

Original Research Article**Clinical and immunological evaluation of application of Ronkoleukin
in nonspecific vulvovaginitis at adolescent girls****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.

Our research has shown that the inclusion of Ronkoleukin in the complex therapy has clinically significant effects: rapid relief of symptoms of local inflammation, accelerated elimination of the causative pathogen and significantly shortening periods of treatment.

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 the research

Prospective study

Methodology

Within 2006-2010 years due to the adolescent therapist's advice and periodic screening the examination of request adolescent girls with a variety of complaints of genitals was carried out on the basis of the children's and adolescent consulting room in Municipal Polyclinic № 11 and "The City Center of Human Reproduction" in Almaty.

Microbiological and immunological studies were performed on the bases of Almaty: "The Scientific Center for Obstetrics, Gynecology and Perinatology", "The Scientific Center of Pediatrics and Pediatric Surgery" of the Ministry of Health of the Republic of Kazakhstan, "The City Center of Human Reproduction", Municipal Polyclinic № 11, "The City Skin and Venereal Diseases Dispensary of Almaty".

1. Analysis of the complaints of the anamnesis and clinical symptoms.

2. Microbiological analysis includes the bacterioscopic and bacteriological examination of the discharge from the urethra, vagina and vestibule of vagina.

3. For exclusion specific pathogens of vulvovaginitis there is held an enzyme-linked

36 immunoelectrodiffusion essay for sexually transmitted infections.

37 4.Immunological research

38 The quantitative study of the main lymphocyte populations and subpopulations of
39 lymphocytes was performed with the application of laser flow cytometry with monoclonal
40 antibodies “Status”-manufactured. Analysis of the samples was carried out on a flow cytometer
41 FacsCalibur (Becton Dickenson) in the program Cell Quest.

42 The next panel of antibodies was used in the research:

- 43 • CD45/CD14 (to identify the lymphocyte population and discharge of lymphocytes in low-
44 angle light scattering and SSC);
- 45 • IgG1/IgG2 (to control non-specific binding of lymphocytes with anti-human antibodies and
46 lymphocytic discharge with negative fluorescence);
- 47 • CD3/CD19 (for determination of containing of the thymus-processed lymphocytes and
48 bursal lymphocyte respectively);
- 49 • CD4/CD8 (to determine the number of t-helper cells and cytotoxic t-lymphocytes);
- 50 • CD3/HLA-DR (to determine the number of thymus-processed lymphocytes);
- 51 • CD3/CD16+56+ (for the determination of natural killers).

52 5.Cytokine study.

53 To determine IL-6 was used test system for enzyme-linked immunoassay, manufactured by
54 LLP "Cytokine" (St. Petersburg/Russia).

55 To define IFN- γ , TNF- α were used the proper test systems for enzyme immunoassay of IFN-
56 γ – EIA-BEST and TNF- α - EIA-BEST, manufactured CJSC "Vector-Best" (Koltsovo, Novosibirsk
57 region of Russian Federation).

58 6. Evaluation of phagocytic activity of neutrophils of vaginal secretions.

59 7. Transabdominal ultrasound was conducted by "ALOKA SSD-500".

60 For accomplishing the above objective there were selected 124 menstruant adolescent girls
61 from 11 to 18 years, not sexually active.

62 I group-20 healthy patients (control)

63 II group -62 patients with per-acute nonspecific vulvovaginitis

64 III group - 42 patients with sub-acute nonspecific vulvovaginitis

65 The selection criteria in the II and III groups were the lack of systemic and local antimicrobial
66 therapy during one month before this examination, clinical and microbiological confirmation of the
67 diagnosis, excluded sexually transmitted infections, vaginal foreign body, complaints, consent of
68 the mothers of patients for research and treatment.

69 In cooperation with the scientific consultant and immunologist, M.D. A. Kurmanova there was
70 developed a dosage schedule of Roncoleukin in the complex therapy, based on the results of the
71 analyses of the immune and cytokine status and phagocytic system of the vagina.

72 II group 62 patients with peracute nonspecific vulvovaginitis

73 IIA– 20 patients with subacute nonspecific vulvovaginitis

74 (Standard therapy + Roncoleukin 250 thousand U. 1 ml of vaginal irrigation once).

75 IIB– 25 adolescent girls with subacute

76 (Standard therapy + Roncoleukin 250 thousand U. 1 ml subcutaneously twice alternate days).

77 IIC – 17 adolescent girls with sub-acute (standard therapy).

78 III group - 42 patients with acute nonspecific vulvovaginitis

79 (standard therapy + Roncoleukin 250 thousand U. 1 ml subcutaneously twice alternate days).

80 Standard therapy of 7-10 days included (1;2):

81 - compliance with hygiene rules, restricted diet of digestible carbohydrates and irritating
82 substances, and increase of consumption of fresh fruits and vegetables;

83 - the local treatment (washing of vulva, vaginal disinfection processing, application of drugs
84 with anesthetic, astringent desensitizing and/or other effects on the external genitals);-
85 antimicrobials (in compliance with receptiveness of microbial population);

86 - antimycotics (for prevention of dysbacteriosis);

87 - decongestant (for the main indication);

88 - vitamin product;

89 - biogenic stimulant;

90 - eubiotics.

91 The immunomodulatory drug Roncoleukin was included into the complex therapy.

92 The drug Roncoleukin (interleukin-2 human recombinant) has been considered and
93 recommended to the registration by the Pharmacopoeia State Committee of the Republic of
94 Kazakhstan (Protocol No. 92 dated 28/11/2001).

95 The drug is produced by LLC "BIOTECH Ltd.", St Petersburg (3) Ingredients: 1 ampoule (1 ml)
96 contains interleukin-2 human recombinant -0.25 mg (250000 IU) and excipients: sodium lauryl
97 sulfate, mannitol, dithiothreitol, ammonium hydrogen carbonate, water for injection.

98 Patients' mothers were informed and obtained their consent to the treatment according to the Law of
99 the Republic of Kazakhstan dated 07.07.2006 №170-3 «National Health Protection Act» Article 31.

100 Efficacy of the drug was assessed by patient complaints, inspection, data of microbiological
101 studies of vaginal discharge, immune state, cytokine status, and local phagocytic system on the
102 7th and 14th days of treatment.

103 We did not observe any serious side effects, common reactions to the drug administration in

104 the form of anaphylaxis, urticaria; in 2 cases the patients had low-grade pyrexia, which took place
105 independently, and in 3 cases the local reaction to the drug administration was expressed by
106 moderate abnormality that was stopped by applying hot compress.

107 The statistical processing of the material collected was held by using the licensed software
108 packages StatSoft Statistica 6.0 and Microsoft Excel XP, generally accepted methods of the variation
109 statistics with the calculation of the arithmetic mean (M), the moderate mean (m); the confidence level
110 results were observed within 95% ($p < 0.05$) on the criteria of Student (t) with respect to the medical,
111 biological and mathematical studies.

112 To examine the relationship between values the correlation coefficient r was used. The weak link
113 was for $r \leq 0.3$, moderate 0.31-0.5, a-0.7, strong-0.51 > 0.7 .

114 **Keywords:**

115 Nonspecific vulvovaginitis, girl, adolescent, immunity, cytokines, phagocytosis, recombinant
116 interleukin -2 persons (Roncoleukin).

117 **Introduction**

118 In the structure of gynecological disorders at girls and adolescents vulvovaginitis occupies a
119 leading place (4).

120 According to scientific studies, the frequency of vulvovaginitis up to 93 per cent for girls,
121 for adolescents it goes up to 53%, while 60% has a recurrent character (5;6;7;8;9;10;11;12).

122 At the present stage there is no doubt that the microbial factor prevails in the pathogenesis of
123 nonspecific vulvovaginitis of the adolescent girls (13;14;15;16;17).

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

126 Common infectious disease accompanied by decrease in immunity, as well as the hormonal
127 disease break the qualitative and quantitative composition of the vaginal microflora that facilitates
128 the invasion of pathogenic microorganisms and can lead to the development of inflammatory
129 processes caused by opportunistic pathogenic bacterium (18).

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

134 The practical significance of vulvovaginitis defined by the fact that they lead to the
135 formation of synechia of the labia, genital infection, disruption of menstrual function, which can
136 cause serious disorders of reproductive function in the future (19;20).

137 The necessity of the clinical relevance of the study of the immunological aspects
138 at vulvovaginitis is that each immune imbalance increases the probability of the progression of the

139 disease, favours the development of complications, the allergization of the organism and the
140 chronization of the process.

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

144 Opportunistic microorganisms which take part in an inflammatory process do not contain any
145 high-toxic poisons, but they are dangerous due to their excessive activation of the inflammatory
146 mediators of the microorganism.

147 At present time the diagnostic significance of the evaluation of the cytokines concentration
148 level consists in the statement of the very fact of its increasing or reducing during a concrete disease,
149 at that it is appropriate to detect the concentration of pro-inflammatory as well as of anti-
150 inflammatory cytokines in the dynamic of the disease development to be able to evaluate the
151 severity and to make prognosis of the course of disease (21;22;23).

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

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

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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)
Painfulurination	-	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)
Hyperaemiaofthe urethral meatus	-	13(65,0±7,5)	9(36,0±5,7)	7(41,2±3,9)	16 (38,1±5,7)
Oedemataofthelabia majoria	-	4(20,0±4,4)	4(16,0±3,9)	4(23,5±3,0)	13(30,9±5,2)
Oedemataof the vestibular mucous membrane	-	8(40,0±6,1)	11(44,0±6,3)	11(64,7±4,8)	30 (71,4±7,1)
Oedemataoftheurethral meatus	-	4(20,0±4,4)	6(24,0±4,7)	5(29,4±3,4)	11 (26,2±4,8)
Hyperaemiaofthe perineum	-	6(30,0±5,3)	8(32,0±5,4)	6(35,3±3,7)	16 (38,1±5,7)
Pathologicdischargefrom 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)

162 The clinical picture of sub-acute and acute nonspecific vulvovaginitis was presented by the
163 symptoms of vulvitis and urethritis.

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

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

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

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

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

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

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

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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.			

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

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

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

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

200 Therefore, the further analysis of cytokine output was conducted by the percentage of
 201 occurrence of elevated and high value.

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Table 3

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

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

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

208 In the second group, there was an increase in output of IFN-γ at 15% of the patients, with
 209 adequately high content (in 4 times), recorded only at one patient (5%). The elevated level of
 210 TNF-α output was registered at 20% of the patients; herewith there was no any highest production
 211 (in 4 or more times). At the same time there was the increased output of IL-6 at 20%. It should
 212 be noted that high (in 5-13 times) output of IL-6 was at 15% (3/20). When comparing the rates of
 213 occurrence of high content with I group, the increased output of IFN-γ and IL-6 was more often

214 registered in the 2nd group. However, adequate cytokine explosion with the activation of
215 decreasing immunity was observed only in 1/6 cases.

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

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

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

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

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

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

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

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

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Table 4

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

Analysed indices	until the treatment	on the 7 th day	on the 14 th 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%)	-
Painfulurination	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%)
Hyperaemiaofthe urethral meatus	13(65.0±7.5%)	-*	-
Oedemataofthelabia majoria	4(20.0,0±4.4%)	-*	-
Oedemataof the vestibular mucous membrane	8(40.0±6.1%)	2(10±3.1%)*	-
Oedemataoftheurethral meatus	4(20.0±4.4%)	-*	-
Hyperaemiaofthe perineum	6(30.0±5.3%)	3(15±3.8%)*	2(10±3.1%)
Pathologicdischargefrom 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 7 th day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 th day			

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

287 Before the treatment in cytokine composition the averages did not differ significantly from
288 the control group average.

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

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

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

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

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

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Table 6

Dynamic of complaints and of clinical signs in the Group IIB (M ± m)

Analysed indices	until the treatment	on the 7 th day	on the 14 th 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%)	_*	_***
Painfulurination	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 7 th day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 th day			

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

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316 Before treatment in the group II B there was recorded a decrease of CD3 + and CD20 +
317 lymphocytes in the value of lymphocyte subpopulation composition. On the 7th day rate of CD3 +
318 increased slightly, but continued to be significantly reduced, and the content of CD20 + turned up to
319 stated value.

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

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

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Table 8

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

Analysed indices	until the treatment	on the 7 th day	on the 14 th 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)	_*	-**
Painfulurination	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)*	-**
Hyperaemiaofthe urethral meatus	7(41,2±3,9)	_*	-**
Oedemataofthelabia majoria	5(29,4±3,4)	_*	-**
Oedemataof the vestibular mucous membrane	13(76,5±5,2)	_*	-**
Oedemataoftheurethral meatus	5(29,4±3,4)	_*	-**
Hyperaemiaofthe perineum	5(29,4±3,4)	2(11,8±2,2)*	-**
Pathologicdischargefrom 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 7 th day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 th day			

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Table 9

Dynamic of the immunological indices, Group II C(M ± m)

Indices	Group I	Group II C		
		until the treatment	on the 7 th day	on the 14 th 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

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Table 10

Dynamic of complaints and of clinical signs in theGroupIII(M ± m)

Analysed indices	until the treatment	on the 7 th day	on the 14 th 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%)	-*	-**
Painfulurination	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%)*	-**
Hyperaemiaofthe urethral meatus	16(38.1±5.7%)	-*	-**

Oedemataofthelabia majoria	13(31.0±5.3%)	-*	-**
Oedemataof the vestibular mucous membrane	30(71.4±7.1%)	2(4.8±2.2%)*	-**
Oedemataoftheurethral meatus	11(26.2±4.8%)	-*	-**
Hyperaemiaofthe perineum	16(38.1±5.7%)	4 (49.5±3.0%)*	2 (4.8±2.2%)**
Pathologicdischargefrom 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 7 th day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 th day			

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Table 11

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

Indices	Group I	Group III		
		until treatment	on the 7 th day	on the 14 th 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

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

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

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

354 The profuse discharge were completely absent on the 7th treatment day, corresponding to the
355 data of objective examination.

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

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

364 **Conclusion**

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

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

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

371 The high percentage of IFN- γ value before treatment was observed at 15% of the patients,
372 TNF and IL-6 (at 20%, after treatment for 7th day it was reported an increased production of TNF-at
373 25% of patients, and on the 14th day the increased production of cytokines were not observed. These
374 changes may indicate a decrement of inflammation, which was preceded by the activation of
375 cytokine.

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

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

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

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

385 After the local application of Ronkoleukin through 7 days reaction of the local secret sharply
386 increased performance spontaneous and induced by pyrogenal of phagocytic index, phagocytic
387 number, NBTR-test.

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

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

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

396 Application of Ronkoleukin can reduce treatment costs and shorten the time of treatment.

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

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

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