Original Article

Correlation of Tertiary Lymphoid Structures in Breast Ductal Carcinoma in Situ and Adverse Pathological Parameters

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ABSTRACT

Objective: To evaluate the tertiary lymphoid structures in ductal carcinoma in situ of breast their association with clinical outcomes, immune cell markers and pathological features.

Study Design: Prospective study

Place and Duration of Study: This study was conducted at the Pathology Department, Faisalabad Medical University, Faisalabad in one year duration from June 2022 to May 2023.

Materials and Methods: A total of 210 patients who were diagnosed with DCIS were enrolled in study. Positive tumors were labeled when there was one or more TLS in the lesion area and it was negative when no TLS found. Classification of TLS-positive tumors was made as tumors with TLSs covering less than 5% of the lesional area was categorized as having a low TLSs area percentage, while tumors with TLSs covering 5% or more of the lesion area were categorized as having a high TLSs area percentage.

Results: There were 69.0% patients with TLSs negativity and 31.0% patients with TLSs positivity. It was noted that the presence of TLSs were associated with HER2 positivity (p=0.001), triple negativity (p=0.047), TILs (25% cut-off) (p<0.001) and FoxP3 (1% cut-off) (p<0.001). Further, high TLSs (≥5%) was associated with calcifications (p<0.001), HER2 positivity (p=0.012), triple negativity (p<0.001), TILs (25% cut-off) (p<0.001), FoxP3 (1% cut-off) (p<0.001), CD4/CD8 ratio (1% cut-off) (p=0.016) and PD-L1 in TILs (1% cut-off) (p=0.043).

Conclusion: Tertiary lymphoid structures are significantly associated with negative hormone receptors, triple negativity, HER2 positivity, necrosis in situ but significance was not appropriate with recurrence and invasive behavior.

Key Words: DCIS, Breast, triple negativity, HER2, tertiary lymphoid structures

Citation of article: Naeem N, Taqi I, Shamsi FB, Saeed A, Alam A. Correlation of Tertiary Lymphoid Structures in Breast Ductal Carcinoma in Situ and Adverse Pathological Parameters. Med Forum 2023;34(7):50-54. doi:10.60110/medforum.340712.

INTRODUCTION

Tertiary lymphoid structures (TLS) are immune cell aggregates that can form in certain tissues, including tumors. They resemble lymph nodes and contain various immune cells, such as T cells, B cells, and dendritic cells[1]. The presence of TLS within tumor tissues has been a subject of research interest in recent years. Breast ductal carcinoma in situ (DCIS) is a non-invasive form of breast cancer where abnormal cells are found in the lining of the breast ducts[2]. The correlation between TLS and adverse pathological parameters in DCIS has been investigated in several studies[3].

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Received: June, 2023
Accepted: June, 2023
Printed: July, 2023

Adverse pathological parameters refer to features of the tumor that are associated with a worse prognosis or more aggressive disease[4]. Several studies have suggested that the presence of TLS in breast DCIS is associated with adverse pathological parameters. These parameters may include larger tumor size, higher histological grade, presence of necrosis, and increased proliferation of cancer cells[5]. In other words, the presence of TLS in DCIS tumors may indicate a more advanced or aggressive form of the disease[6]. Furthermore, the presence of TLS in DCIS has also been associated with an increased risk of recurrence and progression to invasive breast cancer[7,8]. This suggests that TLS may play a role in promoting the progression of DCIS to invasive disease[9]. It is important to note that the research on TLS in breast DCIS is still evolving, and the exact mechanisms underlying their formation and their impact on disease progression are not yet fully understood[10].

There is an increase in the significance of detailed assessment of radiological, histopathological and immunohistochemical features in DCIS for optimal risk stratification.

These features along with accurate estimation of margin status in excised specimens is helpful for oncologist to decide management options and reliably predict outcomes.

MATERIALS AND METHODS

The study was carried out at Pathology Department, Faisalabad Medical University in one year duration from June 2022 to May 2023. Study was started after ethical approval from hospital ethical board and consent was obtained from patients after ensuring about confidentiality of their data. A total of 210 cases of DCIS were enrolled and clinicopathological parameters like tumor size, microinvasion, nuclear grade, ethnicity necrosis and calcification status were recorded. Slides (haematoxylin and eosin H & E) were made and viewed under IMS (image management system). TLSs quantity was observed around DCIS through these digital images. A pathologist having maximum experience in observation of TLSs slides was appointed for immunohistochemistry of lymphoid markers. Positive tumors were labeled when there was one or more TLS in the lesion area and it was negative when no TLS found.

Further classify TLS-positive tumor was done, tumors with TLSs covering less than 5% of the lesional area were categorized as having a low TLSs area percentage, while tumors with TLSs covering 5% or more of the lesion area were categorized as having a high TLSs area percentage. Evaluation of antibodies like progesterone receptors PR, oestrogen receptors (ER), CD4, CD8, human epidermal growth factor receptor 2 (HER2), FoxP3, CD68, PD-L1 were made.

SPSS version 25 for windows was used for data analysis and fisher's exact test applied to see association among TLS and clinicopathological parameters and biological markers. P value less than or equal to 0.05 was taken as significant.

RESULTS

Overall, 210 females were included in this study. There were 145 (69.0%) patients with TLSs negative and 65 (31.0%) patients with TLSs positive. It was noted that the presence of TLSs were associated with HER2 (p=0.001), triple negativity (p=0.047), TILs (25% cut-off) (p<0.001) and FoxP3 (1% cut-off) (p<0.001). (Table. 1).

Further, high TLSs (≥5%) was associated with calcifications (p<0.001), HER2 positivity (p=0.012), triple negativity (p<0.001), TILs (25% cut-off) (p<0.001), FoxP3 (1% cut-off) (p<0.001), CD4/CD8 ratio (1% cut-off) (p=0.016) and PD-L1 expression in TILs (1% cut-off) (p=0.043). (Table. 2).

Table No. 1: Association of TLSs and clinical parameters & biomarkers

Parameters and biomarkers	TLSs (-) N (%)	TLSs (+) N (%)	p-value
Age: <50 years	88 (60.7)	45 (69.2)	0.235
≥50 years	57 (39.3)	20 (30.8)	
Size: ≤50 mm	108 (74.5)	47 (72.3)	0.740
>20 mm	37 (25.5)	18 (27.7)	
Nuclear grade: Low	106 (73.1)	41 (63.1)	0.123
Intermediate	30 (20.7)	19 (29.2)	
High	9 (6.2)	5 (7.7)	
Necrosis			
Absent	93 (64.1)	42 (64.6)	0.947
Present	52 (35.9)	23 (35.4)	
Calcifications			
Absent	91 (62.8)	31 (47.7)	0.041
Present	54 (37.2)	34 (52.3)	
Micro invasion			
Absent	99 (68.3)	40 (61.5)	0.340
Present	46 (31.7)	25 (38.5)	
Recurrence			
No	96 (66.2)	37 (56.9)	0.197
Yes	49 (33.8)	28 (43.1)	
Invasive recurrence			
No	83 (57.2)	35 (53.8)	0.647
Yes	62 (42.8)	30 (46.2)	
ER			
Negative	70 (48.3)	34 (52.3)	0.589
Positive	75 (51.7)	31 (47.7)	
PR			
Negative	80 (55.2)	30 (46.2)	0.226
Positive	65 (44.8)	35 (53.8)	
HER2			
Negative	123 (84.8)	42 (64.6)	0.001
Positive	22 (15.2)	23 (35.4)	
Triple negativity			
No	108 (74.5)	40 (61.5)	0.047
Yes	37 (25.5)	25 (38.5)	
Basal-like: No	98 (67.6)	40 (61.5)	0.393
Yes	47 (32.4)	25 (38.5)	
TILs (25% cut-off)			
Low	125 (86.2)	41 (63.1)	<0.001
High	20 (13.8)	24 (36.9)	
FoxP3 (1% cut-off)			
Negative	122 (84.1)	35 (53.8)	<0.001
Positive	23 (15.9)	30 (46.2)	
CD68 (10% cut-off)			
Low	110 (75.9)	43 (66.2)	0.144
High	35 (24.1)	22 (33.8)	
CD163 with cut-off 10%			
Low	99 (68.3)	37 (56.9)	0.111
High	46 (31.7)	28 (43.1)	
CD4 with cut-off value 20%			
Low	101 (69.7)	41 (63.1)	0.346
High	44 (30.3)	24 (36.9)	
CD4/CD8 ratio with cut-off value 1%			
Low	119 (82.1)	49 (75.4)	0.263
High	26 (17.9)	16 (24.6)	
PD-L1 in TILs with cut-off value 1%			
Negative	113 (77.9)	47 (72.3)	0.376
Positive	32 (22.1)	18 (27.7)	

Table No. 2: Association of area of TLSs and clinical parameters & biomarkers

Biomarkers and Parameters	Low percentage of TLSs (<5%)	High percentage of TLSs (≥5%)	p-value
Age			
<50 years	105 (62.5)	28 (66.7)	0.616
≥50 years	63 (37.5)	14 (33.3)	
Size			
≤50 mm	122 (72.6)	33 (78.6)	0.433
>20 mm	46 (27.4)	9 (21.4)	
Nuclear grade			
Low	120 (71.4)	27 (64.3)	0.366
Intermediate	40 ()	10 (23.8)	
High	8 ()	5 (11.9)	
Necrosis			
Absent	110 (65.5)	25 (59.5)	0.471
Present	58 (34.5)	17 (40.5)	
Calcifications			
Absent	109 (64.9)	13 (31.0)	<0.001
Present	59 (35.1)	29 (69.0)	
Microinvasion			
Absent	116 (69.0)	23 (54.8)	0.080
Present	52 (31.0)	19 (45.2)	
Recurrence			
No	110 (65.5)	23 (54.8)	0.197
Yes	58 (34.5)	19 (45.2)	
Invasive recurrence			
No	99 (58.9)	19 (45.2)	0.110
Yes	69 (41.1)	23 (54.8)	
ER			
Negative	84 (50.0)	20 (47.6)	0.783
Positive	84 (50.0)	22 (52.4)	
PR			
Negative	91 (54.2)	19 (45.2)	0.300
Positive	77 (45.8)	23 (54.8)	
HER2			
Negative	138 (82.1)	27 (64.3)	0.012
Positive	30 (17.9)	15 (35.7)	
Triple negativity			
No	128 (76.2)	20 (47.6)	<0.001
Yes	40 (23.8)	22 (52.4)	
Basal-like			
No	114 (67.9)	24 (57.1)	0.191
Yes	54 (32.1)	18 (42.9)	
TILs (25% cut-off)			
Low	145 (86.3)	21 (50.0)	<0.001
High	23 (13.7)	21 (50.0)	
FoxP3 (1% cut-off)			
Negative	144 (85.7)	21 (50.0)	<0.001
Positive	24 (14.3)	21 (50.0)	
CD68 (10% cut-off)			
Low	126 (75.0)	27 (64.3)	0.163
High	42 (25.0)	15 (35.7)	
CD163 (10% cut-off)			
Low	114 (67.9)	22 (52.4)	0.060
High	54 (32.1)	20 (47.6)	
CD4 (20% cut-off)			
Low	116 (69.0)	26 (61.9)	0.376
High	52 (31.0)	16 (38.1)	
CD4/CD8 ratio with cut-off value 1%			
Low	140 (83.3)	28 (66.7)	0.016
High	28 (16.7)	14 (33.3)	
PD-L1 in TILs with cut-off value 1%			
Negative	133 (79.2)	27 (64.3)	0.043
Positive	35 (20.8)	15 (35.7)	

DISCUSSION

In this study 31.0% patients were TLSs positive and TLSs were associated with HER2 positivity, triple negativity, TILs (25% cut-off) and FoxP3 expression (1% cut-off). Zeng et al[11] conducted a study and reported that presence of TLSs in (DCIS) was associated with unfavorable prognostic features. Specifically, TLSs with high levels of certain immune cell markers, such as FoxP3+, high CD4, high (CD4/CD8) ratio, and high CD68, were identified as independent factors for poorer disease-free survival (DFS) in patients with invasive, in-situ recurrence (IIR).

A cohort study conducted by Kim et al[12] on 204 Korean patients with DCIS. The study found that a higher percentage of tumor-infiltrating lymphocytes (TLSs) were associated with HER2+ status, negative hormone receptors and triple-negative DCIS. Additionally, they observed that more TLSs were associated with cases of DCIS with microinvasion. It is noted that the TLS-positive rate in Kim et al.'s study was 60.3%, which is significantly higher than the rate mentioned in our study.

Positive rate of tumor-infiltrating lymphocytes (TLSs) in 248 patients with invasive breast cancer were observed in a study by Liu et al[13], that was 37.5%. TLSs were predominantly found in the vicinity of the tumor or at the invasive tumor front. Additionally, it is mentioned that cases of ductal carcinoma in situ (DCIS) with high and low nuclear grades exhibit distinct genetic characteristics.

The study conducted by Hendry et al[14] examined the relationship between programmed death-ligand 1 (PD-L1) expression, tumor-infiltrating lymphocytes (TILs), and various clinicopathological features in a cohort of pure ductal carcinoma in situ (DCIS). It was found that the presence of TILs and/or PD-L1 expression in DCIS was associated with comedonecrosis, positivity of HER2, high nuclear grade and TP53 mutations.

The study conducted by Chen et al[15] investigated the immunohistochemical expression of CD4, CD8, and PD-L1 in ductal carcinoma in situ (DCIS). Their findings suggested that immune infiltrating cells, specifically CD4 and CD8 lymphocytes, as well as the expression of PD-L1, were associated with certain characteristics of DCIS and had prognostic implications for disease recurrence.

Thike et al[16] also reported similar findings and reported that presence of CD4 and CD8 lymphocytes, was found to be associated with high nuclear grade, negative

hormone receptor, HER2 positivity, and triple-negative status of DCIS. These characteristics are known to be associated with more aggressive tumor behavior and worse clinical outcomes.

There is a lack of a standardized method for identifying TLSs, with different studies utilizing diverse approaches ranging from morphological observation to the application of molecular markers[17]. Morphology combined with immunohistochemistry is suggested as a relatively reasonable method that has the potential for standardization in routine use. This approach involves examining the structural characteristics of TLSs under a microscope and utilizing immunohistochemistry to detect specific molecular markers associated with lymphoid tissue[18, 19].

CONCLUSION

Tertiary lymphoid structures in ductal carcinoma in situ of breast are significantly associated with negative hormone receptors, HER2 positivity, triple negativity, necrosis but significance was not appropriate with recurrence and invasive behavior. Furthermore, it is also associated with immune markers like CD4/CD8, PD-L1, FoxP3 and TILs.

Limitations: Unawareness about adverse consequences of disease, its late diagnosis, delay in treatment initiation and refusal of inclusion in research process from patient side are main limitations of study.

Recommendations: Further studies are needed to better understand the relationship between TLS and adverse pathological parameters in DCIS and to explore their potential as prognostic markers or therapeutic targets.

Author's Contribution:

Concept & Design of Study:	Namra Naeem
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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