

Pre-Operative Levels of Serum Immunoglobulins, Circulating Immune Complexes and Complement Proteins in Patients with Different Types of Neoplasms

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Abstract. In the sera of 30 neoplasm patients without metastases, the average IgG level was higher than in the control group (CG) (18.16 ± 5.10 vs. 12.62 ± 2.14 g/l or 12.22 ± 2.14 after excluding an outlier). Average concentrations of circulating immune complexes (CIC), IgM, complement 1 inhibitor (C1i), C3c and C4 did not statistically differ between the groups. Dividing the patients' group into: breast or ovary cancer (BC), melanoma (M), digestive tract cancer (DT) and other neoplasms (ON) subgroups revealed that the IgG increase did not apply to the BC group. Relatively decreased CIC concentrations in the BC and DT group and an increased C1i in the DT group were found. Several diversities detected in the humoral immunity indices' distributions and correlations suggest activation of different mechanisms depending on the neoplasm types.

Key words: neoplasm(s); immunoglobulin(s); complement; immune complexes.

Introduction

Tumors can be antigenic for their host and elicit both humoral and cell-mediated responses²³. For several years it has been expected that the immunological phenomena accompanying neoplasia could be useful in cancer diagnostics (exclusion or support for malignant neoplasm diagnosis, detection of metastases, evaluation of the disease's stage, malignancy grading) and treat-

ment (antibodies against neoplastic antigens/cells, vaccines, activation of cellular immunity mechanisms). However, because of several reasons, neither the search for a single, specific factor playing the role of a neoplastic marker, nor the attempt to find a single molecule/molecule fraction or a cell kind has resulted in a striking breakthrough in diagnostics and neoplasm treatment¹⁵.

The lack of tumor cells' immunogenicity is one of

Abbreviations used: C – complement, C1i – complement 1 inhibitor, CG – control group, BC – breast or ovary cancer group, DT – digestive tract cancer group, M – melanoma patients group, ON – other neoplasms, CIC – circulating immune complexes, PEG – polyethylene glycol, SD – standard deviation.

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the most important problems to be overcome^{6, 24}. Another phenomenon responsible for blocking immunological rescue mechanisms is complement resistance, related to CD46, CD55 and CD59^{11, 13, 14, 21}.

Current studies are directed to analyzing the correlations among the different factors regulating humoral and cellular reactions, and between the effectiveness and progress of the two types of immunological reaction. It seems reasonable to postulate that the levels of the serum immunoglobulins and other humoral immunity indices and their correlations can be better suited to characterize antineoplastic resistance¹⁸. This kind of study can possibly also be used to distinguish the presence or lack of immunogenicity of tumor cells.

Therefore, selected indices of humoral immunity in a group of 30 patients qualified for surgical treatment of neoplastic disease, in generally good condition, without signs of metastasis, are analyzed in the present paper. Concomitantly, the same kind of studies were performed with a group of 10 persons without malignancy.

Materials and Methods

The studies were performed with sera from 30 neoplasm patients qualified for surgical treatment (blood samples were drawn before surgery) and 10 persons without any malignancy. The oncological patients were later divided into 4 subgroups, depending on the affected organ: breast or ovary cancer (BC), digestive tract (DT), i.e. stomach or colorectal cancers, melanoma cutis (M) and other neoplasms (ON), i.e. lymphoma and liposarcoma. All these neoplasms were of stage I or II according to clinical staging as described by BERNER et al.¹. The neoplasm patients, who were between 38 and 85 years old, were treated in the Department of Surgical Oncology, Medical University of Łódź, between February 25, 1997, till June 6, 1997, and this was the first antineoplastic treatment in their case histories. Pre-operative estimations were confirmed during operation.

Persons with no malignancy made the control group (CG); these were volunteers recruited from the outpatients complaining of mild heart symptoms. The characterization of the groups is given in Table 1.

Serum levels of C1 inhibitor (C1i), C3c, C4, IgG and IgM were quantified with the radial immunodiffusion method using Sanofi Pasteur RID test kits. All the measurements were performed in duplicate according to the instructions of the manufacturer. At the end of the diffusion period, the diameters of the precipitation rings were measured with a suitable device allowing ± 0.1 mm accuracy. Comparison with reference values gave the sample concentration readings of the measured factors. Circulating immune complexes (CIC) were estimated with the polyethylene glycol (PEG) precipitation test⁴. Briefly, the sera were diluted 1:25 in borate buffer, pH 8.4 (250 μ l serum added to 6 ml buffer). The diluted serum was mixed with an equal volume of 3.5% PEG solution (4 ml serum + 4 ml PEG solution). Next, the mixture was incubated at 4°C for 18 h, then centrifuged at 16 000 g for 40 min. The supernate was decanted and the precipitate was washed with the PEG solution, then suspended in 5 ml of 0.1 N NaOH and incubated in a shaking water-bath at 25°C. The optical density of the solution at 280 nm gave information on the protein concentration (0.1 optical density unit was read as 0.07 mg/ml of CIC protein).

The results were analyzed with the Q-Dixon's test to detect out-liers and exclude them when calculating average values²⁸. Next, average values and standard deviations (SD) were calculated and plotted using the Excel computer program. In the figures, the error brackets bars represent SD. Variances were compared by means of the F-Fisher-Snedecore's test²⁸. In case of statistical significance of the differences, as compared with CG, calculated by means of the F-test²⁷, asterisks are used for indicating this fact in the figures.

For the comparison of average values, the non-paired Student's *t*-test was used (Excel program) and the detected statistical significance of the differences are shown with "p <" or "p =" the appropriate value, while

Table 1. Characteristics of the studied groups of persons

Group	Diagnosis	Number of persons	Mean age	Men	Women
BC	breast cancer	12	57	0	13
	ovary cancer	1			
DT	cancer of stomach	2	66	6	5
	colorectal cancer	8			
	other digestive tract cancers	1			
M	melanoma	4	63	1	3
ON	other neoplasms	2	63	1	1
CG	control group	10	52	6	4

in case of insignificance of the differences, “ $p > 0.05$ ” or no information on p is indicated in the appropriate place on the figures.

Correlations between the distributions of the values were calculated with the Excel program and their significance was assessed according to ZAR²⁷.

Results

In the neoplasm patients the average IgG level was higher (18.16 ± 5.10 g/l) than in the control group (12.62 ± 2.14 g/l), $p < 0.0023$. After excluding outliers, the neoplasm group remained unchanged while the IgG level in the CG was 12.22 ± 2.14 g/l. Average values of CIC concentrations were lower in the DT ($p < 0.0021$) and BC ($p < 0.01$) groups than in the CG (Fig. 1). Additionally, by using Student's t -test, higher CIC concentrations in the M group, compared with DT group, appeared statistically significant ($p < 0.03$) (Fig. 1).

The average results of IgG measurements for the DT group ($p < 0.002$) and the M group ($p = 0.00054$), shown in the Fig. 2, appeared higher than in the CG. Surprisingly, IgG concentrations in the BC group were lower than in the M group ($p < 0.000008$) and also lower than in the DT group ($p < 0.0047$).

Another immunoglobulin assay, quantifying IgM levels, showed an increased average concentration of this fraction in the BC group compared with the DT group ($p < 0.024$). No significant differences between oncological patients and the CG were detected (Fig. 3).

The results of the measurements of complement factors are summarized in Fig. 4. Only C1i was shown to increase in one of the patient groups (DT) significantly ($p < 0.004$), as compared with the CG. This increase in

C1i in DT patients also appeared significant in comparison with the BC group ($p < 0.0045$).

No significant differences were observed between the studied groups' C3c or C4 average levels (Fig. 4). Applying F-Fisher-Snedecor's test allowed the detection of differences (indicated with asterisks) between variances calculated for IgG concentrations in the DT and BC groups ($p < 0.02$).

As suggested in the Introduction, concomitant changes in the different parameter levels can be of special meaning, showing a generalized picture of humoral immune reactions (Table 2). In the CG, a positive correlation between CIC and IgG levels appeared significant ($r = 0.694$; $p < 0.05$). In the DT group, significant positive correlations could be detected between C1i and C3c ($r = 0.652$; $p < 0.05$) and between IgM and C4 ($r = 0.634$; $p < 0.05$).

Characteristic of the BC group were positive correlations between the levels of IgG and C3c ($r = 0.810$; $p < 0.001$), C1i and C4 ($r = 0.685$; $p < 0.01$) and between C3c and C4 ($r = 0.634$; $p < 0.05$) (Table 2). In melanoma (M) patients' sera, CIC and IgM concentrations were negatively correlated ($r = -1$; $p < 0.002$) (Table 2). Since only 2 patients were included in the ON group, we did not take their results into account while calculating the significance of differences between the groups.

In neoplasm patients, no significant decrease in humoral immunity indices was detected. High average levels of IgG, IgM and complement factors seem rather to indicate an activation of humoral immunity. The significant differences in the levels of the studied indices and the observed correlations within the groups may suggest differences in the mechanisms of humoral immunity activation depending on the neoplasm type.

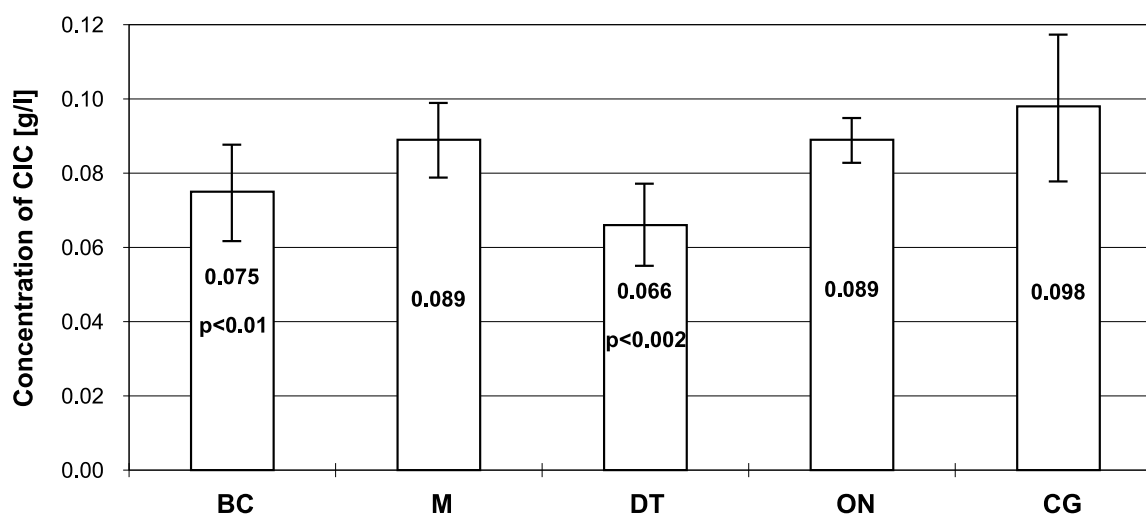


Fig 1. Average CIC concentrations (norm < 0.084 g/l); statistical significance of the differences as compared with the concentration in the CG group by means of the Student's t -test shown by “ $p < \dots$ ” values

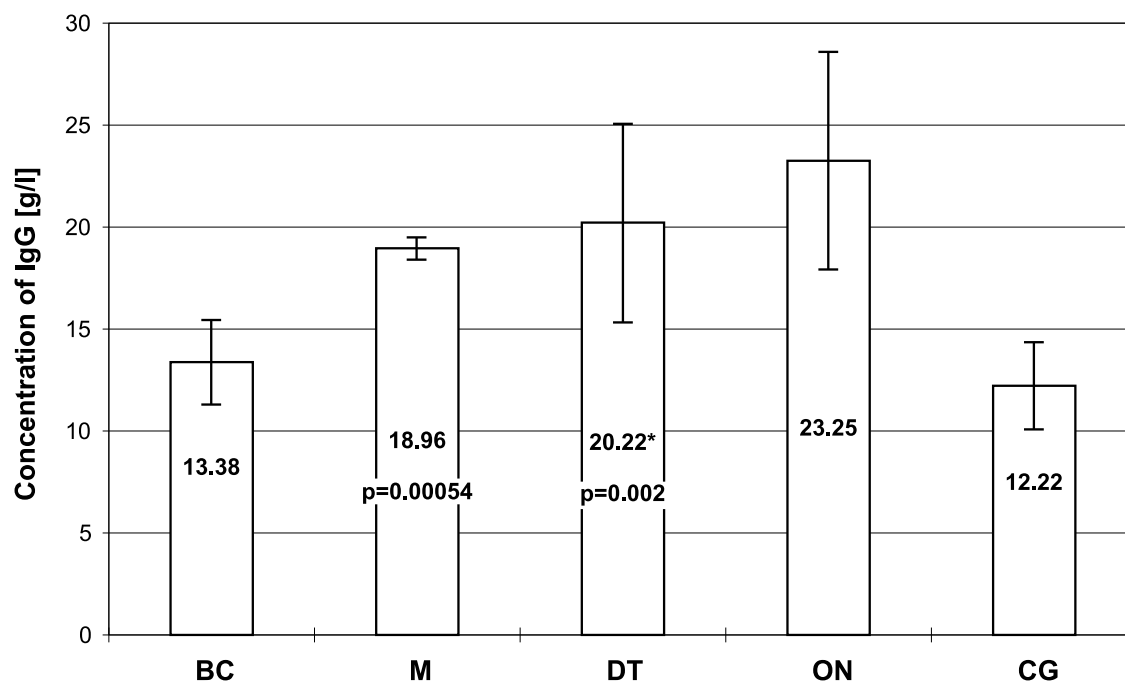


Fig. 2. Average concentrations of IgG (norm 8.13–18.96 g/l); statistical significance of the differences as compared with the concentration in the CG group by means of the Student's *t*-test shown by “p=...” values; * Significant difference of variances as compared with the CG value by means of the F-Fisher-Snedecore's test

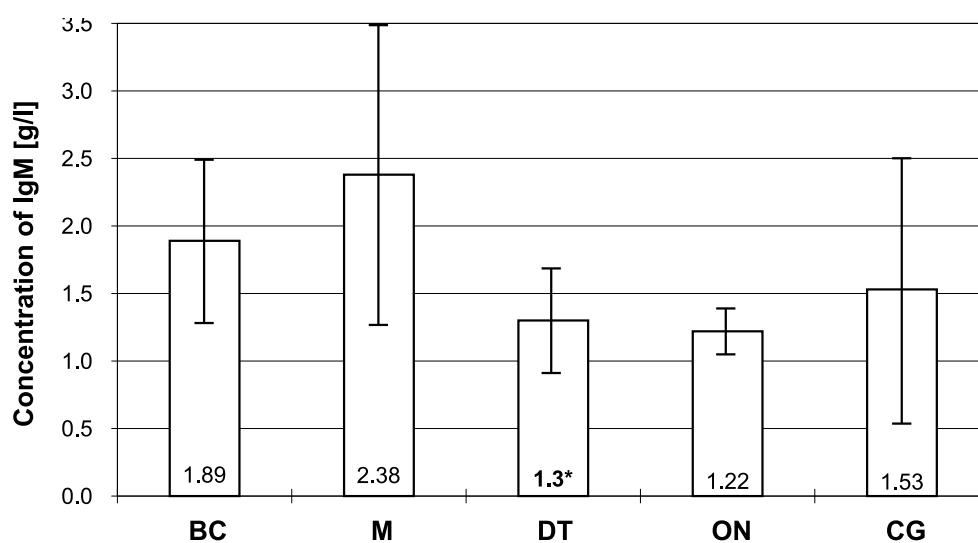


Fig. 3. Average IgM concentrations (norm 0.46–2.06 g/l); * Significant difference of variances as compared with the CG value by means of the F-Fisher-Snedecore's test

Table 2. Significant correlations between the studied index levels

Group	Indices	Coefficient of correlation (r)	Significance
CG	C1C/IgG	0.694	p<0.05
DT	C1i/C3c	0.652	p<0.05
	IgM/C4	0.634	p<0.05
BC	IgG/C3c	0.810	p<0.001
	C1i/C4	0.685	p<0.01
	C3c/C4	0.634	p<0.05
M	C1C/IgM	–1.000	p<0.002

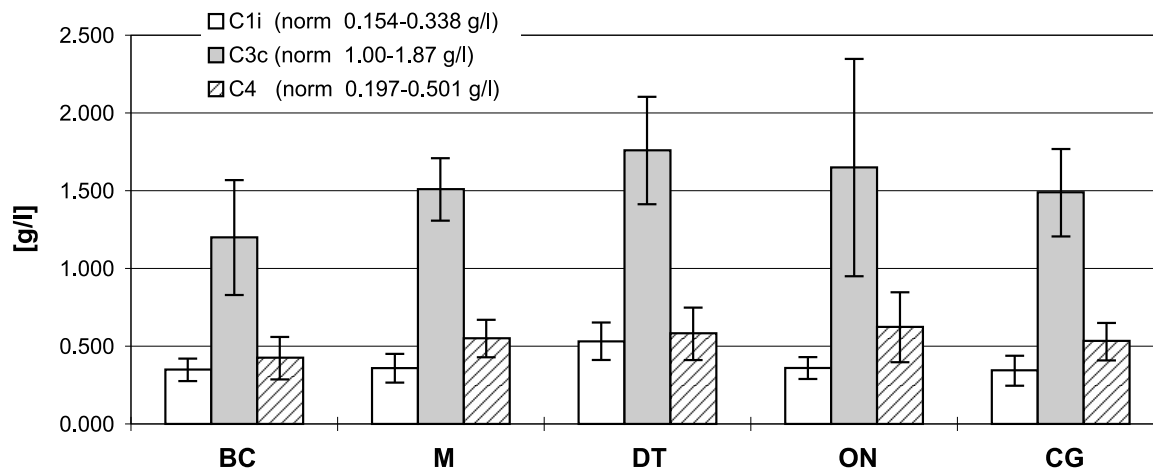


Fig. 4. Average concentrations of complement factors

Discussion

Detailed data concerning differences among mean values of CIC concentrations in several types of neoplasms were already described in 1979²³. Statistical analysis applied to compare the frequency of CIC in the studied populations revealed significant differences between patients with melanoma, colon carcinoma, osteogenic sarcoma, breast carcinoma and healthy persons²³. Surprisingly, there were studies in which no differences in CIC levels between neoplasm patients and healthy persons were found^{2, 3}; however, post-mastectomy CIC levels were found to be significantly lower than the premastectomy ones³.

Most available papers give support to the thesis that serum immunological complex concentrations in neoplasm patients are higher or at least equal to the levels observed in persons without any malignancy^{10, 23}. On the other hand, it is well known that there are diseases other than neoplasms, i.e. collagenoses or some inflammatory diseases, shown to proceed with increased concentrations of CIC, quite often with levels meaningfully exceeding the highest values observed in neoplastic patients^{25, 26}. Among these non-malignant diseases, the best correlation with disease activity was seen in systemic lupus erythematosus and infective endocarditis, where serial immune complex determinations were clearly of value in monitoring therapy²⁵.

Our results seem not to support reports on increased CIC concentrations in neoplastic patients compared with healthy persons. This may result from different reasons, such as neoplasm type or the early stage of neoplastic disease, but it may also be a result of the fact that we did not compare sick people with healthy ones, but neoplastic patients to persons without malignancy. It should be additionally noted that neoplastic patients

are not usually excluded from a study if suffer from another disease besides cancer. However, according to our knowledge, no person suffering from an acute inflammatory disease (flu, angina, or the ones mentioned above) was included in any of the groups studied.

While it is very difficult to define health, there are several criteria describing stages of neoplastic diseases. As far as neoplasms and CIC are concerned, the most striking and common literature observation seems to be a positive correlation of CIC concentrations and disease stage: patients with extended lung cancer had more immune complexes than patients with limited disease^{9, 17}. In the case of breast cancer, CIC levels of patients with stage III cancer were significantly higher than those of patients with stage I or stage II cancer³.

Our studies, reported in the present paper, were performed only with sera from non-metastatic patients with operable neoplasms (stage I and stage II). This selection of patients could be the reason for the lack of correlation of CIC levels with the presence of the neoplasm, though, on the other hand, some other authors have already reported a lack of correlation between breast cancer stage and CIC level²².

The diversified data on CIC frequencies in neoplasm patient's populations may also depend on the method applied to detect and quantify them^{19, 23}. The PEG method detects CIC of another proportion of antibody to antigen molecules than, for example, the latex agglutination inhibition (LAI) test⁵. The PEG method detects complexes which are formed in antigen excess, while the LAI test can detect those formed over an extended range of antigen-antibody ratio. An additional problem arises from the fact that CIC can be bound by neoplastic cells and, thus, their serum level decreases⁵. CIC in human cancers are known to be very heterogeneous in size and composition, with a very diversified

potential for complement factor and immunoglobulin (IgG or IgM) binding, so CIC can contain very diversified proportions of all these constituents²⁰.

We consider the possibility that the significant difference in the average CIC contents between the M and DT groups observed by us can be illustrative of meaningful differences between melanoma and digestive tract cancer in this respect. However, it cannot be excluded that the observed differences are a result of the enormous diversity in the sizes and compositions of CIC in all neoplasms reported by other authors²⁰, and so analyses in very large groups of patients could give a completely different result (for example, no difference between M and DT patients).

It seems that the role of the complement system in the organism's antineoplastic activity can be more meaningful than has been widely suggested. Complement-mediated cytotoxic injury results in a reduction of tumor cell mass and a subsequent decrease in CIC⁵.

In surgically excised tumors, all the breast cancer cells were found to contain molecules of CD59, a molecule protecting cells from the destructive action of the membrane attack complex of complement (MAC)¹¹. It appears that, to date, the interdependences between CIC and other humoral immunity indices in neoplastic diseases have not been conclusively elucidated and that, for example, the lack of correlation between C3 and C4 levels in breast cancer metastatic patients³ seems difficult to explain.

A more characteristic alteration than changes in mean values appears to be the increased diversity of levels of humoral indices in neoplastic patient populations compared with persons without any malignancy.

The course of monocyte C1i synthesis is stimulated by immune complexes as well as interferons¹⁶. It has been reported that malignancy induces increased C-reactive protein⁸ and C1 esterase inhibitor levels^{8, 12}. In our studies, a significant increase in C1i was found only in the DT group as compared with CG. This change appeared significant in the DT group, both relative to the CG group as well as to the BC and M groups, thus suggesting that different neoplasms can cause diversified effects on C1i production. We did not find any correlation between serum immunological complex levels and C1i, neither in the CG nor in neoplastic patients.

Our results do not support the observations of JANSSEN et al.¹² on an IgG level correlation with C1i concentration in digestive tract neoplasms. However, in our studies on the DT group, patients with different digestive tract localizations of the cancer were included, while the JANSSEN et al. studies¹² concerned only gastric carcinoma patients.

IgG level is not necessarily a simple reflection of the antineoplastic immunity state at a given time, though a growing tumor can induce an increase in serum IgG concentration. We suggest that our results of a low IgG level in the BC group, relative to its concentrations in the DT and M neoplasm groups as well as in the CG, accompanied by low serum CIC concentration and complement factor levels, can indicate absorption of IgG and complement compounds containing IC on neoplasm cell surfaces. These reactions lead to vasculitis and, thus, are responsible for spontaneous tumor destruction more in the BC group than in the other neoplasm patient populations studied by us. These suggestions are consistent with the mechanism of breast tumor destruction proposed by other authors⁵.

An increased IgG in the DT and M groups, together with a low CIC level, can indicate an increase in unbound, natural antibodies or a presence of CIC undetectable by the PEG method. Free, unbound antibodies can belong to anti-idiotypic antibodies, which can be responsible for the so-called "immunological promotion" during a neoplastic disease and immunologically support neoplasm growth. However, significant differences detected between the DT or BC groups relative to the CG indicate a diversity in the distribution of results in these populations and, thus, suggest that IgG plays a significant role in tumors of these localizations.

Significantly higher levels of IgM in the BC, as compared with the DT group, can suggest a predisposition for the production of this immunoglobulin in breast cancer. In the available literature, no report on such an observation has been found.

We suggest that the distributions of the individual serum immunoglobulins and the different humoral immunity indices levels' correlations can be better suited to characterize antineoplastic resistance than the average values (Table 2). Among the numerous differences in the distributions of the values studied as calculated with Fisher's F test, the most significant was between the BC and DT groups in IgG levels ($p < 0.02$).

A positive correlation between IgM and C3c in both the M and the DT groups can imply the formation of IgM- and complement-containing CIC in these groups.

Significant distribution differences and correlations of the studied humoral immunity indices in different groups of neoplasm patients may indicate differences in the mechanisms of humoral immunity activated depending on the neoplasm types.

We are planning to extend our studies in two directions. The first is to confirm or exclude the correlations and trends indicated in this paper while performing analyses using larger groups of patients. The second line

of study is to assess the effects of the studied sera introduced into experimental animals and their potency to induce paraneoplastic effects⁷.

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