Cancer Chemother Pharmacol (2006) 57: 663–670
DOI 10.1007/s00280-005-0086-4

ORIGINAL ARTICLE

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Failure of neutrophil chemotactic function in breast cancer patients treated with chemotherapy

Received: 10 May 2005 / Accepted: 25 July 2005 / Published online: 27 August 2005

Abstract Neutrophil migration is a key host event against infection. Chemotherapy may alter neutrophil function and favor increased risk of infection. Herein, we investigated the effect of chemotherapy on the migration capacity of circulating neutrophils obtained from breast cancer patients and mechanisms involved in this event. Breast cancer women ($n=23$) at disease stage I–III and healthy control women ($n=25$) were prospectively enrolled. No differences in the in vitro migratory responses towards the chemotactic stimuli *N*-formyl- *L*-methionyl- *L*-leucyl- *L*-phenylalanine (fMLP), leukotriene B_4 (LTB_4) and interleukin (IL)-8 were observed in purified neutrophils from controls and patients, in a microchemotaxis chamber assay. However, the migration capacity evaluated upon chemotherapy (5-fluoruracil, adriamycin and cyclophosphamide, 21-day intervals between cycles, total leukocyte count $\geq 2{,}000/mm^3$), on the day immediately before the beginning of the sixth cycle, showed that patient neutrophils ($n=14$) failed to migrate in response to fMLP compared to response observed upon diagnosis. Considering patients ($n=8$) with documented bacterial infection between cycles, the number of migrated neutrophils (mean ± SD) compared to response at diagnosis was markedly reduced upon chemotherapy to either fMLP (30.1 ± 8.26 vs. 2.81 ± 1.28) or LTB_4 (15.72 ± 4.8 vs. 2.8 ± 1.64) stimuli respectively. Treatment of control neutrophils with sera of chemotherapy-treated patients with infective episodes, to test for the presence of circulating immunosuppressive factors, significantly reduced the migratory capacity of healthy neutrophils to fMLP, LTB_4 and IL-8, in a dose-dependent way. But no significant differences were found in the serum levels of nitric oxide (NO) metabolites, tumor necrosis factor (TNF)-α, IL-6, IL-8 and IL-10 collected at the same time as the collection of blood for neutrophil migration experiments. In conclusion, breast cancer patients showed suppressed neutrophil migratory response upon chemotherapy, accompanied by bacterial infection episodes. Circulating factors are involved, at least partially, in the inhibitory mechanism on neutrophil migration.

Keywords Breast cancer · Neutrophil migration · Chemotherapy · Infection

Introduction

Neoplastic disorders constitute a paradox in terms of inflammatory response. Although tumors are infiltrated by leukocytes attracted by chemotactic mediators released by tumor tissues, patients can be defective in the capacity to mount an inflammatory response, in sites other than the tumor, due to altered systemic leukocyte functions [1].

Neutrophils play the first line in the host defense against microorganisms, being promptly recruited to the inflammatory sites by chemoattractants such as leukotriene B_4 (LTB_4) and chemokines such as interleukin (IL)-8 [2, 3]. Once emigrated to tissue, these leukocytes are able to phagocytose, to release lytic enzymes and antimicrobial products and to generate

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large amounts of reactive oxygen and nitrogen species, such as hydrogen peroxide and nitric oxide (NO), which are crucial products for the microbicidal activity of these cells [4, 5]. In septic patients, failure of neutrophil migration was associated with poor prognosis [6]. In patients with gastrointestinal cancer, it was reported depressed neutrophil migration capacity compared to healthy volunteers, already upon diagnosis [7]; while in a more recent work, preserved neutrophil phagocytosis was observed compared to controls [8].

The use of chemotherapeutic drugs in cancer patients leads to a variety of adverse reactions, one of the most common being myelosuppression with consequent neutropenia, mainly manifested as increased infection risk [9]. Besides, it is well known that chemotherapeutic drugs alter in vitro functions of healthy neutrophils such as chemotaxis, phagocytosis and superoxide production [10]. Studies performed in patients with acute leukemia have demonstrated altered neutrophil chemotactic and phagocytic functions between chemotherapy cycles that could be an additional predisposing factor for infections [11]. Nevertheless, since it is known in leukemia that the function of mature neutrophils can be impaired by disease itself [12], the effect of chemotherapy on these neutrophil functions could be overestimated.

The host response to malignant tumors comprises not only changes in tumor microenvironment but also systemic findings. The production of mediators such as cytokines in cancer patients has been used as a tool to investigate possible alterations in the immune response. It has reported higher amounts of the anti-inflammatory cytokine IL-10, produced by mononuclear cells from breast cancer patients [13]. Also, serum levels of tumor necrosis factor (TNF)-α, IL-8 and NO were reported to have increased in patients compared to controls [14–16]. Evidence from experimental models of endotoxemia and sepsis has demonstrated that the high levels of circulating cytokines such as TNF-α and IL-8 are involved in the reduction of neutrophil migration [17, 18]. Besides, this effect could be mediated by NO since previous administering of an NO synthesis inhibitor prevented the failure of neutrophil migration [17–19].

In the present study, we hypothesized that during chemotherapy cycles, although cancer patients may present recovered leukocyte numbers to undergo the next cycle, defective leukocyte function could exist thus favoring an increased risk of infection. To address this issue, we investigated the ability of neutrophils from breast cancer patients to migrate towards different chemoattractants during chemotherapy, at time points upon diagnosis and on follow-up, immediately before drug administration in the next cycle. We also tested for the presence of immunosuppressive factors in the sera of cancer patients on neutrophil chemotaxis and quantified the systemic concentrations of cytokines and NO.

Patients and methods

Patients

Women attending the Outpatient Mastology Service of the Faculty of Medicine of Triângulo Mineiro (FMTM), from public health system, were prospectively diagnosed with breast cancer at different stages. Patients were excluded if they had received previous treatment for tumor or were using immunosuppressive drugs. The anathomopathological diagnosis followed the recommendation of the American Joint Committee on Cancer and the Committee of the International Union against Cancer, considering tumor extension, presence of axillar nodes and/or metastasis. Healthy female volunteers served as controls. We compared the neutrophil migratory response from controls and patients at diagnosis and, in a second part of the study, we investigated the possible suppressive effect of chemotherapy on the neutrophil migration capacity of breast cancer patients.

The study protocol was approved by the FMTM Committee on the Use of Human Subjects, and written informed consent was obtained from patients and volunteers.

Neoadjuvant chemotherapy and evaluation of infection episodes

Patients received neoadjuvant chemotherapy with 5-fluoruracil (600 mg/m^2), adriamycin (50 mg/m^2) and cyclophosphamide (500 mg/m^2), accomplished by 6 or 8 cycles with 21-day intervals between them. Patients were interviewed immediately before the next cycle about bacterial infection episodes between cycles, confirmed by urine and/or blood sample and/or thorax radiography. In all cases, patients underwent the next cycle of chemotherapy with total leukocyte count ≥2,000/mm^3.

Blood Collection

Peripheral venous blood was collected upon diagnosis and on follow-up, immediately before the sixth chemotherapy cycle. At each time, two samples (5 ml) were collected with (100 IU/ml heparine) or without anticoagulant, which were used for chemotaxis assay and quantification of serum mediators, respectively. For obtaining of serum, blood was centrifuged (180× *g* for 15 min) and the supernatants stored at −70°C until required.

In vitro Neutrophil Chemotaxis

Preparation of neutrophils

Neutrophils obtained from heparinized blood of healthy volunteers and breast cancer patients were purified using

ficoll-hypaque (density 1.114) gradient density centrifugation, according to manufacturer's instructions and as reported [20]; such a procedure lasting around 1 h. Viable neutrophils were >95%, determined by trypan blue exclusion, and viability was not significantly altered at the final period of investigation. Aggregates were not formed or were not important. Purified neutrophils were washed three times (180*g*, 10 min) with RPMI medium containing 0.01% bovine serum albumin (RPMI-BSA) and then resuspended in the same medium.

Chemotaxis assay

Chemotaxis was studied in a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, MD, USA) containing 5 μm pore size polyvinylpyrrolidone-free polycarbonate membranes. In brief, 28 μl of one of the chemoattractants (10^{-7} M, all purchased from Sigma): N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), LTB_4 and IL-8 diluted in RPMI-BSA or medium alone (control) were added to the bottom compartment, and 50 μl of the neutrophil suspension (10^6 cells/ml) were added to the top compartment. The chamber was incubated for 1 h (37°C, 5% CO_2), after which the polycarbonate membrane was removed, fixed and stained with Hema 3 Stain set (stain kit, Biochemical Sciences, Bridgeport, NJ, USA). The neutrophils which migrated to the lower side of the filter were counted (100x objective) in four random fields in triplicate. Neutrophils from one healthy donor were included in each assay. Neutrophils migrated towards RPMI-BSA indicating alleatory migration. The results are expressed as the absolute number of neutrophils migrating toward the chemoattractants minus the number of neutrophils migrating to RPMI medium.

Pretreatment of healthy neutrophils with patient sera

In a set of experiments as described [21], healthy control neutrophils were previously incubated during 30 min (37°C, 5% CO_2) in sera (diluted 0.5 to 50% v/v in RPMI/BSA) from each chemotherapy-treated patient with infective episodes or sera obtained from other healthy donors. After incubation, these neutrophils were assayed for chemotaxis, as described above.

Cytokine assay

The serum levels of TNF-α, IL-6, IL-8 and IL-10 were determined by ELISA, as reported [17]. Briefly, flat-bottomed 96-wells microtiter plates were coated with antibody (50 μl/well) to each cytokine, diluted (1–3 μg/ml) in buffer binding solution and incubated overnight (4°C). Plates were washed (PBS/Tween 20) and nonspecific binding was blocked (120 min, 37°C) with PBS/BSA (100 μl, 1%). The samples and standards were loaded into plates (50 μl) and incubated overnight (4°C). Plates were washed (PBS/Tween 20) and the appropriate biotinylated monoclonal anti-cytokine antibody was added. The plates were washed 1 h later, avidin peroxidase (diluted 1:5,000) was added and the plates incubated (30 min). These were washed again and substrate (100 μl of *o*-phenylenediamine dihydrochloride [OPD, Sigma, St. Louis, MO, USA]) was added. The plates were then incubated under environment temperature (15 min). The reaction was stopped with H_2SO_4 (50 μl, 1 M) and the optical density was measured at 490 nm in a multiwell plate reader (Multiskan MCC340 MKII, Flow Laboratories). The results were expressed as picograms of cytokine per ml of serum, comparing the optical density in the samples with standard curves.

Determination of serum NO metabolites

The nitrate concentration in serum samples were determined by enzymatically reducing nitrate with nitrate reductase, as described [22]. Briefly, 50 μl of serum samples were incubated with the same volume of reductase buffer (0.1 M potassium phosphate, pH 7.5, containing 1 mM nicotinamide adenine dinucleotide, and 4 U of nitrate reductase/ml) for 20 h at 37°C. A standard nitrate curve was obtained by incubating sodium nitrate (10–200 μM) with the reductase buffer. The total amount of nitrite was then determined by the Griess method [23]. Briefly, samples were incubated with same volume of freshly prepared Griess reagent (1% sulphanilamide, 0,1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid). Absorbance at 550 nm was determined using a multiwell plate reader (Multiskan MCC340 MKII). The results are reported as micromole of $NO_3^- + NO_2^-$.

Statistical analysis

Statistical analysis was performed by SigmaStat 2.03 software. Unpaired groups with non-normally distributed population were compared by Mann-Whitney, while Kruskal-Wallis followed by Dunn was performed for multiple comparisons vs. control. Paired groups were compared by paired *t* test for normal population or Wilcoxon for non-normally distributed population. Statistical significance was set at $P < 0.05$.

Results

Study population

There were 25 control women and 23 breast cancer patients enrolled in this study; of these 16 (69.6%) underwent neoadjuvant chemotherapy, while seven (30.4%) underwent surgery. Differences were not found in the mean (± SE) age (years) of controls (48.21 ± 2.24) and patients (53.25 ± 2.53), nor in patients presenting (51.38 ± 5.55, $n = 8$) or not (52.33 ± 5.30, $n = 6$) presenting infection episodes between chemotherapy cycles.

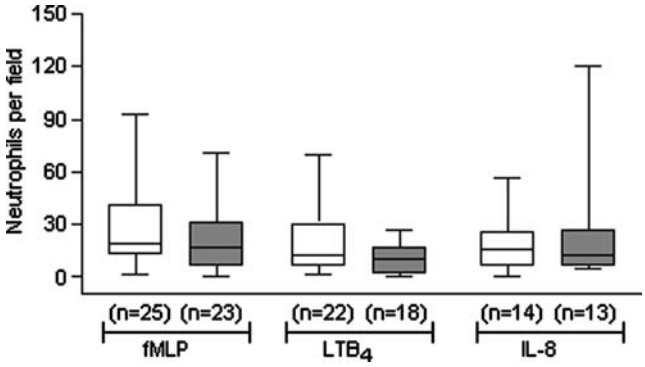

Fig. 1 Neutrophil chemotactic function in controls and patients. The *box plots* indicate the number of emigrated neutrophils obtained from controls (*open bars*) and breast cancer patients (*closed bars*), in response to the chemotactic stimuli (10^{-7} M) fMLP, LTB_4 and IL-8, subtracted from values obtained for random migration. The 25th and 75th percentiles are represented by a bar centered about the median; the minimum and maximum values are error bars. The number of controls and patients is indicated within the parentheses below each bar. *P* not significant (Mann-Whitney test)

Patients did not have other underlying diseases, except for one patient from the infective group who was diagnosed with controlled diabetes mellitus.

Neutrophil chemotactic function

We evaluated the neutrophil chemotaxis from 23 patients and 25 normal volunteers toward fMLP chemoattractant. From these, 18 patients and 22 controls were also evaluated for LTB_4 and 13 patients and 14 controls for IL-8, which were later added in the protocol. Figure 1 shows that neutrophils obtained from controls and breast cancer patients presented similar migration in response to all chemoattractants tested.

Considering the disease stage, ≤ II ($n=15$) or ≥ III ($n=8$), respectively, we also did not find significant differences (Mann-Whitney test) in the number of migrated neutrophils (medians) in response to fMLP (16.6 and 15.4, $P=0.87$) or LTB_4 (8.25 and 11.35, $P=0.54$).

Effect of chemotherapy on the neutrophil chemotactic function

Of the 16 patients who underwent chemotherapy, two refused to participate in the second phase and, therefore, we studied 14 patients before and on follow-up, during chemotherapy. In Fig. 2 significant reduced neutrophil chemotaxis for fMLP can be seen, but not for LTB_4 or IL-8, considering the time points upon diagnosis and before the sixth cycle.

Because eight (57.14%) patients presented one documented infection episode (pneumonia and/or genito-urinary tract infections) between chemotherapy cycles, we next analyzed this group separately. Fig. 3 shows that neutrophils obtained from these patients completely failed to migrate towards fMLP and LTB_4 stimuli, compared to the chemotactic response before treatment. Considering patients without infection episodes, the number of migrated neutrophils (means ± SD) quantified at diagnosis were not reduced upon chemotherapy, respectively, to either fMLP (8.1 ± 3.0 vs. 8.7 ± 7.8; $n=6$, $P=0.85$, paired *t* test) or LTB_4 (10.6 ± 7.7 vs. 17.7 ± 17.6; $n=5$, $P=0.39$, paired t test) inflammatory stimuli.

Effect of patient sera on the chemotactic function of healthy neutrophils

We next asked if soluble factor(s) present in serum from chemotherapy-treated patients which developed infection accounted for this suppressive effect. As shown in Fig. 4, healthy neutrophils incubated with patient sera showed reduced chemotaxis to all chemoattractants tested, apart from 0.5% serum concentration for LTB_4 and IL-8 (panels B and C) and 50% serum concentration for fMLP (panel A), compared with incubation in 50% healthy donor sera.

Serum cytokine and nitrate concentrations

The systemic production of soluble mediators was investigated in samples obtained from chemotherapy-treated patients with infection episodes, upon diagnosis and before the sixth cycle. These samples, obtained at the same time as the collection of blood for chemotaxis, were assayed for NO metabolites, IL-6, IL-8, IL-10 and TNF-α concentrations. Table 1 shows that TNF-α was not detected and only NO metabolites were detected in all samples evaluated. No differences were found in the serum concentrations of the mediators assayed at enrollment and on follow-up, before the sixth cycle of chemotherapy.

Discussion

The main objective of the present work was to evaluate the effect of chemotherapy on the migratory capacity of circulating neutrophils from breast cancer patients. To address this issue, patients with breast cancer at different tumor stages and healthy volunteers, without differences in the mean age of groups, were prospectively studied. Upon diagnosis, we did not find differences in the ability of neutrophils from breast cancer patients to migrate compared to healthy controls. The ability of neutrophils obtained from cancer patients to migrate was observed for structurally different chemoattractants, including bacterial products such as fMLP, chemokines as IL-8 and lipid mediators, as LTB_4.

Different leukocyte functions can be altered in cancer. In breast cancer, neutrophil and monocyte phagocytic

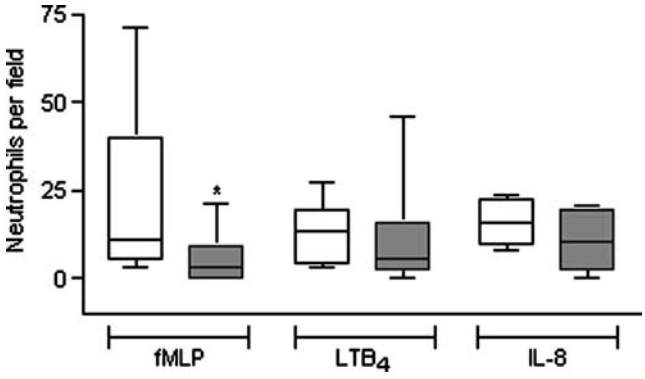

Fig. 2 Neutrophil chemotactic function in patients submitted to chemotherapy. The *box plots* indicate the number of emigrated neutrophils obtained from breast cancer patients upon diagnosis (*open bars*) and before the 6th cycle of chemotherapy (*closed bars*), in response to the chemotactic stimuli (10^{-7} M) fMLP ($n=14$), LTB_4 ($n=9$) and IL-8 ($n=6$), subtracted from values obtained for random migration. The 25th and 75th percentiles are represented by a *bar* centered about the median; the minimum and maximum values are *error bars*. * $P<0.05$ compared to time point at diagnosis (Wilcoxon test)

functions were found to be reduced early in the disease process [24, 25]. It has been hypothesized that a reduction in leukocyte function would mainly be a consequence of the progression of the disease [25]. In women with gynecological cancer of different origins, reduced production of superoxide radicals was found, already at the initial stages and in a more marked way with the evolution of the disease [26]. In our work, we did not find significant differences in the chemotactic function of neutrophils obtained from breast cancer patients at earlier or more advanced tumor stages. Considering that neutrophil chemotaxis is central for all other functions,

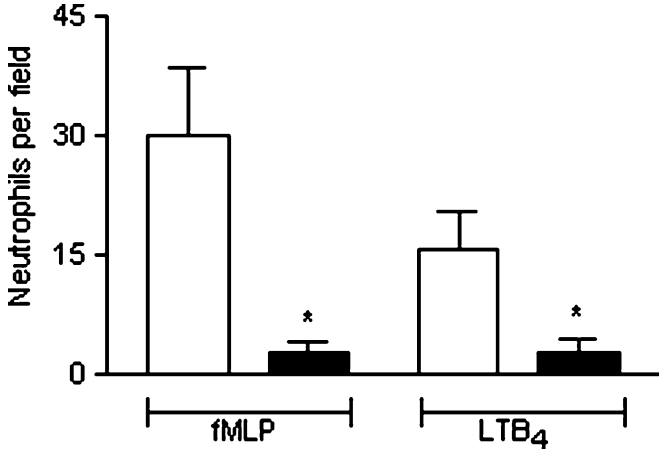

Fig. 3 Neutrophil chemotaxis in breast cancer patients with infection episodes during chemotherapy. The *bars* are means ± SD of the number of emigrated neutrophils obtained from breast cancer patients with infection episodes upon diagnosis (*open bars*) and before the 6th cycle of chemotherapy (*closed bars*), in response to the chemotactic stimuli (10^{-7} M) fMLP ($n=8$) and LTB_4 ($n=4$), subtracted from values obtained for random migration. * $P<0.05$ compared to respective time points at diagnosis (paired t test)

it seems possible that host defense preserves neutrophil responsiveness to the chemotactic stimuli. Nevertheless, since the number of patients studied was less in number, the absence of differences at the disease stage remains to be further validated in a higher number of patients.

In a second set of experiments, when patients were evaluated on follow-up during chemotherapy, significant reduction in the neutrophil chemotactic response was observed to fMLP but was not significant for endogenous mediators such as LTB_4 and IL-8, compared to the response at diagnosis. In the a next step, we separately analyzed patients with confirmed infection episodes between cycles. We then detected that the impairment of neutrophil chemotaxis was evident in this group, but was not observed in patients without infection, suggesting that this defect could be an important factor in determining the development of infections. The presence of other diseases as additional risk factors is not probable since only one patient from infective group had diabetes, which was kept under control. The infections were not severe, which can be explained, at least partially, by a possible preserved host defense mediated by neutrophil phagocytosis and microbicidal activity, functions not investigated here. Our data supports previous reports in that patients with non-Hodgkin lymphoma had reduced random and stimulated neutrophil chemotaxis after chemotherapy [27]. In leukemic children, neutrophil functions are differentially sensitive to the adverse effects of chemotherapy. Although migration capacity was reported to be restored after the recovery of bone marrow aplasia, the persistence of a bactericidal defect could be observed [28]. More recently, in patients with malignant glioma, decreased oxygen radical production was observed and thus impairment of microbicidal activity up to 6 weeks after the completion of drug administration [29].

One may argue why all patients had not developed reduced neutrophil migration with increased risk of infections. One possible explanation is that the toxicity of antineoplastic drugs does not occur in a uniform way with interindividual variance and also with differences between drugs [30].

Our results also demonstrated that the defect in the neutrophil migration of chemotherapy-treated patients should be due, at least partially, to the presence of circulating factors since patient sera significantly reduced the chemotaxis of neutrophils from healthy donors. To reinforce, healthy sera in the highest concentration tested did not affect neutrophil chemotaxis. However, we did not find differences in the systemic production of TNF-α, IL-8, IL-10 or NO metabolites, mediators that have been implicated in the impairment of neutrophil migration in experimental models of endotoxemia and sepsis [17–19, 31]. A problem with the assay is not probable since all cytokines were detected at unless some patient samples, as observed by maximum values, or in the control curve. Maybe these cytokines were really at decreased concentrations and/or their systemic levels were below the limit of sensitivity assay, as it could be

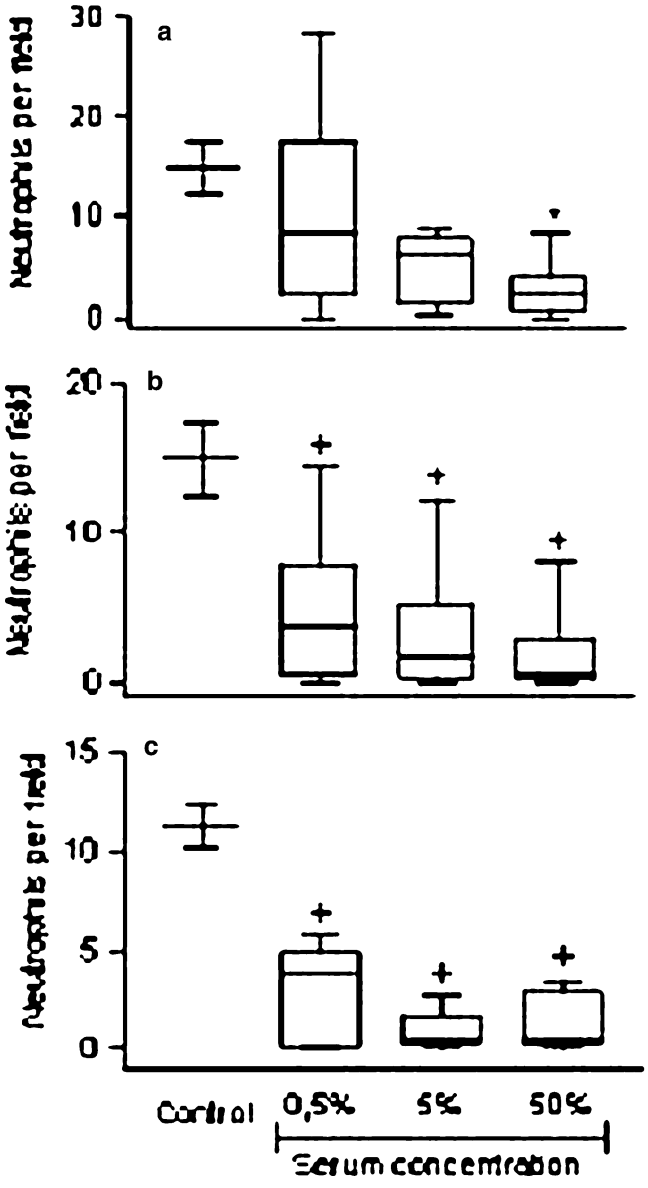

Fig. 4 Sera from breast cancer patients reduced chemotaxis of control neutrophils. The *box plots* indicate the number of emigrated healthy neutrophils, incubated for 30 min in sera (0.5–50% concentration) of chemotherapy-treated patients with infection episodes ($n=8$) or healthy donor sera (50%, $n=2$), in response to the chemoattractants (10^{-7} M) fMLP (panel A), LTB_4 (panel B) and IL-8 (panel C), subtracted from values obtained for random migration. Neutrophils from one healthy donor were included in each assay * $P<0.05$, $^{+}$ $P<0.01$ compared to control (Kruskal-Wallis + Dunn)

for TNF-α. But, it is important to note that the absence of a mediator in the circulation in a single time point does not rule out the possibilty that the neutrophil motility has been affected by the same mediator released earlier, since the time course of cytokine production (especially TNF-α) follows a bell-shape. Other possible explanations for our findings include increased clearance and/or tissue uptake. Moreover, it is possible that in vivo, the involved mediator(s) might modulate the immune response in a paracrine rather than endocrine way. In this context, the ELISPOT assay in that immune cell frequencies can be measured at the single cell level would be useful to monitor immune response in humans and for detection of more than one cytokine released by the same cell [32].

In agreement with our findings, others have not found differences in the systemic levels of IL-6 and IL-8 after chemotherapy with 5-fluoruracil, adriamycin and cyclophosphamide [33], the same drugs evaluated in our study. Nevertheless, for paclitaxel, a dose-dependent effect on the systemic cytokine production was observed in breast cancer patients, since plasma levels of IL-6, IL-8 and IL-10 increased with larger doses of the drug [34]. But in leukemic children, reduced plasma levels of IL-6 and IL-8 was detected after chemotherapy and recovery of bone marrow aplasia, compared to normal children [33].

It was demonstrated that breast cancer patients who had previously undergone chemotherapy showed reduced mobilization of progenitor cells from bone marrow and, since plasma from these patients also inhibited mobilization, the authors suggested that a circulating factor could be responsible for this effect [35]. Although the nature of such inhibitors is uncertain, a role for transforming growth factor (TGF)-β was suggested [35] since this anti-inflammatory cytokine can down-regulate leukocyte activity. Plasma obtained from transfused patients inhibited neutrophil migration of healthy neutrophils, due to the presence of TGF-β1 in these samples [36]. Another mechanism implicated in reduced neutrophil migration involves intracellular signaling. Most chemoattractants such as LTB_4 and IL-8 act via binding to specific G protein-coupled receptors (GPCR) controlling a cascade of signaling events fundamental for neutrophil migration. However, GRKs, specific kinases interacting with GPCR-protein, induce receptor phosphorylation and thereby signal GPCR desensitization in the continuing presence of chemoattractants [37]. Therefore, an increased expression of GRKs could augment chemotactic receptor desensitization and in turn reduces neutrophil migratory response [38]. Intracellular mechanisms were not investigated in the present work but remain an intriguing possibility.

Other effects of chemotherapeutic drugs on cells of the immune system are reported. One such study found a reduced number of B lymphocytes in breast cancer patients treated with chemotherapy, after recovery of bone marrow aplasia [39], at a similar time point used for investigation in our study. More recently, it has been suggested that chemotherapy can alter cell function indirectly, through the destruction of bone marrow microenvironment. Studies analyzing bone marrow 24 months after primary surgery and chemotherapy in breast cancer patients showed a reduced number of activated NK and NK T cells, suggesting a long-lasting negative effect of chemotherapy on the bone marrow immune system [40]. Therefore, taking these findings together, it seems possible that an effect on both bone

Table 1 Concentrations of cytokines and NO metabolites detected in sera from breast cancer patients at diagnosis and on the follow-up

	Mediators				
	IL-6	IL-8	IL-10	TNF-α	NO
Diagnosis	0 (0–224.1)	0 (0–56.7)	0 (0–45.2)	ND	35 (11–140)
Follow-up	0 (0–63.6)	0 (0–16.7)	0 (0–254.4)	ND	34.5 (13–240)

On the follow-up serum samples were obtained from patients with infective episodes ($n=8$), immediately before the sixth cycle of chemotherapy. Data are medians (and range) of the levels of cytokines (pg/ml) and NO metabolites (μM), *ND* non-detected. *P* not significant (Wilcoxon test)

marrow and circulating cells may have been affecting the capacity of neutrophils to migrate, once exposed to chemotherapeutic drugs.

In summary, we did not find altered neutrophil migration capacity in breast cancer patients upon diagnosis, regardless of tumor stage. But we present evidence for the association between the reduction of neutrophil migration during chemotherapy cycles and increased incidence of infections. The presence of circulating factors may account, atleast, in part, for this defect that compromises the ability of neutrophils from chemotherapy-treated patients to migrate towards the injury site. Our study also emphasizes that the mechanisms of long-lasting effects of chemotherapy on the immune system are not understood.

Acknowledgments We thank Januário Barbosa dos Santos Júnior, Douglas Cobo Micheli, Eduardo Schmidt Bachin and Lilian Beatriz Davi for technical assistance, Giuliana Bertozzi for cytokine assay and Beatriz Cibele Rezende Gerolin for confection of figures. We also thank the financial support by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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