

Peculiarities of the State of the Body's Immunoprotective Functions, Bacterioscopic and Cytological Studies in the Presence of a Chronic Inflammatory Process of the Reproductive System

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At the current stage, the state of health of the Ukrainian nation of reproductive age is characterized by a low birth rate. Chronic inflammatory diseases of the reproductive system significantly affect the health of millions of people of childbearing age. Chronic inflammation is characterized by a protracted, often erased course, a tendency to relapse, the presence of complications and resistance to therapy. The development and formation of inflammatory diseases are based on interconnected processes that begin with acute inflammation and end with destructive changes, therefore the assessment of the body's adaptive capabilities is increasingly considered one of the most important health criteria. There is a whole series of integral hematological indicators that allow you to assess the state of various parts of the immune system without resorting to special research methods.

Introduction

There are about a hundred definitions of the concept of "health". Health is understood as

the ability of the body to actively adapt to environmental conditions. The state of human health is dynamic due to changes in the

environment, so health can be defined not as a state, but as a process.

In this regard, the human body, which is subjected to continuous stressful influences in modern conditions, must be considered a dynamic system that continuously adapts to environmental conditions by changing the level of functioning of individual systems and the corresponding tension of regulatory mechanisms [1]. As criteria that quantitatively determine the level of individual health, those criteria that are related to its essential characteristics can be used. These include indicators that to one degree or another reflect the activity of the self-organization of the living system - adaptation, homeostasis, reactivity, and immune-protective functions of the body. The assessment of the adaptation capabilities of the body is increasingly considered one of the most important health criteria. There is a whole series of integral hematological indicators that make it possible to assess the state of various parts of the immune system without resorting to special research methods. Some of these indicators change even in the early stages of the disease, which allows you to use them in assessing the risk of disease prognosis [2].

In clinical practice, during the selection of a human treatment protocol, it is necessary to have available methods that will allow a quick but effective assessment of the body's protective potential. Such a simple and generally available method is a general blood test with the output of

a leukogram, the components of which quickly react to any changes in homeostasis in the body (Figure 1).

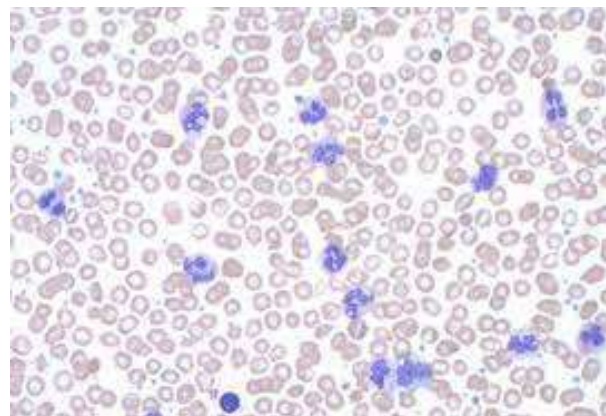


Figure 1. Increased number of circulating neutrophils.

Using the parameters of the leukogram, it is possible to operate with leukocyte indices that have diagnostic and prognostic value, as they allow to evaluate of the work of effective mechanisms of the immune system and the level of immunological reactivity, which determine the process of formation of nonspecific adaptive reactions [3].

The topicality of the topic is due to the significant spread of infectious diseases of the genitourinary tract at the current stage. The problem of inflammatory diseases of the genital organs, especially those caused by sexually transmitted infections, is growing every year. It is known that the state of the body's immunoreactivity is of great importance for the course, development of complications, and effectiveness of treatment of infectious inflammatory diseases. An available method of evaluation is a leukogram, as an indirect sign of the state of the immunocompetent system [4]. An idea about the state of systemic non-specific

reactivity of the body can be obtained by analyzing the quantitative and qualitative indicators of the composition of leukocytes and ESR. In many cases, polymicrobial flora is found in patients with inflammatory diseases of the pelvic organs (**Figure 2**).

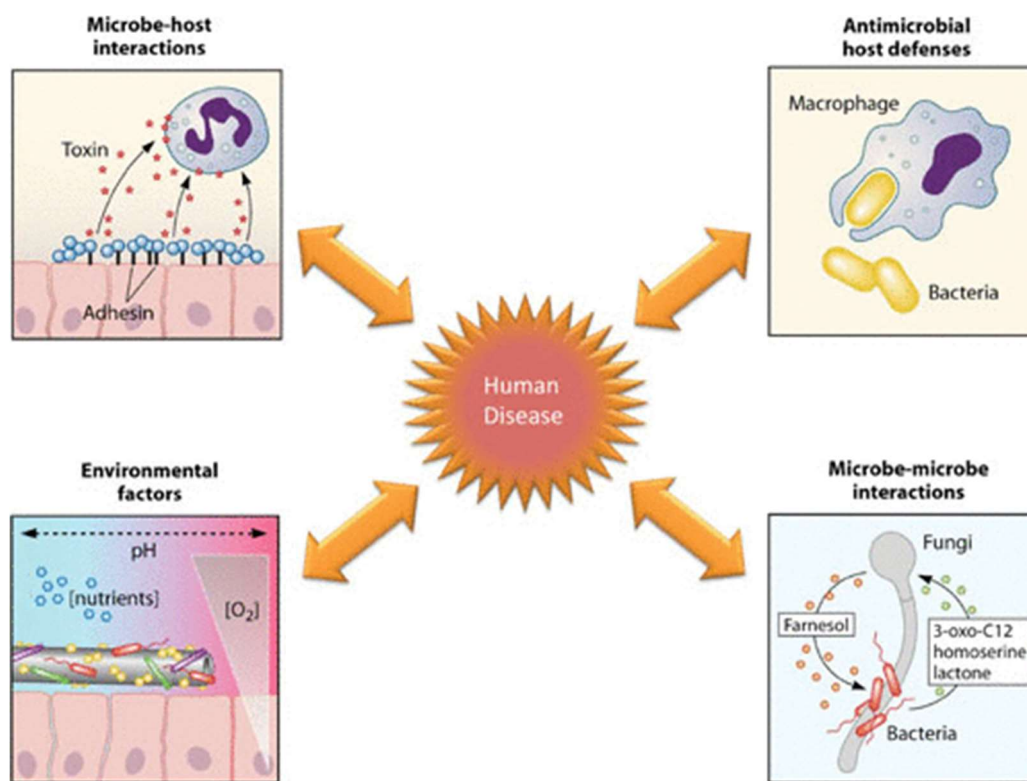


Figure 2. Influence of polymicrobial flora on morbidity. Schematic showing the interdependent relationships required for development of human disease. Infection is influenced by microbe–microbe interactions, microbe–host interactions, antimicrobial host defenses, and environmental factors. Significant changes in any of these factors can lead to the development of or predisposition to infection.

Nevertheless, it is assumed that at the beginning of the pathological process, only one infectious agent initiates the inflammatory process, and changes the functioning of local immune mechanisms, thereby preparing a favorable ground for further infection by other microorganisms [5].

The severity of the inflammatory process can be judged by changes in the leukocyte formula, taking into account other hematological indicators. The diagnostic and prognostic

capabilities of calculated indices are becoming more and more important nowadays since certain combinations of hemogram indicators reflect the integral characteristics of the body's homeostatic systems, which form non-specific adaptive reactions.

The purpose of our study was to study the possibilities of using integrative indicators of the leukogram of peripheral blood for an approximate assessment of the state of the immune protective functions of the body with a

risk assessment of sexually transmitted infections in patients with a chronic nonspecific inflammatory process [6].

Experimental part

Material and methods

Bacterioscopic and cytological studies were carried out in preparations of scrapings from the mucous membrane of the genital system, stained by the Romanovsky-Giemsa method. Bacterial flora, fungal elements, trichomonads were found against the background of epithelial elements and leukocytes. Signs of intracellular parasitism (chlamydial, viral infection) were determined cytoscopically [7]. If it is necessary to determine gram-negative flora, smears of scrapings of the mucous membrane were stained according to the Gram method [8]. If necessary to confirm the etiological diagnosis, cultural studies, polymerase chain reaction, immunological tests, etc. were carried out in the bacteriological laboratory of the Zaporizhzhya city dermatovenerological dispensary with the participation of laboratory employees.

The study of the state of the cells of the mucosa of the urogenital organs was carried out by the method of light microscopy in smears of scrapings of the epithelium after fixation with ethyl alcohol and staining according to Romanovsky-Giemsa (**Figure 3**).

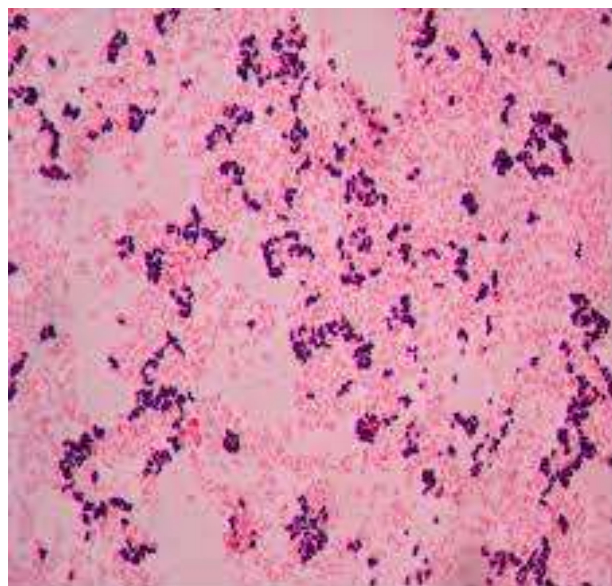


Figure 3. Gram-stained blood smear. Gram stain, also called the Gram method, is a method for differentiating bacterial species into two large groups.

All examined persons underwent a general clinical blood test. Based on the database of leukograms and ESR of peripheral blood, integral indicators were calculated using mathematical formulas [9].

Results and discussion

The control group (K) of the examined consisted of 95 clinically healthy persons (part-time donors), of whom 50 were men and 45 were women (Table 1). By age, the composition of K was as follows: 18-28 years - 30 people, 29-39 years - 35 and 41-50 years - 30 people. The groups of subjects included 162 patients with chronic diseases of the reproductive system, of which 83 were men and 97 were women (Table 1). Patients were divided by age as follows: 18-28 years - 60, 29-39 years - 58, and 40-50 years - 44 patients.

Table 1. Distribution of surveyed persons by age and sex.

Group	K1	K2	K3	1	2	3
Age (years)	18-28	29-39	40-50	18-28	29-39	40-50
Men	15	20	15	28	30	25
Women	15	15	15	32	28	19

Bacterioscopic and cytological studies. Trichomoniasis was one of the most frequently detected sexually transmitted infections (**Figure 4**).

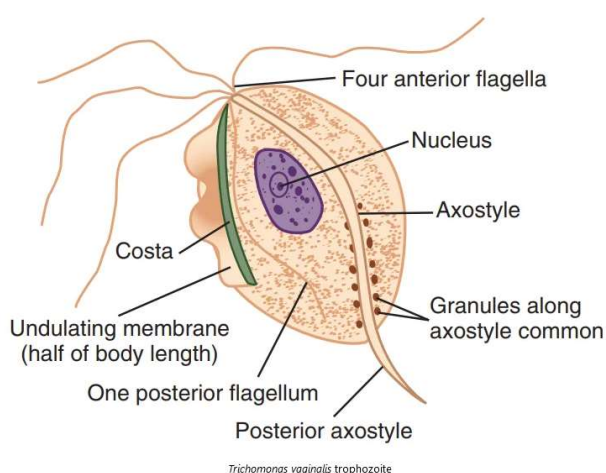


Figure 4. *Trichomonas vaginalis* *T. vaginalis* can be present in the female vagina and cervix, as well as the Bartholin's glands, urethra, and urinary bladder. It often affects men's anterior urethra, but it may also affect the prostate and preputial sac.

This disease was identified in 14% of patients. A thorough microscopic examination was sufficient to make the diagnosis. In native preparations of secretions from the genital organs, a single-celled microorganism of a pear-shaped or oval shape was determined, which in size was equal to or larger than a neutrophil. In our studies, atypical forms of trichomonads were more often encountered: microforms the size of a

leukocyte, or macroforms that resemble cells of the intermediate layer of a flat epithelium. The inflammatory background was usually manifested by a neutrophilic reaction with a large amount of detritus and mucus. In patients of the 3rd group (both men and women), the frequency of detection of trichomonads (7 out of 44) was significantly lower than that in patients of the 1st (19 out of 60) and 2nd (16 out of 58) groups.

Fungal lesions of the genitals occurred in 18% of women and 16% of men (except men of the 1st age group). Pseudomycelium, fungal spores were determined against the background of a more or less pronounced inflammatory reaction with a relatively large number of mononuclear cells. In women of the 3rd age group, fungal lesions (7 out of 22) were observed significantly more often than in women of the 1st (9 out of 38) group. No significant difference in the frequency of fungal lesions was found in different age groups of men.

The presence of gonorrhoea was suspected in 16% of men and 6% of women, when large diplococci were determined, which were gram-negatively stained, had the appearance of coffee beans, and their concave sides were directed towards each other. Diplococci were located extracellularly or intracellularly, including - in neutrophils, in clusters - perpendicular to each other (**Figure 5**).

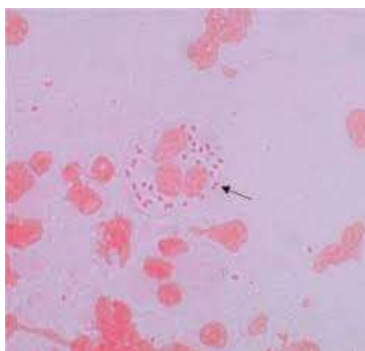


Figure 5. Gram-Negative Cocci. Gonococci cause both localized infections, usually in the genital tract. Gonococci reach these organs via the bloodstream (gonococcal bacteremia). Gonorrhoea in men is characterized primarily by urethritis while in women is cervicitis.

During the microscopic examination of smears of scrapings from the mucous membrane of the genitals of patients, it was found that the etiological factor of the inflammatory process of the genital system in 23% of cases was mixed infection (Table 2), while monoinfection was observed in 77% of cases.

The inflammatory reaction, as a rule, was pronounced, the presence of a large number of polymorphonuclear leukocytes with signs of degeneration of various degrees and phagocytosed diplococci, as well as detritus and mucus were determined. In some patients, diplococci lost their morphological properties, stained gram-variably, the inflammatory reaction was unexpressed, with the presence of a large number of lymphocytes and monocytes. Such a picture indicates a sluggish course of the disease and the chronicity of the inflammatory process.

In sick men and women of the 3rd (9 out of 44) groups, gonococci were detected significantly less often than in patients of the 1st (19 out of 60) and 2nd (19 out of 58) groups.

Table 2. Results of microscopic examination of sexually transmitted infections

Group	Number in the group	The number of patients in whom:								
		Trichomonas	Fungus	Gonococci	Bacteria	Chlamydia	Herpes virus	Cytomegalovirus	Papilloma virus	Mycoplasmosis
1	Men	22	9	0	10	0	9	2	0	0
	Women	38	10	9	9	6	17	5	1	0
2	Men	30	9	1	12	1	14	4	1	0
	Women	28	7	7	7	9	13	4	1	1
3	Men	22	4*	2	5*	1	12	3	1	1
	Women	22	3*	7*	4*	10*	10*	4	2	2

Note. * - $p < 0.05$, in comparison with the data of the 1st group.

Bacterial vaginosis (BV) in women of reproductive age was observed in more than 30% of cases. The diagnosis during microscopic examination of the smear was based on the determination of small coccal flora and the

presence of "key" cells. The inflammatory reaction is not characteristic. Microflora associated with bacterial vaginosis was detected in 3% of cases in smears of scrapings from the urethra of men, and there was no local

inflammatory reaction. In women of the 3rd group, bacterial vaginosis was observed significantly more often (10 out of 22) than in women of the 1st (6 out of 38) group.

Urogenital chlamydiosis was suspected in 16% of observations. (**Figure 6**).



Figure 6. Chlamydia trachomatis. Budding yeast cells in patient urine (urinary tract infections), microscopic analysis, contrast adjustment

At the same time, direct and indirect cytological signs of intracellular infection were determined. Direct cytopathogenic signs included elementary bodies and reticular bodies. Fragmentation of the nucleus, vacuolization of the cytoplasm was attributed to indirect signs. These signs were most often detected in the cells of the cylindrical epithelium of the cervical canal of women or the middle part of the urethra of men.

The inflammatory reaction was unexpressed, manifested by the presence of a relatively large number of mononuclear cells (10-15% of the total pool of tissue leukocytes). No significant difference in the frequency of chlamydia lesions in different age groups was found in men and women.

Genital herpes was suspected in 10% of cases during cytological examination of smears of scrapings from the genitals of men and women. Morphological changes induced by the virus were most clearly manifested in the cells of the intermediate layer of the flat epithelium or in the cells of the cylindrical epithelium. There was an increase in the size of the cells, the presence of basophilic inclusions in the nucleus, surrounded by a zone of illumination. Multinucleated cells were found. Characteristic lymphoplasmacytic reaction. There was no difference in the frequency of lesions between age groups. Signs of papillomavirus and cytomegalovirus infection were determined in single patients of the 2nd and 3rd groups. The presence of koilocytes (large, often rounded cells with pyknotic nuclei and wide areas of vacuolization or luminescence, which are surrounded by a ring of dense cytoplasm, multinucleated cells) was noted.

In isolated cases, signs suspicious of myco-ureaplasmosis were also observed. At the same time, the presence of small, rounded cavities of the same size with even edges of cavities was noted in the cells.

Intracellular infections were usually accompanied by inflammatory reactions with a significant number of mononuclear cells in the focus of inflammation. Inflammatory diseases in 23% developed as a result of mixed infections. The most frequently identified associates are chlamydia and gonorrhoea, gonorrhoea and

trichomoniasis, bacterial vaginosis, and herpes infection.

Pronounced dystrophy of the mucous epithelium of the genital organs was detected in isolated cases (**Figure 7**).

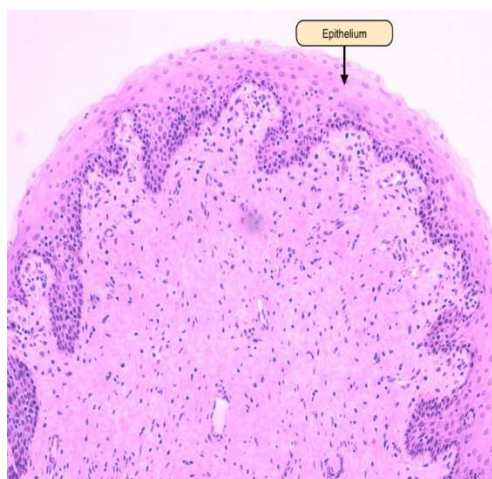


Figure 7. Stratified squamous epithelium of the female reproductive system. The vagina is lined by a stratified squamous epithelium that features a small degree of keratinization. Below the epithelium is a thick layer of dense connective tissue, like that in the dermis of the skin. A layer of loose connective tissue containing many blood vessels and nerves follows this.

Weak dystrophy was manifested as vacuolization of the cytoplasm, fatty degeneration of cells, with moderate dystrophy, degenerative changes of the nuclei were also observed (vacuolization, fragmentation, lysis, chromatin coagulation). In two patients of the 3rd group, pronounced dystrophy of the epithelium was found: a large amount of epithelial detritus, the phenomenon of necrobiosis, was found in the preparation.

Signs of weak dystrophy were observed significantly more often (14 out of 22) in sick men of the 3rd group compared to men of the 1st (9 out of 22) group. In women of the 3rd group,

the frequency of detection of both weak and moderate dystrophy of the epithelium of the mucous membrane of the genitals was significantly higher (21 out of 22) than in women of the 1st group (26 out of 38). In addition, in the 1st man and 1st woman of the 3rd group, pronounced dystrophy of the cells of the epithelium of the mucous membrane of the genital organ (GO) was found.

Thus, in men of the 3rd, the oldest age group, lesions with trichomonads and gonococci were observed significantly less often than in men of the 1st, the youngest age group.

A significant difference in the detection frequency of fungal, chlamydial, viral, etc. no infections were detected. However, signs of mild dystrophy of the urethral mucosa were observed significantly more often in men of the 3rd group, and in the 1st case, signs of severe dystrophy were found. In sick women of the 3rd group, such infections as trichomoniasis and gonorrhoea were detected significantly less often than in women of the 1st group. However, fungal lesions and bacterial vaginosis were detected in significantly more cases.

Dystrophy of the epithelium of the vagina of the first and second degrees of severity was observed significantly more often, severe dystrophy of cells of the mucous membrane of the vagina was found in one woman (**Figure 8**).

Table 3. The degree of dystrophy of the epithelium of the mucous membrane of the genital organs in patients with sexually transmitted infections

Group	Number in the group	The number of patients in whom:		
		1st degree dystrophy	2nd degree dystrophy	3rd degree dystrophy
1	36	14	13	-
2	26	11	11	1
3	30	18	6*	-
4	28	15	4*	-
5	19	10	5*	-
6	41	19	13	1

Note. * - $p < 0.05$, in comparison with the data of the 1st group

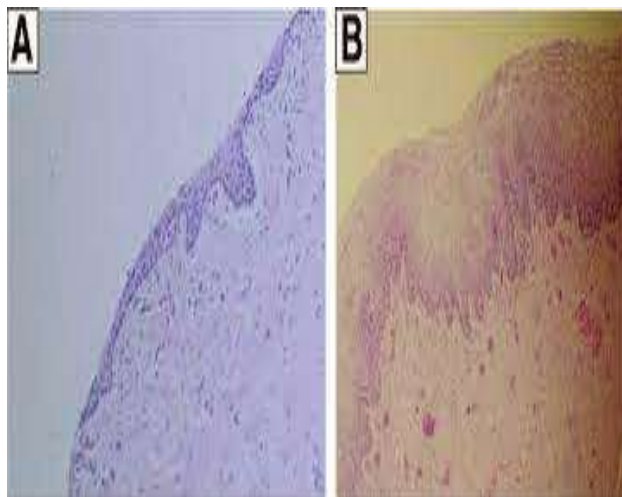


Figure 8. Histological photo of dystrophic (A) and mature epithelium (B) in a woman. Histologic appearance of atrophic and mature vaginal epithelium. (A): Atrophic vaginal epithelium with few epithelial cells; (B): Mature stratified squamous epithelium in the vagina. Note the highly vascular stroma. Photographs courtesy of Murray A. Freedman

A cytological assessment of the epithelium of the mucous membrane of the vagina and cervical canal in women and the

mucous urethra of men with sexually transmitted infections was carried out. Weakly or moderately expressed dystrophy of the epithelium was found in these patients (**Table 3**). When analyzing the frequency and degree of dystrophy of the epithelium of the genital system in patients, depending on the type of inflammatory agent, no significant differences were found (**Table 4**).

Table 4. The degree of dystrophy of the epithelium of the mucous membrane of the genital organs in patients with sexually transmitted infections, depending on the causative agent of inflammation

Group	Number in the group	The number of patients in whom:			
		1st degree dystrophy	2nd degree dystrophy	3rd degree dystrophy	
1	Men	22	9	6	0
	Women	38	15	11	0
2	Men	30	16	10	0
	Women	28	20	8	0
3	Men	22	14*	8	1
	Women	22	12*	9*	1

Note. * - $p < 0.05$, in comparison with the data of the 1st group.

Thus, the difference in the frequency of detection of various sexually transmitted infections, as well as epithelial dystrophy, may be related to age characteristics, lifestyle characteristics, hormonal background, metabolism, general reactivity of the body, previous treatment with antimicrobial and anti-inflammatory agents, etc (**Figure 9**).

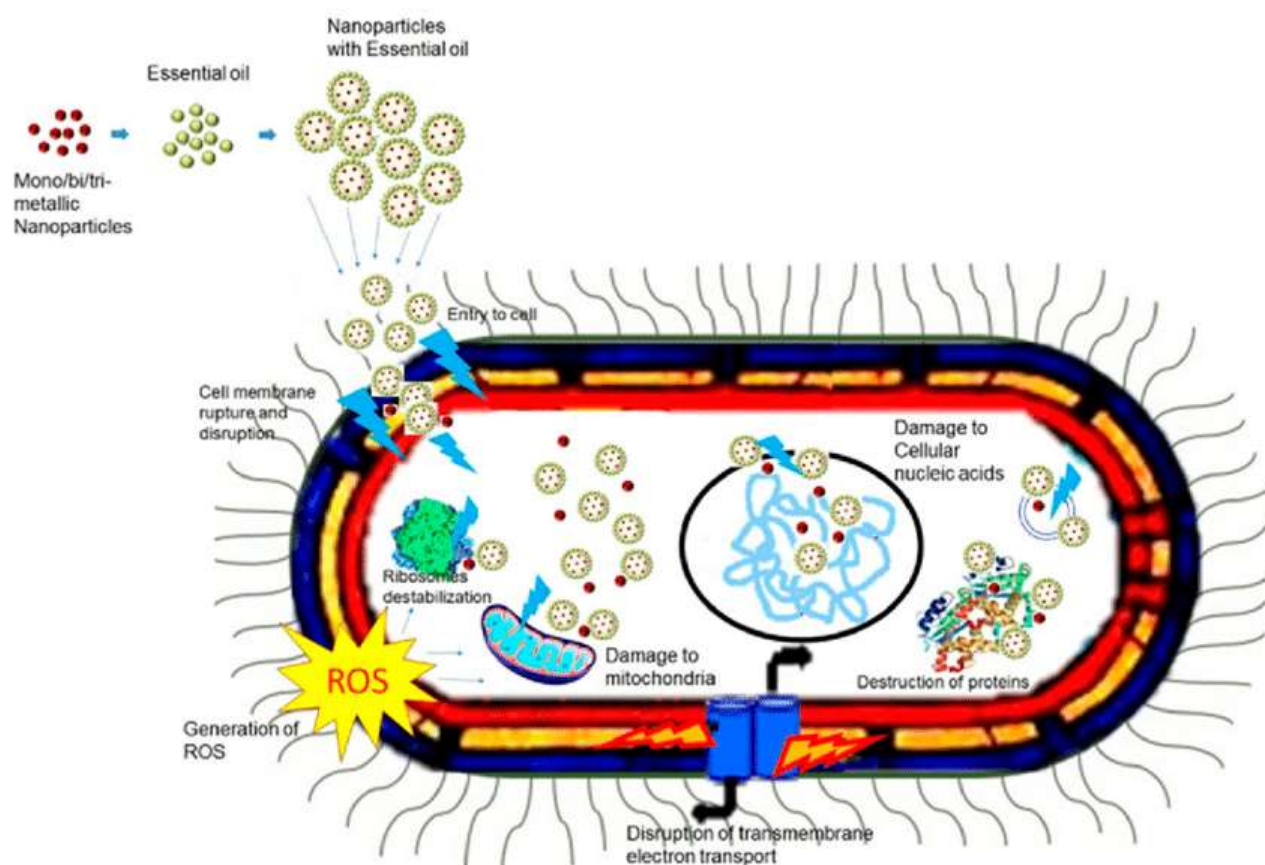


Figure 9. Antibacterial mechanisms. Combination attack bacteria cell through; direct interaction with cell membrane , protein dysfunction, DNA damage, inhibition of the electron transport chain, and the regulation of bacterial metabolic processes.

Integral indicators of leukogram of peripheral blood.

It is known that the state of the body's immunoreactivity is of great importance for the course, development of complications and effectiveness of treatment of infectious inflammatory diseases [10]. An available method of evaluation is a leukogram, as an indirect sign of the state of immunocompetence of the body system [11, 12]. An idea about the state of systemic non-specific reactivity of the body can be obtained by analyzing the quantitative and qualitative indicators of the composition of leukocytes and ESR. Using the parameters of the leukogram, it is possible to operate with

leukocyte indices that have diagnostic and prognostic value, as they allow to evaluate the work of effective mechanisms of the immune system and the level of immunological reactivity, which determine the process of formation of nonspecific adaptive reactions [13, 14]. LD - index of leukocyte displacement, LG - lymphocyte-granulocyte index, LESR - lymphocyte and ESR ratio index, LER - lymphocyte and eosinophil ratio index, LMR- lymphocyte and monocyte ratio index, NLR - neutrophil and lymphocyte ratio index, NMR - neutrophil and monocytes ratio index, GI - general index.

Table 5. Leukogram indicators and integral hematological indices in men with chronic inflammatory diseases

Index	Healthy	Men with chronic diseases of the reproductive system				
		Group 1 (men)		Group 2 (men)		
	X±m	X±m	P	X±m	P	P ₁
LD	1,90±0,08	1,45±0,14	<0,05	1,78±0,10	-	<0,05
LESR	0,96±0,15	1,53±0,28	<0,05	2,74±0,38	<0,05	<0,05
LG	4,74±0,44	6,95±0,53	<0,01	5,82±0,66	<0,05	<0,05
TI	5,30±0,26	8,53±0,70	<0,001	8,63±1,21	<0,01	-
NLR	2,26±0,08	1,43±0,09	<0,001	1,98±0,23	-	<0,05
NMR	14,5±1,4	11,3±1,3	-	12,0±0,9	-	-
LMR	7,06±0,80	8,15±0,70	-	7,10±0,80	-	-
LER	15,0±1,9	27,6±3,50	<0,01	25,1±3,4	<0,05	-

Table 6. Leukogram indicators and integral hematological indices in women with chronic inflammatory diseases

Index	Healthy	Women with chronic diseases of the reproductive system				
		Group 1 (women)		Group 2 (women)		
	X±m	X±m	P	X±m	P	P ₁
LD	2,10±0,12	1,70±0,15	<0,01	1,76±0,24	-	-
WLESR	1,30±0,16	1,96±0,30	<0,05	2,30±0,95	<0,05	-
ILG	4,60±0,30	6,80±0,60	<0,01	5,80±0,37	<0,05	<0,05
3TI	5,60±0,35	8,40±0,65	<0,001	8,10±1,10	<0,05	-
INLR	1,94±0,15	1,48±0,07	<0,001	1,80±0,17	-	<0,05
INMR	19,4±2,5	14,5±4,5	-	12,6±1,0	<0,05	-
ILMR	9,50±1,00	9,10±1,70	-	7,30±1,04	<0,05	<0,05
ILER	17,6±2,4	17,8±2,6	-	18,4±4,7	-	-

The index of leukocyte displacement (LD) in sick men and women is lower than that of the control group (Tables 5, 6), and in patients of the 1st group the decrease was significant (by 1.3 times; $p < 0.05$ in men and 1.4 times; $p < 0.01$ in women). In patients of the 2nd group, an increase in LG was observed in comparison with such patients of the 1st group, which was significant in men (by 1.2 times; $p < 0.05$). These changes indicate a more active inflammatory process in patients of the 2nd group. The lymphocyte/ESR index (LESR) in patients of the 1st group is significantly higher than that of the

control group (by 1.5 times; $p < 0.05$). In people of the 2nd group, the growth of LESR is even more significant (2.8 times in men and 1.8 times in women; $p < 0.05$).

Note. P-reliability of differences between indicators of healthy persons and patients of this group; P₁-reliability of differences between indicators of patients of the 1st and 2nd groups. In men of the 2nd group, LESR is also significantly higher than that in the 1st group (by 1.8 times; $p < 0.05$).

Thus, patients with a chronic inflammatory process suffer from

autointoxication, which is more pronounced in patients of the 2nd group. The presence of autointoxication also confirms the growth of the lymphocyte/granulocyte index (LG) and the total index (TI).

However, there is a decrease in LG in patients of the 2nd group compared to patients of the 1st group (by 1.2 times; $p < 0.05$), which

confirms the assumption of greater activity of the inflammatory process in the patients of the 2nd group. The neutrophil-to-lymphocyte ratio index (NLR) in the 1st group was significantly reduced (by 1.5 times in men and 1.3 times in women; $p < 0.001$), i.e. increased activity of a specific link of immune protection (**Figure 10**).

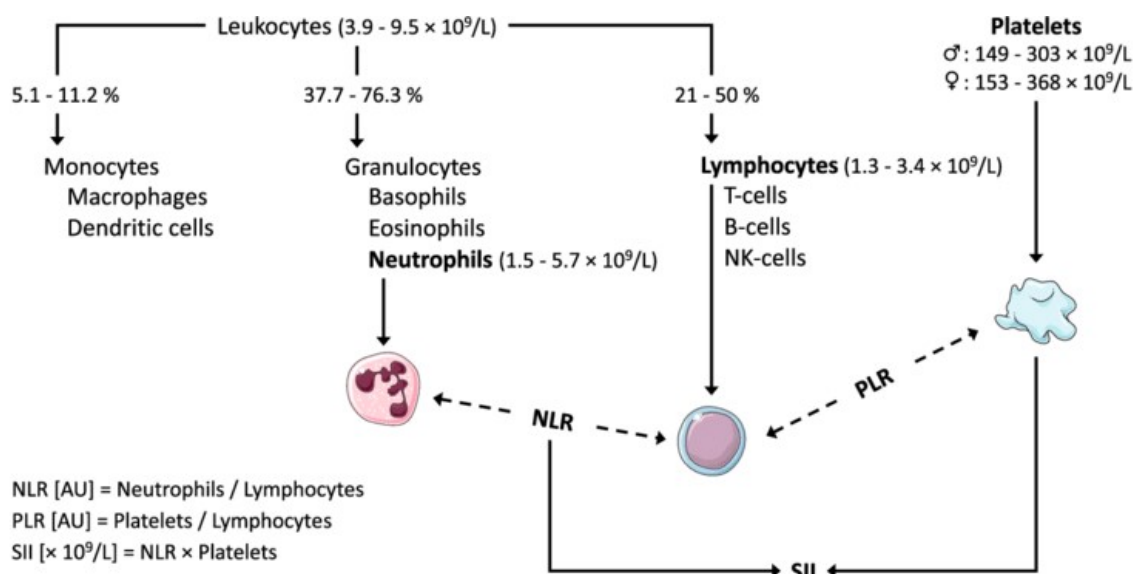


Figure 10. Calculation of the cellular immune inflammation markers neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII). Altered concentrations of the underlying blood cell populations in response to the inflammatory process.

In patients of the 2nd group, this indicator was significantly lower than in patients of the 1st group (1.3 times in men and 1.2 times in women; $p < 0.05$), which indicates a higher activity of nonspecific protection

On the other hand, the index of the ratio of neutrophils and monocytes (NMR) in sick men showed a tendency to decrease. In sick women, NMR decreased, and more significantly in persons of the 2nd group (1.5 times; $p < 0.05$), which indicates the superiority of the macrophage link in the phagocytosis system.

The index of the ratio of lymphocytes and monocytes (LMR) in men and women of the 1st group did not change significantly. In sick women of the 2nd group, LMR was significantly lower than that of the control and 1st groups (by 1.3 and 1.2 times, respectively; $p < 0.05$). These shifts also suggest a greater activity of the macrophage system.

The index of the ratio of lymphocytes and eosinophils (LER) in sick men significantly increased (by 1.8 times; $p < 0.01$ in the 1st group of patients and by 1.6 times; $p < 0.05$ in the 2nd

group), that is, delayed-type hypersensitivity reactions predominated.

Thus, in patients with chronic diseases of the genital organs, shifts in the integral indicators of the leukogram and the sedimentation rate of peripheral blood erythrocytes were found, which have features in different degrees of epithelial dystrophy, as well as depending on gender. Probably, immunological reactions are hormone-dependent. And this is natural, because the immune system, together with the nervous and endocrine system, is the only system for regulating homeostasis in the body. The obtained results show that the use of integral formalized indicators of the leukocytes count of peripheral blood and the sedimentation rate of erythrocytes allows for an approximate assessment of the state of immunological reactivity in patients with a chronic non-specific inflammatory process, which contributes to the correct appointment of immunotropic agents

Conclusions

1. In patients with chronic non-specific inflammatory diseases of the genital organs, there is a violation of the immunological reactivity of the body.

2. A tendency to autointoxication is observed, the greater the degree of epithelial dystrophy, with a low degree of epithelial dystrophy, the activation of a specific link of immunity is more characteristic.

3. In patients with a high degree of dystrophy, the system of non-specific protection

is more activated, and in men, higher activity was found on the side of the microphagocytic link, and in women - the macrophagocytic link. In men with a chronic inflammatory process, there is a tendency to hypersensitivity reactions of the delayed type. That is, for an approximate assessment of the state of the body's immunological reactivity, it is advisable to use the integral indicators of the peripheral blood leukogram and the erythrocyte sedimentation rate

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