Tumor-infiltrating lymphocytes (TILs) in early breast cancer patients: High CD3+, CD8+, and Immunoscore® are associated with a pathological Complete Response

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Background

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 tumour and lymph nodes was made using bi-dimensional caliper measurements of the primary tumour and axillary nodes.
- Sonographical assessments of the primary tumour 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 tumour 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.
- 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 tumour tissue samples were analyzed by immunohistochemistry for density (cells/mm^3) 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 tumour 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 tumours with invasive margin and was adapted when no invasion was identified on the specimen.

Figure 1. Immunoscore® Assessment.

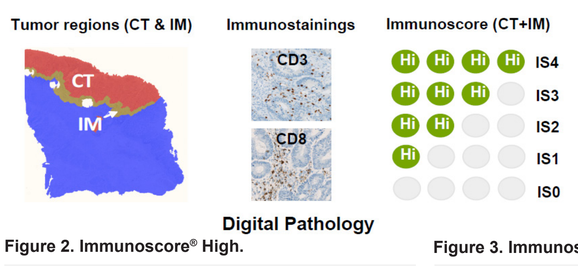


Figure 2. Immunoscore® High.

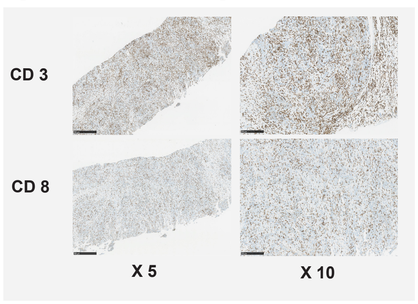


Figure 3. Immunoscore® Low.

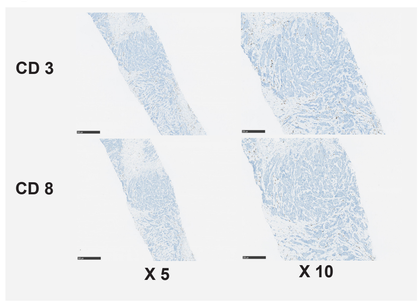
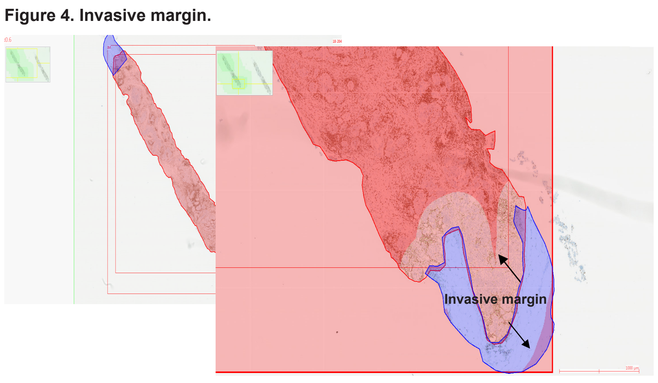


Figure 4. Invasive margin.



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.
- The Mann Whitney U-test was used to compare the cell density between TNBC and Non-TNBC patients.
- 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%
Tumour 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%
3	35	34%
4	3	3%

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/mm^3	**Range Cells/mm^3**
CD3 centre of tumour	884	25-5771
CD3 invasive margin	1409	53-6197
CD8 centre of tumour	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%

Results

T-Cell densities compare between TNBC vs Non-TNBC patients

Figure 5. CD3 - Centre of Tumour.

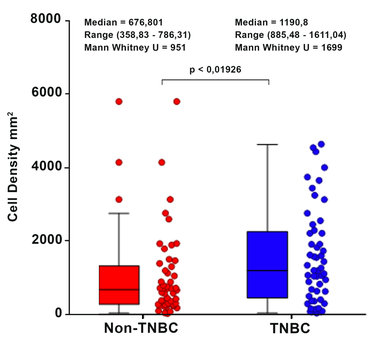


Figure 6. CD3 - Invasive Margin.

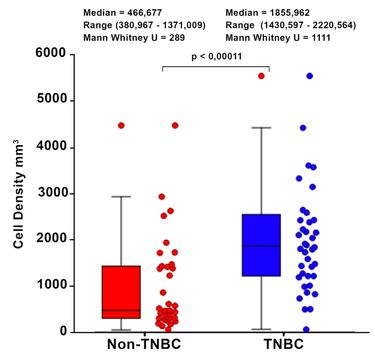


Figure 7. CD8 - Centre of Tumour.

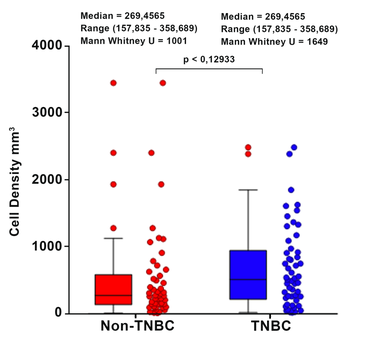
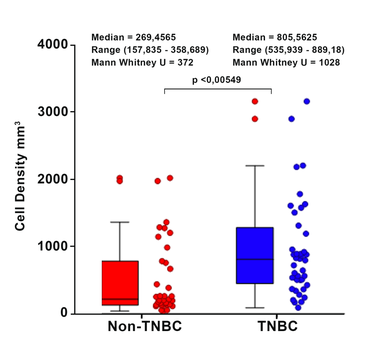


Figure 8. CD8 - Invasive Margin.



ROC Curves

Figure 9. ROC Curve – CD3-Centre of Tumour.

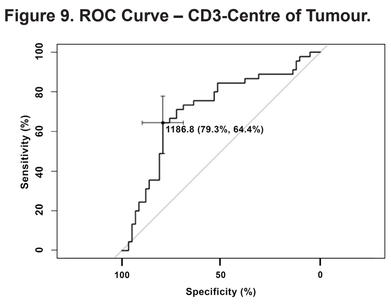


Figure 10. ROC Curve – CD8 – Centre of Tumour.

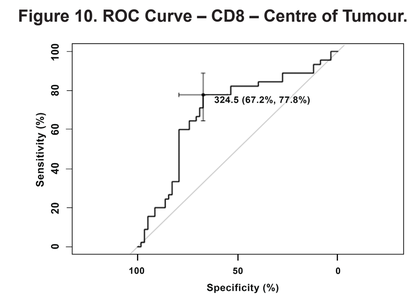


Figure 11. ROC Curve – CD3 – Invasive Margin.

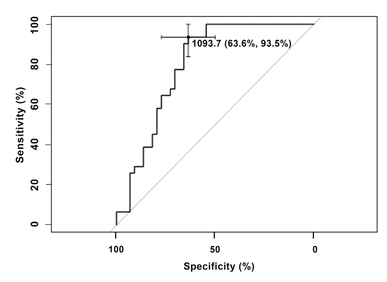


Figure 12. ROC Curve – CD8 – Invasive Margin.

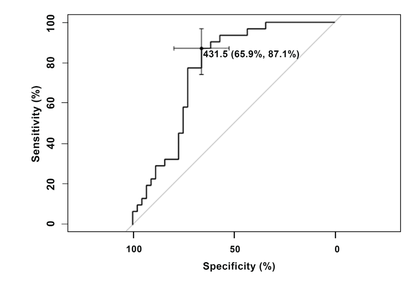
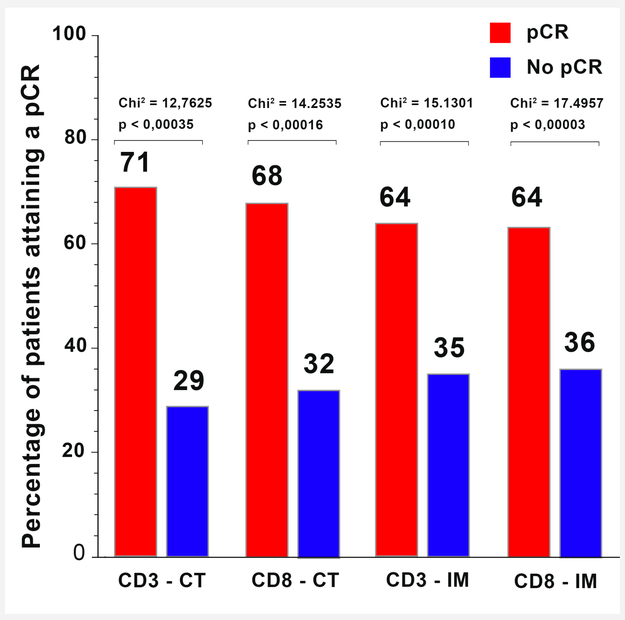


Figure 13. Percentage of patients attaining a pCR.



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%		
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%		

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

Figure 14. Median cell density in patients with pCR vs non-pCR patients.

Table 2. Median cell density in patients with pCR vs non-pCR patients.

	Outcome	Median	CI (95,0%)	p-value
CD3 Centre of Tumour	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 Tumour	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	

Conclusions

- These results revealed a significant prognostic role for the spatial distributions of the CD3+, and CD8+ lymphocytes, as well as the ISCR in relation to pCR following neo-adjuvant chemotherapy

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