Tumor Infiltrating Lymphocytes (TILs) in triple-negative breast cancer: High Immunoscore is associated with pathological CR in patients receiving neoadjuvant chemotherapy

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Introduction

Background

- Neoadjuvant chemotherapy is widely used to downstage breast cancers prior to surgery.
- Pathologic complete response (pCR) rate is a strong predictor of outcome for breast cancer.

Immunoscore®

- The Immunoscore® assay is the first standardized immune-based assay for classification of cancer [Hermitte et al., 2016]. It assesses the host immune response by measuring intra- and peri-tumoral T cell infiltration in formalin-fixed paraffin-embedded (FFPE) tissue sections.
- Originally developed for colon cancer indication, it is intended to be widely used in solid cancer indications for diagnostic and prognostic purposes, as well as a pharmacodynamic biomarker during drug development processes. As a first clinical validation in breast cancer, we assessed the Immunoscore in a cohort of 103 breast cancer patients, that previously received neo-adjuvant chemotherapy.

Methods

Pathological and clinical assessment

- Clinical assessment of the primary tumor and lymph nodes was made using bi-dimensional caliper measurements of the primary tumor and axillary nodes.
- Sonographical assessments of the primary tumor and lymph nodes were performed regularly.
- Immunohistochemical staining was performed for ER, PR, HER-2 and Ki67 .
- Fluorescence in situ hybridization (FISH) was used to confirm HER-2 positivity.
- We analyzed data retrospectively/prospectively on 103 breast cancer patients undergoing neoadjuvant chemotherapy.
- Pathological complete response (pCR) was defined as the complete disappearance of the invasive cancer in the breast and absence of tumor in the axillary lymph nodes.
- Ethics approval was obtained from Pharma-Ethics, Pretoria, South Africa (ethics committee working according to the South African Ethics regulations).
- NCSS software version 11 for Windows (USA) was used for statistical analyses.

Methods

- Outcome assessments: Associations of clinical and pathological characteristics including Ki67, CD8+ cytotoxic T cells and CD3+ T cells with pCR.
- All patients were treated with anthracycline and/or taxane-based neoadjuvant chemotherapy.

Immunoscore® Assessment

- In this retrospective analysis, 103 pre-treatment tumor tissue samples were analyzed by immunohistochemistry for density (cells/mm²) of T-cell subsets (CD3+,CD8+).
- CD3 and CD8 staining was performed using Benchmark® XT station on 2 consecutive formalin-fixed paraffin-embedded (FFPE) slides (4 µm).
- Digital pathology-dedicated software permitted the measurement of positive cell densities into interest area (core of the tumor and invasive margin).
- A prespecified bioinformatics algorithm was used to generate a numerical index (Immunoscore®) and analysis cut-offs. Immunoscore® assay measures the density of CD8+ cytotoxic T cells and CD3+ T cells of resected or biopsied cancer samples and performed on FFPE tissue slides.
- Immunoscore® provides 3 score levels (high / intermediary / low).
- Immunoscore® was applied to tumors with invasive margin and was adapted when no invasion was identified on the specimen.

Figure 1. Immunoscore® Assessment.

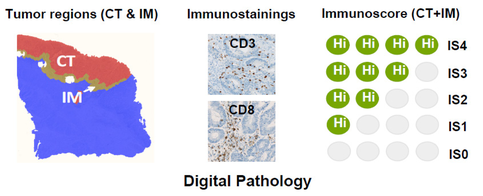


Figure 2. Immunoscore® High.

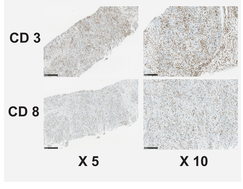


Figure 3. Immunoscore® Low.

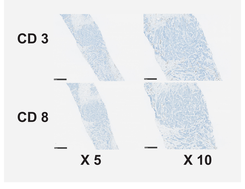
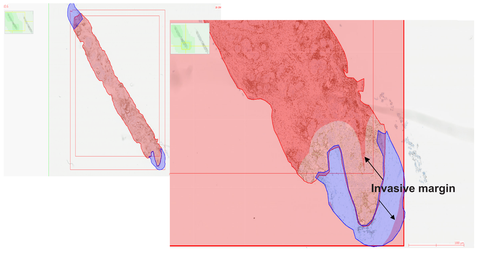


Figure 4. Invasive margin.



Statistical Methods

- The primary hypothesis was that higher levels of CD8+ cytotoxic T cells, CD3+ T cells and Immunoscore® would be associated with a better overall prognosis, independent of anti-cancer therapy.
- Receiver-operating characteristic (ROC) curve analysis was used to determine the optimal cut-point for Ki67, CD8+ cytotoxic T cells, CD3+ T cells and Immunoscore®.
- Fisher's exact or Chi-squared tests were used for the analysis of categorical variables.
- Logistic regression multivariate models included only variables that exhibited a univariate association with the dependent variable, pCR ($p < 0.1$).
- NCSS software version 11 for Windows (USA) was used for statistical analyses.

Patient Characteristics

Table 1. Patient Characteristics.

Age (n=103)		
Median Age	52	
Range	26-84	
Histology		
	Total	**%**
Ductal	99	96%
Lobular	2	2%
Other	2	2%
Menepausal Status		
	Total	**%**
Pre	41	40%
Post	62	60%
Tumor Size		
	Total	**%**
T1	23	22%
T2	65	63%
T3 + T4	15	15%
Nodal Status		
	Total	**%**
Negative	45	44%
Positive	54	52%
Unknown	4	4%
Stage		
	Total	**%**
A1	8	7%
A2	1	1%
B1	49	48%
B2	26	25%
C1	10	10%
C2	7	7%
C3	2	2%
Ki-67 Pre-chemo		
Median	40	
Range	5-90	
	Total	**%**
≥ 40%	51	50%
15-39%	37	35%
< 15%	13	13%
Unknown	2	2%
Molecular type		
	Total	**%**
Luminal A	9	9%
Luminal B	23	22%
HER2 Positive	18	18%
TNBC	53	51%
CD3 and CD8 Count		
	Median Cells/ mm²	**Range Cells/ mm²**
CD3 centre of tumor	884	25-5771
CD3 invasive margin	1409	53-6197
CD8 centre of tumor	358	10-3448
CD8 invasive margin	535	38-3117
Immunoscore		
	Total	**%**
0	8	8%
1	9	8%
2	48	47%
3	35	34%
4	3	3%

ROC Curves for Prediction of pCR

Figure 5. CD3 centre of tumor.

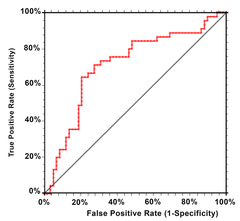


Figure 6. CD3 invasive margin.

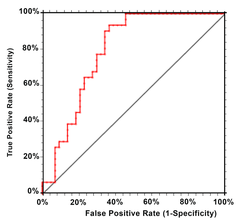


Figure 7. CD8 centre of tumor.

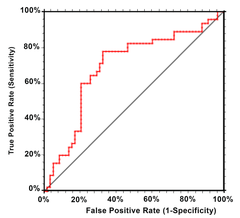
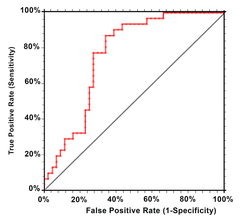
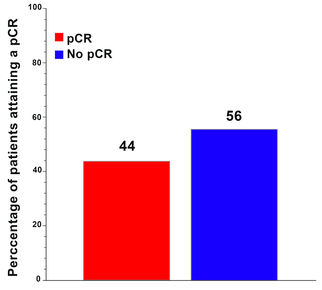


Figure 8. CD8 invasive margin.



Results

Figure 9. Response to Neo-Adjuvant Chemotherapy.



Univariate Analysis

Table 2. Univariate Analysis - Significant factors assosiated with pCR.

Stage			
	pCR	**Chi²**	**pValue**
1	67%	9,03	0,02885
2A	51%		
2B	42%		
3	16%		
ER			
	pCR	**Chi²**	**pValue**
Positive	18%	21.80	0.00001
Negative	64%		
PR			
	pCR	**Chi²**	**pValue**
Positive	13%	22.81	0.00001
Negative	61%		
HER2			
	pCR	**Chi²**	**pValue**
Postive	67%	0.3531	0.55237
Negative	51%		
Molecular type			
	pCR	**Chi²**	**pValue**
Luminal	9%	23,03	0,00001
HER2 Positive	50%		
TNBC	62%		
Ki-67			
	pCR	**Chi²**	**pValue**
≥ 40%	57%	13,84	0,00099
15-39%	41%		
< 15%	0%		

CD3 centre of tumor			
	pCR	**Chi²**	**pValue**
≥ 800 cells/ mm³	73%	12,76	0,00035
< 800 cells/ mm³	27%		
CD3 invasive margin			
	pCR	**Chi²**	**pValue**
≥ 1400 cells/ mm³	77%	15.13	0,00010
< 1400 cells/ mm³	23%		
CD8 centre of tumor			
	pCR	**Chi²**	**pValue**
≥ 800 cells/ mm³	66%	14,25	0,00016
< 800 cells/ mm³	33%		
CD8 invasive margin			
	pCR	**Chi²**	**pValue**
≥ 500cells/ mm³	84%	17,49	0,00003
< 500cells/ mm³	16%		
Immunoscore®			
	pCR	**Chi²**	**pValue**
High	63%	9,99	0,00674
Intermediate	35%		
Low	23%		
Immunoscore®			
	pCR	**Chi²**	**pValue**
High	63%	9,27	0,00010
Intermediate + Low	32%		

Logistic regression analysis

Table 3. Logic regression analysis.

Coefficient Significance Tests					
Independent	**Regression Coefficient**	**Standard Error**	**Wald Z-value**	**Wald Prob**	**Odds Ratio**
Ki-67 (Continuous)	5,84051	1,83561	3,182	0,00146	343,95612
Biological Type - Luminal	-2,79292	1,17165	-2,384	0,01714	0,06124
Immunoscore Intermediate	-1,80059	0,77698	-2,317	0,02048	0,1652
Immunoscore Low	-1,99918	0,98812	-2,023	0,04305	0,13545
Tumor 2-5cm	2,17458	1,09489	1,986	0,04702	8,79853
Biological Type - TNBC	-3,2585	1,66519	-1,957	0,05037	0,03845
Stage 2B	-2,58973	1,5177	-1,706	0,08794	0,07504
Stage 2A	-2,01162	1,25775	-1,599	0,10974	0,13377
Intercept	2,63261	1,67336	1,573	0,11566	13,91008
Stage 3	-2,83108	1,84867	-1,531	0,12567	0,05895
ER Positive	-1,63232	1,72928	-0,944	0,34521	0,19548
Tumor > 5cm	-1,37975	1,78558	-0,773	0,43969	0,25164
PR Positive	-0,85124	1,1142	-0,764	0,44487	0,42688

Conclusions

- Ki-67, Biological type, Immunoscore® and tumor size are independent prognostic factors of pCR in patients with early breast cancer undergoing neoadjuvant chemotherapy.

Median cell density in patients with pCR vs non-pCR patients

Figure 10. Median cell density in patients with pCR vs non-pCR patients.

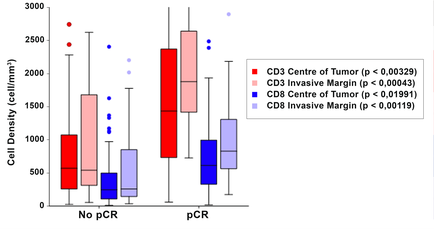


Table 4. Median cell density in patients with pCR vs non-pCR patients.

Median cell density in patients with pCR vs non-pCR patients				
	Outcome	**Median**	**CI (95,0%)**	**p-value**
CD3 Centre of Tumor	No pCR	567,559	358,83 - 753,29	0,00329
	pCR	1432,01	1103,19 - 1900	
CD3 Invasive Margin	No pCR	540,828	431,97 - 1211,749	0,00043
	pCR	1877,745	1430,597 - 2418,445	
CD8 Centre of Tumor	No pCR	246,0505	154,086 - 307,483	0,01991
	pCR	614,485	450,177 - 749,512	
CD8 Invasive Margin	No pCR	255,148	175,811 - 425,343	0,00119
	pCR	827,267	643,216 - 1189,143	

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