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Changes in monocyte phagocyting activity after multi-agent chemotherapy in non-small cell lung cancer

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Abstract. – **Changes in monocyte functions have been described in several human malignancies. The monocyte/macrophage system is known to play a crucial role in the rejection of tumor cells and phagocytosis represents an important defense mechanism used by these cells. This paper reports the adherence power and phagocyting ability (latex beads) of circulating monocytes in 20 patients with unresectable non-small cell lung cancer (NSCLC), stage IIIB or stage IV, before and after multiagent chemotherapy (carboplatin + etoposide + ifosfamide or cisplatinum + etoposide).**

We demonstrated that both monocyte adherence and phagocytosis were not affected in lung cancer patients before chemotherapy in comparison with healthy controls. After chemotherapy, a statistically significant decrease in monocyte count on day 4 ($p < 0.05$) and in their phagocyting ability on day 4 and 15 ($p < 0.001$ and $p < 0.05$ respectively) was showed. In addition, a statistically reduced monocyte adherence was found on day 4 ($p < 0.05$). The described impairment was prolonged but reversible. These changes in monocyte functions after chemotherapy could be due to a direct effect of the chemotherapy on these cells or to functionally immature cells circulating after myelodepression. The in vitro assessment of monocyte functions may be useful to better clarify mechanisms by which anti-neoplastic agents may act on immune functions and prevent adverse side effects.

Key Words:

Lung cancer chemotherapy, Monocytes, Phagocytosis, Antineoplastic agents, Immunosuppression.

Introduction

Treatment of lung cancer, is still carried out in terms of surgery and/or chemotherapy and/or radiotherapy. Multi-agent chemotherapy is currently under evaluation in several prospective, multicentre studies and it has been found to have a small, short-term but significant survival benefit[1]. On the other hand, chemotherapy can induce serious haematological and non-haematological side-effects, in particular on host immunity[2-10]. The monocyte/macrophage system, alone or in association with other cells and secretion substances, represents the main defense of the immune system that is able to recognize and kill tumor cells[2,11]. Activated monocytes release several reactive oxidative metabolites including hydrogen peroxide and superoxide anion that have been implicated as killing agents[11-12]. Some studies on monocytes/macrophages in patients with cancer report conflicting results about their functions[2,3-5,7-8,10,13-25]. So, it is described that monocyte/macrophage functions can be depressed both before[14-17,25] and after chemotherapy[4,10,18]. Cytotoxic agents can induce: myelosuppression, with consequent leukopenia and anaemia[4,6,9-10], a decrease in hydrogen peroxide release by monocytes[4-5,13] and in monocyte-mediated cytotoxicity[10,18] and phagocytosis[19]. In contrast, other authors have described restoration of defective baseline monocyte/macrophage cytotoxicty in cancer patients receiving cisplatinum chemotherapy[19-23].

The aim of the present study was to analyze the phagocyting ability of circulating monocytes and their adherence to plastic cover-slips in patients affected by unresectable lung cancer before and after anti-neoplastic multiagent chemotherapy.

Materials and Methods

Patients

Twenty patients (all smokers and male; age range 44-69, mean 58), with histologically proven NSCLC, were recruited for the study. According to standard criteria[26], fourteen were classified as having the disease in stage IIIB and six in stage IV. The histological diagnosis was made on biopsies taken during fiberoptic bronchoscopy. Eleven subjects were found to have squamous cell carcinoma and nine adenocarcinoma. Nine age-matched healthy male smoking subjects (age range 35-60, mean 54) made up the control group.

After informed consent, patients (pts) with cancer received chemotherapy with the purpose of reducing the tumor mass and controlling tumor growth. Patients were divided into two groups. The first group (10 pts) was treated with carboplatin (120 mg/m^2), etoposide (120 mg/m^2) and ifosfamide (1000 mg/m^2) for three consecutive days, while the second group (10 pts) was treated with cisplatinum (60 mg/m^2) on the first day and etoposide (120 mg/m^2) for three consecutive days. In addition to anticancer drugs, all patients received a daily dose of anti-emetic drugs (ondansetron 8 mg), steroids (betamethasone 4 mg) and anti-H_2 receptor antagonist (ranitidine 100 mg). Both chemotherapy regimens used were scheduled to be repeated every 28 days for six consecutive courses.

For the purpose of the present study only the first course was considered. Total and differential peripheral blood cells were counted, adherence to cover-slips and the ability of peripheral blood monocytes to phagocytose latex beads were measured in healthy controls and in patients with cancer before chemotherapy (T0) and three times after chemotherapy administration, on day 4 (T4), on day 15 (T15), at the time of maximal granulocytic suppression (nadir), and on day 28 (T28).

Human peripheral blood mononuclear cell preparation and in vitro phagocytosis assay

Thirty ml of venous blood were drawn in plastic test tubes containing 0.2 ml of heparin. The blood was diluted 1:1 with a phosphate buffer solution (PBS). Five aliquots of diluted blood were gently layered upon 3 ml Lymphoprep®, taking care not to mix the two liquids. The tubes were centrifuged at 800 × g for 20 min at room temperature in a swing-out rotor. The surface mononuclear cell band that formed was removed with a Pasteur pipette. The harvest fraction of mononuclear cells, separated from red cells, was washed three times with PBS. An aliquot of these cells was cytocentrifuged (Hettich Universal, Tuttlingen, Germany) and stained with May-Grünwald-Giemsa. Another three aliquots were incubated for four hours in RPMI 1640 (added with serum calf 10% and streptomycin 1%) on plastic cover-slips at 37° C in 5% CO_2 atmosphere. Each aliquot contained 1×10^5 cells. Supernatant and non-adherent cells were discharged and the attached monocytes were kept in touch with 50 µl of latex beads (Sigma Chemical Co, St Louis, MO, USA), measuring 3 microns in diameter diluted in 1 ml RPMI 1640 for two hours in the above mentioned conditions. At the end of incubation the cover-slips were gently washed, dried and stained with May-Grünwald-Giemsa.

Each slide was used for counting the cells attached in twenty pre-arranged microscopic fields (400 × magnification) for establishing monocyte adherence, expressed as the mean number of the adherent cells in each field.

The number of latex particles phagocyted was counted with the aid of an image analyzer connected via a TV camera to a bright-field photomicroscope (Microimage and Microphot FXA, Nikon Instruments SPA, Florence, Italy) in a sample of almost 400 cells for each slide. Phagocytosis ability was expressed as the mean number of particles phagocyted by each cell.

Statistical analysis

Data are shown as mean and standard deviation (SD). Results before and after chemotherapy, at the different steps of the study as mentioned above, were analyzed by unpaired or paired Student's t-test. Distribution of monocytes in controls and in patients prior to chemotherapy, in regard to phagocyting ability, was analyzed by the chi-square (χ^2). A p value < 0.05 was considered significant.

Results

All patients received and showed good tolerance to the first cycle of chemotherapy regimen assigned. Side effects were restricted and transient; none of the patients had leukopenia or anaemia such as to require growth factors. Total leukocyte count in cancer patients was not statistically different before chemotherapy in comparison with healthy controls ($8.6 \pm 2.83*10^3$ *vs* $8.1 \pm 2.3*10^3/mm^3$). After chemotherapy, there was a mild decrease in leukocyte number on day 4 (7.1 ± 1.8; $p > 0.05$) which became significant on day 15 (5.24 ± 1.8; $p < .001$); on day 28 the data returned to baseline value (6.8 ± 2.4; $p > 0.05$). In contrast, chemotherapy caused a statistically significant decrease, in comparison with baseline value, in the number of monocytes/µl (Figure 1). These changes were first detected on day 4 (T4 *vs* baseline, $p < 0.001$) and were still documented on day 15 (T15 *vs* baseline, $p < 0.05$). The number of monocytes was not statistically different from the pre-chemotherapy values on day 28. In all patients studied, the monocyte counts on days 4 and 15 have never fallen under 150/microl value that was reported as predictor for neutropenia[3].

Monocyte adherence, expressed as mean number of adherent cells counted in each microscopic field of plastic cover-slips where 1×10^5 cells had been incubated, did not display statistically significant difference prior to the first course of chemotherapy in comparison with healthy controls. In contrast, a statistically significant impairment ($p < 0.05$) was found on day 4 after the administration of chemotherapy (T4) when compared to the pre-chemotherapy data. No statistically significant differences were found on days 15 and 28 (Figure 2).

Prior to the first course of chemotherapy the quantitative analysis of monocyte phagocytosis, expressed as the mean number of latex particles phagocyted by each monocyte, was not statistically different in neoplastic patients compared with healthy controls (Figure 3). After chemotherapy, a considerable decrease in monocyte phagocyting ability was displayed. This decrease was documented immediately after the first course of chemotherapy (T4, $p < 0.001$) and was present also on day 15 (T15, $p < 0.05$). On day 28, the monocytes recovered their phagocyting ability although it remained lower than baseline.

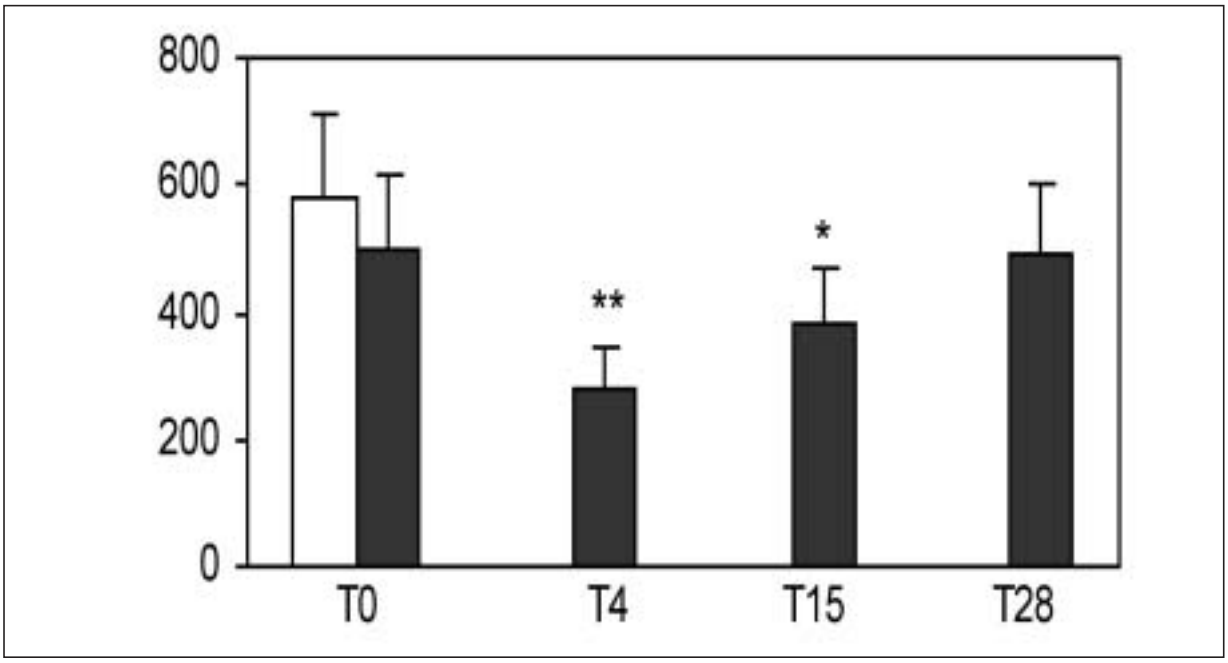

Figure 1. Circulating monocytes (mean + SD) in healthy controls (white column) and in lung cancer patients (black columns) before (T0) and after chemotherapy, on days 4 (T4), 15 (T15) and 28 (T28). Statistical analysis was performed by unpaired and paired Student's t test (* and **: $p < 0.05$ and $p < 0.001$).

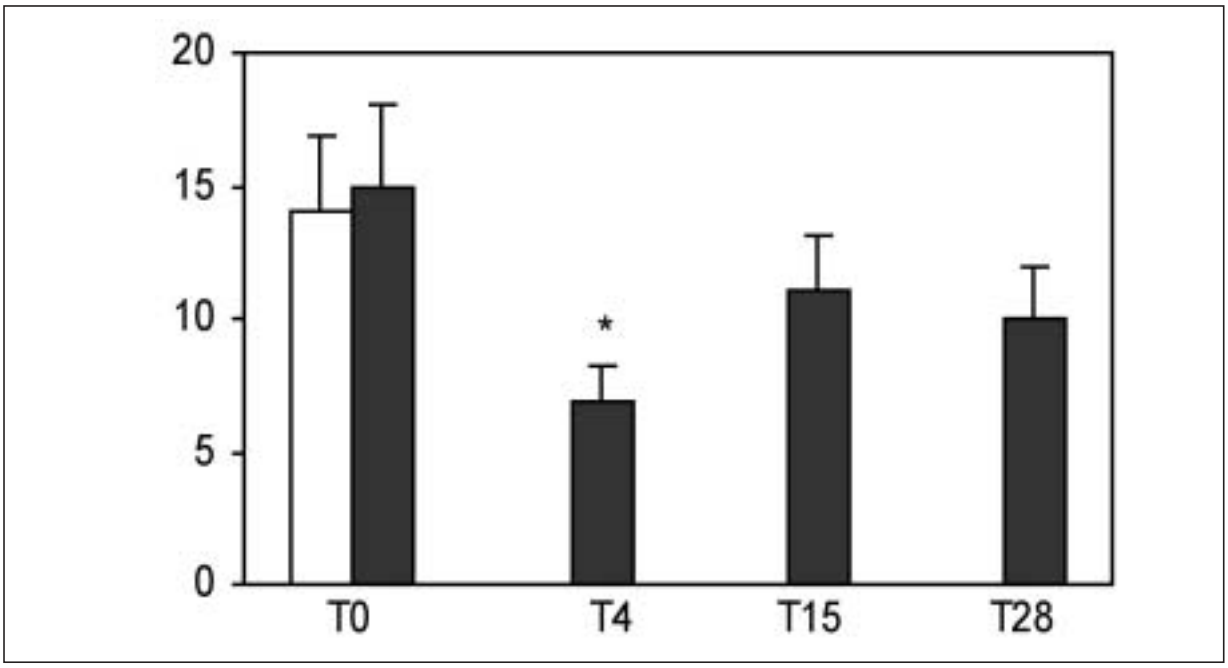

Figure 2. Monocyte adherence in healthy controls (white column) and in lung cancer patients (black columns) before and after administration of anticancer drugs. The histogram represents the mean number of adherent monocytes in prearranged light microscope fields before (T0) and on day 4, 15, 28 (T4, T15, T28) after chemotherapy. Statistical analysis was performed by unpaired and paired Student's t test (*: $p < 0.05$).

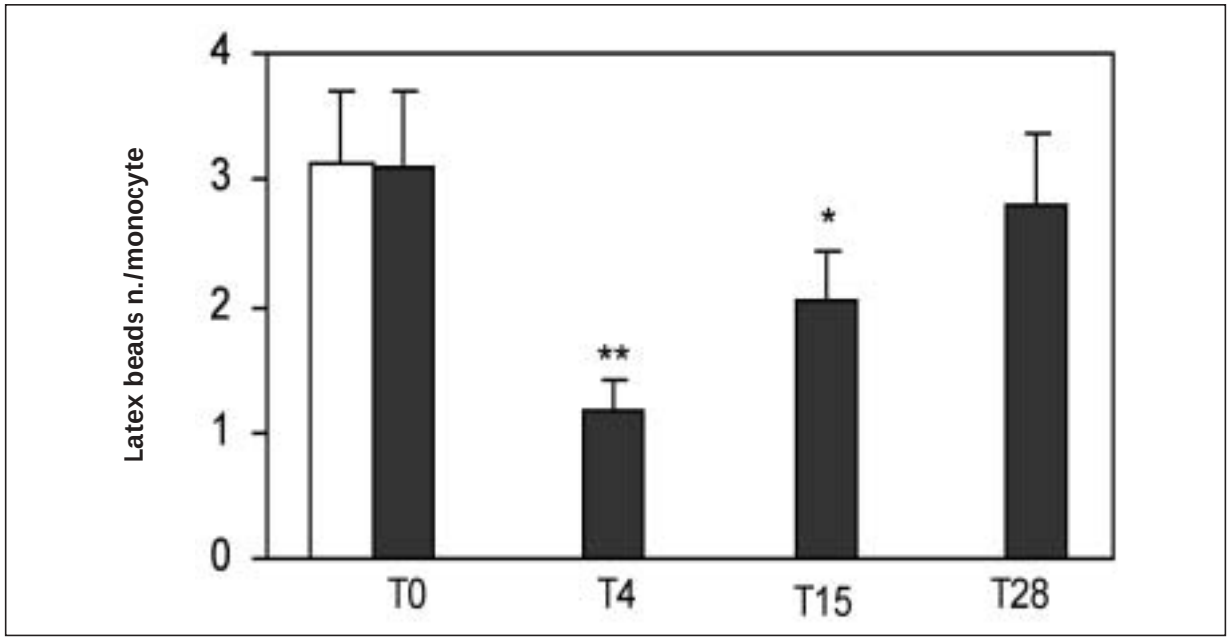

Figure 3. Monocyte phagocyting activity in controls (white column) and in lung cancer patients before (T0) and after chemotherapy (black columns; T4, T15, T28). Phagocyting activity was espressed as mean number of latex beads phagocyted by 400 cells per slide in prearranged fields. Note the decrease of phagocyted particles on days 4 (T4) and 15 (T15) and the recovery on day 28 (T28). Statistical analysis was performed by unpaired and paired Student's t test (* and **: $p < 0.05$ and $p < 0.001$).

Likewise, when we analyzed the distribution of monocytes, in regard to the number of latex particles phagocyted, on baseline, we observed a similar distribution of monocytes in neoplastic patients as well as in healthy controls (Figure 4). On day 4 after chemotherapy, this pattern was found to be statistically different, in regard to baseline and controls (χ^2; $p < 0.05$), with a high number of monocytes showing a low phagocyting ability. On day 15 and 28 no statistically significant difference was found.

The two different chemotherapy regimens used affected in the same manner monocyte number and functions (data not shown).

Discussion

The monocyte/macrophage system alone and/or in association with lymphocytes and the cytokine represent a strong cellular defense system against external and internal injuries. In neoplastic disease, the immune system is cheated and cancer cells survive and develop to the extreme consequences for the host. Several studies on monocyte function at diagnosis have shown that their chemotaxis[14,16,22] or natural cytotoxicity[15,21] is impaired, while others reported phagocyting activity or adherence within the normal range.

Multi-agent chemotherapy is a useful strategy for the treatment of lung cancer even if not resolutive. Side-effects are considerable especially on a wide range of immune functions[2-8,10,15,18]. In vitro and in vivo studies on monocyte functions after chemotherapy are limited and have often shown conflicting results reporting both immunosuppression or restoration of their activity, impaired at diagnosis[4,10,18-20].

In the present study, the effects of anticancer chemotherapy on monocyte phagocytosis and adherence were evaluated at various times from the administration of drugs emphasizing maximum depression and its recovery. At baseline, there were no differences in the number, adherence and phagocyting ability of monocytes in lung cancer patients as compared with healthy controls. Monocytes, in relation to phagocyted latex beads, showed a good activity and cells phagocyting a low-to-high number of parti-

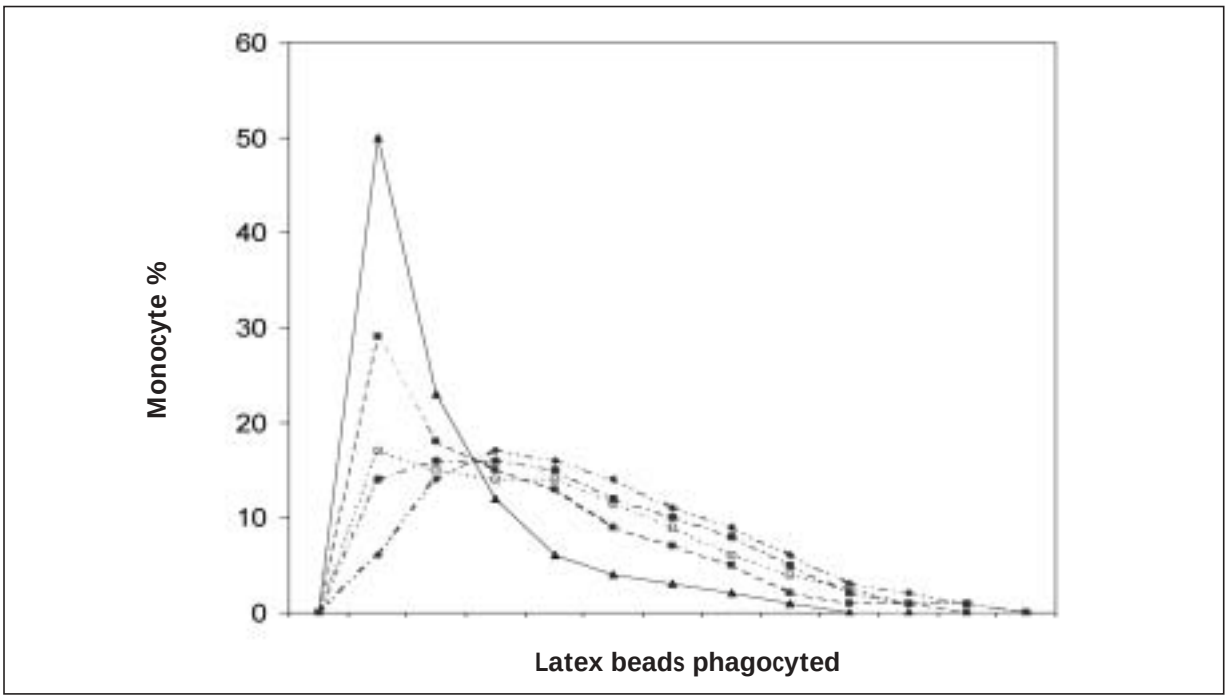

Figure 4. Monocyte distribution, in regard to the number of latex beads phagocyted, in healthy controls (♦) and in lung cancer patients before (●) and after chemotherapy administration on day 4 (▲), day 15 (■), and day 28 (○). Statistical analysis was performed by Chi-square [on day 4 (▲), $p < 0.05$ versus controls(♦) and baseline(●)].

cles had a balanced distribution in the investigated groups. After chemotherapy, a significant and prolonged decrease, on days 4 and 15, in monocyte count was found. The early monocytopenia with values less than 150/µl on day 6 to 8 after chemotherapy was demonstrated to be a predictive factor for grade 3 or 4 neutropenia at nadir during cancer chemotherapy at 3- or 4-week intervals[3]. Powell et al[10] found in mice, after chemotherapy, a reduced activity of natural cytotoxic cells with a behavior similar in time to the phagocyting activity found in our patients. This reduction in natural cytotoxic activity coincided with a reduction in circulating monocytes. Also Lower and Baughman[4] found a significant decrease in hydrogen peroxide release from monocytes two weeks after chemotherapy.

In our study, monocyte adherence did not show any differences between patients and controls before chemotherapy while after chemotherapy, on day 4, a significant impairment was found. Other authors[4] do not report the influence of chemotherapy on this step of monocyte defense.

Some conflicting results could be explained by different times and cycles of chemotherapy regimens when these functions were tested; furthermore, some authors showed different changes with different treatments. On the other hand a restoration of defective monocyte functions, chemotaxis or cytotoxic activity has also been reported, after administration of chemotherapy regimens containing cisplatinum[20-23] underlining an interaction between monocyte activity and cytostatic drugs[2].

The mechanisms that induce the impairment of monocyte phagocytosis or of hydrogen peroxide release after chemotherapy are still controversial. Direct cytotoxicity of anticancer agents does not seem to be the only cause that affects monocyte functions. It has been documented that the half life of circulating monocytes is only three days and cytotoxic agents are quickly metabolized and excreted[2]. In our study, the impairment of monocyte functions on day 4, after the end of chemotherapy, may be related to a direct effect of chemotherapy while the decreased phagocytosis ability detected on day 15 could be caused by different mechanisms. Recent studies have revealed a reduced phagocyting ability of immature granulocytes with decreased cell membrane Fc receptors during the cycles of chemotherapy[24]. It can be assumed that as far as the granulocytes are concerned, after bone marrow suppression due to chemotherapy toxicity, there is an increased number of circulating immature monocytes that show a reduced phagocytosing ability. However, impairment of monocyte functions is prolonged but reversible. Phagocyting activity on day 28 after chemotherapy and prior to the subsequent cycle was normal suggesting further that the impairment in monocyte functions, following chemotherapy, may be due to immature cells. In this regard, Lower and Baughman[4] found a transient decrease in hydrogen peroxide release, a mediator of the killing activity, by adherent monocytes two weeks after chemotherapy in breast and lung cancer patients. Vukovic-Dekic et al[18] compared monocyte phagocytosis three weeks after the 2nd and 4th cycles of chemotherapy; an impairment of this function was found only after the 4th cycle. Both chemotherapy regimens, administered to our patients, seem to affect monocyte phagocytosis with the same modality. This observation further suggests that the impairment of monocyte functions can be related to an aspecific cytotoxic activity of the compounds used. The in vitro assessment of monocyte functions may help to better clarify the mechanism of action of antineoplastic agents on immune functions and to test drugs capable of modulating the side effects of anticancer drugs on these cells.

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