THE CONTENT OF SOLUBLE HLA CLASS I AND HLA-DR MOLECULES IN SERUM IN PATIENTS WITH UTERINE CERVIX AND BODY PATHOLOGY

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The aim of the investigation was to assess the correlation of the content of soluble HLA (sHLAI) class I and HLA-DR (sHLA-DR) molecules in blood serum of patients with uterine cervix and body pathologies, and their pathology type, tumor grade, the number and localization of myomatous nodes.

Materials and Methods. 142 women with uterine cervix and body pathology aged 31–79 years (median — 52 years) were under study. Serum level of sHLAI and sHLA-DR molecules was determined by enzyme immunoassay using monoclonal antibodies. Blood samples were drawn from cubital vein. All tests were performed before and after the treatment course. For enzyme immunoassay we used mouse monoclonal antibodies to reveal soluble differentiated molecules.

Results. Malignant pathologies of uterine cervix and body and myomas were found to be accompanied by an increased serum level of sHLAI и sHLA-DR molecules, its degree depending on the type of pathology diagnosed in patients with cervical cancer and hysterocarcinomas, and differentiation degree of adenocarcinoma in hysterocarcinoma patients, the number and localization of myomatous nodes. Initial concentration of soluble HLA class I and HLA-DR molecules in a preoperative period can serve as an additional diagnostic test in patients with uterine tumors for their further selection for surgery.

Key words: HLA class I and HLA-DR soluble molecules; sHLAI, sHLA-DR; cervical cancer; hysterocarcinoma; myomas; endometriosis.

The human leucocyte antigens system (HLA) provides immune response regulation and has such primary physiological functions as the cooperation of all immunocompetent cells, self-non-self recognition including modified cells, the start and realization of immune response. This system generally provides human survival under conditions of exogenous and endogenous aggression [1]. HLA I class and HLA-DR molecules can exist not only in a membrane form, but soluble as well (sHLAI and sHLA-DR) and be found in human blood serum. The elevated sHLAI level in blood serum was described in patients with acute myeloid leukemia [2], malignant lymphadenosis [3], HIV-infection [4], different liver disorders [5, 6], burn disease [7]; the decreased level in patients with brain neoplasms, stomach cancer, insulin dependent diabetes mellitus [8], in one of syphilis forms [9]. The increase of sHLA-DR serum level was found in patients with rheumatoid arthritis [10], acute lymphoblastic leukemia [11], HIV-infection [4]. However, there is no available data about the sHLAI and sHLA-DR molecules concentration in patients with uterine cervix and body pathologies. K.A. Korovushkina reported [12] that the sHLAI molecule level in patients with hysteromyomas and endometrial cancer remained the same.

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correlation of the content of soluble HLA (sHLAI) class I and HLA-DR (sHLA-DR) molecules in blood serum of patients with uterine cervix and body pathologies with their pathology type, tumor grade, the number and localization of myomatous nodes.

Materials and Methods. 142 women with uterine cervix and body pathology aged 31–79 years (median — 52 years) were under study. Among them there were 53 women (37%) with hysteromyomas, 18 (13%) with uterine cervix cancer (UCC), 65 (46%) with malignant uterine body tumor, 6 (4%) with endometriosis. In accordance with clinical, instrumental examination and the postoperative classification for UCC and uterine body tumor stage I was diagnosed in 61 patients out of 83 (73%), stage II in 17 (21%), stage III in 5 patients (6%). In every case the diagnosis was confirmed by histological pathological study of postoperative material. In every case of UCC the squamous cell carcinoma was found. The histological variant of adenocarcinoma prevailed in patients with uterine body tumor — 50 (77%). The high grade tumor was registered most often (32 patients, 49%) with no connection to stage and localization, the moderately differentiated tumor was found less frequently (19 patients, 29%) and the low grade tumors (14 patients, 22%). The performed treatment depended on tumor stage, histological tumor type and included radical hysterectomy (in most cases — 79, 56%) and supracervical hysterectomy (25 cases, 18%). Other surgeries were performed significantly less frequently: conservative myomectomy — 17 (12%), supracervical hysterectomy with bilateral oophorectomy — 15 (10%) and hysterectomy — 6 (4%). The control group consisted of 45 women with no gynecological pathology comparable in age with the tested group.

The study was performed in accordance with the Declaration of Helsinki (accepted in June of 1964 in Helsinki, Finland, revised in October of 2000 in Edinburgh, Scotland) and approved by the Ethics Committees of State Medical Academy of Nizhny Novgorod. In every case the informed consent was received.

Blood samples were drawn from cubital vein. For clotting reaction the blood samples were hold sequentially in a thermostat at +37°C for 30 minutes and in a refrigerator at +4°C. Then the clotted blood was centrifugated over 15 minutes at 200 g. The serum was gathered into dry clean plastic test tubes. Obtained serum was kept at a temperature of –40…–60°C in a refrigerator up to 6 months.

All tests were performed in dynamics: before and after the treatment course. For enzyme immunoassay we used mouse monoclonal antibodies (MMA) of IKO series (Research Institute, N.N. Blokhin Cancer Research Center, Moscow) produced by the gybrodomas given by N.N. Blokhin Cancer Research Center to reveal soluble differentiated molecules. Serum level of soluble differentiated molecules was determined by two-sited enzyme immunoassay using polyclonal antibodies as a packing and monoclonal horseradish-peroxidase-conjugated antibodies. The spectrophotometrical result count was assessed at a wave length of 492 nm using photometer Multiscan EX (LabSystems, Finland). The results were assessed in standard units of optical density (U/ml).

The software Statistica 6.0. was used for the study results processing.

Results and Discussion. The performed studies demonstrated some differences of the initial content of soluble HLA I class and HLA-DR molecules in blood serum in patients with uterine cervix and body pathology and in the group with no gynecological diseases (Table 1).

In patients with UCC the sHLAI molecules level was significantly higher than in the control group — by 2.3 times, sHLA-DR level — by 2.5 times; in patients with malignant uterine body tumors — by 2.1 and 1.8 times; with hysteromyomas — by 1.7 and 2.0 times relatively. In patients with endometriosis the sHLAI and sHLA-DR molecules concentration was similar to relevant values in the control group.

The high-differentiated uterine body tumors were associated with the statistically significant increase of sHLAI and sHLA-DR molecules content (by 2.3 times for each parameter) in comparison with the control group (Fig. 1). In patients with moderately differentiated tumors the sHLAI significant differences with the control group (by 1.8 time) were found but the sHLA-DR molecules level was close to normal and significantly lower (by 3.4 times) than in patients with high-grade tumor. The sHLAI

Table 1

Content of soluble HLA I class and HLA-DR molecules in blood serum in patients with uterine cervix and body pathology, U/ml

Tumor localization	Soluble HLA I class molecules	Soluble HLA-DR molecules
Uterine cervix cancer (n=18)	2357.1±260.4*	245.3±20.2*
Malignant uterine body tumor (n=65)	2147.88±204.89*	184.39±34.13*
Hysteromyoma (n=53)	1751.6±148.3*	196.0±14.4*
Endometriosis (n=6)	1277.6±210.5	123.7±21.0
Control group (n=45)	1012.0±214.0	99.5±18.4

* — the value differences are statistically significant in comparison with the control, $p<0.05$.

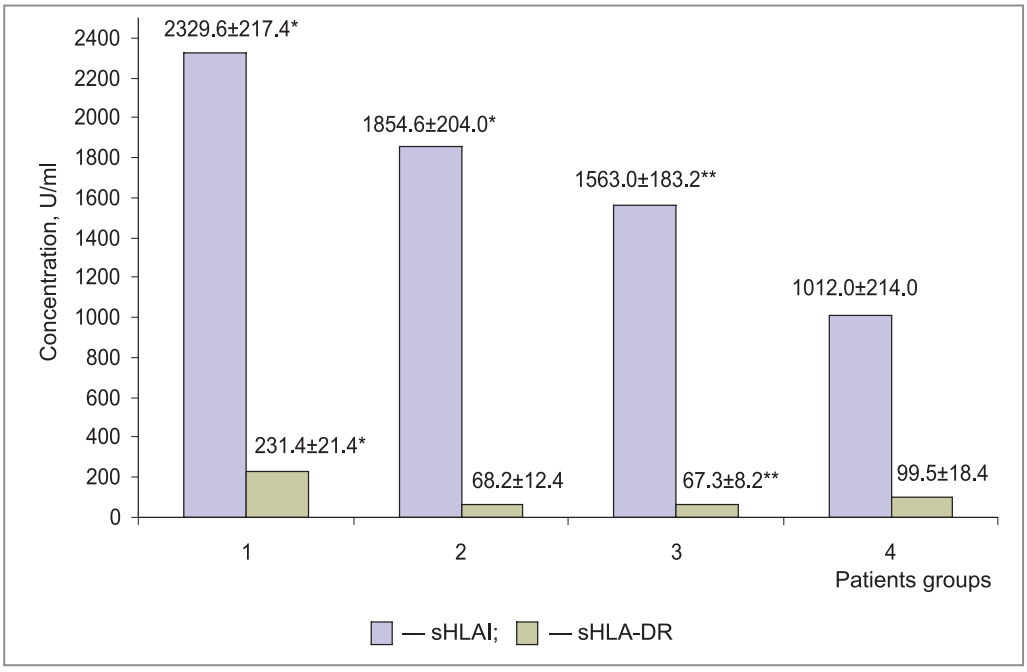

Fig. 1. The content of soluble HLA class I and HLA-DR molecules in blood serum in patients with malignant uterine body tumor depending on tumor grade: *1* — patients with high grade adenocarcinomas (n=32); *2* — patients with moderate grade adenocarcinomas (n=19); *3* — patients with low adenocarcinomas (n=14); *4* — control group (n=45); * — significant differences in comparison with the control ($p<0.05$); ** — in comparison with the group with high grade tumors ($p<0.05$)

and sHLA-DR molecule content of serum in patients with low grade tumor remained at a normal rate. However in regard of tumor growth the lack of significant increase of both molecule type levels indicated the functional exhaustion of immune system instead of well-being under condition of the most aggressive disease variant.

The product of the main human histocompatibility complex — a membrane HLA I class molecule — is known to participate in the presentation of peptide-fragmented cytotoxic T-lymphocyte antigens [13]. A.K. Golenkova et al. [14] reported that the sHLAI content can be considered as an integral criterion of tumor weight in patients with chronic lymphatic leukemia and multiple myeloma. All these data show the important role of this molecule in the oncogenesis. The functional role of HLA-DR membrane form consists in binding of foreign cell protein fragments entered a cell by endocytosis. This form takes part in the presentation of fragmented foreign antigen $CD4^+$ to lymphocytes and their activation. The sHLA-DR molecules are believed [1] to protect the organism against a foreign antigen as well and their connection induces a signal transduction. The lack of significant elevation of soluble HLA I class and HLA-DR molecules in response to the formation of a blastomatous bud can cause more aggressive neoplasm with poorer prognosis. By contrast the tumors with relatively better course are associated with significant elevation of HLA I class and HLA-DR membrane forms expression and correspondingly the higher levels of their soluble forms. In should be noticed that the sHLAI and sHLA-DR molecules concentration in patients with low grade tumors was statistically lower than in patients with high grade tumors (by 1.5 and 3.4 times). However the reverse correlation is possible — initially a more aggressive tumor has more disintegrating effect on the immune system that is manifested in particular in decreased concentration of soluble HLA I class and HLA-DR molecules in blood serum.

The study showed that the changes of sHLAI and sHLA-DR molecules levels were related to tumor localization in patients with hysteromyomas (Fig. 2).

In patients with subserous myomatous nodules the sHLAI molecules concentration was not significantly different from norm before treatment. In patients with interstitial-subserous nodules the sHLAI molecules level was by 1.6 times higher than in the control group ($p<0.05$). In patients with interstitial, interstitial-submucous and submucous myomatous nodules the significant elevation of the sHLAI molecules content (by 1.8; 2.2 and 2.4 times relatively) was observed compared with the control.

The comparison of the serum sHLAI molecules levels in patients with different localization of myomatous nodules showed that these values in most patients with interstitial-subserous and subserous nodules localizations were similar. However in patients with interstitial, interstitial-submucous or submucous localization of myomatous nodules the significant elevation of the sHLAI molecules content was observed in comparison with the group with mostly subserous nodules (by 1.8; 2.2 and 2.4

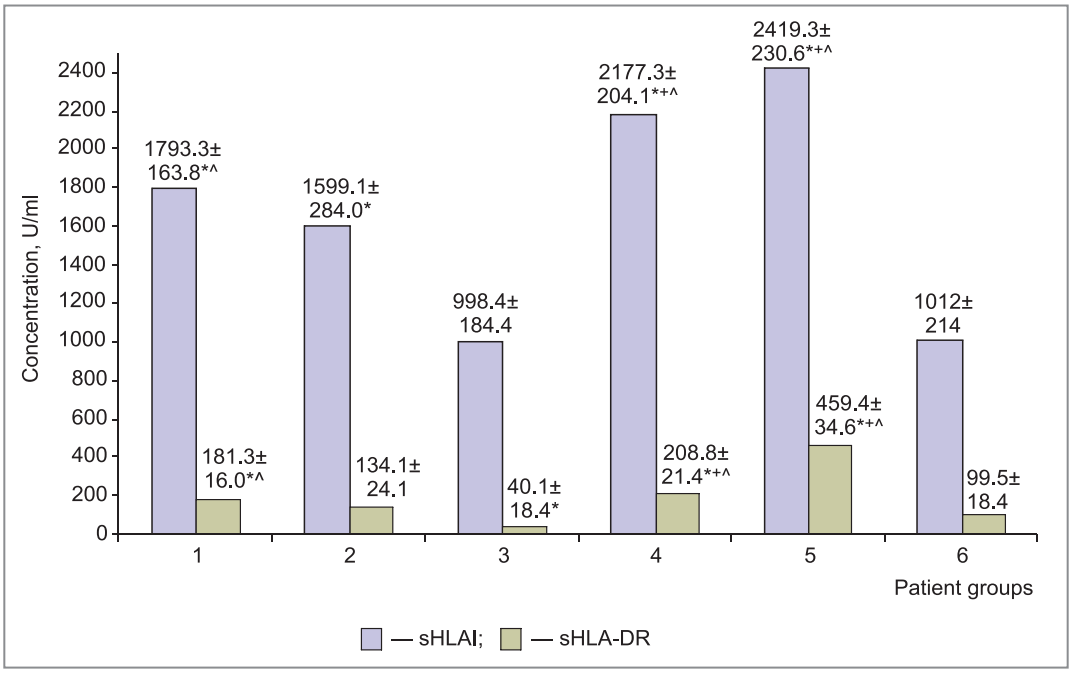

Fig. 2. The concentration of soluble HLA class I and HLA-DR molecules in blood serum in patients with different number of myomatous nodules: *1* — patients with mainly interstitial myomatous nodules (n=8); *2* — patients with mainly interstitial-suserous myomatous nodules (n=15); *3* — patients with mainly serous myomatous nodules (n=12); *4* — patients with mainly interstitial-submucous myomatous nodules (n=9); *5* — patients with mainly submucous myomatous nodules (n=9); *6* — control group (n=45); * — significant differences in comparison with the control ($p<0.05$); $^{+}$ — in comparison with interstitial-subserous localization ($p<0.05$); ^ — in comparison with subserous localization ($p<0.05$)

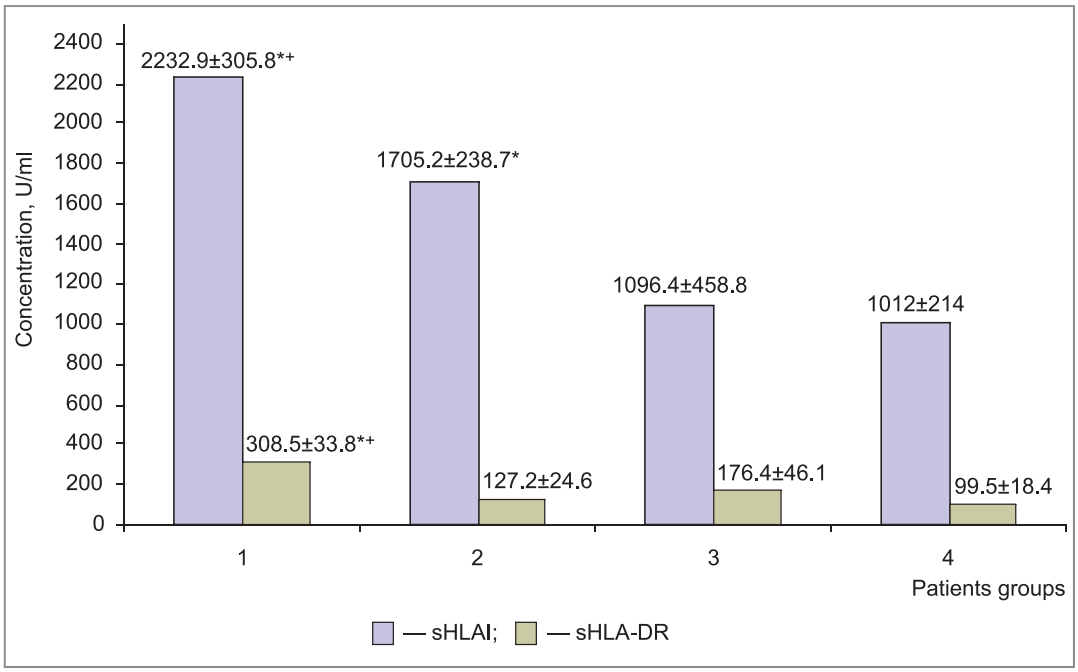

Fig. 3. The content of soluble HLA class I and HLA-DR molecules in blood serum in patients with different number of myomatous nodules: *1* — patients with one nodule (n=15); *2* — patients with 2–3 nodules (n=28); *3* — patients with 4–6 nodules (n=10); *4* — control group (n=45); * — significant differences in comparison with the control ($p<0.05$); $^{+}$ — in comparison with the group with 4–6 myomatous nodules ($p<0.05$)

times relatively). Furthermore, in patients with interstitial-submucous or submucous tumors the increase of sHLAI molecules level was significant in comparison with the mostly interstitial-subserous myomas (by 1.4 and 1.5 times relatively).

The content of soluble HLA-DR molecules in patients

Table 2

Influence of a surgery type on the changes of the soluble HLA I class and HLA-DR molecules content in patients with uterine cervix and body pathology (n=142)

Tumor localization		Type of surgery	HLA I class molecules concentration, U/ml		HLA-DR molecules concentration, U/ml	
			Before treatment	After treatment	Before treatment	After treatment
Uterine cervix cancer (n=18)	All		2357.1±260.4*	2461.60±175.01*	245.3±20.2*	159.46±43.76
Malignant tumor of uterine body (n=65)	All		2147.88±204.89*	2047.23±181.73*	184.39±34.13*	194.46±35.98*
Hysteromyoma (n=53)		Conservative myomectomia (n=16)	1477.62±80.21*+	1402.00±96.59*+	182.0±56.06*+	91.06±14.36+
		Supracervical uterus amputation (n=25)	1862.76±145.61*	1851.00±78.34*	145.40±36.24+	153.88±36.20+
		Supracervical uterus amputation with bilateral oophorectomy (n=12)	2328.00±134.23*	2225.58±103.85*	324.00±44.23*	331.33±39.32*
		All	1751.6±148.3*	1700.26±86.63*	196.0±14.4*	175.09±30.45*
Endometriosis (n=6)	All		1277.6±210.5	1566.00±189.98	123.7±21.0	174.40±49.59
Control group (n=45)	—		1012.0±214.0		99.5±18.4	

* — significant differences in comparison with the control ($p<0.05$); + — in comparison with the subgroup with hysteromyoma treated with supracervical uterus amputation with bilateral oophorectomy ($p<0.05$).

with interstitial-subserous localization of nodules was no different from norm and in patients with mostly subserous nodules was significantly lower than in the control (by 2.5 times). In patients with other localization (interstitial, interstitial-submucous or submucous) the sHLA-DR level was significantly higher than the norm (by 1.8; 2.1 and 4.6 times relatively). The differences of the sHLA-DR molecules levels in patients with distinct tumor localization revealed for the sHLAI molecules content measurement persisted. In patients with dominating interstitial-subserous or subserous nodules there was no difference in the sHLA-DR molecules content. However this parameter was higher in patients with interstitial-submucous or submucous tumors than in patients with dominating interstitial-subserous myomas (by 1.6 and 3.4 times), and in patients with interstitial, interstitial-submucous or submucous nodules the sHLA-DR level was higher than in patients with subserous localization of tumor (by 4.5; 5.2 and 11.5 times relatively). It should be noticed that in patients with dominating subserous tumors the serum content of both sHLAI and sHLA-DR molecules was the lowest. The subserous tumor location could cause stronger immune response disorders than any other locations. It may be assumed that the identified protein level deviations indicate the clinical diversity of one or another disease variant, because they act as endogenous immunomodulators and directly participate in the realization of immune response, which is different in different myoma locations.

The differences of soluble HLA I class and HLA-DR molecules levels among patients with different number of myomatous nodules were analyzed before treatment (Fig. 3). Their concentration in patients with one nodule was higher ($p<0.05$) by 2.2 and 3.1 times than in the control. In patients with 2–3 nodules the sHLAI molecules content was 1.7 times as high as in healthy women and the sHLA-DR content was no different from the control group. In patients with 4–6 myomatous nodules the sHLAI and sHLA-DR molecules content was normal but significantly lower than in patients with only one nodule (by 2.0 and 1.7 times).

The revealed changes of serum concentration of the tested proteins seem to reflect immune reaction particularities at different stages of blastomatous process. The benign tumor growth was accompanied with the quantitative changes of sHLAI and sHLA-DR molecules that were most significant at the stage of 4–6 nodules formation. The direct intervention in the production of soluble tumor antigen may be a possible reason. The performed studies [13, 15, 16] suggest that tumor cells express on their surface receptors which are similar to the immunocompetent cells' receptors and take an active part in immune reactions, all that results in the cytotoxic cells neutralization, first of all T-lymphocytes. The difference of sHLAI and sHLA-DR molecules concentration may be a factor determining the probability of multifocal nodule localization.

Any surgery has no significant influence on the HLA I class and HLA-DR levels (Table 2). However the concentrations of soluble proteins elevated compared to the control before surgery was no different from norm in patients with UCC after surgery. The similar changes of the sHLAI and HLA-DR molecules levels were observed in patients with hysteromyoma after conservative myomectomy. The sHLAI and sHLA-DR molecules content were significant different in women planning conservative myomectomy or supracervical hysterectomy with bilateral oophorectomy before surgery. In the latter case the serum sHLAI and sHLA-DR levels were 1.6 and 1.8 times as higher than in patients planning conservative myomectomy before surgery. The sHLA-DR molecules level in patients planning supracervical hysterectomy was significant lower (in 2.2 times) before surgery than in patients planning supracervical hysterectomy with bilateral oophorectomy. The presented data show that in patients with uterine cervix and body pathology the soluble HLA I class molecules definition (especially sHLA-DR molecules) may serve as an additional diagnostic test for their further selection for a surgery.

Conclusion. The content of soluble HLA class I and HLA-DR molecules in patients with uterine cervix and body pathologies correlate with their pathology type, tumor grade, the number and localization of myomatous nodes. There was no connection of the proteins concentration with a surgery type.

The initial concentration of soluble HLA class I and HLA-DR molecules in a preoperative period can serve as an additional diagnostic test in patients with uterine tumors for their further selection for surgery.

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Conflict of Interest. The authors have no conflict of interests.

References

1. Khaitov R.M., Alekseev L.P. Physiological role of human major histocompatibility complex. *Immunologiya* 2001; 3: 4–12.

2. Countini P., Zocchi M.R., Pierri I., Albarello A., Poggi A. In vivo apoptosis of CD8(+) lymphocytes in acute myeloid leukemia patients: involvement of soluble HLAI and Fas ligand. *Leukaemia* 2007; 21(2): 253–260, http://dx.doi.org/10.1038/sj.leu.2404494.

3. Nocito M., Montalban C., Gonzales-Porque P., Villar L.M. Increased soluble serum HLA class I antigens in patients with lymphoma. *Hum Immunol* 1997; 58(2): 106–111, http://dx.doi.org/10.1016/S0198-8859(97)00227-9.

4. Khudiakova N.E., Novikov V.V., Kravchenko G.A., Ivanova N.I., Ptitsyna I.S., Nosov N.N. Soluble HLA class I and HLA class II antigens in the blood of HIV infected patients. *Zhurnal mikrobiologii, epidemiologii i immunobiologii* 2004; 1: 42–45.

5. Kravchenko G.A., Novikov D.V., Ptitsyna Yu.S., Novikov V.V. Soluble immune cell membrane antigen levels in serum of carriers of viral hepatitis G markers. *Voprosy virusologii* 2005; 50(5): 19–22.

6. Novikov V.V., Evsegneeva I.V., Karaulov A.V., Baryshnikov A.Yu. Soluble immune cell membrane antigens in socially important infections. *Rossiyskiy bioterapevticheskiy zhurnal* 2005; 4(2): 100–105.

7. Lebedev M.Ju., Vilkov S.A., Sholkina M.N., Krizhanova M.A., Novikov V.V., Vyasmina E.S., Baryshnikov A.Ju. Peripheral blood lymphocytes immunophenotype and serum concentration of soluble HLA class I in burn patients. *Burns* 2003; 29(2): 123–128, http://dx.doi.org/10.1016/S0305-4179(02)00245-0.

8. Novikov V.V., Karaulov A.V., Baryshnikov A.Yu., Kravchenko G.A., Babaev A.A., Gostyuzhova E.A., Evsegneeva I.V. The characteristics of structural condition of soluble immune cell membrane antigen pool. *Molekulyarnaya meditsina* 2009; 4: 27–33.

9. Novikov V.V., Evsegneeva I.V., Karaulov A.V., Baryshnikov A.Yu. Soluble immune cell membrane antigen in socially important infections. Report 2. The study of their role in viral infections. *Rossiyskiy bioterapevticheskiy zhurnal* 2005; 4(3): 131–145.

10. Verbuggen L.A., Dumarey N., Van de Velde H. Soluble HLA–DR antigen levels in serum correlate with rheumatoid arthritis disease activity and the presence of disease — associated epitopes. *Tissue Antigens* 2000; 56(5): 436–440.

11. Stevenson F.K., George A.J., Walters M.T., Hamblin T.J. Analysis of soluble HLA class II antigenic material in patients with immunological diseases using monoclonal antibodies. *J Immunol Methods* 1986; 86(2): 187–190.

12. Korovushkina K.A. *Sravnitel'naya otsenka biokhimicheskikh i immunologicheskikh pokazateley pri dobrokachestvennykh i zlokachestvennykh opukholyakh zhenskoy polovoy sfery.* Avtoref. dis. … kand. biol. nauk [Comparative assessment of biochemical and immunological indices in benign and malignant tumors of female genital zone. Abstract of Dissertation for the degree of Candidate of Biological science]. Nizhny Novgorod; 2010.

13. Yarilin A.A. *Osnovy immunologii* [Basics of immunology]. Moscow: Meditsina; 1999; 608 p.

14. Golenkov A.K., Mitina T.A., Novikov V.V., et al. Clinical value of soluble adhesion (sCD50–ICAM-3), apoptosis (sCD95) and sHLA class I molecules in lymphoprofilerative diseases. *Rossiyskiy bioterapevticheskiy zhurnal* 2002, 1: 60–64.

15. Golovizin M.V. Intervention of tumor cells in T-lymphocyte maturation and selection processes as a neoplastic proliferation factor. *Immunologiya* 2001; 6: 4–10.

16. Perenkov A.D., Novikov D.V., Sakharnov N.A., Alyasova A.V., Utkin O.V., Baryshnikov A.Yu., Novikov V.V. Heterogeneous CD38 expression in tumor tissues of patients with colorectal cancer. *Molecular Biology* 2012; 46(5): 705–709.