

# Dendritic cells in peripheral blood of patients with breast and lung cancer - a pilot study

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**Abstract:** Dendritic cells (DCs) are regarded as the most potent antigen presenting cells that are well suited to activate T cells toward various antigens, such as tumor-associated antigens, due to their costimulatory activity. There is evidence that DCs are of diverse origin, with at least two types of myeloid and lymphoid precursors implicated in their generation. The recent reports demonstrated that the number and function of dendritic cells might change dramatically in cancer patients. In the present study we evaluated the percentage of myeloid and lymphoid DCs in patients with breast cancer, non-small cell lung cancer (NSCLC) and in the healthy donors. The percentage of both DC populations was significantly lower in patients with NSCLC than in the control group. In patients with breast cancer, the number of lymphoid DCs was significantly higher than in NSCLC patients. The obtained results suggest influence of pathological states on host immune system. The decrease in the number of DCs in the peripheral blood from cancer patients may be closely correlated with the type of tumour.

**Key words:** Dendritic cells - Immunophenotype - Breast cancer - Lung cancer

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## Introduction

Dendritic cells (DCs) constitute a family of professional antigen presenting cells (APC) defined by their morphology and their unique capacity to generate and regulate primary immune responses and maintain immunological memory [2, 7]. The main functions of DCs include the ability to take up, process and present antigens, to migrate selectively through tissues, and to interact with, stimulate and direct T- and B-lymphocytes [2, 9]. DCs represent a system of hematopoietic cells that are rare but ubiquitously distributed throughout the peripheral tissues. Their journey starts in the bone marrow where, at least, two DC lineages have been identified so far, namely the conventional myeloid-related DCs and the newly defined lymphoid-related DCs [11, 12]. Both populations migrate to the blood and become immature DCs, which wander through tissues where they monitor the invading pathogens [9, 12]. Upon pathogen capture, immature DCs receive activation signals, which initiate their maturation and migration to secondary lymphoid organs in order to induce antigen-specific immune responses [9, 12].

In this report we focused on subpopulation of DCs circulating in peripheral blood. We investigated circulating myeloid- and lymphoid- related DCs using markers specific for blood dendritic cells: BDCA-1 and BDCA-2, respectively. BDCA-1 (blood dendritic cell antigen), which in cluster differentiation corresponds to CD1c antigen, is expressed on human myeloid subpopulation of peripheral blood dendritic cells (PBDCs), which are CD4<sup>+</sup>, CD2<sup>+</sup>, Lin<sup>-</sup>, CD123<sup>dim</sup> and CD45RO<sup>+</sup> [2, 7, 9]. BDCA-1-positive cells express myeloid markers (CD11c<sup>+</sup>, CD13, CD33), are able to phagocytose because of the high expression of Fc receptors (CD32, CD64, FcεRI) and have monocytoid morphology [9, 12]. It is thought that BDCA-1-positive cells express CD1a and after entering tissues give rise to DCs resembling Langerhans cells and epithelial DCs [12]. BDCA-2 is specifically expressed on human plasmacytoid (lymphoid) dendritic cells [3, 4]. This apparently corresponds to a cell type that previously has been erroneously designated plasmacytoid T cell or plasmacytoid monocyte due to its peculiar morphology [4]. BDCA-2-positive cells are CD4<sup>+</sup>, Lin<sup>-</sup>, CD11c<sup>-</sup> and CD45RA<sup>+</sup> [4]. They express CCR5 (CD195) receptor and CD123 (IL-3Rα), just as activated lymphocytes, and critically depend on IL-3 [4, 8]. These cells express neither myeloid lineage markers (CD13, CD33) nor Fc receptors and show low phagocytic potential compared to myeloid DCs. The

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main function of lymphoid-related DCs is the secretion of large amounts of type I interferons upon viral infection, although they are postulated to enter lymph nodes through the high endothelial venules and differentiate into plasmacytoid DCs [2, 4, 12].

During the evolution of pathological condition, the host immune system is restrained from defence [6, 9]. Tumor cells are poor initiators of immune reaction and tumor microenvironment can directly inhibit immune reactivity. It is well established that dendritic cells play a central role in induction of antitumor immune responses [5, 6, 10]. The important role of DCs in cancer is underscored by number of reports in which the presence of dendritic cells in tumor tissues was associated with good clinical prognosis of disease [5, 9, 10]. However, in recent years several groups have described defective function of DCs in tumor-bearing mice and in cancer patients [1, 5, 6].

These findings call our attention to circulating population of dendritic cells. In the present study we assess quantitatively myeloid- and lymphoid-related DCs in peripheral blood of patients with non-small cell lung cancer (NSCLC) and breast cancer.

## Materials and methods

**Subjects.** The study population consisted of 23 patients with breast cancer and 13 patients with non-small cell lung cancer (8 male and 5 female). All patients had advanced cancer with no prior chemotherapy. 21 healthy age-matched volunteers served as controls. Informed consent was obtained from each individual.

**Preparation of the mononuclear cells.** All peripheral blood samples were collected in heparinized tubes and immediately processed. Mononuclear cells were isolated by density gradient centrifugation on Gradisol L (Aqua Medica, Poland). Interphase cells were removed, washed twice in PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Biomed, Poland) containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA (Sigma, Germany) and then resuspended in the same buffer for future immunostaining.

**Phenotyping of the cells.** Non-specific staining was inhibited by adding 20  $\mu\text{l}$  of FcR Blocking Reagent to  $10^7$  or fewer cells resuspended in the buffer prior the labelling. Immunofluorescence studies were performed using combination of the following monoclonal antibodies (mAbs): mouse anti-human BDCA-1 FITC (Miltenyi-Biotec, Germany), mouse anti-human BDCA-2 FITC (Miltenyi-Biotec, Germany), anti-CD123 PE (Becton Dickinson, USA) and anti-CD19 Cy-Chrome (PharMingen, USA). The cells were incubated for 10 min in dark at  $4^\circ\text{C}$  and washed in the buffer afterwards.

**Flow cytometric analysis.** Cells were analysed using a FACSCalibur flow cytometer (Becton Dickinson) equipped with 488-nm argon laser. An acquisition gate was established based on FSC and SSC that included both the lymphocyte and monocyte populations (mononuclear cells) and excluded dead cells and debris. Each measurement contained 300 000 total events. After acquisition cells were analysed with CellQuest software.

**Statistical analysis.** Statistical analysis was performed using Wilcoxon-non-parametric test, U Mann-Whitney test and Statistica 5.0 software.

## Results

In our study we defined subpopulation of myeloid dendritic cells as BDCA-1-positive and CD19-negative cells and lymphoid DCs as BDCA-2 and CD123 double positive cells. The count of DCs was expressed as percentage of the peripheral blood mononuclear cells (PBMC).

In the control group, the total number of myeloid and lymphoid dendritic cells was  $0.402\% \pm 0.23$  and  $0.22\% \pm 0.14$ , respectively, of peripheral blood mononuclear cells. The percentage of myeloid DCs was significantly higher ( $p < 0.01$ ) than that of lymphoid DCs (Figs. 1, 2).

In the group of patients with breast cancer, the percentage of myeloid DCs ( $0.403\% \pm 0.325$  of PBMC) was significantly higher ( $p < 0.01$ ) than that of lymphoid DCs ( $0.23\% \pm 0.11$  of PBMC) (Figs. 1, 2).

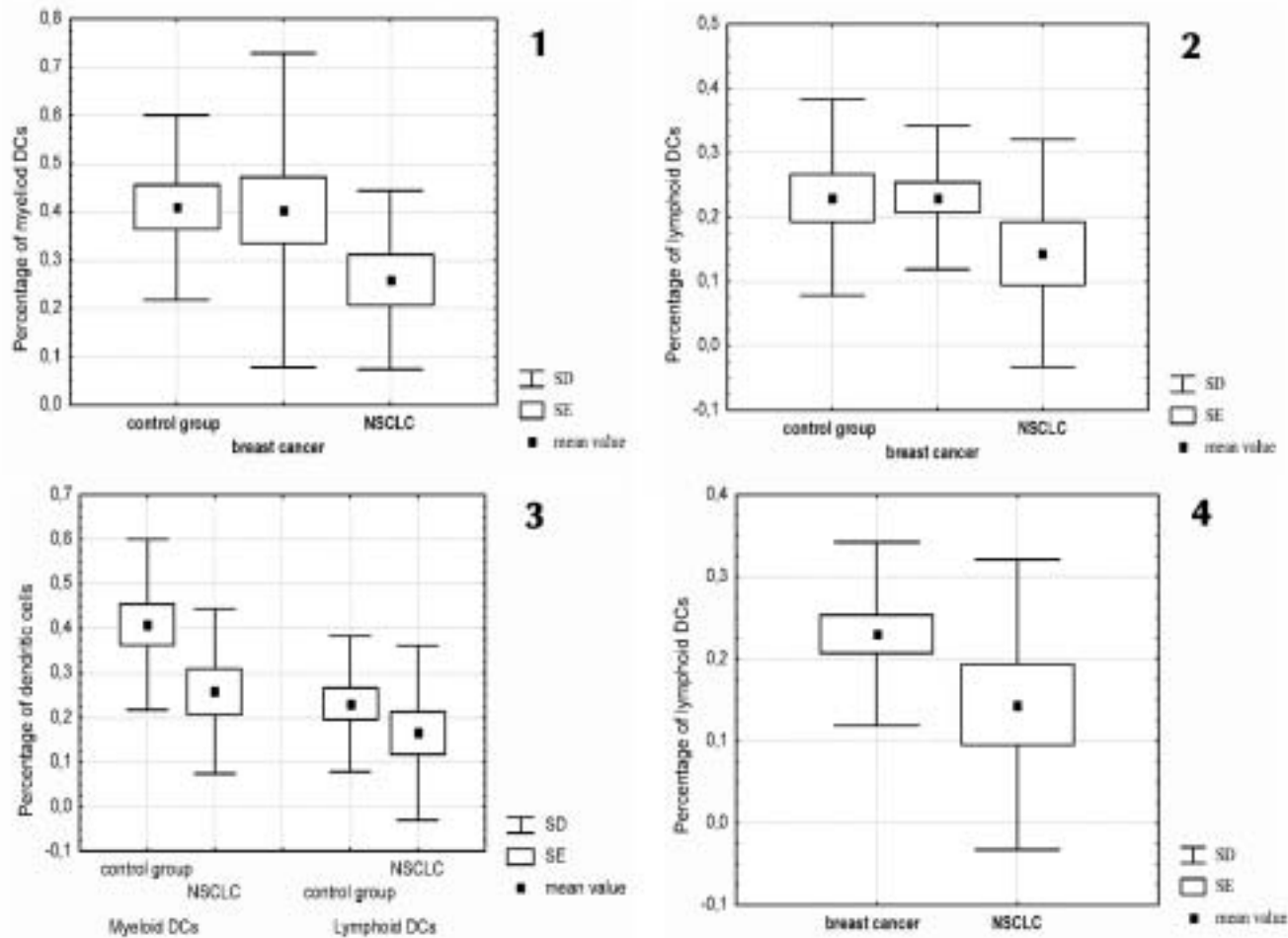
In patients with non-small cell lung cancer, the percentage of myeloid- and lymphoid-related DCs was  $0.253\% \pm 0.2$  and  $0.193\% \pm 0.166$  of PBMC, respectively. The percentage of myeloid-related DCs was also significantly higher ( $p < 0.001$ ) than that of lymphoid DCs (Figs. 1, 2).

Comparison of dendritic cell subsets between NSCLC patients and healthy donors demonstrates that the percentage of both myeloid and lymphoid DCs was significantly lower ( $p < 0.05$ ) in patients with non-small cell lung cancer than in the control group (Fig. 3). No statistically significant differences in the percentage of myeloid and lymphoid DCs between control groups and breast cancer patients were found. We also noticed that in blood from patients with NSCLC, the number of lymphoid DCs was significantly lower ( $p < 0.05$ ) than in patients with breast cancer (Fig. 4).

## Discussion

It is clear that in cancer patients, the immune response to cancer cells has failed [1]. Cancers probably acquire many characteristics that allow them to evade an effective immune response [6, 9]. The presence of functional, competent DCs is critical for effective antitumor control and for the success of cancer immunotherapy. There is ample evidence of inadequate function of these cells in tumor-bearing hosts [1, 6]. Tumor cells may produce several growth factors and cytokines able to affect hematopoiesis, differentiation and accumulation of effector cells at the site of neoplastic lesion [1, 6].

In our study, we investigated differences in myeloid and lymphoid DCs circulating in peripheral blood of cancer patients. We found that in all examined groups the percentages of myeloid DCs was significantly higher than that of lymphoid DCs. According to the function of myeloid DCs we conclude that abundance of myeloid DCs in cancer patients may correspond to predominance of Th1 type of immune response what is comparable



**Fig. 1.** Comparison of myeloid DCs between control group, breast cancer and NSCLC patients. **Fig. 2.** Comparison of lymphoid DCs between control group, breast cancer and NSCLC patients. **Fig. 3.** Comparison of both subpopulations of dendritic cells between control group and NSCLC patients. **Fig. 4.** Comparison of percentage of lymphoid DCs between breast cancer and NSCLC patients.

with healthy donors. We found noteworthy unchanged ratio but decrease in percentage of both populations of DCs in NSCLC patients. On the contrary, Almand *et al.* found increased production of immature myeloid cells in cancer patients and these cells were able to directly inhibit antigen-specific T-cell responses [1]. Gabrilovich *et al.* isolated DCs from the peripheral blood of patients with breast cancer. They demonstrated significantly reduced ability to cluster and to stimulate immune responses by this population of DCs [5]. We found no significant differences in both populations of DCs between breast cancer patients and healthy donors. The higher percentage of lymphoid DCs in breast cancer patients compared to NSCLC patients may result from different sex of the examined subjects [14]. The level of estrogens, predominant hormones in females, corresponds to higher levels of lymphoid DCs [13].

There is now enough evidence that defective DC function is an important mechanism of tumor escape

from the immune system control [6]. Our observation presented in this paper is a pilot study on the quantitative analysis of dendritic cells and allows us to conclude that the amount of DCs in peripheral blood of cancer patients may correspond to the type of neoplasm. It faces us with the necessity to expand the examined groups and to include the other pathological conditions. The further study should be aimed at comparisons of the stage, duration and localization of neoplasm with the number of dendritic cells.

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