Ann Surg Oncol (2019) 26:3337–3343
https://doi.org/10.1245/s10434-019-07562-x

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – BREAST ONCOLOGY

Tumor-Infiltrating Lymphocytes in a Contemporary Cohort of Women with Ductal Carcinoma In Situ (DCIS)

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ABSTRACT

Background. Growing evidence suggests that the tumor immune microenvironment influences breast cancer development and prognosis. Density of tumor-infiltrating lymphocytes (TILs) within invasive breast cancer is correlated with response to therapy, especially in triple-negative disease. The clinical relevance and outcomes of TILs within ductal carcinoma in situ (DCIS) are less understood.

Methods. Our institutional database of 668 patients with pure DCIS from 2010 to 2018 was queried. TILs were evaluated by International TILs Working Group guidelines. Percentage of TILs was assessed from the densest focus (hotspot) in one high-power field of stroma touching the basement membrane. Statistical methods included cluster analyses (to define sparse versus dense TILs), logistic, and Cox regression models.

Results. Sixty-nine patients with DCIS and TILs were evaluated, of whom 54 (78%) were treated by breast-conserving surgery. Thirteen (19%) patients had ipsilateral recurrence. Each recurrence ($n = 13$) was matched to four controls ($n = 56$) based on date of surgery. Median follow-up was 6.7 years. TILs were defined as sparse (< 45%) or dense (≥ 45%). Dense TILs were associated with younger age ($p = 0.045$), larger tumor size ($p < 0.001$), high nuclear grade ($p = 0.010$), comedo histology ($p = 0.033$), necrosis ($p = 0.027$), estrogen receptor (ER) negativity ($p = 0.037$), and ipsilateral recurrence ($p = 0.001$). Nine patients with dense TILs had mean time to recurrence of 73.5 months compared with four patients with sparse TILs with mean time to recurrence of 97.9 months ($p = 0.003$).

Conclusions. Dense TILs were significantly associated with age, tumor size, nuclear grade, comedo histology, necrosis, and ER status and was a significant predictor of recurrence in patients with pure DCIS.

BACKGROUND

Approximately 63,410 women were diagnosed with ductal carcinoma in situ (DCIS) this past year, accounting for 20% of all newly diagnosed breast cancers in the USA.[1] With the increasing use of screening mammography, the number of women diagnosed with DCIS continues to increase. There have been many controversial discussions on whether to define DCIS as a precursor or a risk factor for development of invasive carcinoma[2–5] as well as whether DCIS is being overdiagnosed and/or overtreated[6–9] Fifty percent of all DCIS recurrences are in the form of invasive carcinoma.[10] Narod et al.[11] found that women diagnosed with DCIS who then developed invasive recurrence were 18.1 times more likely to die of breast cancer than women who did not. The main challenge lies in identifying those women with DCIS who might be at higher risk of developing subsequent invasive cancer.

This article was presented at The American Society of Breast Surgeons 20th Annual Meeting in Dallas, TX; April 30–May 5, 2019.

First Received: 29 April 2019;
Published Online: 25 June 2019

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Given the abundance of literature available regarding treatment of DCIS and its excellent outcome, current research is focused on stratifying risk and limiting treatment. The main focus in research has been determining which patients would receive minimal benefit from adjuvant therapies. These low-risk patients are usually identified based on clinicopathologic factors and then offered omission of adjuvant therapy. ECOG E5194 and RTOG9804 studied ipsilateral breast tumor recurrence after omission of radiotherapy.[12,13] Both studies support previous literature and found the following characteristics associated with recurrence: nuclear grade, histologic type, margins, size, and age. Several studies emphasize tumor size as one of the most important factors contributing to recurrence in DCIS,[14,15] while other studies emphasize the importance of nuclear grade.[16,17]

The prognostic factors for local recurrence for DCIS for patients who had breast-conserving surgery (BCS) include age, tumor size, margin width, histologic type, and grade.[18,19] The Van Nuys Prognostic Index (VNPI) is a useful decision-making tool to help determine which patients are at increased risk of local recurrence and who may benefit from adjuvant radiotherapy. This algorithm incorporates the following prognostic factors known to be important in predicting local recurrence in patients with DCIS and BCS: tumor size, margin width, nuclear grade, age, and necrosis.[18]

Further efforts are needed to help characterize the tumor biology and genomic heterogeneity of DCIS. Recently, there has been increasing evidence suggesting that the tumor immune microenvironment influences breast cancer development and prognosis. Density of tumor-infiltrating lymphocytes (TILs) in invasive breast cancer has been associated with high grade, human epidermal growth factor receptor (HER)2-positive invasive carcinoma, as well as triple-negative breast cancer (TNBC)[20–23] TILs have been shown to correlate with response to therapy in invasive breast cancer, especially in the TNBC subgroup.[23,24] TILs have been shown to favorably prognosticate breast cancers secondary to their role in adaptive immune response. However, evidence of the clinical relevance of TILs in pure DCIS and outcomes is lacking. The purpose of this study is to investigate the prevalence of TILs in pure DCIS and its association with clinicopathologic characteristics and recurrence.

METHODS

Study Population

The institutional Breast Cancer Database was established in January 2010 and includes all patients undergoing definitive breast cancer surgery at our medical center. The database was queried for all patients who were newly diagnosed with DCIS. Each recurrent case ($n = 13$) was matched to four controls ($n = 56$) for a 1:4 ratio based on date of surgery. The variables of interest included age, race, strong family history of breast cancer (at least one first-degree relative), method of presentation, palpability, tumor size, multifocality, nuclear grade, histologic type (comedo, noncomedo), necrosis, margin status, hormone receptor status including ER and progesterone receptor (PR), atypical ductal hyperplasia (ADH), presence of atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS), and details of treatment (hormone therapy and radiation therapy) and outcomes (ipsilateral recurrences). All clinical data were obtained from detailed questionnaires filled out by patients who gave written consent for the database studies and electronic medical record review. This study was approved by the NYU Institutional Review Board.

Pathology Assessment

All DCIS was classified according to the well-established criteria including nuclear grade, necrosis, and comedo versus noncomedo subtypes.[25] All tumor characteristics, including size, multifocality, concomitant presence of ADH, ALH, LCIS, and estrogen and progesterone receptor status were obtained. TILs were assessed and analyzed according to the guidelines set forth by The International Immuno-Oncology Biomarker Working Group for evaluating TILs in DCIS.[26,27] The TILs were scored using both random-field and hotspot methods. Since the random-field method had poor reproducibility from case to case, the guidelines were modified by applying the hotspot method of TIL assessment. In brief, two pathologists (F.D. and U.O.), blinded to the outcome, identified the densest field ("hotspot") on scanning magnification and scored the mononuclear cells including lymphocytes and plasma cells in one high-power field (40 × objective, BX53; Olympus) of stroma touching the basement membrane of the DCIS (Fig. 1). TILs were defined as sparse (highest % $< 45\%$) or dense (highest % $\geq 45\%$) (Fig. 2) based on cluster analyses (described below).

Statistical Analyses

Statistical methods included hierarchical and *k*-means cluster analyses to define sparse versus dense TILs. The hierarchical cluster analysis gives a dendogram plot, which shows longer horizontal lines where the values between groups appear to be the least related. In the DCIS data ($n = 69$), the hierarchical cluster analysis showed two distinct clusters for the TILs highest %, which were the

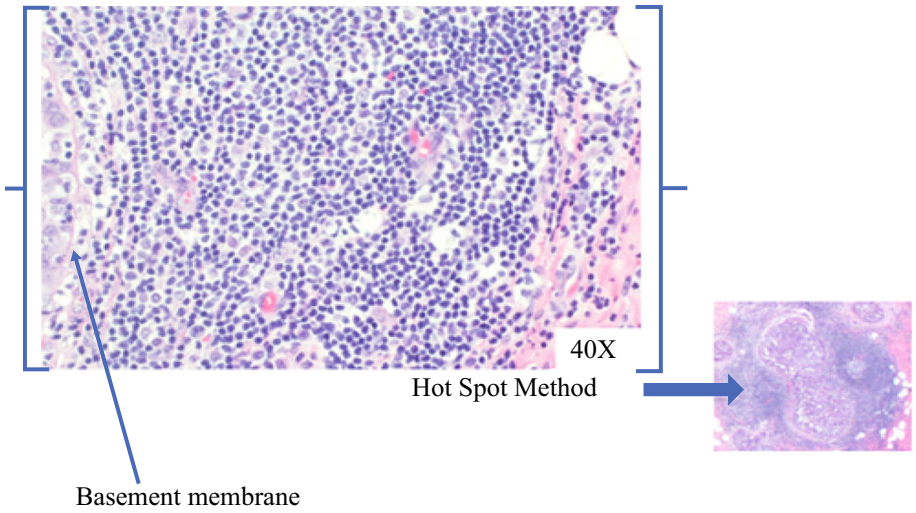

FIG. 1 The hotspot method for scoring TILs. The field with the densest TILs was identified on scanning magnification (inset; arrowhead = TIL). The high-power field (40 ×, objective) was adjusted so that one side of the field touches the DCIS basement membrane. The percentage of the area occupied by the mononuclear inflammatory cells was then scored over total stromal area

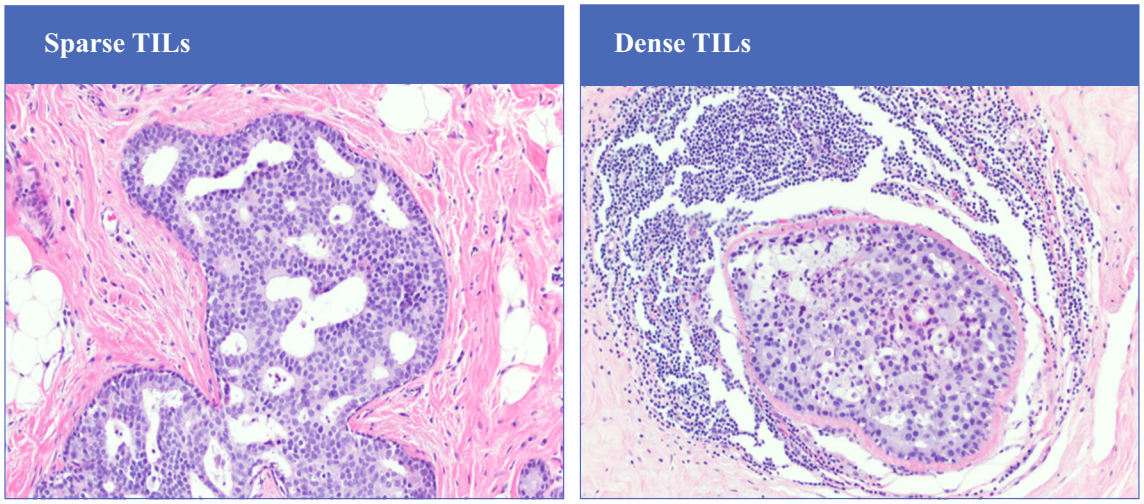

FIG. 2 DCIS with cribriform pattern and intermediate nuclear grade showing sparse TILs (left); DCIS with solid pattern and high nuclear grade enveloped by thick basement membrane and dense cuff of TILs (right)

same two clusters when the TILs mean % was also included. However, because the clusters for the TILs mean % alone were not as distinct, the TILs highest % was used for the cluster definitions. The *k*-means cluster analysis was in complete agreement with the hierarchical cluster analysis for the TILs highest % in terms of cluster membership. The two clusters identified by the *k*-means and the hierarchical cluster analyses were displayed in the scatter plot of the TILs mean and highest %, with the dense cluster defined as TILs highest % > 45 and the sparse cluster as TILs highest % < 45. Age-adjusted logistic regression models were performed to compare the clinical and tumor characteristics for sparse and dense groups. To evaluate which variables were predictors of ipsilateral recurrence, stepwise forward selection was used in a logistic regression model including all of the variables from Table 1 and the TILs clusters as independent variables.

Overall disease-free survival was estimated according to the Cox proportional hazards regression models with the same independent variables that were selected in the logistic model. All analyses were performed using SPSS version 25.0 (released 2017; IBM Corp., Armonk, NY) and SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Out of a total of 2816 patients with a breast cancer diagnosis in the institutional database, 581 (21%) patients had pure DCIS. Of those, sixty-nine patients with pure DCIS were evaluated, of whom 54 (78%) were treated by breast-conserving surgery. The median age for this cohort was 60.2 years, and the median follow-up was 6.7 years. The majority of patients were Caucasian (74%) and did not have strong family history of breast cancer (67%)

TABLE 1 Clinicopathologic characteristics of sparse versus dense TILs

Variable	Total	Sparse (TILs highest % < 45) $N = 47$	Dense (TILs highest % > 45) $N = 22$	p value
Median age at diagnosis (years; range)	62.0 (34–88)	65.0 (34–88)	54.5 (35–86)	0.045*
Race				
African American	6 (9%)	4 (9%)	2 (9%)	0.502
Asian	8 (12%)	3 (6%)	5 (23%)	
Hispanic	3 (4%)	3 (6%)	0 (0%)	
White	51 (74%)	37 (79%)	14 (64%)	
Other	1 (1%)	0 (0%)	1 (5%)	
Strong family history of breast cancer				
Yes	23 (33%)	16 (34%)	7 (32%)	0.899
No	46 (67%)	31 (66%)	15 (68%)	
Method of presentation				0.997
Breast exam	3 (4%)	3 (6%)	0 (0%)	
Mammography	63 (91%)	42 (90%)	21 (95%)	
Ultrasound	1 (1%)	1 (2%)	0 (0)	
MRI	2 (3%)	1 (2%)	1 (5%)	
Palpability				
Yes	3 (4%)	3 (6%)	0 (0%)	0.976
No	66 (96%)	44 (94%)	22 (100%)	
Tumor size (cm; range)	12.0 (0.1–8.0)	1.31 (0.1–5.0)	3.38 (0.8–8.0)	< 0.001*
Multifocal				
Yes	22 (32%)	15 (32%)	7 (32%)	0.854
No	47 (68%)	32 (68%)	15 (68%)	
Nuclear grade				
Low	4 (6%)	4 (9%)	0 (0%)	0.010*
Intermediate	26 (38%)	24 (51%)	2 (9%)	
High	39 (56%)	19 (40%)	20 (91%)	
Comedo histology				
Yes	21 (30%)	10 (21%)	11 (50%)	0.033*
No	48 (70%)	37 (79%)	11 (50%)	
Necrosis				
Yes	48 (70%)	29 (62%)	19 (86%)	0.027*
No	21 (30%)	18 (38%)	3 (14%)	
Margin status				
Positive	2 (2.9%)	0 (0%)	2 (9.1%)	0.884
Close	10 (14.5%)	7 (14.9%)	3 (13.6%)	
Negative	57 (82.6%)	40 (85.1%)	17 (77.3%)	
Estrogen receptor				
Positive	56 (81%)	41 (87%)	15 (68%)	0.037*
Negative	13 (19%)	6 (13%)	7 (32%)	
Progesterone receptor				
Positive	52 (75%)	38 (81%)	14 (64%)	0.081
Negative	17 (25%)	9 (19%)	8 (36%)	
ADH				
Yes	18 (26%)	13 (28%)	5 (23%)	0.865
No	51 (74%)	34 (72%)	17 (77%)	

TABLE 1 continued

Variable	Total	Sparse (TILs highest % < 45) $N = 47$	Dense (TILs highest % > 45) $N = 22$	p value
ALH				
Yes	15 (22%)	10 (21%)	5 (23%)	0.867
No	54 (78%)	37 (79%)	17 (77%)	
LCIS				
Yes	10 (14%)	9 (19%)	1 (5%)	0.121
No	59 (86%)	38 (81%)	21 (95%)	
Hormone therapy				
Yes	22 (32%)	18 (38%)	4 (18%)	0.083
No	47 (68%)	29 (62%)	18 (82%)	
Radiation therapy				
Yes	41 (59%)	26 (55%)	15 (68%)	0.219
No	28 (41%)	21 (45%)	7 (32%)	
Recurrence				
Yes	13 (19%)	4 (9%)	9 (41%)	0.008*
No	56 (81%)	43 (91%)	13 (59%)	

*Age-adjusted logistic regression with a signficance level of $p < 0.05$

MRI magnetic resonance imaging

(Table 1). Also, the majority of lesions were detected by mammography (91%) and were nonpalpable (96%). Within a relatively short follow-up period, 13 patients with DCIS had ipsilateral recurrence. Ten out of the 13 (77%) had radiation therapy. Out of the 13 recurrences, 9 (69%) were DCIS and 4 (31%) were invasive breast cancer. Of those ER-positive patients who recurred, there was a higher proportion of women on antiendocrine therapy compared with those who did not take antiendocrine therapy (56% vs. 44% respectively). Risk of recurrence did not differ significantly between ER-positive patients who took antiendocrine therapy and those who did not ($p = 0.294$).

After adjusting for age, dense TILs was significantly associated with younger age ($p = 0.045$), larger tumor size ($p < 0.001$), high nuclear grade ($p = 0.010$), comedo histology ($p = 0.033$), necrosis ($p = 0.027$), ER negativity ($p = 0.037$), and ipsilateral recurrence ($p = 0.008$) (Table 1). The following variables were not significantly associated with dense TILs: race ($p = 0.502$), strong family history of breast cancer ($p = 0.899$), method of presentation ($p = 0.997$), palpability ($p = 0.976$), multifocality ($p = 0.854$), margin status ($p = 0.884$), PR status ($p = 0.081$), ADH ($p = 0.865$), ALH ($p = 0.867$), LCIS ($p = 0.121$), hormone therapy ($p = 0.083$), and radiation therapy ($p = 0.219$).

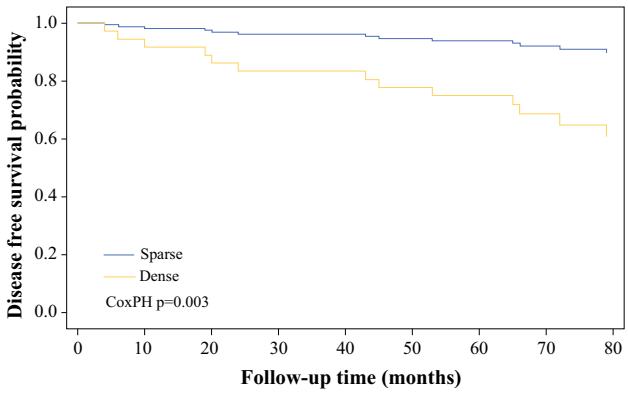

FIG. 3 Disease-free survival analysis of dense verses sparse TILs

After running the forward stepwise selection in the logistic regression model predicting ipsilateral recurrence, the only significant predictor remaining in the model was TILs density [odds ratio (OR) = 7.4, 95% confidence interval (CI) 2.0–28.2, Wald $\chi^2 = 8.7$, $p = 0.003$]. In the Cox proportional hazards model, the dense cluster also showed significantly shorter time to recurrence, with average time to recurrence of 73.5 months compared with 97.9 months for the sparse cluster [hazard ratio (HR) = 5.9, 95% CI 1.8–19.4, Wald $\chi^2 = 8.7$, $p = 0.003$] (Fig. 3).

DISCUSSION

Histopathologic parameters including nuclear grade, necrosis, size, and margin status have been successfully used to stratify outcomes in patients with DCIS.[28] Recent research suggests that assessment of the immune microenvironment may add information beyond the traditional histopathologic parameters and help to predict risk of recurrence.[29–31] The results of the current study demonstrate that dense TILs in pure DCIS was significantly associated with ipsilateral recurrence.

For the purpose of this study, we modified the guidelines[26,27] by applying the hotspot method of TILs assessment. We initially analyzed TILs using both the hotspot method and the random method. For the latter, we scored multiple ducts (range 1–47; median 11) exhibiting a range of TILs from low to high. After statistical analyses, the hotspot method proved to correlate with the histopathologic parameters while the random method did not. We also found the hotspot method to be more straightforward and reproducible.

To explain the correlation between dense TILs and ipsilateral recurrence, it can be postulated that dense TILs are a harbinger of microinvasion, thereby increasing the likelihood of local recurrence. In fact, in an elegant cluster analysis comparing a cohort with pure DCIS with a cohort with DCIS and microinvasion, Beguinot et al. showed that DCIS with high TILs density (> 30%) was more tightly clustered with the microinvasive cohort than with the DCIS with less dense TILs (< 30%).[30] The authors ascribed the similarity between the two groups to the higher rate of HER2 positivity in the dense TILs DCIS group compared with the nondense TILs DCIS. They proposed a two-tier classification of biologically distinct DCIS based on TILs density with potential implications for immunotherapy-based preventive treatment.

Alternatively, it can be hypothesized that TILs density corresponds to the antigenicity of the neoplastic cells in DCIS. There is evidence to suggest that HER2-positive and triple-negative invasive breast cancers are highly immunogenic.[30,32,33] Similarly, in DCIS, TILs density appears to correlate with HER2 positivity and ER negativity.[26,34] Our data indicate that, after age adjustment, ER positivity was significantly correlated with sparse TILs, supporting the notion that ER-negative DCIS is more antigenic, in line with current literature. We did not analyze HER2 status in this study.

We showed that dense TILs was significantly associated with younger age, higher nuclear grade, presence of necrosis, DCIS size, and comedo-type histology in addition to ipsilateral recurrence. Multivariate analysis also demonstrated that dense TILs was still significantly associated with ipsilateral recurrence after a logistic regression model ($p = 0.003$). In addition, dense TILs was significantly associated with shorter time to recurrence and lower disease-free survival probability ($p = 0.044$) (Fig. 3). The value of TILs as a prognostic indicator of recurrence in DCIS has been studied by other researchers, with mixed results. In their study of 1488 patients, Pruneri et al. did not observe any association between high number of TILs and ipsilateral recurrence.[26] Similarly, Thompson et al. demonstrated that DCIS cases with moderate to diffuse TILs (> 5%) were more likely to have PD-L1-positive TILs, but the TILs density did not correlate with the clinical outcome.[35] Other studies with subset analysis of TILs have shown an association between low CD8 + TILs and low CD8 +/FOXP3 + ratio (with +HLADR) and higher risk of ipsilateral recurrence.[31,36] These results indicate that, as the lymphocytic composition of the DCIS immune microenvironment shifts from a proinflammatory (high CD8, low FOXP3) to an antiinflammatory (low CD8, high FOXP3) signature, the immunosurveillance weakens, leading to higher risk of recurrence.[29]

CONCLUSIONS

The results of this study show that dense TILs are a significant predictor of recurrence in patients with pure DCIS treated by breast-conserving surgery. This may have meaningful implications for developing effective strategies for clinical management and identifying patients at risk for ipsilateral recurrence and who may benefit from adjuvant therapies. Further studies, including TILs subset analysis and immune checkpoint expression, are currently underway to further characterize the tumor microenvironment of DCIS and correlate those findings with outcomes.

ACKNOWLEDGMENT We would like to acknowledge the Shifrin–Myers Breast Cancer Discovery Fund for funding this study.

DISCLOSURES None.

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