ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 01 | January - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ROLE OF TUMOR INFILTRATING CD3, CD4, CD8, CD20 AND MPO IN TRIPLE NEGATIVE BREAST CANCER

Oncology

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ABSTRACT

Objective: Immune cells are not only chief of immune system but also potent to modulate microenvironment favourably leads to immune rejection triggering enhanced tumor growth. Aim of the present study was to evaluate the prognostic significance of CD3+, CD4+, CD8+, CD20+and MPO+ in triple-negative breast cancer (TNBC). **Method:** Immunohistochemistry was performed and scored in tumor core, invasive margin and stroma separately. **Results:** Univariate survival analysis revealed that high CD3+ and CD4+ and CD8+infiltration in tumor core, invasive margin and stroma was associated with better disease-free and overall survival. Also, high CD20+ infiltration in tumor core alone was associated with better disease-free and overall survival. Multivariate cox regression analysis revealed that highCD4+ infiltration in tumor core was independent good prognosticator of DFS and high CD3+ infiltration in tumor core was independent good prognosticator of OS. No correlation was found of MPO+ with survival. **Conclusion:** This may indicate that high infiltration of CD3+, CD4+, CD8+ and CD20+ may serve as a good prognostic marker overall. These results may need further validation on larger cohort.

KEYWORDS

Tumor microenvironment, Tumor infiltrating lymphocyte, Myeloperoxidase, Invasive margin, Triple negative breast cancer, CD3, CD8, CD20.

INTRODUCTION

Breast cancer is one of the most common cancers in India with 13.5% of all cancer cases diagnosed in 2020 with highest death rate of 10% (Globocan, 2020). Triple negative breast cancer (TNBC) is most heterogeneous subtype of breast cancer accounts for 12-20% of all breast cancer cases. Steady improvements in screening methods and therapy, awareness and early detection have led to excellent prognosis for disease. It is a challenge to treat TNBC as it is relatively complex due to poor cell differentiation, molecular heterogeneity, and prone to metastasis, leads to chemo resistance and recurrence of the disease(Chang-Qing et al.,2020). Survival rates of this subtype are poor, only 15% of patients diagnosed with stage IV breast cancer with metastatic spread to other organs of the body will survive their disease for up to five years (Breast cancer statistics, 2017). Lack for routine markers for early detection of metastases, emphasises the need for new treatment strategies and novel therapeutic approaches to improve the long-term survival of patients. Over the last decade, research has moved away from the hypothesis that cancer cells alone result in development and progression of the disease.

The tumour microenvironment (TME)is a cellular environment in which dynamic communication between tumor cells and immune cells occurs. In elimination phase, cancer cells are knocking down by a competent immune system able to activate a strong immune response against cancer cells. Cancer cells which survived the elimination phase and in equilibrium phase immune cells reciprocally shape each other which establish a balance between the tumor and the immune system with a selection pressure on tumor cells, which are genetically unstable and rapidly mutating. This leads to tumor escape from immune cells and establish an immunosuppressive microenvironment (Folkman et al., 1992, Folkman et al., 1974, Hanahan et al., 2011). This complex interaction leads to more invasive tumor cells that can demolish the connective tissue and metastasize to survive, proliferate and migrate (Hinshaw et al., 2019). Understanding the complexity of how tumor Infiltrating Lymphocytes (TILS) co-operate with cancer cells is instrumental to ultimately establish more successful anti-cancer treatment. Tumor infiltrating lymphocytes growing incidence revealed that TIL-T, TIL-B, and tumor infiltrating myeloid cells have a positive impact on breast cancer prognosis.CD3 is a receptor glycoprotein present on mature T lymphocytes. High CD3 tumor infiltration is reported with favourable outcome in oropharyngeal cancer and low CD3 tumor infiltration has been reported to predict a shorter disease-free survival in colon and cervical cancer. CD4 is a glycoprotein found on helper T cells and CD8 is found on surface of cytotoxic T cells. There are studies which indicate that CD4 T cells are adequate to remove cancer cell alone however both CD3 and CD8 are necessary for effective tumor elimination (Galon et al., 2007). CD20 is a membrance protein found on surface of B cells. The role of infiltrative B lymphocytes is less explored and its prognostic importance is yet to be reached. MPO (myeloperoxidase) accumulate by neutrophilic granulocytes during their early maturation phase. It has been reported that high MPO infiltration is associated with better overall survival in breast cancer although there is lack of studies which evaluated role of T cell, B cell and MPO altogether (Pretscher et al., 2009). Hence, the present study explored thepresence of TILs CD3, CD4, CD8, CD20 and myeloid cell MPO by immunohistochemistry.

MATERIALS AND METHOD

This retrospective study included 100 TNBC patients who had been diagnosed and treated at the Institute in the year 2014 to 2019. The detailed history retrieved from case files of patients maintained at Medical Record Department of the institute. Formalin fixed paraffin embedded tumor tissue (FFPE) blocks were retrieved from Histopathology Department of the institute. Staging of disease was done according to UICC TNM classification. Disease status was obtained by clinical, biochemical and radiological investigations. Patients were followed up for 75 months. The mean follow-up period was 37 months. During the follow-up period, 15 developed metastases and 15 died due to cancer. Patients other than TNBC subtype and subjected to neoadjuvant therapy were excluded from the study. The study was approved by Institutional Scientific Review Board and Ethics Committee.

Immunohistochemistry

Immunohistochemical localization of CD3+TILs, Cd4+TILs, CD8+TILs, CD20+TILs, and MPO+ neutrophils were performed on FFPE tissue blocks containing primary tumor. The 4µm thin sections were cut on microtome (Leica, Germany) and transferred on 3-aminopropyl triethoxysilane (APES) coated glass slides and processed on Ventana Benchmark XT auto-immuno-Stainer using Ventana reagents (Ventana, USA). The protocol includes following steps of deparaffinization using EZ solution, antigen retrieval using cell conditioning (CC1), incubation with ultraview DAB inhibitor for 4 minutes, 100µl of primary antibody, ultraview HRP multimer for 8 minutes, ultraview DAB detection kit for 8 minutes, counter stain with haematoxylin for 8 minutes, bluing reagent for 4 minutes and mounted with DPX. The details of primary antibody used are as follow.

Microscopic evaluation

All slides are morphologically analysed on low power field to identify the field of interest. Immune stained positive cells were counted in three randomly selected high-power fields (HPF) at 40X magnification in tumor core which is area within the tumor border. Invasive margin of tumor which is border separating the malignant cell nests from the host

tissue and stroma separately. Cells which are immediately adjacent to the invasive margin are counted as cells of invasive margin. Absolute counts for the three fields were averaged. Results are expressed as count/HPF and the Median score of the averaged cell count was calculated. Cell count below median score were considered as low expression and cell count above the median score considered as high expression.

Statistical analysis:
Statistical analysis was carried out using SPSS statistical software version 26 (SPSS Inc, USA). Disease free survival (DFS) duration was calculated from date of disease diagnosed to the date of disease relapse or last follow up date. Overall survival (OS) time was calculated from the date of disease diagnosed to the death of patient due to cancer. Univariate survival analysis was performed using log rank test using Kaplan-Meier method. Multivariate analysis was performed using cox regression method with 95% confidence interval. p value ≤0.05 were considered significant.

RESULTS

Incidence and infiltration of Immune cells in tumor microenvironment and their correlation with clinical and pathological parameters

CD3+TILs

In microenvironment of TNBC tumors, in tumor core, invasive margin and stroma infiltration of CD3+TILswere noted in 89% (89/100), 77% (77/100) and 87% (87/100) of TNBC. In comparison with tumor core and stroma invasive margin had less infiltration of CD3+TILs.Further infiltrating CD3+TILs were correlated with clinical and pathological parameters. With tumor size, smaller tumors noted significant high infiltration of CD3+TILsas compared to larger tumors in tumor core (64%, 49/77vs. 0%, 0/23, p=<0.001), invasive margin (58%, 45/77vs. 17%, 4/23 p=<0.001) and stroma (53%, 41/77vs. 26%, 6/23, p=0.02). With lymph node negative status, significant high infiltration of CD3+TILS was observed in tumor core (62%,36/58, p=0.002), along with invasive margin (59%,34/58, p=0.02) however such difference was not observed with stromal infiltration. With a disease stage significant incidence of high CD3+TILs infiltration in stage I as compared stage II and III was observed in tumor core (100%, 2/2, p=<0.001vs. stage II, 63%,47/& stage III, 0%0/23), invasive margin (100%, 2/2,vs. stage II, 57%,43/75 & stage III, 17%, 4/23 p=<0.001), and stroma (50%,1/2vs. stage II, 47% 35/75 & stage III, 26%, 6/23 p=0.03).Majority of the patients had invasive ductal carcinoma subtype (81%), followed by IDC+DCIS subtype (17%) and IDC+Medullary subtype (2%). IDC+Medullary subtype had higher stromal infiltration (100%, 2/2, p=0.02) of CD3+TILs than IDC alone and IDC+Medullary subtype. With BR score a significant high infiltration of CD3+TILsin stroma were observed in patients with high BR score (53%, 33/62, p=0.01) although no such significance observed in tumor core and invasive margin. With absence of perinodal extension significant highCD3+TILs infiltration in tumor core (58%, 58/78, p=<0.001) and invasive margin (56%, 56/78, p=0.009) was noted; however no such difference was observed with stromal infiltration. With a disease status, a group of patients who were not developed metastasis had significant higherCD3+TILs infiltration in tumor core (54%, 46/85, p=0.01 and stroma (52%, 44/85, p=0.02) and a trend of high infiltration was observed in invasive margin (53%, 45/85,p=0.06), (Table-II).

CD4+TILs

We observed CD4+TILs rich microenvironment in TNBC tumors. Infiltration of CD4+TILs in tumor core was noted in 68% (68/100) of TNBC patients. Infiltration of invasive margin was noted in 72% (72/100) patients and infiltration in stroma was noted in 81% (81/100) of TNBC patients. In comparison with invasive margin and stroma tumor core has less infiltration of CD+ T cells. With tumor size smaller tumors noted significant high CD4+TILs infiltration in tumor core (60%, 46/77vs. 4%1/23, p<0.001), invasive margin (38%, 29/77vs. 4%, 1/23p=0.002) and stroma (42%, 32/77vs.17%, 4/23 p=0.03) as compared to larger tumors. With lymph node negative status, a significant higher CD4+TILs infiltration in tumor core (57%, 33/58, p=0.02) was observed however no such difference was observed in invasive margin and stromal infiltration. Similarly, stage-I patients showed high CD4+TILs infiltration in tumor core (100%, 2/2, p=<0.001), invasive margin (50%, 1/1, p=0.002), and stroma (100%, 2/2, p=0.01). With absence of perinodal extension, significant high CD4+TILs infiltration in tumor core (56%, 44/79, p=0.001), and a trend of high CD4+TILs infiltration in invasive margin (34%, 27/79, p=0.07*) was observed, however no such difference was noted with stromal infiltration. In patients with absence of perineural invasion significant high CD4+TILs infiltration in stroma (38%, 36/94, p=0.05) was noted while no such difference was noted with tumor core and invasive margin infiltration. With a disease status, patient who were not developed metastasis had significant high CD4+TILs infiltration in tumor core (53%, 45/85, p=0.004), invasive margin (34%, 29/85, p=0.03) and stroma (40%, 34/85, p=0.04), (Table-III).

CD8+TILs

In our study, we observed infiltration of CD8+TILs in tumor core were noted in 64% (64/100), in invasive margin was noted in 67% (67/100) patients and in stroma was noted in 73% (73/100) of TNBC patients. In comparison with invasive margin and stroma, tumor core had less CD8+ infiltration. With tumor size, smaller tumors noted significant high CD8+TILs infiltration in tumor core (61%, 47/77, p=<0.001), invasive margin (60%, 46/77, p=<0.001) and stroma (60%, 46/77, p=<0.001). With absence of lymph node negative status, significant high CD8+TILs infiltration in tumor core (60%, 35/58, p=0.003) and trend of high infiltration in invasive margin (57%, 34/58, p=0.006) and stroma(59%, 34/8, p=0.006) was noted in patients with stage-I disease were showed low CD8+ TILs infiltration in tumor core (96%, 22/23, p=<0.001), invasive margin (96%, 22/23, p=<0.001) and stroma (96%, 22/23, p=<0.001). Patients with absence of perinodal extension exhibited high CD8+TILs infiltration in tumor core (56%, 44/79, p=0.002), invasive margin (53%,42/79, p=0.003) and stroma (54%, 43/79, p=0.003). With a disease metastasis, patient who were not developed metastasis had significant higher CD8+TILs infiltration in tumor core (53%, 45/85, p=0.01), invasive margin (52%, 44/85, p=0.02) and stroma (52%44/85, p=0.02), (Table-IV).

CD20+ TILs

CD20+TILsinfiltration in microenvironment of TNBC patients noted in tumor core of 52% (52/100) patients, invasive margin of 51%(51/100) patients and stroma of 82%(82/100) patients. In comparison with tumor core and stroma, invasive margin had less infiltration of CD20+TILs. With histological subtype, significant high incidence of stromal CD20+ TILs infiltration (54%, 54/81, p=0.02) was observed along with trend of high infiltration in tumor core (42%, 34/81, p=0.07*) in patients with Infiltrative ductal carcinoma subtype, however no such difference was seen with invasive margin in filtration. Patients with high BR score exhibited high CD20+TILs infiltration in invasive margin (57%, 35/62, p=0.01), while no such difference was observed with tumor core and stromal infiltration. In a group of patients free from disease metastasis had significant high CD20+TILs infiltration in tumor core(42%, 36/85, p=0.03) however no such difference was seen with invasive margin and stroma, (Table-V).

Tumor infiltrating Myeloperoxidase cells

We observed infiltration of myeloperoxidase (MPO) in tumor core of 63% (63/100), invasive margin of 72% (72/100) and stroma of 73% (73/100) patients. In comparison with invasive margin and stroma, tumor core had less infiltration of MPO. Patients with pre-menopausal status had significant high incidence of MPO infiltration in tumor core (61%, 28/46, p=0.03), however it was not observed with invasive margin and stroma. With histological subtype, patients with infiltrative ducal carcinoma subtype had high MPO infiltration of tumor core (52%,42/81,p=0.03) which is notwithstanding with invasive margin and stroma. In group of patients with absence of perinodal extension exhibited high MPO infiltration in tumor core (52%, 41/79, p=0.05), however no such difference was seen with infiltration of invasive margin and stroma, (Table-VI).

Inter-marker correlation of T cell markers

The nonparametric Pearson's correlation revealed that CD3+ T cell infiltration in tumor core, invasive margin and stroma exhibited significant positive correlation with CD4+ T cell infiltration in tumor core, invasive margin and stroma (p=<0.001). With regard to CD8+ T cell infiltration in tumor core, invasive margin and stroma showed significant positive correlation with CD3+ T cell infiltration in tumor core, invasive margin and stroma (p=<0.001). Moreover, CD4+ T cell infiltration in tumor core, invasive margin and stroma was significantly positively correlated with CD8+ T cell infiltration in tumor core, invasive margin and stroma (p=<0.001)

Univariate survival analysis

Disease free survival

The prognostic value of infiltrating immune cells with respect to

Disease free survival was determined using Kaplan Meier survival curves. In univariate survival analysis patients with high CD3+TILsinfiltration in tumor core (49%, 67.23±2.06 months, χ^2=6.72, p=0.01) had better longer disease-free survival as compared to invasive margin (47%, 68.63±2.07 months, χ^2=8.9, p=0.003), and stroma (47%, 66.13±2.13 months, χ^2=6.14, p=0.01). Also, CD4+TILS infiltration in tumor core (47%, 68.63±2.07 months, χ^2=8.9, p=0.003) invasive margin (47%, 68.63±2.07months, χ^2=8.9, p=0.003) and stroma (36%, 66.63±2.30 months, χ^2=4.3, p=0.03) showed a significant longer disease-free survival. Patients expressing highCD8+TILsinfiltration in tumor core (48%, 66.89±2.38 months, χ^2=5.27, p=0.02), invasive margin (47%, 66.83±2.31 months, χ^2=4.99, p=0.02) and stroma (47%, 66.83±2.31 months, χ^2=4.99, p=0.02) depicted longer disease-free survival. Patients expressing high CD20+tumor core infiltration (38%, 66.50±1.47 months, χ^2=5.33, p=0.02) had significant longer disease-free survival; however such a difference was not seen with infiltration of invasive margin and stroma.

Overall survival

Patients with high incidence of CD3+TILsinfiltration in tumor core (49%, 68.38±1.78 months, χ^2=8.23, p=0.004), invasive margin (49%, 67.35±1.99 months, χ^2=7.66, p=0.006) and stroma (47%, 67.28±1.84 months, χ^2=8.00, p=0.005) reported significant better overall survival.CD4+TILs infiltration in tumor core (47%,68.47±1.72 months, χ^2=8.12, p=0.004),invasive margin (30%, 69.22±1.73 months, χ^2=5.42, p=0.02) and stroma(36%, 66.51±2.33 months, χ^2=3.8,p=0.05)was associated with significant better overall survival.Also,CD8+TILs infiltration in tumor core (48%, 66.38±1.78 months, χ^2=6.52, p=0.01), invasive margin (47%, 68.34±1.81 months, χ^2=6.24, p=0.01) and stroma (47%, 68.34±1.81 months, χ^2=6.24, p=0.01) was associated with significant better overall survival. High CD20+TILs infiltration in tumor core was also significantly associated with better overall survival (38%, 66.47±1.48 months, χ^2=5.26, p=0.02) however, infiltration in invasive margin and stroma had no significant relation to overall survival. Infiltration of MPO did not show any significant relation with overall survival.

3.8 Multivariate survival analysis

Multivariate Cox regression analysis was performed using forward selection method revealed that greater tumor size (Wald=9.47, HR=0.18, 95% CI=0.06-0.05, P=0.002) entered at step 1 and high BR score (Wald=0.56, HR=4.90, 95% CI=1.61-14.93, P=0.005) entered at step 2 are significant poor prognostic factors for predicting disease free survival. Regarding OS, greater tumor size (Wald=8.60, HR=0.15, 95% CI=0.04-0.54, P=0.003) entered at step 1, high BR score (Wald=7.76, HR=6.13, 95% CI=1.71-21.95, P=0.005) at step 2, and high grade at step 3(Wald=5.51, HR=0.08, 95% CI=0.01-0.66, P=0.019) as significant poor prognostic factors for predicting overall survival.

Multivariate analysis with only infiltrating immune cells was performed revealed that high infiltration of CD4+TILs in tumor core (Wald=6.56, HR=7.00, 95%CI=1.58-31.08, P=0.01) entered at step 1 found to be an independent good prognostic factor for disease free survival and with regard to overall survival, infiltration of CD3+TILs in tumor core (Wald=6.15, HR=6.59, 95%CI=1.48-29.23, P=0.013) entered at step 1found to be an independent good prognostic factor for overall survival.

DISCUSSION

It is noteworthy to mention that immune cell interactions with other components in the TME can impact tumor fate. Studies manifest that tumor brilliantly controls its surrounding environment to promote its establishment, growth, survival, and spread by reprogramming innate immune cells to stimulate tumor growth and survival, leaving the patient with a weakened shielding against disease and sometimes a worse prognosis (Hinshaw et al., 2019) In the present study we evaluated the localization of CD3+. We observed that high CD3+TILs infiltration in stroma was associated with the patients expressing both invasive ducal and medullary subtype. Similar findings were obtained in breast cancer patients (Wang et al., 2016). There was no significance observed in Infiltration in tumor core and invasive margin. Also, our study reported that patients having lymph nodes free of tumor showed high CD3+TILs infiltration in both tumor core and invasive margin. Similar finding were reported in breast cancer patients (Blenman et al., 2018). However stromal infiltration has no significant association with lymph node involvement. This indicates CD3+TILs traffic in tumor might have a successful control over extravasation of tumor cells to be associated lymph nodes. Moreover, it is observed that patients having high BR score had more infiltration ofCD3+TILs in stroma. Similar results were reported in breast cancer patients (Lee et al., 2013). In our study, we found significant correlation between the smaller tumor size and high CD3+TILs infiltration in tumor core, invasive margin as well as stroma. Similar findings were reported in breast cancer (Rathore et al., 2014), Similar results indicating the inverse association between tumor size and CD3+TILs infiltration in colorectal cancer (Galon et al., 2007), bladder cancer (Sjödahl et al., 2014) and in squamous cell carcinoma (Mukherjee et al., 2020) were reported. Which indicates CD3+TILs might be a robust tumor suppressor immune cell. Further, it was observed that patients with early stage of tumors are high CD3+TILs infiltration in all three, tumor core, invasive margin and stroma. Supportive findings to our results were reported in TNBC and breast cancer (Balkenhol et al., 2021; Tsiatas et al., 2018). It was observed that high infiltration of CD3+TILs in tumor core, and stroma of patients with absence of metastasis. Patients who developed metastasis had poor infiltration of CD3+TILs. Similar results were reported in breast cancer (Rathore et al,. 2014; Kim et al., 2013). Additionally, we found that patients with absence of perinodal extension had higher CD3+TILs infiltration in tumor as well as invasive margin which is up to our knowledge reported first time in any study.

Furthermore, on evaluating expression of CD4+ Helper TILs in TNBC, it has been observed that high infiltration in tumor core of patients with lymph node negative status. Supportive findings to these results were reported in TNBC (Sikandar et al., 2017; Chen et al., 2020; Quintana et al., 2021).However, there was no significant correlation found with invasive margin and stromal infiltration. In consistent to CD3+TILs expression, CD4+TILswere also found to be high in tumor core, invasive margin and stroma in patients with smaller tumor size. Similar results were reported in breast cancer (Schmidt et al., 2018). Likewise, CD3+TILs expression, patients with early stage of disease exhibited high CD4+TILs infiltration in tumor core, invasive margin and stroma. Similar findings were reported in TNBC and breast cancer (Jagtap et al., 2018; shi et al., 2018). In our study, absence of metastasis was also associated with high CD4+TILs infiltration in tumor core, invasive margin and stroma. Similar results were reported in breast cancer (matkowaki et al., 2009; zhang et al., 2020). Moreover, patients with absence of perinodal extension had high CD4+TILs infiltration in tumor core and patients with absence of perineural extension had high CD4+TILs infiltration in stroma are first time reported in any study as per our knowledge. However, there have been limited studies of CD4+TILs in breast cancer. We also observed patients with absence of perinodal extension exhibited high expression of CD8+TILs in tumor core, invasive margin and stroma which is a novel finding of this study. In our study we observed high CD8+TILs infiltration in tumor core, invasive margin and stroma of patients with absence of metastatic lymph node. Similar findings were reported in rectal cancer and oral squamous cell carcinoma (Däster et al., 2014; Mukherjee et al., 2020). Further we observed high CD8+TILs infiltration in tumor core, invasive margin and stroma was associated with smaller tumor size. Similar findings in breast and oral cancer reported (Rathore et al., 2014, Mukherjee et al., 2020). We observed low infiltration of CD8+TILs in tumor core, invasive margin and stroma of advanced stage patients. Similar findings were observed in breast cancer (Rathore et al., 2014; Abdou et al., 2020). In present study high CD8+TILs infiltration in tumor core, invasive margin and stroma was observed in patients with absence of metastatic lymph node. Similar finding was reported in breast cancer patients (Li et. al., 2021).

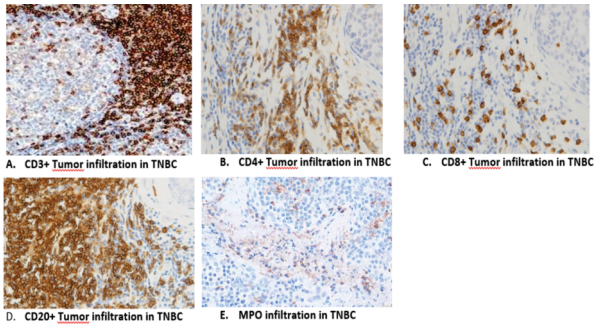
We also examined the expression of CD20+ TILs in TNBC and evaluated high CD20+ TILs stromal infiltration associated with IDC subtype which is similar to other studies. However, no significant infiltration in tumor core and stroma observed (Xu et al., 2018; Bai et al., 2020). Also, patients with high BR score expressed high CD20+ TILs invasive margin infiltration which is contradictory to other study (Eiró et al., 2012). However, no significant finding was observed in infiltration of tumor core and stroma. In addition, we also observed high CD20+TILstumor core infiltration in patients with absence of metastasis. Supportive finding was reported in TNBC and in head and neck cancer (Kuroda et al., 2021; pretscher et al., 2009). However infiltration of invasive margin and stroma are not significant.

In present study we observed high MPO infiltration in tumor core of premenopausal patients as compared to post and peri menopausal patients. These results are parallel with the findings in ovarian cancer

(Castillo-Tong et al., 2014). Also, we observed that high infiltration of MPO in tumor core is associated with infiltrative ductal carcinoma subtype and absence of perinodal extension. However due to lake of studies there are no literature consistent with our results. Present study observed significant positive correlation between CD3+ T cell, CD4+ T cell and CD8+ T cell, which indicates that helper T cells help to activate cytotoxic T cells to kill target cells.

4.1 CONCLUSION

This study noted that patients with smaller tumor size and lymph node negative status had high CD3+, CD4+ and CD8+ TILs and were found to be good prognosticators of disease-free survival and overall survival of TNBC.This may offer new biomarkers and drug targets which leads to precision-personalized therapies for TNBC. Although quantifying immune cells by histopathology and immunohistochemistry is a frequently used application, the methodological factors may astonish the exact magnitude on prognosis. The study needs to be done in larger sample size, including the cytokines for a better understanding of role of TILs.



Representative Microphotographs of Immunohistochemical staining

REFERENCES:

1. GLOBOCAN(2020): New Global Cancer Data retrived from: https://www.uicc.org/news/globocan-2020-new-global-cancer-data
2. Chang-Qing, Y., Jie, L., Shi-Qi, Z., Kun, Z., Zi-Qian, G., Ran, X., ...& Chen-Yan, Z. (2020). Recent treatment progress of triple negative breast cancer. *Progress in biophysics and molecular biology*, *151*, 40-53.
3. Breast cancer statistics (2017): https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/survival
4. Folkman J. The role of angiogenesis in tumor growth. InSeminars in cancer biology 1992 Apr 1 (Vol. 3, No. 2, pp. 65-71).
5. Folkman J. Tumor angiogenesis. Advances in cancer research. 1974 Jan 1;19:331-58.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell. 2011 Mar 4;144(5):646-74.
7. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. Cancer research. 2019 Sep 15;79(18):4557-66.
8. Wang XX, Jiang YZ, Liu XY, Li JJ, Song CG, Shao ZM. Difference in characteristics and outcomes between medullary breast carcinoma and invasive ductal carcinoma: a population based study from SEER 18 database. Oncotarget. 2016 Apr 4;7(16):22665.
9. Blenman KR, He TF, Frankel PH, Ruel NH, Schwartz EJ, Krag DN, Tan LK, Yim JH, Mortimer JE, Yuan Y, Lee PP. Sentinel lymph node B cells can predict disease-free survival in breast cancer patients. NPJ breast cancer. 2018 Aug 23;4(1):1-7.
10. Lee HJ, Seo JY, Ahn JH, Ahn SH, Gong G. Tumor-associated lymphocytes predict response to neoadjuvant chemotherapy in breast cancer patients. Journal of breast cancer. 2013 Mar 1;16(1):32-9.
11. Rathore AS, Kumar S, Konwar R, Makker A, Negi MP, Goel MM. CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. The Indian journal of medical research. 2014 Sep;140(3):361.
12. Galon J, Fridman WH, Pages F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer research. 2007 Mar 1;67(5):1883-6.
13. Sjödahl G, Lövgren K, Lauss M, Chebil G, Patschan O, Gudjonsson S, Månsson W, Fernö M, Leandersson K, Lindgren D, Liedberg F. Infiltration of CD3+ and CD68+ cells in bladder cancer is subtype specific and affects the outcome of patients with muscle-invasive tumors. InUrologic Oncology: Seminars and Original Investigations 2014 Aug 1 (Vol. 32, No. 6, pp. 791-797). Elsevier.
14. Mukherjee G, Bag S, Chakraborty P, Dey D, Roy S, Jain P, Roy P, Soong R, Majumder PP, Dutt S. Density of CD3+ and CD8+ cells in gingivo-buccal oral squamous cell carcinoma is associated with lymph node metastases and survival. PloS one. 2020 Nov 19;15(11):e0242058.
15. Balkenhol MC, Ciompi F, Świderska-Chadaj Ż, van de Loo R, Intezar M, Otte-Höller I, Geijs D, Lotz J, Weiss N, de Bel T, Litjens G. Optimized tumour infiltrating lymphocyte assessment for triple negative breast cancer prognostics. The Breast. 2021 Apr 1;56:7887.
16. Tsiatas M, Kalogeras KT, Manousou K, Wirtz RM, Gogas H, Veltrup E, Zagouri F, Lazaridis G, Koutras A, Christodoulou C, Pentheroudakis G. Evaluation of the prognostic value of CD3, CD8, and FOXP3 mRNA expression in early.stage breast cancer patients treated with anthracycline.based adjuvant chemotherapy. Cancer medicine. 2018 Oct;7(10):5066-82.
17. Kim ST, Jeong H, Woo OH, Seo JH, Kim A, Lee ES, Shin SW, Kim YH, Kim JS, Park KH. Tumor-infiltrating lymphocytes, tumor characteristics, and recurrence in patients with early breast cancer. American journal of clinical oncology. 2013 Jun 1;36(3):22431.
18. Sikandar B, Qureshi MA, Naseem S, Khan S, Mirza T. Increased tumour infiltration of CD4+ and CD8+ T-lymphocytes in patients with triple negative breast cancer suggests susceptibility to immune therapy. Asian Pacific Journal of Cancer Prevention: APJCP. 2017;18(7):1827.
19. Chen YY, Ge JY, Ma D, Yu KD. Immune-Activated Regional Lymph Nodes Predict Favorable Survival in Early-Stage Triple-Negative Breast Cancer. Frontiers in Oncology. 2020 Oct 9;10:570981.
20. Quintana Á, Peg V, Prat A, Moliné T, Villacampa G, Paré L, Galván P, Dientsmann R, Schmid P, Curigliano G, Muñoz-Couselo E. Immune analysis of lymph nodes in relation to the presence or absence of tumor infiltrating lymphocytes in triple-negative breast cancer. European Journal of Cancer. 2021 May 1;148:134-45.
21. Schmidt M, Weyer-Elberich V, Hengstler JG, Heimes AS, Almstedt K, Gerhold-Ay A, Lebrecht A, Battista MJ, Hasenburg A, Sahin U, Kalogeras KT. Prognostic impact of CD4-positive T cell subsets in early breast cancer: a study based on the FinHer trial patient population. Breast Cancer Research. 2018 Dec;20(1):1-0.
22. Jagtap SV. Evaluation of CD4+ T-cells and CD8+ T-cells in triple-negative invasive breast cancer. Indian Journal of Pathology and Microbiology. 2018 Oct 1;61(4):477.
23. Shi F, Chang H, Zhou Q, Zhao YJ, Wu GJ, Song QK. Distribution of CD4+ and CD8+ exhausted tumor-infiltrating lymphocytes in molecular subtypes of Chinese breast cancer patients. OncoTargets and therapy. 2018;11:6139.
24. Matkowski R, Gisterek I, Halon A, Lacko A, Szewczyk K, Staszek U, Pudelko M, Szynglarewicz B, Szelachowska J, Zolnierek A, Kornafel J. The prognostic role of tumor-infiltrating CD4 and CD8 T lymphocytes in breast cancer. Anticancer research. 2009 Jul 1;29(7):2445-51.
25. Zhang T, Duan F, Su D, Ma L, Yang J, Shi B, He X, Ma R, Sun S, Yao X. Analysis of the Heterogeneity of CD4+ CD25+ T Cell TCR β CDR3 Repertoires in Breast Tumor Tissues, Lung Metastatic Tissues, and Spleens from 4T1 Tumor-Bearing BALB/c Mice. Journal of immunology research. 2020 Sep 24;2020.
26. Däster S, Eppenberger-Castori S, Hirt C, Zlobec I, Delko T, Nebiker CA, Soysal SD, Amicarella F, Iezzi G, Sconocchia G, Heberer M. High frequency of CD8 positive lymphocyte infiltration correlates with lack of lymph node involvement in early rectal cancer. Disease markers. 2014 Dec 30;2014.
27. Abdou Y, Attwood K, Cheng TY, Yao S, Bandera EV, Zirpoli GR, Ondracek RP, Stein L, Bshara W, Khoury T, Ambrosone CB. Racial differences in CD8+ T cell infiltration in breast tumors from Black and White women. Breast Cancer Research. 2020 Dec;22(1):1-0.
28. Li K, Li T, Feng Z, Huang M, Wei L, Yan Z, Long M, Hu Q, Wang J, Liu S, Sgroi DC. CD8+ T cell immunity blocks the metastasis of carcinogen-exposed breast cancer. Science Advances. 2021 Jun 18;7(25):eabd8936.
29. Xu Y, Lan S, Zheng Q. Prognostic significance of infiltrating immune cell subtypes in invasive ductal carcinoma of the breast. Tumori Journal. 2018 Jun;104(3):196-201.
30. Bai YG, Gao GX, Zhang H, Zhang S, Liu YH, Duan XN, Xu L. Prognostic value of tumor-infiltrating lymphocyte subtypes in residual tumors of patients with triple-negative breast cancer after neoadjuvant chemotherapy. Chinese Medical Journal. 2020 Mar 5;133(05):552-60.
31. Eiró N, Pidal I, Fernandez-Garcia B, Junquera S, Lamelas ML, del Casar JM, Gonzalez LO, Lopez-Muniz A, Vizoso FJ. Impact of CD68/(CD3+ CD20) ratio at the invasive front of primary tumors on distant metastasis development in breast cancer. PLOS one. 2012 Dec 26;7(12):e52796.
32. Kuroda H, Jamiyan T, Yamaguchi R, Kakumoto A, Abe A, Harada O, Masunaga A. Tumor-infiltrating B cells and T cells correlate with postoperative prognosis in triple-negative carcinoma of the breast. BMC cancer. 2021 Dec;21(1):1-0.
33. Pretscher D, Distel LV, Grabenbauer GG, Wittlinger M, Buettner M, Niedobitek G. Distribution of immune cells in head and neck cancer: CD8+ T-cells and CD20+ B-cells in metastatic lymph nodes are associated with favourable outcome in patients with oro-and hypopharyngeal carcinoma. BMC cancer. 2009 Dec;9(1):1-0.
34. Castillo-Tong DC, Pils D, Heinze G, Braicu I, Sehouli J, Reinthaller A, Schuster E, Wolf A, Watrowski R, Maki RA, Zeillinger R. Association of myeloperoxidase with ovarian cancer. Tumor Biology. 2014 Jan;35(1):141-8.