Evaluation of CD30/CD4/CD8 in triple-negative invasive ductal carcinoma of breast in association with clinicopathological prognostic factors

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Abstract

Background: Triple-negative breast cancer (TNBC) lacks the benefits of receptor-targeted therapeutic strategies. The limitations in treatment options along with poor patients' outcome heighten the need for novel approaches. Due to recent concentration on the role of biomarkers in prognosis, treatment, and survival of various cancer subtypes, this study involves an investigation of CD4, CD8, and CD30 markers detected by immunohistochemistry in TNBCs and their association with clinicopathological and prognostic factors.

Materials and Methods: Tissue samples of 85 hormone receptor- and human epidermal growth factor receptor-2-negative ductal breast carcinomas extracted from the archive of pathology department. Regarding CD4/CD8 ratio, the infiltrated T-lymphocytes were investigated. The tumoral tissue regions were also identified to be immunohistochemically assessed for the CD30 expression levels.

Results: With an elevated CD4/CD8 ratio, a significant increase in lymph node involvement was observed (P < 0.05); in contrast, increased expression levels of CD30 were related to significant reduction of lymph node involvement. CD30 overexpression was found to be significantly associated with shortened overall survival (OS) and highly infiltrated lymphocytes.

Conclusion: Following the progression in stage and grade of tumor, CD4/CD8 ratio and CD30 expression levels are increased and are accompanied by adverse prognosis and poor OS, while CD8-enhanced expression carries a favorable prognostic impact as it improves OS status. Therefore, all these findings could be of interest in the field of target therapy.

Materials and Methods

In this cross-sectional study, a total of 85 patients, who underwent mastectomy from 2009 to 2015 were included in the study. The cases were diagnosed for TNBC based on IHC findings of estrogen, progesterone, and HER2 receptors. Formalin-fixed paraffin blocks were isolated from pathology department archive; blocks with sufficient tumor tissue and stroma were selected. All samples were reviewed by two expert pathologists to confirm tumor grade. They also defined the suitable region for CD4, CD8 T-lymphocytes, and CD30 cells in tumoral tissue for IHC tests. According to computerized documents and medical records, patients' information and phone numbers were determined to assess survival status. Patients were then contacted and their clinical outcome (death/alive) was questioned. Information of patients who survived more than 4 weeks after surgery was used in the study; cases with incomplete information and insufficient tissue were excluded from the study. Time period between definite diagnosis and death or latest follow-up in living patients was applied for OS analysis. Paraffin-embedded tissue sections with a thickness of 3–4 microns were cut and IHC was done according to the manufacturer protocols (Biosystem, UK). CD4 and CD8 staining for infiltrated lymphocytes and CD30 staining for tumor cells were performed; staining percentage of involved cells was classified into five groups as following: <1%, 1%–25%, 25%–50%, 50%–75%, and 75%–100%. Staining intensity (SI) was categorized into four groups: negative, weak, moderate, and severe. Staining percentage and intensity were scored from 0 to 4 in colorectal cancers is associated with increased survival rates and significant prognostic impact as well as better response to treatment and survival status in breast cancers. Reverse effects have been demonstrated with the dominance of infiltrated CD4+ T-lymphocytes in breast cancer. It is worth to say that the presence of CD8+ T-cells along with the absence of CD4+ T-cells is associated with better overall survival (OS).

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Introduction

Breast cancer is known as a global health burden responsible for 21% of all cancers; in Iran, it continues to be the first leading cause of cancer among females, accounting for nearly 24% of all malignancies.[1],[2] Given its heterogeneous nature, various subtypes have so far been recognized.[3] Among all forms, triple-negative breast cancer (TNBC) does not or rarely (<1%) expresses estrogen receptors (ERs), progesterone receptors, and human epidermal growth factor receptor-2 (HER2).[4] It is not responsive to conventional receptor-targeted therapies, mostly found in younger women with more advanced clinical stages, and has a greater possibility of distant metastasis, especially in the first 5 years after definitive diagnosis.[5],[6],[7] Abundant data regarding predictive validity of tumor-infiltrating lymphocytes (TILs) in several cancers is available.[8] Highly expressed level of infiltrative CD8+ T-cells in colorectal cancers is associated with increased survival rates and significant prognostic impact.[9],[10] as well as better response to treatment and survival status in breast cancers.[11],[12] Reverse effects have been demonstrated with the dominance of infiltrated CD4+ T-lymphocytes in breast cancer. It is worth to say that the presence of CD8+ T-cells along with the absence of CD4+ T-cells is associated with better overall survival (OS).[13] CD30 targeted therapy with Brentuximab Vedotin (Adcetris) has also gained attraction in treatment of patients with refractory Hodgkin's lymphoma (HL) and Anaplastic large cell lymphoma (ALCL).[14] Hence, the aim of this study is to focus on the expression of markers such as CD30, CD4, and CD8 using immunohistochemistry (IHC) in TNBCs and their correlation with prognostic clinicopathological factors and survival status.
Results

In this study, 85 patients were entered with mean age of 50.93 ± 12.17 with the minimum of 26 and maximum of 88 years old. The average number of months for follow-up was 17.15 ± 8.61 with a minimum of 3 and maximum of 38 months. Two patients (2.4%) were in Stage I, 36 patients (42.4%) were in Stage II, 36 patients (42.4%) were in Stage III, and 11 patients (12.9%) were in Stage IV. Seven patients (8.2%) were in Grade I of disease, 33 patients (38.8%) in Grade II, and 45 patients (52.9%) in Grade III. Histopathologic variant of all samples was “invasive ductal carcinoma (NOS type).” ANOVA test results showed that disease distribution is not correlated with age (P = 0.405).

The mean age of patients in Grades I, II, and III was 54.43 ± 11.02, 50.52 ± 11.51, and 50.69 ± 12.95, respectively. ANOVA test results showed no correlation between age distribution and disease grade (P = 0.733).

Kruskal-Wallis test results revealed significant differences between CD4/8 ratio and CD8 and CD30 expression in various stages of disease (P = 0.006, P < 0.001, P < 0.001). Table 1 Table 2

The same test results showed that CD4/8 and CD8 distributions were not significantly different in various grades of disease (P = 0.167, P = 161), while CD30 distribution was significantly different in different grades (P = 0.017). Spearman’s correlation coefficient results demonstrated that with elevated CD4/8 ratio, lymph nodes involvement increased significantly (P = 0.005). As it is shown in Figure 2, CD8 increased expression levels are associated with significantly reduced lymph node involvement (P < 0.001). Table 3 Table 4

Correlation coefficient results indicated that with advancing in disease stage and grade, lymph node involvement, CD4/8 T-cell ratio, and CD30 expression levels are increased, and survival rate decreases significantly while CD8 increased levels were associated with higher survival rate. The results in Table 4 imply that 66% of TNBCs express CD30, which could be promising in terms of target therapy. Different SI of markers by IHC in TNBC is shown in Figure 4 and Figure 5.

Discussion

TNBCs, comprising 10%–20% of all breast cancer subtypes, tend to have aggressive clinical manifestations, adverse metastasis, low survival rate, and higher chance of recurrence during the first 3 years following diagnosis. Therefore, they require more invasive interventions.[8] According to a recent study in Iran, approximately 23% of all breast cancers (60 out of 267) were diagnosed with TNBC, while lymph node involvement, clinical stage (with most cases categorized in Stage 3), and lymph node involvement (70%) found to be higher among Iranian population.[15]

Pathophysiological significance of tumor-infiltrating T-cells has been investigated in many other carcinomas such as colon, pancreas, and ovarian carcinoma.[16,17] Prognostic significance of TILs has also been reported in breast cancer in several literatures but has not yet provided any comprehensive results.[18]

These findings suggest that immune markers can be applied as suitable predictors of relapse or weakness of immune system. The present study is a prospective evaluation of TNBC in Iranian population with a sample size that was calculated based on a previous study regarding positive CD4, CD8, and CD30 intratumoral T-cells (with 95% confidence level and 0.05 degree of accuracy).

Iwase et al. reported TNBC in younger population.[19] However, Akhtar showed that there were no significant age differences in TNBC patients compared to other groups; no such correlations were found between age and disease grade in our study, and the mean ages were also similar to Akhtar’s study.[20]

Liu et al. recently showed an IHC analysis of CD8 staining on more than 3000 breast cancers of different subtypes. Such evidence implies that basal TNBC may be the most immunotherapy responsive as it has more intratumoral T-cell regulation among ER-negative breast cancers. They revealed that CD8 can be considered as an independent prognostic factor for survival improvement in basal-like breast cancer. CD8-positive patients had mean survival of 3.5 years longer than those who lack the presence of CD8; these results were completely compatible with our study, and our results showed that CD8 expression increment is accompanied with significantly reduced lymph node involvement.[20]

Macchetti performed IHC method for infiltrated T-lymphocytes detection in 23 patients; he found that the infiltrations of CD4+ and CD8+ cells were higher in patients with lymph node metastases and were associated with worse prognosis.[21] In our study, following an increase in ratio of CD4/CD8 of involved lymph node, survivals were significantly decreased (P < 0.05). Another research mentioned that the presence of CD8 positive in infiltrated T-cells is generally associated with better prognosis; CD8-positive T-cells which include T-regulatory cells and tumor-associated macrophages (TAMs) bring worse outcomes.[3] In a recent study by DeNardo et al., intratumoral T-cells and macrophages’ correlation with clinical outcome of patients was assessed. IHC analysis of tissue microarrays derived from 179 treated naïve breast cancers showed that increased levels of infiltrated CD4+ T-cells and macrophages were associated with reduced OS; however, favorable OS was observed in high levels of CD8+ T-cells combined with low levels of macrophages and CD4+ T-cells.[13] Naito’s investigation in colorectal carcinoma and Sato’s study in ovarian carcinoma disagree with this study; they have mentioned that an increase in infiltrated T-cells in stroma, epithelium and margin cancers is associated with better survival.[17] Eireo et al. showed similar results in small cell lung carcinoma and mentioned that in patients with increased TIL, tumor sizes show reduction and the grade is lower; it will be associated with better prognosis as well. The biological differences between these four cancers can be due to differences in tissue microenvironment of breast cancer, small cell carcinoma of the lung, colorectal, and ovarian.[22] CD30 found in cancer, especially in lymphoid malignancies such as ALL and HL, is accompanied with poor prognosis.[23] The researchers had particular intention to CD30 roles, due to limited expression in normal cells and high expression in cancer cells and its significantly importance in lymphoma and other cancers’ development.

Li’s study reported CD30 as a prognostic factor for OS and progression-free survival (PFS) determination in patients with extranodal natural killer/T-cell lymphoma in nasal form. OS and PFS of patients with positive CD30 cells were significantly lower in comparison with negative CD30 patients; survival was 5 years higher in CD30+ cases.[24] In another study done on 903 diffuse large B-cell lymphoma patients, CD30+ individuals had higher OS and PFS.[25] In our study, with increasing levels of CD30+ in cells, lymph node involvement increased and OS rate decreased significantly.

Conclusion

Following the progression in stage and grade of tumor, CD4/CD8 ratio and CD30 expression levels are increased and are accompanied by adverse prognosis and poor OS, while CD8 enhanced expression carries a favorable prognostic impact as it improves overall survival status. Therefore, all these findings could be of interest in the field of target therapy.

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Conflicts of interest

There are no conflicts of interest.
References


