Prognostic Value of Blood Serum Content of Soluble CD50 and CD54 Molecules in Patients with Uterine and Cervical Cancer

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© **M.E. Mamaeva,** Head of Gynecology Department, Clinical Hospital No.1[1]; PhD Student, Oncology Department, Postgraduate Faculty[2];
A.V. Alyasova, MD, DSc, Professor, Oncology Department, Postgraduate Faculty[2];
S.V. Shumilova, PhD, Researcher, Scientific Research Institute of Molecular Biology and Regional Ecology[3];
Zh.A. Kazatskaya, PhD, Senior Researcher, Scientific Research Institute of Molecular Biology and Regional Ecology[3];
M.V. Khazov, Chief Doctor of Clinical Hospital No.1[1];
N.N. Churkina, Physician Assistant, Clinical Hospital No.1[1];
V.V. Novikov, DSc, Professor, Director of Scientific Research Institute of Molecular Biology and Regional Ecology, Head of the Department of Molecular Biology and Immunology[3]

[1]Privolzhsky District Medical Center of Federal Medico-Biologic Agency of Russia, 14 Ilyinskaya St., Nizhny Novgorod, 603109, Russian Federation;
[2]Nizhny Novgorod State Medical Academy, 10/1 Minin and Pozharsky Square, Nizhny Novgorod, 603005, Russian Federation;
[3]Lobachevsky State University of Nizhni Novgorod, 23 Prospekt Gagarina, Nizhny Novgorod, 603950, Russian Federation

The aim of the investigation was to reveal the correlation of blood serum content of soluble CD50 and CD54 molecules in patients suffering from uterine and cervical cancer with oncological pathology localization, histological structure of the tumor, degree of tumor differentiation, and the extent of tumor invasion in the surrounding tissues, and to evaluate its significance for the disease prognosis.

Materials and Methods. 83 patients aged 31 to 79 years were under observation. The serum level of soluble CD50 and CD54 molecules was determined by enzyme immunoassay with mouse monoclonal antibodies. Blood from the cubital vein was taken to obtain serum samples.

Results. The development of malignant pathology of uterine and cervix was found to be accompanied by alteration of the serum level of CD50 and CD54 molecules. This alteration depends on oncological pathology localization, histological structure of the tumor, degree of tumor differentiation, and the extent of tumor invasion in the surrounding tissues. The serum level of soluble CD50 and oligomeric fractions of CD54 molecules was proved to decrease in patients with a worse prognosis. The initial concentration of soluble CD50 and CD54 molecules in the preoperative period can be considered an additional test allowing prediction of the disease progression in patients with uterine tumors.

Key words: soluble CD50 and CD54 molecules; cervical cancer; uterine tumors.

A vast group of endogenous immunity regulators, the so-called soluble differentiation molecules, includes several dozens of protein molecules performing the functions of immune reaction suppression and stimulation [1–5]. Along with differentiation molecules modulating apoptotic processes, immune response initiation and intercellular signaling it comprises numerous molecules involved in cell adhesion regulation, including molecules of ICAM family — CD50, CD54 [6, 7].

Soluble adhesion molecules are capable of modulating immune response, acting not only as activators but also as immune reaction inhibitors [8, 9]. Their content in blood serum significantly changes in many diseases and is used as a monitoring indicator [10–13]. There have been obtained the data showing alteration of the serum level of soluble CD50 and CD54 molecules in colon, lung and breast cancer [14–18]. The serum content of soluble adhesion molecules was found to alter in female malignant and benign genital tumors [19]. However, the results of earlier investigations provide no conclusions about the relationship between the serum level of soluble adhesion molecules and progression of these diseases. Using modern technologies for assessment of the serum level of soluble adhesion molecules and comparing the obtained results with different clinical and histological characteristics of neoplasms make it possible to reveal new prognostic factors in the period of preoperative examination of patients.

The aim of the investigation was to reveal the correlation of blood serum content of soluble CD50 and CD54 molecules in patients suffering from uterine and cervical cancer with oncological pathology localization, histological structure of the tumor, degree of tumor differentiation, and the extent of tumor invasion in the

For contacts: Mamaeva Marina Eugenievna, e-mail: mamaevame@yandex.ru

surrounding tissues, and to evaluate its significance for the disease prognosis.

Materials and Methods. Eighty-three patients aged 31 to 79 years were under study. Cervical cancer (CC) was diagnosed in 18 patients, the diagnosis of malignant uterine tumor was made in 65 patients. In all cases the disease diagnosis was histologically confirmed by postoperative material examination.

The study complies with the Declaration of Helsinki (adopted in June 1964 (Helsinki, Finland) and revised in October 2000 (Edinburgh, Scotland)) and approved by the local ethics committee. Written informed consent was obtained from all patients.

In all CC patients squamous cell carcinoma was revealed and it had high differentiation degree in 16 patients (88.9%). Adenocarcinoma prevailed in 55 patients (84.5%) with uterine body tumors, while uterine sarcoma (6 patients) and adenosquamous carcinoma (4 patients) were less frequent. Twenty-two adenocarcinoma patients (40.0%) were reported to have high degree of tumor differentiation, moderately differentiated (19 patients — 34.5%) and low-differentiated (14 patients — 25.5%) neoplasms were less frequent. In 10 patients (18.2%) the tumor invaded only the endometrium, it penetrated half the myometrium thickness in 22 patients (40.0%) and 2/3 of the myometrium thickness in 23 patients (41.8%). Administered treatment depended on the disease stage, histological structure of the tumor and included different types of operative intervention, most often, complete hystero-oophorectomy.

Control group comprised 45 women without gynecological pathology and the age comparable to the studied patients.

Blood from cubital vein was used to obtain serum samples. Blood analysis was performed prior to operative intervention. Blood samples were consistently incubated at +37°C for 30 min and kept in a refrigerator at +4°C to form a clot. After that, the clotted blood was centrifuged at 200 g for 15 min. Sera were collected in dry pure plastic test tubes and stored at 40–60°C in a refrigerator up to 6 months. To detect soluble differentiation molecules by enzyme immunoassay mouse monoclonal antibodies of ICO series, produced by hybridomas obtained from N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences (Moscow) were used. The serum level of soluble differentiation molecules was determined by two-site enzyme immunoassay. The total antigen fractions were determined using polyclonal antibodies as a substrate and monoclonal horseradish peroxidase-conjugated antibodies [5, 6, 8]. The results were analyzed spectrophotometrically using Multiscan EX photometer (LabSystems, Finland). The findings were calculated in conditional units of optical density (U/ml) and processed using a statistical software package Statistica 6.0.

Results and Discussion. In CC patients the serum level of soluble CD50 (sCD50) molecules was 3.5 times higher ($p<0.05$) than that in the control group (Table 1). At the same time there was statistically significant increase ($p<0.05$) in the level of oligomeric fraction of soluble CD54 (ol.sCD54) — by 4.3 times and their total fraction (sCD54) — by 4.9 times, compared to healthy patients' indices.

In the group of women with malignant uterine tumors sCD50, ol.sCD54, sCD54 levels were quite normal but statistically significantly lower ($p<0.05$) than in CC patients — by 4.2, 2.9 and 2.4 times, respectively.

The present data give evidence that alteration in the levels of sCD50, ol.sCD54, sCD54 molecules in patients with different gynecological pathology are divergent. In CC there is increase in serum level of all three molecules while in patients with malignant uterine tumors the content of these proteins remains at the level of healthy subjects' indices. Such changes may reflect functional peculiarities of patients' immune system and be associated with a specific disease development.

An unquestionable etiopathogenetic factor of CC development is persisting infection caused by human papilloma virus. A number of studies show high significance of soluble differentiation adhesion molecules and histocompatibility molecules for the protection against viral agents [2, 3, 6]. Evidently, elevated sCD50, ol.sCD54 and sCD54 levels modulating adhesion processes contribute to the formation of slowly developing immune response to human papilloma virus.

The content of sCD50, ol.sCD54 and sCD54 molecules in different types of histological structure of the tumor was studied in patients with malignant uterine tumor (Figure 1).

In adenocarcinoma patients sCD50 levels were 1.4

Table 1

The content of soluble CD50 and CD54 molecules in patients with uterine and cervical cancer (U/ml)

Tumor localization	sCD50	ol.sCD54	sCD54
Cervical cancer (n=18)	1244.3±143.5*	566.8±76.5*	325.5±43.5*
Malignant tumors of uterine (n=65)	295.58±49.62+	193.65±25.32+	135.50±15.56+
Control group (n=45)	353.7±48.2	131.4±27.8	65.3±10.4

* statistically significant value differences ($p<0.05$) compared to the control; + compared to the group of cervical cancer patients.

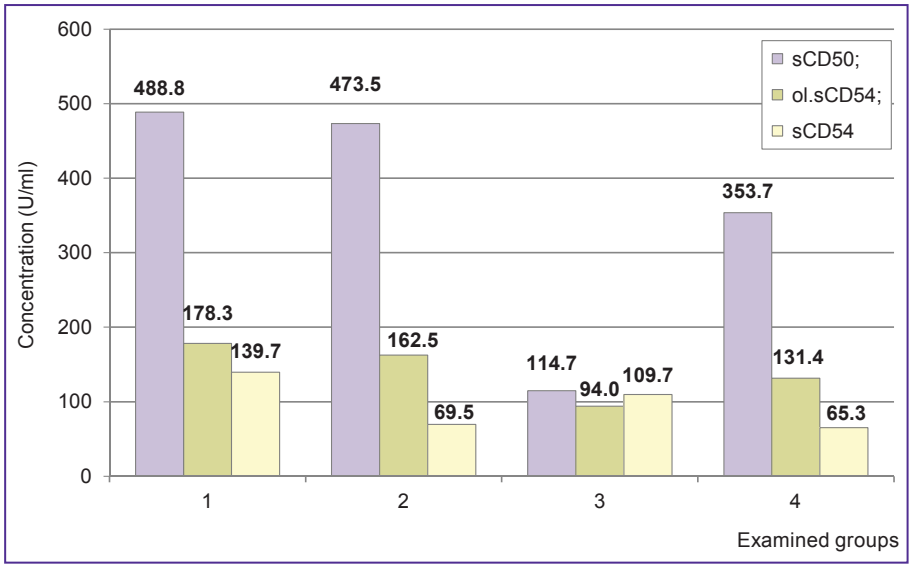

Figure 1. The content of soluble CD50, CD54 molecules in different types of histological structure of the tumor: group 1 — adenocarcinoma; group 2 — adenosquamous carcinoma; group 3 — uterine sarcoma; group 4 — control

times higher (p<0.05) than in the control group, ol.sCD54 content was proved to be normal, and sCD54 was 2.1 times higher (p<0.05) than in the healthy subjects. In case of adenosquamous carcinoma the level of sCD50 statistically significantly increased (1.3 times) compared to the control, while ol.sCD54 and sCD54 indices did not differ from those of the healthy subjects. In uterine sarcoma patients sCD50 levels were statistically significantly lower than in the control group (by 3 times), sCD54 levels were 1.6 times higher than the norm (p<0.05).

It should be noted that in uterine sarcoma patients the level of sCD50 molecules appeared to be statistically significantly lower than in adenocarcinoma patients — by 4.2, and 4.1 times lower than in adenosquamous carcinoma patients. Uterine sarcoma is characterized by highly aggressive progression of neoplastic process. Low sCD50 content in this group of patients may speak of decreasing CD50 expression on mononuclear cell membranes and marked impairments of immune response initiation in the given disease.

The serum content of the studied proteins was also found to correlate with the degree of neoplastic process differentiation (Table 2). In patients with highly differentiated adenocarcinomas the serum level of sCD50 appeared to be 1.3 times higher than the norm (p<0.05), ol.sCD54 and sCD54 levels were higher by 1.6 (p<0.05) and 2.5 times (p<0.05), respectively.

In women with moderately differentiated tumors sCD50 content was statistically significantly lower than in the control — by 1.6 times, ol.sCD54 content did not differ from the norm, and sCD54 levels were 2.2 times higher than the norm (p<0.05). It should be noted that sCD50 and ol.sCD54 levels in this group were found to be statistically significantly lower than in the subjects with highly differentiated tumors, by 2.2 and 1.4 times, respectively.

Low-differentiated adenocarcinomas were notable for the most considerable decrease in the level of sCD50: their content appeared to be statistically significantly lower than the norm — by 3.1 times and 4.2 and 1.9 times lower than in patients with highly and moderately differentiated adenocarcinomas. The levels of ol.sCD54 did not differ from the norm but were 1.5 times lower than in patients with highly differentiated tumors (p<0.05). sCD54 levels were 1.6 times above the norm (p<0.05), yet statistically significantly lower than in the subjects with highly and moderately differentiated tumors — by 1.5 and 1.4 times, respectively.

The analysis showed that in the group of highly differentiated adenocarcinoma patients (group 1) the proportion of subjects with elevated level of soluble sCD50 molecules appeared to be statistically significantly higher than in the group with low-differentiated adenocarcinomas — group 2 (7 out of 22 and 0 out of 14, respectively, p<0.001). Simultaneously, significant increase in the proportion of subjects with

Table 2

The content of soluble CD50 and CD54 molecules in different degrees of tumor differentiation (U/ml)

Degree of tumor differentiation	sCD50	ol.sCD54	sCD54
High (n=22)	479.25±63.0*	218.0±24.3*	166.0±23.1*
Moderate (n=19)	218.55±38.06*+	153.7±17.2+	147.2±16.9*
Low (n=14)	112.6±32.84*+v	144.8±15.4+	106.2±16.7*+v
Control group (n=45)	353.7±48.2	131.4±27.8	65.3±10.4

* statistically significant value differences (p<0.05) compared to the control; + compared to the patients with highly differentiated tumors; v compared to the patients with moderately differentiated tumors.

reduced level of this protein was observed (14 out of 22 and 14 out of 14, respectively, p<0.001). The elevated level of ol.sCD54 was also statistically significantly more frequent in group 1 (5 out of 22 and 0 out of 14, respectively, p=0.006). By contrast, the normal protein level prevailed in subjects suffering from low-differentiated adenocarcinomas (19 out of 22 and 14 out of 14, respectively, p=0.03). Analyzing the content of sCD54 molecules in group 2 the proportion of subjects with normal level of the protein was found to be low (4 out of 22 and 0 out of 14, respectively, p=0.01).

The present data strongly suggest, that the development of the most aggressive disease progression liable to recurrences and distant metastases is accompanied by the most significant abnormalities of immunity indices manifesting themselves, in particular, by divergent alterations in the content of certain soluble adhesion molecules. The development of low-differentiated tumor with the worst prognosis is accompanied by statistically significant decrease in the proportion of subjects having elevated serum levels of sCD50 molecules, elevated levels of ol.sCD54 molecules and normal levels of sCD54 molecules.

The serum levels of proteins under study also correlate with the extent of malignant uterine tumor invasion in the surrounding tissues (Figure 2).

In patients with tumors penetrating only the endometrium (group 1) sCD50 level statistically significantly exceeded the norm (group 4) by 1.3 times, ol.sCD54 content did not differ from the norm, and sCD54 level was statistically significantly (2.1 times) higher than in the control group.

In the group of women whose tumor penetrated half the myometrium thickness (group 2) sCD50 and ol.sCD54 levels did not differ from the norm, while sCD54 level 1.8 times exceeded the norm. Yet sCD50 level was 1.2 times lower (p<0.05) than in the group of patients with affected endometrium.

In cases of adenocarcinoma penetrating 2/3 of the myometrium thickness (group 3) sCD50 was 1.4 times below the norm (p<0.05), ol.sCD54 level did not differ from the control, sCD54 level remained statistically significantly (2.5 times) higher than in the control. sCD50 level in this group appeared to be statistically significantly (1.8 times) lower than in the patients with affected endometrium and 1.5 times lower than in those whose tumor penetrated half the myometrium thickness.

The obtained results give evidence that a greater extent of neoplastic process penetration is accompanied by significant decrease in sCD50 level, unchanged of ol.sCD54 level and persisting elevation of sCD54 level. Decrease in CD50 serum level may suggest the development of more severe immune system disorders in patients with adenocarcinoma penetrating 2/3 of the myometrium thickness compared to the patients having a less extent of neoplastic process invasion and a better prognosis.

Conclusion. The changes in the serum levels of soluble CD50 molecules, oligomeric fraction of soluble CD54 molecules and persisting elevation of total

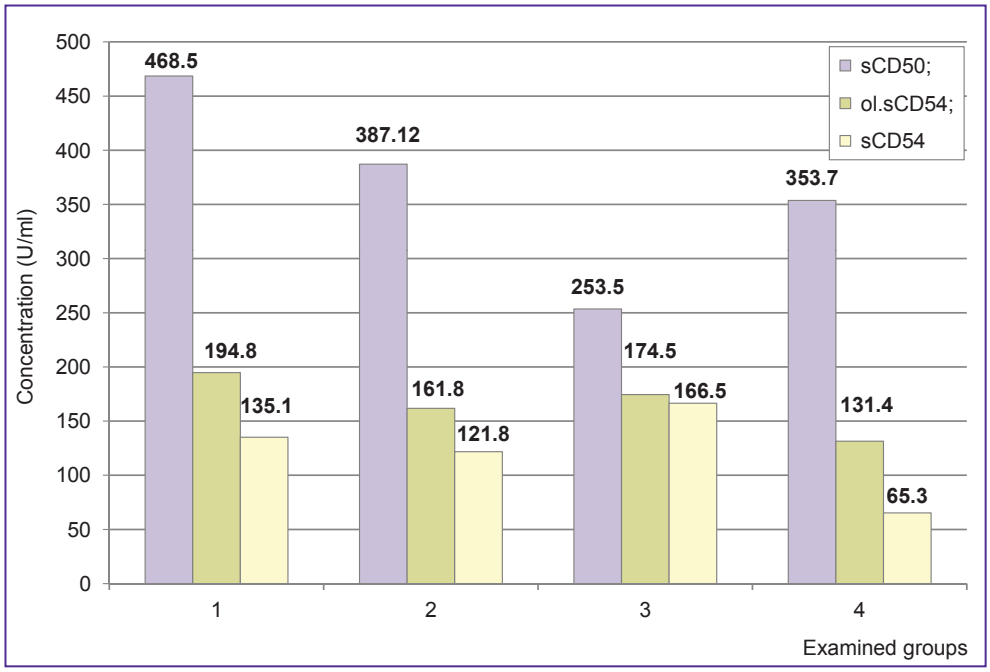

Figure 2. The content of soluble CD50, CD54 molecules in different extent of malignant uterine tumor invasion into the surrounding tissues: group 1 — invasion in the endometrium; group 2 — half the myometrium thickness; group 3 — 2/3 of the myometrium thickness; group 4 — control

fraction of soluble CD54 molecules in patients with malignant pathology of uterine and cervix correlate with the histological structure of the disease, differentiation degree and the extent of tumor invasion in the surrounding tissues. Along with increasing neoplastic process aggressiveness there appear and progress diverse alterations in the content of soluble proteins whose membrane forms are included in the group of adhesion antigens. In patients with the worst prognosis the serum level of soluble CD50 and oligomeric fractions of CD54 molecules is proved to decrease. Determining the level of these proteins in the preoperative period can be considered an additional method for prognosis of the disease progression.

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Conflicts of Interest. There is no conflict of interests related to the present study.

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