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USING CLUSTER OF DIFFERENTIATION CD 38, CD 45 AND CD 95 AS A METHOD OF PRIMARY DIAGNOSIS OF UTERINE FIBROIDS

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Abstract

Uterine myoma is one of the first places among gynecological diseases. There is a rise of the number of pathology diagnoses among young women. Currently in Ukraine there is no single approved laboratory method for screening of uterine myomas.

The aim of our work is the studying of the efficiency of CD38, CD45, and CD95 leukocyte differentiation clusters for early detection of uterine myomas.

Determination of a subpopulation of lymphocytes in urine with immune complex of peroxidase-antiperoxidase. The features of the number of leukocytes with CD 38, CD 45, CD 95 antigens were analyzed among women with uterine myoma compared with women who did not have gynecological diseases and underwent an appropriate research. Each sample consisted of 50 women aged 30 to 65 years (average age in both samples was 45 years). The Student's test for independent samples was used for statistical methods of comparison.

The results we've got indicate that the most statistically significant differences were established by SD 95, so it can be assumed that it is the one that is the most informative as a

possible marker for the presence of uterine fibroids. At the same time, during the analyzingof the clusters of CD38 and CD45 differentiation, there were also found highly significant differences between the group of patients with uterine myoma and healthy women.

Key words: uterine myoma, CD 38, CD45, CD95, screening method.

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ВИКОРИСТАННЯ КЛАСТЕРІВ ДИФЕРЕНЦІЮВАННЯ CD 38, CD45 ТА CD95, ЯК МЕТОД ПЕРВИННОЇ ДІАГНОСТИКИ МІОМ МАТКИ

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Реферат

Міома матки займає одне з перших місць серед гінекологічних захворювань. Відмічається збільшення кількості випадків діагностування патології у жінок молодого віку. На даний час в Україні не існує єдиного затвердженого лабораторно методу для скринінгу міом матки.

Метою нашої роботи є дослідження ефективності кластерів диференціювання лейкоцитів CD 38, CD45 та CD95 для ранньої діагностики міом матки.

Визначення субпопуляції лімфоцитів у сечі за допомогою імунного комплексу пероксидаза-антипероксидаза. Аналізували особливості кількості лейкоцитів з антигенами CD 38, CD 45, CD 95 у жінок, хворих на міому матки у порівнянні з жінками, які не хворіли на гінекологічні захворювання та пройшли відповідне дослідження. Кожна вибірка нараховувала 50 жінок віком від 30 до 65 років (середній вік у обох вибірках склав 45 років). В якості статистичних методів порівняння використовували критерій Стьюдента для незалежних вибірок.

Отримані результати свідчать про те, що найбільш статистично значущі відмінності були встановлені за СД 95, таким чином можна припустити, що саме він є найбільш інформативним як можливий маркер наявності міоми матки. В той же час при аналізі кластерів диференціювання CD 38 та CD45 також були виявлені високо значущі відмінності між групою хворих на міому матки та здоровими жінками.

Ключові слова: міома матки, CD 38, CD45, CD95, скринінговий метод дослідження.

ИСПОЛЬЗОВАНИЕ КЛАСТЕРОВ ДИФФЕРЕНЦИРОВКИ CD 38, CD45 И CD95, КАК МЕТОД ПЕРВИЧНОЙ ДИАГНОСТИКИ МИОМ МАТКИ

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Реферат

Миома матки занимает одно из первых мест среди гинекологических заболеваний. Отмечается увеличение количества случаев диагностирования патологии у женщин молодого возраста. В настоящее время в Украине не существует единого утвержденного лабораторно метода для скрининга миом матки.

Целью нашей работы является исследование эффективности кластеров дифференцировки лейкоцитов CD 38, CD45 и CD95 для ранней диагностики миомы матки.

Определение субпопуляции лимфоцитов в моче с помощью иммунного комплекса пероксидаза-антипероксидаза. Анализировали особенности количества лейкоцитов с антигенами CD 38, CD 45, CD 95 у женщин, больных миомой матки по сравнению с женщинами, которые не болели гинекологические заболевания и прошли соответствующее исследование. Каждая выборка насчитывала 50 женщин в возрасте от 30 до 65 лет (средний возраст в обеих выборках составил 45 лет). В качестве

статистических методов сравнения использовали критерий Стьюдента для независимых выборок.

Полученные результаты свидетельствуют о том, что наиболее статистически значимые различия были установлены по СД 95, таким образом можно предположить, что именно он является наиболее информативным как возможный маркер наличия миомы матки. В то же время при анализе кластеров дифференцировки CD 38 и CD45 также были обнаружены высоко значимые различия между группой больных миомой матки и здоровыми женщинами.

Ключевые слова: миома матки, CD 38, CD45, CD95, скрининговый метод исследования.

Relevance. Uterine cancer is one of the most common diseases in women. The structure of gynecological pathology is one of the first places [1]. Statistics show that the incidence of uterine fibroids is 12-25% of all gynecological diseases and reaches maximum values in later reproductive and premenopausal age [2, 3]. Uterine fibroids most often after the age of 32 years, in recent years, the growth rate of this disease among young women of reproductive age [4, 5]. It is believed that the epidemiology of uterine fibroids based only on data from clinical studies unreliable. Additional information, including post-mortem pathological studies suggest that the true prevalence of this disease reaches 77% [2, 6, 7].

The most common cause of planned surgery in gynecologic practice - a surgery for uterine fibroids. In most cases carried histeroektomiya with increasing percent of patients younger, at best - conservative myomectomy without comprehensive treatment. But due to lack of primary screening methods, this pathology detected by chance (in diagnostic interventions on pelvic organs, pregnancy diagnosis, etc.), Or in the later stages of the disease when the disease progressing leads to a significant deterioration in the quality of life and health. However, for the treatment of these conditions carry bulky surgery [8]. Modern medicine in recent years goes mainly to the use of conservative treatment instead of organ traumatic surgery, except for acute emergency surgical pathology. The main problem in this situation is to late diagnosis, when pathogenetic processes already in full swing and irreversible processes predominate.

Currently, in Ukraine there is no single laboratory approved method for screening for uterine fibroids. Protocol MOH and WHO recommendations diagnosis is performed using instrumental methods [9]. Also in the records is not spelled out method of primary screening patients.

Instrumental methods used for primary screening of uterine fibroids (US) are not always effective for diagnosis of this disease in the early stages of the disease when nodes are small in size and have a low growth rate. To clarify use of MRI or CT [10]. These more accurate diagnostic methods to differentiate precisely this pathology. Using these methods and associated main drawback of instrumental methods used for primary and mass screening of the population. The first disadvantage of this system is its great need for a financially secure due to the large cost of the devices and need special conditions of use.

This leads to high prices for the service, and not all patients can afford it.

The second drawback is not very high throughput capability of devices that can not diagnose large amounts of people.

The third drawback that each device requires specialist with regard to financial security again and time for training.

Therefore the invention and implementation of population screening laboratory method with further refinement of diagnosis using instrumental methods more effective and appropriate to optimize and improve healthcare delivery.

Our method is based on immune response based on development is atypical cells. The method is used in clusters of differentiation of white blood cells that are receptor proteins in the membrane of immune cells.

CD38. Hydrolase cyclic ADP-ribose. By nature - glycoprotein that is present in many cells, including T-killer cells and T helper cells, B-lymphocytes. Key features - of calcium signals and cell-cell adhesion [11]. Repeatedly firmly established the role of CD38 in the development and pathogenesis of benign tumors. By increasing the soluble CD38-antigen, could limit the immune response to a tumor in the area of oncology female reproductive system [12].

CD45. Proteintyrosinephosphokinase. Leukocyte antigen that performs signaling function, resulting in cell growth, differentiation, regulation of mitotic cycle, indirectly resulting in oncogenic transformation [13]. The study showed the same combined participation of CD31, CD45, CD73, CD90 and CD105 in the growth of fibroids [14].

CD95. (FasR, APO-1, APT, TNFRSF6), 1 antigen apoptosis, membrane protein, tumor necrosis factor receptor. Initiates Fas-dependent apoptosis way [15]. Increased Fas-ligand stimulation results in tumor suppression [13]. Found increase serum concentration of CD95-protein in patients with malignant lymphoma, osteosarcoma, cancer of the liver, stomach, breast. In some other diseases recorded a decrease in its serum levels displayed on the molecular mechanisms of initiation of apoptosis. According to the submitted data, the

development of tumors of female genitals in most cases accompanied by increased serum concentrations of soluble CD38 and CD95 antigens. The most pronounced increase seen in patients with cervical cancer. Increased serum concentrations of soluble CD38 and CD95 antigens occur not only in cancer but also in benign tumors. Performing surgery does not make a significant impact on the level of soluble CD38 and CD95 antigens, except myomectony, leading to normalization of content CD38 antigen [16].

Objective: Research by effective of clusters CD 38, CD 45 and CD 95 for difference of leukocytes for early diagnostic of uterine fibroids.

Materials and methods:

Determination of a subpopulation of lymphocytes in urine with the help of an immune complex of peroxidase-antiperoxidase.

After the corresponding hygiene of the external genital organs, urine was sampled in Eppendorf tube in a volume of up to 10 ml.

Then, from a given amount of urine, 4 ml of urine is collected and diluted with physiological saline in a ratio of 1: 2 and centrifuged for 15 minutes, at 1800 rpm, at room temperature. The supernatant was drained, physiological saline added, resuspended and centrifuged at 1000 rpm, at room temperature. Washed leukocytes were diluted with physiological saline, bringing them to a concentration of 2-4 * 10 cells / μl. 100 μl of monoclonal CD38 and CD45 antibodies, as well as a complex of horseradish peroxidase-antiperoxidase were successively applied on the cytological preparation. The finished preparations were stained with methylene green and the activity of horseradish peroxidase was determined to identify various subpopulations of lymphocytes. Cells that have an antigen bound to horseradish peroxidase had a dark rim of brown color along the edge of the cytoplasm.

To prepare a buffered saline solution, 6 ml of 0.5 M phosphate buffer are added to 100 ml of standard saline and NaCl 0.9% (using a buffer manufactured by Simko Ltd in Lviv, pH 7.2-7.4). To prepare the ficoll solution, 9.9 g of dry ficoll are dissolved in 100 ml of distilled water, after which 20 ml are added. 40% solution of Verografins (Verografin in ampoules, manufactured by KRKA, Slovakia), the density of the solution is checked with a hydrometer, it is 1,077. Measurements are carried out in the following order: 1 ml of urine is diluted with physiological solution in a ratio of 1 to 2 and layered on the medium for the release of leukocytes (ficoll-verografin, density 1,077) from the calculation of 2 parts of urine into 2 parts of ficolls. The mixture is centrifuged for 40 minutes at 2000 rpm. min., at room temperature. The cell layer is carefully collected from the urine-ficoll interface, it is

suspended in 5 volumes of saline and centrifuged for 15 minutes, at 1800 rpm, at room temperature. The supernatant is drained, physiological saline is added, resuspended and centrifuged at 1000 rpm, at room temperature. Washed leukocytes are resuspended and diluted with physiological saline, bringing them to a concentration of 2-4 * 10 cells μl. A cytological preparation is prepared, which is applied to the reaction zone with 100 μl of horseradish peroxidase complex-monoclonal antibodies to horseradish peroxidase and incubated for one hour at room temperature. Preparations are washed by successively immersing the glass in 2 cups with buffered saline.

Excess of washed solution is carefully discarded by filter paper. The drug is dyed methylene green.

Determination of horseradish peroxidase activity for identification of different subpopulations of leukocytes.

1. The reaction is recorded by microscopy with a magnification of 100 times.

2. Cells that are antigen bound to horseradish peroxidase have a dark rim of brown color along the edge of the cytoplasm. [17]

Results: In study are analyzed the features of the number of leukocytes with CD 38, CD 45, CD 95 antigens in women with uterine myoma compared to women who did not have gynecological diseases and underwent a corresponding study. Each selection consisted of 50 women aged 30 to 65 years (mean age in both samples was 45 years).

As statistical methods of comparison, the Student's test was used for independent selection for each marker. Data were also presented graphically, using arithmetic mean, standard deviation and 95% confidence interval.

For CD 38, the mean ± standard deviation for the control group and for the comparison group are 4.98 ± 0.94 and 17.36 ± 1.55, respectively. Values and statistical significance of the Student's test are $t = 48.37$, $p < 10\text{-}5$.

Point and interval estimates for CD 38 are shown in Fig. 1.

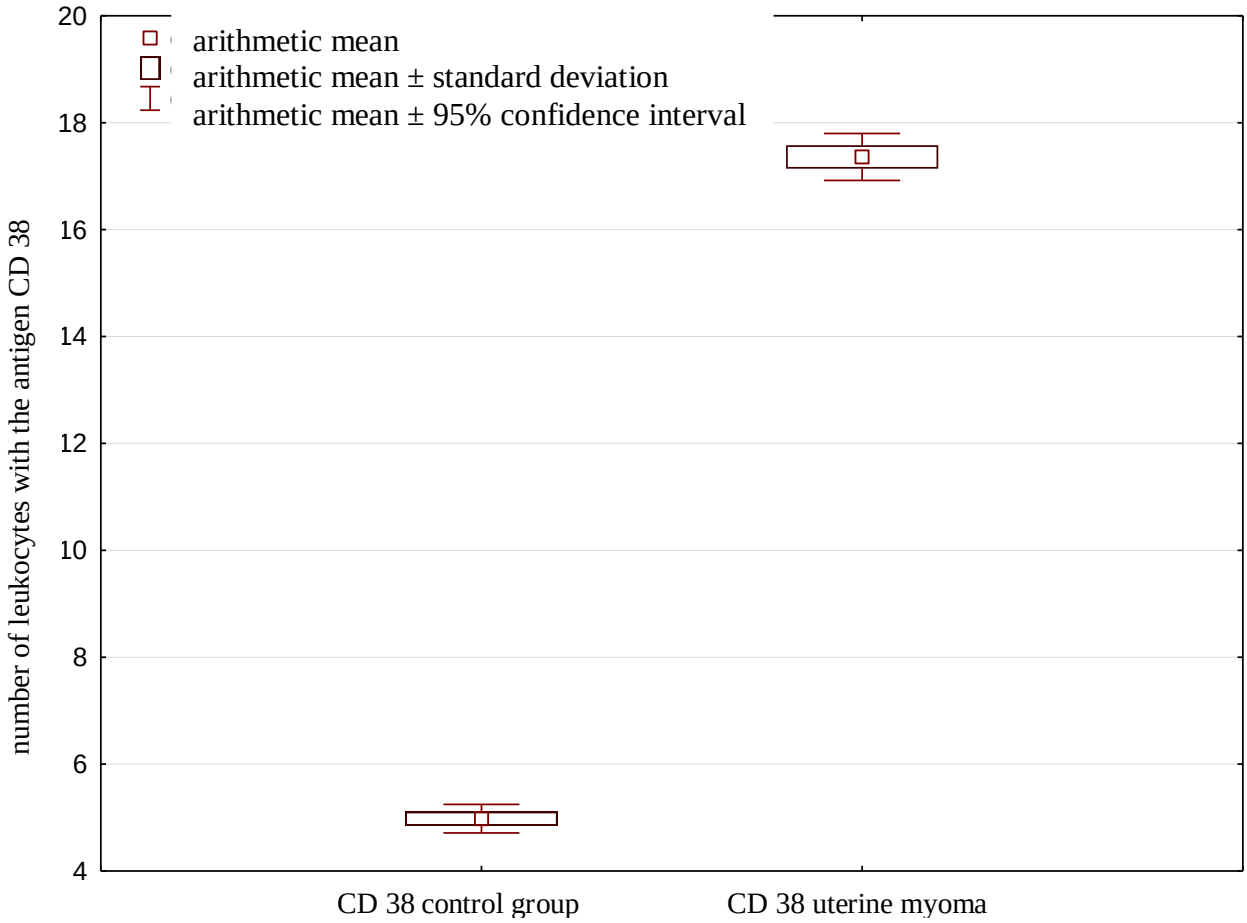

Fig. 1. Comparison of the number of leukocytes with the antigen of CD38 in healthy women (control group) and in patients with uterine myoma (comparison group).

The value of CD 45 in the control group was 5.00 ± 0.93, and in the comparison group 14.84 ± 2.32 (t = 27.81, p <10-5), Fig. 2.

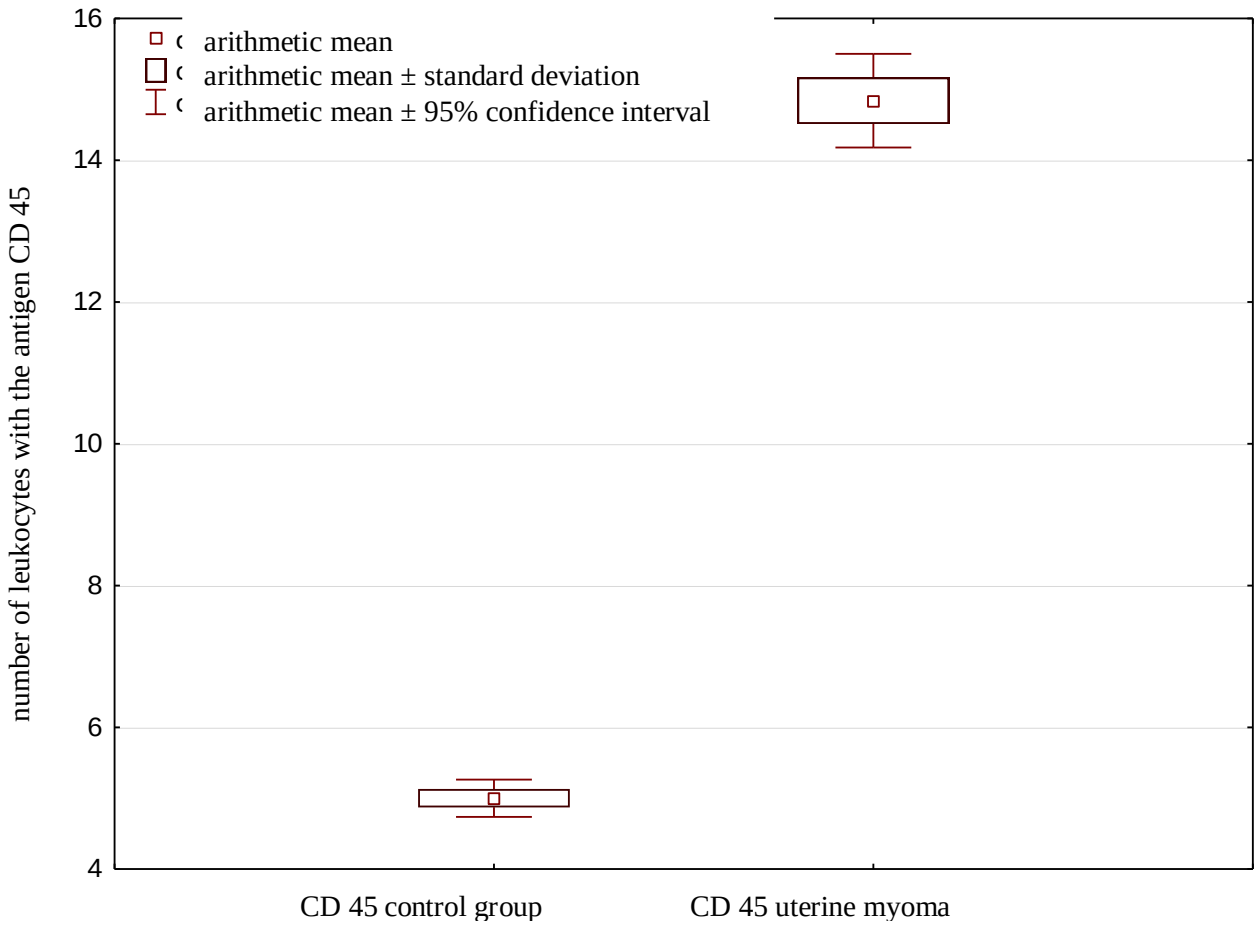

Fig. 2. Comparison of the number of leukocytes with the antigen CD 45 in healthy women (control group) and in patients with uterine myoma (comparison group).

In the control group, the value of CD 95 was 5.20 ± 0.70, and in the comparison group 19.40 ± 1.74 (t = 53.59, p <10-5), Fig. 3.

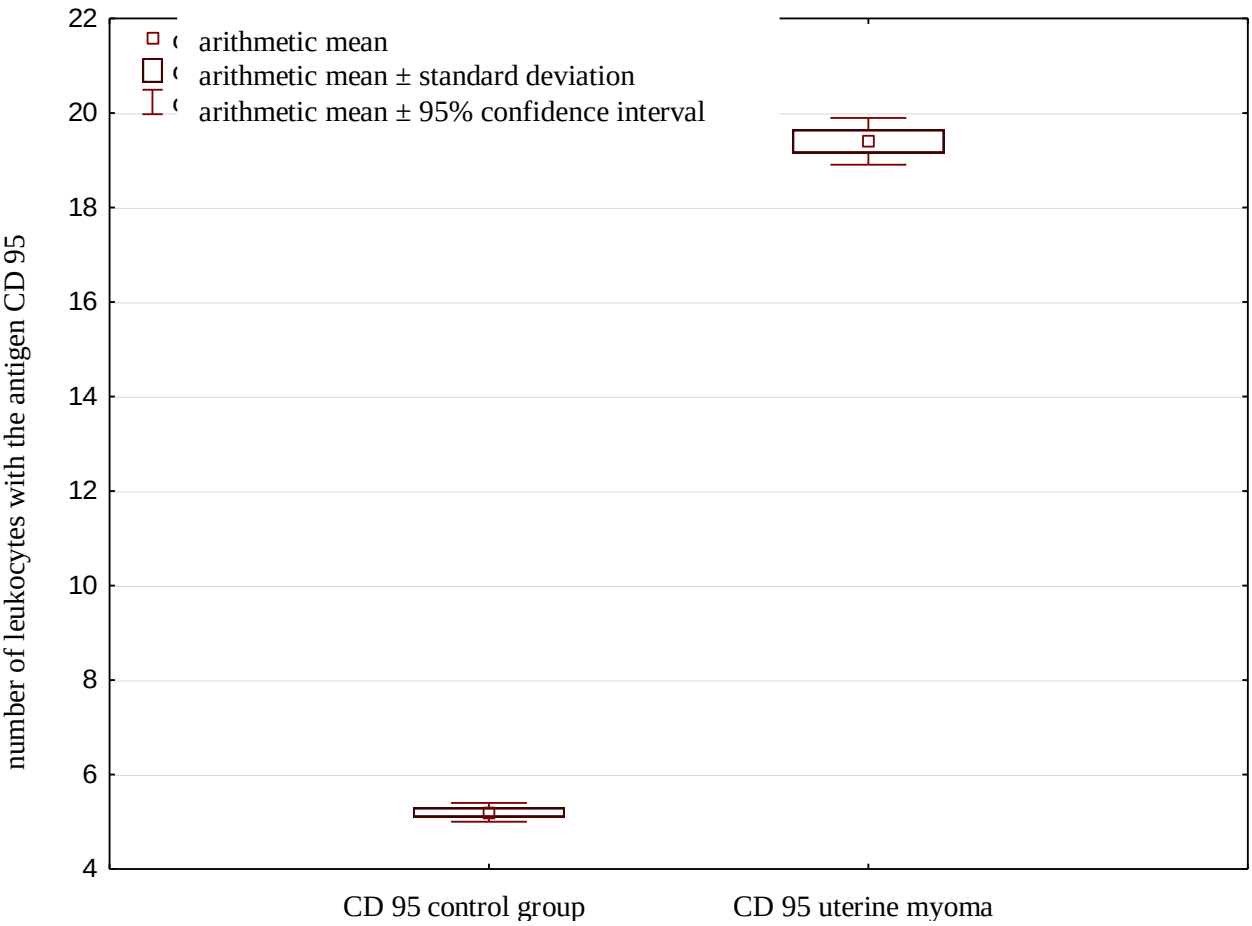

Fig. 3. Comparison of leukocyte count with CD 95 antigen in healthy women (control group) and in women with uterine myoma (comparison group).

Conclusion: In all parameters, extremely clinically and statistically significant differences in the number of leukocytes with the corresponding antigens between a group of patients with uterine myoma and healthy women were obtained. This gives grounds for using all these parameters or even any of them as a screening method for identifying women with uterine myoma. The most statistically significant differences were established by CD 95, so it can be assumed that it is the most informative as a possible marker for the presence of uterine fibroids in women in the population.

Diagnosis of uterine fibroids using differentiation clusters CD 38, CD45 and CD95 will allow to diagnose pathology with a high degree of probability and to prescribe quantitative and qualitative pathogenetic therapy in a timely manner.

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