CME/SAM

Biomarkers Predicting Pathologic Complete Response to Neoadjuvant Chemotherapy in Breast Cancer

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ABSTRACT

Objectives: *Recent studies have shown strong correlation of pathologic complete response (pCR) to neoadjuvant chemotherapy with survival and prognosis in breast cancers.*

Methods: *Clinical data from 237 breast cancer patients who received neoadjuvant chemotherapy between 2012 and 2014 were reviewed. Correlations were sought between pCR and estrogen receptor (ER), progesterone receptor (PR), and HER2 status; Nottingham and nuclear grades; tumor tubule formation; mitotic score; Ki67 index; and tumoral and stromal lymphocytic infiltration (TLI and SLI, respectively).*

Results: *Of the 237 cases, 104 (43.9%) achieved pCR. The HER2+ and triple negative breast cancer (TNBC) subtypes had higher pCR rates compared with the luminal subtype (ER+ or PR+ and HER2–). ER and PR negativity, HER2 positivity, Nottingham grade 3, increased TLI and SLI, high mitotic count and Ki67 score correlated significantly with pCR in the overall cohort. TLI and SLI correlated significantly with pCR in the HER2+ and TNBC subtypes in multivariate analysis, whereas no biomarkers correlated with pCR in the luminal subtype.*

Conclusions: *In addition to the pathologic parameters and biomarkers already routinely assessed, evaluation of TLI and SLI may help to better select patients with HER2+ and TNBC for neoadjuvant chemotherapy.*

Upon completion of this activity you will be able to:
- know the current definition of pathologic complete response (pCR) of breast cancer in a neoadjuvant therapy setting.
- describe the pCR rates associated with different major subtypes of breast cancer.
- discuss the association of tumor-infiltrating lymphocytes in pCR in HER2+ and triple-negative breast cancer.

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Breast cancer is the most prevalent malignancy and second deadliest cancer in women. Currently, the main treatments include surgery, chemotherapy, radiation and targeted therapies. Chemotherapy as a systemic control regimen has dramatically increased the disease-free survival and overall survival rate.[1,2] Chemotherapy can be administrated before or after surgery. When chemotherapy is given before surgery, it is referred as neoadjuvant therapy. For decades, neoadjuvant therapy has been used to treat locally advanced tumors to be operable. Recent research and clinical trials have shown strong correlation of breast cancer responses to neoadjuvant therapies with survival and prognosis.[3-5] Patients who achieve pathologic complete response (pCR) to neoadjuvant therapy tend to have improved disease-free and overall survival compared with patients with residual invasive disease.[6,7] pCR is defined as no invasive carcinoma in the breast and lymph nodes at the time of surgery. Because of the strong correlation between pCR and survival, the US Food and Drug Administration (FDA) now considers pCR to neoadjuvant chemotherapy a surrogate endpoint for clinical trials and drug approval.[8] The strongest correlation between pCR and outcomes is found within the triple negative breast cancer (TNBC) and HER2+ breast cancers.[9]

Predicting which patients will achieve pCR to neoadjuvant chemotherapy is important because neoadjuvant chemotherapy is not without risk. For instance, although neoadjuvant chemotherapy can prolong disease-free and overall survival, it may increase the rate of ipsilateral tumor recurrence compared with adjuvant therapy.[2] In addition, delaying surgery may decrease survival.[10] A variety of methodologies have been investigated, such as magnetic resonance imaging,[11] positron emission tomography,[12] and gene expression profiling.[13] However, none has been universally accepted, and these modalities are expensive and not routinely performed. We conducted a comprehensive evaluation of tumor morphology and biomarker status, and correlated these parameters with pCR rate in a neoadjuvant setting.

Material and Methods

Patient Selection and Clinicopathologic Characteristics

A total of 2,691 consecutive excisional specimen cases were retrieved from the archives of the Department of Pathology and Laboratory Medicine at Emory University from 2012 to 2014 after protocol approval from the Emory Institutional Review Board. All surgical procedures were performed at two major teaching hospitals of Emory University. Among the 2,691 cases, 237 patients had neoadjuvant therapies. Of the 237 cases, 229 cases had the status of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression available, and 195 cases had information of biopsy diagnoses. Among the 195 cases, 129 had core biopsy slides available, and all of these 129 cases were reviewed by a pathologist (XL). For the cases without biopsy slides, information was retrieved from the patient's medical record and pathology report. The majority of the patients received four cycles of neoadjuvant chemotherapies. HER2+ cancers received HER2 targeted therapies in addition to neoadjuvant chemotherapies. Clinicopathologic characteristics of all patients were summarized in **Table 1**.

Table 1
Clinicopathologic Characteristics[a]

Characteristics	No. of Cases	Percentage
Age, y		
20-29	2	0.84
30-39	27	11.40
40-49	62	26.16
50-59	66	27.85
60-69	58	24.47
70+	22	9.28
Race		
Black	121	51.10
White	105	44.30
Other	11	4.64
Tumor grade		
1	21	9.01
2	89	38.20
3	123	52.79
Clinical stage		
I/II	158	66.95
III/IV	78	33.05
Nodal status		
Positive	130	54.85
Negative	107	45.15
Lymphocytic infiltration		
SLI	88	68.22
TLI	72	55.81
Hormone receptor		
ER+	108	47.16
PR+	74	32.31
HER2 expression		
Positive	79	33.33
Negative	158	66.67
Subtypes		
HER2+	79	34.50
Triple negative	78	34.06
Luminal	72	31.44
Response		
pCR	104	43.88
Non-pCR	133	56.12

ER, estrogen receptor; pCR, pathologic complete response; PR, progesterone receptor; SLI, stromal lymphocytic infiltration; TLI, tumoral lymphocytic infiltration;
[a]Some information was missing in a few cases.

Pathologic Evaluation

The following morphological features and biomarkers were evaluated in the biopsy specimen: tubule formation (score 1-3), nuclear grade (score 1-3), mitotic count (score 1-3) per the College of American Pathologists (CAP) recommendation, Nottingham histologic grade (score 1-3), stromal and tumoral lymphocytic infiltration (SLI and TLI, respectively), fibrosis (scored 1-3 as follows: 1, mild; 2, moderate; 3, severe), ER and PR expression, and HER2 amplification (positive or negative per American Society of Clinical Oncology recommendation) and Ki67 score (high: ≥15%; low: <15%). TLI was evaluated as percentage of tumor cells infiltrated with lymphocytes. SLI was evaluated as percentage of stromal area covered by lymphocytes. The stroma included both intratumoral stroma as well as the stroma adjacent to the periphery of the tumor.

Breast Cancer Classification and Definition of pCR

Tumors were classified as luminal, HER2+, or TNBC. The luminal subtype was defined as ER+ and/or PR+ and HER2–. HER2+ cancer was defined by either a score of

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3+ from immunohistochemical (IHC) study or positive *HER2* amplification by fluorescence in situ hybridization (FISH) regardless of ER or PR status. TNBC was defined by the absence of ER and PR expression and HER2 overexpression by IHC or FISH. Standard 1% expression rate was used as the cutoff to define positivity in ER and PR expression. pCR was defined as no invasive carcinoma in both breast and lymph nodes at the time of surgery. In situ carcinoma was allowed in the pCR cases.

Statistical Analysis

Logistic regression was performed on the total patient cohort as well as the subtypes with a pCR case indicating an event. Odds ratios (ORs), which indicate a multiplicative effect in the odds of achieving a pCR, were compared between categorical groups (using the lowest risk group as the reference). Multivariate models were fit via a backwards selection method, in which the full model was reduced and refit stepwise by removing the least significant variable based on Wald test for the individual parameters, until either all variables had been removed or all had a *P* value <.10. Due to the variability in multivariate data completeness, we also used the Firth penalized likelihood approach. Correlations were analyzed using the Pearson product-moment correlation coefficient. statistical analysis software was used for all statistical analysis.

Results

Clinicopathologic Features of the Cases

Of the total 237 patients, 91 (38.4%) were younger than 50 years of age, 78 (32.9%) had advanced-stage disease (pT3 or pT4), and 104 (43.9%) had pCR. Among the 229 cases with available biomarker information, 72 were luminal, 79 were HER2+ and 78 were TNBC (Table 1). In the non-pCR patients, the size of residual invasive carcinoma ranged from 0.5 to 9 cm.

HER2+ and TNBCs Have Higher pCR Rate Than Luminal Breast Cancer

Subtype analysis revealed that 20 of 72 (27.8%) luminal type, 46 of 79 (58.2%) HER2+ and 37 of 78 (47.4%) TNBC exhibited pCR. On univariate analysis, the HER2+ and TNBC had 3.6 and 2.4 times the odds of achieving pCR compared with the luminal subtype (95% confidence interval [CI] = 1.83-7.17, $P < .001$ and 1.19-4.64, $P = .014$, respectively) **Table 2**. Univariate analysis failed to identify any parameter or biomarker as significantly correlated with pCR rate in the luminal subtype.

High TLI and SLI Are Significantly Associated With pCR Rate in HER2+ Breast Cancers and TNBCs

TLI (both with 3% as threshold and as a continuous increasing value) as well as SLI (both with 5% as threshold and as a continuous increasing value) were significantly associated with pCR in the overall cohort (Table 2). The categorical cutpoints (3% in TLI and 5% in SLI) were chosen by using the threshold that was at the intersection of specificity and sensitivity when identifying pCR patients vs non-pCR patients. On univariate analysis, high TLI and SLI were both found to be significantly associated with a greater probability of pCR in HER2+ and TNBC cancers (Table 2). This significant correlation persisted in HER2+ and TNBCs in multivariate analysis **Table 3**. No correlation between TLI or SLI and pCR was seen in the luminal type.

ER and PR Expression Is Associated With Decreased pCR in the Overall Cohort and HER2+ Breast Cancers

Positivity of ER and PR expression was found to be associated with decreased pCR in the overall cohort and within the HER2+ subtype (Table 2). With increased ER or PR expression, there was a small decrease in the odds of a pCR.

High Mitotic Count and Ki67 Score and Nottingham Histologic Grade 3 Are Significantly Associated With pCR Rate in the Overall Cohort

Nottingham histologic grade 3 and high mitotic count had a positive correlation with the probability of pCR in the overall cohort (OR 3.47; $P = .022$ and OR 3.44; $P < .001$, respectively; Table 2). Along with the positive correlation of mitotic count and pCR, Ki67 score (both ≥15% as threshold and as increasing absolute value) was significantly associated with pCR (Table 2).

The mitotic count remained a significant predictor of pCR in the overall cohort in multivariate analysis (Table 3).

High Correlation Between Stromal and Intratumoral Lymphocytic Infiltration

TLI and SLI are very highly correlated in the overall cohort ($r = 0.68$, $P < .001$) and within each subtype, HER2+ ($r = 0.52$, $P < .001$), luminal ($r = 0.85$, $P < .001$), triple negative ($r = 0.69$, $P < .001$). Such strong correlation was seen in multivariate analysis model. When we swapped SLI with TLI in the final multivariate model, very similar ORs (0.96 vs 0.95) and OR *P* values (.013 vs .017) with correlation of pCR were obtained.

Luminal Type Breast Cancers Have Low pCR Rate and No Parameter Is Strongly Associated With pCR

Among all 3 subtypes, the luminal type had the lowest pCR rate of 27.8%, compared with 58.2% in HER2+ and

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Table 2
Univariate Logistic Analysis of pCR in the Total Cohort and Within Subtypes[a]

Covariate		Overall Cohort				HER2+			
Level	n	Odds Ratio (95% CI)	OR *P* Value	Type 3 *P* Value	n	Odds Ratio (95% CI)	OR *P* Value	Type3 *P* Value	n
Mitotic score									
1	75	-	-	**<.001**	22	-	-	.075	36
2	41	0.94 (0.41-2.15)	.876		12	2.02 (0.48-8.43)	.334		12
3	58	3.44 (1.68-7.06)	**<.01**		18	5.06 (1.25-20.48)	**.023**		12
Nuclear grade									
1	6	-	-	*	0	-	-	.967	6
2	71	*	*		28	-	-		32
3	101	*	*		26	1.02 (0.35-3.01)	.967		25
Tubular formation									
1	3	-	-	.966	3	-	-	.614	
2	17	1.40 (0.11-18.61)	.799		10	2.00(0.13-29.81)	.872		4
3	155	1.30 (0.12-14.62)	.833		40	3.00 (0.25-35.91)	.323		57
Nottingham grade									
1	21	-	-	**.017**	5	-	-	.17	15
2	89	1.89 (0.63-5.62)	.255		35	1.42 (0.21-9.55)	.721		29
3	123	3.47 (1.20-10.07)	**.022**		38	3.25 (0.48-22.07)	.228		27
PR intensity									
0	153	-	-	**.012**	54	-	-	.127	21
1	16	0.71 (0.25-2.00)	.517		5	0.75 (0.11-4.90)	.764		11
2	21	0.37 (0.13-0.99)	.048		8	0.30 (0.06-1.40)	.125		13
3	39	0.31 (0.14-0.69)	**.004**		12	0.25 (0.07-0.94)	.041		27
PR %	229	0.99 (0.98-1.00)	**.014**	**.014**	79	0.98 (0.96-1.00)	**.014**	**.014**	72
PR WI (H score)	229	1.00 (0.99-1.00)	**.018**	**.018**	79	0.99 (0.99-1.00)	**.019**	**.019**	72
ER intensity									
0	122	-	-	.085	39	-	-	0.168	5
1	23	0.70 (0.28-1.71)	.431		9	1.96 (0.36-10.75)	.438		14
2	15	0.60 (0.20-1.80)	.366		2	*	*		13
3	69	0.45 (0.25-0.84)	**.011**		29	0.40 (0.15-1.06)	.065		40
ER %	229	0.99 (0.99-1.00)	**.008**	**.008**	79	0.99 (0.98-1.00)	**.028**	**.028**	72
ER WI (H score)	229	1.00 (1.00-1.00)	**.009**	**.009**	79	1.00 (0.99-1.00)	**.018**	**.018**	72
HER2 receptor status									
Negative	158	-	-	**.003**	-	-	-	-	-
Positive	79	2.34 (1.35-4.06)	**.003**		-	-	-		-
Subtype									
HER2+	79	3.62 (1.83-7.17)	**<.001**	**<.01**	-	-	-	-	-
Triple negative	78	2.35 (1.19-4.64)	**.014**		-	-	-		-
Luminal	72	-	-		-	-	-		-
Ki67 threshold									
Below 15%	36	-	-	**.024**	8	-	-	.234	21
Above 15%	145	2.58 (1.13-5.86)	**.024**		53	2.54 (0.55-11.77)	.234		40
Ki67%	181	1.01 (1.00-1.02)	**.008**	**.008**	61	1.00 (0.99-1.02)	.714	0.714	61
Stage group									
I/II	158	-	-	0917	45	-	-	.713	53
III/IV	78	0.97 (0.56-1.68)	.917		34	0.84 (0.34-2.08)	.713		19
Race									
White	105	-	-	.757	37	-	-	.794	36
Other	11	1.48 (0.43-5.16)	.537		2	0.61 (0.04-10.53)	.733		4
Black	121	0.93 (0.55-1.57)	.787		40	0.74 (0.30-1.85)	.524		32
SLI threshold									
Above 5%	88	-	-	**.001**	33	-	-	.228	23
Below 5%	41	0.27 (0.12-0.60)	**.001**		12	0.43 (0.11-1.68)	.228		23
SLI	129	1.04 (1.01-1.06)	**.001**	**.001**	45	1.06 (1.01-1.12)	**.028**	**.028**	46
TLI threshold									
Above 3%	72	-	-	**.003**	29	-	-	**.036**	18
Below 3%	57	0.34 (0.16-0.70)	**.003**		16	0.25 (0.07-0.91)	**.036**		28
TLI	129	1.26 (0.87-1.82)	**.016**	**.016**	45	1.10 (0.98-1.23)	.119	.119	46
Fibrosis	128	1.26 (0.87-1.82)	.228	.228	46	1.25 (0.65-2.43)	.503	.503	44
Mitosis	139	1.02 (1.00-1.04)	.069	.069	46	1.01 (0.98-1.04)	.539	.539	53
Age	237	0.98 (0.96-1.00)	.054	.054	79	0.97 (0.93-1.01)	.172	.172	72

CI, confidence interval; ER, estrogen receptor; OR, odds ratio; PR, progesterone receptor; SLI, stromal lymphocytic infiltration; TLI, tumoral lymphocytic infiltration.
[a]A dash (-) indicates a missing value while a (*) indicates nonconversion for that variable. Bold values indicate significance.

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Luminal				Triple Negative		
Odds Ratio (95% CI)	OR *P* Value	Type3 *P* Value	n	Odds Ratio (95% CI)	OR *P* Value	Type3 *P* Value
-	-	.615	16	-	-	.069
0.70 (0.13-3.87)	.683		13	0.50 (0.10-2.58)	.407	
1.75 (0.42-7.35)	.445		28	2.58 (0.73-9.12)	.143	
-	-	*	0	-	-	.475
*	*		9	-	-	
*	*		47	0.59 (0.14-2.49)	.475	
			0	-	-	*
-	-	.209	3	-	-	
0.27 (0.03-2.09)	.209		53	*	*	
-	-	0.418	0	-	-	.383
1.27 (0.28-5.85)	.757		23	-	-	
2.35 (0.53-10.41)	.259		54	1.56 (0.58-4.20)	.383	
-	-	.736	-	-	-	-
1.14 (0.25-5.26)	.864		-	-	-	
0.60 (0.12-2.91)	.526		-	-	-	
0.57 (0.16-2.06)	.393					
1.00 (0.99-1.02)	.832	.832	-	-	-	-
1.00 (1.00-1.01)	.947	.947	-	-	-	-
-	-	.873	-	-	-	-
0.41 (0.05-3.68)	.425		-	-	-	
0.67 (0.08-5.68)	.71		-	-	-	
0.57 (0.08-3.88)	.564					
1.00 (0.99-1.02)	.856	.856	-	-	-	-
1.00 (1.00-1.01)	.947	.947	-	-	-	-
-	-	-	-	-	-	-
-	-		-	-	-	
-	-	-	-	-	-	-
-	-		-	-	-	
-	-		-	-	-	
-	-	.763	7	-	-	.963
0.83 (0.25-2.73)	.763		52	519500.5 (0.00-1)	.963	
1.02 (1.00-1.04)	.082	.082	59	1.01 (0.99-1.03)	.224	.224
-	-	.184	53	-	-	.913
0.40 (0.10-1.55)	.184		24	0.95 (0.36-2.49)	.913	
-	-	.591	29	-	-	.656
3.00 (0.37-24.49)	.305		5	1.40 (0.20-9.66)	.733	
1.17 (0.40-3.45)	.771		44	0.71 (0.28-1.82)	.475	
-	-	.477	31	-	-	**.032**
0.60 (0.14-2.48)	.477		6	0.08 (0.01-0.80)	**.032**	
0.99 (0.95-1.04)	.667	.667	37	1.05 (1.00-1.10)	**.036**	**.036**
-	-	.429	24	-	-	.445
0.57 (0.14-2.32)	.429		13	0.58 (0.15-2.32)	.445	
1.00 (0.94-1.06)	.989	.989	37	1.09 (1.00-1.19)	**.042**	**.042**
0.74 (0.36-1.48)	.389	.389	37	1.66 (0.77-3.56)	.196	.196
1.01 (0.98-1.04)	.607	.607	39	1.01 (0.97-1.06)	.567	.567
0.99 (0.95-1.03)	.592	.592	78	0.97 (0.94-1.01)	.173	.173

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Table 3
Multivariate Logistic Regression Analysis for pCR in the Overall Cohort and HER2+ and TNBC Subtypes[a]

	Covariate	Level	Odds Ratio (95% CI)	Odds Ratio *P* Value	Type 3 *P* Value
Overall cohort	Stage	I/II	reference	–	**.015**
		III/IV	0.20 (0.063-0.625)	0.015	
	HER2	+	9.40 (2.897-30.295)	<.001	**<.001**
	Mitotic score	1	reference	–	**.012**
		2	18.60 (2.521-136.510)	0.009	
		3	94.89 (7.316->999.999)	0.004	
TNBC	PR	+	0.77 (0.632-0.943)	0.045	**.045**
	TLI/SLI		1.10 (1.025-1.122)	0.017	**.017**
HER2	TLI/SLI		9.10 (1.352-60.684)	0.023	**.023**

pCR, pathologic complete response; PR, progesterone receptor; SLI, stromal lymphocytic infiltration; TLI, tumoral lymphocytic infiltration; TNBC, triple negative breast cancer.
[a]Backward elimination ($\alpha = 0.10$) was used for covariate selection. Bold values indicate significance.

47.4% in TNBC. No pathologic parameter or biomarker was identified to have significant association with pCR in the luminal type.

Discussion

In our study, we have performed a comprehensive evaluation of tumor morphology and biomarker statuses, and correlated them with pCR rate in the neoadjuvant setting. We found that pCR was significantly higher in the HER2+ and triple-negative subtypes (58.2% and 47.4%, respectively) compared with the luminal subtype (27.8%). The odds of achieving a pCR in HER2+ cancers were 3.6 times higher than in luminal cancers. This result is similar to that found in the metaanalysis by Houssami et al of 30 studies encompassing 11,695 patients, which estimated that pCR occurred in 8.3% of hormonal receptor positive (HR+)/HER2–, 18.7% of HR+/HER2+, 38.9% of HR–/HER2+, and 31.1% of TNBCs. The pCR rates in our study were slightly higher than those in the metaanalysis by Houssami et al, but high pCR rates of TNBC and HER2+ breast cancers have been reported.[14] The different pCR rates in different studies might be due to variation of subtypes of breast cancers. For example, TNBC has been classified into six subtypes, and these subtypes might have different clinicopathologic characteristics and respond differently to chemotherapies.[15-19] Furthermore, Houssami et al found that the odds of achieving a pCR was ~7 times higher for patients with HR–/HER2+ breast cancer and ~5 times higher for patients with TNBC in comparison with patients with the HR+ subtype.[9] Several other studies that investigated the use of anti-HER2 therapies in the neoadjuvant setting have found that the pCR rate varies from ~20% to 65% in HER2+ cancers.[1,20-24] Other studies have also reported an increased pCR rate in TNBCs as compared with non-TNBCs (22% vs 11%).[1,25-27] While Tan et al reported that ER and PR negativity significantly correlated with pCR in multivariate analysis, we found that ER and PR negativity were only associated with pCR in univariate analysis, indicating that ER and PR negativity is associated with HER2+ or triple-negative status.[28] In our study, HER2+ cases included HR+ as well as HR– cases. Within the HER2+ group, we found that pCR positively correlated with ER and PR negativity. Von Minckwitz et al suggest that pCR may not be a suitable endpoint for the luminal subtypes. Specifically, they found that in low-proliferative subgroups (which included lobular, grade 1 and HR+ tumors) pCR conferred no predictive power in disease-free or overall survival, in contrast with the high-proliferative subgroup (which included ductal, grade 2/3, and HR– tumors) in which pCR was associated with improved disease-free and overall survival.[7] We too determined that pCR is significantly associated with high histologic grade and mitotic activity in both univariate and multivariate analyses across the entire cohort.

We report a strong correlation between pCR and Ki67 score both as a categorical variable (specifically, when ≥15% is set as the threshold for defining high proliferation index) and as a continuously increasing variable, similar to the findings of other studies.[1,29-32] Brown et al, who measured Ki67 expression using quantitative automated quantitative analysis by immunofluorescence (AQUA), found that both average and maximum AQUA scores were significant predictors of pCR to neoadjuvant therapy in multivariate analysis.[32] Kim et al found Ki67 expression to be the only independent predictor of pCR and also discovered that a Ki67 value of greater than 25% was a significant predictive factor for pCR.[29] Yoshioka et al found that high Ki67 was a predictive marker for pCR and that all patients achieving pCR were disease-free by the study's end.[31] Furthermore, high Ki67 expression in tumors of posttreatment was strongly correlated with poor disease-free and overall survival regardless of subtype in their study. We found high mitotic score positively correlated with pCR. A mitotic count of >9/mm^2 was reported to be significantly correlated with pCR by Balmativola et al.[33] Based on these compelling data, mitotic count and Ki67 should be considered for inclusion in routine

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clinical evaluation of patients with HER2+ or TNBC to help determine whether neoadjuvant chemotherapy is indicated.

Herein, we additionally found TLI and SLI to be significantly correlated with pCR in univariate analysis in HER2+ breast cancers (OR = 0.94, $P = .028$). Our results are similar to those reported by Mao et al, who carried out a systematic review and metaanalysis to evaluate the predictive roles of tumor infiltrating lymphocytes (TILs) in response to neoadjuvant chemotherapy in breast cancer.[34] They evaluated a total of 13 studies that included 3,251 patients. In pooled analysis, they found that the detection of higher TIL numbers in the pretreatment biopsy was correlated with better pCR to neoadjuvant chemotherapy (OR 3.93; 95% CI = 3.26-4.73). Moreover, TILs predicted higher pCR rates in HER2+ and TNBCs (OR = 2.49 [95% CI = 1.61-3.83] and OR = 5.05 [95% CI = 2.86-8.92], respectively), but not in ER+ breast cancer (OR = 6.21 [95%CI: 0.86-45.15]). In multivariate analysis, they found that TILs were still an independent predictor for high pCR rate (OR = 1.41 [95% CI= 1.19-1.66]).[34] Furthermore, they found that, in three studies that examined lymphocyte-predominant breast cancer (LPBC; defined as having >50% or 60% lymphocytic infiltration of the tumor bed or stroma), LPBC patients had higher pCR rates compared with non-LPBC patients (OR = 3.64 [95% CI = 2.70-4.90]).[35,36] Other studies have evaluated the density of TILs as per 10% increase in the number of lymphocytes infiltrating either intratumoral or stromal compartments, both of which predicted better pCR (OR = 1.35 [95% CI= 1.27-1.44] and OR = 1.26 [95% CI, 1.20-1.32], respectively). Altogether, their analysis indicated that TIL infiltration was an independent predictive marker for higher pCR rate (OR = 1.41 [95% CI = 1.19-1.66]), whether TILs were detected in intratumoral (OR = 1.23 [95% CI = 1.12-1.34]) or stromal (OR = 51.22 [95% CI = 1.09-1.36]) compartments.[34] While other studies have used higher thresholds in evaluating TLI and SLI, we report significantly higher pCR rates even with very low thresholds (3% and 5%, respectively).

Our study reveals that TLI and SLI are highly correlated with each other, and both are significantly associated with pCR in univariate analysis. Furthermore, TLI and SLI are each individually correlated with pCR in multivariate analysis, and assessment of both variables together does not give any additive value in predicting pCR. During our evaluation of the H&E slides, we found that TLI was challenging to assess, usually requiring tedious evaluation at high power. Therefore, evaluating SLI alone might be sufficient to assist in predicting pCR.

In conclusion, our comprehensive evaluation of pathologic features in conjunction with biomarker statuses shows that HER2 positivity, TNBC, high Ki67 and mitotic indices, and TLI and SLI are strongly associated with pCR in patients receiving neoadjuvant chemotherapy. While evaluation of ER, PR and HER2 statuses is mandated by CAP, routine testing of breast cancers for Ki67 expression is not currently required by either the American Society of Clinical Oncology or the National Comprehensive Cancer Network. It might be useful to report SLI and Ki67 in addition to Nottingham histologic grade and receptor statuses. The combination of all these parameters might help to better predict response to neoadjuvant chemotherapy and select patients for neoadjuvant therapy. Patients who are unlikely to respond to neoadjuvant chemotherapy may thus be prescribed alternate therapy regimens and spared the side effects of a probably futile treatment program that would delay a more successful strategy.

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