Journal of BUON **16:** 444-449, 2011

ORIGINAL ARTICLE

Simplified scoring system predicting HER-2 status in patients with breast carcinoma

M. Jovicic-Milentijevic[1], T. Tokic[2], N. Zivkovic[1], T. Dencic[1]

[1]University of Nis, Medical Faculty, Institute of Pathology, Nis; [2]University of Nis, Faculty of Electronic Engineering, Department of Computer Science, Nis, Serbia

Summary

Purpose: *The existing scoring systems HERSI (Kajo 2007) and DIVER (Taucher 2003) are based on the assumption that tumor grade, estrogen (ER) and progesterone receptors (PR) equally affect HER-2 status. Our idea was to propose a simplified scoring system (SIMPLY) which employs tumor characteristics according to their significance.*

Methods: *A total of 621 cases of invasive breast carcinoma with determined tumor grade, size, histological type, ER, PR, lymph node metastasis, lymphovascular invasion, age and HER-2 status were included in the study. The main features of the proposed system were simplicity in the scoring of ER and PR and emphasizing the role of tumor grade as the most important predictor of HER-2.*

Results: *The percentage of cases for which HER-2 assessment could be postponed was 33.98% for HERSI, 42.51% for DIVER, 53.62% for SIMPLY (score 5), and 66.34% for SIMPLY with a subgroup (score 3.5-5), when all scoring systems were applied to the same data set.*

Conclusion: *In the SIMPLY group with the highest score, HER-2 assessment could be delayed, since the results from HER-2 assessment were not expected to affect patient management. SIMPLY could be helpful in improving patient care in countries lacking financial resources.*

Key words: breast cancer, estrogen receptors, grading, HER-2, progesterone receptors, scoring index

Introduction

Breast carcinoma is the most common malignancy among women. The increasing incidence and significant cancer mortality highlight the need for new therapeutic developments, especially targeted treatment. Herceptin (trastuzumab), a humanised monoclonal antibody targeting the HER-2/*neu* gene product, is a prime example of this new class of treatment.

The existing scoring systems, HERSI [1] and DIVER [2], are based on the assumption that tumor grade, ER and PR equally affect HER-2 status. Therefore, while assigning the points in scoring they treat the mentioned factors equally.

Our idea was to propose a simplified scoring system (SIMPLY) which employs in scoring the tumor characteristics according to their correlation to HER-2 status. Both in HERSI and DIVER scoring systems, the percentage or intensity of ER/PR positive tumor cells should be taken into account. Unlike the mentioned approaches, we suggest only recognition of positive or negative ER/PR receptors for HER-2 status prediction, avoiding interpersonal variability.

Aiming at defining subgroups of patients in whom HER-2 assessment could be of minor value in the decision-making concerning adjuvant therapy, we tried to develop SIMPLY. The goal was to develop a model to select patients with HER-2 negative breast carcinomas.

Methods

Patients with invasive breast carcinoma (n=621), whose specimens were studied at the Institute of Pathology, Medical Faculty, University of Nis, between 2007 and 2009, were included in this study. For the final evaluation only cases with the known clinicopathologic characteristics of primary breast carcinoma (tumor

Correspondence to: Maja Jovicic-Milentijevic, PhD. Institute of Pathology, Clinical Center Nis, Novoprojektovana bb, 18000, Nis, Serbia.
Tel: +381 63 104 58 31, E-mail: majajmi@gmail.com

Received 01-02-2011; Accepted 17-04-2011

grade, tumor size, histological type, hormonal receptor status, axillary lymph node metastasis, lymphovascular invasion, patient age and HER-2 status) were included in our study (n=621). Histological types of breast carcinomas were: 395 infiltrating ductal no special type, 75 infiltrating lobular, 118 mixed tumors and 33 other types (14 mucinous, 8 papillary, 7 tubular and 4 cribriform). Cases with medullary carcinomas were excluded. Histological tumor grading was performed according to Elston and Ellis study [3]. Tumor grading, ER and PR were carried out within the frame of primary diagnosis where several pathologists were included analysing 621 cases separately. All cases were reviewed by one pathologist.

Histological examination

Histological tumor examination was performed on 4-5 μm thick H&E-stained sections of formalin-fixed, paraffin-embedded tumors.

Immunohistochemical analysis

Immunohistochemical (IHC) analysis was performed on tissue sections from the paraffin blocks according to the manufacturer`s instructions. For the detection of ER we used anti-ER (clone 1D5, Dako) and for the detection of PR we used anti-PR (clone PgR 636, Dako). The ER and PR status was interpreted using the quick (Allred) score. This is based on the assessment of the proportion and the average intensity of nuclear staining. The scores are summated to give a maximum of 8 [4,5]. Score 0 was negative, 2-5 low positive and 6-8 high positive.

HER-2 IHC analysis

HER-2 IHC analysis was performed using the HercepTest kit (Dako). The recommended scoring method is based on the intensity of membrane staining in more than 10% of tumor cells. Samples scoring 3+ are regarded as positive and 0/1+ as negative. Borderline 2+ results require confirmation using another analysis method [4,5].

Chromogenic in situ hybridization

Chromogenic *in situ* hybridization (CISH) was performed only for patients with 2+ results using IHC (n=39). Unstained 4 μm thick formalin-fixed and paraffin-embedded specimens were used and prepared for evaluation according to the manufacturer`s instructions (Zymed Laboratories, USA).

Statistical analysis

Chi-square test was used to examine the categorical variables and the association between HER-2 overexpression with other clinicopathologic characteristics of breast carcinoma (the significance level was set to $p<0.05$). The correlation coefficients (r) were used to analyze the degree of association between quantitative variants. A positive value of r indicated a positive correlation and a negative value an inverse one. Kappa values (k) were computed to demonstrate discrepancies detected in the histological grading or hormone receptors status between the examining pathologist and the previous routine diagnosis. Thresholds in scoring system were checked by ROC curves. Complete statistical analysis was performed by MedCalc, version 11.4.4 (Belgium).

Results

The median patient age was 61 years (range 29-87). The predominant histological type was ductal infiltrating carcinoma (63.61%), and tumor grade 2 (57.81%). Most of the tumors were pT2 (54.11%) and exhibited no lymph node metastasis (57.16%). Positive ER and PR status was present in 478 (76.97%) and 393 (63.28%) cases, respectively. HER-2 membrane receptor was overexpressed (IHC score 3+) in 95 (15.29%) cases. HER-2 gene amplification assessed by CISH was positive in 8 of 39 (20.51%) cases, which in the final analysis represented 103 cases (16.59%) with positive HER-2 status. HER-2 positivity was predominantly found in invasive ductal carcinomas (19.75%), whereas none of special types of breast carcinomas (mucinous, papillary, tubular and cribriform) expressed HER-2 positivity.

The discrepancies in tumor grade, ER and PR were demonstrated through the following kappa values: $k_G=0.91$ (SE=0.01), $k_{ER}=0.88$ (SE=0.01) and $k_{PR}=0.83$ (SE=0.02).

Basic clinicopathologic characteristics compared with HER-2 status and statistical parameters are shown in Table 1. The corresponding figures showed that tumor grade, ER and PR status of invasive breast carcinomas correlated significantly with a positive HER-2 status ($p<0.0005$). HER-2 status was not affected by other clinicopathologic characteristics.

No tumor grade 1 case showed positive HER-2 status. In contrast, 60.87% of high grade tumors were HER-2 positive. Our study showed a trend of increasing HER-2 positive tumors with increasing from well to poorly differentiated breast carcinomas. An inverse relationship was observed between hormone receptor status and HER-2 status. Only 1.92% (7 out of 365 cases

Table 1. Association between clinicopathological characteristics of the studied patients (n=621) and HER-2 status

Characteristics	*HER-2 status*		*Statistical parameters*	
	Negative n (%)	*Positive n (%)*	*Chi - square*	*Correlation coefficient*
Tumor grade				
G1	124 (100.00)	0 (0.00)	x^2= 253.357	r_1=0.5447
G2	340 (94.71)	19 (5.29)	p<0.0005	
G3	54 (39.13)	84 (60.87)		
Histological type				
Ductal	317 (80.25)	78 (19.75)	x^2= 12.417	r_2=−0.0396
Lobular	68 (90.67)	7 (9.33)	p>0.001	
Mixed	100 (84.75)	18 (15.25)		
Other	33 (100.00)	0 (0.00)		
Tumor size				
pT1	154 (86.03)	25 (13.97)	x^2= 1.398	r_3=0.0461
pT2	278 (82.74)	58 (17.26)	P>0.05	
pT3	86 (81.13)	20 (18.87)		
ER				
Negative	74 (51.75)	69 (48.25)	x^2= 164.765	r_4=−0.5070
Low positive	86 (76.11)	27 (23.89)	p<0.0005	
Highly positive	358 (98.08)	7 (1.92)		
PR				
Negative	151 (66.23)	77 (33.77)	x^2= 92.886	r_5=−0.3929
Low positive	146 (84.88)	26 (15.12)	p<0.0005	
Highly positive	221 (100.00)	0 (0.00)		
Lymph nodes				
Negative	291 (81.97)	64 (18.03)	x^2= 1.246	r_6=−0.0448
Positive	227 (85.34)	39 (14.66)	p>0.2	
LVI				
Negative	250 (84.75)	45 (15.25)	x^2= 0.721	r_7=0.0341
Positive	268 (82.21)	58 (17.79)	p>0.2	
Age (years)				
Range	29-87	x^2= 0.00050	r_8=−0.0240	
Median	61		p>0.5	
>50	427 (82.4)	85 (82.5)		
≤50	91 (17.6)	18 (17.5)		
Total	518 (100.0)	103 (100.0)		

ER: estrogen receptor, PR: progesterone receptor, LVI: lymphovascular invasion

with highly positive ER) and none of the patients with highly positive PR (quick score 6-8) status had HER-2 positive tumors and none of the patients with strongly positive (quick score 6-8) ER or PR status, respectively, had HER-2 positive tumors. The correlation coefficients showed that the best correlations with HER-2 status were found for tumor grade (0.545), ER (-0.507) and PR status (-0.393). HER-2 status (0, 1+, 2+ and 3+) in breast carcinomas is depicted in Table 2.

Association between HER-2 status and the combined hormone receptor status is shown in Table 3. Tumors having both receptors (ER and PR) positive, were less likely to be HER-2 positive (5.73%).

Tumor grade exhibited the strongest correlation with HER-2 status, compared with ER and PR (Table 1), which influenced individual scores: G1 or G2 = 2 points, G3 = 0 points. Individual scorings for both ER and PR were identical: negative = 0, while positive = 1.5 points. We created a simple scoring system to determine the probability of HER-2 status (SIMPLY): G (0-2) + ER (0-1.5) + PR (0-1.5). The classification according to the summarised score in the range 0-5 points is presented in Table 4, as well as the analysis of the

Table 2. HER-2 status (0, 1+, 2+ and 3+) in breast carcinomas

	HER-2 status	
	Negative n (%)	*Positive n (%)*
0	90 (17.37)	–
1+	397 (76.64)	–
2+	31 (5.98)	8 (7.77)
3+	–	95 (92.23)
	518 (100.00)	103 (100.00)

Table 3. Association between HER-2 status and combined hormone receptor status

HER-2	*ER+/PR+ n (%)*	*ER+/PR- n (%)*	*ER-/PR+ n (%)*	*ER-/PR- n (%)*
Negative	362 (94.27)	82 (87.23)	5 (55.56)	69 (51.49)
Positive	22 (5.73)	12 (12.77)	4 (44.44)	65 (48.51)
Total	384 (100.00)	94 (100.00)	9 (100.00)	134 (100.00)

Table 4. Classification according to the SIMPLY scoring system in respect to HER-2 status

SIMPLY score	*HER-2 status*						
	Negative n (%)	*Positive n (%)*	*Total*	*ACC*	a_s	b_s	*c (%)*
0	17 (3.28)	48 (46.60)	65	–	621	103	–
1.5	8 (1.54)	14 (13.59)	22	0.88	556	55	9.89
2	52 (10.04)	17 (16.50)	69	0.89	534	41	7.67
3	29 (5.60)	22 (21.36)	51	0.84	465	24	5.16
3.5	79 (15.25)	2 (1.94)	81	0.83	414	2	0.48
5	333 (64.29)	0 (0.00)	333	0.7	333	0	0.00
Total	518 (100.00)	103 (100.00)					

$x^2 = 192.474$, $p<0.0005$

ACC: accuracy of threshold obtained by ROC curve analysis
a_s: the number of patients with $S \geq t_s$
b_s: the number of HER-2 positive patients with $S \geq t_s$
c (%): the percentage of HER-2 positive patients in respect to a_s

number of HER-2 positive patients with a score greater or equal to the threshold. Thresholds (t) in scoring were checked by ROC curves. The following values for accuracy (ACC) and percentage of HER-2 positive patients were obtained for each threshold: t=1.5, ACC=0.88, 9.89%; t=2, ACC=0.89, 7.67%; t=3, ACC=0.84, 5.16%; t=3.5, ACC=0.83, 0.48%; and t=5, ACC=0.70, 0.00%. The best ratio between sensitivity and specificity was observed for t=2, but the percentage of HER-2 positive cases with SIMPLY Score (S) ≥2 (7.67%) was unacceptable for prediction. An acceptable accuracy of 0.70 was achieved for t=5, with no HER-2 positive patients. That's why we accepted a threshold of 5 to predict HER-2 status. Furthermore, another acceptable accuracy was obtained for threshold 3.5 with only 0.48% of HER-2 positive cases what was acceptable in respect to the resolution of the experiment (621 cases) and we created subgroups using t=3.5 for HER-2 prediction. SIMPLY scoring subgroups compared with HER-2 status are shown in Table 5.

Table 5. SIMPLY scoring subgroups in respect to HER-2 status

SIMPLY subgroup	*HER-2 status*		*Total*
	Negative n (%)	*Positive n (%)*	
I (0-1.5)	25 (4.83)	62 (60.19)	87
II (2-3)	81 (15.64)	39 (37.86)	120
III (3.5-5)	412 (79.54)	2 (1.94)	414
Total	518 (100.00)	103 (100.00)	

$x^2 = 287.563$, $p<0.0005$

Discussion

The development of a system for preliminary evaluation of HER-2 status at the time of primary diagnosis becomes an interesting issue when financial resources are insufficient or lacking. This system would identify a group of patients with a high probability of negative HER-2 status or in whom treatment with trastuzumab is not indicated.

The present study failed to reveal a significant relationship between HER-2 status and following clinicopathologic characteristics: tumor size, histological type, lymph node metastasis, lymphovascular invasion, and patient's age [6-13].

Tumor grade showed the best correlation with HER-2 status (0.545), and was the most suitable HER-2 predictor. Similar results were also achieved in other studies, where a proportion of HER-2 positive cases increased with poorer grade [2,9,10,13].

The inverse relationship between HER-2 status and hormone receptors has been widely described in the literature [1,2,14]. We found that both ER and PR positive tumors had a low incidence of HER-2 overex-

Table 6. HERSI scoring applied to the same data set as SIMPLY

HERSI		
	HER-2 status	
Score	*Negative n (%)*	*Positive n (%)*
0	17 (3.28)	48 (46.60)
1-2	74 (14.29)	52 (50.49)
3-4	216 (41.70)	3 (2.91)
5-6	211 (40.73)	0 (0.00)
Total	518 (100.00)	103 (100.00)

x^2=288.1344, p<0.0005

Table 7. DIVER scoring applied to the same data set as SIMPLY

DIVER		
	HER-2 status	
Score	*Negative n (%)*	*Positive n (%)*
0-6	254 (49.03)	103 (100.00)
7-9	264 (50.97)	0 (0.00)
Total	518 (100.00)	103 (100.00)

x^2=91.313, p<0.0005

pression (5.73%), whereas 48.51% of ER/PR negative tumors were HER-2 positive. The association between HER-2 and hormone receptor status in breast cancer showed that overexpression of HER-2 was associated with lower ER/PR tumor levels [15]. These results are similar to the data presented in our study.

Both scoring systems (HERSI and DIVER) are able to predict HER-2 positive status in daily practice. For the sake of comparison we applied both HERSI and DIVER to our data set. The results are given in Tables 6 and 7, respectively. Let us note the following:

1. The percentage of HER-2 negative status by HERSI in group 5-6 was 40.73%, while by DIVER in the group with highest score 7-9 was 50.97%. SIMPLY gave higher percentage (64.29%) for score 5, and with additional clustering (3.5 and 5 are joined in the same subgroup) it was increased to 79.54%, but with 1.94% HER-2 positive cases. In respect to the total number of patients (621), the percentage of cases for whom HER-2 assessment could be postponed was 33.98% for HERSI, 42.51% for DIVER, 53.62% for SIMPLY (score 5), and 66.34% for SIMPLY in the subgroup with score 3.5-5.

2. Point assignment was easier by SIMPLY, especially for ER and PR, because we reduced their influence by scoring negative-0, and positive-1.5; at the same time the effect of tumor grade was amplified by G1/G2-2, and G3-0. To estimate the likelihood of HER-2 status, it is not necessary to determine the intensity of ER/PR like in DIVER (weakly, medium and strongly positive), or the percentage of positive cells in HERSI (10-50%, and >50%). Simplification for ER and PR (positive, negative) may increase inter-observer consistency.

Therefore, SIMPLY scoring system provides a valuable tool for estimating the HER-2 status in patients with breast carcinoma. The assessment of tumor grade and hormone receptor status is confirmed in the majority of breast units at primary diagnosis in the first step, whereas HER-2 status comes second, mostly in referral centers. The accurate assessment of HER-2 status, which is cost-intensive and time-consuming, should be performed in patients who are most likely to have positive HER-2 status (SIMPLY subgroup I and II), particularly if therapeutic decisions may be based on these results. HER-2 testing may not necessarily be performed for SIMPLY subgroup III, increasing the overall cost effectiveness without losing relevant predictive information.

The proposed scoring system, SIMPLY, is a simple method for selection of patients with HER-2 negative breast carcinomas. SIMPLY gives higher percentage of cases with HER-2 negative status in comparison with HERSI and DIVER on the same data set. Assessment of HER-2 status of cases in the SIMPLY group with the highest score could be delayed, since the results from HER-2 assessment are not expected to affect patient management. In our opinion, SIMPLY scoring system could be helpful in improving patient care, particularly in countries lacking economical, technical and personnel support for breast cancer patients, as it is the case in Serbia and almost in all Western Balkan countries.

References

1. Kajo K, Zubor P, Barthova M, Plank L. Scoring index for prediction of HER-2 status in the invasive breast carcinoma. Exp Oncology 2007; 29: 281-286.
2. Taucher S, Rudas M, Mader RM et al. Do we need HER-2/neu testing for all patients with primary breast carcinoma? Cancer 2003; 98: 2547-2553.
3. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19: 403-410.
4. European guidelines for quality assurance in breast cancer screening and diagnosis (4th Edn). ISBN 92-79-01258-4, European Communities, 2006.
5. Goldhirsch A, Ingle JN, Gelber RD et al. panel members: Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer. Ann Oncol 2009; 1319-1329.
6. Ayadi L, Khabir A, Amouri H et al. Correlation of HER-2 over-expression with clinico-pathological parameters in Tunisian breast carcinoma. World J Surg Oncol 2008; 6: 112. DOI:

10.1186/1477-7819-6-112.
7. Naeem M, Nasir A, Aman Z, Ahmad T, Samad A. Frequency of HER-2/neu receptor positivity and its association with other features of breast cancer. J Ayub Med Coll Abbottabad 2008; 20: 23-26.
8. Bilous M, Ades C, Armes J et al. Predicting the HER2 status of breast cancer from basic histopathology data: an analysis of 1500 breast cancers as part of the HER2000 international study. Breast 2003; 12: 92-98.
9. Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P. Correlation of Her2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. Breast J 2005; 11: 278-280.
10. Huang HJ, Neven P, Drijkoningen M et al. Association between tumor characteristic and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. J Clin Pathol 2005; 58: 611-616.
11. Seo BK, Pisano ED, Kusimak CM et al. Correlation of HER-2/neu overexpression with mammography and age distribution in primary breast carcinomas. Acad Radiol 2006; 13: 1211-1218.
12. Traina A, Agostara B, Marasa L, Calabro M, Zarcone M, Carruba G. HER-2/neu expression in relation to clinicopathologic features of breast cancer patients. Ann N Y Acad Sci 2006; 1089: 159-167.
13. Prati R, Apple SK, He J, Gornbein JA, Chan HR. Histopathologic characteristics predicting HER-2/neu amplification in breast cancer. Breast J 2005; 11: 433-439.
14. Lal P, Tan LK, Chen B. Correlation of HER-2 status with estrogen and progesterone receptors and histological features in 3655 invasive breast carcinomas. Am J Clin Pathol 2005; 123: 541-546.
15. Konency G, Pauletti G, Pegram M et al. Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. J Natl Cancer Inst 2003; 95: 142-153.