Захаров В.К. *Мета роботи – вивчення неспецифічних факторів захисту у хворих на прихований ранній сифіліс. Результати дослідження засновані на даних комплексного обстеження до лікування 142 пацієнтів з раннім прихованим сифілісом і 20 пацієнтів групи порівняння. Методи обстеження: серологічні методи діагностики сифілісу (комплекс серологічних реакцій, імуноферментний аналіз (IgM, IgG), реакція імунофлюоресценції (РІФ-200, РІФ-abs); визначення в сироватці крові цитокінів IL-2, IL-6, IL-10, TNFα і INFγ методом імуноферментного аналізу; визначення фенотипу лімфоцитів (CD-рецептори), концентрації циркулюючих імунних комплексів, фагоцитарного числа, фагоцитарного індексу, тесту відновлення нітросинього тетразолію. У сироватці крові пацієнтів була істотно підвищена концентрація IL-2, IL-6, IL-10 і TNFα, INFγ. Концентрація IL-10 і IL-6 також була значно підвищена у хворих з давністю інфікування більше одного року. Найбільш значущі порушення показників метаболічної активності нейтрофілів були виявлені у хворих з давністю зараження більше одного року; також було встановлено дисбаланс концентрації циркулюючих імунних комплексів. При збільшенні давності зараження концентрація CD16+ у сироватці крові прогресивно зменшується. Вміст у сироватці крові хворих на ранній прихований сифіліс прозапальних та протизапальних цитокінів був суттєво підвищеним і залежав від строків інфікування. Ця залежність була найбільш виражена для IL-6 та IL-10. У хворих на ранній прихований сифіліс також спостерігається дисбаланс CD25+ та пізнього фактора активації HLA-DR на тлі прогресивного зменшення кількості CD16+ лімфоцитів. Установлено пряму залежність між терміном інфікування та кількістю NK-клітин. Виявлені порушення можуть бути наслідком значних порушень з боку нейтрофілів і можуть бути одним з чинників латентного перебігу сифілітичної інфекції.*

The urgency of the early latent syphilis problem is due to the fact that its share among all forms of infectious syphilis is more than 50% and the mechanisms of this form of syphilis pathogenesis are not yet completely clarified [3, 6, 13].

Among the possible factors of increasing the incidence of early latent syphilis are: the widespread use of antibiotics by the population, both for the treatment of syphilis and other STIs, the introduction of new susceptible tests for syphilis (enzyme-linked immunosorbent assay (ELISA), reaction of passive hemagglutination (RPHA), immune blotting), reduction of pathogenicity and virulence of pale treponema and its resistance to antibiotics [2, 3, 10].

Currently, scientists who have studied the mechanisms of pathogenesis of syphilis believe that one of the causes of latent syphilis may be L- and cyst forms of pale treponema [10]. At the same time, none of the researchers have any doubts about the disorders of the immune system in this form of syphilis. To date, there is no unanimous opinion of experts on the patterns of non-specific and specific immunity disorders in syphilis, including latent: there are almost no scientific papers about a comprehensive study of all immunity levels [8, 9, 12].

The above data suggest that the problems of classification of identified disorders of the immune system in early latent syphilis remain not only unresolved, but also contradict each other [6, 8, 14, 15].

In the case of chronic inflammatory process, which is a characteristic of syphilitic infection, not only the cytokine profile is disturbed, but also the metabolic activity of neutrophils and dysregulation of the T-link of immunity is observed [4, 11].

A comprehensive study of nonspecific protective factors in patients with early latent syphilis, in our opinion, will provide more in-depth and detailed understanding of some mechanisms of latent syphilis and furthermore will allow to develop the prognostic algorithms of the disease and its classification.

The objective of the paper is to study the concentration of interleukins IL-2, IL-6, IL-10 and TNFα, INFγ; CD16, CD25, HLA-DR, circulating immune complexes (CICs), Nitroblue tetrazolium test (NBT-test), phagocytic number and phagocytic index in the serum of patients with early latent syphilis before treatment.

MATERIALS AND METHODS OF RESEARCH

The study included 142 patients with early latent syphilis aged 18 to 42 years, who were treated at the Dnipropetrovsk Regional Dermatological and Venereological Dispensary, which is the clinical base of the Dnipro State Medical University. According to the gender distribution, there were 82 women and 60 men. The mean age of patients was 28.5±0.5 years. The comparison group consisted of 20 practically healthy individuals, which corresponded to

the group of patients with early latent syphilis according to gender distribution and age.

Criteria for inclusion in the study were: age from 18 to 45 years, the diagnosis of early latent syphilis. Exclusion criteria: age over 45 years, patients with late latent syphilis and false-positive serological reactions to syphilis, pregnant women, patients with tuberculosis, diabetes, viral hepatitis, HIV/AIDS, and patients taking antibiotics for the last 3 months.

Serologic methods to diagnose syphilis were used: complex of serological reactions (CSR), enzyme-linked immunosorbent assay (ELISA) (IgM and IgG), indirect immunofluorescence reaction (RIF) RIF-200, RIF-abs [7]. Cytokines IL-2, IL-6, IL-10 and TNFα, INFγ were determined in blood serum by enzyme-linked immunosorbent assay using certified in Ukraine sets of reagents for enzyme-linked immunosorbent assay of diagnostic test systems of CJSC "Vector-Best" (Novosibirsk, RF) and Human IL-23 Platinum ELISA.

We have also conducted studies of standardized tests provided by the Order of the Ministry of Health of Ukraine No. 422 of 19.11.2002 "On the development of clinical immunology in Ukraine" to determine the phenotype of lymphocytes that differentiate receptors, concentration of circulating immune complexes (CICs), phagocytic index, Nitro-blue tetrazolium test (NBT-test) (spontaneous).

The study of the total and relative number of lymphocytes and leukocyte formula was performed using automatic analyzer AVH-Pentra 60 C ("Horiba ABX") S.A.S., France, certificate No. 6455/2007 from 18.05.2007. Order of the Ministry of Health of Ukraine No. 41 from 18.05.2007.

The mathematical treatment of the resulting figures has been carried out using a software package for processing and analysis of statistical information with the use of methods of biometric analysis implemented in packages of licensed software EXCEL 2003® and STATISTIKA 6.1 (Stat Soft Inc., S/N HGAR 909 E-415882 FA) [5].

The difference in indices with $p<0.05$ was considered as probable one.

To identify the relationships between the indices, Friedman's nonparametric variance analysis with the definition of χ^2 was used. The coupling between indices was considered significant if χ^2 value exceeded the critical one ($\chi^2=3.84$) [1].

The materials presented in the article correspond to the principles of bioethics set out in the Declaration of Helsinki "Ethical principles of medical research with human participation", developed by the World Medical Association, "Universal Declaration of Bioethics and Human Rights (UNESCO)"

RESULTS AND DISCUSSION

The concentration of IL-10 in serum of patients with early latent syphilis was significantly ($p<0.05$) increased to 14.9±0.9 pg/ml compared to the control group 4.11±0.2 pg/ml, that is by 3.7 times. In the analysis of this index, it was exceeded by 5.7 times in patients with a term of infection over 1 year (23.4±0.6 and 4.11±0.2, respectively, $p<0.05$) depending on the term of infection.

In patients before treatment the concentration of IL-6 in the serum was 31.3±1.3 pg/ml i.e. was exceeded by 6.5 times compared with the control group of 4.81±0.3 pg/ml ($p<0.05$). In a detailed analysis of interleukin depending on the duration of infection, it was found that in patients with a history of infection up to 1 year it was exceeded by 10 times (48.3 ±0.8 and 4.81±0.3 ($p<0.05$) and vice versa in patients with a history of infection over 1 year the concentration of IL-6 in the serum was by 3 times lower (15.6±0.5 pg/ml $p<0.05$).

The concentration of IL-2 in the serum of patients with early latent syphilis before treatment was 37.8±1.4 pg/ml while the normal level in healthy individuals is 16.5±3.2 pg/ml, meaning it was exceeded by 2.3 times ($p<0.05$). The concentration of interleukin did not depend on the duration of the infection.

The concentration of TNFα in the serum of patients was exceeded 3.6 times (18.8±0.6 pg/ml, and in healthy people 5.2±0.2 pg/ml $p<0.05$) and INFγ concentration was exceeded 3.8 times (53.7±3.1 pg/ml and in healthy individuals it was 16.1±1.3 pg/ml $p<0.05$).

The number of CD25+ lymphocytes in patients with early latent syphilis before treatment was significantly higher (2.56 times) than in the control group: $(0.43\pm0.02)\times10^9$ vs. $(0.17\pm0.01)\times10^9$. The most significant changes in the number of CD25+ lymphocytes were observed in patients with a term of infection up to 6 months (3.15 times). Regarding the HLA-DR + indicator (late activation factor) it was found that this indicator was lower than in the control group of healthy people $(14.1\pm1.2)\times10^9$ in infections of up to 6 months $(9.3\pm1.3)\times10^9$ ($p<0.05$), and on the contrary in patients with a term of infection more than 1 year, this indicator was $(27.4\pm1.8)\times10^9$, i.e. was increased by 1.9 times ($p<0.05$).

The level of NK lymphocytes (CD16+) in patients with early latent syphilis was by 2.75 times lower than in the control group of healthy individuals (0.10±0.01 vs. 0.29±0.02, $p<0.05$). In a detailed analysis of the CD16+ lymphocytes content it was found that this indicator depended on the duration of infectious process. As the time of infection increased, the content of CD16+ lymphocytes progressively decreased. We found inhibition

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of phagocytic activity of neutrophils in comparison with the group of healthy individuals namely the phagocytic number by 1.3 times (6.0±0.2 vs. 4.6±0.1, $p<0.05$) and phagocytic index – by 1.6 times (72.3±3.9 vs. 45.0±2.7, $p<0.05$). The metabolic activity of neutrophils (NBT-test) increased by 1.5 times in patients with early latent syphilis compared with the control group (24.3±2.1 vs. 36.2±1.9 $p<0.05$). The most significant changes in the indicators which characterize functional capacity of neutrophils were observed in patients with an infection period of up to 1 year.

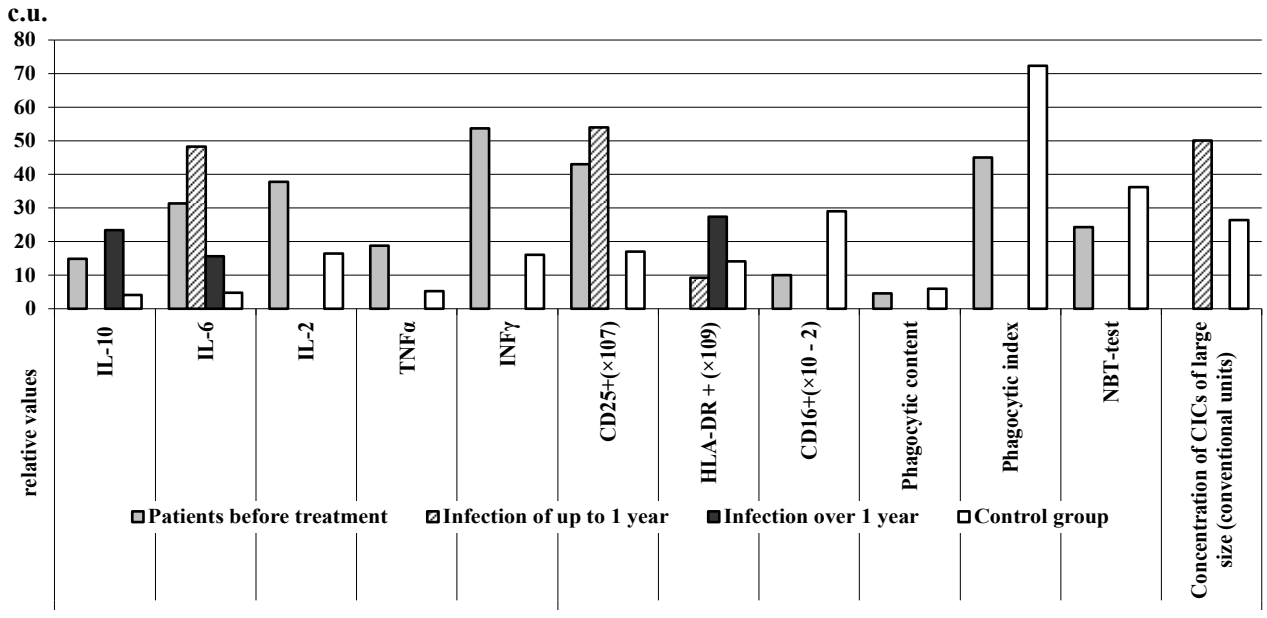
The study of the activated lymphocytes content in the serum of patients with early latent syphilis made it possible to assess the disorders of the corresponding subpopulations of T-lymphocytes. The number of CD25+ lymphocytes in patients with early latent syphilis before treatment was significantly higher (2.56 times, $p<0.05$) than in the control group. The most significant changes in the amount of CD25+ were observed in patients with infection of up to 6 months (3.15 times).

In patients with early latent syphilis, significant ($p_1<0.05$ and $p_2<0.05$) reductions in late activation factor (HLA-DR +) were found at the time of infection of up to 6 months. Increases of HLA-DR+ at the time of infection of over 1 year were noted. The figures were respectively 9.3±1.3 and 27.4±1.8, while the normal level in healthy individuals is 14.1±1.2.

In patients with early latent syphilis the amount of CD16+ lymphocytes are 2.75 times less than in healthy donors ($p<0.01$) (0.10±0.01 and 0.29±0.02). In further analysis, it was found that the amount of CD16+ depended on the timing of infection, namely, the amount of NK cells progressively decreased with disease continuation. We also found suppression of phagocytic activity of neutrophils ($p<0.05$) in comparison with the group of healthy individuals, namely, a decrease in the phagocytic number by 1.3 times and the phagocytic index by 1.6 times. Regarding the metabolic activity of neutrophils (NBT-test), it was found to increase by 1.2 times ($p<0.05$) compared with the control group. The most significant changes in phagocytic count and phagocytic index, as well as NBT-test were found in patients with early latent syphilis with a duration of infection of up to 1 year.

In patients with early latent syphilis, some imbalance was found in the concentration of CICs of different molecular weight in the serum, namely CICs with large molecular size were significantly increased in patients with infection of up to 6 months (50.1±2.7 conventional units against 26.4±2.1 in the control group, $p<0.05$). The content of pathogenic CICs of medium and small size was increased by 1.5 and 2.7 times ($p_1<0.03$; $p_2<0.01$)in patients with early latent syphilis with an infection period of more than 1 year.

The Figure illustrates the serum content of proinflammatory and anti-inflammatory cytokines in patients with early latent syphilis.



The serum content of patients with early latent syphilis

CONCLUSIONS

1. The serum content of proinflammatory and anti-inflammatory cytokines in patients with early latent syphilis was significantly elevated and depended on the timing of infection. This dependence was most significant for IL-6 and IL-10.
2. Patients with early latent syphilis have an imbalance of CD25+ and late activation factor.
3. For HLA-DR against the background of a progressive decrease in the number of CD16+ lymphocytes, a direct relationship between the duration of infection and the number of NK cells was found.
4. Detected disorders, namely discoordination of the CICs content, may be the result of significant disorders of neutrophils, which could be one of the factors in the latent course of syphilitic infection.
5. Our study found disorders of non-specific protective factors in the serum of patients with early latent syphilis which can be influenced by dysfunction of the immune system caused by chronic infection.

Conflict of interests. The authors declare no conflict of interest.

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