

The prevalence of ER-low-positive breast cancer and its relation to tumor characteristics in Syria

Research Article

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Abstract: Background: Breast cancer is a heterogeneous disease with varying clinical behaviors and responses to endocrine therapy. In particular, estrogen receptor (ER)-positive breast cancer can present with various subtypes, and the effectiveness of anti-hormone therapy on ER-low-positive tumors is unclear. This study aimed to evaluate and characterize ER-positive subtypes in patients admitted to the Surgical Oncology Clinic in the National Hospital of Jableh in Syria between 2020 and 2022. Patients and Methods: This study included 120 patients diagnosed with breast cancer; the data from each patient's report were collected to classify them according to grade, stage, LNR, tumor size, and nodal stage. Results: The prevalence of ER-low-positive subtype was 23.33%. There was a significant difference between ER-negative and ER-positive subtypes concerning age, grade, stage, and LNR. Additionally, we confirmed a variation between ER-low-positive and ER-high-positive tumors, which may explain the difference in therapeutic response in ER-positive tumor patients taking anti-ER drugs. Conclusion: Further research is necessary to study the association between therapeutic response and ER-staining intensity breast cancer subtypes.

Keywords: breast cancer • ER-low-positive breast cancer • CRP • lymph node ratio—LNR • invasive ductal carcinoma—IDC

1. Background

Breast cancer (BC) is the most common malignancy in women (38.5%) and the second-leading cause of cancer-related mortalities (13.8%) [1]. In Syria, the Global Cancer Observatory (GCO) reported 4144 new cases in 2022, comprising 18.8% of all cancer cases, with 1781 new deaths [2].

The molecular mechanism of tumor development in some women is still not well understood, but many studies have pointed to the existence of a relationship between the chronic inflammatory state observed in some diseases and increasing the risk of its occurrence [3].

The response of each breast cancer patient to hormone therapy is influenced by various factors, such as the status of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her-2), age, stage, histological grade, and involvement of lymph nodes [4, 5]. Therefore, evaluation of ER status on invasive carcinomas is a common practice in therapeutic procedures and management of BC tumors.

Women with advanced stage and negative ER expression (ER-) are at high risk and pose a challenge when it comes to selecting appropriate treatment protocols. In such cases, target therapy must be carefully tailored to maximize benefits while minimizing toxic effects [6].

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Inflammation is the first sign of malignant changes in the tumor microenvironment. In response to cytokines, hepatocytes produce C-reactive protein (CRP), which is then released from leukocytes in the tumor microenvironment. Several studies have shown that higher serum levels of CRP are observed in invasive cancer compared to that in non-invasive cancer, indicating its potential as a diagnostic marker [7, 8].

The definition of estrogen receptor-positive (ER+) tumors is established by the College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO) as those tumors expressing ER in 1% or more of cancer nuclei, as determined by immunohistochemistry (IHC) staining [9]. About 60–65% of primary breast cancers are ER+, which has also proven to be a successful target for the treatment of ER+ breast carcinomas. On the other hand, depending on the patient's age and lymph node status, ER- tumors tend to be related to higher histological grade, poorly differentiated, and decreased overall survival (OS) [10].

Different phenotypes display various clinical behaviors based on the fact that BC is a heterogeneous disease on the morphologic, immune-histologic, and molecular levels [11]. Practically, large clinical studies, which only refer to cancer subtypes as ER+/ER- frequently, fail to determine the degree of ER positivity as an additional useful element of information. As a result, there is a clear need for determining the intensity of ER staining when evaluating the effectiveness of treatments.

Recent studies show that tumors with low ER expression can behave similarly to tumors with negative ER expression as they have a poor prognosis and treatment options. The prognosis for patients with lower levels of ER has recently been found to be poor and is comparable to that of patients with strictly defined triple-negative BC (TNBC) [12].

2. Objective

The aims of this study were the classification of breast cancer patients according to the degree of ER-staining intensity and the comparison of tumor and patient characteristics between ER+ subtypes and ER- tumors in the Syrian population.

3. Patients and Methods

3.1. Population of Study

The study involved a review of patient charts, including pathology reports, operative reports, and clinical

outcomes. All patients provided a written informed consent, and the Institutional Ethics Board approved the study. The study population consisted of 120 patients, aged between 35 and 80 years, admitted to the Surgical Oncology Clinic in the National Hospital of Jableh, Latakia, Syria, between December 2020 and December 2022. Basic blood investigations and chest x-rays were performed for all patients, and a diagnosis of breast cancer was confirmed and classified according to the grade and stage (as written in reports).

3.2. Clinical History

1. Specimen type and surgical reports

Patients underwent a total or subtotal (left or right) mastectomy with axillary lymph node dissection, and the number of lymph nodes excised was different in patients.

2. Pathology reports

Data based on pathology reports, which were done in a Pathology Laboratory by the same pathologist, including tumor size (cm), stage, histologic grade, and number of lymph nodes excised (positive and total number), were obtained prospectively.

3. Histological diagnosis

Patients were diagnosed as invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and mucinous carcinoma (MC).

4. Breast cancer staging system

According to the pathological TNM staging according to the American Joint Committee on Cancer (AJCC), patients were assessed as following:

- T; Tumor size: T1 ≤2 cm, T2: 2.1–5 cm, T3 >5 cm, T4 = chest wall or skin infiltration
- N; Nodal staging: pN1: 1–3 nodes, pN2: 4–9 nodes, pN3: ≥ 10 nodes
- M; Metastasis: absence (M0) or presence (M1) of metastasis

5. Immune-histochemical (IHC) analysis

Expression of estrogen receptor (ER) was confirmed by immune-histochemical analysis (IHC) and considered positive if more than 1% of tumor cells [6]. The staining intensity of ER+ was classified into three categories concerning ER score ranging from 1 to 4 as follows:

- Low-staining intensity (1–9.99%): with score 1/4.
- Moderate-staining intensity (10–20%): with score 1–2 or 2/4.
- High-staining intensity (>20%): with score 2–3 or 3 or 4/4.

6. Lymph node ratio (LNR): Lymph node ratio (LNR) was calculated as follows: the ratio of the number of positive nodes to the total number of nodes excised, and LNR had been categorized as low (0–0.2), intermediate (>0.2–0.65), and high risk (>0.65–1) [13].

7. Assays for serum CRP levels: The samples were collected (before any surgical procedures) and measured by immunoturbidimetric determination using Erba Lachema Kit.

3.3. Statistical analysis

Descriptive statistics using frequencies (%) for categorical data was calculated. The chi-square test was used to compare percentages between groups. Two-sample Wilcoxon rank-sum (Mann–Whitney) test was used to compare differences between two independent groups when the dependent variable is either ordinal or continuous, but not normally distributed, while Kruskal–Wallis test was used to compare differences between more than two independent groups when the dependent variable is not normally distributed. Both of these statistical tests were used to compare means of serum CRP levels. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM Corporation). As the analysis involves multiple subgroups, significance was set at the 5% level ($P < 0.05$).

4. Results

1. Characteristics of patients and tumor status:

In this study, the most common histologic type of BC was invasive ductal carcinoma (90%), followed by invasive lobular carcinoma (6.67%) and mucinous carcinoma (3.33%). The patients were divided into two age groups, with 46 years being the cutoff point. The mean age at diagnosis was 54.5 years. Tumor size was categorized into T1, T2, and T3, with a mean size of 2.38 cm. The majority of patients were classified as grade II tumors (83.33%) and stage III (48.33%). Additional details on patient characteristics and tumor status are provided in Table 1.

2. Classification of breast cancer according to estrogen-receptor-staining intensity

Negative ER breast cancer was the most common type of BC subtypes in this study (31.67%), while low ER, moderate ER, and high ER subtypes were less frequent (23.33%, 20%, and 25%, respectively).

Table 1: General characteristics of tumor and study population.

Classification	n	%
Diagnosis	IDC	108
	ILC	8
	Mucinous	4
Age (years)	≤46	56
	>46	64
Tumor size (cm)	T1: ≤2cm	28
	T2: 2.1 to 5	74
	T3: >5	18
Histologic grade	I	2
	II	100
	III	18
Stage	I	6
	IIA	18
	IIB	38
	IIIA	48
	IIIB	10

In patients aged 46 years and younger, negative ER subtype (57.89%), low ER subtype (50%), and moderate-positive ER subtype (66.67%) were more common, while high-positive-ER subtype was less common (13.33%). In comparison, patients over 46 years old were more likely to have high-positive-ER subtype (86.67%). There was a statistically significant difference related to age between ER-staining intensity groups ($P=0.018$). These findings are presented in Table 2 and Figure 1.

Concerning tumor size, histological stage, and diagnosis, there was no statistical difference (P -value > 0.05). While the difference was statistically important for the histological grade (P -value = 0.01), most of the patients were grade II (89.47%, 57.14%, 91.67%, and 93.33% for negative, low, moderate, and high ER subtypes, respectively).

The highest nodal stage was in the low ER subtype (92.86%) with more than 4 positive lymph nodes, while this ratio decreased to 58.33% and 53.33% for moderate and high ER breast cancer subtypes, and the difference was significant between groups.

Furthermore, patients were classified into three groups based on lymph node ratio (LNR) risk. The majority of negative, low, and moderate ER-staining intensity subtypes had intermediate risk, and there was a statistically significant difference in LNR risk according to ER status (P -value = 0.045).

Figure 1 shows that patients with high ER+ had higher levels of CRP (mean=8.06 mg/l), while patients

Table 2: Characteristics of patients and tumor according to ER status.

		ER-staining intensity subtypes N=120								
Parameter		Negative N=38 (31.67%)		Low N=28 (23.33%)		Moderate N=24 (20%)		High N=30 (25%)		P value
		n	%	n	%	n	%	n	%	
Age, years	≤46	22	57.89	14	50	16	66.67	4	13.33	0.018
	>46	16	42.11	14	50	8	33.33	26	86.67	
Tumor Size, cm	≤2cm	2	5.26	10	35.71	10	41.67	6	23.33	0.18
	2.1 to 5	30	78.95	12	42.86	12	50	20	61.67	
	>5	6	15.79	6	21.43	2	8.33	4	15	
Diagnosis	IDC	34	89.47	26	92.86	22	91.67	26	86.67	0.812
	ILC	4	10.53	2	7.14	0	0	2	6.67	
	Mucinous	0	0	0	0	2	8.33	2	6.67	
Histological grade	I	0	0	0	0	2	8.33	0	0	0.01
	II	34	89.47	16	57.14	22	91.67	28	93.33	
	III	4	10.53	6	42.86	0	0	2	6.67	
Stage	I	2	5.26	0	0	2	8.33	2	6.67	0.131
	II	24	63.16	6	21.43	10	41.67	16	53.33	
	III	12	31.58	22	78.57	12	50	12	40	
Nodal stage, pN	1 to 4	20	52.63	2	7.14	10	41.67	14	46.67	0.035
	>4	18	47.37	26	92.86	14	58.33	16	53.33	
LNR	Low risk (0–0.2)	20	52.63	2	7.14	10	41.67	16	53.33	0.046
	Intermediate risk (>0.2–0.65)	18	47.37	22	78.57	18	50	12	40	
	High risk (>0.65–1)	0	0	4	14.29	2	8.33	2	6.67	

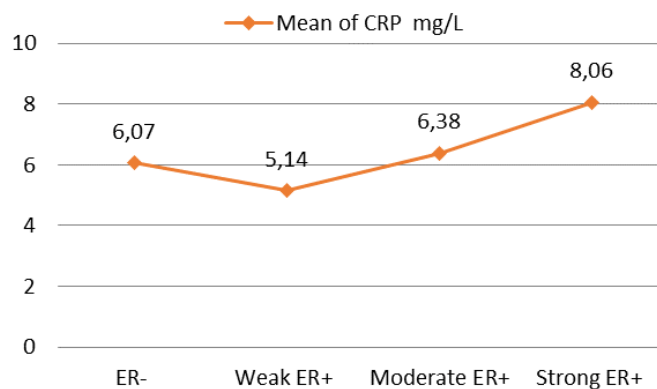


Figure 1: Mean of CRP levels according to ER status in BC patients.

with ER- or low ER+ had lower levels (6.07 and 5.14 mg/l, respectively), and the difference was statistically significant according to the ER status (P-value=0.021).

Receiver operating characteristic (ROC) curve was generated to obtain the area under the curve (AUC) and to determine the cutoff point of CRP level for predicting high ER+ as shown in Figure 2. Results showed that

CRP could predict the high ER+ with a cutoff of 4.7 mg/l as shown in Figure 2.

To compare studied tumor characteristics between low and high ER subtypes, the distribution of patients in both groups was analyzed, and the results showed that there was a difference according to age, stage, nodal stage, and LNR as shown in Table 3.

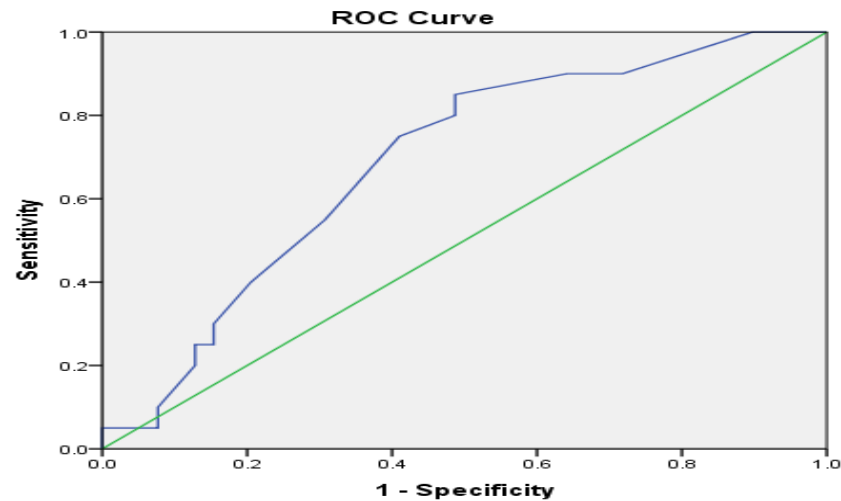


Figure 2: ROC curve of CRP levels for predicting high ER+. Cutoff value of CRP was 4.7mg/l with specificity 52%, sensitivity 80%, and AUC 0.68 ($P < 0.05$).

Table 3: Comparison between ER-low-positive and ER-high-positive BC.

ER-staining intensity subtypes						
Parameter		Low N=28 (23.33%)		High N=30 (25%)		P value
		n	%	n	%	
Age, years	≤46	14	50	4	13.33	0.003
	>46	14	50	26	86.67	
Tumor size, cm	≤2cm	10	35.71	6	23.33	0.189
	2.1 to 5	12	42.86	20	61.67	
	>5	6	21.43	4	15	
Diagnosis	IDC	26	92.86	26	86.67	0.695
	ILC	2	7.14	2	6.67	
	Mucinous	0	0	2	6.67	
Histological grade	I	0	0	0	0	0.05
	II	16	57.14	28	93.33	
	III	6	42.86	2	6.67	
Stage	I	0	0	2	6.67	0.009
	II	6	21.43	16	53.33	
	III	22	78.57	12	40	
Nodal stage, pN	1 to 4	2	7.14	14	46.67	0.001
	>4	26	92.86	16	53.33	
LNR	Low risk (0–0.2)	2	7.14	16	53.33	0.001
	Intermediate risk (>0.2–0.65)	22	78.57	12	40	
	High risk (>0.65–1)	4	14.29	2	6.67	

5. Discussion

BC is a common disease that affects millions of people worldwide, and it can be serious and life threatening. However, with advances in treatment and early detection,

there have been improvements in outcomes and survival rates for those affected. It could be classified according to hormone receptor testing as ER-negative BC or ER-positive BC^[14]. ER-positive BC is the most common type, approximately 70% of all cases, and it is more sensitive

to endocrine therapy compared to ER-negative BC^[15]. ER-low-positive BC is defined as tumors with ER 1–10% expression^[9]. This small subgroup accounts up to 2–7% of all patients and is likely to have unique features, and therefore, it has distinct therapeutic response compared with ER-moderate-positive BC and ER-high-positive BC^[16].

The prevalence of ER-low-positive BC in this study was higher (23.33%) in comparison with that in the previous study^[17]. Unfortunately, ER-low-positive BC has a more similar outcome to ER-negative BC than to ER-positive BC in terms of overall survival (OS)^[18].

This study suggested that ER-low-positive BC is more prevalent among females who were diagnosed as IDC with tumor size (2.5–5 cm), histological grade II, stage III, nodal stage >4, and LNR (**>0.2–0.65**).

In comparison to ER-high-positive BC, there was a significant difference based on certain tumor characteristics. Specifically, it was found that ER-high-positive BC is more common in females aged over than 46 years, while the prevalence was equal in ER-low-positive BC among both age groups (P-value=0.003). Furthermore, ER-low-positive BC was found to be more prevalent among patients with stage III disease (78.57%), higher nodal stage (92.86%), and higher LNR (14.29%) compared to ER-high-positive BC (P-value=0.009, 0.001, and 0.001 respectively). These unique differences in tumor characteristics may explain why ER-low-positive BC responds differently to endocrine therapy and has a different overall survival rate compared to ER-high-positive BC.

CRP levels for all patients were measured to estimate the degree of inflammation present in the tumor microenvironment. The results revealed that patients with ER-high-positive BC and ER-moderate-positive BC had higher CRP levels compared to those with ER-low-positive BC (8.06, 6.38, and 5.14 mg/L, respectively). In addition, ER-low-positive BC patients had lower CRP levels compared to ER-negative patients (6.07 mg/L).

It is important to note that our study has a retrospective, single-center design, which can limit the availability of data when compared to multi-center studies. However, this design offers more control over potential confounding variables that could influence the study outcomes by minimizing variability in patient characteristics and treatment approaches.

6. Conclusion

This study found a significant difference between ER-low-positive and ER-high-positive BC according to some tumor characteristics, which may explain the variation

in response to treatment and increased the mortality of some ER-positive BC taking selective estrogen receptor modulators.

Abbreviations

Breast cancer, BC; global cancer observatory, GCO; estrogen receptor, ER; progesterone receptor, PR; human epidermal growth factor receptor-2, Her-2; C-reactive protein, CRP; College of American Pathologists, CAP; American Society of Clinical Oncology, ASCO; immunohistochemistry, IHC; overall survival, OS; triple-negative BC, TNBC; invasive ductal carcinoma, IDC; invasive lobular carcinoma, ILC; mucinous carcinoma, MC; American Joint Committee on Cancer, AJCC; lymph node ration, LNR; receiver operating characteristic, ROC; area under the curve, AUC.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Scientific Research Ethics Committee at the Tishreen University. All patients have provided a written informed consent for the case details to be published, and all patients provided an informed consent to have their medical records reviewed for this study.

Statement of Ethics

We confirm that we have read and understood the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of Interest Statement

The authors declare that there is no conflict of interest associated with this study.

Author Contributions

Design: R. A (Remal Abdulaziz Asaad), H. S (Hla Sudan), B. H (Bailasan Hasan); Supervision: R. A, S. A (Siraj Saadaldin Abdullah); Materials: S.A; Data collection and/or processing: S.A; Analysis and/or interpretation: R. A, H. S; Literature search: R. A, H.

S; Writing: R. A, H. S, B. H; Critical reviews: R. A, H. A, B. H, S. A. All authors have read and approved the manuscript.

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