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Efficacy of preoperative immunoprophylaxis in patients with neoplastic diseases

I. Phagocyte activity and bactericidal properties of peripheral blood granulocytes

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SUMMARY

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To evaluate the immunological system it is essential to examine neutrophils, their quantity, phagocyte and bactericidal activity. Patients with a neoplasm and at chemotherapy are a group at risk for infections. Attemps to prevent postoperative infectious complications are fully justfied.

Key words: antihomotoxic drugs; neoplastic diseases; immunodeficiency; cell-mediated phenomena

STRESZCZENIE

Skuteczność okołooperacyjnej immunoprofilaktyki pacjentów z chorobą nowotworową

I. Aktywność fagocytarna i właściwości bakteriobójcze granulocytów krwi obwodowej

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Zbadanie aktywności układu immunologicznego to przede wszystkim zbadanie granulocytów obojętnochłonnych, ich liczby, aktywności fagocytarnej i właściwości bąkteriobójczych. Pacjenci z chorobą nowotworową, zwiaszcza w przebiegu chemioterapii, stanowią grupę ryzyka wystąpienia infekcji. Postępowanie mające na celu zapobieganie zakażeniom w przebiegu pooperacyjnym jest w pełni uzasadnione.

Słowa kluczowe: leki antyhomotoksyczne; choroba nowotworowa; odporność komórkowa; zaburzenia odporności

The development of a neoplasm is a multi-stage and long-lasting process as carcinogenic factors are not a direct cause of the neoplasm formation. These factors induce many indirect factors such as excessive number of free radicals continually destroying cell membranes, deoxyribonucleic acid and other cell structures (1, 4, 12, 16).

The immune system is a complex of strictly cooperating cells and particles directed at the recognition and elimination of "strange antigenic" substances. It is effective in relation to many different pathogenic microorganism, their toxins and also eliminates neoplatic cells (10, 22). The development of a neoplasm is accompanied by a certain immunological activity against neoplastic cells which is not always effective (22).

This reactivity means the development of antibodies against neoplastic cells or the increase of the lymphocyte ability for cytotoxic activity against neoplastic cells (6, 7, 9). However, not all immunological processes in patients with a developing neoplasm are favourable. Some may even promote neoplastic development.

Immunosuppression caused by drugs, virus infections, extensive injuries, increase of suppressive cell activity, mass release of antineoplastic antigens, and creation of immunological complexes composed of neoplastic antigens and specific antibodies belong to such mechanism (13). Because of that these patients are a group at high risk of infections which is related both to the neoplastic process and antineoplastic agressive chemotherapy.

Investigations concerning the inability of phagocytic cells to react to chemotactic stimuli and disorders in killing functions are particularly important. Low nonspecific cell resistance makes it difficult or impossible to control the infection (32).

Operative injury decreasing immunological reactivity is another infection-favouring factor.

MATERIALS AND METHODS

Sixty one patients, aged from 30 to 77 years, with *Ca mammae* (some of them also after chemotherapy), *Ca recti, Ca ventriculi* were divided into two groups: experimental and control (Tab. 1).

Table 1. Structure of investigated groups

	Control group (without immunostimulation) (n=23)			Investigated group (with immunostimulation) (n=23)		
	F	M	Σ	F	M	Σ
Breast carcinoma	14	–	14	13	–	13
Abdominal cavity organs carcinoma	4	5	9	11	14	25
Σ	18	5	23	24	14	38

F – females, M – males.

In the experimental group, patients received Gripp-Heel and Engystol'N preparations intramuscularly every day for 5 days (increasing the efficacy of the treatment). On the first day of hospitalization, after drug administration and a week after the last injection different immunological parameters were estimated.

Venous blood taken the elbow vein in fasting state in the morning was used for investigations. Most investigations were carried out on whole blood with anticoagulant (heparin, natrium citrate, dipotassium versenate), the rest was carried out on serum after fibrin coagulation.

Phagocyte activity of neutrophils, leucocytosis and NBT test were estimated in whole blood. The ability of granulocytes for bacteria phagocytosis, infracellular killing and generating oxydative burst were estimated using the flow cytometry assay. Spontaneus and stimulated chemoluminiscence of granulocytes was done with formylo-methionylo-leucylo-phenolalanine (fMPL), phorbol 12-myristate 13-acetate (PMA) and zymosan.

Investigations of humoral reaction included estimation of antibody titres against influenza viruses A and B (directed against neuraminidase and haemagglutinin).

In a morphological study leucocytosis was measured.

Mean values of the estimated parameters are presented graphically marking the significances of differences both among individual groups in successive studies and in relation to the first examination of each group. As the examined groups were not homogenous in view of age and sex, they were treated as a whole.

Statistical analyses of the obtained results were done using the statistical package: Statistica (Stat Soft Inc.) and Excel (Microsoft Co.)

RESULTS

The increase in the granulocyte number in immunostimulated patients was observed in the investigations. The rise of the functional activity of neutrophils was accompanied by the growth of granulocytes which was observed in NBT test, in phagocytosis investigations and phagocytic index, in chemoluminiscence test and phagotest.

The change of neutrophil defensive functions was observed using the test with nitrotetrazol blue (NBT) which in healthy people is destroyed to formazan by macrophages in pulmonary alveoli. The ability for intracellular killing depends on the normal production of oxidizing factors and other mechanisms (21, 23).

Figure 1 presents metabolic activity of neutrophils. Highly significant increase ($p<0.001$) of neutrophil metabolic activity was observed in patients after immunostimulation operated on because of carcinoma of abdominal cavity organs. In patients with breast carcinoma, the neutrophil metabolic activity was significantly higher ($p<0.01$) in the group treated with antihomotoxic preparations.

Phagotest of the phagocyte activity of neutrophils in peripheral blood after contact with model *S. aureus* strain ATCC 25923 (2, 3) in the group after immunostimulation, in patients operated on because of a neoplasm in the abdominal cavity and highly significant in patients with breast carcinoma (Fig. 2).

Phagocytic index was also defined, i.e. the number of bacteria phagocytized by one macrophage in at least 100 phagocytes (18, 20). The value of the phagocytic index was defined according to the formula:

$$\text{phagocytic index} = \frac{\text{the number of phagocytized bacteria}}{\text{the number of phagocytizing granulocytes}}$$

Figure 3 shows the changes of the phagocytic index, patients were divided into patients with breast carcinoma and those with abdominal cavity carcinomna. A Significant tendency of the phagocytic index growth in Experiment I was found only in patients after immunostimulation operated on because of an abdominal cavity neoplasm. An extremely significant increase ($p<0.001$) was observed in these patients in the 3rd week of hospitalization.

The quantity of neutrophils is dominating among the macrophage system in peripheral blood. They function as forces of fast response in the organism. The future of investigations on neutrophils will consist in replacing the present allowing an effective evaluation of several parameters from a small sample at the same time (24, 26). Flow cytometry assay enables fast, quantitative and qualitative measurement of physical and biological properties of cells when their suspension is flowing through the measuring system.

The investigation of neutrophil chemoluminiscence deepens the analysis of the behaviour of these cells after immunostimulation. The intensity of granulocyte chemoluminiscence was investigated without stimulation *in vitro* or stimulation *in vitro* with PMA, fMLP and zymosan (11, 15). Two values were estimated: the peak-corresponding to the highest light intensity and the sum which is equal to 15 points of light.

Statistically significant increase of values was observed in patients after immunostimulation. Chemoluminicent activity was observed in neutrophils without stimulation *in vitro* in patients operated on because of breast and abdominal cavity carcinoma.

In experiment III a significant increase ($p<0.01$) of chemoluminiscence after neutrophil stimulation *in vitro*

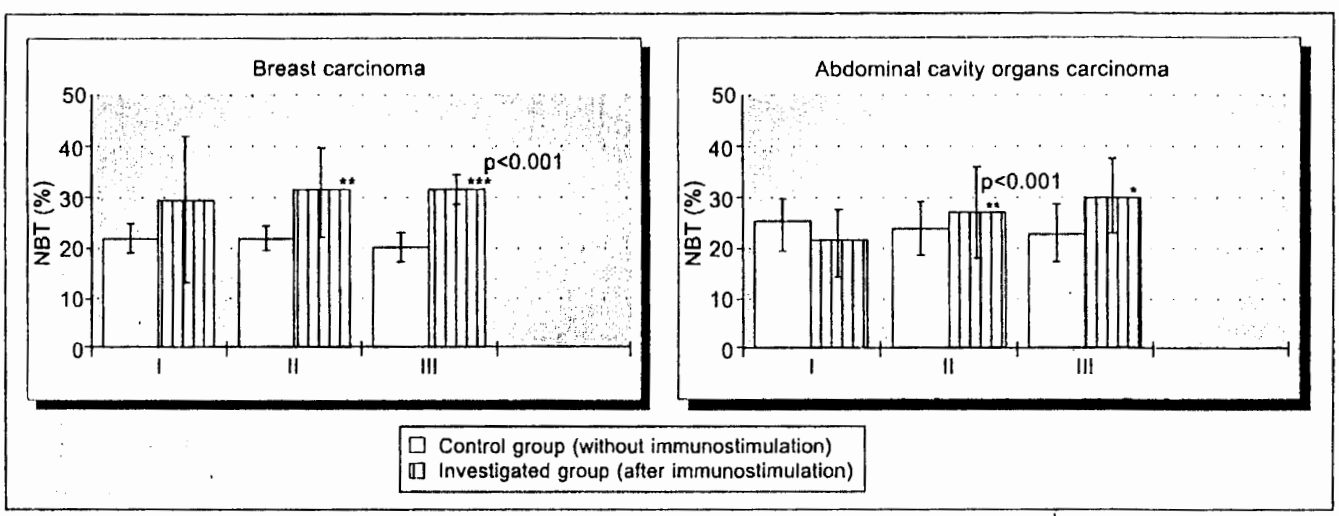

Fig. 1. Mean value of NBT test in patients with breast carcionoma and abdominal cavity organs carcinoma without and after immunostimulation.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

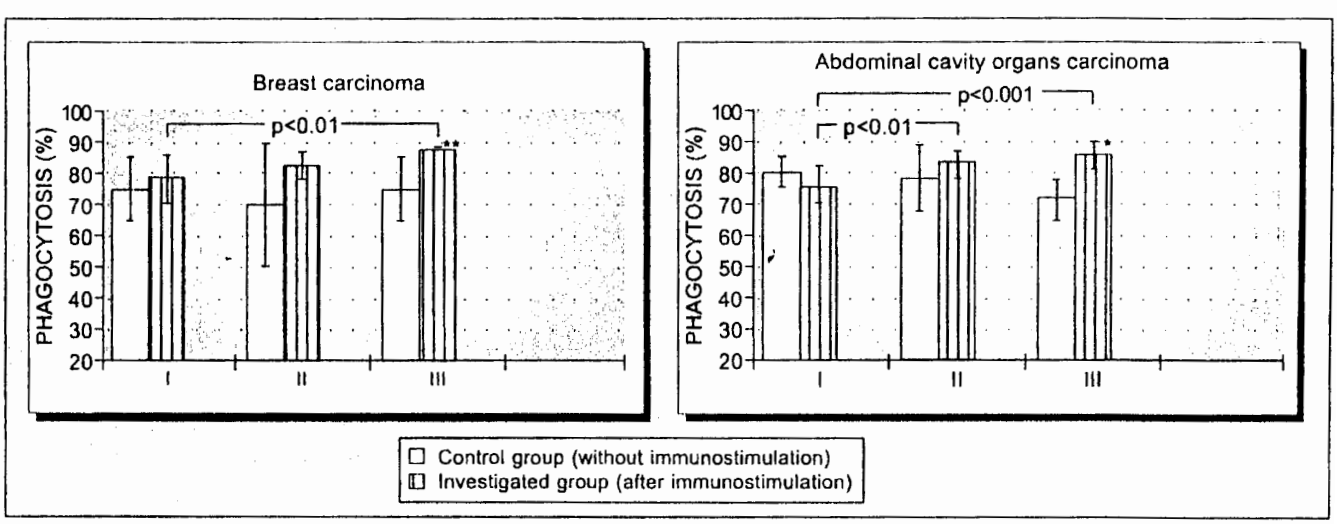

Fig. 2. Mean values of phagocyte activity of neutrophils blood in patients with breast carcinoma and abdominal cavity organs carcinoma without and after immunostimulation.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

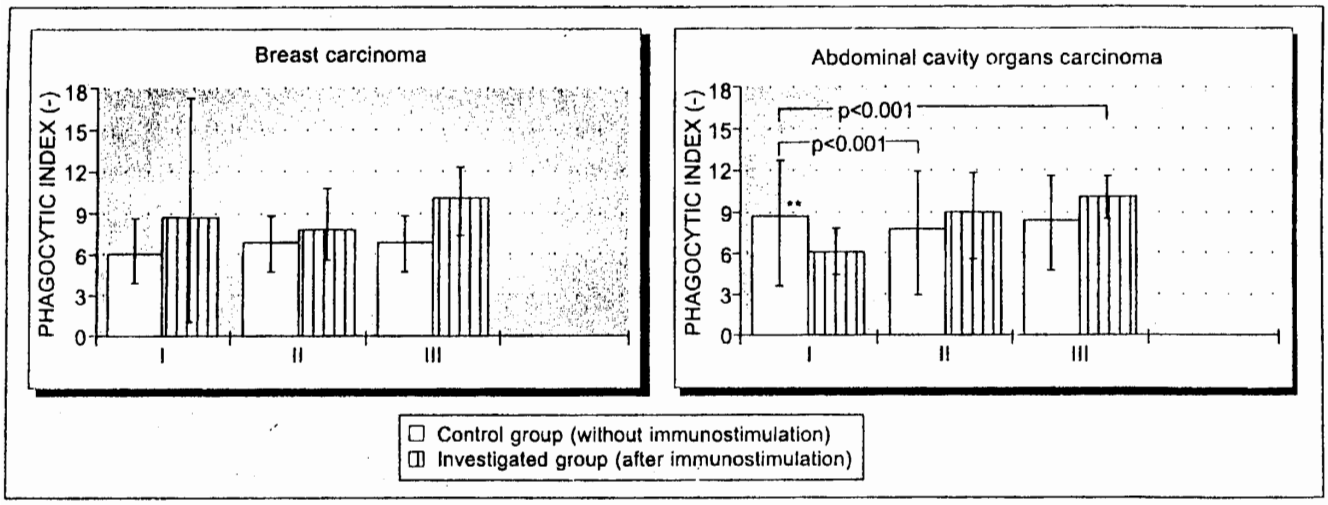

Fig. 3. Mean value of phagocytic index of neutrophils in patients with breast carcinoma and abdominal cavity organs carcinoma without and after immunostimulation.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

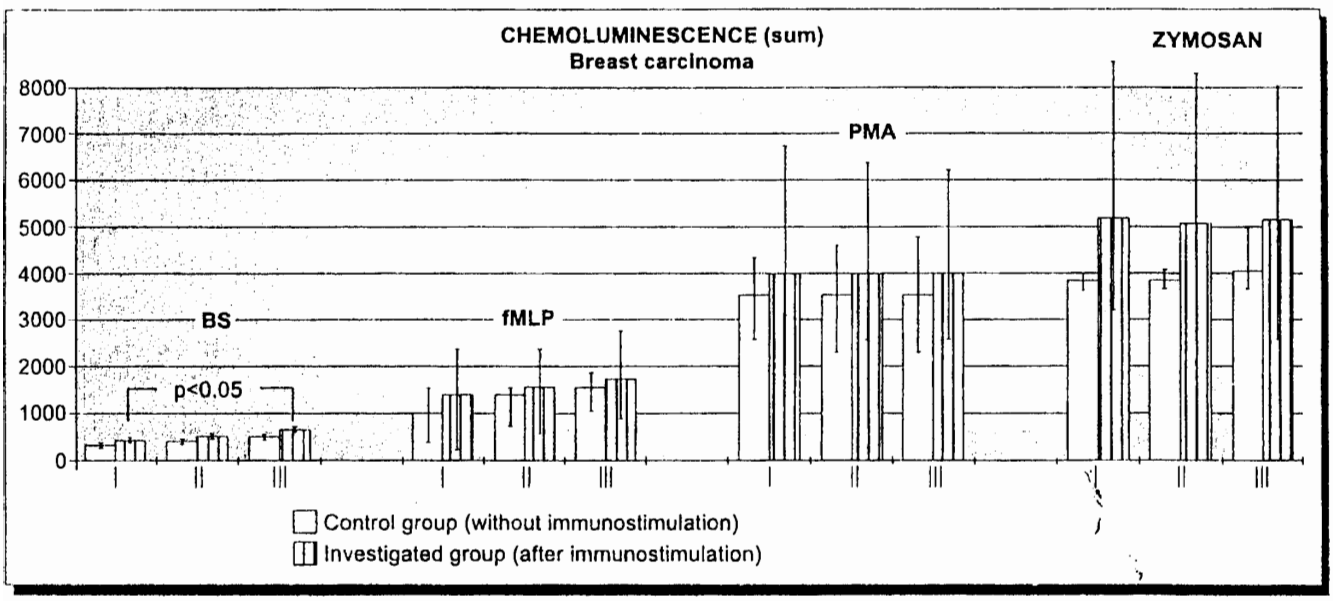

Fig. 4. Mean values of granulocytes chemoluminescence (sum) in peripheral blood in patients with breast carcinoma.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

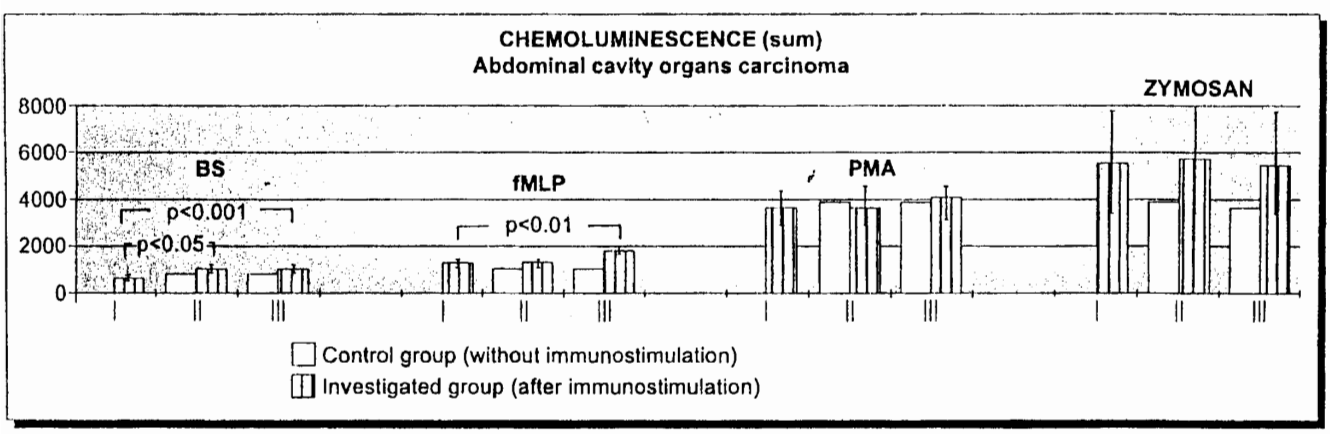

Fig. 5. Mean values of granulocytes chemoluminescence (sum) in peripheral blood in patients with abdominal cavity organs carcinoma.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

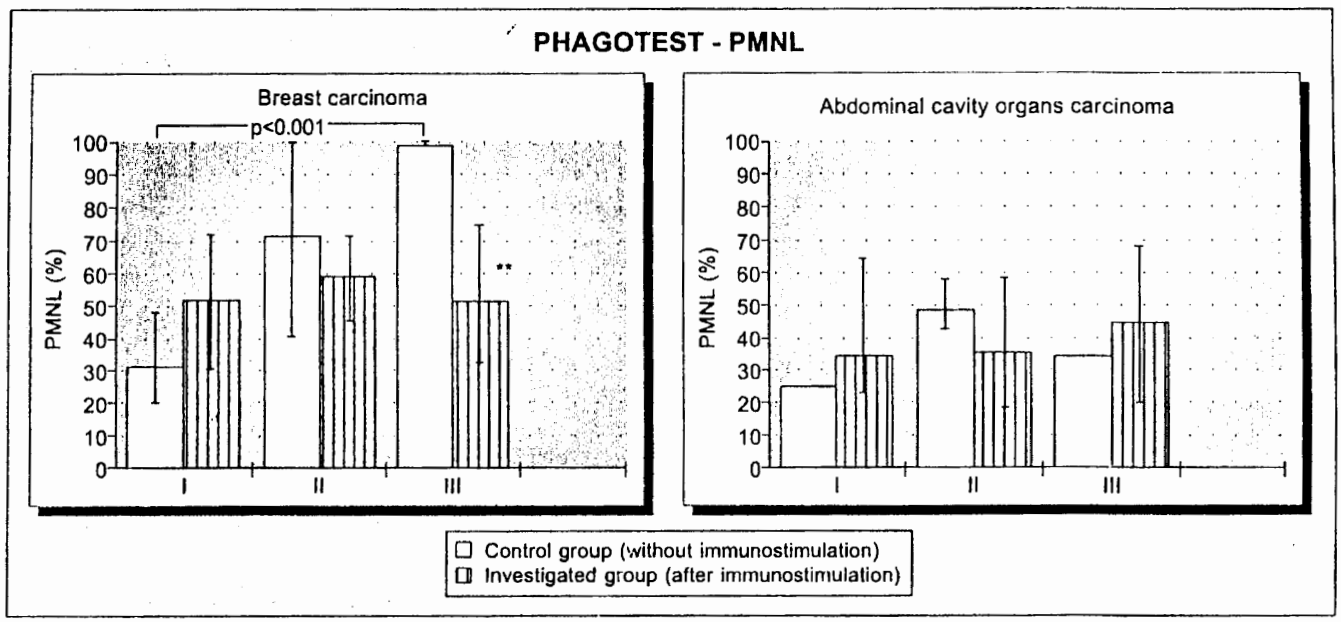

Fig. 6. Mean values of granulocytes taking part in bacteria phagocytosis.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

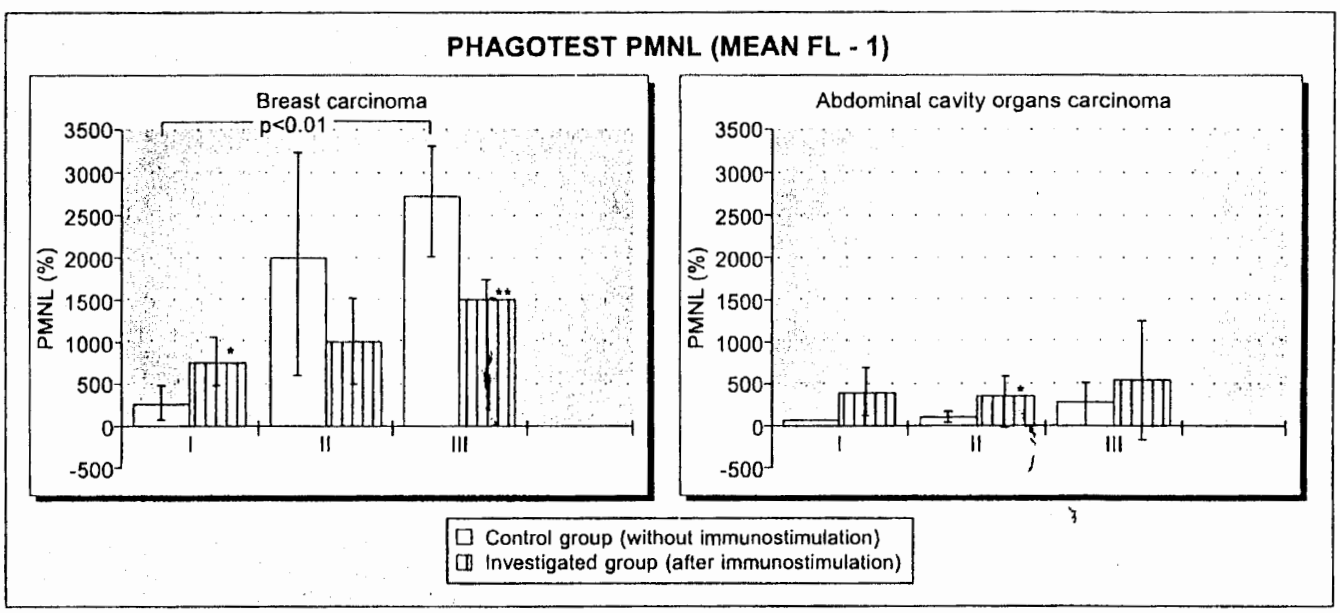

Fig. 7. Mean number or bacteria phagocytized by macrophage – granulocyte.

*p<0.05; **p<0.01; ***p<0.001 (related to groups without and after immunostimulation)

with fMLP in patients with abdominal cavity carsinoma was obseved. At PMA stimulation no statistical significance of either the sum or the peak was observed. After zymosan application, mean values of chemoluminiscence in patients after immunostimulation were statistically higher than in patients without immunostimulation at each stage of investigation, however, no changes were shown under observation.

PHAGOTEST was the last test analysing the percentage of granulocytes active in killing bacteria cells and the mean number of bacteria phagocytized by a macrophage. The phagocyte activity of granulocytes is dependent on factors reacting with receptors of these cells in early stages of phagocytosis.

In successive investigations a significant increase of granulocytes (PMNL) taking part in phagocytosis in patients without immunostimulation was observed. This tendency was shown particularly in patients with a breast neoplasm where a highly significant increase ($p<0.01$) of the granulocyte number was observed in experiment III. Similar changes were observed in the same group of patients in relation to the mean number of bacteria phagocytized by one granulocyte (PMNL MEAN FL-1).

The number of neutrophils in peripheral blood may also depend on the progression of the neoplastic disease. The growth of a neoplasm is usually accompanied by a decrease of the neutrophil number in

peripheral blood. It proves that the neoplastic disease is accompanied by immunosuppression and therefore immunostimulating preparations were used.

Antibiotic prophylaxis in surgery is vital in preventing perioperative infections. It applies not only to planned operations in the abdominal cavity, chest, to open and dirty wounds but also to so called "clean operations". Besides obvious advantages of such procedures, i.e. operating (traumatizing of tissues) in the environment with the level of antibiotics sufficient to stop the growth of microorganisms in the wound, there are also disadvantages – the probability of developing resistant strains in the ward.

Immunoprophylaxis, i.e. use of different parameters with immunomodulating or immunostimulating properties in the preoperative period is the alternative. Propylactic administration of preparations from this group in patients with extensive injuries is important.

Infectious complications in the respiratory system are most common in standard conditions of health care. Pneumonia is a particularly dangerous infection, so is septicaemia – another infectious complication.

The investigations of the prophylactic efficacy of preparations from this group are based on the functional condition of the patients', immune system. As there is a relationship between the condition of the system and the possibility of infectious complications (17, 19, 27, 31).

Reports on the efficacy of prophylaxis against pneumonia and septicaemia by preparations obtained from bacteria – lysates and other preparations such as OK. -432, 1-3 polyglucose, stress their good results. Lower frequency of pneumonia and septicaemia was observed in patients treated with these preparations (5, 8).

Patients operated on because of neoplastic diseases are a group at risk. Both previous treatment with preparations with unfavourable influence on the immune system such as cytostatic drugs and irradiation increase the probability of infectious complications (14, 25, 29).

General anaesthesia and operation itself disturb neutrophil functions, lower their ability to adherence, chemotaxis and phagocytosis [28]. it was also shown in our results where an operation had an unfavourable influence on the investigated parameters which was demonstrated in the lowering of NBT values in the 2nd week of hospitalization in patients without and after immunostimulation. Similar observations apply to the phagocytic reaction and phagotest in relation to the mean number of bacteria phagozytized by one neutrophil in patients with a neoplasm of organs in the abdominal cavity after immunostimulation.

Increase of the number of granulocytes was observed in patients after immunostimulation. The quantitative increase was accompanied by a functional increase of neutrophils observed in all tests. The highest values were observed in experiment III.

CONCLUSIONS

1. Postoperative trauma causes a decreas of values of the investigated parameters which are characterised by a decrease in the number and metabolic activity of phagocytizing cells.
2. Gripp-Heel and Engystol'N used before operation show immunostimulating properties characterized by an increase of the number of cells involved in phagocytic reactions and their increased antimicroorganism activity.

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