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The Medical Oncology Centre of Rosebank
Personalised Cancer Care

Tumour 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 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/mm³) 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.

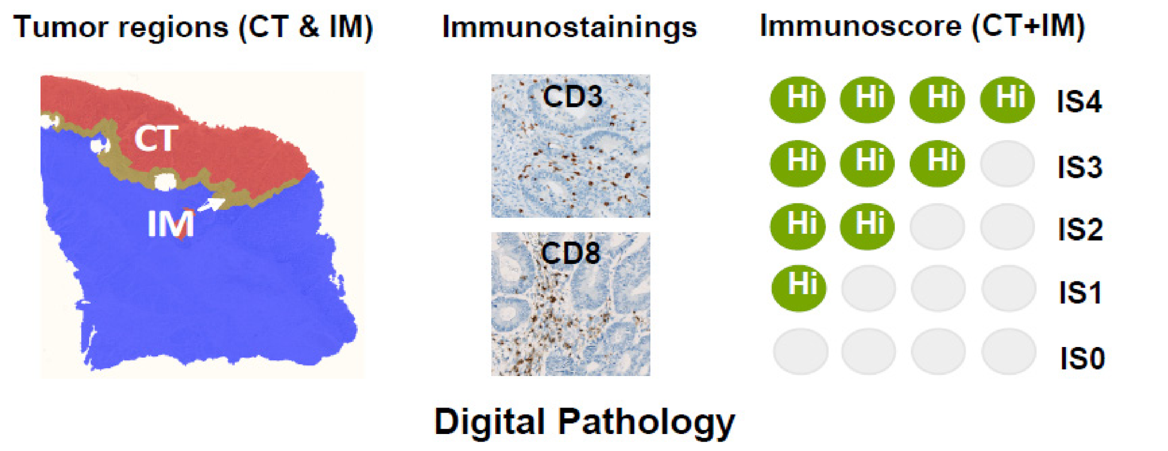

Tumor regions (CT & IM)
CT
IM
Immunostainings
CD3
CD8
Immunoscore (CT+IM)
Hi Hi Hi Hi IS4
Hi Hi Hi IS3
Hi Hi IS2
Hi IS1
IS0
Digital Pathology

Figure 2. Immunoscore® High.

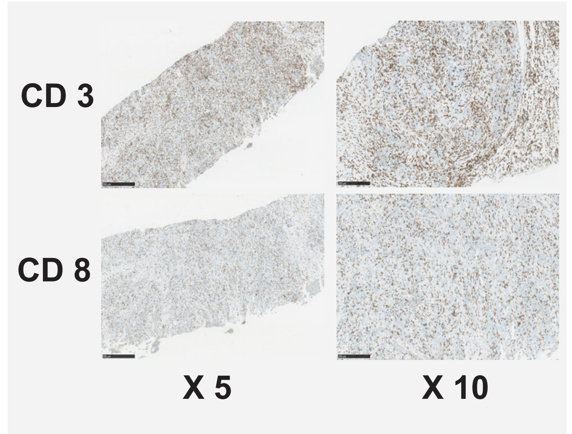

CD 3
CD 8
X 5
X 10

Figure 3. Immunoscore® Low.

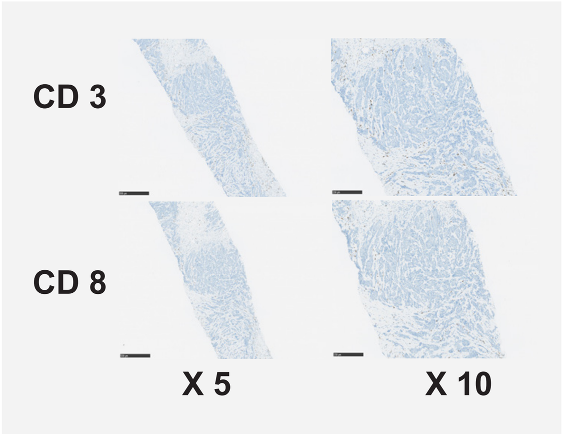
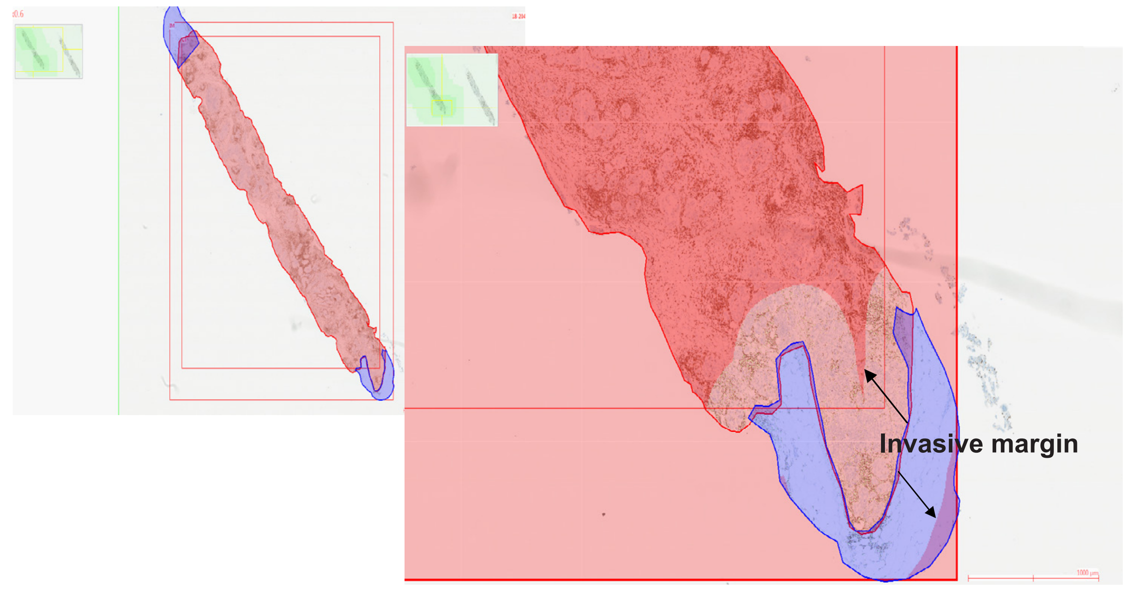

CD 3
CD 8
X 5
X 10

Figure 4. Invasive margin.


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%
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/ mm³	Range Cells/ mm³
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

ROC Curves for Prediction of pCR

Figure 5. ROC Curve – CD3-Centre of Tumour.

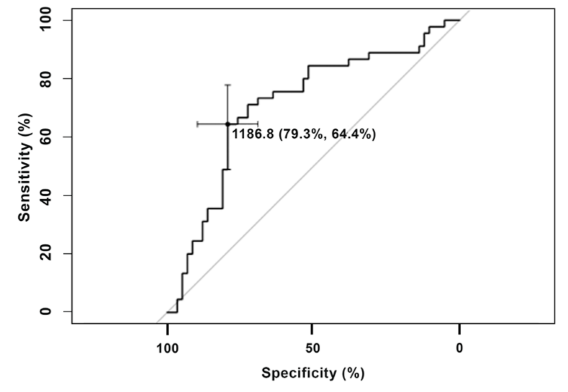

1186.8 (79.3%, 64.4%)
Sensitivity (%)
Specificity (%)

Figure 6. ROC Curve – CD8 – Centre of Tumour.

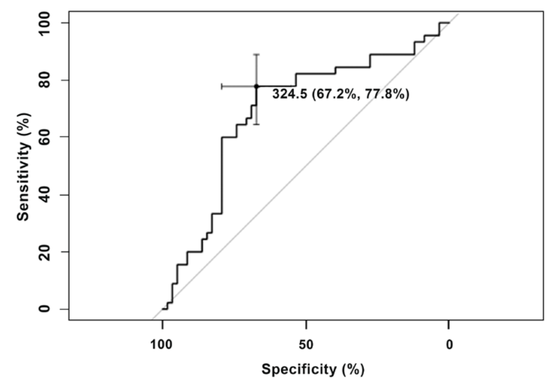

324.5 (67.2%, 77.8%)
Sensitivity (%)
Specificity (%)

Figure 7. ROC Curve – CD3 – Invasive Margin.

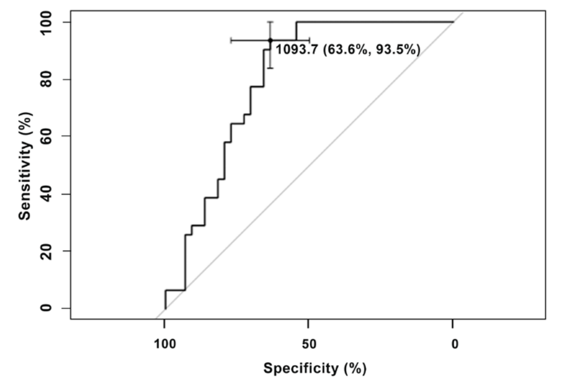

1093.7 (63.6%, 93.5%)
Sensitivity (%)
Specificity (%)

Figure 8. ROC Curve – CD8 – Invasive Margin.

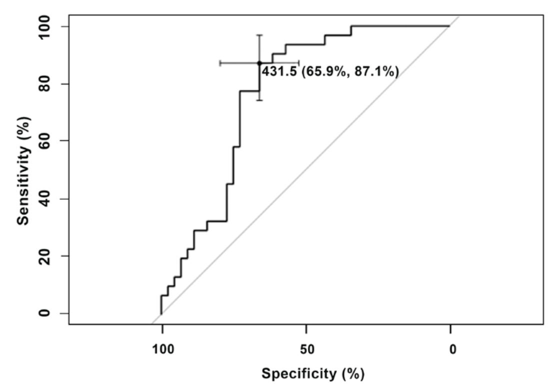

431.5 (65.9%, 87.1%)
Sensitivity (%)
Specificity (%)

Figure 9. Percentage of patients with cell density below/over 1200 mm³ (Centre of Tumour).

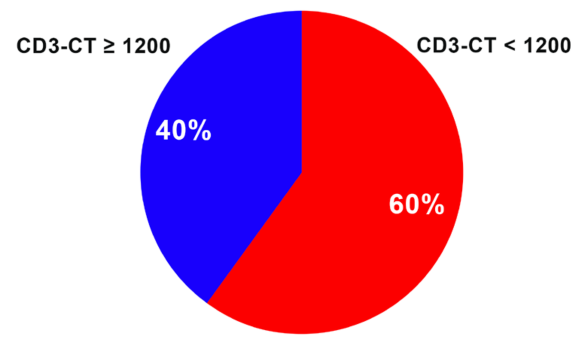

CD3-CT ≥ 1200
CD3-CT < 1200
40%
60%

Figure 10. Percentage of patients with cell density below/over 1100 mm³ (Invasive Margin).

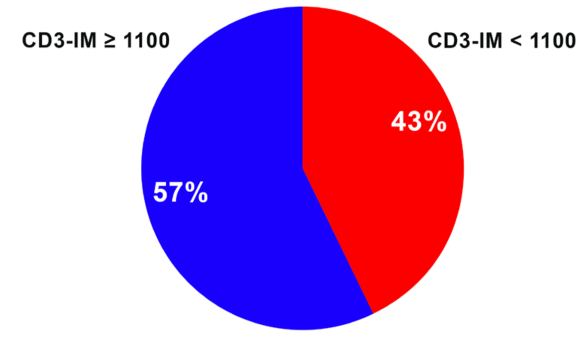

CD3-IM ≥ 1100
CD3-IM < 1100
57%
43%

Figure 11. Percentage of patients with cell density below/over 300 mm³ (Centre of Tumour).

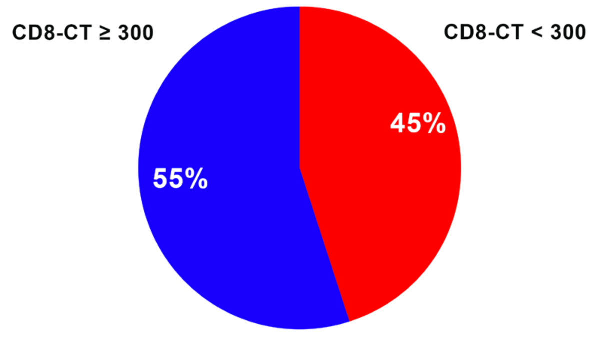
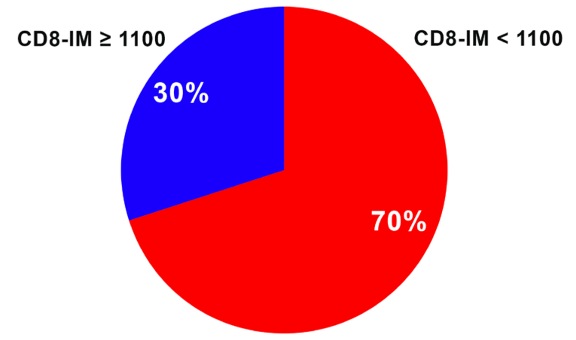

CD8-CT ≥ 300
CD8-CT < 300
55%
45%

Figure 12. Percentage of patients with cell density below/over 300 mm³ (Centre of Tumour).


CD8-IM ≥ 1100
CD8-IM < 1100
30%
70%

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

Figure 13a. CD3 - Centre of Tumour.

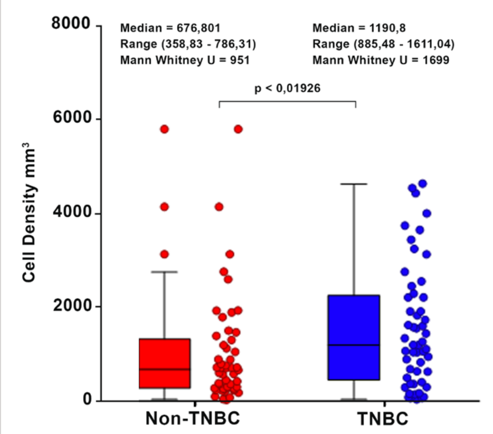

Median = 676,801
Range (358,83 - 786,31)
Mann Whitney U = 951
Median = 1190,8
Range (885,48 - 1611,04)
Mann Whitney U = 1699
p < 0,01926
Cell Density mm³
Non-TNBC
TNBC

Figure 13b. CD3 - Invasive Margin.

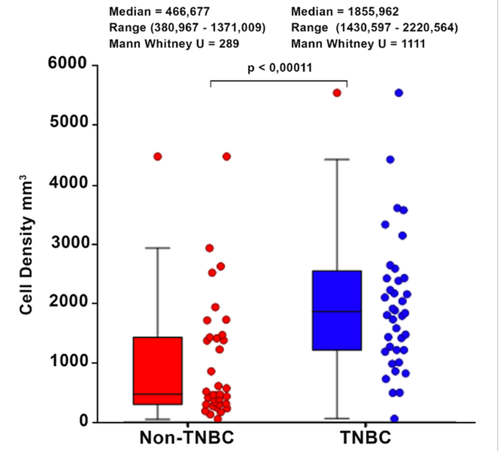

Median = 466,677
Range (380,967 - 1371,009)
Mann Whitney U = 289
Median = 1855,962
Range (1430,597 - 2220,564)
Mann Whitney U = 1111
p < 0,00011
Cell Density mm³
Non-TNBC
TNBC

Figure 13c. CD8 - Centre of Tumour.

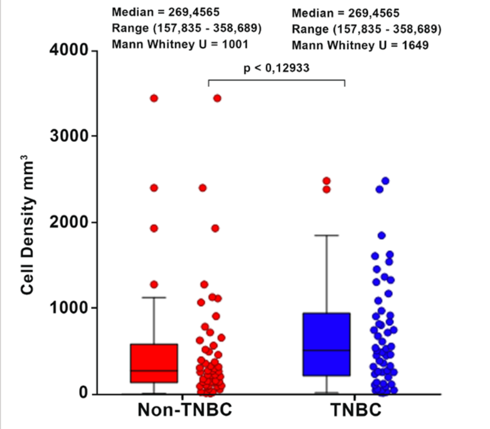
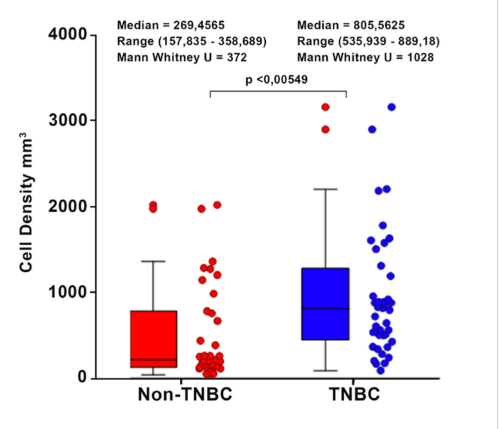

Median = 269,4565
Range (157,835 - 358,689)
Mann Whitney U = 1001
Median = 269,4565
Range (157,835 - 358,689)
Mann Whitney U = 1649
p < 0,12933
Cell Density mm³
Non-TNBC
TNBC

Figure 13d. CD8 - Invasive Margin.


Median = 269,4565
Range (157,835 - 358,689)
Mann Whitney U = 372
Median = 805,5625
Range (535,939 - 889,18)
Mann Whitney U = 1028
p <0,00549
Cell Density mm³
Non-TNBC
TNBC

Univariate Analysis

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

	pCR	Chi²	pValue
Stage			
1	67%	9,03	0.02885
2A	51%		
2B	42%		
3	16%		
ER			
Positive	18%	21.80	0.00001
Negative	64%		
PR			
Positive	13%	22.81	0.00001
Negative	61%		
HER2			
Postive	67%	0.3531	0.55237
Negative	51%		
Molecular type			
Luminal	9%	23,03	0,00001
HER2 Positive	50%		
TNBC	62%		
Ki-67			
≥ 40%	57%	13,84	0,00099
15-39%	41%		
< 15%	0%		
Immunoscore®			
High	63%	9,99	0,00674
Intermediate	35%		
Low	23%		
Immunoscore®			
High	63%	9,27	0,00010
Intermediate + Low	32%		

Figure 14. Response to Neo-Adjuvant Chemotherapy.

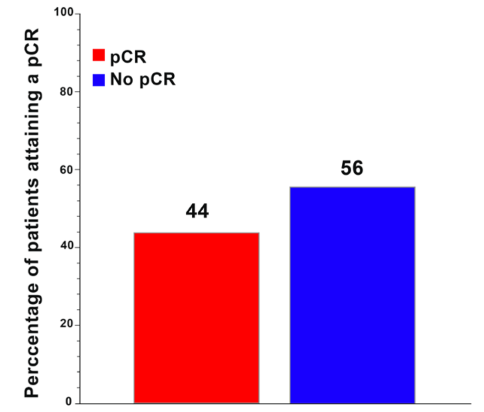
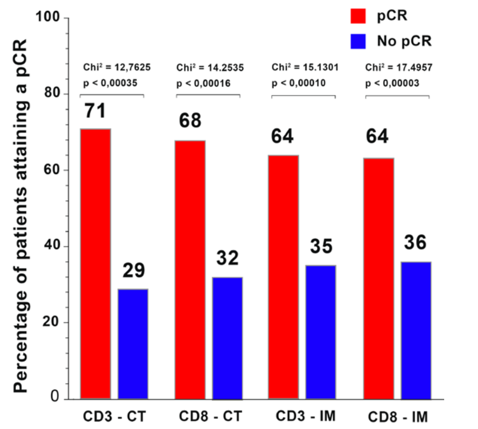

pCR
No pCR
44
56
Perccentage of patients attaining a pCR

Figure 15. Percentage of patients attaining a pCR.


pCR
No pCR
Chi² = 12,7625 p < 0,00035
Chi² = 14.2535 p < 0,00016
Chi² = 15.1301 p < 0,00010
Chi² = 17.4957 p < 0,00003
71
29
68
32
64
35
64
36
Percentage of patients attaining a pCR
CD3 - CT
CD8 - CT
CD3 - IM
CD8 - IM

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

Figure 16. Median cell density in patients with pCR vs non-pCR patients.

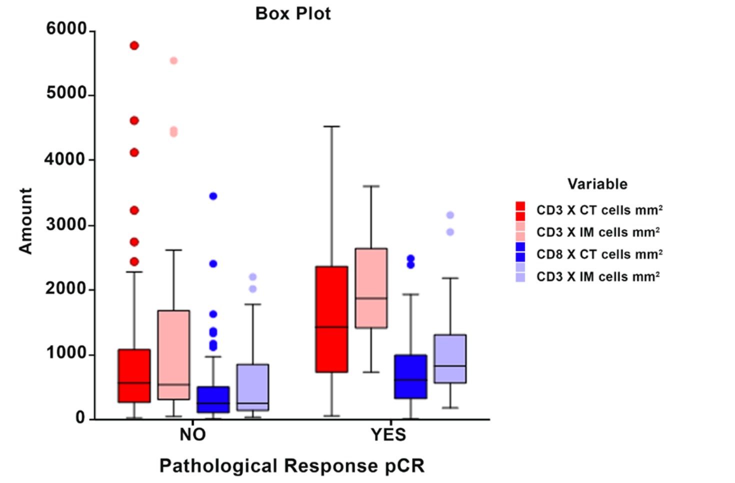

Box Plot
Amount
Variable
CD3 X CT cells mm²
CD3 X IM cells mm²
CD8 X CT cells mm²
CD3 X IM cells mm²
NO
YES
Pathological Response pCR

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	

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	343,95612
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
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.

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