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Clinical and Immunological Evaluation of Application of Ronkoleukin in Nonspecific Vulvovaginitis at Adolescent Girls

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Package Insert: According to immunological parameters there was found that during the sub-acute there is the secondary immune deficiency and the immunodeficiency is absent during the acute. During the acute phagocytic function of the local secret is satisfactory, and during the sub-acute against satisfactory absorption function there is a decrease of bacterial growth-inhibitory activity of vaginal secretions, indicating the necessity of correction of phagocytic component of these patients.
The Purpose of the Research: Clinical and immunological evaluation of ronkoleukin using depending on the routes of entry in the treatment of non-specific vulvovaginitis among adolescent girls in different variants of the disease state.
Design of their Search: Prospective study.
Methodology: From 2006 to 2010 years by the assignment of adolescent therapist and upon periodic screening the adolescent girls with a variety of complains for genitalia were examined in the child and adolescent consulting room in Municipal Polyclinic № 11 and «The City Center of Human

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Reproduction» in Almaty.
124 menstruate adolescent girls from 11 to 18 years, not sexually active, were selected to accomplish the target objective.
I group -20 healthy (control).
II group - 62 with per-acute nonspecific vulvovaginitis.
III group - 42 with sub-acute nonspecific vulvovaginitis.
In cooperation with the scientific consultant and immunologist, M.D. Prof. A. Kurmanova there was developed a dosage schedule of roncoleukine in the complex therapy, based on the results of the analyses of the immune and cytokine status and phagocytic vaginal system.
II group - 62 patients with per-acute nonspecific vulvovaginitis.
II A– 20 patients with sub-acute nonspecific vulvovaginitis.
(Standard therapy + roncoleukine 250 000 U/ml vaginal irrigation once a day).
II B– 25 adolescent girls with sub-acute.
(Standard therapy + roncoleukine 250 000 U/ml twice subcutaneously, every other day).
II C – 17 adolescent girls with sub-acute (standard therapy).
III group - 42 patients with acute nonspecific vulvovaginitis.
(standard therapy + roncoleukine 250 000 U/ml twice subcutaneously, every other day).
Standard therapy of 7-10 days included [1,2]:
Efficacy of the drug was assessed by patient complaints, inspection, and data of microbiological studies of vaginal discharge, immune state, cytokine status, and local phagocytic system on the 7th and 14th days of treatment.

Keywords: Nonspecific vulvovaginitis; girl; adolescent; immunity; cytokines; phagocytosis; recombinant human interleukin (roncoleukine).

1. INTRODUCTION

In the structure of gynecological disorders at girls and adolescents vulvovaginitis occupies a leading place [3].

According to scientific studies, the frequency of vulvovaginitis is up to 93 per cent for girls, for adolescents it goes up to 53%, while 60% has a recurrent character [4-11].

At the present stage there is no doubt that the microbial factor prevails in the pathogenesis of nonspecific vulvovaginitis of the adolescent girls [12-16].

Local protection of female genital organs is due to their anatomical and physiological characteristics, the presence of normal microflora, humoral and cellular factors of immunity.

Common infectious disease accompanied by decrease in immunity, as well as the hormonal disease break the qualitative and quantitative composition of the vaginal microflora that facilitates the invasion of pathogenic microorganisms and can lead to the development of inflammatory processes caused by opportunistic pathogenic bacterium [17].

The opportunistic pathogens, involved in the inflammatory process, do not contain highly toxic poisons, but they are dangerous for hypernormal promoting of inflammation mediators of micronychia microorganism.

The practical significance of vulvovaginitis is defined by the fact that they lead to the formation of synechia of the labia, genital infection, disruption of menstrual function, which can cause serious disorders of reproductive function in the future [18,19].

The necessity of the clinical relevance of the study of the immunological aspects of vulvovaginitis is that each immune imbalance increases the probability of the progression of the disease, favours the development of complications, the allergization of the organism and the chronization of the process.

The evaluation of the general and of the local immunity at vulvovaginitis is one of the challenging issues, taking into account the essential role of the immune-pathological mechanisms at the chronization and at the retrocessions of the process.

Opportunistic microorganisms which take part in an inflammatory process do not contain any

high-toxic poisons, but they are dangerous due to their excessive activation of the inflammatory mediators of the microorganism.

At present time the diagnostic significance of the evaluation of the cytokines concentration level consists in the statement of the very fact of its increasing or reducing during a concrete disease, at that it is appropriate to detect the concentration of pro-inflammatory as well as of anti-inflammatory cytokines in the dynamic of the disease development to be able to evaluate the severity and to make prognosis of the course of disease [20-22].

In this connection the search of the new immunological drugs in the complex therapy of vulvovaginitis allowed to examine the use of Interleukin-2 human recombinant (ronkoleukin) [23] in the complex therapy of nonspecific vulvovaginitis at adolescent girls with different kinds of diseases and introduction of the drug.

For the diagnosis and treatment of vulvovaginitisit is not possible to rely solely only on a visual assessment of the genitalia and the discharge from the vagina; it is also necessary to provide the microbiological research and study of the immune status, local cytokine, and phagocytic system.

2. RESULTS AND DISCUSSION

The clinical picture of sub-acute and acute nonspecific vulvovaginitis was presented by the symptoms of vulvitis and urethritis (Table 1).

The most common complaints in the acute period were redness in the genital area, amyctic, burning and discharge from the genital tract, and on examination there were frequently identified hyperemia and swelling of the pronaus.

In the sub-acute the main complaints were redness, amyctic, and on examination there was seen hyperemia of the urethral meatus and pronaus.

In the clinical picture in acute course of the disease the moderate discharge from the reproductive tracts was more evident, and the sub-acute discharge was low.

It drew attention to the fact that the patients in both groups had the discharge of pathological character from the genital tract during several weeks, and only when the amyctic, burning, discomfort had appeared in the genitals, they turned to a specialist.

On examination of the patients there was noticed the dissonance in the intensity of inflammatory symptoms in the sub-acute.

Objectively sub-acute vulvovaginitis was characterized by isolated hyperemia of internal surfaces of the labia and labia majoria, pronaus and injected vessels of the vulva that indicated the long-term inflammation.

The absence of subjective sensations, the limit of inflammation by inflammation of glands and urethral lacunes, hyperemia of stasis and swelling of pronaus confirm that vulvovaginitis is sub-acute in nature.

It was found (Table 2) that the patients with sub-acute disease had a significant downstream of the relative content of Cd3+, indicating the oppression of their differentiation. There was also recorded a significant reduction of Cd20+. With regard to the value of CD4+ and CD8+ compared to the control group there was a tendency to decrease.

Study of immunological parameters with acute disease has shown that there are no reliable differences in comparison with the control group, although there was watched a trend towards downstream of CD4 +, Cd8 + and Cd20 + cells and the trend of increase in Cd3 +, due to the dispersion of individual value.

On the basis of the revealed violations by the parameters of the immune status, it can be assumed that in the sub-acute there is a recurrent immunodeficiency, and there is no deficiency during acute.

In all studied groups the average cytokine profile's value in peripheral blood did not differ significantly from the average value of the control group in connection with a wide range of value.

Therefore, the further analysis of cytokine output was conducted by the percentage of occurrence of elevated and high value (Table 3).

In the I group the high output of cytokines IFN-γ, TNF-α and IL-6 were not observed. The increased output of IFN-γ was recorded at 10% of examined patients.

In the second group, there was an increase in output of IFN-γ at 15% of the patients, with

adequately high content (in 4 times), recorded only at one patient (5%. The elevated level of TNF-α output was registered at 20% of the patients; herewith there was no any highest production (in 4 or more times). At the same time there was the increased output of IL-6 at 20%. It should be noted that high (in 5-13 times) output of IL-6 was at 15% (3/20). When comparing the rates of occurrence of high content with I group, the increased output of IFN-γ and IL-6 was more often registered in the 2nd group. However, adequate cytokine explosion with the activation of decreasing immunity was observed only in 1/6 cases.

In the III group the enhancement of IFN-γ output was observed at 23.8% of the surveyed. Herewith, the adequately high content (in 4 times) was recorded at 3/42 (7.1%) of the surveyed. An elevated output of TNF-α was at 21.4% of surveyed, with the highest (in 4 or more times) was not a product of one. The increased production of IL-6 was observed at 14.3% of the patients, while high production was recorded at 4.7% of the patients. In comparing values of occurrence of high content with group I, increased production of IFN-γ and IL-6 was significantly recorded in group III ($p \leq 0.05$).

Table 1. The main complaints and clinical manifestations of nonspecific vulvovaginitis adolescent girls (M ± m)

Indices	Group I	Group II A	Group II B	Group II C	Group III
Complaints					
Discharge	-	3(15,0±3,8)	6(24,0±4,7)	7 (41,2±3,9)	19 (45,2±6,0)
Redness	-	11(55,0±7,0)	15(60,0±7,1)	11 (64,7±4,8)	30 (71,4±7,1)
Vulvovaginal pruritus	-	7(35,0±5,7)	12(48,0±6,5)	12 (70,6±5,0)	24 (57,1±6,6)
Heat	-	3(15,0±3,8)	11(44,0±6,3)	11 (64,7±4,8)	22 (52,4±6,4)
Vulvovaginal pain	-	3(15,0±3,8)	8(32,0±5,4)	10 (58,8±4,6)	18 (42,9±5,9)
Painful urination	-	1(5,0±2,2)	3(12,0±3,4)	2 (11,8±2,2)	5 (11,9±3,4)
Examination					
Hyperaemia of the labia majoria	-	6(30,0±5,3)	7(28,0±5,1)	5 (29,4±3,4)	23 (54,8±6,5)
Hyperaemia of the vestibule of vagina	-	11(55,0±7,0)	22(88,0±8,3)	17(100,0±5,8)	39 (92,9±7,5)
Hyperaemia of the urethral meatus	-	13(65,0±7,5)	9(36,0±5,7)	7 (41,2±3,9)	16 (38,1±5,7)
Oedemata of the labia majoria	-	4(20,0±4,4)	4(16,0±3,9)	4(23,5±3,0)	13(30,9±5,2)
Oedemata of the vestibular mucous membrane	-	8(40,0±6,1)	11(44,0±6,3)	11(64,7±4,8)	30 (71,4±7,1)
Oedemata of the urethral meatus	-	4(20,0±4,4)	6(24,0±4,7)	5(29,4±3,4)	11 (26,2±4,8)
Hyperaemia of the perineum	-	6(30,0±5,3)	8(32,0±5,4)	6(35,3±3,7)	16 (38,1±5,7)
Pathologic discharge from the genital tracts					
The value of discharge					
Low	6(30,0±5,3)	14(70,0±7,8)	18(72,0±7,7)	12(70,6±5,0)	-
Moderate	14(70,0±7,8)	6(30,0±5,3)	5(20,0±4,4)	3(17,6±2,6)	25 (59,5±6,7)
Plethorical	-	-	2(8,0±2,8)	2(11,8±2,2)	17 (40,5±5,8)
Colour of discharge					
Off-white	20(100,0±8,9)	-	-	-	-
Off-white–xanthic	-	13(65,0±7,5)	8(32,0±5,4)	6(35,3±3,7)	8 (19,0±4,2)
Pyromucous (xanthic)	-	7(35,0±5,7)	7(28,0±5,1)	8(47,1±4,2)	17 (40,5±5,8)
Pyogenic (xanthic-greenish)	-	-	10(40,0±6,0)	3(17,6±2,6)	17 (40,5±5,8)

Table 2. Immune status and phagocytic function of vaginal discharge in the examined before treatment (M ± m)

Indices	Group I healthy patients (n=20)	Group II with sub-acute form (n=62)	Group III with acute form (n=42)
CD3+(%)	64,5 ± 0,3	59,32±1,9*	69,2±6,4
CD4+(%)	53,9±1,0	49,4 ±5,1	53,0±8,1
CD8+(%)	23,2±0,7	20,8 ±2,7	22,0±5,3
CD20+(%)	15,3±0,7	12,4±0,6*	14,9±4,1
ИНФγ (pg/ml)	11,02±0,74	12,49±6,49	17,32±12,2
ФНОα (pg/ml)	4,48±0,57	3,99±0,70	4,14±1,7
ИЛ-6 (pg/ml)	22,62±1,80	38,24±3,22	36,5±26,7
Phagocytic index, idiopathic, %	22,4±5,4	35,8±5,8	47,2±15,2
Phagocytic number, idiopathic	8,0±0,2	5,9±1,4	6,7±1,5
Phagocytic index, induced by pyrogenal, %	36,3±4,7	48,8±8,4	50,0±10,0
Phagocytic number, induced by pyrogenal	10,0±0,3	6,6±1,2*	8,7±0,7
HCT idiopathic, %	24,5±13,0	10,1±4,12	14,2±6,2
HCT stimulated by pyrogenal, %	44,1±5,7	12,4±4,9*	32,3±2,3***

** The disparity is accurate at P≤0.05 between the Groups I and II . ** The disparity is accurate at P≤0.05 between the Groups I and III. *** The disparity is accurate at P≤0.05 between the Groups II and* III

Consequently, nearly 1.6 times more girls and adolescents with acute nonspecific vulvovaginitis have the increased output of proinflammatory cytokine IFN-γ than with sub-acute, indicating the direct dependence of the activity level of proinflammatory cytokine from the clinical course of the inflammatory process. Although, its high output does not exist in both variants of the disease.

Table 3. The frequency of the increased cytokine concentration in the examined patients (M ± m)

Indices Pg/ml	Group I	Group II	Group III
γ-Interferon	0	15,0±8,0*	23,8±6,6**
TNF α	10,0±6,7	20,0±8,9	21,4±6,3
IL-6	0	20,0±8,9*	14,3±5,4**

** The disparity is accurate at P≤0.05 between the 1st and the 2nd groups. ** The disparity is accurate at P≤0.05 between the 1st and the 3rd groups*

The data obtained give the evidence of the oppression of anticontagious immunity both cellular and humoral, as TNF-α has the co-stimulant function for T-cell activation and activation of mononuclear phagocytes, also it helps the antibody formation by B-cells, and IL-6 is responsible for the specificity and the adequacy of immune reactions. This fact is due, first of all, with the presence of different pathological changes of immune system.

In the group I the absorptive function of the vaginal discharge complies with the similar parameters of healthy women of reproductive age, while digestible function of the vaginal discharge is more evident in adolescence.

The phagocytic function of vaginal discharge at the adolescent girls of the II group in comparison with I group was observed in some improvement of the average absorption capacity of vaginal discharge, but did not differ significantly. Herewith, the digestible ability of the vaginal discharge was decreased, resulting in decreasing the NBTR-test value in the stimulated version (NBTR-test stim. - 12.4±4.9%, $p \leq 0.05$). The spontaneous value of NBTR-test was below the equivalent control values, but not significantly different, due to the wide scatter in values.

In the III group all values both the absorption and digestive functions of vaginal discharge did not differ significantly from those in the I group, however, there has been a tendency to increase of the value both spontaneous and stimulated phagocyte index, and to the downstream of the phagocyte number.

By comparison of the sub-acute value there was no substantial difference in absorption capacity, but was noted a significant reduction of bactericidal activity in sub-acute in comparison with acute.

Thus, in the acute with nonspecific vulvovaginitis at young girls and adolescents the phagocytic function of the local discharge is satisfactory; and during the sub-acute against satisfactory absorption function there is observed the decrease of bactericidal activity of the vaginal discharge, which demonstrates the need for the phagocyte correction of these patients.

Before the treatment in cytokine composition the averages did not differ significantly from the control group average (Tables 4,5).

The same pattern for mean values was observed on the 7th and 14th days after treatment. But when comparing the percentage of occurrence of high levels before treatment there was observed the high output of IFN-γat 15% of the patients, output of TNF-α and IL-6 - at 20%, of the patients, output of TNF-α at 25% of the patients increased on the 7th day after treatment, and increasing output of cytokines was observed on the 14th day. These changes may indicate an inflammatory process remitting, preceded by activation of exogenous cytokine.

Before the treatment in the group II (A) in comparison with the group I the average absorption capacity of vaginal discharge was not different; the digestion ability of vaginal discharge was reduced, that resulted in reduction of the NBTR-test value in the Pirogenal stimulated version. In loading tests with ronkoleukin in vitro, there was a sharp increase of phagocyte index, phagocyte number and NBTR-test, which indicated a positive response of vaginal mucus.

Through 7 days after the vaginal irrigation by ronkoleukin a local secret reaction was the following: the value of spontaneous and induced by Pirogenal the phagocyte index, phagocyte number, and NBTR-test sharply increased.

In comparison with the individual changes, it was observed a significant increase in spontaneous phagocytic number to 139.2%, phagocyte number induced by ronkoleukinto 113.5%, spontaneous NBTR-test, induced by Pirogenal and Ronkoleukinto 353.3%, 220 percent and 161.9% respectively.

Thus, in the sub-acute the local application of Roncoleukin leads to the increased absorption and oxygen-dependent bactericidal ability of vaginal secretion, which contributes to the rapid involution of clinical aspects.

Table 4. Dynamic of complaints and of clinical signs in the Group IIA (M ± m)

Analysed indices	Until the treatment	On the 7th day	On the 14th day
Complaints			
Discharge	3(15.0±3.8%)	7(35±5.7%)*	3(15±3.8%)
Redness	11(55.0±7.0%)	8(40±6.1%)*	2(10±3.1%)
Vulvovaginal pruritus	7(35.0±5.7%)	5(25±4.9%)	2(10±3.1%)
Heat	3(15.0±3.8%)	1(5±2.2%)*	-
Vulvovaginal pain	3(15.0±3.8%)	2(10±3.1%)	-
Painful urination	1(5.0±2.2%)	-*	-
Examination			
Hyperaemia of the labia majoria	6(30.0±5.3%)	3(15±3.8%)*	1(5±2.2%)**
Hyperaemia of the vestibule of vagina	11(55.0±7.0%)	5(25±4.9%)*	3(15±3.8%)
Hyperaemia of the urethral meatus	13(65.0±7.5%)	-*	-
Oedemata of the labia majoria	4(20.0,0±4.4%)	-*	-
Oedemata of the vestibular mucous membrane	8(40.0±6.1%)	2(10±3.1%)*	-
Oedemata of the urethral meatus	4(20.0±4.4%)	-*	-
Hyperaemia of the perineum	6(30.0±5.3%)	3(15±3.8%)*	2(10±3.1%)
Pathologic discharge from the genital tracts			
The value of discharge	14(70.0±7.8%)	11(55±7.0%)	5(25±4.9%)
Low	6(30.0±5.3%)	9(45±6.4%)*	15(75±8.0%)
Moderate	-	-	-
Plethorical			
Colour of discharge	-	7(35±5.7%)*	11(55±7.0%)
Off-white	13(65.0±7.5%)	9(45±6.4%)*	9(45±6.4%)
Off-white–xanthic	7(35.0±5.7%)	4(20±4.4%)*	-
Pyromucous (xanthic)	-	-	-

** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day; ** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day*

Before treatment (Tables 6,7) in the group II B there was record of decrease of CD3 + and CD20 + lymphocytes in the value of lymphocyte subpopulation composition. On the 7th day rate of Cd3 + increased slightly, but continued to be significantly reduced, and the content of Cd20 + turned up to stated value.

An increase of CD3+lymphocytes to the level of stated value was recorded on the 14th day, and by comparing value before and after the treatment there was registered a significant improvement of this indicator.

Thus, the appointment of roncoleukin subcutaneously twice alternate days in the sub-acute leads to the normalization of main value of lymphocyte subpopulation composition.

On the 7th and 14th days the value of lymphocyte subpopulation composition did not undergo any significant changes, but the dynamics tended to decrease (normalization) of CD3+ lymphocytes (Table 11).

Thus, in the acute period twice alternate days subcutaneous injection of roncoleukin has no effect on the normal values of the immune status, i.e. it has a modulatory effect depending on the initial status.

On the 7th day of the treatment there were no any complaints to dysuria and objectively there were no swelling and hyperemia of external urethral opening and swelling of the labia majoria.

The profuse discharge were completely absent on the 7th treatment day, corresponding to the data of objective examination (Table 10).

According to the patient's words there was an increased vaginal discharge, but the nature of this discharge has changed to ordinary fluor albuswith a gradual transition to the normal vaginal discharge to the 10th day of the treatment.

Table 5. Dynamic of the immunological indices and of the indices of the local phagocytic system, Group II A (M ± m)

Indices	Control group (n=20)	Group IIA		
		Until the treatment	On the 7th day	On the 14th day
CD3+ (%)	64.5±0.3	59.32±1.9*	61.3±1.6**	64.6±1.4 #
CD4+ (%)	53.9±1.0	49.4±5.1	49.6±1.6	49.5±2.0
CD8+ (%)	23.2±0.7	20.8±2.7	21.4±1.2	21.6±1.1
CD20+ (%)	15.3±0.7	12.4±0.6*	13.2±1.1	13.6±0.86
IL-6 (pg/ml)	22.6±1.8	38.2±3.22	23.9±0.87	22.3±1.3
Interferon (pg/ml)	11.02±0.74	12.5±6.5	12.04±0.37	12.1±0.23
TNF (pg/ml)	4.48±0.6	3.9 ±0.7	4.57±0.36	4.4±0.2
** The disparity is accurate at P≤0.05 between the examination and until treatment; ** The disparity is accurate at P≤0.05 between the examination and on the 7th day; *** The disparity is accurate at P≤0.05 between the examination and on the 14th day; # The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day*				
Phagocytic index, idiopathic, %	22.4±5.4	35.8±5.8	-	60.2±11.4**
Phagocytic number, idiopathic	8.0±0.2	5.9±1.4	-	7.9±1.6***
Phagocytic index, induced by pyrogenal, %	36.3±4.7	48.8±8.4	-	65.8±9.8**,***
Phagocytic number, induced by pyrogenal	10.0±0.3	6.6±1.2*	-	9.8±0.7***
Phagocytic index, induced by ronkoleukine, %	44.5±4.7	92.6±3.3*	-	99.3±0.4**
Phagocytic number, induced by ronkoleukine	10.0±0.3	14.0±1.9*	-	16.3±1.07**
HCT idiopathic, %	24.5±13.0	10.1±4.12	-	20.0±2.7
HCT stimulated by pyrogenal, %	44.1±5.7	12.4±4.9*	-	22.3±6.2**
HCT stimulated by ronkoleukine, %	44.1±5.7	35.5±7.25	-	52.0±4.6
** The disparity is accurate at P≤0.05 between the control and until the therapy. ** The disparity is accurate at P≤0.05 between the control and after the therapy. ** The disparity is accurate at P≤0.05 between until and after the therapy*				

Table 6. Dynamic of complaints and of clinical signs in the Group II B(M ± m)

Analysed indices	Until the treatment	On the 7th day	On the 14th day
Complaints			
Discharge	12(48.0±6.5%)	7(28.0±5.1%)*	2(8.0±2.8%)**
Redness	19(76.0±7.8%)	8(32.0±5.4%)*	-**
Vulvovaginal pruritus	12(48.0±6.5%)	4(16.0±3.9%)*	2(8.0±2.8%)**
Heat	11(44.0±6.3%)	-*	-**
Vulvovaginal pain	8(32.0±5.4%)	-*	-**
Painful urination	3(12.0±3.4%)	-*	-**
Examination			
Hyperaemia of the labia majoria	14(56.0±6.9%)	4(16.0±3.9%)*	1(4.0±2.0%)**
Hyperaemia of the vestibule of vagina	22(88.0±8.3%)	9(36.0±5.7%)*	-**
Hyperaemia of the urethral meatus	9(36.0±5.7%)	-*	-**
Oedemata of the labia majoria	8(32.0±5.4%)	-*	-**
Oedemata of the vestibular mucous membrane	17(68.0±7.5%)	2(8.0±2.8%)*	-**
Oedemata of the urethral meatus	6(24.0±4.7%)	-*	-**
Hyperaemia of the perineum	11(44.0±6.3%)	2(8.0±2.8%)*	2(8.0±2.8%)**
Pathologic discharge from the genital tracts			
The value of discharge	-	18(72.0±7.8%)*	9(36.0±5.7%)**
Low	14(56.0±6.9%)	7(28.0±5.1%)*	16(64.0±7.3%)**
Moderate	11(44,0±6,3%)	-*	-**
Plethorical			
Colour of discharge	-	6(24.0±4.7%)*	14(56.0±6.9%)
Off-white	8(32.0±5.4%)	11(44.0±6.3%)	11(44.0±6.3%)**
Off-white–xanthic	7(28.0±5.1%)	8(32.0±5.4%)	-**
Pyromucous (xanthic)	10(40.0±6.0%)	-*	-**

** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day*
*** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day*

Table 7. Dynamic of the lymphocytes subpopulations rates in the setting of the treatment, the Group II B (M ± m)

Indices	Group I A	Group II B		
		Until the treatment		Until the treatment
CD3+ (%)	64,5±0,3	59,32±1,9*	61,3±1,6**	64,6±1,4 #
CD4+ (%)	53,9±1,0	49,4±5,1	49,6±1,6	49,5±2,0
CD8+ (%)	23,2±0,7	20,8±2,7	21,4±1,2	21,6±1,1
CD20+ (%)	15,3±0,7	12,4±0,6*	13,2±1,1	13,6±0,86
IL-6 (pg/ml)	22,6±1,8	20,75±1,54	24,17±0,62	20,64±1,54
Interferon (pg/ml)	11,02±0,74	10,44±0,57	11,50±0,28	12,01±0,23
TNF (pg/ml)	4,48±0,6	4,88±0,29	5,07±0,22	4,64±0,20

** The disparity is accurate at P≤0.05 between the examination and until treatment; ** The disparity is accurate at P≤0.05 between the examination and on the 7th day; # The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day*

Table 8. Dynamic of complaints and of clinical signs in the Group II C (M ± m)

Analysed indices	Until the treatment	On the 7th day	On the 14th day
Complaints			
Discharge	7(41,2±3,9)	7(41,1±6,1)	-**
Redness	11(64,7±4,8)	3(17,6±2,6)*	-**
Vulvovaginal pruritus	12(70,5±5,0)	4(23,5±3,0)*	1 (5,9±1,5)**
Heat	11(64,7±4,8)	-*	-**
Vulvovaginal pain	10(58,8±4,6)	-*	-**
Painful urination	2(11,8±2,2)	-*	-**
Examination			
Hyperaemia of the labia majoria	9(52,9±4,4)	-*	-**
Hyperaemia of the vestibule of vagina	17(100±5,8)	2(11,7±3,3)*	-**

Analysed indices	Until the treatment	On the 7th day	On the 14th day
Hyperaemia of the urethral meatus	7(41,2±3,9)	-*	-**
Oedemata of the labia majoria	5(29,4±3,4)	-*	-**
Oedemata of the vestibular mucous membrane	13(76,5±5,2)	-*	-**
Oedemata of the urethral meatus	5(29,4±3,4)	-*	-**
Hyperaemia of the perineum	5(29,4±3,4)	2(11,8±2,2)*	-**
Pathologic discharge from the genital tracts			
The value of discharge	-	9(52,9±4,4)*	7(41,2±3,9)**
Low	11(64,7±4,8)	8(47,1±4,2)*	10(58,8±4,6)
Moderate	6(35,3±3,7)	-*	-**
Plethorical			
Colour of discharge	-	7(41,2±3,9)*	15(88,2±5,5)**
Off-white	-	7(41,2±3,9)*	2(11,2±2,2)**
Off-white–xanthic	10(58,8±4,6)	3(17,6±2,6)*	-**
Pyromucous (xanthic)	7(41,2±3,9)	-*	-**

** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day*
*** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day*

Table 9. Dynamic of the immunological indices, group II C(M ± m)

Indices	Group I	Group II C		
		Until the treatment	On the 7th day	On the 14th day
CD3+(%)	64,5±0,29	63,19±0,80	62.48±0,94	66,25±0,71
CD4+(%)	53,95±0,9	47.13±2,43	50.25±1,25	46,18±1,06
CD8+(%)	23,2±0,7	19.94±1.00	22.78±0,84	24,83±1,45
CD20+(%)	15,3±0,7	11,52±0,55	16.61±1,04	16.06±0,79
IL-6 (pg/ml)	22,6±1,8	19,77±1,46	26,25±0,65	24,22±0,49
Interferon (pg/ml)	11,02±0,74	10.35±0,45	12.02±0,33	12.68±0,71
TNF(pg/ml)	4,48±0,57	4.32±0,30	5.55±0,13	3.95±0,15

Table 10. Dynamic of complaints and of clinical signs in the Group III (M ± m)

Analysed indices	Until the treatment	On the 7th day	On the 14th day
Complaints			
Discharge	19(45.2±6.1%)	14(33.3±5.4%)	2(4.8±2.2%)**
Redness	30(71.4±7.1%)	11(26.2±4.8%)*	-**
Vulvovaginal pruritus	24(57.1±6.6%)	8(19.0±4.2%)*	3(7.1±2.6%)**
Heat	22(52.4±6.4%)	-*	-**
Vulvovaginal pain	18(42.9±5.9%)	-*	-**
Painful urination	5 (11.9±3.4%)	-*	-**
Examination			
Hyperaemia of the labia majoria	23(54.8±6.5%)	4(9.5±3.0%)*	1(2.4±1.5%)**
Hyperaemia of the vestibule of vagina	39(92.9±7.5%)	11(26.2±4.8%)*	-**
Hyperaemia of the urethral meatus	16(38.1±5.7%)	-*	-**
Oedemata of the labia majoria	13(31.0±5.3%)	-*	-**
Oedemata of the vestibular mucous membrane	30(71.4±7.1%)	2(4.8±2.2%)*	-**
Oedemata of the urethral meatus	11(26.2±4.8%)	-*	-**
Hyperaemia of the perineum	16(38.1±5.7%)	4 (49.5±3.0%)*	2 (4.8±2.2%)**
Pathologic discharge from the genital tracts			
The value of discharge	-	27 (64.3±6.9%)*	16(38.1±5.7%)**
Low	25(59.5±6.7%)	15((35.7±5.5%)*	26(61.9±6.8%)
Moderate	17(40.5±5.8%)	-	-**
Plethorical			
Colour of discharge	-	13(31.0±5.2%)*	29(69.0±7.0%)**
Off-white	8 (19.0±4.2%)	18 (42.9±5.9%)*	13(31.0±5.2%)**
Off-white–xanthic	17(40.5±5.8%)	11 (26.2±4.8%)	-
Pyromucous (xanthic)	17(40.5±5.8%)	-*	-**

** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day; ** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day*

Table 11. Dynamic of the immunological indices, Group III (M ± m)

Indices	Group I	Group III		
		Until treatment	On the 7th day	On the 14th day
CD3+(%)	64,5±0,29	69,2±6,4	62,5±1,6	66,8±1,1
CD4+(%)	53,95±0,9	53,0±8,1	50,6±1,6	49,1±2,7
CD8+(%)	23,2±0,7	22,0±5,3	22,3±1,1	24,04±1,2
CD20+(%)	15,3±0,7	14,9±4,1	14,6±1,9	15,3±0,8
IL-6 (pg/ml)	22,6±1,8	36,5±26,7	25,3±1,1	22,5±1,9
Interferon (pg/ml)	11,02±0,74	17,32±12,2	11,8±0,4	12,3±0,5
TNF (pg/ml)	4,48±0,57	4,14±1,7	5,3±0,28	4,3±0,4

Thus, the major clinical criteria of inflammatory process remitting were the quantity reduction of the pathologic discharge from the reproductive tract, appearance of light whitish discharge in moderate and scarce quantities, disappearance of vulvovaginal pain, burning sensation in the genitals, decurrence of dysuric syndrome, the blushed mucous membranes of the vagina with an absence of any pathological changes.

3. CONCLUSION

Thus, in the subacute the appointment of twice alternate days subcutaneous injection of Roncoleukin leads to the normalization of main value of lymphocyte subpopulation.

In the acute Ronkoleukin has no influence on the normal values of the immune status, i.e. it has an effective immune protection depending on the initial status.

Prior to treatment with cytokin composition the mean value did not differ significantly from the control group value in the sub-acute.

The high percentage of IFN-γ value before treatment was observed at 15% of the patients, TNF and IL-6 (at 20%, after treatment for 7th day it was reported an increased production of TNF- at 25% of patients, and on the 14th day the increased production of cytokines were not observed. These changes may indicate a decrement of inflammation, which was preceded by the activation of cytokine.

In the acute period prior to treatment in the cytokine composition the value did not differ significantly from the average of the control group.

Interest occurrence of increased production value IFN-γ was observed at 23.8% of patients, TNF - at 21.4% of patients, IL-6 - at 14.3% of patients, which was significantly more frequently than in the control group.

On the 7th day there was evaluated an increased production of IL-6 at 14.2% of the patients, TNF at 78.6%, and on the 14th day the increasing of IL-6 was only 4.8%. In this case the introduction of roncoleukin has also helped to reduce the inflammatory response.

Changes of phagocytic activity of the local secretion were observed in the sub-acute.

After the local application of ronkoleukin through 7 days reaction of the local secret sharply increased performance spontaneous and induced by pirogenal of phagocytic index, phagocytic number, NBTR-test.

Thus, locally administering of preparation greatly increases the efficiency of patients' treatment with a positive effect on the clinical course of the disease, providing immunomodulatory effects.

All patients with the sub-acute and acute vulvovaginitis should be made an assessment, in addition to clinical examination, for the state of the immune and cytokine status, as well as phagocytic system of vaginal contents in order to address the issue of immune system correction.

Immune system correction by ronkoleukin locally can be recommended to patients with sub-acute disease.

Application of ronkoleukin can reduce treatment costs and shorten the time of treatment.

These phenomena were leveled out independently at the 2nd day and were not accompanied by abnormality of general well-being.

In view of the high tolerability and rare complications, the local application can be performed on an outpatient basis.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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