Clinico-pathological relationship between androgen receptor (AR) and tumor infiltrating lymphocytes (TILs) in triple negative breast cancer (TNBC)

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Abstract

Background

Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer (BC) with ill-defined therapeutic targets. Androgen receptor (AR) and tumor-infiltrating lymphocytes (TILs) had a prognostic and predictive value in TNBC. The relationship between AR, TILs and clinical behavior is still not fully understood.

Methods

Thirty-six TNBC patients were evaluated for AR (positive if ≥ 1% expression), CD3, CD4, CD8 and CD20 by immunohistochemistry. Stromal TILs were quantified following TILs Working Group recommendations. Lymphocyte-predominant breast cancer (LPBC) was defined as having stromal TILs ≥ 50%, whereas lymphocyte-deficient breast cancer (LDBC) was defined as < 50%.

Results

The mean age was 52.5 years and 27.8% were ≥ 60 years. Seven patients (21.2%) were AR+. All AR + cases were postmenopausal (≥ 50 years old). No statistical difference was found in median overall survival (OS) between AR- and AR + groups (31.5 *vs*. 25 months, p = 0.77). LPBC was 32.2%. Median TILs was 37.5% and 10% (p = 0.1) and median CD20 was 20% and 7.5% (p = 0.008) in AR- and AR+, respectively. Mean CD3 was 80.7% and 93.3% (p = 0.007) and CD8 was 75% and 80.8% (p = 0.41) in AR- and AR + respectively. All patients who were ≥ 60 years old expressed CD20. LDBC was found to be significantly higher in N + *vs*. N- patients (p = 0.03) with median TILs of 20% *vs*. 50% in N + *vs*. N-, respectively (p = 0.03). LDBC was associated with higher risk of lymph node involvement (OR = 6, 95% CI = 1.05–34.21, p = 0.04).

Conclusions

AR expression was evident in older age (≥ 50 years). Median CD20 was higher in AR- TNBC, while mean CD3 was higher in AR + tumors. LDBC was associated with higher risk of lymph node involvement. Larger studies are needed to focus on the clinical impact of the relation between AR and TILs in TNBC.

Introduction

Triple negative breast cancer (TNBC) is a challenging heterogeneous disease with distinct molecular subtypes that does not have receptors for estrogen, progesterone hormones and the HER2 protein. TNBC was grouped into six molecular subtypes: basal-like (BL) 1, BL2, mesenchymal (M), mesenchymal stem-like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR) [1]. But thereafter, Lehmann et al. [2] found that transcripts in the previously defined IM and MSL subtypes came from tumor-infiltrating lymphocytes (TILs) and tumor-associated stromal cells, respectively, and they reduced the number of TNBC molecular subtypes to four (BL1, BL2, M, and LAR).

TILs play an essential role in predicting response to chemotherapy and improving clinical outcomes in breast cancer (BC). Moreover, as the immunotherapy landscape continues to evolve, there is interest in whether the immune system could be playing a more substantial role in TNBC specifically. The association between TNBC subtypes and the impact of TILs is still not fully understood. However, accumulating evidence from several studies indicates that intra-tumoral levels of TILs in TNBC is: a) predictive for response to neo-adjuvant chemotherapy (NACT) and b) prognostic in patients treated with adjuvant chemotherapy, being correlated with improved overall survival (OS) and disease free survival (DFS) [3].

Besides the immune cell markers, other biomarkers have been recognized as valuable in TNBC such as, the androgen receptor (AR). AR controls the transcription of different genes including immune response genes [4]. The AR expression was correlated with better survival outcomes in TNBC [5], albeit its clinical utility and immunological impact remains unclear. However, many opened questions still need to be answered such as what is the prevalence of AR positivity in TNBC and if AR expression correlates with the mean TILs or with CD4/8/3/20 expression. Also, it remains unknown whether there is any relationship between the predominance of TILs and the age, stage and survival. Here we addressed these questions and explored the correlation between the AR expression and the total and differential TILs in TNBC.

Methodology

In this cross-sectional study, patients' records were reviewed retrospectively to select patients with TNBC. TNBC was defined as tumors with negative estrogen receptor (ER), progesterone receptor (PR), and HER2. From a cohort of 800 BC patients who were diagnosed in 2012, at the clinical oncology department, Ain shams University; thirty-six patients with TNBC were identified with their tumor paraffin tissue and medical records available. The clinico-pathological data and survival outcomes were collected. TNM staging was done according to the 7th edition of AJCC. The study protocol was approved by the Research ethical committee, faculty of medicine, Ain shams University, Cairo, Egypt.

Pathological evaluation was performed by a dedicated pathologist (T H), who was blinded for the clinical data. Hematoxylin and eosin stained sections were revised for the negativity of ER, PR and Her2 by the pathologist and assessed for the histologic type and grade of the tumor. They were also examined for quantification of the percentage of stromal TILs according to the 2014 TILs International Working Group [6], where it was defined as the percentage of lymphocytes in direct contact with tumor cells. Lymphocyte-predominant breast cancer (LPBC) was defined as TILs $\geq$ 50%, while lymphocytic deficient breast cancer (LDBC) was defined as TILs $<$ 50%.

Formalin-fixed, paraffin-embedded (FFPE) tissue specimens were available for the evaluation of both of AR and TILs in 28 patients, AR alone in 5 patients and TILs alone in 3 patients. The AR expression (Code 200M-18) was evaluated by Immuno-histochemistry (IHC), and considered positive if ≥ 1% nuclear staining of the tumor cells [4]. Also, immunostaining was performed for T cell markers CD3 (Code 00000 51564), CD4 (Code 104R-28), CD8 (Code 108M-98), and B cell marker CD20 (Code 00000 27500). All antibodies were ready to use, Cell Marque, California, USA. CD3, CD4, CD8, CD20 immunostaining results were evaluated as mean percentage of the stained lymphocytes in relation to the total lymphocytes in the whole tissue section then the mean (for CD3 and CD8) and median (for CD4 and CD20) were calculated. The primary aim of our study was to describe the expression of AR and immune cells (CD3, 4, 8, 20) in TNBC, and the percentage of TILs as well. The secondary aim was to correlate the clinico-pathological parameters with these biomarkers.

Statistical Analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance and Mann-Whitney (z) test were used to compare two means and non-parametric data respectively. ANOVA test and Kruskall-Wallis test were used to compare more than two means and multiple-group comparisons in non-parametric data respectively. Chi-square (x2) test was used in order to compare proportions between qualitative parameters. Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables. The p-value was considered significant if ≤ 0.05.

Results

Patient characteristics

Thirty-six TNBC patients with available enough tumor material were identified for analysis. The patients' characteristics are shown in supplementary material, Table 1. The mean age at diagnosis was 52.5 years (range: 30–75 years), and 27.8% of cases were ≥ 60 years old. Most of the tumors (58.3%) were of invasive duct carcinoma (IDC) type, while medullary carcinoma accounted for 22.2%. A high histological grade was observed in most of the cases, with 30.6 % and 52.8% graded as grade II and III tumors respectively. Stages I, II, III, IV represented 5.6%, 30.5%, 52.7%, and 8.3% respectively. Lymph nodes were positive in 77.8% (28 patients). After a median follow up of 39 months, 9 patients had developed a disease progression and the 3 years OS was reached in 44.4% of the patients.

AR expression and its relation with the clinico-pathological and survival parameters

AR was tested in 33 patients and it was expressed in 21.2% (7 patients). All AR + cases (100%) were postmenopausal (≥ 50 years old). Although patients with AR + tumors were older than those who were AR- (mean age: 55 years *vs*. 51.6 years), there was no statistically significant difference in age between the two groups. Lymph nodes were involved in 77% and 85.7% in AR- and AR + respectively (p = 0.61). No statistical difference was found in median OS between AR- and AR + groups (31.5 *vs.* 25 months, p = 0.77). The clinico-pathological parameters according to AR expression are shown in Table 1.

The majority of AR + tumors (85.7%) was LDBC subtype, with median percentage of TILs was 37.5% and 10% in AR- and AR + tumors respectively (p = 0.10). Median CD20 was significantly higher in AR- versus AR+ (20% *vs* 7.5% respectively, p = 0.008), as depicted in Fig. 1.A. While mean CD3 was significantly lower in AR-versus AR+ (80.7% *vs* 93.3% respectively, p = 0.007), as depicted in Fig. 1.B. On the other side, median CD4 and mean CD8 were not statistically different between AR- and AR + tumors. Table 2 illustrated the correlation between the AR and total & differential TILs expression.

Table 1
The clinico-pathological parameters according to AR expression

Clinico-pathological parameters		**AR- (26)**		**AR+ (7)**		**Chi-square test**	**P-value**
		No.	**%**	**No.**	**%**		
Age	Mean ± SD Range	51.65 ± 11.76 30–75		55.00 ± 4.40 50–63		-0.732•	0.470
Age category	< 60 years	18	69.2	6	85.7	0.755	0.385
	≥ 60 years	8	30.8	1	14.3		
Menopausal status	Pre-menopausal	9	34.6	0	0.0	3.332	0.068
	Post-menopausal	17	65.4	7	100		
Laterality	Right	12	46.2	4	57.1	0.689	0.876
	Left	12	46.2	3	42.9		
	Bilateral	1	3.8	0	0.0		
	Unknown	1	3.8	0	0.0		
Pathology	IDC	14	53.9	5	71.4	3.476	0.627
	Medullary carcinoma	7	26.9	1	14.3		
	ILC	3	11.6	1	14.3		
	Adenoid cystic	1	3.8	0	0.0		
	Unknown	1	3.8	0	0.0		
Grade	Grade II	9	34.6	2	28.6	2.640	0.267
	Grade III	11	42.3	5	71.4		
	Unknown	6	23.1	0	0.0		
TNM staging	I	2	7.7	0	0.0	1.539	0.820
	II	7	26.9	3	42.9		
	III	15	57.8	4	57.1		
	IV	1	3.8	0	0.0		
	Unknown	1	3.8	0	0.0		

Abbreviations: IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, LN: lymph node, IQR: interquartile range, OS: overall survival, •: Independent t-test; ‡: Mann Whitney test

Clinico-pathological parameters		AR- (26)		AR+ (7)		Chi-square test	P-value
		No.	%	No.	%		
T stage	T1	5	19.2	0	0.0	3.139	0.535
	T2	10	38.5	5	71.4		
	T3	6	23.1	1	14.3		
	T4	4	15.4	1	14.3		
	Unknown	1	3.8	0	0.0		
LN category	N0	6	23.1	1	14.3	0.255	0.614
	N+	20	76.9	6	85.7		
Relapse/progression	Negative	19	73.1	6	85.7	0.480	0.489
	Positive	7	26.9	1	14.3		
	Unknown	0	0.0	0	0.0		
Median OS	Median (IQR)	31.5 (18–44)		25 (17–77)		-0.286‡	0.775
	Range	1.5–216		4–86			
3-years OS	< 3 years	14	53.8	4	57.1	0.024	0.876
	≥ 3 years	12	46.2	3	42.9		

Abbreviations: IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, LN: lymph node, IQR: interquartile range, OS: overall survival, •: Independent t-test; ‡: Mann Whitney test

Table 2
The correlation between AR and total & differential TILs expression

Variables		AR- (26)	AR+ (7)	Test value	P-value
Median TILs (%)	Median (IQR)	37.5 (10–50)	10 (5–20)	-1.607‡	0.108
	Range	1– 70	3–40		
LPBC vs LDBC	LDBC	14 (53.8%)	6 (85.7%)	3.082*	0.214
	LPBC	8 (30.8%)	0 (0.0%)		
	Unknown	4 (15.4%)	1 (14.3%)		
Median CD20 (%)	Median (IQR)	20 (10–25)	7.5 (5–10)	-2.643‡	**0.008**
	Range	0–40	0–10		
CD20 expression	Negative	2 (7.7%)	1 (14.3%)	0.290*	0.865
	Positive	20 (76.9%)	5 (71.4%)		
	Unknown	4 (15.4%)	1 (14.3%)		
Mean CD3 (%)	Mean ± SD	80.7 ± 10.1	93.3 ± 4.1	-2.954•	**0.007**
	Range	60–100	90–100		
CD3 expression	Negative	0 (0.0%)	0 (0.0%)	0.005*	0.943
	Positive	22 (84.6%)	6 (85.7%)		
	Unknown	4 (15.4%)	1 (14.3%)		
Median CD4 (%)	Median (IQR)	0 (0–10)	12.5 (0–20)	-1.435‡	0.151
	Range	0–20	0–30		
CD4 expression	Negative	14 (53.8%)	2 (28.6%)	1.786*	0.409
	Positive	8 (30.8%)	4 (57.1%)		
	Unknown	4 (15.4%)	1 (14.3%)		
Mean CD8 (%)	Mean ± SD	75.0 ± 16.3	80.8 ± 10.2	-0.825•	0.417
	Range	40–100	70–95		
CD8 expression	Negative	0 (0.0%)	0 (0.0%)	0.005*	0.943
	Positive	22 (84.6%)	6 (85.7%)		
	Unknown	4 (15.4%)	1 (14.3%)		

Abbreviations: IQR: interquartile range, LPBC: lymphocytic predominance breast cancer, LDBC: lymphocytic deficient breast cancer,*:Chi-square test; •: Independent t-test; ‡: Mann Whitney test

Total and differential TILs expression and its relation with the clinico-pathological parameters

In the 31 patients, where TILs were evaluated, the median TILs were 30% (range = 1–70%), while LDBC and LPBC were 67.7% and 32.2% respectively. CD20 and CD4 were negative in 9.6% and 54.8% respectively. Table 3 showed descriptive analysis of the total and differential TILs expression. When correlating the lymphocytic predominance with the clinico-pathological parameters (shown in Table 4), LDBC type was found to be significantly higher in N + *vs*. N- patients ($p = 0.03$), as depicted in Fig. 2. Median TILs was 20% *vs*. 50% in N + *vs*. N- respectively ($p = 0.03$) as illustrated in Table 5A, B. Total TILs expression < 50% (LDBC) was associated with higher risk of lymph node involvement (Odds Ratio (OR) = 6, 95% CI = 1.05–34.21, $p = 0.04$).

On the other side when analyzing the relationship between the age and TILs (shown in supplementary material, Table 2), it was found that median TILs was lower in patients ≥ 60 years old in spite statistically not significant, (median TILs = 10 *vs.* 38 % in ≥ 60 years old *vs*. <60 years old respectively, $p = 0.45$). Moreover, all patients who were ≥ 60 years old expressed B- cell marker (100%), as shown in supplementary material, Table 3. Furthermore, a significant positive correlation was present between CD8 and CD3 (correlation coefficient (r) = 0.591, p□0.001) while significant inverse correlations were present between CD3 and CD20 (r= -0.814, p□0.001), CD8 and CD20 (r= -0.382, $p = 0.03$) and CD8 and CD4 (r= -0.52, $p = 0.002$), as illustrated in Fig. 3. A, B, C and supplementary material, Table 4.

Table 3
Descriptive analysis of the total and differential TILs expression.

Variables		**No. = 31**
Median TILs (%)	Median (IQR)	30 (10–50)
	Range	1–70
LDBC *Vs.* LPBC	LDBC	21 (67.7%)
	LPBC	10 (32.3%)
Median CD20 (%)	Median (IQR)	15 (10–20)
	Range	0–40
CD20 expression	Negative	3 (9.6%)
	Positive	28 (90.4%)
Mean CD3 (%)	Mean ± SD	80.5 ± 17.7
	Range	2–100
CD3 expression	Negative	0 (0.0%)
	Positive	31 (100%)
Median CD4 (%)	Median (IQR)	0 (0–15)
	Range	0–30
CD4 expression	Negative	17 (54.8%)
	Positive	14 (45.2%)
Mean CD8 (%)	Mean ± SD	73.4 ± 19.7
	Range	2–100
CD8 expression	Negative	0 (0.0%)
	Positive	31 (100%)

Abbreviations: IQR: interquartile range, SD: standard deviation, LPBC: lymphocytic predominance breast cancer, LDBC: lymphocytic deficient breast cancer

Table 4
Relation between lymphocytic predominance and clinico-pathological parameters

Variables		**LDBC (= 21)**		**LPBC (= 10)**		**Chi-square test**	**P-value**
Age	Age < 60	15	71.4%	7	70.0%	0.007	0.935
	Age ≥ 60	6	28.6%	3	30.0%		
Morphology	IDC	12	57.1%	6	60.0%	0.873	0.928
	Medullary	1	4.8%	1	10.0%		
	IDC with medullary	4	19.0%	2	20.0%		
	ILC	3	14.3%	1	10.0%		
	Adenoid cystic	1	4.8%	0	0.0%		
TNM staging	I	2	9.5%	0	0.0%	2.045	0.563
	II	6	28.6%	5	50.0%		
	III	11	52.4%	4	40.0%		
	IV	2	9.5%	1	10.0%		
	Unknown	0	0.0%	0	0.0%		
N stage	N0	3	14.3%	5	50.0%	4.762	0.190
	N1	7	33.3%	2	20.0%		
	N2	9	42.9%	2	20.0%		
	N3	2	9.5%	1	10.0%		
	Unknown	0	0.0%	0	0.0%		
LN category	N-	3	14.3%	5	50.0%	4.513	**0.034**
	N+	18	85.7%	5	50.0%		
OS	Median (IQR)	32	(18–72)	25	(12–39)	-0.993‡	0.320
	Range	2-216		9–48`			
3 years OS	< 3 years OS	11	52.4%	6	60.0%	0.159	0.690
	≥ 3 years OS	10	47.6%	4	40.0%		

Abbreviations: IDC: invasive duct carcinoma, ILC: invasive lobular carcinoma, LN: lymph node, IQR: interquartile range, OS: overall survival, ‡: Mann Whitney test

Table 5

A. Relation between lymph node involvement and the total and differential TILs

Total and differential TILs		LN involvement		Test value	P-value
		N- (= 8)	**N+ (= 23)**		
Total TILs (%)	Median (IQR)	50 (38–55)	20 (5–40)	-2.159‡	**0.031**
	Range	10–60	1–70		
Median CD20 (%)	Median (IQR)	15 (3–20)	15 (10–25)	-1.089‡	0.276
	Range	0–20	0–40		
Mean CD3 (%)	Mean ± SD	85.6 ± 9.8	78.8 ± 19.6	0.940•	0.355
	Range	70–100	2–100		
Median CD4 (%)	Median (IQR)	5 (0–13)	0 (0–20)	-0.074‡	0.941
	Range	0–20	0–30		
Mean CD8 (%)	Mean ± SD	74.4 ± 15.0	73.1 ± 21.4	0.151•	0.881
	Range	50–100	2–100		

LN: lymph node, IQR: interquartile range, SD: standard deviation, •: Independent t-test; ‡: Mann Whitney test

Table 5B. Relation between lymph node involvement and the total and differential TILs

Total and differential TILs		LN involvement				Chi-square test	P-value
		N-		N+			
		No.	%	No.	%		
LDBC vs LPBC	LDBC	3	37.5	18	78.3	4.513	**0.034**
	LPBC	5	62.5	5	21.7		
CD20 expression	Negative	2	25.0	1	4.3	2.896	0.089
	Positive	6	75.0	22	95.7		
CD3 expression	Negative	0	0.0	0	0.0	NA	NA
	Positive	8	100	23	100.0		
CD4 expression	Negative	4	50.0	13	56.5	0.102	0.750
	Positive	4	50.0	10	43.5		
CD8 expression	Negative	0	0.0	0	0.0	NA	NA
	Positive	8	100	23	100		

NA: not applicable

Discussion

It is well established that the expression of AR differs according to molecular subtypes with more frequent expression in ER negative cancers. The prevalence of AR + expressing tumors is generally ranging from 10 to 41% in TNBC cases [1,7–13], with rare reports showing rates up to 79% [14,15]. In accordance with most published reports, our rate of AR expression in TNBC was 21.2%.

Whether clinico-pathologic characteristics of TNBC vary based on AR expression status has been extensively studied [7,12,14,16,17]. Some studies showed that patients with AR + tumors were significantly older, exhibited tumors with significantly lower grades (I-II), more frequent nodal involvement, non-ductal histology, and lower Ki67 [13,14,16,17]. Other reports described reduced lymph node metastases in AR + TNBCs [7], or just similar clinico-pathologic profile between AR + and AR-TNBC [12]. Herein, there was no statistical significant difference in the clinico-pathological parameters according to AR expression. However, AR + cases were older in age and exhibit more regional nodal spread. Despite statistically insignificant, this profile was analogous to the LAR subtype described by Lehmann and colleagues [2].

Available evidence about the prognostic value of AR in TNBC is controversial. Some reports suggested that AR-positivity was associated with good outcomes [7,12]. Whereas, others concluded that AR status conferred worse prognosis[18] or had no significant impact on disease prognosis [4,19,20]. Many factors may explain these discrepant results across studies including the sample size limited cohorts, differences in the ethnic origin, the anti-AR antibodies used for staining, staining/scoring method, as well as variability in the thresholds used to define AR positivity [4]. A meta-analysis published in 2017, demonstrated that AR-positive status was associated with better DFS and OS in TNBC (Hazard ratio (HR) = 0.64, 95% CI = 0.51–0.81, p < 0.001 and HR = 0.64, 95%CI = 0.49–0.88, p < 0.001, respectively), in univariate analysis[5]. Of note, no multivariate analysis was provided and this meta-analysis included heterogeneous studies in terms of methods of AR scoring, clinical cohorts' characteristics, therapies received and length of follow up. A large multi-institutional study including about 1,407 TNBC tumors issued after this meta-analysis concluded that the AR-positivity was a marker of good prognosis in US and Nigerian cohorts, whereas it conferred poor prognosis in Norway, Ireland and Indian cohorts, and was neutral in UK cohort [4].Whereas a more recent meta-analysis (2020), [20] demonstrated that AR expression in TNBC was not associated with DFS (HR = 0.92; 95% CI = 0.67–1.27; p = 0.63), OS (HR = 0.91; 95% CI = 0.67–1.22; p = 0.53), distant- DFS (HR = 1.02; 95% CI = 0.96–1.08; p = 0.48), or recurrence-free survival (HR = 0.95; 95% CI = 0.46–1.98; p = 0.90), regardless of confounding factors and heterogeneity that existed among included studies. Our study results had matched the latter meta-analysis results, where no statistical difference in median OS (31.5*vs.* 25 months, p = 0.77) or relapse/progression rate (26.9% *vs.* 14.3%, p = 0.48) was found between AR- and AR + groups.

Compared to other subtypes, TNBC was shown to exhibit higher levels of TILs [21]. There is heterogeneity of TILs cut-off used in published studies in order to distinguish between LPBC and LDBC. Some studies defined LPBC as showing more than 50% of lymphocyte infiltration [22,23], whereas others used different cut-offs [24]. In our cohort, median TILs was 30% (range: 1–70%), with a LPBC prevalence of 32.2%, which is not in full agreement with other reports. Adams et al. [22] reported much lower median TILs percentage (10%), and using the same cut-off of ≥ 50% TILs, only 4.4% were LPBC; whereas, Pruneri et al. [23] described a median TILs level of 20%, with LPBC prevalence of 22% of cases.

Little is known about the association between TNBC clinico-pathologic features and lymphocytic predominance. A pooled analysis of nine large studies by Loi et al. [24] demonstrated that TILs were significantly lower in older age. Whilst, Adams et al.[22] reported no strong associations between TILs scores and age or menopausal status. Despite not statistically significant, we showed lower median TILs in patients ≥ 60 years vs < 60 years old (10% *vs.* 38 %, p = 0.45).

Interestingly, we found that patients with lymph node positive tumors were significantly more likely to be LDBC, where a total TILs expression < 50% (LDBC) was associated with higher risk of lymph node involvement (OR = 6, 95%CI = 1.05–34.21, p = 0.04). This is in agreement with Loi et al.[24], but in contrast with a recent meta-analysis that concluded no significant association between increased TILs and lymph node metastasis risk [25].

Our knowledge about the association between TILs and AR is still limited. In a large cohort study about non-metastatic TNBC of LAR subtype, this tumor subset was found to exhibit lower median stromal TILs and to be less likely LPBC (≥ 50% TILs) compared to non LAR, although this not reached statistical significance [26], similarly to our study. However, we did not examine the genetic profiles of our AR + tumors to classify them into the LAR subtype. Other reports using IHC, described significant association between AR expression and lower levels of stromal TILs [10,16].

Studies about the cells subsets composition of TILs according to AR expression are very scarce. We found that median CD20 was significantly higher in AR- tumors compared to those AR+ (20% *vs* 7.5% respectively, p = 0.008). Whereas, mean CD3 was significantly lower in AR- *vs.* AR+ (80.7% *vs* 93.3% respectively, p = 0.007). Previous publications reported that CD8 + were more frequent in AR + than AR- tumors [11,27,28]. In contrast, neither CD8 nor CD4 were statistically different between AR + and AR- tumors, in our study.

Based on two large-scale BC genomics data, evidence from a comprehensive analysis of 26 immune gene-sets including 15 immune cell type and function suggested that TNBC had the strongest tumor immunogenicity. Comparison of the immune infiltrate densities of different immune cell subpopulations demonstrated higher degree of infiltration in TNBC than non-TNBC, including CD3, CD8 and CD20 and others[29].

T-lymphocytes represent the main lymphocytes type in the tumor microenvironment, and the majority of T lymphocytes express a cytotoxic effector phenotype (CD8+). Intra-tumoral and adjacent stromal CD8 + T-cell infiltration have been found to be significantly associated with ER negativity and basal phenotype [30,31]. Infiltrating CD8 + T-cells have been reported in more than 60% of TNBC cases [31,32]. In our study, CD8 was expressed in 100% of the cases with the mean of its expression was 73.4%.

The role of tumor-infiltrating B cells (CD20) as components of TILs in BC subtypes is still unclear. A positive correlation between higher numbers of total CD20 + B cells and ER and PR negativity, and basal phenotype has been reported [33]. In our study, CD20 was expressed in 90.3% of the tumors and its median expression was significantly higher in AR- *vs.* AR + TNBC (20% *vs.* 7.5% respectively, p = 0.008)

Using a digital pathology computational workflow to quantify the spatial patterns of five immune markers (CD3, CD4, CD8, CD20, and FoxP3) in TNBC, Mi et al.[34] demonstrated positive correlations between CD3 and CD8 cells. Similarly, we also showed a significant positive correlation between CD3 and CD8. Data from a study that used multiplexed ion beam imaging to simultaneously quantify *in situ* expression of 36 proteins in 41 TNBC patients suggested that all patients with B cells had also CD4 T cells and CD8 T cells [35]. In contrast, we found in our study significant inverse correlations between CD20 and CD8, CD20 and CD3 as well as CD8 and CD4.

Immune cellular subpopulations in BC representing the innate immunity (natural killer, CD68 + and CD11c + cells) and adaptive immunity (CD3+ (CD8+, CD4+) cells and CD20 + cells) [36], worth thorough evaluation in TNBC, with the aim of understanding its clinical implications in BC management.

This study had comprehensively described the expression patterns of AR and TILs in TNBC. Moreover, the correlation between AR and the total and different TILs subpopulations was illustrated. TILs were evaluated by one pathologist who was blinded to the clinical characteristics and according to the International Working Group. However, our findings should be interpreted carefully. The limitations of our study include: i) the retrospective nature, ii) the small sample size, and iii) our survival data was not mature due to the short follow-up duration (median: 39 months).

Conclusion

This study highlighted the probable relationship between the AR and total and differential TILs expression in TNBC; and the clinico-pathological characteristics as well. Understanding the immune micro-environment in a subset of tumors with poor prognosis and less identified therapeutic targets like TNBC, may pave the way for the advent of immunotherapy in specific group of patients. Moreover, lower TILs density may identify a subpopulation of TNBC who warrants more radical regional lymph nodes management or prophylactic lymphatic radiation. The prognostic relevance and the potential predictive impact of AR and TILs in TNBCs merit further evaluation in a larger scale studies.

Declarations

Ethical Approval and consent to participate

This research protocol was approved by the Research Ethics Committee, Faculty of medicine, Ain Shams University, Cairo, Egypt. All methods were carried out in accordance with relevant guidelines and regulations. The informed consent was obtained from all participants.

Consent for publication

Not applicable

Availability of data and materials

All datasets used or analyzed for this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that there are no financial or commercial conflicts of interest.

Declaration of interests: None

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Authors' contributions

HE: conceptualization, interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the manuscript.

JB: conceptualization, interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the manuscript.

TH: conceptualization, interpretation of data, critical revision for important intellectual content, and final approval of the manuscript.

A M. A: investigation and resources, drafting the article, final approval of the manuscript.

MK: acquisition of data, drafting the article, final approval of the manuscript.

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References

1. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011 Jul;121(7):2750–67.

2. Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS One. 2016 Jun;11(6):e0157368.
3. Gao G, Wang Z, Qu X, Zhang Z. Prognostic value of tumor-infiltrating lymphocytes in patients with triple-negative breast cancer: a systematic review and meta-analysis. BMC Cancer. 2020 Mar;20(1):179.
4. Bhattarai S, Klimov S, Mittal K, Krishnamurti U, Li XB, Oprea-Ilies G, et al. Prognostic Role of Androgen Receptor in Triple Negative Breast Cancer: A Multi-Institutional Study. Cancers (Basel). 2019 Jul;11(7):E995.
5. Bozovic-Spasojevic I, Zardavas D, Brohée S, Ameye L, Fumagalli D, Ades F, et al. The Prognostic Role of Androgen Receptor in Patients with Early-Stage Breast Cancer: A Meta-analysis of Clinical and Gene Expression Data. Clin Cancer Res. 2017 Jun;23(11):2702–12.
6. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al.; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015 Feb;26(2):259–71.
7. He J, Peng R, Yuan Z, Wang S, Peng J, Lin G, et al. Prognostic value of androgen receptor expression in operable triple-negative breast cancer: a retrospective analysis based on a tissue microarray. Med Oncol. 2012 Jun;29(2):406–10.
8. Astvatsaturyan K, Yue Y, Walts AE, Bose S. Androgen receptor positive triple negative breast cancer: Clinicopathologic, prognostic, and predictive features. PLoS One. 2018 Jun;13(6):e0197827.
9. Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, et al.; Translational Breast Cancer Research Consortium (TBCRC 011). Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic Breast Cancer. Clin Cancer Res. 2013 Oct;19(19):5505–12.
10. Leon-Ferre RA, Polley MY, Liu H, et al. Tumor-infiltrating lymphocytes in androgen receptor-positive, triple-negative breast cancer. J Clin Oncol. 2018;36(5 suppl):5–5.
11. Castaneda CA, Castillo M, Enciso JA, Enciso N, Bernabe LA, Sanchez J, et al. Role of undifferentiation markers and androgen receptor expression in triple-negative breast cancer. Breast J. 2019 Nov;25(6):1316–9.
12. Asano Y, Kashiwagi S, Goto W, Tanaka S, Morisaki T, Takashima T, et al. Expression and Clinical Significance of Androgen Receptor in Triple-Negative Breast Cancer. Cancers (Basel). 2017 Jan;9(1):E4.
13. Jongen L, Floris G, Wildiers H, et al. The prognostic role of the androgen receptor in patients with triple-negative early breast cancers and primary surgery. J Clin Oncol. 2019;37(15 suppl):e12042–12042.
14. Guiu S, Mollevi C, Charon-Barra C, Boissière F, Crapez E, Chartron E, et al. Prognostic value of androgen receptor and FOXA1 co-expression in non-metastatic triple negative breast cancer and correlation with other biomarkers. Br J Cancer. 2018 Jul;119(1):76–9.

15. Traina TA, Miller K, Yardley DA, et al. Results from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR + triple-negative breast cancer (TNBC). J Clin Oncol. 2015;33(15 suppl):1003–1003.
16. Dieci MV, Tsvetkova V, Griguolo G, Miglietta F, Mantiero M, Tasca G, et al. Androgen Receptor Expression and Association With Distant Disease-Free Survival in Triple Negative Breast Cancer: Analysis of 263 Patients Treated With Standard Therapy for Stage I-III Disease. Front Oncol. 2019 Jun;9:452.
17. Sutton LM, Cao D, Sarode V, Molberg KH, Torgbe K, Haley B, et al. Decreased androgen receptor expression is associated with distant metastases in patients with androgen receptor-expressing triple-negative breast carcinoma. Am J Clin Pathol. 2012 Oct;138(4):511–6.
18. Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clin Cancer Res. 2011 Apr;17(7):1867–74.
19. McGhan LJ, McCullough AE, Protheroe CA, Dueck AC, Lee JJ, Nunez-Nateras R, et al. Androgen receptor-positive triple negative breast cancer: a unique breast cancer subtype. Ann Surg Oncol. 2014 Feb;21(2):361–7.
20. Xu M, Yuan Y, Yan P, Jiang J, Ma P, Niu X, et al. Prognostic Significance of Androgen Receptor Expression in Triple Negative Breast Cancer: A Systematic Review and Meta-Analysis. Clin Breast Cancer. 2020 Aug;20(4):e385–96.
21. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013 Mar;31(7):860–7.
22. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol. 2014 Sep;32(27):2959–66.
23. Pruneri G, Vingiani A, Bagnardi V, Rotmensz N, De Rose A, Palazzo A, et al. Clinical validity of tumor-infiltrating lymphocytes analysis in patients with triple-negative breast cancer. Ann Oncol. 2016 Feb;27(2):249–56.
24. Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, et al. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. J Clin Oncol. 2019 Mar;37(7):559–69.
25. Lotfinejad P, Asghari Jafarabadi M, Abdoli Shadbad M, Kazemi T, Pashazadeh F, Sandoghchian Shotorbani S, et al. Prognostic Role and Clinical Significance of Tumor-Infiltrating Lymphocyte (TIL) and Programmed Death Ligand 1 (PD-L1) Expression in Triple-Negative Breast Cancer (TNBC): A Systematic Review and Meta-Analysis Study. Diagnostics (Basel). 2020 Sep;10(9):704.
26. Leon-Ferre RA, Polley M-Y, Liu H, et al. Abstract P3-08-01: Characteristics, outcomes and prognostic factors of luminal androgen receptor (LAR) triple-negative breast cancer (TNBC). Cancer Res. 2019;79(4_suppl):P3-08-01. doi:https://doi.org/10.1158/1538-7445.. SABCS18-P3-08-01.

27. Sánchez-Cousido LF, López-González A, Franco EH, et al. Relationship between androgen receptor and tumor-infiltrating lymphocytes in triple-negative breast cancer. Ann Oncol. 2018;29:viii80. doi:https://doi.org/10.1093/annonc/mdy270.246.
28. Sánchez-Cousido LF, Franco EH, Guadarrama ÓS, et al. 219P Relationship among PDL1, androgen receptor (AR) and tumour infiltrating lymphocytes (TILs) in early triple negative breast cancer. Ann Oncol. 2020;31:S328–9.
29. Liu Z, Li M, Jiang Z, Wang X. A Comprehensive Immunologic Portrait of Triple-Negative Breast Cancer. Transl Oncol. 2018 Apr;11(2):311–29.
30. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8 + lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011 May;29(15):1949–55.
31. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO. CD8 + lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res. 2012 Mar;14(2):R48.
32. Stanton SE, Adams S, Disis ML. Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review. JAMA Oncol. 2016 Oct;2(10):1354–60.
33. Mahmoud SM, Lee AH, Paish EC, Macmillan RD, Ellis IO, Green AR. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. Breast Cancer Res Treat. 2012 Apr;132(2):545–53.
34. Mi H, Gong C, Sulam J, Fertig EJ, Szalay AS, Jaffee EM, et al. Digital Pathology Analysis Quantifies Spatial Heterogeneity of CD3, CD4, CD8, CD20, and FoxP3 Immune Markers in Triple-Negative Breast Cancer. Front Physiol. 2020 Oct;11:583333.
35. Keren L, Bosse M, Marquez D, Angoshtari R, Jain S, Varma S, et al. A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging. Cell. 2018 Sep;174(6):1373–1387.e19.
36. Goff SL, Danforth DN. The Role of Immune Cells in Breast Tissue and Immunotherapy for the Treatment of Breast Cancer [published online ahead of print, 2020 Jul 2]. Clin Breast Cancer. 2020; S1526-8209(20)30155-5.