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Macrophage density is an adverse prognosticator for ipsilateral recurrence in ductal carcinoma in situ[1]

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ABSTRACT

Introduction: There is evidence that supports the association of dense tumor infiltrating lymphocyte (TILs) with an increased risk of ipsilateral recurrence in ductal carcinoma in situ (DCIS). However, the association of cellular composition of DCIS immune microenvironment with the histopathologic parameters and outcome is not well understood.

Methods: We queried our institutional database for patients with pure DCIS diagnosed between 2010 and 2019. Immunohistochemical studies for CD8, CD4, CD68, CD163, and FOXP3 were performed and evaluated in the DCIS microenvironment using tissue microarrays. Statistical methods included Fisher's exact test for categorical variables and the two-sample *t*-test or the Wilcoxon Rank-Sum test for continuous variables.

Results: The analytic sample included 67 patients. Median age was 62 years (range = 53 to 66) and median follow up was 6.7 years (range = 5.3 to 7.8). Thirteen patients had ipsilateral recurrence. Of all the clinicopathologic variables, only the DCIS size and TIL density were significantly associated with recurrence ($p = 0.023$ and 0.006, respectively). After adjusting for age and TIL density, only high CD68 (>50) and high CD68/CD163 ratio (>0.46) correlated with ipsilateral recurrence ($p = 0.026$ and 0.013, respectively) and shorter time to recurrence [hazard ratio 4.87 (95% CI: 1.24–19, $p = 0.023$) and 10.32 (95% CI: 1.34–80, $p = 0.025$), respectively].

Conclusions: In addition to DCIS size and TIL density, high $CD68^+$ tumor-associated macrophages predict ipsilateral recurrence in DCIS. High $CD68^+$ macrophage density and CD68/CD163 ratio also predict a shorter time to recurrence.

1. Introduction

Evidence of the role the immune microenvironment plays in the development and progression of ductal carcinoma in situ (DCIS) is limited compared to the invasive carcinoma of the breast [1–5]. We previously showed that dense tumor infiltrating lymphocytes (TILs), defined as ≥45% TIL, were associated with an increased risk of ipsilateral recurrence in DCIS [6]. In addition, dense TILs were associated with other parameters of unfavorable behavior including younger age, larger size, high nuclear grade, comedo morphology and estrogen receptor (ER) negativity. Other investigators have similarly demonstrated the association of TILs with high nuclear grade, comedo necrosis, high Van Nuys Prognostic Index (VNPI) and ER and progesterone (PR) negative status [1–5]. It is likely that the explanation for the association of immune response with the histopathologic parameters of aggressiveness and unfavorable outcome lies in the composition of TILs and tumor-associated macrophages (TAM) in the tumor microenvironment [7].

The immune response, or cancer immunoediting, has been classified into three dynamic processes: elimination, equilibrium and escape [8]. The elimination process, which is mounted to survey and eradicate the developing cancer, primarily consists of pro-inflammatory immune

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response including M1 macrophages, dendritic cells, natural killer cells, $CD8^+$ cytotoxic T cell and $CD4^+$ T helper 1 cells. In the equilibrium process, the interplay between the immune system and any surviving tumor cell reaches a dynamic equilibrium, in which the tumor may stay dormant for a long time. In the escape process, the surviving tumor cells that have either acquired low antigenicity and insensitivity to immunologic detection and/or have escaped elimination begin to expand in an uncontrolled manner [8]. The escape process shows an anti-inflammatory profile in the tumor microenvironment, characterized by suppressor cells, CD4+/FOXP3+ regulatory T cells, and CD163+ M2 macrophages [1,9].

Exploring the interplay of the DCIS and its microenvironment status may identify immune cellular targets that can potentially be modulated toward achieving a better outcome for patients with DCIS. We sought to investigate the immune status of the DCIS microenvironment and its association with the clinicopathologic parameters in a contemporary cohort of women with pure DCIS.

2. Methods

2.1. Study population

The institutional Breast Cancer Database was queried for all patients who were newly diagnosed with DCIS between January 2010 and January 2019. Each recurrent case (n = 13) was matched to at least four controls (n = 54) based on date of surgery. The variables of interest included age, race, family history, *BRCA1* and *BRCA2* gene status, DCIS size, multifocality, nuclear grade, comedo type, presence of necrosis, atypical ductal hyperplasia (ADH), lobular neoplasia including atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS), classic type, ER and PR status, hormone therapy, radiation therapy, and TIL status (see below).

2.2. Pathology assessment

The TIL status was previously evaluated and reported using a binary system of sparse (<45%) and dense (≥45%) [6]. Three tissue microarray (TMA) blocks were created with 2-mm cores in triplicate from formalin-fixed, paraffin-embedded blocks of DCIS. ER/PR status was obtained from the pathology reports. One pathologist (FD), blinded to the outcome, evaluated CD8 (Ventana, SP57, neat), CD4 (Ventana, SP35, neat), FOXP3 (Ebioscience, 236 A/E7, 1:75), CD68 (Ventana, KP1, neat), and CD163 (Ventana, MRQ-26, neat) antibody stains using "hot-spot" method by counting all the positive cells in one high power field in the stroma immediately adjacent to the DCIS (40X objective, BX53; Olympus). In addition, CD8/FOXP3 ratio and CD68/CD163 ratio were determined using the absolute counts of the respective stain. ER, PR, and human epidermal growth factor receptor 2 (her2) (Ventana, 4B5, neat) were assessed based on the guidelines set forth by the American Society of Clinical Oncology (ASCO)/College of American Pathologist (CAP) [10,11]. Ki67 (Spring Biosciences, SP6, 1:400) was scored by counting 100 DCIS cells in the hotspot and determining the percentage of positive nuclei.

2.3. Statistical analyses

Counts and percentages were calculated for categorical variables. Means and standard deviations (or medians and interquartile range (IQR), as appropriate) were calculated for continuous variables. Association with recurrence was performed using Fisher's exact test for categorical variables and the two-sample *t*-test (or the Wilcoxon Rank-Sum test, as appropriate) for continuous variables. Due to skewed distributions of marker variables (CD8, CD4, CD68, CD163, FOXP3, CD8/FOXP3 ratio and CD68/CD163 ratio), dichotomized marker variables were created as an approach of transformations. When calculating CD8/FOXP3 and CD68/CD163 ratios, the zero denominator was imputed with 0.5. According to preliminary analyses, higher values of all marker variables were assumed to be associated with higher odds of recurrence. A cutoff for a marker variable was chosen to maximize the sensitivity and specificity of predicting recurrence corresponding to the furthest perpendicular point to the diagonal line on the ROC curve. For each of the binary outcomes, namely ipsilateral recurrence, ER, PR, her2, and ki67, the association of each of the binary marker variables with the outcome was examined using the Fisher's exact test. Logistic regression models with adjustments for patient age and TIL density were fitted to estimate adjusted associations. TIL density was defined as previously described (see above). A disease-free Kaplan-Meier survival curve was displayed for each of the binary markers that had a statistically significant association with recurrence in the logistic model. A Cox proportional hazards regression model was then fitted to examine the association between the marker and time to recurrence, adjusted for age and TIL density. Finally, we conducted a sensitivity analysis to assess the robustness of our results to different approaches to variable transformations; instead of using binary marker variables, each marker variable was treated as continuous with values between 1 and 4 according to the quantiles of its distribution. Results based on this sensitivity analysis were presented in the supplementary document. All analyses were performed in R version 3.5.0 for Windows [12], using packages 'cutpoint' for ROC curves [13] and choosing optimal cut points, and 'survival' for survival models [14].

3. Results

The analytic sample included 67 patients with a DCIS diagnosis in the institutional database (Table 1). The median age was 62 years (IQR = 53 to 66), and the median follow-up was 6.7 years (IQR = 5.3 to 7.8). Among the 67 patients, 13 patients had ipsilateral recurrence within the follow-up period. Of the 13 patients with ipsilateral recurrence, nine (70%) recurred with DCIS, three (24%) recurred with invasive carcinoma and one (8%) recurred with DCIS and microinvasion. The 13 patients with recurrence were on average younger than those without recurrence (mean ± standard deviation: 54.6 ± 13.4 vs. 61.5 ± 11.8, respectively, p = 0.07). Of all the clinicopathologic variables, only the DCIS size and TIL density differed significantly between patients who recurred and those who did not (p = 0.023 and 0.006, respectively).

The distributions of marker variables and cut offs are presented in Table 2. Associations of each dichotomized marker variable with each outcome variable are presented in Table 3. After adjusting for age and TILs density, high CD8 (>70) was significantly associated with negative ER (p = 0.033) and high ki67 (p = 0.014); high CD4 (>90) with negative ER (p = 0.006) and negative PR (p = 0.003); high CD68 (>50) and high CD68/CD163 ratio (>0.46) with ipsilateral recurrence (p = 0.026, p = 0.013, respectively); and high FOXP3 (>6) with negative ER (p = 0.044), negative PR (p = 0.032) and high ki67 (p = 0.01). CD163 and CD8/FOXP3 were not significantly associated with any outcome. These results were confirmed in the sensitivity analyses based on quantile marker variables.

Disease-free Kaplan-Meier survival curves are displayed in Fig. 1. Figs. 1 and 2 for dichotomized CD68 and CD68/CD163 ratio variables, respectively. The average time to recurrence for patients with high CD68 (>50) was 64.8 months compared with 78.8 months for those with low CD68 (≤50). The average time to recurrence for patients with high CD68/CD163 ratio (>0.46) was 70.7 months compared with 77.4 months for the low CD68/CD163 ratio group. In Cox proportional hazards ratio models that were adjusted for age and TIL density, high CD68 (>50) and high CD68/CD163 ratio (>0.46) were associated with shorter time to recurrence with hazard ratios (HR) of 4.87 (95% CI: 1.24–19, p = 0.023) and 10.32 (95% CI: 1.34–80, p = 0.025), respectively. We did not see any correlation between her2 positivity and high Ki67 with ipsilateral recurrence (data not shown; adjusted p values: 0.42 and 0.30, respectively).

Table 1
Comparisons on sample characteristics by recurrence.

	Overall	Recurrence		p
		No	Yes	
N	67	54	13	
Age (mean (sd))	60.18 (12.36)	61.52 (11.84)	54.62 (13.43)	0.07
Race (%)				0.092
African American	6 (9.0)	4 (7.4)	2 (15.4)	
Asian	8 (11.9)	4 (7.4)	4 (30.8)	
Hispanic	3 (4.5)	3 (5.6)	0 (0.0)	
Other	1 (1.5)	1 (1.9)	0 (0.0)	
White	49 (73.1)	42 (77.8)	7 (53.8)	
Family History of BC = Yes (%)	22 (32.8)	20 (37.0)	2 (15.4)	0.194
BRCA.1.2 (%)				0.886
No	18 (26.9)	14 (25.9)	4 (30.8)	
Unknown	45 (67.2)	36 (66.7)	9 (69.2)	
Yes	4 (6.0)	4 (7.4)	0 (0.0)	
DCIS Tumor Size (median [IQR])	1.40 [0.70, 2.35]	1.25 [0.70, 2.00]	2.80 [0.80, 4.90]	0.023
DCIS Multifocal = Yes (%)	22 (32.8)	19 (35.2)	3 (23.1)	0.521
DCIS Tumor Nuclear Grade (%)				1.00
high grade	38 (56.7)	30 (55.6)	8 (61.5)	
intermediate grade	26 (38.8)	21 (38.9)	5 (38.5)	
low grade	3 (4.5)	3 (5.6)	0 (0.0)	
DCIS Comedo = Yes (%)	21 (31.3)	15 (27.8)	6 (46.2)	0.317
Necrosis = Yes (%)	44 (67.7)	34 (64.2)	10 (83.3)	0.309
ADH = Yes (%)	16 (23.9)	14 (25.9)	2 (15.4)	0.718
ALH = Yes (%)	14 (20.9)	11 (20.4)	3 (23.1)	1.00
ER = positive (%)	54 (80.6)	45 (83.3)	9 (69.2)	0.26
PR = positive (%)	51 (76.1)	43 (79.6)	8 (61.5)	0.274
LCIS lobular neoplasia = Yes (%)	10 (14.9)	8 (14.8)	2 (15.4)	1.00
Hormone Therapy = Yes (%)	21 (31.3)	16 (29.6)	5 (38.5)	0.526
Radiation therapy = Yes (%)	40 (59.7)	30 (55.6)	10 (76.9)	0.214
TILs highest % > 45 = Yes (%)	22 (32.8)	13 (24.1)	9 (69.2)	0.006

Means (standard deviations) (Median [IQR], as appropriate) are presented for continuous variables; Frequencies (%) are presented for categorical variables. Comparisons by recurrence were performed by the Fisher's exact test for categorical variables and the two-sample test (Wilcoxon Rank-Sum test, as appropriate) for continuous variables.

Table 2
Distributions of markers and optimal cut points.

Marker	Percentiles					Optimal cut off	Missing
	Min	25th	50th	75th	Max		
CD8	1.0	10.5	46.0	106.5	330.0	70	N = 0
CD4	2.0	20.0	85.0	162.5	420.0	90	N = 0
CD68	1.0	18.0	40.0	65.0	124.0	50	N = 1
CD163	3.0	47.0	70.0	105.0	230.0	70	N = 0
FOXP3	0.0	1.0	10.5	36.0	140.0	6	N = 1
CD8/FOXP3 ratio	1.0	2.5	4.5	20.8	900.0	5	N = 1
CD68/CD163 ratio	0.1	0.3	0.5	0.9	4.0	0.46	N = 1

4. Discussion

The immune infiltrate of the tumor microenvironment can be classified into two interacting components. The first component is the tumor infiltrating lymphocytes (TIL), which, in turn, is a component of the host's adaptive immunity. TILs can be readily recognized on light microscopy and further subtyped based on their immunohistochemical properties. The second component is the tumor-associated macrophages

Table 3
Associations of binary markers with recurrence, ER, PR, her2 status and binary ki67 (above median 12 or below median 12), results from bivariate association analysis by the Fisher's exact test and logistic regression with adjustments for the effects of age and TILs density (>45% or≤45%).

	Recurrence				
	No	Yes	P [1]	Odds ratio [95% C.I.]	p.adj [2]
N	54	13			
CD8 > 70 (%)	17 (31.5)	9 (69.2)	0.024	1.54 [0.26, 8.83]	0.622
CD4 > 90 (%)	21 (38.9)	10 (76.9)	0.027	1.93 [0.35, 11.72]	0.449
CD68 > 50 (%)	13 (24.5)	10 (76.9)	0.001	5.79 [1.32, 31.56]	0.026
CD163 > 70 (%)	23 (42.6)	9 (69.2)	0.123	1.58 [0.37, 7.14]	0.532
FOXP3 > 6 (%)	24 (45.3)	11 (84.6)	0.014	2.84 [0.42, 23.94]	0.287
CD8/FOXP3 ratio >5 (%)	25 (47.2)	2 (15.4)	0.057	0.56 [0.06, 5.15]	0.587
CD68/CD163 ratio >0.46 (%)	22 (41.5)	12 (92.3)	0.001	16.73 [2.63, 338.79]	0.013
	ER negative	positive	P [1]	Odds ratio [95% C.I.]	p.adj [2]
N	13	54			
CD8 > 70 (%)	9 (69.2)	17 (31.5)	0.024	0.14 [0.02, 0.80]	0.033
CD4 > 90 (%)	11 (84.6)	20 (37.0)	0.004	0.06 [0.01, 0.37]	0.006
CD68 > 50 (%)	4 (30.8)	19 (35.8)	1.00	2.32 [0.50, 12.76]	0.301
CD163 > 70 (%)	7 (53.8)	25 (46.3)	0.76	1.03 [0.25, 4.30]	0.969
FOXP3 > 6 (%)	11 (84.6)	24 (45.3)	0.014	0.15 [0.02, 0.86]	0.044
CD8/FOXP3 ratio >5 (%)	2 (15.4)	25 (47.2)	0.057	3.73 [0.63, 29.92]	0.161
CD68/CD163 ratio >0.46 (%)	9 (69.2)	25 (47.2)	0.218	0.38 [0.09, 1.46]	0.175
	PR negative	positive	P [1]	Odds ratio [95% C.I.]	p.adj [2]
N	16	51			
CD8 > 70 (%)	10 (62.5)	16 (31.4)	0.039	0.23 [0.04, 1.08]	0.068
CD4 > 90 (%)	13 (81.2)	18 (35.3)	0.002	0.07 [0.01, 0.37]	0.003
CD68 > 50 (%)	5 (31.2)	18 (36.0)	1.00	2.12 [0.52, 10.09]	0.316
CD163 > 70 (%)	8 (50.0)	24 (47.1)	1.00	1.22 [0.34, 4.60]	0.758
FOXP3 > 6 (%)	13 (81.2)	22 (44.0)	0.011	0.17 [0.03, 0.81]	0.032
CD8/FOXP3 ratio >5 (%)	3 (18.8)	24 (48.0)	0.045	3.25 [0.68, 18.26]	0.148
CD68/CD163 ratio >0.46 (%)	11 (68.8)	23 (46.0)	0.154	0.38 [0.10, 1.29]	0.132
	her2 negative	positive	P [1]	Odds ratio [95% C.I.]	p.adj [2]
N	48	12			
CD8 > 70 (%)	18 (37.5)	8 (66.7)	0.104	0.93 [0.14, 5.65]	0.942
CD4 > 90 (%)	22 (45.8)	8 (66.7)	0.333	0.53 [0.06, 3.34]	0.511
CD68 > 50 (%)	16 (34.0)	6 (50.0)	0.334	0.77 [0.15, 3.48]	0.734
CD163 > 70 (%)	22 (45.8)	8 (66.7)	0.333	1.21 [0.26, 5.72]	0.803
FOXP3 > 6 (%)	24 (50.0)	10 (83.3)	0.052	1.54 [0.17, 14.26]	0.684

(*continued on next page*)

Table 3 (*continued*)

	Recurrence				
	No	Yes	P [1]	Odds ratio [95% C.I.]	p.adj [2]
CD8/FOXP3 ratio >5 (%)	21 (43.8)	2 (16.7)	0.107	1.30 [0.11, 29.67]	0.837
CD68/CD163 ratio >0.46 (%)	23 (48.9)	7 (58.3)	0.748	0.98 [0.23, 4.15]	0.973
	ki67 [3] ≤12	>12	P [1]	Odds ratio [95% C.I.]	p.adj [2]
N	33	29			
CD8 > 70 (%)	6 (18.2)	20 (69.0)	<0.001	6.05 [1.48, 27.67]	0.014
CD4 > 90 (%)	10 (30.3)	20 (69.0)	0.005	2.34 [0.61, 8.85]	0.206
CD68 > 50 (%)	7 (21.9)	16 (55.2)	0.009	2.58 [0.71, 9.67]	0.148
CD163 > 70 (%)	12 (36.4)	19 (65.5)	0.041	2.06 [0.62, 6.79]	0.23
FOXP3 > 6 (%)	10 (30.3)	24 (82.8)	<0.001	6.28 [1.60, 27.61]	0.01
CD8/FOXP3 ratio >5 (%)	18 (54.5)	6 (20.7)	0.009	0.54 [0.13, 2.16]	0.381
CD68/CD163 ratio >0.46 (%)	13 (40.6)	19 (65.5)	0.073	2.37 [0.74, 7.94]	0.148

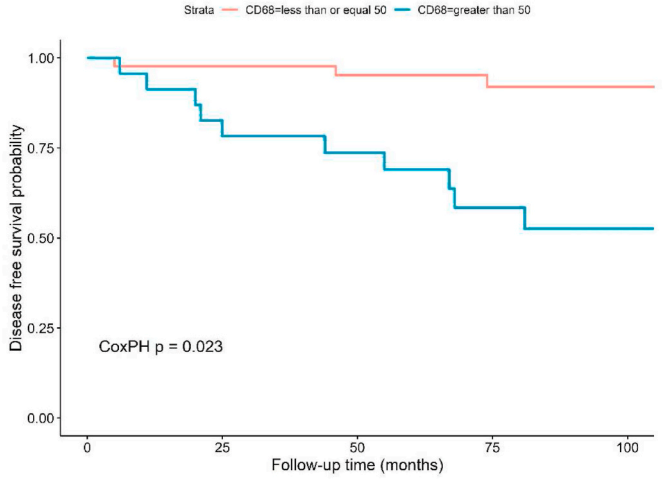

Fig. 1. Disease-free survival analysis of CD68 marker. The Cox PH model has been adjusted for age and TILs density.

(TAM), an integral part of the host's innate immune response. TAMs are the circulating monocytes that are recruited in the tumor microenvironment and differentiated into either pro-inflammatory M1 macrophages or anti-inflammatory M2 macrophages depending on the interplay between TAMs and other inflammatory cells and tumor cells in the tumor microenvironment [8]. M1 macrophages activate type I T helper cells and are tumoricidal. M2 macrophages downregulate immune response and promote angiogenesis. While TAMs are not readily quantifiable on routine histologic sections, they can be highlighted and characterized based on their immunophenotype. CD68 is a pan-macrophage marker recognizing both M1 and M2 macrophages and CD163 specifically highlights M2 macrophages [15,16].

Our analysis demonstrated that after controlling for TIL density and age, high CD68 (>50) and high CD68/CD163 (>0.46) were significantly associated with ipsilateral DCIS recurrence (p = 0.026 and 0.013, respectively; Fig. 3). We also noted a shorter time to ipsilateral DCIS recurrence for both CD68 and CD68/CD163 (Figs. 1 and 2). We did not find a significant association between high CD163 and outcome (p = 0.532). These findings indicate that the high density of TAMs, regardless

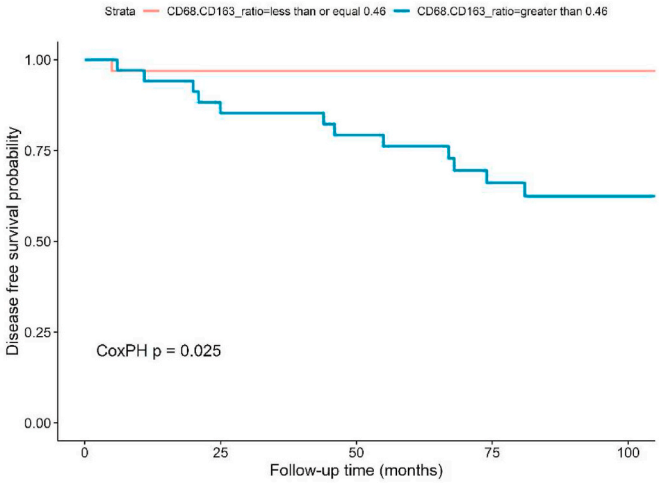

Fig. 2. Disease-free survival analysis of CD68/CD163 ratio. The Cox PH model has been adjusted for age and TILs density.

of their M1/M2 differentiation, is associated with ipsilateral recurrence. Other investigators have demonstrated the association of $CD68^+$ macrophage density with adverse prognostic markers including high VNPI, palpability, high nuclear grade, presence of necrosis, and ER and PR negativity in DCIS [2,3]. Our review demonstrates that the only study that reports the correlation between TAM and DCIS outcome is by Chen at al. Which showed that $CD68^+$ and CD163+ macrophages were significantly associated with ipsilateral invasive recurrence on multivariate analysis (p = 0.023 and 0.024, respectively) [17]. In this paper the authors demonstrated that while both M1 and M2 macrophages prognosticate ipsilateral invasive recurrence, only CD163+ TAM, M2 macrophage, was associated with all recurrences on multivariate analysis (p = 0.005).

The correlation of high $CD68^+$ TAMs and worse outcome in invasive breast cancer has been previously established [15,18,19]. Similarly in DCIS, Campbell et al. showed a significant correlation between $CD68^+$ and CD68+/PCNA + TAMs with poor prognostic variable including high VNPI, high nuclear grade, comedonecrosis, and high proliferative index (high Ki67). They did not find the same correlation between their CD68 + MRC1+ cells (M2-type macrophage) and adverse clinicopathologic variables consistent with our results [2]. It appears that the balance between TAM's pro-inflammatory and anti-inflammatory functional status in the tumor microenvironment is crucial in the biological course of the neoplasm. For example, TAMs promote angiogenesis by producing VEGF and other angiogenic factors and produce growth factors and proteases that enhance tumor progression. TAMs can secrete a variety of proteases including matrix metalloproteinase 7 and 9, which can facilitate the breakdown of basement membrane and tumor cell escape, a process of utmost interest in the progression of DCIS to invasive carcinoma [19]. In other words, dense TAMs can be the harbinger of microinvasion in DCIS. TAMs have also been shown to be capable of releasing ferritin into the breast tumor microenvironment directly stimulating tumorigenesis [20]. The significant correlation of $CD68^+$ TAM density and the ratio of CD68+/CD163+ TAM with ipsilateral recurrence in our analysis suggests that the dynamic status of TAM in the DCIS microenvironment leans toward an anti-inflammatory and tumor-promoting milieu in the subset of DCIS with the worse outcome.

We previously demonstrated that high TIL density was a significant predictor of ipsilateral recurrence in patients with pure DCIS [6]. In the current study, we expanded our investigation to TIL subset analysis. Our results showed that after controlling for TIL density and age, the density of CD8, CD4, FOXP3 and the ratio of CD8/FOXP3 were not significantly associated with ipsilateral recurrence. However, high $CD8^+$ T cell, high $CD4^+$ T cell and high FOXP3+ T regulatory cell density significantly

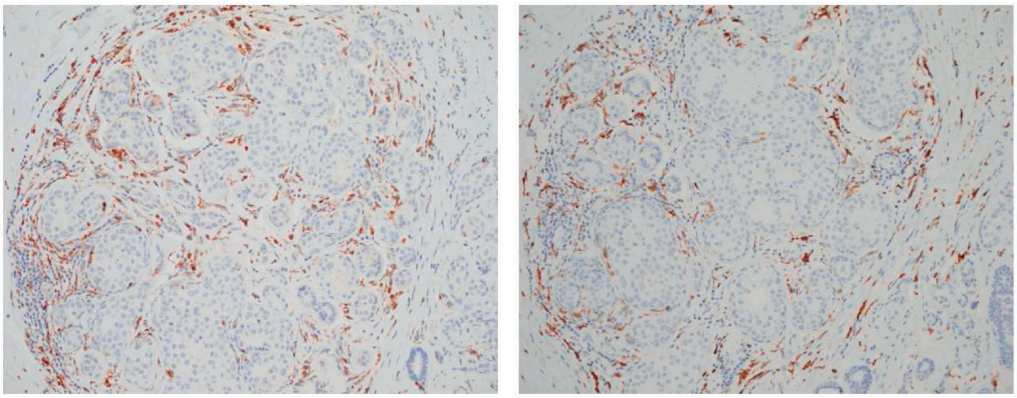

Fig. 3. CD68 immunostain showing a macrophage infiltrate with high density (>50) in the microenvironment of a high-grade ductal carcinoma in situ (left; immunohistochemistry; 20x); CD163 immunostain showing a presumed M2 macrophage infiltrate in the same case as above (right; immunohistochemistry; 20x). The CD68/CD163 in this case is 0.65.

correlated with hormone receptor negativity. In addition, high $CD8^+$ T cell and FOXP3+ T regulatory cell density correlated with high ki67 proliferation index. Other investigators have shown that $CD8^+$ cytotoxic T cells generally confer a favorable phenotype in ER-negative invasive breast cancer [21,22]. Similarly, $CD8^+$ T cell dense microenvironment is typically a protective phenotype in DCIS. Low density of $CD8^+$ T cells and an unfavorable CD8+/FOXP3+ ratio have been shown to be associated with increased risk of ipsilateral recurrence [5]. The favorable process of "spontaneous healing" in DCIS also appears to be driven by $CD8^+$ T cells [23].

$CD4^+$ T cells have been shown to be associated with high grade DCIS, an unfavorable pathologic feature [2]. The association of $CD4^+$ T cells with poor pathologic prognosticators including high nuclear grade and hormone receptor negativity in our study may be attributable to the preponderance of T regulatory cells, a subset of $CD4^+$ T cells that also co-expresses FOXP3. In our study FOXP3+ cells were significantly associated with high proliferation index, another adverse prognostic phenotype in line with the above observation. In fact, CD4+FOXP3+ regulatory T cell infiltration has been shown to be a poor prognostic indicator in ER + invasive carcinoma [24].

One of the limitations of this study is the use of TMA as opposed to whole tissue section for immunohistochemical studies. However, we mitigated this limiting factor by arraying each case in triplicates. Since our interest was in the microenvironment in the immediate vicinity of DCIS, we believe that our results are minimally affected by examining TMA sections. Another limitation of this study is the relatively small sample size which can be partly attributed to the strict criteria we used to ensure adequate follow-up data and available tissue samples for all patients.

In summary, we conclude that in addition to large size of DCIS and dense TILs, the presence of $CD68^+$ TAMs in the DCIS microenvironment is an adverse prognosticator of ipsilateral recurrence. This may have implications as well for identifying those DCIS lesions at risk of progression to invasive cancer.

Declaration of competing interest

The authors have nothing to declare. They have no conflict of interest. There were no ethical issues involved in the preparation of this script.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2022.04.004.

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