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ORIGINAL ARTICLE

Preliminary study of mononuclear phagocytosis during breast cancer therapy

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Summary

Purpose: *To evaluate the eventual changes in the number and phagocytic functions of blood monocytes in breast cancer patients during surgical treatment and chemotherapy.*

Materials and methods: *The absolute and relative number of peripheral blood leukocytes and monocyte phagocytic functions were determined at the time of diagnosis (I), following surgery (II), during (III) and after chemotherapy (IV) in 30 patients diagnosed with breast cancer. The control group consisted of 30 age-matched healthy women.*

Results: *The mean number of monocytes was significantly lower in cancer patients at diagnosis, while they increased following surgery reaching the control values. There were no postchemotherapy changes in the number of monocytes. Monocyte phagocytic activity was decreased at the time of diagnosis. Following surgery, the capacity of phagocytosis (CP) recovered to normal values, but the index of phagocytosis (IP) remained decreased. During and after chemotherapy, as well as one year after surgery, the IP still remained decreased.*

Conclusion: *Our results showed that some properties of monocytes' phagocytic activity in cancer patients were decreased at diagnosis, returning back to normal range after surgical therapy. However, time is needed to confirm whether the alteration of IP may provide additional information when monitoring breast cancer patients.*

Key words: breast cancer, macrophages, monocytes, phagocytosis

Introduction

Mononuclear phagocytes play an important role in the immunobiology of neoplastic tissues. Virchow's observations from 1863 and Hardley's interpretation from 1907, opened many dilemmas associated with the interaction of malignant cells and leukocytes present in/around human tumors. Virchow thought that the presence of inflammatory cells pointed out that malignant tumor always appeared at sites of previous chronic inflammations. On the contrary, Hardley claimed that the presence of these cells was a sign of possible regression of the malignant process. Such opposed views have already pointed to the ambiguous role of leukocytes in human malignancies.

Phenotypic and functional examinations of leukocytes that infiltrate most of the human and animal malignant tumors have shown that these infiltrates are mostly composed of mononuclear leukocytes [1] although, at the same time, there is significant presence of polymorphonuclear leukocytes [2-4]. Tumor-infiltrating mononuclear leukocytes are composed of tumor-infiltrating lymphocytes and cells of the monocyte/macrophage lineage, so called tumor-associated macrophages (TAM) [5].

The level of macrophages in the neoplastic tissue is regulated by factors associated with the tumor cell itself [6-8]. There are very complex and mani-

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fold interactions between TAM and the malignant cell. TAM can inhibit tumor growth and destroy neoplastic cells, and on the other hand TAM can induce and promote tumor growth [9]. Results from recent research confirm the ambivalent nature of the relationship between TAM and the malignant cell [10,11].

In some studies, the phagocytic activity of TAM, observed through the index of phagocytosis, is directly correlated with tumor progression [12]. In the same way, the prognosis of a malignant disease may be directly correlated with the ability of TAM to phagocytose malignant cells. Some tumors, in which phagocytosis of malignant cells is noted (unlike those in which such activity does not exist), do not give distant metastases [13].

On the other hand, the number of monocytes in patients who have breast cancer can be equal, lower or higher compared with healthy controls [14]. Likewise, their functionality varies, from an increased [15] to a decreased [16] degree of phagocytosis. Therefore, some assays of monocyte function were unbalanced in certain types of cancer, but were fully normal in others and did not show consistent correlations with tumor type or stage.

Although mononuclear phagocytes of cancer patients have been extensively studied over the past decades, their pleiotropic, ambivalent function, as well as the prognostic relevance of their numerical and functional properties are still unclear and require further studies. The present study was designed to elucidate eventual changes in number and phagocytic function of monocytes in breast cancer patients before and after surgical treatment and chemotherapy.

Materials and methods

Patients

We analyzed a group of 30 female patients with breast cancer. The control group consisted of 30 age-matched healthy women. Patients with biochemical evidence of inflammation or known inflammatory disease were excluded from the study. The absolute and relative number of peripheral blood leukocytes and monocyte phagocytic function were determined at the time of breast cancer diagnosis (I), following surgery (II), during (III) and after chemotherapy (IV).

Preparation of peripheral blood monocytes

Mononuclear cells were isolated from heparinized venous blood using a density gradient (Lymphoprep 1.077, NYCOMED PHARMA AS, Oslo, Norway). Adherent monocyte monolayer was obtained using the plastic adherence technique [17]. The absolute and relative number of monocytes was determined by nonspecific esterase staining.

Phagocytosis assay

We used an assay developed by Vujanovic et al. with minor modifications [18]. In brief, the isolated monocytes were resuspended in Haemacel medium (Jugoremedia, Zrenjanin, Yu) at a concentration of 1 x 10^6 cells/400 ml. Heat-inactivated yeast particles, labeled with Neutral red (Merck, Germany) were then added at a 1:12 Effector: Target ratio, and cells were centrifuged at room temperature for 5 min at 50 g. The mixed suspension was incubated for 1h at 37° C. Non-ingested yeast particles were removed by washing twice with ice cold 0.02% EDTA. At least 300 cells per well were assessed, and each experiment used duplicate sample wells per condition. The average number of yeast particles ingested per one cell was defined as the IP. CP represented a number of yeast particles ingested per monocytes from 1 ml of blood (CP=number of monocytes/1 ml blood X IP).

Statistics

Statistical analyses were performed using commercially available software (SPSS version 8.0; SPSS Inc., Chicago, IL). Data were expressed as mean + SD. The distributions of data were evaluated for normality using the Kolmogorov-Smirnov test and then retested with x^2 test. Student's t-test was used for comparisons between two groups, for normally distributed parameters. For nonparametric variables, differences between two independent groups were determined by the Mann-Whitney U-test. Comparison between three and more groups of nonparametric variables was evaluated using the Kruskal-Wallis test. A p-value < 0.05, from two-sided tests, was considered statistically significant.

Results

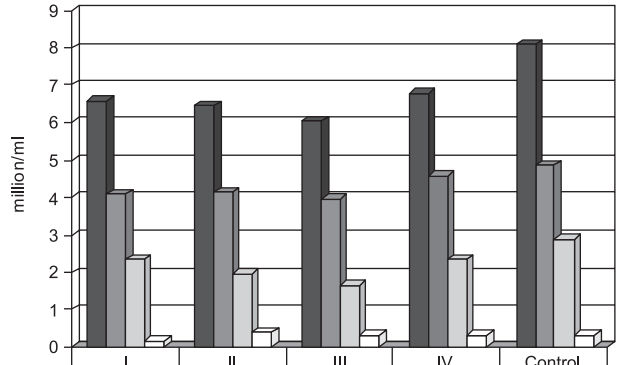
White blood cell count

At the time of diagnosis the total count of circulating leukocytes, as well as the absolute and relative number of polymorphonuclear cells and lymphocytes were lower in breast cancer patients compared to controls, but this difference was not statistically sig-

nificant. However, the mean number of monocytes was significantly lower in cancer patients (0.16 ± 0.06 *versus* 0.34 ± 0.15; $p < 0.001$). Comparing the samples taken after surgery and during chemotherapy no changes were observed in the number of total leukocytes, polymorphonuclear cells and lymphocytes, while monocytes increased following surgical treatment and chemotherapy, reaching the control values (Figure 1).

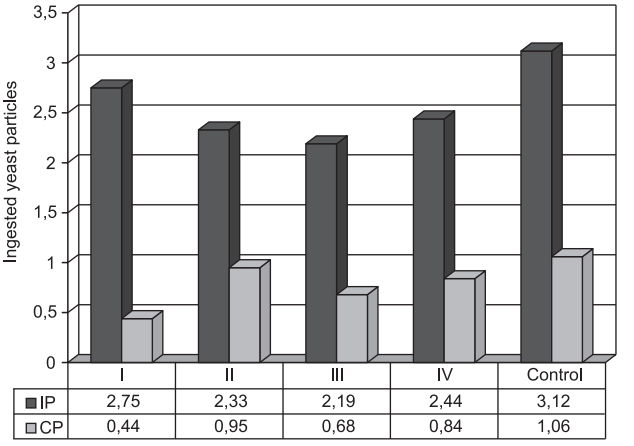
Phagocytosis assay

The phagocytic activity of breast cancer patients' monocytes was decreased at the time of diagnosis. We noted a two-fold decrease in CP (0.44 ± 0.26 *versus* 1.06 ± 0.48; $p < 0.001$) and marked decrease in IP in cancer patients compared to controls (2.75 ± 0.45 *versus* 3.12 ± 0.41; $p < 0.001$). However, the CP reached normal values following surgery, but the IP remained decreased. During chemotherapy the CP remained stable, while the IP further decreased, reaching values significantly lower than those found before the start of chemotherapy (2.19 ± 0.36 *versus* 2.75 ± 0.45; $p < 0.001$). Three months after the last cycle of chemotherapy and one year after surgery the CP returned to normal (control) values, while the IP remained decreased (Figure 2).



	I	II	III	IV	Control
Le	6,59	6,46	6,02	6,78	8,08
PMN	4,08	4,14	3,96	4,58	4,85
Ly	2,35	1,94	1,67	2,34	2,89
Mo	0,16	0,38	0,31	0,34	0,34

Figure 1. Differential count of circulating leukocytes during therapy. Differential count of peripheral blood leukocytes (Le) was determined at the time of diagnosis (I), following surgery (II), during (III) and after chemotherapy (IV). Absolute and relative count of polymorphonuclears (PMN) and lymphocytes (Ly) were not significantly different between investigated groups ($p > 0.05$). The mean number of monocytes (Mo) was significantly lower in cancer patients at diagnosis ($p < 0.001$), while they increased following surgery reaching the control values at the end of treatment.



	I	II	III	IV	Control
IP	2,75	2,33	2,19	2,44	3,12
CP	0,44	0,95	0,68	0,84	1,06

Figure 2. Monocyte phagocytosis during therapy. IP and CP were determined using light microscope at the time of diagnosis (I), following surgery (II), during (III) and after chemotherapy (IV). During and after therapy CP recovered to normal values and remained stable, while IP further decreased reaching values significantly lower than those found before the start of therapy ($p < 0.001$).

Discussion

More than 30 years ago, Chambers and Weiser pointed out that, if adequately activated, monocyte/macrophage cells were powerful killers of malignant cells [19]. The multiple nature of the monocyte cytocidal effects is independent from immune specificity. Moreover, Fiumara and his associates showed *in situ* evidence of neoplastic cell phagocytosis by macrophages in papillary thyroid cancer [13].

Our study showed that there was a difference in the number of leukocytes, polymorphonuclear cells and lymphocytes between breast cancer patients and healthy controls, although without statistical significance. Similarly, there was no marked difference in the number of leukocytes, polymorphonuclear cells and lymphocytes in patients with breast cancer at different clinical stages or in different phases of treatment. However, in breast cancer patients the number of monocytes was significantly decreased ($p < 0.001$). Great increase in the number of monocytes was noted four weeks after tumor removal compared with values observed before the operation. This phenomenon is probably a result of the removal of the malignant mass, leading to decreased levels of neoplastic cell inhibitory products, such as PGE-2 or phosphatidyl serine [20,21]. On the other hand, it can be the consequence of the stimulating effect of operative trauma on macrophages. It is well-known that trauma increases the synthesis and secretion of stimulating cytokines (IFN-γ, GM-CSF, M-CSF) [22]. Ever-

son et al. have shown that, until the tenth postoperative day, the absolute number and functional ability (mobility, phagocytosis, lysosome production) of monocytes were significantly increased compared with preoperative values [23].

Except the decreased number of peripheral blood monocytes, we also noticed a significant decrease of the phagocytic function of monocytes in breast cancer patients. Moreover, we found that the phagocytic function of monocytes was statistically lower in patients with advanced disease. In the group of patients with metastatic breast cancer, the phagocytic activity of monocytes was mostly inhibited. These findings are in accordance with our previous results, based on which we proposed that an increase in tumor burden leads to a great decrease in the number and function of monocytes [24]. Spillert and associates drew the same conclusion [25], but these results are opposite to the findings of Valdez et al. who showed that the IP was significantly higher in cancer patients than in healthy controls [26]. If we consider these contradictory, at first sight, data through new knowledge, such as, for example, the fact that mammary tumor produces several factors (PGE-2, GM-CSF and phosphatidyl serine) capable of altering the level of synthesis of IFN-γ in CD4+T cells, as well as the level of secretion of IL-12 and IL-18 in mononuclear phagocytes [27], it becomes rather clear that malignant cells significantly influence the function of monocyte/macrophage cells of the peripheral blood.

However, during and after therapy CP is stabilized at a level approximately to normal values (values registered in the control group). Yet, the IP values through all phases of treatment, as well as three months after the last cycle of chemotherapy, are significantly lower than those in the control group ($p < 0.001$). This contradictory result can be explained by the powerful suppression of the peripheral blood leukocytes in patients who are treated with chemotherapy. The decrease of the mean number of all the observed elements can be seen clearly in Figure 2, but it is well known that among them monocytes are the least sensitive to the applied therapy. However, chemotherapy influences monocytes, especially their functional ability, a fact documented by the extremely low IP. For this reason, a significant fall of CP during chemotherapy was, above all, the consequence of low functional ability of macrophages (expressed through IP).

Our results showed that the number of monocytes, decreased at the time of diagnosis, returned within normal range, but the IP remained decreased one year after surgical therapy. However, time is needed to confirm whether the alteration of phagocytic activity in peripheral blood monocytes may provide additional information when monitoring surgically treated breast cancer patients.

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