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ORIGINAL ARTICLE

A new predictive marker for predicting response after neoadjuvant chemotherapy in hormone receptor positive/ HER2-negative patients: a logarithmic model

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Summary

Purpose: *Estrogen receptor (ER) and progesterone receptor (PgR) levels as well as Ki-67 expression levels are independent predictive markers in patients with hormone receptor-positive breast cancer. In this study, we investigated the predictive significance of the formula of log (ER)*log (PgR)/Ki-67, which was created using 3 independent predictive markers, for the pathological complete response of the Hormone Receptor (HR)-positive/HER2-negative breast cancer patients receiving neoadjuvant chemotherapy (NACT).*

Methods: *This retrospective study included 126 patients with HR-positive/HER2-negative breast cancer and axillary lymph node metastasis who received NACT. The log (ER)*log (PgR)/Ki-67 value was calculated from the pre-NACT pathological evaluation results in all patients. We determined the ideal predictive cut-off value, which separates patients into 2 groups according to pathological complete response (pCR) and pathological non-complete response (non-pCR), using Receiver Operating Characteristic (ROC) curve analysis. According to this cut-off point, patients were divided into 2 groups as cut-off ratio^{high} and cut-off ratio^{low} and were compared using logistic regression analysis along with clinicopathological features.*

Results: *According to the predictive model, we estimated the ideal cut-off value that distinguishes patients as pCR and non-pCR to be 0.12 (p=0.015). According to this cut off value, %54.8 of the patients were categorized as cut-off value^{high} and %46.2 were cut-off value^{low}. The non-pCR rates of the groups were 91.3% and %71.9, respectively(p=0.004). A cut-off value of 0.12 provided the feature of being a predictive marker in the univariate analysis for distinguishing between pCR and non-pCR (OR=4.09 95% CI 1.48-11.33, p=0.007), and it preserved this feature in the multivariate analysis. (OR=3.27, 95% CI 1.12-9.56, p=0.030).*

Conclusion: *The formula of log (ER)*log (PgR)/Ki-67 can be used as a simple and easy-to-use predictive marker for response to neoadjuvant therapy in patients with HR-positive/ HER2-negative breast cancer receiving NACT.*

Key words: *breast cancer, neoadjuvant chemotherapy, new predictive marker, hormone positive, pathologic complete response*

Introduction

Breast tumors are biologically diverse according to their molecular features such as estrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor (HER2), and exhibit different clinical behaviours due to this diversity [1]. Hormone receptor (HR)-negative (ER

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and PgR negative) subgroup is associated with a high response rate to neoadjuvant chemotherapy (NACT), while the response of HR-positive (ER or PgR positive) subtype to neoadjuvant chemotherapy (NACT) is variable [2,3]. Still, NACT can be a preferred treatment strategy for the HR-positive patients due to its potential advantages such as axillary downstaging, higher rates of breast-conserving surgery, and assessment of early *in vivo* response to systemic treatment [4].

With predictive significance in ER, PgR and Ki-67-based classifications, the European Society for Medical Oncology (ESMO) recommends a cut-off value of 20% with insufficient evidence for PgR in the distinction between Luminal A and Luminal B, and also there is great uncertainty for Ki-67 cut off values in terms of categorization. In the breast cancer pathological classification of ESMO, all patients having ER level 1% or more are considered hormone positive and ER expression is not considered to have an pivotal role for distinguishing between luminal A and luminal B [5]. However, studies have shown the predictive importance of the degree of hormone receptor expression in terms of response to NACT[6-8]. As a result, classical luminal classification remains insufficient for guidance of NACT decision making process. A more precise predictive model is required which evaluates ER, PgR and ki-67 expression in combination, and delineates the importance of hormone receptor expression levels and also resolves the existing cut-off problems.

The log-transformation method is recommended for skewed data in predictive markers for breast cancer [9]. This method is commonly applied during statistical analysis in many of the contemporary studies [10-12]. This method, which can also be applied for quantitative determination of estrogen receptor levels, enables standardization of measurements [13].

In this study, we aimed to investigate the predictive significance of the value of log (ER)*log (PgR)/Ki-67 estimated before chemotherapy for response to NACT in patients receiving NACT and to determine an ideal cut-off value for this new formula.

Methods

Patients

We retrospectively analyzed the data of hormone receptor-positive (ER or PgR-positive), HER2-negative and positive clinical or pathological lymph nodes of 126 patients who received NACT followed by surgery between February 2014 and December 2019 at Tekirdag Namik Kemal University Hospital. Tissue and lymph node biopsies of all patients were evaluated immunohistochemically by the same pathologist. In patients without lymph node biopsy, lymph node positivity was determined according to the involvement on PET-CT or magnetic resonance imaging performed before NACT. The study included patients who received either 4 cycles of docetaxel every 3 weeks (75mg/m^2) or 12 cycles of paclitaxel weekly (80mg/m^2) after 4 cycles of cyclophosphamide+epirubicin. The exclusion criteria were considered as follows: HER2 positivity, hormone receptor (HR) negativity, receiving different neoadjuvant therapy, lymph node negativity or metastasis during the NACT period. Clinical and pathological tumor staging was based on the Union Internationale Contre le Cancer (UICC), TNM classification of malignant tumours, 8th edition.

The local institutional review board approved the project and this study conformed to the provisions of the 1995 Declaration of Helsinki. All patients provided informed consent, and the Local Ethics Committee of Tekirdag Namik Kemal University gave formal approval to this retrospective study (approval no: 2020.238.10.06 on 27th October, 2020). This study adheres to the REMARK guidelines [14].

Pathological assessments

All breast cancer tru-cut biopsies and post-chemotherapy surgical specimens (if they contained tumor tissue) were processed for immunohistochemistry (IHC). Staining was performed by applying Estrogen (SP1. Ventana), Progesterone (1E2. Ventana), c-ErbB2 (anti-HER-2 / neu; 4B5. Ventana), and Ki-67 (30-9. Ventana) antibodies using a BenchMark XT automated slide-staining system. Estrogen, Progesterone, HER-2 and Ki-67 results were evaluated under an Olympus CX41 microscope according to the CAP Breast Biomarker Template (Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Breast-2020). In cases of score 2+ HER2 by immunohistochemistry, dual in-situ hybridization test was performed on the same material.

Our pathology laboratory reported the Ki-67 cut-off value as 18 for luminal separations for our center, and this cut-off value was used for categorization of tumors according to the luminal subtypes and Luminal B and Luminal B distinction. Surrogate molecular subtypes were defined as Luminal A, Luminal B/HER2-negative, Luminal B/HER2-positive, HER2-enriched, and Triple-negative, as already described [15].

Predictive model and data collection

In the formula of log (ER)*log (PgR)/Ki-67, log (ER) defines the base-10 logarithm of the estrogen receptor level, log (PgR) defines the base-10 logarithm of the progesterone receptor level, and Ki-67 defines the proliferation index without "%". Values with ER zero (0) or PgR zero (0) are considered 0 as they do not cut the logarithm curve (Appendix 1; Figure S1).

The clinical data, treatments administered, and their results along with the pathological data of all patients were retrospectively gathered and entered in an anonymized dedicated database.

Study endpoint

The primary objective of the study was to verify the possible predictive value of log (ER)*log (PgR)/Ki-67 for non-pCR in HR-positive and HER2-negative patients and to determine the ideal cut-off for this.

We defined pathological complete response (pCR) from the postoperative surgical specimen as no residual invasive tumor and absence of any invasive tumor in lymph nodes (ypT0/ypTis, ypN0). All patients with pathological T stages and N stages, the presence of isolated tumor cells and micrometastases in the lymph node and breast tissue was defined as non-PCR.

Statistics

Since this formula has not yet been defined in the literature, a specific cut-off has not been validated. Therefore, the best cut-off points were calculated considering the maximum (sensitivity and 1-specificity) point of the Receiver Operating Characteristic (ROC) curve for the prediction of non-pCR.

The correlations between log (ER)*log (PgR)/Ki-67, non-pCR, and other key clinical-pathological features were evaluated by Pearson's correlation analysis. Univariate and multivariate analyses were performed using a logistic regression model. Odds Ratio (OR) was reported with the corresponding 95% confidence intervals (95% CI), and a p-value of <0.05 was considered statistically significant. Statistical analyses were performed using the SPSS Statistical software version 24 (SPSS Inc., Chicago, III).

Results

Patient and tumor baseline characteristics

In this study, the data of 126 patients with hormone receptor (HR) positivity, HER2 negativity, and lymph node metastasis who received NACT and who were subsequently then operated were analyzed.

The median age of the patients was 50 (range 28-79) years. When patients were divided into molecular subtypes, 31 patients (24.6%) were luminal A and 95 (75.4%) were luminal B/HER2-negative. Prevalent histology was invasive ductal carcinoma (83.3%), and the majority of cases was grade 2 at the time of diagnosis (60.3%) (Table 1).

Table 1. Distribution of clinical and pathological features of all patients according to the predictive formula derived cut-off value

Clinical and pathological features	*Total (n=126) n (%)*	*New Formula <0,12 (%) (n=57) n (%)*	*New Formula ≥0,12 (%) (n=69) n (%)*	*p*
Age				
<40 (young adult)	29 (23.0)	23 (79.3)	6 (20.7)	0.001
≥40	97 (77.0)	34 35.1)	63 (64.9)	
Molecular subtype				
Luminal A*	31 (24.6)	0 (0.0)	31 (100.0)	<0.001
Luminal B/HER2-negative	95 (75.4)	57 (60.0)	38 (40.0)	
Ki-67				
<18	43 (34.1)	7 (16.3)	36 (83.7)	<0.001
≥18	83 (65.9)	50 (60.2)	33 (39.8)	
Histologic type				
Invasive ductal	105 (83.3)	49 (46.7)	56 (53.3)	0.471
Others	21 (16.7)	8 (38.1)	13 (61.9)	
Grade				
Grade 1	12 (9.5)	5 (41.7)	7 (58.3)	0.399
Grade 2	76 (60.3)	38 (50.0)	38 (50.0)	
Grade 3	38 (30.2)	14 (36.8)	24 (63.2)	
Clinical T stage				
T1	25 (19.8)	11 (44.0)	14 (56.0)	0.890
T2-T3	101 (80.2)	46 (45.5)	55 (54.5)	
Complete response (pCR) (T0N0)				
No	104 (82.5)	41 (39.4)	63 (27.3)	0.004
Yes	22 (17.5)	16 (72.7)	6 (60.6)	

*Luminal-A; ER (+), HER2 (-) and either PgR ≥20% or Ki-67<18

*Relationship between baseline characteristics and log (ER)*log (Pgr)/Ki-67*

The best good predictive value to distinguish non-pCR from pCR in patients receiving NACT is determined as 0.12 using ROC curve (Figure 1). This value allowed identifying two separate populations: cut-off ratio ^{low} (<0.12), 57 patients (45.2%), and cut-off ratio ^{high} (≥0.12) 69 patients (54.8%) (n=126, AUC=0.665, p=0.015). The sensitivity and specificity of this value to identify non-PCR patients were 60.5% and 72.7%, respectively.

According to this cut-off value, the pathological and clinical characteristics of the patients were determined. There was no significant difference in their clinical T (p=0.890), histologic type (p=0.471) and grade (p=0.399) in the intergroup comparison carried out according to the cut-off value (Table 1) .

Relationship between baseline characteristics and non-pCR or pCR

Of the patients who underwent surgery following NACT, 104 (82.5%) achieved non-pCR and

Table 2. Baseline patient characteristics (n=126) and corresponding pCR and non-pCR rates

Clinicopathologic characteristics	*pCR (%) n=22 n (%)*	*non-pCR n=104 n (%)*
Age		
<40 (young adult)	9 (31)	20 (69.0)
≥40	13 (13.4)	84 (86.6)
Molecular subtype		
Luminal A	3 (9.7)	28 (90.3)
Luminal B/HER2-negative	19 (20.0)	76 (80.0)
Histologic type		
Ductal	19 (18.1)	86 (81.9)
Others	3 (14.3)	18 (85.7)
PgR		
Negative	5 (27.8)	13 (72.2)
Positive	17 (15.7)	91 (84.3)
Ki-67		
<18	5 (11.6)	38 (88.4)
≥18	17 (20.5)	66 (79.5)
Grade		
Grade 1	3 (25.0)	9 (75.0)
Grade 2	12 (15.8)	64 (84.2)
Grade3	7 (18.4)	31 (81.6)
Clinical T stage		
T1	4 (16.0)	21 (84.0)
T2-T3	18 (17.8)	83 (82.2)
Log (ER)*log(PgR)/Ki-67		
Cut-off ^{low} (<0,12)	16 (28.1)	41 (71.9)
Cut-off ^{high} (≥0,12))	6 (8.7)	63 (91.3)

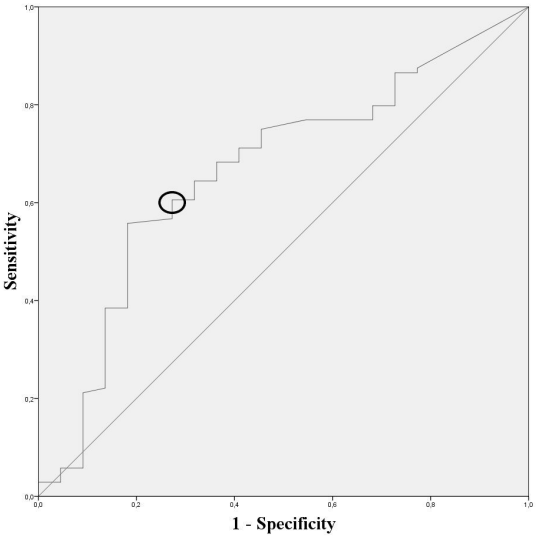

Figure 1. Receiver operating characteristic to determine the ideal cut-off. Circle represents the cut-off point of 0.12 and diagonal segments are produced by ties.

Table 3. Univariate and multivariate analyses of factors for pathological non-complete response (non-pCR) in HR-positive and HER2-negative patients with neoadjuvant chemotherapy (n=126)

Variables	*Category*	*Univariate analysis*		*Multivariate analysis*	
		OR (95% CI)	*p*	*OR (95% CI)*	*p*
Age	Continuous	1.04 (1.00-1.09)	0.034	1.02 (0.98-1.07)	0.199
ER	Continuous	1.01 (0.99-1.02)	0.208		
PgR	Continuous	1.00 (0.99-1.01)	0.449		
Ki67	<18/≥18	0.51 (0.17-1.49)	0.220		
Luminal type	A/B (her2 -)	0.42 (0.11-1.56)	0.199		
Histologic type	ductal/others	1.32 (0.35-4.95)	0.675		
Nuclear grade	1/2/3	1.08 (0.50-2.35)	0.832		
Clinical T Stage	t1/t2-t3	0.87 (0.26-2.87)	0.830		
Log(ER).log(PgR)/ki67	low/high	4.09 (1.48-11.33)	0.007	3.27 (1.12-9.56)	0.030

22 (17.5%) achieved pCR. When the treatment response was evaluated according to the clinical and pathological characteristics of the patients, the rate of non-PCR was the highest in patients with Luminal A subtype (90.3%), while this rate was the lowest in young adult patients (69.0%) (Table 2).

When the treatment responses were analyzed by univariate logistic regression analysis, the number of patients with non-pCR increased as the age increased, with a positive correlation between them (OR 1.046, 95% CI 1.004-1.091, p=0.034). No statistical significance of histological type (p=0.199), nuclear grade (p=0.832), preoperative clinical T stage (p=0.830), and luminal type (p=0.199) was determined in non-pCR and pCR distinction (Table 3).

The new formula of log (ER)*log (PgR)/Ki-67 had a predictive value in treatment response, and those with a cut-off ratio^{high} had approximately 4-fold more non-pCR than those with a cut-off ratio^{low} . (OR=4.09, 95% CI 1.48-11.33, p=0.007). Our new formula preserved its predictive significance when evaluated by multivariate analysis with age. (OR=3.27, 95% CI 1.12-9.56, p=0.030).

Discussion

In this study, 126 patients with hormone receptor-positive, HER2-negative breast cancer and axillary lymph node metastasis were retrospectively evaluated. We estimated the predictive numerical value according to the log (ER)*log (PgR)/Ki-67 formula from the pathology samples of these patients and we found the ideal cut-off ratio value for non-pCR using ROC curve analysis (AUC 0.665, p=0.015). We demonstrated that according to the formula based on ER, PgR and Ki-67, pre-NACT pathology specimens with a cut-off ratio^{high} were good predictive markers for response to treatment in both univariate and multivariate analysis compared to those with a cut-off ratio^{low} (Table 3).

HR-positive breast cancer is the most common subtype of breast cancer, accounting for approximately 78% of all cases [16]. Molecular subtype is significantly associated with response to NACT and HR-positive group has a very poor rate of pCR [17]. In the study by Dave et al, the pCR rate was 6% in luminal A patients and 21% in luminal B/HER2-negative group. In the study by Minckwitz et al, on the other hand, it was 8.9 for luminal A, while it was 15.4% for luminal B/HER2 negative [18]. In our study, the pCR rates were lower in the luminal A group (9.7%), while they were higher in the luminal B (HR + HER2 -) group (20.0%); however, this difference was statistically insignificant (p=0.199).

An ideal predictive marker that will predict the treatment response in HR-positive/HER2-negative patients receiving NACT has not yet been revealed, and research has focused on addressing this [19-27]. Only recently the use of next generation sequencing methods such as Oncotype DX and Mammaprint have been introduced as predictive markers for selection of patients for NACT. There are no standardized cut off values for either method in the context of pCR prediction, however literature supports increasing use of these methods in the coming years [28]. Additionally, both NGS methods

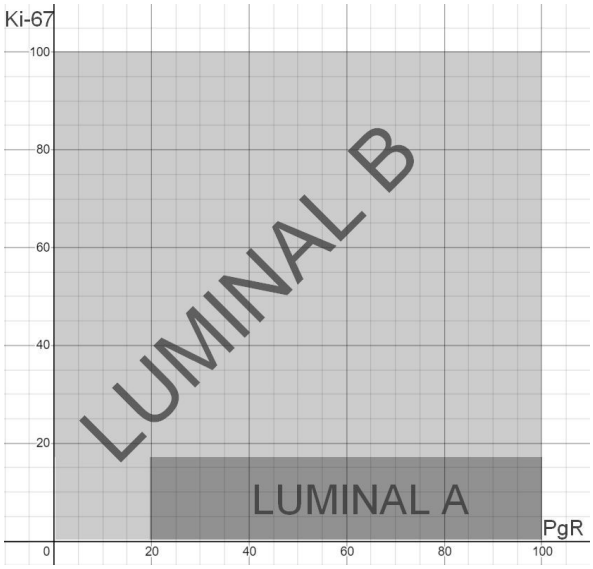

Figure 2. Classical luminal classification for ER≥1 patients.

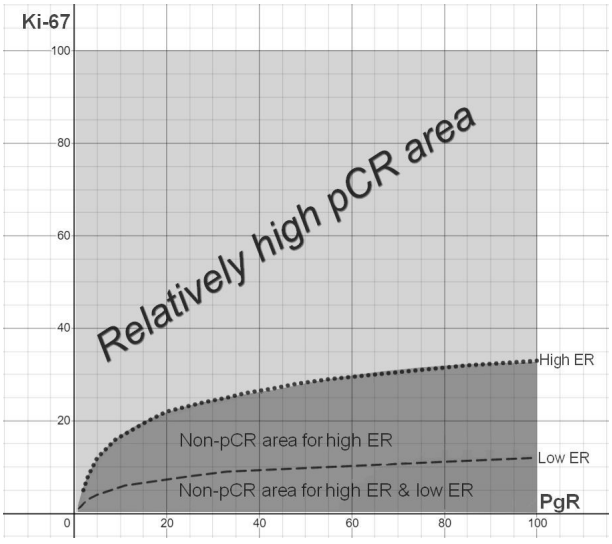

Figure 3. Classification of patients according to the predictive model utilizing ER, PgR and Ki-67 values.

are inaccessible for the majority of breast cancer patients due to high costs and lack of reimbursement. Therefore, there is still a need for development of low cost , standardized and easy to perform tests with a high predictive value for pCR in breast cancer patients [29].

Extensive studies have been conducted on the cellular proliferation marker Ki-67 considered as a predictive marker [30,31]. Although low Ki-67 levels produce a poor response to NACT, retrospective studies have demonstrated that high or very high Ki-67 levels are controversially associated with increased pCR [32,33].In conjunction with Ki-67 ER and PgR expression levels also have predictive roles. Increased expression levels of both ER and PgR are associated with higher non-PCR rates [34,35].

Classical luminal classification, which is based on ER, PgR and Ki-67 expression levels is insufficient in terms of predicting response to NACT. The fact that classification does not discriminate between levels of ER expression may be one of the reasons for the inadequacy of the luminal classification (Figure 2). The predictive model that we developed based on literature data; removes the cut-off uncertainty of Ki-67, as well as integrates ER levels expression as a continuous parameter into the formula (Table S1). The predictive model can sensitively distinguish chemosensitive patients in the luminal B group from the unresponsive ones. (Figure 3) In our analysis, we categorized 54.7% of the HR positive / HER-2 negative patients as cut-off high group with a 91.3% positive predictive value for being non-PCR [36].

In our study, we investigated the treatment responses with the formula of (log (ER)*log (PgR) / Ki67) that we developed using a logarithm in HR-positive/HER2-negative patients receiving NACT. Since this model, which is simple and easy-to-use, was defined by us for the first time, it is the first study in this field.

With the univariate analysis, we concluded that those with a cut-off ratio ^{high} had significantly poorer treatment response and more non-PCR than those with a cut-off ratio ^{low} (OR 4.09, 95% CI 1.48-11.33, p=0.007). The new formula also provided the feature of being an independent predictive factor for pathologic complete response in the multivariate analysis (OR 3.27, 95% CI 1.12-9.56, p=0.030).

Our study has several limitations. First of all, it was a single center, retrospectively designed study. It was not always possible to determine whether a patient would be a candidate for BCS or total mastectomy before and after NACT. Although the treatment choice for all the patients has been discussed in the multidisciplinary tumor board, our study couldn't rule out the possibility of selection bias. HER2+ and triple negative patients were not included in this study, and this predictive model is not applicable for this patient group.

The same neoadjuvant treatment regimen was administered at a single institution, all of the patients pathological examinations were performed by the same pathologist team which is specialized in evaluation of breast cancer tumors therefore the clinicopathologic parameters of the patient population was homogeneous which is the main strength of our analysis.

In conclusion, we demonstrated that the formula of log (ER)*log (PgR)/Ki-67 could be a predictive marker of treatment response in patients with luminal type breast cancer who would receive NACT. Recruitment of larger patient populations would enable construction of a more sensitive and specific predictive model by inclusion of other independent parameters associated with treatment response to neoadjuvant therapy in the hormone positive patient population.

Author contributions

Author contributions: YI, ESS participated in study design, model design, statistics and coordination, and helped interpret the data and wrote the manuscript. MO reported biopsy samples. OA, SOGi HT participated in the data analysis and interpretation, statistics, and critically revised the manuscript. EC, SYT, KK, AY participated in the study design and oriented the data collection. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Local Ethics Committee of Tekirdag Namik Kemal University gave formal approval to this retrospective study (approval no: 2020.238.10.06 on 27th October, 2020).

Additional information

Supplementary Figures and Tables are available at: https://jbuon.com/archive/26-6-C25093-Supplementary-materials.pdf.

Conflict of interests

The authors declare no conflict of interests.

References

1. Perou CM, Sørile T, Eisen MB et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
2. Anderson E. The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Cancer Res 2002;4:197-200.
3. Liedtke C, Mazouni C, Hess K, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008;26:1275-81.
4. Schott AF, Hayes DF. Defining the Benefits of Neoadjuvant Chemotherapy for Breast Cancer. J Clin Oncol [Internet]. 2012;30:1747-9. Available from: https://doi.org/10.1200/JCO.2011.41.3161
5. Cardoso F, Kyriakides S, Ohno S et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019;30:1194-1220.
6. Landmann A, Farrugia DJ, Zhu L et al. Low Estrogen Receptor (ER)-Positive Breast Cancer and Neoadjuvant Systemic Chemotherapy: Is Response Similar to Typical ER-Positive or ER-Negative Disease? Am J Clin Pathol 2018;150:34-42.
7. Ding Y, Ding K, Yu K et al. Prognosis and endocrine therapy selection for patients with low hormone receptor-positive breast cancer following neoadjuvant chemotherapy: A retrospective study of 570 patients in China. Oncol Lett 2019;18:6690-6.
8. Colleoni M, Bagnardi V, Rotmensz N et al. Increasing steroid hormone receptors expression defines breast cancer subtypes non responsive to preoperative chemotherapy. Breast Cancer Res Treat [Internet]. 2009;116:359-69. Available from: https://doi.org/10.1007/s10549-008-0223-y
9. Chapman JW, Murray D, McCready DR. An improved statistical approach: can it clarify the role of new prognostic factors for breast cancer? Eur J Cancer 1996;32:1949-56.
10. Changyong F, Hongyue W, Naiji LU, Tian C, Hua HE, Ying LU. Log-transformation and its implications for data analysis. Shanghai Arch Psychiatry 2014;26:105.
11. Van den Eynden G, Colpaert CG, Vermeulen PB et al. Comparative analysis of the biochemical and immunohistochemical determination of hormone receptors in invasive breast carcinoma influence of the tumor-stroma ratio. Pathol Pract 2002;198:517-24.
12. Nome ME, Euceda LR, Jabeen S et al. Serum levels of inflammation-related markers and metabolites predict response to neoadjuvant chemotherapy with and without bevacizumab in breast cancers. Int J Cancer 2020;146:223-35.
13. Chapman J-AW, Mobbs BG, Hanna WM et al. The standardization of estrogen receptors. J Steroid Biochem Mol Biol 1993;45:367-73.
14. Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An abridged explanation and elaboration. J Natl Cancer Inst 2018;110;803-11.
15. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ. Strategies for subtypes-dealing with the diversity of breast cancer: Highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer 2011. Ann Oncol 2011;22:1736-47.
16. Cancer Stat Facts: Female Breast Cancer Subtypes. Surveillance, Epidemiol End Results Progr [Internet]. Available from: https://seer.cancer.gov/statfacts/html/breast-subtypes.html
17. Houssami N, MacAskill P, Von Minckwitz G, Marinovich ML, Mamounas E. Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. Eur J Cancer 2012;48:3342-54.
18. Von Minckwitz G, Untch M, Blohmer J-U et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796-804.
19. Ueno T, Masuda N, Yamanaka T et al. Evaluating the 21-gene assay Recurrence Score® as a predictor of clinical response to 24 weeks of neoadjuvant exemestane in estrogen receptor-positive breast cancer. Int J Clin Oncol 2014;19:607-13.
20. Denkert C, von Minckwitz G, Darb-Esfahani S et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018;19:40-50.
21. Lips EH, Mulder L, De Ronde JJ, Mandjes IAM, Vincent A, Vrancken Peeters MTFD, et al. Neoadjuvant chemotherapy in ER+ HER2- breast cancer: Response prediction based on immunohistochemical and molecular characteristics. Breast Cancer Res Treat 2012;131:827-36.
22. Groheux D, Hatt M, Hindié E et al. Estrogen receptor-positive/human epidermal growth factor receptor 2-negative breast tumors: early prediction of chemosensitivity with (18)F-fluorodeoxyglucose positron emission tomography/computed tomography during neoadjuvant chemotherapy. Cancer 2013;119:1960-8.
23. Gentile LF, Plitas G, Zabor EC, Stempel M, Morrow M, Barrio A V. Tumor Biology Predicts Pathologic Complete Response to Neoadjuvant Chemotherapy in Patients Presenting with Locally Advanced Breast Cancer. Ann Surg Oncol 2017;3896-902.
24. Naoi Y, Kishi K, Tsunashima R et al. Comparison of efficacy of 95-gene and 21-gene classifier (Oncotype DX) for prediction of recurrence in ER-positive and node-negative breast cancer patients. Breast Cancer Res Treat 2013;140:299-306.
25. Chae SY, Kim SB, Ahn SH et al. A randomized feasibility study of 18F-fluoroestradiol PET to predict pathologic response to neoadjuvant therapy in estrogen receptor-rich postmenopausal breast cancer. J Nucl Med 2017;58:563-8.
26. Xu C, Wei Q, Guo J et al. FOXA1 Expression Significantly Predict Response to Chemotherapy in Estrogen Receptor-Positive Breast Cancer Patients. Ann Surg Oncol 2015;22:2034-9.

27. Al-Saleh K, Abd El-Aziz N, Ali A, Abozeed W, Abd El-Warith A, Ibraheem A, et al. Predictive and prognostic significance of CD8(+) tumor-infiltrating lymphocytes in patients with luminal B/HER 2 negative breast cancer treated with neoadjuvant chemotherapy. Oncol Lett 2017;14:337-44.
28. Fayanju OM, Park KU, Lucci A. Molecular genomic testing for breast cancer: utility for surgeons. Ann Surg Oncol 2018;25:512-9.
29. Chandler Y, Schechter CB, Jayasekera J et al. Cost effectiveness of gene expression profile testing in community practice. J Clin Oncol 2018;36:554.
30. Luporsi E, André F, Spyratos F et al. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: analytical and critical review. Breast Cancer Res Treat 2012;132:895-915.
31. Stuart-Harris R, Caldas C, Pinder SE, Pharoah P. Proliferation markers and survival in early breast cancer: A systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 2008;17:323-34.
32. Ács B, Zámbó V, Vízkeleti L, Szász AM, Madaras L, Szentmártoni G, et al. Ki-67 as a controversial predictive and prognostic marker in breast cancer patients treated with neoadjuvant chemotherapy. Diagn Pathol 2017;12:1-12.
33. Fasching PA, Heusinger K, Haeberle L et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer 2011;11-13.
34. Petit T, Wilt M, Velten M et al. Semi-quantitative evaluation of estrogen receptor expression is a strong predictive factor of pathological complete response after anthracycline-based neo-adjuvant chemotherapy in hormonal-sensitive breast cancer. Breast Cancer Res Treat 2010;124:387-91.
35. van Mackelenbergh MT, Denkert C, Nekljudova V et al. Outcome after neoadjuvant chemotherapy in estrogen receptor-positive and progesterone receptor-negative breast cancer patients: a pooled analysis of individual patient data from ten prospectively randomized controlled neoadjuvant trials. Breast Cancer Res Treat 2018;167:59-71.
36. Haque W, Verma V, Hatch S, Suzanne Klimberg V, Brian Butler E, Teh BS. Response rates and pathologic complete response by breast cancer molecular subtype following neoadjuvant chemotherapy. Breast Cancer Res Treat [Internet]. 2018;170:559-67. Available from: https://doi.org/10.1007/s10549-018-4801-3.