Tumor infiltrating lymphocytes in early breast cancer: High levels of CD3, CD8 cells and Immunoscore® are associated with pathological CR and time to progression in patients undergoing neo-adjuvant chemotherapy.

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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/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).

Figure 1. Immunoscore® Assessment.

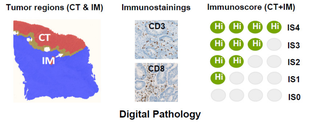


Figure 2. Immunoscore® High.

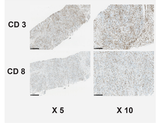


Figure 3. Immunoscore® Low.

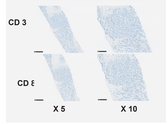
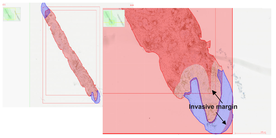


Figure 4. Invasive margin.



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.

Percentage of patients with cell density below/over 1200 mm² (Centre of Tumour)	
CD3 CT ≥ 1200	40%
CD3 CT < 1200	60%
Percentage of patients with cell density below/over 1100 mm² (Invasive Margin)	
CD3 IM ≥ 1100	57%
CD3 IM < 1100	43%
Percentage of patients with cell density below/over 300 mm² (Centre of Tumour)	
CD3 CD8 ≥ 300	55%
CD3 CD8 < 300	45%
Percentage of patients with cell density below/over 1100 mm² (Invasive Margin)	
CD8 IM ≥ 1100	30%
CD8 IM < 1100	70%

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

Figure 5. CD3 - Centre of Tumour.

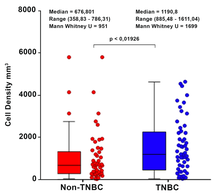


Figure 6. CD3 - Invasive Margin.

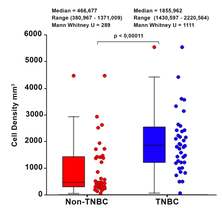


Figure 7. CD8 - Centre of Tumour.

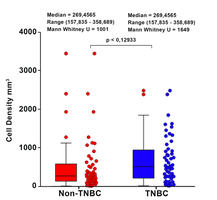


Figure 8. CD8 - Invasive Margin.

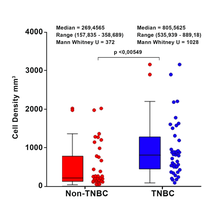


Figure 9. Response to Neo-Adjuvant.

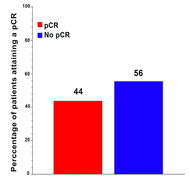
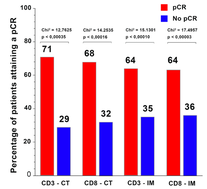


Figure 10. Percentage of patients attaining a pCR.



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%		

Results

Figure 11. ROC Curve: CD3 – Centre of Tumour.

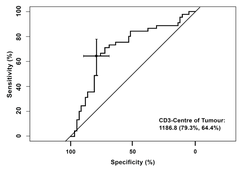


Figure 12. ROC Curve: CD8 – Centre of Tumour.

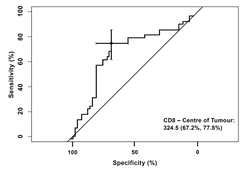


Figure 13. ROC Curve: CD3 – Invasive Margin.

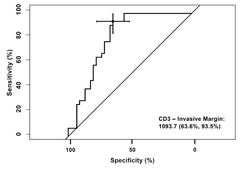
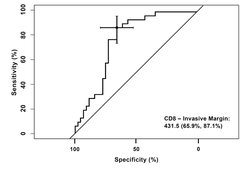


Figure 14. ROC Curve: CD8 – Invasive Margin.



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

Figure 15. Median cell density in patients with pCR vs non-pCR patients.

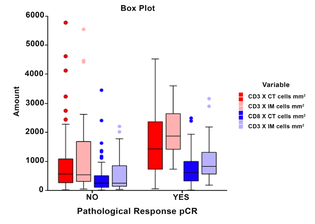


Table 3. Median cell density in patients with pCR vs non-pCR patients.

Median cell density in patients with pCR vs non-pCR patients				
	Outcome	**Median**	**CI (95,0%)**	**p-value**
CD3 Centre of 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	

Figure 16. Progression Free Survival by Immunoscore.

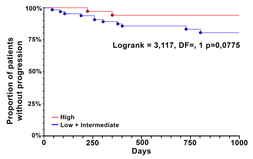


Figure 17. Progression Free Survival in TNBC subset by TIL's.

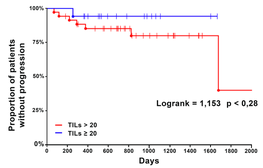


Figure 18. Progression Free Survival (PFS) CD8 CT. Progression Free Survival (PFS) by CD8 CT.

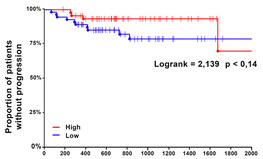


Figure 19. Progression Free Survival (PFS) CD8 IM. Progression Free Survival (PFS) by CD8 IM.

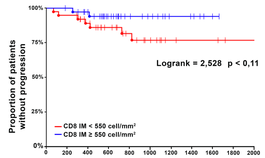


Figure 20. Progression Free Survival (PFS) CD3 IM. Progression Free Survival (PFS) by CD3 IM.

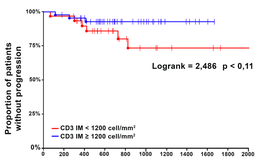
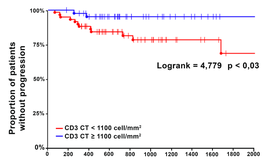


Figure 21. Progression Free Survival (PFS) CT3 CT. Progression Free Survival (PFS) by CD3 CT.



Logistic regression analysis

Table 4. 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	343,96612
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
Tumour 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
Tumour > 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 tumour size are independent prognostic factors of pCR in patients with early breast cancer undergoing neoadjuvant chemotherapy.
- Low CD3 and CD8 in the CT and IM is associated with a decreased time to progression in early breast cancer patients, however, further follow-up is required.

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