Prognostic Significance of Peripheral Blood Flow Cytometry Parameters in Patients with Non-Metastatic Breast Cancer

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Abstract

Background: Immune functions and their relation to prognosis in breast cancer patients have become areas of great interest in recent years. Correlations between survival outcomes and peripheral blood flow cytometry parameters are therefore of interest. Here we focused on patients with non-metastatic breast cancer (BC).

Materials and Methods: A total of 29 patients with pathological confirmed breast carcinoma and flow cytometry data were assessed for overall survival (OS) and progression free survival (PFS).

Results: The median age of the patients was 54 years (range, 29-83). Multivariate analysis revealed that OS was significantly associated with absolute cytotoxic T cell count (95%CI, coef 2.26, p=0.035), tumor size (95%CI, coef -14.5, p 0.004), chemotherapy (95%CI, coef 12.9, p 0.0001), MFI of CD4 (95%CI, coef -5.1, P 0.04), MFI of HLA DR (95%CI, coef -5.9, p 0.008) and tumor grade (95%CI, coef -13, P 0.049) with R-Sq(adj)=67%. Similar findings were obtained for PFS.

Conclusions: OS and PFS were significantly associated with tumor grade, tumor size, chemotherapy, MFI of CD4, HLA DR and absolute cytotoxic T cell count. The study revealed that MFI of basic CD markers and absolute cytotoxic T cell number may be a prognostic factors in women with non-metastatic BC.

Keywords: Breast cancer - flow cytometry parameters - cytotoxic T cells
levels in early stage breast cancer patients (Gokmen et al., 2011). To date there are few studies that have investigated the T, B and NK cells by peripheral blood flow cytometry in patients with breast cancer but there are no data about the correlation between the overall survival and recurrence risk of breast cancer. So in this study we aimed to examine these relationships in postoperative patients with non-metastatic breast cancer.

Materials and Methods

This retrospective study was done in the outpatient oncology clinic of Bulent Ecevit University, Zonguldak between the period of 2004-2011. Our analysis included 29 patients with pathologically confirmed breast carcinoma.

Inclusion criteria’s were: Patients who had the Eastern Cooperative Oncology Group performance status of 0 or 1; baseline left ventricular ejection fraction measured by echocardiography greater than 50%; adequate organ function by measured and normal levels of laboratory of institute, complete blood count (neutrophils ≥1.5x10⁸/L, platelets ≥150x10⁹/L), liver function (serum bilirubin <1.5 times the upper limit of normal (ULN), transaminases ≤2.5 times ULN, alkaline phosphatase ≤2.5 times ULN), renal function (serum creatinine <1.5 times ULN).

Metastases were excluded by chest and abdomino-pelvic CT routinely and bone scan if needed. Patients’ morning blood samples were drawn before the treatment. Blood samples were measured by flow cytometry using monoclonal antibodies for cell surface antigens. Samples were measured by flow cytometry using monoclonal antibodies for cell surface antigens for percentages and absolute numbers of CTLs (CD3/CD8), total lymphocytes, T cells (CD3), activated T cells (CD3+ HLA-DR), helper T cells (CD3/CD4), cytotoxic T cells (CD8+CD28+), suppressor T cells (CD8+CD28-) and total white blood cell (TWC) count.

Statistical analyses

Treatment outcomes were estimated as response rate (RR), disease control rate, OS and progression-free survival (PFS). OS was defined as the time between the date of the diagnosis and the date of death from any cause. PFS was defined as the time from the date of the diagnosis to the date of disease progression or death from any cause. Also second OS and PFS were defined by the same way after the diagnosis of progression for patients whom received second line chemotherapy. CTLs counts were examined for their predictive relationship to survival time using Cox regressions analysis. Although the primary focus of this article was on the relationship between CTLs count and survival we examined the relationships between CTLs count and total lymphocyte count and subsets using Spearman rank correlations. Spearman correlations were also conducted to assess the relationship of CTL count with other markers of disease status, including age, metastases, TWC count, estrogen receptor (ER) status, progesteron recetor (PR) status, HER 2 status and ECOG performance status. All statistical analyses were done by the SPSS 17.0 software program.

Results

Patients’ characteristics

From 2004 to 2011, 29 BC patients were treated with systemic adjuvant chemotherapy as first-line therapy and flow cytometry analyses were done. The general characteristics of these patients are shown in Table 1. The median age of the patients was 54 years (range, 29-83). Four patients (14.0%) had diabetes mellitus and 5 patients (17%) had hypertension. Twenty one patients had modified radical mastectomy (MRM) and remaining 8 patients had breast conserving surgery (BCS) before the chemotherapy. None of the patient had metastases.

CTL counts were analyzed and the mean absolute (abs) CD19 count was 181/µl (95%CI, 60-1418), the mean absolute CD4 was 552/ µl (±190), mean abs CD8 was 845/µl (±338), mean abs NK 460/µl (±200), mean abs activated T cell was 3.6/µl (±1.2), mean abs cytotoxic T cell was 8.51 (±3.2) and mean abs suppressor T cell was 7.8 (±2.9). Mean florescence intensity (MFI) analysis was done and MFI of CD3 was 11.4 (±1.9), MFI of CD4 was 9.9 (4.9-11.1), MFI of CD8 was 15.6 (±5.3), MFI of HLADR was 3.2 (2.5-8.2), MFI of CD28 was 3.17 (±0.6), MFI of CD45 was 25 (12-58) and MFI of TCR γδ was 7.1 (±1.8).

Treatment

As first-line chemotherapy, 25 patients received polychemotherapy and the remaining 4 patients received hormonal therapy as letrozole, anastrozole or tamoxifen. Among the polychemotherapy regimens 6 patients received...
FEC-T regimen (5-FU 500 mg/m² iv d1+Epirubicin 100 mg/m² iv d1+Cyclophosphamide 500 mg/m² iv d1 Q3w ×3 cycles followed by Docetaxel 100 mg/m² iv Q3w ×3 cycles), 4 patients received CE regimen (Cyclophosphamide 75 mg/m² po qd d1-14+Epirubicin 60 mg/m² iv d1 and 8+5-FU 500 mg/m² iv d1 and 8 Q4w ×6 cycles), 5 patients received CMF regimen (Cyclophosphamide 100 mg/m²/d po d1-14+Methotrexate (MTX) 40 mg/m² iv d1 and 8+5-FU 600 mg/m² iv d1 and 8 Q4w ×6 cycles), 4 patients received AC-Docetaxel regimen (Doxorubicin 60 mg/m² iv push over 5-15 min d1+Cyclophosphamide 600 mg/m² iv over 30-60 min d1 Q3w ×4 cycles followed by Docetaxel 100 mg/m² iv over 1 hour Q3w ×4 cycles), 6 patients received AC-Paclitaxel-Trastuzumab regimen (Doxorubicin 60 mg/m² iv push over 5-15 min d1+Cyclophosphamide 600 mg/m² iv over 30-60 min d1 Q3w ×4 cycles followed by Paclitaxel 80 mg/m² iv over 1 hour Qw ×12 cycles+Trastuzumab 4 mg/kg loading dose beginning with paclitaxel, then 2 mg/kg iv qw ×1 year. All hormone positive patients received hormonal treatments such as tamoxifen, letrozole or anastrazole for at least 5 years. Twenty two patients received radiation therapy following the chemotherapy.

Survival and prognostic factors for first line chemotherapy

All 29 patients were included in the survival analysis. The median PFS for 29 patients was 50 months (95% CI, 1-84), median OS was 60 months (95% CI, 2-84) and the median DFS was 52 months (0-78) also showed in Figure 1. Multivariate analysis revealed that OS was significantly associated with absolute cytotoxic T cell count (95% CI, coef 2.26, p 0.035), tumor size (95% CI, coef -14.5, p 0.004), chemotherapy (95% CI, coef 12.9, P 0.0001), MFI of CD4 (95% CI, coef -5.1, p=0.04), MFI of HLA DR (95% CI, coef -5.9, P 0.008) and tumor grade (95% CI, coef -13, P 0.049) with R-Sq(adj)=67%. Multivariate analysis revealed that PFS was significantly associated with absolute cytotoxic T cell count (95% CI, coef 2.9, p 0.01), tumor size (95% CI, coef -14.1, p 0.004), chemotherapy (95% CI, coef 12.95, p 0.0001), MFI of CD4 (95% CI, coef -7, p 0.008) and MFI of HLA DR (95% CI, coef -5.4, p 0.018) with R-Sq(adj)=65%. For DFS multivariate analysis revealed that there was a significant association with absolute cytotoxic T cell count (95% CI, coef 2.9, p 0.007), tumor size (95% CI, coef -13, p 0.006), chemotherapy (95% CI, coef 13, p=0.0001), MFI of CD4 (95% CI, coef -5.9, p 0.019), MFI of HLA DR (95% CI, coef -4.7, p 0.026) and tumor grade (95% CI, coef -13.8, p 0.048) with R-Sq(adj)=68%.

Age, tumor markers, hemoglobin levels, WBC counts, absolute CD3, CD4, CD8, CD19, NK and TCRγδ were not significant independent prognostic factors for OS, PFS and DFS. Eight patients developed metastatic disease during the follow up period. Three of them had positive Her-2 and 2 patients had positive ER/PR status. Metastatic patients had statically significant higher levels of absolute CD3 count (1583 vs 1328, p 0.02) and CD8 counts (884 vs 831, p 0.023).

Discussion

In the present study we found that; OS and PFS were significantly associated with tumor grade, tumor size, chemotherapy, MFI of CD4, MFI of HLA DR and absolute cytotoxic T cell number. This is the first study in the literature which revealed that MFI of CD4, MFI of HLA DR and absolute cytotoxic T cell number may be a prognostic factor in women with breast cancer without metastases.

It is well known that tumor size, histological type and tumor grade are prognostic factors for breast cancer and chemotherapy decreases the recurrence risk in the adjuvant setting and is responsible, at least in part, for the reduction in cause-specific mortality from breast cancer in women. According to statistics from the American Cancer Society, the five-year relative survival based on tumor size alone is 95, 82, and 63 percent for tumors ≤2 cm, 2.1-5 cm, and >5 cm, respectively. Pathologic tumor size (>2 cm) was significantly associated with tumor grade, tumor size alone the five-year relative survival based on tumor size alone is 95, 82, and 63 percent for tumors ≤2 cm, 2.1-5 cm, and >5 cm, respectively. Pathologic tumor size (>2 cm) was associated with both distant DFS for recurrence and OS. Tumor grade is a strong predictor of outcome in patients with invasive breast cancer (Berry et al., 2005; Schwartz et al., 2008; Van der Hage et al., 2011).

There is evidence that CTL count (CD3/CD8) can have antitumor activity. The role of T cells mediate cytotoxicity is not clear in the tumor behavior (Kagi et al., 1994; 1996). T cells have extensive diversity in terms of phenotype and function. Functions of T cells are controlled by various categories of regulatory cells, these cells are dendritic cells (DC), CD4+CD25+ regulatory T cells (Tregs) and
CD8+CD28− suppressor cells via either the secretion of inhibitory cytokines or contact-mediated inhibition (Effros et al., 2004; Fehervari et al., 2004). CD8+CD28+ T cells are effector T cells (22) whereas CD4+CD25+ and CD8+CD28− T cells have either regulatory or suppressor functions. Previous reports have suggested suppressor T cells play a key role in the progression of cancer (Effros et al., 2004; Leong et al., 2006). In a study GC patients had a significant increase in levels of CD8+CD28− T cells in peripheral blood and percentage of CD8+CD28− cells among lymphocytes was higher in GC patients with LN metastasis than those without LN metastasis. The percentage of CD8+CD28− cells was also related to tumor infiltration and size, but not to the degree of differentiation of cancer cells (Shen et al., 2012). We couldn’t find a significant relation between OS and CD8+CD28− in breast cancer but found a significant relation between OS and CD8+CD28− T cells. Mortimer et al. revealed that there is a robust relationship between CTL count and survival independent of the effects of medical treatment in patients with metastatic breast cancer (Blake-Mortimer et al., 2004). Also, a significant reduction in the percentages of peripheral CTLs has been found in breast cancer patients with metastases compared to those without metastases (Liang et al., 1993; Alkhateeb et al., 2013). Consistent with these findings, a progressive decrease in both CD4 and CD8 cells has been associated with the spread of disease in patients with breast, malignant melanoma, lung, and colorectal cancers (Blake-Mortimer et al., 2004). Melichar et al found that CD8+CD28− T cell counts were significantly lower in breast cancer patients then controls but CD8+CD28+ T cell levels increased following doxorubicin-paclitaxel chemotherapy. Systemic chemotherapy may thus potentiate the effects of subsequent manipulation of the immune system both by decreasing the tumor mass and, simultaneously, indirectly by increasing the number of immunocompetent cells through still undefined mechanisms, e.g. alteration of the profile of macrophage cytokine production (Melichar et al., 2001). In our study we did not have a control group but increased CD8+CD28− T cell can explain the relationship with OS and PFS.

MFI of CD4 and HLA DR levels have not been studied in women with breast cancer by peripheral blood flow cytometry. Mean fluorescence intensity (MFI) was used to reflect the expression levels of these molecules. In a study a comparable percentage of DCs expressing CD86+ (B7-2), CD40+, and HLA-DR+ were detected in both cultures, higher expression levels were detected in DCs derived from bulk culture (Hinkel et al., 2000). In a study the authors observed lower levels of HLA DR, CD 80, CD 86 and CD 40 cells in the sentinel lymph node positive breast cancer (SN) compared to the lymph node-negative patients (Matsuura et al., 2006). Also we found a negative significant correlation between the MFI of HLA DR and CD4 levels with OS and PFS but we need more studies to put forth the prognostic importance of MFI in breast cancer. Our HLA DR results about lymphocytes, especially B cells and activated T cells. We can speculate that augmentation of HLA DR indicates increased antigen presentation to CD4 positive T cells by B lymphocytes via MHC Class II pathway. Also, augmentation of CD4 molecules serves the same purpose. But, this result is not indicates cellular anti-tumor activity. It is show just helping activity for immunoglobulin class switching on B cells by T helper cells. This switching may be due the blocking antibody generation. The tumor microenvironment may be a trigger for the immune tolerance. We have no data about this situation.

In this study we have some limitations. Very small study population and the retrospective design are the major limitations but we hope that this can be a preliminary study. Also we did not have a control group to compare the base line characteristics.

In conclusion, OS and PFS were significantly associated with tumor grade, tumor size, chemotherapy, MFI of CD4, MFI of HLA DR and absolute cytotoxic T cell count. This is the first study in the literature which revealed that MFI of basic CD markers and absolute cytotoxic T cell number may be a prognostic factor in women with non-metastatic breast cancer.

References
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