



# Correlation of Allred Score with Tumor Behavior in Breast Cancer Patients Referred to Shahid-Mohammadi Hospital From 2010 to 2017

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## Abstract

**Background:** Breast cancer is one of the most common malignancies in women around the world, which makes it essential to identify the behavior of tumors regarding cost-effectiveness and highly diagnostic methods.

**Objectives:** The aim of this study was to determine the relationship between Allred score and tumor behavior in breast cancer patients.

**Methods:** This study was performed on 100 patients with breast cancer. The following data were obtained for each participant: Patient satisfaction and demographic data, tumor size, tumor grade, lymph node involvement and histology of tumor, and Allred score. Chi-square, T student and analysis of variance (ANOVA) tests were used to compare the data.

**Results:** The results of this study showed that Allred score based on PR and ER had a reverse and significant correlation with tumor size ( $P < 0.05$ ). On the other hand, Allred negative findings were found to be higher in patients with lymph nodes involvement ( $P < 0.05$ ).

**Conclusions:** Considering the benefits and potentials of Allred score based on PR and ER in detecting tumor behavior and according to the high prevalence of breast cancer, it is suggested for the results of this study to be provided to specialists from different health centers to improve the correct prediction of tumor behavior.

**Keywords:** Breast Cancer, Allred Score, Tumor Behavior

## 1. Background

Breast cancer is the most common cancer in women, leading to death among women (1), where 520 000 individuals died in 2012 from breast cancer (2). Breast cancer therapeutic decision is mostly guided by histomorphological features of tumor tissue, such as expression of hormonal receptors (estrogen (ER) and progesterone receptors (PR)) and HER2 (3). Some studies proved that patients with ER-/PR+ and ER+/PR+ breast cancer are candidates for endocrine therapy, such as tamoxifen, raloxifene, and aromatase inhibitors drugs (including anastrozole and letrozole); on the other hand, ER-/PR+ individuals seem to benefit less from adjuvant tamoxifen than ER+/PR+ (4). Moreover, histomorphological features predict the behavior of breast cancer and overall survival, for example ER expression confers better prognosis (5, 6) and PR- or HER2- breast cancers are more likely to be aggressive (7, 8). Thus, according to the acceptable accuracy and accessibility of the evaluation of breast cancer HR status following Immuno-

histochemistry (IHC) analysis and providing the most accurate scoring method is needed for evaluating breast cancer clinical outcomes, which includes ER and PR statuses (9). Since various scoring systems were used at different centers of the world for this purpose, the Allred scoring system was introduced for assimilation of these systems (10). Moreover, considering the need for a non-invasive method for evaluating the lymphatic status of patients with breast cancer, and that the Allred scoring system can be measured for all patients with breast cancer (11-13), and since there is not enough prospective studies on this issue.

## 2. Objectives

The current study was designed to evaluate the correlation of Allred score with tumor behavior in breast cancer patients.

### 3. Methods

#### 3.1. Participants

This cross-sectional study was conducted at the Surgery and Pathology Department of Bandar Abbas Shahid Mohammadi Hospital, from January 2010 to March 2017. All cases were revised by a pathologist, and breast cancer diagnosis was confirmed. Inclusion criteria consisted of presence of breast cancer and having been referred to the Surgery and Pathology Department of Bandar Abbas Shahid Mohammadi Hospital, and having undergone IHC staining for ER and PR. Exclusion criteria consisted of physically inapplicable LAM, as well as triple negative breast cancer and ER, and PR negativity (Figure 1).

#### 3.2. Study Design

The current study evaluated 110 LAMs and, one hundred cases with breast cancer, who had been diagnosed by a pathologist and based on inclusion and exclusion criteria were included. The study received ethics approval from the Ethics Committee of Hormozgan University of Medical Sciences.

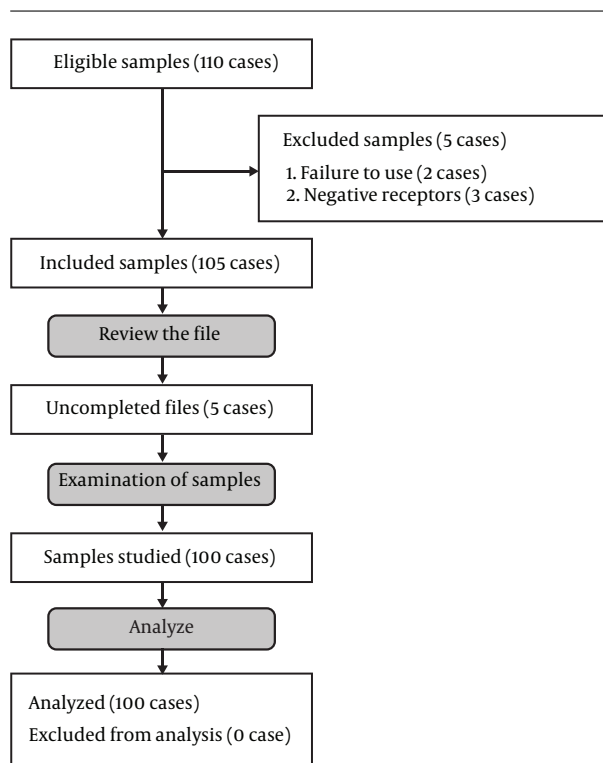


Figure 1. Study flowchart

Demographic data, disease information, and tumor characteristics were recorded in a prepared questionnaire.

From formalin-fixed, paraffin-embedded sample tissues from breast cancer, eight 4- $\mu$ m sections were cut serially and mounted on pre-coated monoclonal antibody slides. IHC assays were performed as described in previous reports (5, 6). Anti-ER and anti-PR mouse monoclonal antibody were used for IHC by the BioGenex system, according to the manufacturer's instructions (BioGenex Corp) (Using an automated i6000 immunostainer).

Tumor Allred ER and PR scores were scored based on previous studies performed by Daltoe et al. (9). Allred score was based on the proportion of colored cells (score 0: negative; score 1: < 1%; score 2: 1% - 10%; score 3: 10% - 33.3%; score 4: 33.3% - 66.6%; and score 5: > 66.6%) and the intensity of colored cells (score 0: negative; score 1: weak; score 2: intermediate; and score 3: strong). Finally, the two scores (proportion score and intensity score) were combined, and the total score of Allred was given, considering an A-score of 0 to 8, and score 0 and 2 were negative scores, and 3 to 8 were positive scores.

#### 3.3. Data Analysis

Statistical analysis of data was performed using the SPSS version 24 software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact test were used to compare qualitative variables between groups. Kolmogorov-Smirnov test was used in order to evaluate the normal distribution of all quantitative studied parameters. Student *t*-test was used for variables with normal distribution, on the other hand Mann-Whitney and Wilcoxon tests were used for variables without a normal distribution. In order to evaluate the differences between more than two groups, analysis of variance (ANOVA) was used. A two tailed P value of less than 0.05 was considered significant.

### 4. Results

This study was performed on 100 patients. The mean age of the patients was  $50.07 \pm 13.97$  years old (21 to 92 years). Eighty-eight cases (88%) had an invasive ductal carcinoma and 65.2% had grade II tumors and 63% had lymph node involvement and 47% had HER2 positive tissue.

The ER and PR Allred score based on grouping age, tumor type, tumor grade, and lymph node involvement did not differ statistically ( $P > 0.05$ ). However, it was found that the mean score of Allred (based on ER and PR) in patients with tumor size of < 20 ( $\text{mm}^3$ ) (multiplication of length, width, and height) were significantly higher than patients with tumor size < 20 ( $\text{mm}^3$ ) (5.14 versus 3.98,  $P =$

0.049 and 4.24 versus 2.96,  $P = 0.032$ , respectively). Moreover, the mean score of Allred (based on ER and PR) in patients with HER2-positive tissues were significantly higher than patients with HER2 negative tissues (5.66 versus 3.31,  $P < 0.001$  and 4.9 versus 2.23,  $P < 0.001$ , respectively) (Table 1).

By performing the Pearson correlation analysis, allred score based on ER had a reverse and significant relationship with tumor size ( $r = -0.226$  and  $P = 0.024$ ). However, allred score based on PR was not significantly associated with any of the quantitative variables ( $P > 0.05$ ).

It was also found that the frequency of Allred ER in patients with different types of tumors (including secretion, medullary, and mucinous breast cancer) was significantly higher than Allred ER+ (13.8% versus 1.4%) ( $P = 0.028$ ). Also, the frequency of Allred ER- in patients with lymph node involvement was significantly higher than Allred ER+ (79.3% versus 56.3%) ( $P = 0.031$ ). However, there was no significant difference in the frequency of Allred PR based on the tumor's behavior ( $P > 0.05$ ) (Table 2).

Also, there was no significant difference in the frequency of HER2/Allred ER and HER2/Allred PR on the basis of tumor behavior ( $P > 0.05$ ).

## 5. Discussion

The results of this study showed that Allred score based on PR and ER had a reverse and significant relationship with tumor size, and it was found that negative Allred/ER was more in patients with lymph node involvement.

In a study performed by Qureshi and Pervez it was shown that the sensitivity and specificity of Allred method based on ER were 99.4% and 99.5% whereas for the conventional scoring system these were 88.0% and 84%, respectively (10). Although the diagnostic accuracy of Allred score in the current study was not investigated, yet it was found that in 100 patients, Allred index based on ER was significantly higher than PR and had a direct and significant relationship with low-grade tumors. As the size of the tumor was lower, this index increased, and the Allred index decreases in patients with HER2 positivity. Moreover, Yamashita et al. demonstrated that the proportion of Allred score alone might be enough to predict post-relapse survival in metastatic breast cancer and hormone responsiveness (14). Although the current study found that Allred index based on ER had a significant correlation with tumor behavior, a longitudinal study is needed on the Iranian population in order to find the diagnostic accuracy of Allred index based on ER on predicting treatment response and five years overall survival in breast cancer pa-

tients. Horii et al. showed that based on the proportion and the intensity of Allred score, a significant difference was observed in five years and overall survival for a cut-off in total scores between four and five points, which emphasized on the role of Allred index in predicting tumor behavior (15).

In another study performed by Tang et al. showed that lower Allred score for PR, luminal B subtype, tubal formation, and mitosis are strongly correlated with a higher recurrence score (16). However, this study found that negative Allred/ER was higher in patients with lymph node involvement and Allred/PR did not have such correlation. These differences may be due to different sample sizes, different types of breast cancer, different inclusion and exclusion criteria, and different study type. Ahmed showed that increased tumor size and grade, ER and PR negativity based on Allred score and HER2 positivity were strong indicators of axillary lymph node involvement, and only tumor size and HER2 expression were independent predictors of axillary lymph node involvement on multivariate analysis (12). However, it was found that negative Allred score based on ER was greater in patients with lymph node involvement and Allred/PR did not have a significant correlation with lymph node involvement. On the other hand, it was found that tumor size is significantly correlated with Allred score based on PR and ER. Lee et al. showed that the percentage of ductal carcinoma in situ within the breast tumor was negatively correlated with Allred score based on ER, yet positively correlated with average HER2 copy number and HER2/CEP17 ratio, and ratio of metastatic lymph node number was significantly correlated with average HER2 copy number HR negativity hormone receptor (17).

### 5.1. Conclusions

The results showed that Allred score based on PR and ER had a reverse and significant relationship with tumor size, and it was found that negative Allred/ER was more in patients with lymph node involvement. This allows the prediction of tumor behavior and response to treatment immediately in surgery department and more critically ill patients may benefit from these advantages of this scoring system, therefore, training this method to pathologists should be considered in Iran or other countries. Further studies are required to confirm the findings.

### Supplementary Material

Supplementary material(s) is available [here](#) [To read supplementary materials, please refer to the journal website and open PDF/HTML].

**Table 1.** Allred Score Based on Tumor Behavior

Variables	Allred Score			
	ER	P Value	PR	P Value
<b>Age, y</b>				0.163
< 50	4.61 ± 2.71	0.847	4.05 ± 3.13	
≥ 50	4.5 ± 3.25		3.17 ± 3.13	
<b>Type</b>				0.44
Lobular carcinoma	5.57 ± 2.63	0.1	4.28 ± 3.19	
Invasive ductal carcinoma	4.62 ± 2.92		3.67 ± 3.13	
Etc.	2 ± 3.46		2 ± 3.46	
<b>Grade</b>				0.11
I	5.66 ± 1.58	0.41	5.75 ± 1.98	
II	4.74 ± 2.98		3.58 ± 3.12	
III	4.13 ± 3.19		3.04 ± 3.31	
<b>Size</b>				0.032
≤ 20 (mm <sup>3</sup> )	5.14 ± 2.84	0.049	4.32 ± 3.13	
> 20 (mm <sup>3</sup> )	3.98 ± 3.01		2.96 ± 3.05	
<b>Lymph node involvement</b>				0.082
Negative	5.27 ± 2.44	0.161	4.35 ± 3.02	
Positive	4.14 ± 3.18		3.2 ± 3.16	
<b>HER2</b>				< 0.001
Negative	5.66 ± 2.19	< 0.001	4.9 ± 2.85	
Positive	3.31 ± 3.25		2.23 ± 2.87	

**Table 2.** Frequency of Allred ER/PR Based on Tumor Behavior<sup>a</sup>

Variables	Allred Score					
	Allred ER-	Allred ER+	P Value	Allred PR-	Allred PR+	P Value
<b>Type</b>			0.028			0.176
Lobular carcinoma	1 (3.4)	6 (8.5)		5 (4.8)	5 (8.8)	
Invasive ductal carcinoma	24 (82.8)	64 (90.1)		36 (85.7)	51 (89.5)	
Etc.	4 (13.8)	1 (1.4)		4 (9.5)	1 (1.8)	
<b>Grade</b>			0.115			0.117
I	0	9 (13.8)		1 (2.7)	7 (13.7)	
II	16 (66.7)	42 (64.6)		24 (64.9)	34 (66.7)	
III	8 (33.3)	14 (21.5)		12 (32.4)	10 (19.6)	
<b>Lymph node involvement</b>			0.031			0.12
Negative	6 (20.7)	31 (43.7)		12 (28.6)	25 (43.9)	
Positive	23 (79.3)	40 (56.3)		30 (71.4)	32 (56.1)	
<b>Size</b>			0.123			0.051
≤ 20 (mm <sup>3</sup> )	11 (37.9)	39 (54.9)		16 (38.1)	33 (57.9)	
> 20 (mm <sup>3</sup> )	18 (62.1)	32 (45.1)		26 (61.9)	24 (42.1)	

<sup>a</sup> Values are expressed as No. (%).

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## Footnotes

**Conflicts of Interests:** The authors have indicated that they had no conflict of interest regarding the content of this article.

**Ethical Considerations:** The study received ethics approval from the Ethics Committee of Hormozgan University of Medical Sciences.

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