

# **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 there 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 Reproduction» in Almaty.

Microbiological and immunological studies were performed in the hospitals 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 anamnestic data of complaints and clinical symptoms

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

3. Enzyme immunoassay for sexually transmitted diseases to exclude specific pathogens of

## vulvovaginitis

### 4. Immunoassay

The quantitative study of the main lymphocyte populations and subpopulations was performed with the help of double label with monoclonal antibodies on the laser flow cytometry manufactured by «Status» (LLC «Sorbent»/ Podolsk, Russia). Analysis of the samples was carried out on a flow cytometer FacsCalibur (Becton Dickenson) in the Cell Quest program

The next panel was used in the study of antibodies:

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

### 5. Cytokine study

The relevant enzyme immunoassay test system, manufactured by LLP "Cytokine" (St. Petersburg/Russia) we used to determine IL-6.

To define IFN- $\gamma$  and TNF- $\alpha$  the relevant test-systems for enzyme immunoassay of IFN- $\gamma$  – EIA-BEST and TNF- $\alpha$  - EIA-BEST were used, manufactured by CJSC "Vector-Best" (Koltsovo, Novosibirsk region of Russian Federation).

### 6. Evaluation of neutrophil phagocytic rate of vaginal discharge

### 7. Trans-abdominal ultrasound was held by using «ALOKA SSD-500»

124 menstruate adolescent girls from 11 to 18 years, not sexually active, were selected to accomplish the target objective.

I group -20 healthy patients (control)

II group - 62 patients with per-acute nonspecific vulvovaginitis

III group - 42 patients with sub-acute nonspecific vulvovaginitis

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

In cooperation with the scientific consultant and immunologist, M.D.Professor A. Kurmanova there was developed a dosage schedule of roncoleukine in the complex therapy,

71 based on the results of the analyses of the immune and cytokine status and phagocytic  
72 vaginal.system.

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

74 II A– 20 patients with sub-acute nonspecific vulvovaginitis

75 (Standard therapy + roncoleukine 250 000 U/ml vaginal irrigation once a day).

76 II B– 25 adolescent girls with sub-acute

77 (Standard therapy + roncoleukine 250 000 U/ml twice subcutaneously, every other day).

78 II C – 17 adolescent girls with sub-acute (standard therapy)

79 III group - 42 patients with acute nonspecific vulvovaginitis

80 (standard therapy + roncoleukine 250 000 U/ml twice subcutaneously, every other day).

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

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

84 - the local treatment (washing of the vulva, vaginal disinfection processing, application of  
85 drugs with anesthetic, astringent desensitizing and/or other effects on the vulva);

86 - antimicrobials (in compliance with receptiveness of microbial population);

87 - antimycotics (for prevention of dysbacteriosis);

88 - decongestant (for the main indication);

89 - vitamin product;

90 - biogenic stimulant;

91 - eubiotics.

92 The immunomodulatory drug roncoleukine was included into the complex therapy.

93 The drug Roncoleukin (interleukin-2 human recombinant) has been considered and  
94 recommended to the registration by the Pharmacopoeia State Committee of the Republic of  
95 Kazakhstan (Protocol No. 92 dated 28.11.2001).

96 The drug is produced by LLC “BIOTECH Ltd.”, St Petersburg (3).

97 Ingredients: 1 ampoule (1 ml) contains recombinant human interleukin - 0.25 mg (250 000  
98 IU) and excipients: sodium lauryl sulfate, mannitol, dithiothreitol, ammonium hydrogen carbonate,  
99 water for injection.

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

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

106 We did not observe any serious side effects, common reactions to the drug administration in  
107 the form of anaphylaxis, urticaria; in 2 cases the patients had low-grade pyrexia, which took place  
108 independently, and in 3 cases the local reaction to the drug administration was expressed by  
109 moderate abnormality that was stopped by applying hot compress.

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

116 The weak link was observed when  $r \leq 0,3$ , moderate - 0,31-0,5, noticeable - 0,51-0,7, strong -  
117  $> 0.7$ .

#### 118 **Keywords:**

119 Nonspecific vulvovaginitis, girl, adolescent, immunity, cytokines, phagocytosis, recombinant  
120 human interleukin (roncoleukine).

#### 121 **Introduction**

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

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

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

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

130 Common infectious disease accompanied by decrease in immunity, as well as the hormonal  
131 diseasebreak the qualitative and quantitative composition of the vaginalmicroflora that facilitates  
132 the invasion of pathogenic microorganisms and can lead to the development of inflammatory  
133 processes caused by opportunistic pathogenic bacterium (18).

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

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

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

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

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

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

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

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

162 **Results and Discussion**

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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
<b>Complaints</b>					
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)
<b>Examination</b>					
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)
<b>Pathologicdischargefrom the genital tracts</b>					
<b>The value of discharge</b>					
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)
<b>Colour of discharge</b>					
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)

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

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

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

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

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

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

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

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

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Table 2

Immune status and phagocytic function of vaginal discharge in the examined before treatment ( $M \pm m$ )

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
НСТ idiopathic, %	24,5±13,0	10,1±4,12	14,2±6,2
НСТ stimulated by pyrogenal, %	44,1±5,7	12,4±4,9*	32,3±2,3***
* The disparity is accurate at $P \leq 0.05$ between the Groups I and II .			
** The disparity is accurate at $P \leq 0.05$ between the Groups I and III.			
*** The disparity is accurate at $P \leq 0.05$ between the Groups II and III.			



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

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

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

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

204 Therefore, the further analysis of cytokine output was conducted by the percentage of  
 205 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 <sup>st</sup> and the 2 <sup>nd</sup> groups. ** The disparity is accurate at P≤0.05 between the 1 <sup>st</sup> and the 3 <sup>rd</sup> groups.			

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

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

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

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

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

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

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

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

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

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

254 Thus, in the acute with nonspecific vulvovaginitis at young girls and adolescents  
255 thephagocytic function of the local dischargeis satisfactory; and during the sub-acute against  
256 satisfactory absorption function thereisobservedthe decrease of bactericidal activity of the  
257 vaginal discharge, which demonstrates the need for the phagocyte correction of these patients.

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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 <sup>th</sup> day	on the 14 <sup>th</sup> day
<b>Complaints</b>			
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%)	-*	-
<b>Examination</b>			
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%)
<b>Pathologicdischargefrom the genital tracts</b>			
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	-	-	-
<b>Plethorical</b>			
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 <sup>th</sup> day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 <sup>th</sup> day			

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

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

293 The same pattern for mean values was observed on the 7<sup>th</sup> and 14<sup>th</sup> days after treatment. But  
294 when comparing the percentage of occurrence of high levels before treatment there was observed  
295 the high output of IFN- $\gamma$  at 15% of the patients, output of TNF- $\alpha$  and IL-6 - at 20%, of the patients,  
296 output of TNF- $\alpha$  at 25% of the patients increased on the 7<sup>th</sup> day after treatment, and increasing output  
297 of cytokines was observed on the 14<sup>th</sup> day. These changes may indicate an inflammatory process  
298 remitting, preceded by activation of exogenous cytokine.

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

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

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

311 Thus, in the sub-acute the local application of Roncoleukin leads to the increased absorption  
312 and oxygen-dependent bactericidal ability of vaginal secretion, which contributes to the rapid  
313 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 <sup>th</sup> day	on the 14 <sup>th</sup> day
<b>Complaints</b>			
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%)	_*	_***
<b>Examination</b>			
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%)**
<b>Pathologic discharge from the genital tracts</b>			
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%)	_*	_***
<b>Plethorical</b>			
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 <sup>th</sup> day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 <sup>th</sup> 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 <sup>th</sup> day # The disparity is accurate at $P \leq 0.05$ between the beginning of the treatment and on the 14 <sup>th</sup> day				



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320 Before treatment in the group II B there was record of decrease of CD3 + and CD20 +  
321 lymphocytes in the value of lymphocyte subpopulation composition. On the 7<sup>th</sup> day rate of CD3 +  
322 increased slightly, but continued to be significantly reduced, and the content of CD20 + turned up to  
323 stated value.

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

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

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

Analysed indices	until the treatment	on the 7 <sup>th</sup> day	on the 14 <sup>th</sup> day
<b>Complaints</b>			
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)	_*	-**
<b>Examination</b>			
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)*	-**
<b>Pathologicdischargefrom the genital tracts</b>			
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)	_*	-**
<b>Plethorical</b>			
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 <sup>th</sup> day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 <sup>th</sup> 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 <sup>th</sup> day	on the 14 <sup>th</sup> 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 <sup>th</sup> day	on the 14 <sup>th</sup> day
<b>Complaints</b>			
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%)	-*	-**
<b>Examination</b>			
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%)**
<b>Pathologicdischargefrom the genital tracts</b>			
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%)	-	-**
<b>Plethorical</b>			
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 <sup>th</sup> day			
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14 <sup>th</sup> 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 <sup>th</sup> day	on the 14 <sup>th</sup> 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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350 On the 7<sup>th</sup> and 14<sup>th</sup> days the value of lymphocyte subpopulation composition did not undergo  
351 any significant changes, but the dynamics tended to decrease (normalization) of CD3+  
352 lymphocytes.

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

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

358 The profuse discharge were completely absent on the 7<sup>th</sup> treatment day, corresponding to the  
359 data of objective examination.

360 According to the patient's words there was an increased vaginal discharge, but the nature of  
361 this discharge has changed to ordinary fluor albus with a gradual transition to the normal vaginal  
362 discharge to the 10<sup>th</sup> day of the treatment.

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

### 368 **Conclusion**

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

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

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

375 The high percentage of IFN- $\gamma$  value before treatment was observed at 15% of the patients,  
376 TNF and IL-6 (at 20%, after treatment for 7<sup>th</sup> day it was reported an increased production of TNF-at  
377 25% of patients, and on the 14<sup>th</sup> day the increased production of cytokines were not observed. These  
378 changes may indicate a decrement of inflammation, which was preceded by the activation of  
379 cytokine.

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

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

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

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

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

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

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

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

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

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

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

## 405 **References**

406 [1]. Uvarova E.V., Tarusin D.A. Manual for inspection of the conditions of the reproductive  
407 system of children and adolescents Triada-X ISBN 5-8249-0145-7; 2009 232 p.

408 [2]. Standard principles of examination and treatment of children and adolescents with  
409 gynecological diseases and disorders of sexual development Edited by E.V. Uvarova. Triada-X.  
410 Series: Handbook of Child gynecologist. ISBN 5-8249-0112-0; 2008.180p.

411 [3]. Interleukin-2: Summary of clinical experience: a commemorative edition for the 20th  
412 anniversary. Ltd. "Biotech" / V.N. Yegorov, A.M. Popovich, I.V. Babachenko [et al.]. - St.  
413 Petersburg: Ultra Print, 2012. P.74-82. ISBN 978-5-905218-11-8

414 [4]. Olejek A1, Kellas-Slecicka S, Kozak-Darmas I, Bilska A, Zamłyński J, Horak S, Nowak  
415 L. Vulvovaginitis in young girls. Ginekol Pol. 2009 Dec;80(12):931-4.

416 [5]. Pediatric, Adolescent, & Young Adult Gynecology. Eds. A. Altchek, L. Deligdisch.  
417 Wiley-Blackwell, 2009.P.60-80. ISBN 978-1-4051-5347-8  
418 <http://onlinelibrary.wiley.com/doi/10.1576/toag.12.1.067a.27566/full>  
419 [6]. Gurkin Y.A. Experience phased treatment nonspecific vulvovaginitis in girls //  
420 Reproductive health of children and adolescents. 2009. 5.P.15-20.  
421 [7]. Gynecology. National leadership. Quick Edition / ed. G.M. Savelevoj, G.T. Sukhikh, I.B.  
422 Manukhina. Moscow. GEOTAR Media, 2013. 704 pp.  
423 [8].Zaharova I.N. Vulvovaginitis in childhood and adolescence (diagnosis, rational treatment,  
424 advice on hygiene) guide for physicians / I.N. Zakharova, N.A. Korovin, T.M. Tvorogova // Second  
425 Edition / Moscow, 2008. P.56.  
426 [9]. Kokolina V.F. The reproductive health of girls and adolescent girls in modern conditions  
427 / V.F. Kokolina, M.Y. Mitin // effective pharmacotherapy in obstetrics and gynecology. 2006. № 1.  
428 P. 6-11.  
429 [10]. Medical and social aspects of reproductive health of adolescent girls /N.A.Buralkina the  
430 Republic of Mordovia, Saransk // E.V.Uvarova, 2010.228pp.  
431 [11]. Kokolina V.F. Pediatric and Adolescent Gynecology: a guide for doctors. Moscow.  
432 Medical Information Agency, 2009. 640pp.  
433 [12] .Kulakov V.I. Guide gynecology children and adolescents / V.I. Kulakov, E.A.  
434 Bogdanova // Moscow. Triada-X, 2005, 336pp.  
435 [13]. Bumbulienė Ž, Venclavičiūtė K, Ramašauskaite D, Arlauskienė A, Bumbul E,  
436 Drašutienė G. Microbiological findings of vulvovaginitis in prepubertal girls. Postgrad Med J  
437 2014;90:8-12 doi:10.1136/postgradmedj-2013-131959)  
438 [14]. Randelović G, Mladenović V, Ristić L, et al. Microbiological aspects of vulvovaginitis  
439 in prepubertal girls. European Journal of Pediatrics.2012. V. 171.№ 8.P.1203-1208.  
440 [15].Sikanić-Dugić N, Pustisek N, Hirsl-Hećej V, Lukić-Grić A. Microbiological findings in  
441 prepubertal girls with vulvovaginitis. Acta Dermatovenerol Croat. 2009;17(4):267-272.  
442 [16].Ayse E. Yilmaz,Nurullah Celik , Gul Soylu, Ahsen Donmez, Cigdem Yuksel.  
443 Comparison of clinical and microbiological features of vulvovaginitis in prepubertal and pubertal  
444 girls. Journal of the Formosan Medical Association (2012) 111.P. 392-396  
445 [17].Heleen van Beekhuizen. Gynecology in Children and Adolescents.24. P. 296-298.  
446 [18].Matytsina L.A., Greydanus D.E., Gurkin Y.A. Vaginal microbiocoenosis and cytology of  
447 prepubertal and adolescent girls: their role in health and disease. World J Pediatr. 2010 Feb;6(1):32-  
448 7. doi: 10.1007/s12519-010-0003-8.  
449 [19].Rome ES. Vulvovaginitis and other common vulvar disorders in children. Endocr Dev.  
450 2012;22:72-83. doi: 10.1159/000326634. Epub 2012 Jul 25.

451 [20]Van Eyk N, Allen L, Giesbrecht E, Jamieson MA, Kives S, Morris M, Ornstein M,  
452 Fleming N. Pediatric vulvovaginal disorders: a diagnostic approach and review of the literature. J  
453 Obstet Gynaecol Can. 2009 Sep;31(9):850-62.

454 [21]. V.G .Galaktionov Immunology. Moscow. The Academy, 2009. 560pp.

455 [22]. Simbirtsev A.S. Cytokines - a new system of regulation of defense reactions //  
456 Cytokines and Inflammation. 2002. №1. P. 9-17.

457 [23]. Khaitov P.M. Modern understanding of the body's defense against infection //  
458 Immunology. 2000. №1. P. 61-64.