Correlations between tumor-infiltrating lymphocytes, CD3, CD8 cells, and Immunoscore®, with pathological CR and time to progression in triple-negative breast cancer patients undergoing neoadjuvant chemotherapy.

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Background

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- High levels of stromal tumor-infiltrating lymphocytes (TILs) have been associated with better prognosis in early triple-negative breast cancer (TNBC).
- The Immunoscore® (IS) is a prognostic tool, which categorizes the densities of spatially positioned CD3 and CD8 cells in both invasive margins (IM) and the center of the tumor (CT), yielding a five-tiered classification (0–4).
- High IS values have been reported to predict improved outcomes in colorectal cancer.
- Originally developed for colon cancer indication, it is intended to be widely used in solid cancer indications for diagnostic and prognostic purposes.
- As a first clinical study in breast cancer, we assessed the Immunoscore® in a cohort of 52 TNBC patients, that previously received neo-adjuvant chemotherapy.

Methods

Pathological and clinical assessment

- Clinical assessment of the primary tumor and lymph nodes were done 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.
- Patients who completed neo-adjuvant chemotherapy were eligible.
- Immunoscore® analysis was performed on 52 TNBC 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.

Outcome Assessment

- 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 analysis, we included 52 TNBC patients who completed neo-adjuvant 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).
- Additionally, we measured stromal TILs according to the International TILs Working Group.
- Digital pathology-dedicated software permitted the measurement of positive cell densities into interest area (core of the tumor and invasive margin).
- A pre-specified 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.
- Quantitative analysis of the immune cells was carried out using computer-assisted image analysis in different tumor locations for CD3 and CD8 T-cell markers.
- 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.

Statistical Analysis

- Descriptive statistics were used to analyze each variable.
- 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®.
- The relationship between various clinical-pathological factors and immune factors were analyzed by Chi² and Fischer exact test.
- The Pearson correlation test was used to analyze the association between various clinical pathological– and immune factors.
- The Mann Whitney U-test was used to compare the cell density.
- The log-rank test and the Kaplan Meyer methods were used to estimate relapse-free survival.
- NCSS software version 11 for Windows (USA) was used for statistical analyses.

Ethics Approval

- Ethics approval was obtained from Pharma-Ethics, Pretoria, South Africa.

Results

Patient Characteristics

Table 1. Patient Characteristics.

Patient Characteristics n=52	
Mean age	50 (27-84)
Nodal Status	
Yes	33 (63%)
No	19 (37%)
Stage	
Stage I	5 (9%)
Stage IIA	32 (62%)
Stage IIB	9 (17%)
Stage III	6 (12%)
Tumor Size	
T1	9 (17%)
T2	40 (77%)
T3	3 (6%)

Ki67	
Median (range)	45% (5-90%)
CD3 and CD8 Count (Median cells/mm²)	
CD3 centre of tumour	1190 (34 - 4614)
CD3 invasive margin	1855 (56 - 6190)
CD8 centre of tumour	508 (17 - 2485)
CD8 invasive margin	805 (90 - 3155)
Immunoscore	
IS = 0	4 (8%)
IS = 1	3 (5%)
IS = 2	20 (38%)
IS = 3	23 (45%)
IS = 4	2 (4%)
Stromal TILs (%)	
Median (range)	5% (0 - 60%)

Figure 1. T-Cell Densities for TNBC patients

Figure 1a. T-Cell Densities CD3 Centre of Tumor.

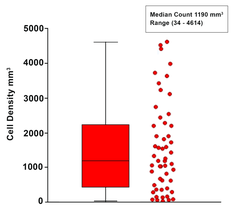


Figure 1b. T-Cell Densities CD3 Invasive Margin.

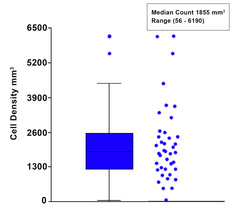


Figure 1c. T-Cell Densities CD8 Centre of Tumor.

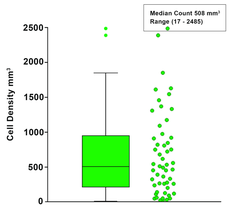


Figure 1d. T-Cell Densities CD8 Invasive Margin.

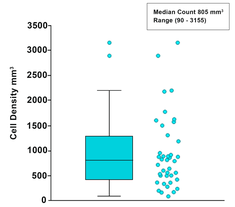


Figure 2. ROC curves.

Figure 2a. CD8 Centre of Tumor.

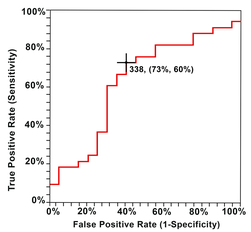


Figure 2b. CD3 Centre of Tumor.

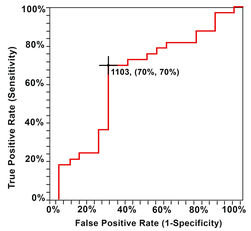


Figure 2c. CD3 Invasive Margin.

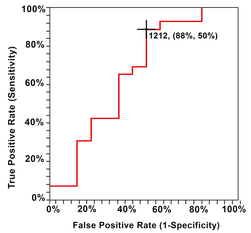


Figure 2d. CD8 Invasive Margin.

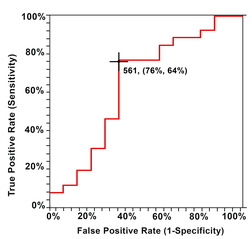
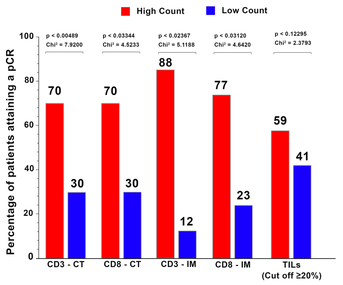


Figure 3. Percentage of patients attaining a pCR by cell density and TILs.



Univariate Analysis

Table 2. Univariate analysis – significant factors associated with pCR.

Nodal Status	pCR	p - value
Positive	42%	p<0.2362
Negative	74%	
Ki-67	**pCR**	**p - value**
<40%	33%	p<0.00235
≥40%	76%	
Cell Count - Centre of Tumor		
CD3 ≥ than 1100mm³	70%	p<0.00489
CD3 < than 1100mm³	30%	
CD8 ≥ than 400mm³	70%	p<0.03344
CD8 < than 400mm³	30%	
Cell Count - Tumor Margin		
CD3 IM ≥ than 1200mm³	88%	p<0.002367
CD3 IM < than 1200mm³	12%	
CD8 IM ≥ than 550mm³	77%	p<0.03
CD8 IM < than 550mm³	23%	

Table 3. Immunoscore and TILs showed numerical difference that did not, however, reach a statistical significance.

Immunoscore	pCR	p - value
High (IS-3 & IS-4)	73%	p<0.111
Intermediate (IS-2)	55%	
Low (IS-0 & IS-1)	43%	
TILs	**pCR**	**p - value**
TILs ≥ 20%	76%	p<0.12295
TILs < 20%	54%	

Figure 4. Response to neo-adjuvant chemotherapy for all patients.

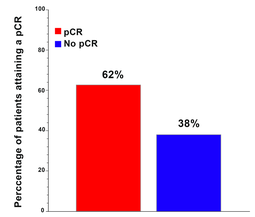


Figure 5. Median cell density in patients with pCR vs patients with non pCR.

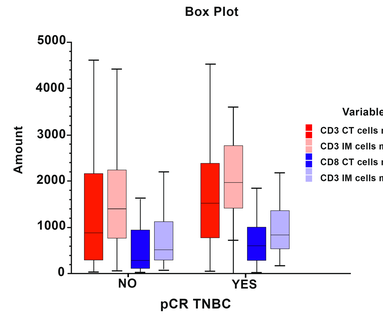


Figure 6. Correlations between stromal TILs, CD3 and CD8.

Figure 6a. Correlation between TILs and CD3 Centre f Tumor.

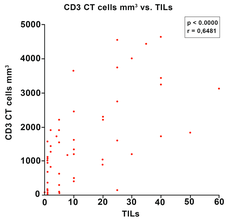


Figure 6b. Correlation between TILs and CD3 Invasive Margin.

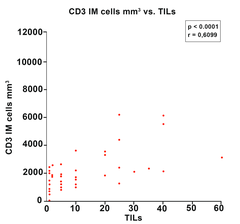


Figure 6c. Correlation between TILs and CD8 Centre of Tumor.

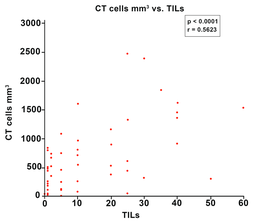


Figure 6d. Correlation between TILs and CD8 Invasive Margin.

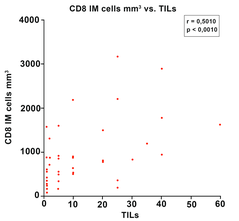


Figure 7. Time to progression plots.

Figure 7a. Time to progression between patients attaining a pCR and patients not attaining a pCR.

The median time to progression (TTP) of patients attaining a pCR was 1600 days compared to those who did attain a pCR with a median PFS not yet reached, but exceeding 1800 days.

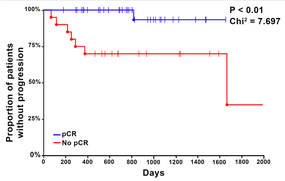


Figure 7b. Time to progression between patients attaining with Ki-67 < 40% and patients with Ki-67 ≥ 40%.

The median time to progression (TTP) of patients with Ki-67 < 40% was 1700 days compared to those with Ki-67 ≥ 40% where TTP have not yet been reached.

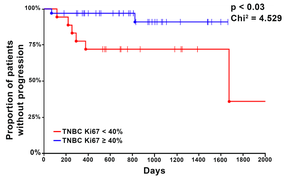


Figure 7c. Time to progression for patients attaining with CD3 CT < 1100mm³ and patients with CD3 CT ≥ 1100mm³.

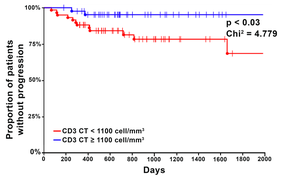
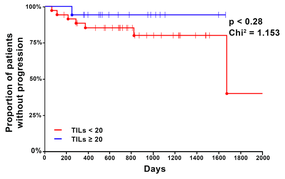


Fig 7d. Time to progression by TILs.



Conclusions

- This exploratory study shows that analyzing CD3 and CD8 cells in the center of the tumor and invasive margin might be more useful than examining TILs for predicting pCR in TNBC patients.
- Further prospective, well-designed, adequately powered studies are required to confirm these findings.

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