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

Amir Hossein Jafarian1, Aida Tasbandi2, Hamed Gilan3, Maryam Sheikhi4, Nema Mohamadian Roshan5

1 Department of Pathology and Laboratory Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2 Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3 Cancer Molecular Pathology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondence Address:
Nema Mohamadian Roshan
Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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 CD8 were related to significant reduction of lymph node involvement. CD30 overexpression was found to be significantly associated with shortened overall survival (OS) and high involvement of lymph nodes. 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.

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 young women with more advanced clinical stages, and has a greater possibility of distant metastasis, especially in the first 5 years after definite diagnosis.5-7 Abundant data regarding predictive validity of tumor-infiltrating lymphocytes (TILs) in several cancers is available.8 Highly expressed levels 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 Vodotin (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.

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 and 0 to 3, respectively; each case got a final score measured by multiplying percentage to intensity, and the outcome was defined as new parameter called CD30 expression score ranging from 0 to 12 for each sample.

Statistical analysis

Patients' information was fully recorded on separate checklists. Data analysis was done by Kruskal–Wallis test and Spearman's correlation coefficient for nonparametric samples and...
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 1)

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) [Table 2](Table 2)

Spearman's correlation coefficient results demonstrated that with elevated CD4/8 ratio, lymph nodes involvement increased significantly (P = 0.005) [Figure 1](Figure 1)

As the results indicated in [Figure 2](Figure 2), CD8 increased expression levels are associated with significantly reduced lymph node involvement (P < 0.001).

As it is shown in [Figure 3](Figure 3), the OS rate during 3 years of follow-up is 96% according to Kaplan-Meier curve.[Figure 3]

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 [Table 3]. The results in [Table 4](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](Figure 4) and [Figure 5](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] 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 (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.[2]

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 intratumor 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]

Macchietti 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; CD4-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 on 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] Eirola 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 ALCL 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.

Financial support and sponsorship

This work is supported by Research Deputyship of Mashhad University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.
References

1. Taheri NS, Baklشهادnorsa S, Talebi MN, Kashani E, Rajaei S, Besharat S, et al. Epidemiological pattern of breast cancer in Iranian women: Is there an ethnic disparity? Asian Pac J Cancer Prev 2012;13:4517-20.

2. Akhtar M, Dasgupta S, Rangwala M. Triple negative breast cancer: An Indian perspective. Breast Cancer Targets Ther 2015;7:239.

3. Stagg J, Altiok B, Inman-Brown KY, Pervolaraki F, Sigalos JP, et al. CD30 expression in triple-negative breast cancer: Latest research and clinical prospects. Ther Adv Med Oncol 2013;5:169-81.

4. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007;131:18-43.

5. Dent R, Hanna WM, Trudeau M, Rawlinson E, Narod SA, et al. Pattern of metastatic spread in triple-negative breast cancer. Breast Cancer Res Treat 2009;115:423-8.

6. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 2007;13:4429-34.

7. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California Cancer Registry. Cancer 2007;109:1721-8.

8. Salgado R, Denkert C, Demaria S, Siritane N, Klausingen F, Pruneng G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:59-71.

9. Miucnik B, Tosolini M, Krivolsky A, Berger A, Bindea G, Meatchi T, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol 2011;29:610-8.

10. Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE, et al. Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer. Oncol Rep 2005;10:309-13.

11. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011;29:1949-55.

12. Seo AN, Lee HJ, Kim EJ, Kim HJ, Jang MH, Lee HE, et al. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. Br J Cancer 2013;109:2705-13.

13. DeNardo DG, Brennan DJ, Resheph E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 2011;1:54-67.

14. Engert A, CD30-positive malignant lymphomas: Time for a change of management? Haematologica 2013;98:1165-8.

15. Mirzania M, Safaee SR, Shahi F, Jahandideh M, Jahanbakhsh H, et al. Treatment outcomes and clinicopathologic characteristics of triple-negative breast cancer: A report from cancer institute of Iran. Int J Hematol Oncol Stem Cell Res 2017;11:47-52.

16. Fukunaga A, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, et al. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. Pancreas 2004;30:26-31.

17. Saty S, Reddy R, Suresh G, Bhyruth B, Nithakaran H, Gann F, et al. Interstitial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005;102:18538-43.

18. Tischkowitz M, Brunet JS, Beglin LR, Huntsman DG, Cheang MC, Akslen LA, et al. Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. BMC Cancer 2007;7:134.

19. Iwase H, Kurobayashi J, Tsuda H, Ohtta T, Kurosumi M, Miyamoto K, et al. Clinicopathological analyses of triple negative breast cancer using surveillance data from the Registration Committee of the Japanese Breast Cancer Society. Breast Cancer 2010;17:118-24.

20. Liu S, Lachapel J, Leung S, Gao D, Fouleks WD, Nielsen TO, et al. CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res Treat 2012;14:R48.

21. Macchialli AH, Marana HR, Silva JS, de Andrade JM, Ribeiro-Silva A, Bighetti S, et al. Tumor-infiltrating CD4+ and T lymphocytes in early breast cancer reflect lymph node involvement. Clinica (Sao Paulo) 2006;61:203-8.

22. Eierla AK, Soini Y, Pääkkö P. A high number of tumor-infiltrating lymphocytes are associated with a small tumor size, low tumor stage, and a favorable prognosis in operated small cell lung carcinoma. Clin Cancer Res 2000;6:1875-81.

23. von Wasielewski R, Werner M, Fischer K, Hansmann ML, Hübner K, Hasenclever D, et al. Lymphocyte-predominant Hodgkin’s disease. An immunohistochemical analysis of 208 reviewed Hodgkin’s disease cases from the German Hodgkin Study Group. Am J Pathol 1987;120:793-803.

24. Li P, Jiang L, Zhang X, Liu J, Wang H. CD30 expression is a novel prognostic indicator in extranodal natural killer/T-cell lymphoma, nasal type. BMC Cancer 2014;14:890.

25. Hu S, Xu-Monette ZY, Balasubramanyam A, Maryam GC, Visco C, Tzankov A, et al. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: A report from the international DLBCL rituximab-GHOP consortium program study. Blood 2013;121:2715-24.