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Prognostic Significance of Tumor Infiltrating Lymphocytes in InvasiveBreast Carcinoma

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Abstract
Background:Breast cancer is a major public health problem for woman. Several studies suggestedthat development and progression of malignant tumors are characterized by an interaction with the cells in the tumor microenvironment including infiltrating immune cells.
Aim:The aim was to investigate relationship between tumor infiltrating lymphocytes(TILs) in infiltrating breast carcinomaand other recognized clinicopathological factors.
Material and Methods: 100 cases of infiltrating breast carcinomas were included in the study.Stromal.TILs status was evaluated using hematoxylin and eosin staining. For all statistical data chi-square test was applied using SPSS and Spearman correlation.P-values of less than 0.05 were considered as significant.
Results: Stromal TILs correlated with well-established prognostic markers:HER2 positivity(P=0.001),higher tumour grade (P=0.016), ER negativity (P=0.015), PR negativity (P=0.008),lymphnode status(P=0.005) and lymphovascular invasion(LVI) (P=0.001), molecular classification (P=0.000) , and p53 positivity (P=0.013). No correlation between CD10 positivity or high proliferative index(KI67)and stromal TILs.
Conclusion:Stromal TILs is directly correlated more aggressive phenotype:HER2 positivity ,ER negativity, PR negativity ,molecular classification, present LVI, higher tumor grade and greater nodal involvement;so TILs could be used as novel predictive and prognostic parameter and used to develop newer drugs (immunotherapy).
Keywords:TILs ,Infiltrating Breast Carcinoma,Stroma, Prognosis.

1.Introduction:

Infiltrating Breast carcinoma is the most common non-skin malignancy in women.[1,2]Breast tissue is composed of glandular formations (epithelial component) and stroma (mesenchymal component). Epithelial growth of tumor depends partly on chemical factors between both components(tumor and stroma).[3]Infiltrating breast carcinoma is amalignant tumour arising in the epithelial cells of the terminal ductal

lobular unit(TDLUs), stromal tumor microenvironment (TME) plays a very important role in breast cancer tumor progression, evolution, invasion and metastasis,also tissue microenvironment plays a key role in controlling cell migration ,survival, proliferation, polarization, and differentiation. [4,5]A better understanding of stromal participation to cancer progression will identify specific pathways and signals that motivate dedifferentiation ,growth, , invasion of malignant cells and may eventually result in the determination and identification of novel therapeutic agents for future treatment .[6]Stromal markers are now emerging as new novel factors to estimate the prognosis of infiltrating breast cancer.

Development and progression of malignant proliferations are characterized by an interaction with the cells in the tumor microenvironment including infiltrating immune cells.[7]

In immune cell infiltration in breast cancer tissue, the expression of tumour-related immune cells differed greatly among different breast cancer subtypes and patients.[8]

Lymphocyte-predominant breast cancer (LPBC) is defined as a presentation wherein at least 50% of the tumour tissue is invaded by tumour-infiltrating lymphocytes (TILs), TIL expression could serve as a strong prognostic marker for colorectal and breast cancer,thereafter, many retrospective studies reported that TIL expression in breast cancer could predict the efficacy of drug therapy and prognosis.[9,10]

Although the methods to quantify TIL expression and cut-off TIL values in breast cancer tissues varied among studies and have not been clearly standardized, the International TILs Working Group published the first guidelines for a TIL evaluation in 2014 [11].

Accordingly, mononuclear immune cells located between tumor sheets, i.e., within the tumor stroma, are defined as stromal TILs (str-TILs).

The International Working Group recommended that str-TIL expression should be graded as :

- low (str-TILs: <10%).
- Intermediate (str-TILs: ≥10 and ≤40%) .
- High (str-TILs: >40%) .

TIL expression shouldbe graded based on their relative abundance within the tumor stroma.[11]

The extent of TILs in lBC is gaining importance as a prognostic marker, for quantifying TILs, it is recommended to follow the scoring recommendations(steps) according to WHO classification of breast tumors 2019.[2]

The steps are:

1-Define the area for evaluation Large areas of central necrosis or fibrosis are not included in theevaluation.

2-Focus only on stromal TILs.

3-Determined the type of inflammatoryinfiltrate include only mononuclear infiltrate (lymphocytesand plasma cells).

4 & 5: Assess and report the percentage of the stromal area involved by TILs,report the average of the stromal area; do not focus on hotspots.[2]

2.Aim and Objectives:

The aim was to investigate relationship between tumor infiltrating lymphocytes(TILs) in infiltrating breast carcinoma other recognized clinicopathological factors.

3.Materials and Methods:

This study was performed on 100 women who hadundergone modified radical mastectomy forinfiltrating breast carcinoma in Al-Assad University Hospital(Damascus ,Syria) during 2017. None of the patientshad received neoadjuvant therapy.Tissue from primary tumor was processed, paraffin blocks were prepared and six slideswere cut from each:

Slide1: Stained with hematoxylin and eosin.
Slide2: Immunohistochemistry for estrogen receptor
Slide 3: Immunohistochemistry for progesterone receptor.
Slide 4: Immunohistochemistry for HER2/neu
Slide 5: Immunohistochemistry for CD10
Slide 6: Immunohistochemistry for KI67 .

Hematoxylinand-eosin stained microscopic slides of the primary mass were reviewed to complete the study, evaluation ofstromal TILs ,definetumor subtype, and perform grading of infiltrating carcinomas according tothe Nottingham modification of the Bloom andRichardson system. [12]**Tumor size** in this study means the greatest tumor diameter in milimeter.**Lymph node status** was defined according to tumor node metastasis (TNM) staging system for breast carcinoma in which 0 correspond negative nodes, 1-3 positivenodes (N1), 4-9 positive nodes(N2), and 10 or more positive nodes (N3), respectively.**Estrogen receptor (ER) and progesterone receptor (PR) markers** were considered as positive when at least 1% of tumor cell nuclei were immunoreactive for the marker.[12]**Expression of human epidermal growth factor receptor 2 (HER2/neu)** was scored as 0 to 3 as Follow: Score of 0 (negative): no staining or membrane staining in less than 10% of the tumor cells, 1+ (negative): a faint/barely perceptible partial staining in the membrane of more than 10% of the tumor cells,2+ (weakly positive): a weak to moderate complete membrane staining in more than 10% of the tumor cells, and 3+ (strongly positive) : a strong complete membrane staining in more than 30% of the tumor cells. [13]

4.Study Design : systematic reviews and meta-analysis

5.Ethics: Our study was performed on paraffin blocks of tumor tissue.No tests, measurements or experiments were performed on humans as apart of this work.

6.Statistics: We used well-known prognostic biomarkers, such as ER, PR, and HER2/neu, as well as clinicopathological prognostic factors including: grade, lymph node status,CD10 expression,p53 and molecular classification to investigate the prognostic potential of stromal TILsin breast carcinoma.

The collected data was analyzed using SPSS. The relationships between stromal TILs and all variables were evaluated by Chi-square. P-values of less than 0.05 were considered as significant.

7.Results:

100 cases of infiltrating breast carcinoma were included in this study. Age of the patients ranged from 33-79 years ,the majority were in 5th decade.The size of the tumors ranged from 11 to more than 50mmand the tumor size was more than 50 mm in 48% of cases.infiltratingductal carcinoma, no special type(NST) comprised the majority ofour study population (60 cases, 60%), followed by 30 cases (30%) of invasive lobular carcinoma (ILC), 4 cases (4%)of micropapillary carcinoma,4 cases (4%)of invasive cribriform carcinomaand 2 cases (2%)of tubular carcinoma. All cases of invasive breast carcinoma were graded based onthe Bloom and Richardson grading system. Most cases(56 cases, 56%) were grade II, followed by 30 (30%)grade III, and 14 (14%) grade I cases Regarding the lymph node status, 44 cases (44%) lacked lymph node involvement.

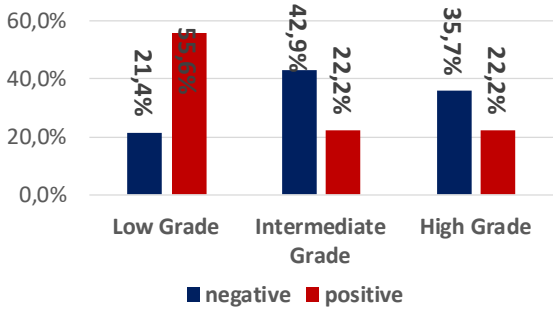
Stromal TILs evaluated according to WHO classification 2019.[2]

CD10 immunostaining was performed on all 100 cases. No stromal expression was detected in thenormal tissue of breast. The myoepithelial cells lining the normal TDLUs components in normal breast parenchyma adjacent to the tumor were considered as the positive control for CD10 expression.

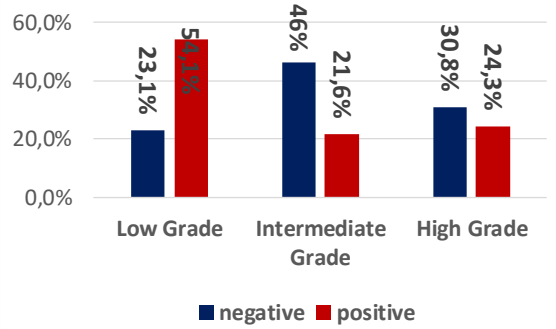
The staining was scored as negative , weak, and strong as described previously in the methods section. CD10 was found to be positive in 60% (60 cases), out of which 28% (28 cases) showed weak immunoreactivity and 32% (32 cases) showed strong immunoreactivity.Most patients in this study were ER positive (74 cases, 74%) and PR positive (72 cases, 72%).

Stromal TILs correlated with well-established prognostic markers:HER2 positivity(P=0.001) higher tumour grade (P=0.016), ER negativity (P=0.015), PR negativity (P=0.008),lymphnode involvement (P=0.005) and lymphovascular invasion(LVI) (P=0.001), molecular classification (P=0.000) , and p53 positivity (P=0.013).

Chi-square test showed a statistically significant correlation between stromal TILs and ER negativity (P = 0.015), the same finding was observed about PR negativity (P = 0.008))(Figures 1,2).



Figure(2): TILs and PR status

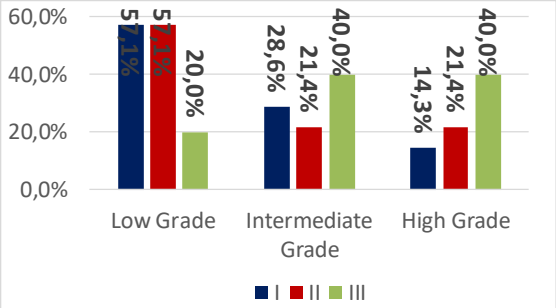


Figure(1): TILs and ER status

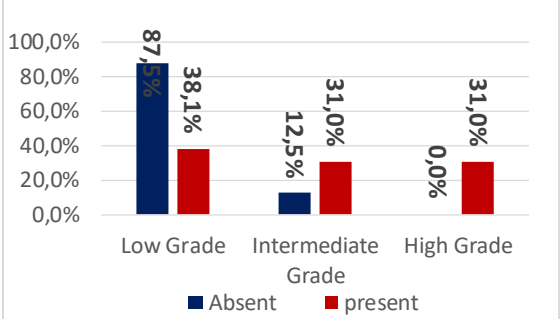
A statistically significantpositiveassociation was seen between stromal TILs and lymph node status (P=0.005), a higher percent in TILswas seen in cases with lymph node metastases(N3: 10 lymph nodes or more 46%).

A statistically significantpositiveassociation was seen between stromal TILs and Lymphovascular invasion (LVI); high gradeTILs was seen in cases with lymphovascular invasion (100%) (Figure 3).

A statistically significant positive association was seen between stromal TILs and tumor grade; a higher percent high gradeTILs was seen in cases classified grade III (40%)(Figure 4)



Figure(4):TILs and Tumor grade

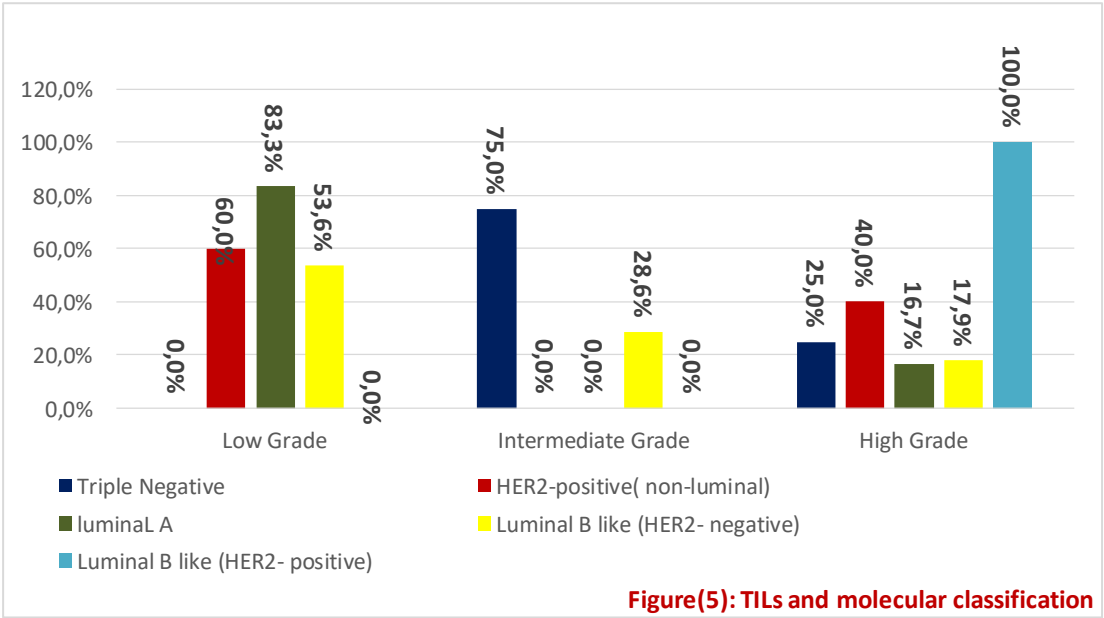


Figure(3):TILs and LVI

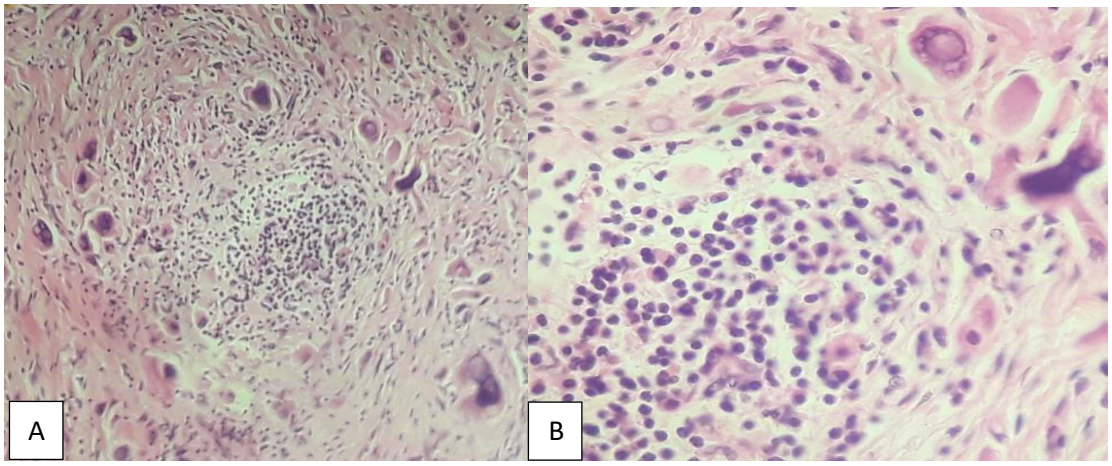
Nostatistically significant positive association was seen between stromal TILs and stromal CD10 positivity (P=0.071).

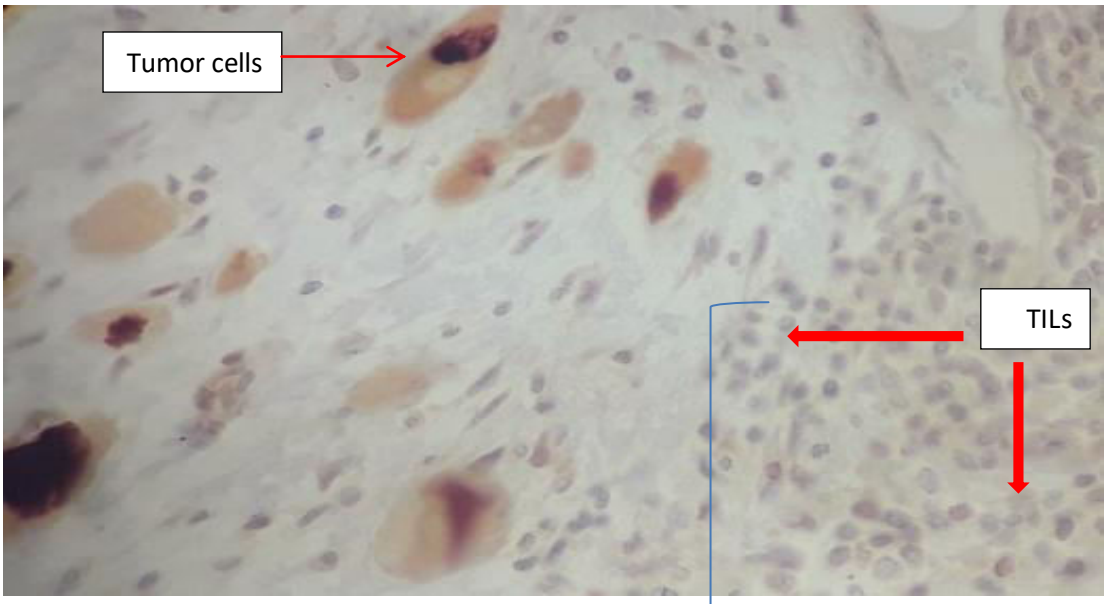
Nostatistically significant positive association was seen between stromal TILs and proliferative index KI67 (P=0.136).

A statistically significantassociation was seen between stromal TILs and molecular classification (P=0.000),a higher percent in high grade TILs was seen in luminal B (HER2-positive) (100%) ,followed by HER2 positive(non-luminal)(40%) and triple negative (25%)(Figure 5).



Figure(5): TILs and molecular classification





ure(6) A+B :Stromal TILs in breast carcinoma(mononuclear cells composed of lymphocytes and plasma cells) in our study , C: Nuclear staining of tumor cells for p53 by IHC stain.

8.Discussion:

Development and progression of malignant tumors are characterized by an interaction with the cells in the tumor microenvironment including infiltrating immune cells. [7]

In immune cell infiltration in breast cancer tissue, the expression of tumour-related immune cells differed greatly among different breast cancer subtypes and patients. [8]

In our study stromal TILs correlated with well-established prognostic markers:HER2 positivity(P=0.001) higher tumour grade (P=0.016), ER negativity (P=0.015).

also the same results found in a study performed by Kurozumi et al in the year 2019[8]

In our study no correlation between stromal TILs and KI67 ,whereas Losurdo et al [14]study revealed positive correlation between stromal TILs andhigh proliferative index (KI67).

Our study showed correlation between stromal TILs and lymphnode status, also the same results found in a study performed by Losurdo et al in year 2020. [14]
Most of the results are similar may be due to the similarity of descriptive statistics between studies.

9.Conclusion:

TILs could be used as novel prognostic and predictivefactor.
Our findings support the use of stromal TILs to identify a more aggressive phenotype of tumors.
A further study to validate the prognostic utility of TILs based on breast cancer subtypes in large cohorts is required.
PD-L1(Programmed death-ligand1) recommended for prognosis and identifying breast carcinoma patients who benefit most from immunotherapy by PDL1 inhibition because the results from the current meta-analysis support integrating immunotherapy with conventional in future breast carcinoma research.
Further studies are needed including larger number of patients to identify the effect between TILs and chemptherapy response.

10.Conflict of interest

The authors declare that they have no potential conflicts of interest to disclose.

11.Funding Statement:Not available

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