TROP-2 expression in triple-negative breast cancer: correlations with tumor-infiltrating lymphocytes, histological subtypes and survival

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Abstract

Background

Limited data exist regarding the associations between TROP-2 protein expression, clinicopathological characteristics, and outcome in triple-negative breast cancer (TNBC).

Methods

TROP-2 expression was determined for patients diagnosed with TNBC between 2000-2017 by immunohistochemistry (IHC) (ab227689, Abcam) on whole slide tumor sections, and assessed as continuous and categorical variables (H-score high, 201-300, medium 100-200 and low <100). Associations between TROP-2 expression and age, BMI, tumor grade and size, lymphovascular invasion (LVI), ductal carcinoma in situ (DCIS) presence, nodal status, stromal tumor-infiltrating lymphocytes (sTILs), androgen receptor (AR), standardized mitotic index (SMI), and outcome were assessed.

Results

We included 685 patients with a median age at diagnosis of 54y (range 22-90y). TROP-2 expression was high, medium and low in 97 (16.5%), 149 (25.3%) and 343 (58.2%) of patients, respectively. The presence of LVI, associated DCIS, nodal involvement, apocrine histology and AR expression were correlated with higher TROP-2 levels. TROP-2 was not associated with pathological complete response (pCR). There were no associations between TROP-2 expression and time-to-event outcomes.

Conclusions

In stage 1-3 TNBC, higher TROP-2 expression was correlated with apocrine histology, higher AR expression, presence of DCIS, LVI, and nodal involvement. There was no correlation between TROP-2 expression and sTILs or outcome.

Introduction

Patients with triple-negative breast cancer (TNBC) account for 10–15% of all breast cancer cases [1]. Their tumors lack both expression of estrogen and progesterone receptors and overexpression of the human epithelial growth factor (HER2) receptor, rendering them non-eligible for endocrine or traditional HER2-targeted therapy. These cancers generally have a worse prognosis and higher recurrence rates within the first 3 years after diagnosis when compared to the other subtypes. The identification of reliable prognostic and predictive factors in TNBC remains an unmet medical need.

Although TNBC is an exclusion diagnosis, consisting of a heterogeneous breast cancer subtype with variable morphology and biology, it is currently treated in a similar way regardless of further differentiation [2]. The intrinsic molecular subtype classifications by Lehmann et al. and Burstein et al. have characterized luminal AR (LAR), mesenchymal-like, immunomodulatory and basal-like subtypes on

the transcriptomic level [3–5]. In some cases, the histological subtype can infer molecular subtype: e.g., apocrine carcinomas are often in the LAR group, while metaplastic carcinomas fit in the mesenchymal group and breast carcinomas with medullary features ($BC^{medullary}$) fall within the immunomodulatory group [6–9]. Each of these subtypes have different prognoses and may require different therapeutic strategies. As each subtype has a different response to treatment and clinical outcome, tailored treatment for patients with TNBC is fundamental.

AR, which is expressed in 10–50% of TNBC, stimulates tumor cell growth in TNBC [10, 11]. sTILs, which can be considered surrogate markers for an anti-tumor immune response, are more prevalent in TNBC compared to other breast cancer subtypes [12]. sTILs have been shown to provide robust prognostic value in early and loco-regionally advanced TNBC, both in patients with and without (neo) adjuvant chemotherapy, predicting pCR after NACT [12–18].

Antibody-drug conjugates (ADCs) have emerged as a new promising treatment option for solid tumors. In the treatment landscape of advanced TNBC, Sacituzumab Govitecan (SG) is now approved by FDA (Food and Drug Administration) and EMA (European Medicine Agency) for the treatment of advanced TNBC after 2 prior regimens, of which at least one for metastatic disease. SG is an ADC targeting TROP-2 (trophoblast cell-surface antigen-2), linked by a cleavable linker to SN38, a topoisomerase-1 inhibitor and the active metabolite of Irinotecan [19]. TROP-2 is an interesting but poorly explored tumor-associated cell-surface antigen, which has been associated with increased tumor aggressiveness and metastatic potential when overexpressed in cancer cells [20–22]. TROP-2, a transmembrane calcium protein belonging to the EpCAM (epithelial cell adhesion molecule) family, is encoded by the tumor-associated calcium signal transducer 2 (TACSTD2) gene on chromosome 1p32. TROP-2 is expressed at high levels by normal human multistratified epithelia and trophoblast cells. Overexpression can be present in several solid tumors, including TNBC. The precise role of TROP-2 in invasion and metastasis is poorly understood, but seems to differ between different cancer types and be modulated by different pathways [23].

TROP-2 expression has been investigated to a limited extend in breast cancer regardless of subtype and more specifically in patients with advanced TNBC [24]. The characteristics and prognostic value of this marker have not been thoroughly evaluated in patients with early and loco-regionally advanced TNBC. Therefore, our aim was to evaluate the expression of TROP-2 in early and loco-regionally advanced TNBC and investigate potential associations with clinical-pathological characteristics and survival outcomes.

Methods

Patients

Patients with primary diagnosis of stage 1–3 TNBC between 1st January 2000 and 31st December 2017 at UHL were included. Selection for the presented analysis required availability of information on TROP-2

expression, sTILs, and AR-IHC. We excluded patients with metastatic disease at time of diagnosis, a synchronous or metachronous non-TNBC or other concurrent metastasized tumors.

Patient and tumor characteristics were retrieved from the clinical database and the medical records (age, body mass index (BMI), family history, menopausal status, tumor size, histological subtype, grade of differentiation, section margins, presence of DCIS, LVI, lymph node involvement, pCR, data on relapse, survival and cause of death).

Histology

A 5µm thick freshly cut slide of formalin-fixed, paraffin-embedded (FFPE) tumor blocks was used to review the tumor type on H&E according to recent literature, measure TILs and assess Standardize Mitotic Index (SMI). Two pathologists (G.F. and L.W.) scored TILs on resection specimens according to the recommendations by the international TILs working group [25]. The intra-class correlation coefficient and Bland-Altman analysis was done to assess the inter-rater variability. Low sTILs were defined as < 30%, intermediate 30–49% and high as ≥ 50% [13, 26].

SMI was counted manually on a representative H&E section by two pathologists (L.W. and G.F.), according to the publication of Collan et al [27]. SMI was assessed with a correction for the proportion of tumor cell nests expressed as % of the area occupied for each microscopic field according to Haapasalo et al [28]. The cutoff for positivity of SMI was based on the median value.

We then assessed protein-level expression of TROP-2 and AR by IHC on the Bond Automatic IHC Stainer (Leica Biosystems). A 1:150 dilution of rabbit monoclonal anti-TROP-2 antibody (Ab227689 by Abcam) was used, pre-treated for 20 minutes at pH 9, and incubated for 30 minutes. Expression of TROP-2 antibody was scored semiquantitatively using the H-score. We subdivided the scores into categories: low scores < 100, medium scores 100–200, and high scores 201–300. A 1:100 dilution of monoclonal mouse anti-human AR (clone AR411, DAKO), was used. Two pathologists (L.W. and G.F.) scored the stains, using both the percentage of positive cells (0-100%) and the intensity of the nuclear staining pattern (0–3). For statistical analysis, we used both 1% and 10% as cut-off scores for positivity.

Statistical analysis

Associations between continuous variables were assessed by the Spearman correlation coefficient, and group differences by the Kruskal-Wallis test for multiple groups or Mann-Whitney U test for two groups. Associations between categorical variables were assessed by means of the Fisher exact test.

BCSS (breast cancer-specific survival) and DRFI (distant recurrence-free interval) were estimated using the cumulative incidence function and IDFS (invasive disease-free survival) using Kaplan- Meier estimates [29]. BCSS (breast cancer specific survival) is defined as the time between diagnosis and death of breast cancer. Death of other causes is considered as a competing event. Patients alive are censored at last follow-up. DRFI (distant recurrence free interval) is defined as the time between diagnosis and metastasis or death of breast cancer. Death of other causes is considered as a competing event. Patients

alive without metastasis are censored at last follow-up. IDFS (invasive disease free survival) is defined as the time between diagnosis and any relapse or death of any cause. Patients alive without relapse are censored at last follow-up.

Cox proportional hazards models were used to assess the association between TROP-2 expression and outcome. Results are presented as hazard ratios (HR) with 95% confidence intervals. Analyses were performed using SAS software (version 9.4 of the SAS System for Windows).

Results

Patients

A total of 685 patients were included for which TROP-2, AR-IHC and sTILs were available. Median age at diagnosis was 54 years (range 22-90y) (Table 1). As expected, most of the tumors in our cohort were invasive breast carcinoma of no special type (IBC-NST) (79.4%), while other histological subtypes were less frequent: $BC^{medullary}$ (5.8%), metaplastic carcinoma (5.0%), apocrine carcinoma (4.5%), other histology (3.5%), or mixed tumors (1.8%). Lymph node involvement and LVI was present in 36.2% and 23.4% of patients, respectively, while associated DCIS was observed in 62.8% of patients.

Table 1
Baseline patient- and tumor characteristics visualized for all patients and separated according to systemic treatment modality

Variable	Statistic	Upfront surgery	Neoadjuvant CT	No CT	Total
N	N	475	64	146	685
Age (y)	Mean	52.0	51.8	72.1	56.3
	Median	51.0	51.0	75.0	54.0
	Range	(22.0; 85.0)	(26.0; 78.0)	(37.0; 90.0)	(22.0; 90.0)
Menopausal status					
pre/perimenopause	n/N (%)	232/466 (49.8%)	30/59 (50.9%)	12/145 (8.3%)	274/670 (40.9%)
postmenopause	n/N (%)	234/466 (50.2%)	29/59 (49.1%)	133/145 (91.7%)	396/670 (59.1%)
BMI (kg/m²)	Mean	25.6	26.0	25.3	25.5
	Median	24.7	24.9	24.4	24.7
	Range	(17.7; 46.0)	(18.6; 39.4)	(14.2; 48.1)	(14.20; 48.1)
T-stage					
T1	n/N (%)	207/475 (43.6%)	7/64 (10.9%)	74/146 (50.7%)	288/685 (42.0%)
T2	n/N (%)	239/475 (50.3%)	26/64 (40.7%)	65/146 (44.5%)	330/685 (48.2%)
T3	n/N (%)	29/475 (6.1%)	10/64 (15.6%)	5/146 (3.4%)	44/685 (6.4%)
T4	n/N (%)	0/475 (0.0%)	21/64 (32.8%)	2/146 (1.4%)	23/685 (3.4%)
N-stage					
N0	n/N (%)	307/474 (64.8%)	19/64 (29.7%)	110/145 (75.8%)	436/683 (63.8%)
N1	n/N (%)	134/474 (28.3%)	21/64 (32.8%)	25/145 (17.2%)	180/683 (26.4%)
N2	n/N (%)	29/474 (6.1%)	6/64 (9.4%)	6/145 (4.2%)	41/683 (6.0%)
N3	n/N (%)	4/474 (0.8%)	18/64 (28.1%)	4/145 (2.8%)	26/683 (3.8%)
N-stage					

Variable	Statistic	Upfront surgery	Neoadjuvant CT	No CT	Total
Negative	n/N (%)	307/474 (64.8%)	19/64 (29.7%)	110/145 (75.9%)	436/683 (63.8%)
Positive	n/N (%)	167/474 (35.2%)	45/64 (70.3%)	35/145 (24.1%)	247/683 (36.2%)
GRADE					
1	n/N (%)	1/475 (0.2%)	0/64 (0.0%)	8/146 (5.5%)	9/685 (1.3%)
2	n/N (%)	23/475 (4.8%)	11/64 (17.2%)	36/146 (24.7%)	70/685 (10.2%)
3	n/N (%)	451/475 (95.0%)	53/64 (82.8%)	102/146 (69.9%)	606/685 (88.5%)
DCIS					
No	n/N (%)	162/475 (34.1%)	39/64 (60.9%)	54/146 (37.0%)	255/685 (37.2%)
Yes	n/N (%)	313/475 (65.9%)	25/64 (39.1%)	92/146 (63.0%)	430/685 (62.8%)
LVI					
No	n/N (%)	306/402 (76.1%)	28/41 (68.3%)	94/116 (81.0%)	428/559 (76.6%)
Yes	n/N (%)	96/402 (23.9%)	13/41 (31.7%)	22/116 (19.0%)	131/559 (23.4%)
Histology					
IBC-NST	n/N (%)	386/475 (81.3%)	61/64 (95.3%)	97/146 (66.4%)	544/685 (79.4%)
Mixed	n/N (%)	6/475 (1.3%)	0/64 (0.00%)	6/146 (4.1%)	12/685 (1.8%)
Apocrine	n/N (%)	15/475 (3.2%)	2/64 (3.1%)	14/146 (9.6%)	31/685 (4.5%)
$BC^{medullary}$	n/N (%)	35/475 (7.4%)	0/64 (0.0%)	5/146 (3.4%)	40/685 (5.8%)
Metaplastic	n/N (%)	24/475 (5.1%)	1/64 (1.6%)	9/146 (6.2%)	34/685 (5.0%)
Other*	n/N (%)	9/475 (1.9%)	0/64 (0.0%)	15/146 (10.3%)	24/685 (3.5%)

Variable	Statistic	Upfront surgery	Neoadjuvant CT	No CT	Total

CT: chemotherapy; N0: no evidence of lymph node involvement; DCIS: ductal carcinoma in situ; LVI: lymphovascular invasion; IBC-NST: invasive breast carcinoma of no special type;

*Other histology (N = 24): N = 7 invasive lobular adenocarcinoma (ILA) N = 4 Pleomorphic ILA, N = 4 adenoid cystic, N = 3 micropapillary, N = 1 mucinous, N = 1 papillary, N = 1 secretory, N = 1 polymorphic, N = 1 myoepithelial, N = 1 small cell

After a median follow-up of 9.6 years, loco-regional and distant relapse, were observed in 5.1% and 17.5% of patients, respectively. Breast cancer-related deaths occurred in 16% of patients, with death due to other causes recorded in 9.5% of patients. Estimates for 2-, 5-, and 10-year IDFS, DRFI and BCSS are available in Supplementary Table 1.

IHC findings

TROP-2 staining showed membranous positivity, which in most cases was variable in intensity throughout the tumor (Fig. 1). In some cases, associated DCIS showed stronger expression of TROP-2 than the invasive carcinoma. Positive internal control was present in normal ductulo-lobular units and was rather weak in intensity. The mean H-score was 88 (range 0-300). Of the 589 patients with TROP-2 staining available, expression was high (H-score 201–300), medium (H-score 100–200) and low (H-score < 100) in 97 (16.5%), 149 (25.3%) and 343 (58.2%) patients, respectively (Table 2). Notably, 151 (25.6%) samples had absent or extremely low staining (H-score 0–10). All apocrine carcinomas showed an intermediate or high TROP-2 score (median H-score 175), while the metaplastic carcinomas showed a low or intermediate score (median H-score 70) ($p < .001$)).

Table 2
TROP-2 expression, AR expression, sTILs and SMI

Variable	Statistic	Upfront surgery	Neoadjuvant CT	No CT	Total
TROP-2 score	N	406	64	119	589
	Mean	96.2	92.5	57.5	88.0
	Median	80.0	100.0	20.0	70.0
	Range	(0.0; 300.0)	(0.00; 220.0)	(0.0; 300.0)	(0.0; 300.0)
TROP-2 categorical					
low H-score < 100	n/N (%)	226/406 (55.7%)	29/64 (45.3%)	88/119 (74.0%)	343/589 (58.2%)
medium H-score 100–200	n/N (%)	96/406 (23.7%)	28/64 (43.8%)	25/119 (21.0%)	149/589 (25.3%)
high H-score 201–300	n/N (%)	84/406 (20.7%)	7/64 (10.9%)	6/119 (5.0%)	97/589 (16.50%)
AR percentage	N	415	64	123	602
	Mean	11.4	16.5	25.6	14.9
	Median	0.0	0.0	0.0	0.0
	Range	(0.0; 100.0)	(0.0; 100.0)	(0.0; 100.0)	(0.0; 100.0)
AR (1% cutoff)					
Negative	n/N (%)	300/415 (72.3%)	42/64 (65.6%)	70/126 (55.6%)	412/605 (68.1%)
Positive	n/N (%)	115/415 (27.7%)	22/64 (34.4%)	56/126 (44.4%)	193/605 (31.9%)
AR (10% cutoff)					
Negative	n/N (%)	330/415 (79.5%)	46/64 (71.9%)	81/126 (64.3%)	457/605 (75.5%)
Positive	n/N (%)	85/415 (20.5%)	18/64 (28.1%)	45/126 (35.7%)	148/605 (24.5%)
sTILs score	N	416	64	126	606
	Mean	28.7	19.5	22.9	26.5
	Median	22.5	9.5	12.5	20.0
	Range	(0.0; 94.0)	(0.0; 86.0)	(0.0; 96.0)	(0.0; 96.0)
sTILs categorical					

Variable	Statistic	Upfront surgery	Neoadjuvant CT	No CT	Total
low (> 30)	n/N (%)	247/416 (59.4%)	48/64 (75.0%)	86/126 (68.3%)	381/606 (62.9%)
medium (30–49)	n/N (%)	86/416 (20.7%)	8/64 (12.5%)	15/126 (11.9%)	109/606 (18.0%)
high (> 50)	n/N (%)	83/416 (20.0%)	8/64 (12.5%)	25/126 (19.8%)	116/606 (19.1%)
SMI	N	408	0	49	457
	Mean	20.0	-	13.8	19.3
	Median	17.3	-	7.7	16.8
	Range	(1.0; 128.5)	-	(0.0; 66.3)	(0.0; 128.5)
SMI (binary)					
< Median (16.8)	n/N (%)	194/408 (47.6%)	-	35/49 (71.4%)	229/457 (50.1%)
≥ Median (16.8)	n/N (%)	214/408 (52.4%)	-	14/49 (28.6%)	228/457 (49.9%)

AR: androgen receptor; sTILs: stromal tumor-infiltrating lymphocytes; SMI: standardized mitotic index

With a 10%-cutoff, 148 (24.5%) of the cases were considered AR-positive, while based on the 1% cutoff, AR-positivity was observed in 193 cases (31.9%). All apocrine carcinomas were strongly AR-positive (Table 2).

Low, intermediate and high sTILs were observed in 62.9%, 18% and 19.1%, with a median score of 20 (range 0–96) (Table 2). The inter-rater reliability defined by intra-class correlation coefficient was 0.87 (95% C.I. 0.73–0.94) in 100 samples. We observed no significant difference between raters from the Bland-Altman analysis (p = 0.5), with a 95% level of agreement 95% lower limit of -26.4, and upper limit of + 25.9.

The median SMI was 16.8. Using this median threshold, 49.9% of patients had a high SMI, while 50.1% had a lower SMI.

Correlations

Higher TROP-2 expression was correlated with the presence of LVI (p = 0.006) and DCIS (p < .001), both as a continuous and categorical variable. TROP-2 expression was significantly associated with lymph node involvement when evaluated as a continuous score (p = 0.02) and a similar trend was observed for the categorical score. Higher TROP-2 expression was associated with higher continuous and categorical AR expression with a 10%-cutoff (continuous: ρ = 0.13, p = 0.002, categorical: p = 0.009) (Fig. 2). No

significant correlations were observed between TROP-2 expression and tumor size, grade, sTILs or SMI. Continuous higher TROP-2 expression showed a correlation with lower continuous BMI (ρ=-0.09, p = 0.03); however, this was not significant when BMI was evaluated as a categorical variable.

When evaluating TROP-2 expression as a continuous variable, we detected no significant differences in long-term time-to-event outcomes according to TROP-2 expression, although a trend towards improved IDFS, DRFI and BCSS for higher TROP-2 expression was observed. After adjustment for age, nodal status, tumor stage, grade, and continuous AR expression, the trend became significant for IDFS (HR 0.91 (95% C.I. 0.96-1.00); p = 0.049), but not for BCSS and DRFI. TROP-2 expression as a categorical variable was not significantly associated with the evaluated time-to-event outcomes in univariable or multivariable analyses. (Table 3)

Table 3
Univariable and Multivariable outcome analysis (continuous and categorical)

	Outcome	Predictor	Test	Hazard Ratio (95% CI)	P-value	N patients	N events
Univariable	**BCSS**	TROP2 continuous	+ 10 units	0.978 (0.953;1.003)	0.089	495	95
	DRFI	TROP2 continuous	+ 10 units	0.978 (0.954;1.002)	0.075	518	104
	IDFS	TROP2 continuous	+ 10 units	0.982 (0.964;1.000)	0.051	517	181
Multivariable	BCSS	TROP2 continuous	+ 10 units	0.983 (0.957;1.009)	0.205	492	94
	DRFI	TROP2 continuous	+ 10 units	0.982 (0.958;1.007)	0.165	514	102
	IDFS	TROP2 continuous	+ 10 units	0.981 (0.963;1.000)	0.049	513	178
Univariable	BCSS	TROP2 categorical	Global test	-	0.434	495	95
	DRFI	TROP2 categorical	Global test	-	0.415	518	104
	IDFS	TROP2 categorical	Global test	-	0.235	517	181
Multivariable	BCSS	TROP2 categorical	Global test	-	0.433	492	94
	DRFI	TROP2 categorical	Global test	-	0.472	514	102
	IDFS	TROP2 categorical	Global test	-	0.264	513	178

HR: hazard ratio, CI: confidence interval, HR>(<)1: increased(decreased) risk with increasing TROP2 level, corrected for: age, N-positive, T-stage, grade, AR percentage.

TROP-2 was not associated with pCR, which was documented in 22 out of 64 patients treated with NACT. There was no significant difference in TROP-2 expression when we compared expression on CNB before NACT, with expression on residual invasive cancer tissue following NACT and surgery ($p = 0.618$).

Higher TILs were associated with higher SMI ($\rho = 0.19$, $p < .001$), younger age ($\rho=-0.14$, $p < .001$), lower BMI ($\rho=-0.1$, $p = 0.01$), lower AR percentage ($\rho=-0.09$, $p = 0.03$), smaller tumor size ($p < .001$), higher tumor grade ($p < .001$) and absence of DCIS ($p < .001$). The highest TILs were observed in $BC^{medullary}$ (median 64), and lowest in the apocrine subtype (median 6) ($p < .001$).

Higher SMI was not correlated with outcome, but correlated with continuous TILs (ρ = 0.19, p < .001), higher grade (p < .001), and with decreasing continuous AR expression (ρ=-0.158, p < .001), age (ρ=-0.25, p < .001) and BMI (ρ=-0.10, p = 0.04).

Discussion

TROP-2 is an emerging biomarker which has raised therapeutic interest as target for ADCs, among which SG, that is currently being studied in a variety of cancer types. We investigated semiquantitative TROP-2 expression in patients with early and loco-regionally advanced TNBC treated in an academic hospital and correlated this with demographics and clinical-pathological characteristics, including sTILs, AR expression, histological subtype, and long-term outcomes.

We found TROP-2 expression in about 86% (n = 589/685) of the cases in our cohort; this large proportion of TROP-2-positivity is consistent with the currently reported literature [20]. Previous studies have shown higher levels of TROP-2 protein and gene (over) expression in TNBCs (88%) compared to other cancer types or other subtypes of breast cancer [20, 23, 30]. However, in advanced TNBC, the proportion of patients with high and medium TROP-2 expression according to the biomarker analysis from ASCENT was considerably higher when compared to those in our series [24]. This can be explained by differences in sensitivity and specificity of the different antibodies used in both studies and by potential differences in TROP-2 expression between the early and advanced TNBC settings.

A previous study by Ambrogi et al. in early breast cancers has shown a worse prognosis in cases with higher membranous expression of TROP-2 [20]. In our series, we could not demonstrate a significant association between TROP-2 expression and BCSS, DRFI or IDFS in univariable and multivariable analyses. A limited number of events warrant caution in the interpretation of the prognostic value of TROP-2 expression. Ambrogi et al. demonstrated differential prognostic value of membranous and cytoplasmic expression using two different antibodies, with cytoplasmic expression as favorable prognostic biomarker. The antibody used in our study stains both membranous and cytoplasmic TROP-2, which can potentially explain the absence of prognostic value for TROP-2 expression in our study.

The application of Trop-2 as a target for ADCs has already been clinically validated considering the successful trial results and approval of SG for patients with advanced TNBC [19]. Uncertainty remains regarding the role of Trop-2 IHC as predictive biomarker for the benefit of SG. The biomarker analysis of ASCENT showed improved outcomes for patients with SG compared to treatment of physician's choice in patients with high, medium and low TROP-2 expression. However, the numerical increase in outcome seemed higher in groups with high and medium TROP-2 expression [24]. Caution in interpretation is warranted, given small numbers precluded formal testing. Additional studies are needed to assess whether higher TROP-2 expression is predictive for better response to SG in advanced TNBC.

Our study showed a correlation of high TROP-2 expression with the presence of LVI and presence of nodal involvement both on the continuous and categorical TROP-2 scoring system. TROP-2 has been described to be involved in the PI3K/AKT pathway, which induces epithelial-mesenchymal transition

(EMT) [23, 30, 31]. Since metaplastic carcinomas show a high grade of EMT, we anticipated higher TROP-2 scores in this subtype. In all metaplastic carcinomas in our series, we found significantly lower TROP-2 expression compared to other subtypes. This suggests that TROP-2 overexpression is unlikely to be associated with EMT in primary early and loco-regionally advanced TNBC. Other studies have also found a possible role in angiogenesis [23, 32], which could potentially explain our observed association between LVI and high TROP-2 expression.

We observed AR-positivity in 31.9% of cases with a 1%-cutoff and 24.5% with a 10%- cutoff. These results are in line with previous studies, which suggest AR-positivity in 10–50% of TNBC [11]. All apocrine carcinomas with known AR status in our cohort were strongly AR-positive. Strong AR-positivity is typical for apocrine carcinomas, making this a good surrogate for the LAR subtype [33]. Additionally, apocrine tumors and non-apocrine tumors with higher AR expression showed significantly higher TROP-2 expression, which could suggest TROP-2 is overexpressed in the LAR subtype. Recent studies in prostate cancer have also shown a co-expression of TROP-2 and AR [34, 35]. TNBCs from the LAR subtype are known to be enriched in PIK3CA, AKT1 and CDH1 mutations, which could also explain the association between TROP-2 and AR expression [36]. In line with previous studies, we also found AR linked to a lower SMI and higher age in our cohort. AR-negative tumors are more likely to show higher mitotic activity and presumably more aggressive clinical behavior. Previous studies have shown a lower proliferation on Ki-67 and lower mitotic activity in AR-positive tumors [33, 37]. A lower response to chemotherapy in the LAR group could possibly be due to these tumors being less mitotically active [33]. Despite correlation between TROP-2 and AR expression, TROP-2 expression was not associated with SMI or grade but was significantly correlated with LVI and nodal involvement, which could indicate a surrogate factor for lymphatic spreading of the metastases. This could be of potential value in tailored treatment and new combination therapies of TROP-2-targeted treatment, AR-targeting agents and other anticancer therapies.

We found high sTILs with a cut-off of 30% in 19.1% of cases. This is in line with literature, which suggests high sTILs in 4–37% of TNBC [38, 39]. We could also confirm previous studies showing a higher number of sTILs in patients with lower age and lower BMI [33, 40]. We did not find any correlation of TROP-2 with sTILs in our cohort. We did find an excellent intra-class correlation coefficient or agreement between the two pathologists scoring sTILs in this study. Further molecular testing is necessary to make a clear classification of TROP-2 within the molecular subtypes as defined by Burstein and Lehmann et al.

In our patient cohort with early and loco-regionally advanced TNBC, higher TROP-2 expression was correlated with apocrine histology, higher AR expression, presence of DCIS, LVI and nodal involvement. Additional research is necessary to confirm our association between baseline characteristics and TROP-2 expression. There was no correlation between TROP-2 expression and sTILs or outcome, but limited numbers warrant caution in interpretation, and the prognostic value of TROP-2 expression in early TNBC remains to be further investigated. With the emergence of TROP-2-directed ADCs such as SG and the potential transition of these agents to early treatment settings, future studies should also focus on the predictive value of TROP-2 expression in early and advanced TNBC.

Abbreviations

ADC	Antibody-drug conjugates
AR	Androgen receptor
BCSS	Breast cancer-specific survival
BMI	Body mass index
CNB	Core needle biopsy
DCIS	Ductal carcinoma in situ
DRFI	Distant recurrence-free interval
EMA	European Medicine Agency
EMT	Epithelial-mesenchymal transition
FDA	Food and Drug Administration
H&E	Hematoxylin and eosin
HER2	Human epithelial growth factor receptor-2
HPF	high power fields
IDFS	Invasive disease-free survival
IHC	Immunohistochemistry
LAR	Luminal Androgen Receptor
LVI	Lymphovascular invasion
NACT	Neoadjuvant chemotherapy
pCR	Pathological Complete Response
TNBC	Triple-negative breast cancer
TROP-2	Trophoblast cell-surface antigen-2
SG	Sacituzumab Govitecan
SMI	Standardized Mitotic Index

sTILs	Stromal tumor-infiltrating lymphocytes
UHL	University Hospitals Leuven

Declarations

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Competing Interests

The Authors declare no Competing Non-Financial Interests but the following Competing Financial Interests: K.P.'s institution received speaker fees, honoraria for advisory/consultancy roles and/or research funding from AstraZeneca, Eli Lilly, Gilead Sciences, Medscape, MSD, Mundi Pharma, Novartis, Pfizer, Pierre Fabre, Hoffmann/La Roche, Sanofi, Teva, Vifor Pharma. K.P. received travel support from AstraZeneca, Novartis, Pfizer, PharmaMar, Hoffmann/La Roche. K.P. received speaker fees and honoraria for advisory/consultancy roles from AstraZeneca, Gilead Sciences, Novartis, Roche and Seattle Genetics (all outside the submitted work)

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Data Availability

The data that support the findings of this study are available upon reasonable request.

Ethics approval and consent to participate

This study involving human participants was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Ethics Committee (IRB) of University Hospitals Leuven approved this study.

Author Contributions

H.I.: Writing- original draft, investigation, visualization

K.P.: Conceptualization, methodology, writing- reviewing & editing

L.W.: Investigation, writing- reviewing & editing

A.L.: Formal analysis

H.W.: Conceptualization

F.V.: Writing- reviewing & editing

C.D.: Conceptualization

J.A.: Investigation

A.S.: Writing- reviewing & editing

S.N.H.: Writing- reviewing & editing

I.S.: Writing- reviewing & editing

P.N.: Supervision, writing- reviewing & editing

G.F.: Investigation, conceptualization, writing- reviewing & editing

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Figures

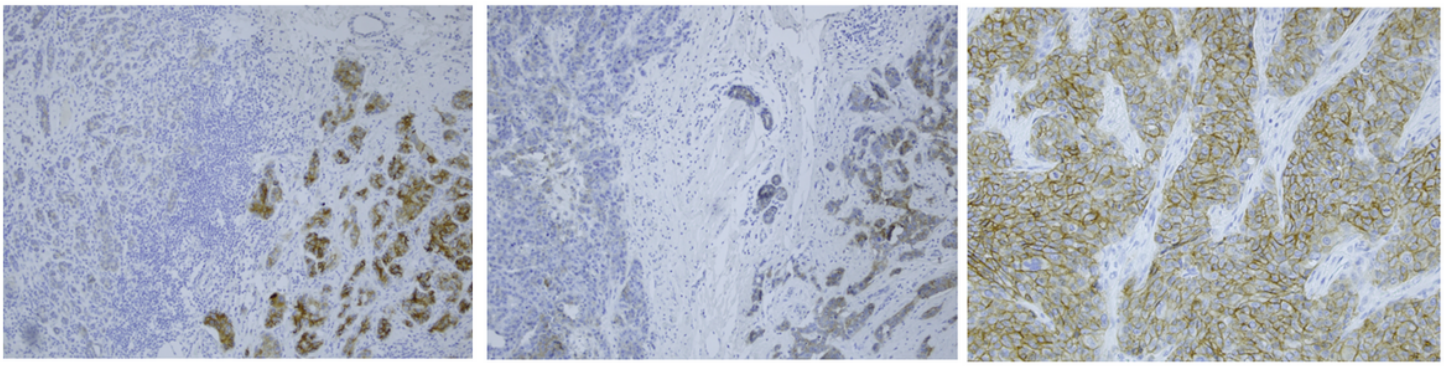

Figure 1

Expression of TROP-2 on pathological specimen. Intermediate expression of Trop-2 (left and middle), and high expression of TROP-2 (right). Positivity of TROP-2 was cytoplasmic, membranous, or both.

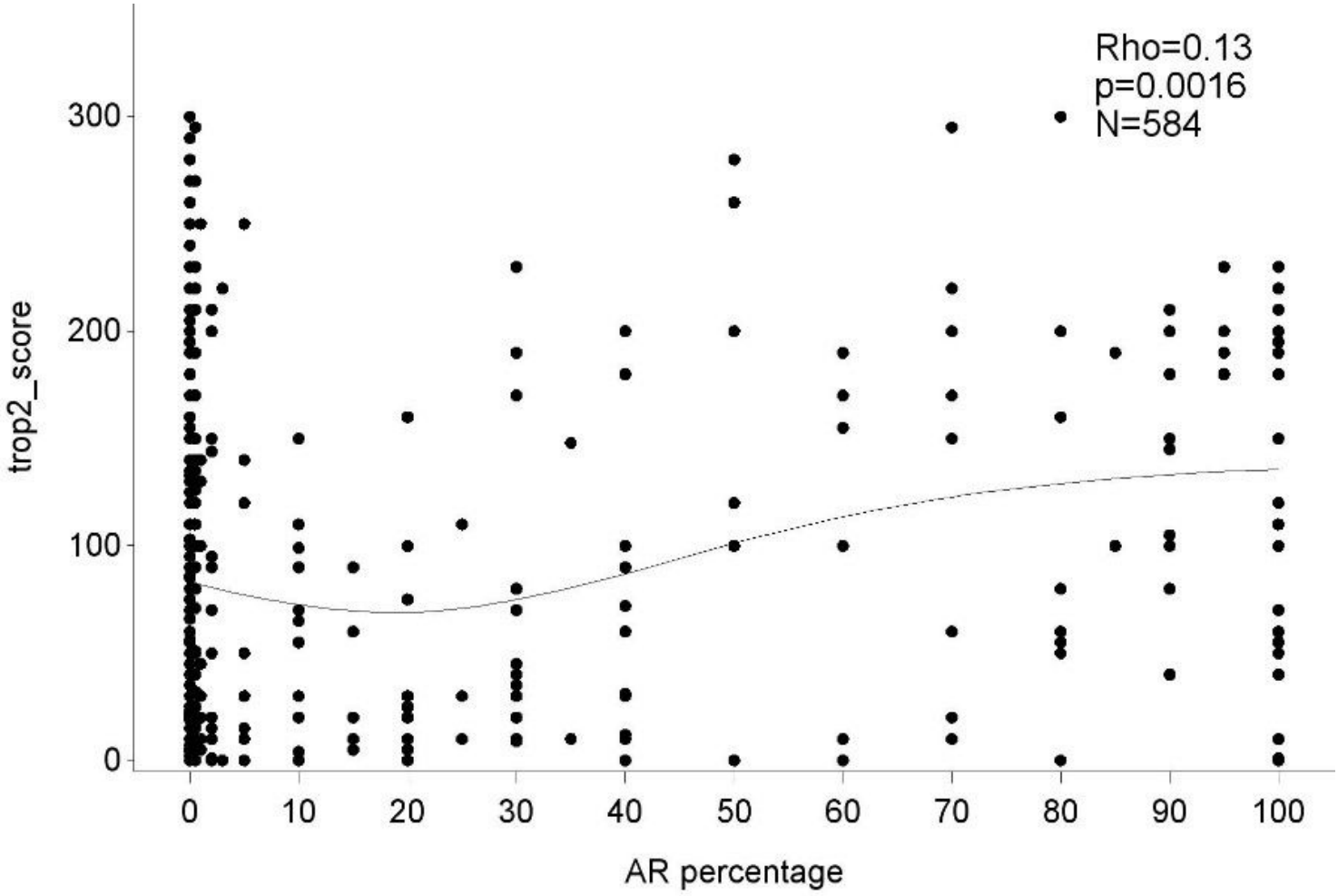

Figure 2

Continuous higher TROP-2 score was correlated with higher AR (10% cutoff). N=584, ρ=0.13, p=0.0016.