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R E P O R T

By topics: "Investigation of the effect of Donovit-VS on cellular, humoral and non-specific immunity in patients with ovarian cancer"

"Investigation of the effect of Donovit-VS on cellular, humoral and non-specific immunity in patients with breast cancer"

KYIV

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Study of the effect of Donovit-VS on cellular, humoral and non-specific immunity in patients with ovarian cancer

Introduction

The incidence of malignant neoplasms in the population of Ukraine, as in all economically developed countries of the world, is characterized by stable growth. Malignant neoplasms occupy the second place (13%) in the general mortality structure of the population of Ukraine after cardiovascular (60%) diseases.

Ovarian cancer (OC) is one of the most common forms of neoplasms and ranks fourth in the incidence structure among women.

Despite the progress achieved in the treatment of patients with ovarian cancer, the death rate from this disease still remains high and amounted to 20.5 people per 100,000 female population in 2008.

The steady growth of the incidence, unsatisfactory results of treatment of patients with ovarian cancer due to the often developing resistance to cytostatics and the high risk of recurrence, even in the early stages of the disease, lead to the search for new approaches and directions of treatment and the search for new drugs that would have an immunostrengthening and oncoprotective effect. One of these directions is immunotherapy.

Immunotherapy in the treatment of cancer patients is a fairly new trend in global oncology. However, he has already established himself as a progressive. Today, immunotherapy is quite actively researched and used by Ukrainian oncologists.

Among many drugs with an immuno-boosting effect, our attention was drawn to a new Ukrainian drug of plant origin, which has an immuno-boosting and oncoprotective effect — Donovit-VS. It showed its oncoprotective effect in a number of tumors, but we did not find data on the use of this drug in malignant ovarian diseases in the available literature.

That is why the purpose of our study was to study the immune status of patients with ovarian cancer when included in the complex antitumor treatment of the plant-derived drug Donovit-VS.

Research objectives :

1. to study indicators of cellular immunity in patients with ovarian cancer with inclusion in the treatment regimen of Donovit-VS;
2. to study indicators of humoral immunity in patients with ovarian cancer with inclusion in the treatment regimen of Donovit-VS;
3. to investigate indicators of non-specific resistance in patients with ovarian cancer.

The object of the study : women with ovarian cancer.

Research methods : generally clinical, histological, cytofluorometric, immunological and statistical.

Practical significance of the obtained results. The results of the research make it possible to recommend the drug Donovit-VS for inclusion in the complex scheme of antitumor treatment of patients with ovarian cancer.

II. Materials and methods

2.1. Characteristics of clinical material

The research, the results of which are presented in the work, was conducted on the basis of the department of immunology and gynecology of the Kyiv City Oncology Hospital (KMOL). The research materials were the results of an immunological examination of 30 women with ovarian cancer, who were treated during 2008-2009 in the gynecological department of the Moscow State Medical University. The control group consisted of 8 practically healthy women who were voluntarily tested for immunological status in the department of immunology. The average age of women in the control group was 45.5 + 1.5 years. The average age of the patients was 62.9 ± 0.8 years (see table 2.1.)

Table 2.1

Distribution of the clinical group of OC patients by age

Grouping by decade	Quantity (%)	The average age of patients in the group
45-55	5(16.7)	52.2 ± 1.5
56-66	13 (43.3)	62.2 ± 0.5
66-76	12(40.0)	74.1 ± 0.6
In total	30(100.0)	62.9 ± 0.8

When examining the patients of the specified groups, general clinical and laboratory research methods were used. In table 2.2. research methods and the number of examined women are presented.

Table 2.2.

Research methods and the number of examined patients

Research methods	Number of women
General clinical	30
Histological	30
Immunological	38
Cytofluorometric	38
Statistical	38

The majority of patients (56-76 years old) (Table 2.3.) were diagnosed with II and III stages of cancer according to the FI GO classification of the tumor process, in 5 (16.7%) the I stage of the disease was detected. In addition to a complex examination, patients with OC underwent complex treatment (according to international standards), which required: radical surgical intervention (extirpation of the uterus with an appendix, omentectomy) with adjuvant chemotherapy (Table 2.4.), some patients underwent similar treatment + immunotherapy with new a Ukrainian drug with antitumor and immunostrengthening effects (table 2.4.).

According to the research plan, intraoperative collection of tumor material and selection of paraffin blocks of removed tumors for current and retrospective analysis were carried out. Histological examination of ovarian tumors was carried out by doctors of the patho-anatomical department of KMOL. Illness histories, outpatient cards, cancer registry materials were processed. Observation of patients after treatment was carried out in the department of oncology and gynecology of the Moscow Medical Center (Table 2.3).

Table 2.3.

Clinical parameters		**Number of patients %**
Stage of the disease	AND	5(16.7)
FI G0	II	10(33.3)
	III	15(50.0)
	In total	30(100.0)
Histological type of OC	Serous	6 (20.0)
	Endometriosis	4(13,3)
	Mucinous	3(10.0)
	Cellular light	3 (10.0)
	Serous papillary	5(16.7)
	Undifferentiated	9(30.0)
	In total	**30**

Table 2.4.

Type of treatment of patients with OC

The treatment was carried out	**Number of patients %**
Operative + chemotherapy	10(33.3)
Operative + chemotherapy + immunotherapy	20 (66.7)
In total	**30(100.0)**

2.2. Method of histological examination of ovarian tumors.

Morphological examination was performed after surgery. According to the classic histological method of tissue processing, fixation in 10% formalin solution and paraffin wiring were used. Histological sections with a thickness of 5-6 μm were stained with hematoxylin and eosin.

After examining the histological preparations under a microscope, the histological structure and degree of differentiated tumors were determined. To characterize the morphological structure of ovarian malignant neoplasms, the histological classification of tumors was used (WHO, No. 9, 1973).

2.3. Methods of studying cellular immunity.

During the last 30 years, the number of E rosette-forming cells and Em rosette-forming cells were considered the most important indicators of the quantitative characteristics of the population of T and B lymphocytes, the method of determining which is based on the fact of the presence of surface receptors of lymphocytes to heterologous erythrocytes. For T-lymphocytes, these are sheep erythrocytes (E - RUK), for B-lymphocytes - mouse erythrocytes (Em - RUK)

The discovery of the hybridoma technology for obtaining monoclonal antibodies and the introduction of automatic analysis based on quantitative ductal cytofluorimetry into the wide practice of clinical and diagnostic laboratories contributed to the gradual displacement of "socket" tests.

CD3+ (T-lymphocytes) and CD19+ (B-lymphocytes, together with CD4+ helper/inducer T-lymphocytes), CD8+ (T-lymphocyte suppressor/cytotoxic), CD16+ and CD56+ (natural killer) antigens are now the primary markers for quantifying population and subpopulation composition of lymphocytes, their expression determines how well a lymphocyte is able to regulate its activation processes and effector functions.

The immunophenotype of lymphocytes (CB3+T-lymphocytes, CD19+-B-lymphocytes, CD16+ CD56+ -NK-cells) was evaluated according to the recommendations of the company "Bekton Dickinson Immynocytometry Systems", USA, using two-parameter cytofluorimetric analysis, a panel of directly labeled monoclonal antibodies.

Measurements were performed on a flow cytofluorimeter "FACS can" equipped with an argon laser (488 nm), using the commercial program "CM Quest" (of the same company). The analysis of cells in the sample was carried out in the lymphocyte gate according to 4 parameters: the intensity of direct and lateral light scattering, FITU and PE fluorescence. To measure fluorescence according to FITU, narrow-band filters of size 530/30 nm, FE-575/26 nm were used, the laser power was 100 mV.

The method of determining the surface markers of lymphocytes using monoclonal antibodies (MA).

1. MCA was diluted with Na azide (0.1% solution) in a ratio of 1:3, stored in a refrigerator for 1-2 months.

2. 5 μl of MCL (CD3+, CD4+, CD8+, CD16+, CD19+) were placed in plastic tubes.
3. 20 ml of genarized blood was added to each test tube, mixed and incubated for 30 min at room temperature.
4. 70-80ml of the treatment solution was added to each test tube for 5-10 minutes.
5. The studied lymphocyte clusters were counted on a cytofluorimeter.

Method of assessment of phagocytic ability and neutrophilic granulocytes.

The method is based on the ability of blood neutrophils to absorb and digest microbes and characterizes their functional activity.

Reagents: isotonic sodium chloride solution (sterile), suspension of killed staphylococcus microbes (Staphylococcus aureus - strain 209).

To prepare a microbial suspension, a small amount of isotonic sodium chloride solution was poured into a test tube or Petri dish with a one-day culture of staphylococcus, mixed, and the diluted culture was transferred to a chemical test tube with a pipette. This test tube was placed in a water bath and Staphylococcus aureus was activated at a temperature of 90°C for 1 hour. Then the contents of the test tube are brought to the degree of turbidity of the bacterial standard with a concentration of 1-5 x 10^3 microbial bodies in 1 ml of isotonic sodium chloride solution.

Performance technique. 100 μl of heparinized blood and an activated culture of Staphylococcus aureus were added to a sterile Vidal test tube. The contents were thoroughly mixed and placed in a thermostat at a temperature of +37°C for 1 hour. After mixing the contents of the test tube, a drop of it was applied to a glass slide and a thin smear was made, which was then dried in air, fixed and stained according to Romanovsky. 100 neutrophil granulocytes were counted along the edge of the stained smear under the immersion system (eyepiece 10 or 15).

2.4. Methodology of research of indicators of humoral and non- specific immunity. Determination of the content of immunoglobulins of individual classes in blood serum.

To assess the functional state of the B-system of immunity in humans, we used the determination of the number of three main classes of immunoglobulins (M, G, A) in blood serum by the method of radial immunodiffusion in agar (according to Manchin).

Reagents and materials:

1. 1.5% agar (preferably Difco or bactoagar) on medinal buffer. After complete melting, the agar was packaged in 8 ml bacteriological test tubes and stored in the refrigerator until use;

2. Medinal buffer: 9 ml of Medinal was dissolved in 0.5 l of distilled water, 65 ml of 0.1N hydrochloric acid solution was added, the volume was brought up to 1 l, pH 8.6.

3. Monospecific antisera against human IgA, IgM, IgG.

Performance technique. Three test tubes with 1.5% agar were placed in a boiling water bath and held until the agar completely melted. Then they were transferred to a bath with a temperature of +50...+56 C. Antisera against individual

classes of immunoglobulins were diluted with distilled water, transferred to bacteriological test tubes and also placed in a water bath at a temperature of $+50^{0}$ ... $^{+56\,0}$ C for 5 minutes. After that, antisera were mixed with agar (the ratio of agar and antisera solution was calculated based on the titer of the latter) and poured between two slightly heated glass plates (9x12), bounded by a frame made of plexiglass or other equivalent material in the form of the letter P.

The thickness of the frame is 1 mm, the width of the walls is 1 cm. The plates were left in a vertical position until the agar completely solidified. Then the fixing clamps were removed and one of the plates was carefully removed, thus obtaining a plateau with a layer of agar 1 mm thick on the other plate. Holes with a diameter of 2 mm at a distance of 2 cm from each other were cut in the agar with a round punch. The agar was sucked out of the wells, and the wells were filled with standard serum and patient sera. In the first 3 wells, a standard (undiluted, diluted 2 and 4 times) with a known concentration of immunoglobulin, which should be determined in sera, was introduced with a thinly drawn Pasteur pipette or a micro syringe. In other wells, studied sera of patients were introduced. The wells were filled with sera up to the upper edge of the agar. To determine the level of IgA, IgG blood serum was previously diluted in a ratio of 1:3. The filled plates were placed in a humid chamber on a strictly horizontal plane and left at room temperature for 24 hours. (for IgA, IgG) and 48 hours. (for IgM).

After measuring the diameter of the rings in a standard preparation with a known level of immunoglobulin, a calibrated curve was constructed on graph paper. The concentration of immunoglobulins was plotted horizontally, and the diameter of the precipitation rings vertically. Based on the calibrated curve, the concentration of immunoglobulins in the tested sample was calculated. If the serum was diluted before setting up the reaction, then the results were multiplied by the value of the dilution.

According to the International Standard of Serum Immunoglobulins, their normal level is: IgA - 3.4 g/l, IgM - 0.8 g/l, lgG - 7.58 g/l.

Determination of the content of immune complexes in **blood serum.**

There are a number of methods for quantitative determination of circulating immune complexes: radioimmunological, method of inhibition of phagocytosis of aggregated IgG by peritoneal macrophages, platelet aggregation test, complement binding reaction, and others.

Comparative studies of the detection of immune complexes in the blood by various methods indicate that the method of precipitation in a 3.75% solution of polyethylene glycol is the simplest, but not inferior to other methods in terms of informativeness.

Reagents: Solution #1 - 0.1 N borate buffer with pH 8.4 (3.41 g of boric acid, 4.275 g of borax in 1 l of distilled water); solution #2 - 10 g of "polyethylene glycol 600" in 240 ml of solution #1.

Performance technique. 0.4 ml of the tested blood serum was introduced into the test tube, 0.8 ml of solution No. 1 was added, 0.4 ml was thoroughly mixed in 2 test tubes, 2.7 ml of solution No. 1 (control) was added to one, and 2.7 ml to the other ml of solution No. 2 (experiment).

The contents of the test tubes were mixed and left for 60 minutes. at room temperature. After that, the optical density of the samples was determined using a spectrophotometer at a wavelength of 450 nm in cuvettes No. 5. The difference in optical density indicators was calculated, the result was multiplied by 1000, and the number of immune complexes in blood serum was obtained, expressed in conventional units.

Methodology for evaluating the phagocytic ability of neutrophil granulocytes.

It is based on the ability of blood neutrophils to absorb and digest microbes and characterizes their functional activity.

Reagents: isotonic sodium chloride solution (sterile), suspension of killed staphylococcus microbes (Staphylococcus aureus - strain 209).

To prepare a microbial suspension, a small amount of isotonic sodium chloride solution was poured into a test tube or Petri dish with a one-day culture of staphylococcus, mixed, and the diluted culture was transferred to a chemical test tube with a pipette. This test tube was placed in a water bath and Staphylococcus aureus was inactivated at a temperature of 90 ^{0} C for 1 hour. Then the contents of the test tube are brought to the degree of turbidity of the bacterial standard with a concentration of 1-5x10 ^{3} microbial bodies in 1 ml of isotonic sodium chloride solution.

Performance technique. 100 μl of heparinized blood and an inactivated culture of Staphylococcus aureus were added to a sterile Vidal test tube. The contents were thoroughly mixed and placed in a thermostat at a temperature of +37 ^{0} C for 1 hour. After mixing the contents of the test tube, a drop of it was applied to a glass slide and a thin smear was made, which was then dried in air, fixed and stained according to Romanovsky.

100 neutrophil granulocytes were counted along the edge of the stained smear under the immersion system (eyepiece 10 or 15). The percentage of phagocytizing neutrophil granulocytes was determined - an indicator of phagocytic activity (FA) and the average number of microbes absorbed by one neutrophil - the phagocytic number.

2.5. Statistical processing of results.

The data obtained from the conducted studies were processed by generally accepted methods of variational statistics using the Student's criterion.

Calculations were carried out using a computer and an application package of statistical programs "Stadia".

Section III

Experimental part

3.1. Study of the effect of Donovit-VS on cellular immunity in women with ovarian cancer.

In this section, the aim of the study was to study a new Ukrainian herbal preparation with oncoprotective and immunostrengthening effects, which is able to influence indicators of cellular immunity, in particular, CD3+ T-lymphocytes, CD19+ B-lymphocytes, CD56+ NK cells, in 50 patients who had I , II and III stages of the disease on OC.

According to the treatment, the patients were divided into groups, the control group consisted of 8 practically healthy women who expressed a desire to be examined voluntarily.

I - group - practically healthy women;

II - group - these are patients who underwent radical surgery + 3 courses of chemotherapy (cyclophosphamide 750.0 mg/m ^{2})

III - group - patients who received similar treatment, but immunotherapy Donovit-VS is included in the scheme of the antitumor complex.

Donovit VS is recommended according to the standard scheme in the intervals between courses of chemotherapy. 3 Donovit-VS courses were held.

Blood sampling for immunological examination was performed before the first course of chemotherapy and 10 days after the last dose of Donovit-VS. It should be noted that the patients of the second group were prescribed traditional pharmaceuticals and a normal food diet with the inclusion of oat decoctions.

As can be seen from the data presented in Table 3.1, CD3+ (T-lymphocytes) significantly decreased in the patients of the second group after complex treatment (3 courses of XT) (53.5±0.6 and 43.1±0.5, respectively). The inclusion of the plant-derived drug Donovit-VS in the scheme of complex treatment ensured the preservation of the number of CD3+ lymphocytes (51.8±0.6 and 59.9±0.4, respectively).

Table 3.1.

The number of CD3+ (lymphocytes) in the blood of patients with OC before and after complex treatment including Donovit-VS immunotherapy

%	And the group	II group		III group	
		Before treatment	After treatment	Before treatment	After treatment
	71.5 ±0.6	53.5 ±0.6	43.1 ±0.5	51.8 ±0.6	59.9 ± 0.4
n	8	10	10	20	20
p	—	<0.05			
R_1					>0.05

In table 3.2. the data of the study of the number of CD19+ (B-lymphocytes) in the blood of patients with OC are presented. The obtained data show that the number of CD19+ (B-lymphocytes) significantly decreases in the patients of the second group in the process of complex antitumor treatment (6.2±0.4 and 3.1±0.6, respectively).

The inclusion of the plant-derived drug Donovit VS in the scheme of complex antitumor treatment significantly reduces the toxic effect of XT and increases the number of CD19+ (B-lymphocytes) above the level before treatment (6.1±0.8 and 10.5±0.3).

Table 3.2.

The number of CD 19+ (lymphocytes) in the blood of patients with OC before and after complex treatment including Donovit-VS immunotherapy

%	And the group	II group		III group	
		Before treatment	After treatment	Before treatment	After treatment
	12 ±0.8	6.2 ± 0.4	3.1 ±0.6	6.1 ±0.8	10.5 ±0.3
n	8	10	10	20	20
p				-	<0.05
R_1	-				>0.05

In table 3.3. presented the data of the study of the number of CD56+ (NC-cells) in the blood of patients with ovarian cancer before and after complex antitumor treatment with the inclusion of immunotherapy with the new Ukrainian plant-derived drug Donovit-VS.

As can be seen from the data presented in Table 3.3. after complex antitumor treatment in the II group of patients, the number of CD56+ lymphocytes (NK cells), which have the properties of natural killers of tumor cells, also significantly decreases.

The inclusion of a plant-derived drug in the scheme of complex antitumor treatment increases the number of CD56+ lymphocytes above the level before treatment (group III) (8.8±0.4 and 14.8±0.5, respectively).

Table 3.3.

The number of CD 56+ (NC cells) in the blood of patients with OC before and after complex treatment including Donovit-VS immunotherapy

%	And the group	II group		III group	
		Before treatment	After treatment	To treatment	After treatment
	21.2 ±0.4	8.4 ± 0.8	6.3 ± 0.6	8.8 ± 0.4	14.8 ±0.5
n	8	10	10	20	20
p		<0.05			
P_1					>0.05

Thus, as a result of the conducted research, it was established that the number of lymphocytes, namely CD3+, CD19+ and CD56+, was reduced compared to that of practically healthy women. In the process of complex antitumor treatment, a further decrease in the number of T, B and NK lymphocytes was observed in patients of group II, and on the contrary, in patients of group III, whose complex treatment scheme included Donovit VS immunotherapy, a preservation of the number of investigated lymphocytes (CD3+, CD19+ and CD56+) was observed to the values before treatment, which indicates the protective effect of Donovit-VS.

3.2. Study of the effect of Donovit-VS on humoral immunity in women with ovarian cancer".

In this section, our research was aimed at studying indicators of humoral immunity in patients with OC, the scheme of complex antitumor treatment of which included radical surgery + chemotherapy (3 courses) - II group and similarly to the antitumor complex, but with the inclusion of a drug of plant origin, which has oncoprotective and immunomodulating effect, Donovit-VS - III group.

Table 3.4 presents the data of the study of circulating immune complexes (CIC) in the blood of women with OC before and after complex treatment with the inclusion of Donovit VS immunotherapy.

As evidenced by the data presented in Table 3.4 before treatment, in patients with OC, the number of CIC in the blood serum was significantly increased compared to the indicators in practically healthy women (94.8 ± 0.6 and 50.5 ± 0.5, respectively).

After complex antitumor treatment in patients of the II group, the amount of CIC in blood serum practically does not change, even a tendency to increase is noted (94.8±0.6 and 98.5±0.4, respectively, before and after treatment).

The inclusion of Donovit-VS in the scheme of complex treatment significantly reduces the amount of CIC in blood serum in patients of group III after treatment (96.5±0.8 and 58.6±0.3, respectively).

Table 3.4.

The amount of CIC in the blood serum of patients with ovarian cancer before and after complex treatment with the inclusion of Donovit-VS immunotherapy

%	And the group	II group		III group group	
		Before treatment	After treatment	To treatment	After treatment
	50.5 ± 0.5	94.8 ± 0.6	98.5 ± 0.4	96.5 ± 0.8	58.6 ± 0.3
n	8	10	10	20	20
p	—	<0.05			<0.05

Table 3.5 presents the data of the study of the number of immunoglobulins of the 3 classes IgA, IgM, IgG in the blood of women with OC before and after complex treatment including Donovit VS immunotherapy.

As can be seen from the data presented in Table 3.5, the number of immunoglobulins of class A and M in groups I and II did not change after treatment, that is, it remained at the same level as the number of practically healthy people. Changes occurred only in class G immunoglobulin, the amount of which decreased in the II group of patients after treatment, and increased in the III group (10.0±0.3 and 7.0±0.5 and 9.8±0.5 and 11, 8±0.2, respectively).

Thus, as a result of the conducted research, it was shown that the studied indicators of humoral immunity in women with cancer during complex anticancer treatment, in particular the number of CYCs and the number of immunoglobulins of the 3 classes IgA, IgM, IgG, the following changes occurred, namely the number of CYCs increased in patients of the II group, the inclusion of Donovit-VS in the treatment regimen led to a decrease in the number of CIC after treatment.

The number of immunoglobulins of the 3 classes Ig A, Ig M, Ig G in women with ovarian cancer before and after complex treatment with the inclusion of immunotherapy Donovit-VS.

Table 3.5.

%	I group			II group						III group					
				before treatment			after treatment			before treatment			after treatment		
	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG
	2.6 ± 0.2	1.30 ± 0.2	12.8 ± 0.2	2.85 ± 0.3	1.1 ± 0.4	10.0 ± 0.3	1.3 ± 0.4	1.2 ± 0.4	7.0 ± 0.5	2.75 ± 0.4	1.2 ± 0.2	9.8 ± 0.5	3.01 ± 0.3	1.7 ± 0.4	11.8 ± 0.2
n	8	8	8	10	10	10	10	10	10	20	20	20	20	20	20

3.3. Study of the effect of Donovit-VS on non-specific immunity - phagocytic activity and phagocytic index in women with cancer.

In this section, the aim of our research was to study non-specific resistance, namely phagocytic activity and phagocytic index in women with OC with inclusion in the scheme of complex treatment of immunotherapy with a new Ukrainian drug with immunostrengthening and antitumor effect - Donovit-VS.

In table 3.6. data of the study of phagocytic activity and phagocytic index and neutrophils in the blood of patients with ovarian cancer before and after complex treatment including Donovit-VS immunotherapy are presented.

As evidenced by the data presented in Table 3.6. phagocytic activity and the phagocytic index of neutrophils in the blood of patients of group II are reduced compared to those of practically healthy people (71.5±0.5 and 60.2±0.4 and 61.1±0.4, respectively).

In the process of complex treatment, there was a significant decrease in FA and FI with indicators before treatment (60.2 ± 0.4 and 42.5 ± 0.3, respectively, FA and 4.6 ± 0.3 and 2.5 ± 0.5, respectively, FI).

The inclusion of Donovit-VS in the scheme of complex antitumor treatment increases the indicators of phagocytic activity and the phagocytic index in the III group of patients almost to the level of practically healthy people (71.5 ± 0.5 and 7.5 ± 0.2 - I group, 68.8 ± 0.6 and 5.6 ± 0.4 - III group, respectively).

Thus, as a result of the research, it was established that the complex antitumor treatment (radical surgery + chemotherapy) significantly reduces the indicators of phagocytic activity and the phagocytic index. Inclusion in the complex treatment scheme of 3 courses of treatment with Donovit-BS significantly improves indicators of phagocytic activity and phagocytic index, which subsequently affects the course of the tumor process in women with OC.

Phagocytic activity and phagocytic index of neutrophils in the blood of patients with ovarian cancer before and after complex treatment including Donovit-VS immunotherapy.

Table 3.6.

%	I group		II group				III group			
			before treatment		after treatment		before treatment		after treatment	
	F	FI	F	FI	F	FI	F	FI	F	FI
	71.5 ± 0.5	7.5 ± 0.2	60.2 ± 0.4	4.6 ± 0.3	42.5 ± 0.3	2.5 ± 0.5	61.1 ± 0.4	4.5 ± 0.5	68.8 ± 0.6	5.6 ± 0.4
n	8	8	10	10	10	10	20	20	20	20
p			<0.05							
p1			_		>0.05					
p2							_		<0.05	
p3										>0.05

Chernenko O.D., Murina V.V., Koren T.M., Samoilenko S.V.

Study of the effect of Donovit-VS on cellular, humoral and non-specific immunity in patients with breast cancer

The purpose of the study is to study the effect of a new Ukrainian plant-derived drug with oncoprotective and immunostrengthening action - Donovit-VS on indicators of cellular, humoral and non-specific immunity, in particular - CD3 + (T-lymphocytes), CD19 + (B-lymphocytes), CD56 + (NK- cells), CYC, phagocytic activity and phagocytic index in 25 patients with stage II, III and IV breast cancer (breast cancer).

The research was conducted on the basis of the laboratory of immunology and the day hospital of the Kyiv City Oncology Hospital (KMOL). The research materials were the results of the immunological examination of 25 patients with breast cancer, who were treated during 2007-2009 in the day hospital of the Moscow Medical Center. The control group consisted of 10 practically healthy women who were voluntarily examined for immunological status in the laboratory of immunology. The average age of the control group was 40.5±2.5 years. The average age of patients is 59.5±5.3 years.

Table 1

Research methods and number of examined patients

Research methods	Number of women
General clinical	25
Histological	25
Immunological	35
Cytofluorometric	35
Statistical	35

In addition to comprehensive examination of patients with breast cancer, complex treatment (according to international standards) was carried out, which included radical surgical intervention followed by chemotherapy and radiation treatment. Part of the patients underwent similar treatment with the inclusion of immunotherapy with a new Ukrainian drug with antitumor and immune-strengthening effects - Donovit-VS.

According to the treatment performed, the patients were divided into groups:

Group I - practically healthy women;

Group II are patients who underwent radical surgery + 3 courses of poly chemotherapy (cyclophosphane 200 mg, mitocoatron 10 mg/m ^{2} , fluorouracil 400 mg/m ^{2} , vincristine 1.4 mg/m ^{2});

Group III - patients who received similar treatment, but immunotherapy Donovit-VS was included in the scheme of the antitumor complex.

Donovit-BS is recommended according to the standard scheme in the intervals between chemotherapy courses. 3 courses with Donovit-VS were conducted.

Blood sampling for immunological examination was performed before the first course of chemotherapy and 10 days after the last dose of Donovit-VS. It should be noted that the patients of the second group were prescribed traditional pharmaceuticals and a normal food diet with the inclusion of oat decoctions.

As can be seen from the data presented in Table 2, the number of CD3 + lymphocytes significantly decreased in the patients of the second group after complex treatment (3 courses of polychemotherapy) (55.4±0.6 and 46.1±0.4, $p<0.05$ in accordance). The inclusion in the scheme of the complex herbal preparation Donovit-BS led to an increase in the number of $CD3^+$ after treatment (53.9±0.3 and 60.1±0.4, $p<0.05$).

Table 2

The number of CD3 + lymphocytes in the blood of women with breast cancer after complex treatment including immunotherapy Donovit-VS

	I group	II group		III group	
		before treatment	after treatment	before treatment	after treatment
%	68.5±0.8	55.4±0.6	46.1±0.4	53.9±0.3	60.1±0.4
n	10	10	10	15	15
p		-	<0.05		
r_1				-	<0.05

Table 3

The number of CD19 + lymphocytes in the blood of women with breast cancer before and after complex treatment including Donovit-VS immunotherapy

	I group	II group		III group	
		before treatment	after treatment	before treatment	after treatment
%	11.8±0.6	6.5±0.5	4.1±0.4	6.2±0.4	9.1±0.5
n	10	10	10	15	15
p		-	<0.05		
r_1				-	<0.05

Table 3 presents data from the study of the number of CD19 + lymphocytes in the blood of patients with breast cancer. The obtained data indicate that the number of CD19 + lymphocytes significantly decreases in the patients of group II in the process of complex antitumor treatment (6.5±0.5 and 4.1±0.4, respectively).

The inclusion of the plant-derived drug Donovit-BS in the scheme of complex antitumor treatment significantly reduces the toxic effect of chemotherapy and increases the number of CD19 + lymphocytes (6.2±0.4 and 9.5±0.5; p <0.05).

Table 4

The number of CD56 + (NC cells) in the blood of women with breast cancer before and after complex treatment including Donovit-VS immunotherapy

	I group	II group		III group	
		before treatment	after treatment	before treatment	after treatment
%	22.4±0.6	9.2±0.6	7.4±0.5	8.6±0.8	15.1±0.6
n	10	10	10	15	15
p	-	<0.05			
r_1				-	<0.05

The data presented in Table 4 indicate that after complex antitumor treatment, the number of CD56 + lymphocytes, which have the properties of natural killers of tumor cells, significantly decreases in patients of group II.

Inclusion in the scheme of complex antitumor treatment of the herbal preparation Donovit-BS significantly increases the number of CD56 + lymphocytes (8.6±0.8 and 15.1±0.6, respectively, before and after treatment, III group of patients).

Table 5 presents data on the determination of circulating immune complexes in the blood of women with breast cancer before and after complex treatment including Donovit-BS immunotherapy.

As evidenced by the data presented in Table 5, the number of CIC in patients with breast cancer increases in comparison with the similar indicator in practically healthy women (80.5±0.8; 83.6±0.5 and 50.8±0.4 in accordance).

Table 5

The amount of CIC in the blood serum of patients with breast cancer before and after comprehensive treatmentwith the inclusion of immunotherapy Donovit-VS

	I group	II group		III group	
		before treatment	after treatment	Before treatment	after treatment
%	50.8±0.4	80.5±0.8	82.4±0.6	82.6±0.5	60.8±0.6
n	10	10	10	15	15
p	-	<0.05			
r_1					<0.05

After complex antitumor treatment in patients of group II, the amount of CIC in blood serum practically does not change (80.5±0.8; 82.4 and 0.6, respectively, before and after treatment).

The inclusion of Donovit-VS in the complex treatment scheme significantly reduces the amount of CIC in blood serum in patients of group III after treatment (82.6±0.5 and 60.8±0.6, p <0.05, respectively).

Table No. 6 presents data on the study of phagocytic activity and the phagocytic index of neutrophils in the blood of patients with breast cancer.

As evidenced by the data presented in Table 6, FA and FI of neutrophils in the blood of patients of group II are reduced compared to those of practically healthy people (62.2±0.4; 61.4±0.6 and 74.5±0.6 in accordance).

Table 6

Phagocytic activity and phagocytic index of neutrophils
in the blood of women with breast cancer before and after complex treatment
with the inclusion of immunotherapy Donovit-VS

	I group		II group				III group			
			before treatment		after treatment		before treatment		after treatment	
	F	FI	F	FI	F	FI	F	FI	F	FI
	74.5±0.6	7.2±0.4	62.2±0.4	4.5±0.3	56.5±0.5	3.6±0.6	61.4±0.6	4.6±0.6	68.4±0.5	5.9±0.3
n	10	10	10	10	10	10	15	15	15	15
p	-		<0.05							
p_1			-		<0.05					
p_2							-		>0.05	
p_3										>0.05

In the process of complex treatment, there was a significant decrease in FA and FI compared to the indicators before treatment (FA – 62.2±0.4 and 56.5±0.5, p <0.5; FI – 4.5±0.3 and 3.1±0.6).

Donovit-VS included in the scheme of complex treatment of women with breast cancer (III group) increased the FA and FI indicators (FA – 61.4±0.6 and 68.4±0.6; FI – 4.6±0, 6 and 5.9±0.3 respectively before and after treatment).

Thus, as a result of the conducted research, it was established for the first time that the inclusion of the new Ukrainian drug Donovit-VS in the scheme of complex treatment of women with breast cancer leads to a significant recovery of the studied indicators of cellular, humoral and non-specific immunity, namely CD3+, CD19+, CD56+, TSK, FA and FI.

In addition, the patients who took Donovit-BS subjectively improved their well-being and tolerated the next polychemotherapy better.

The obtained data allow us to recommend Donovit-VS for inclusion in antitumor treatment regimens, as a drug that has a protective effect on immunocompetent cells and improves the well-being of breast cancer patients.

Conclusions

As a result of the conducted research, it was established that the conducted complex antitumor treatment (radical surgical intervention + chemotherapy) significantly reduces indicators of phagocytic activity and phagocytic index. Inclusion in the complex treatment scheme of 3 courses of Donovit-VS significantly improves indicators of phagocytic activity and phagocytic index, which in the future has a positive effect on the course of the tumor process in women with ovarian cancer.

The obtained data in patients with breast cancer allow us to recommend Donovit-VS for inclusion in antitumor treatment regimens, as a drug that has a protective effect on immunocompetent cells, improves the well-being of patients and allows better tolerance of polychemotherapy.

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