Original article

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The prognostic and predictive value of Ki-67 proliferation index and uPA/PAI-1 complex in serum for patients with early invasive breast cancer

Sadat Pušina[1], Edin Hodžić[1], Mirhan Salibašić[1], Emir Bičakčić[1], Naida Herenda-Pušina[2], Emsad Halilović[1]

[1]Clinical Center of University of Sarajev,o Clinic for General and Abdominal Surgery, Sarajevo, Bosnia and Herzegovina

[2]Clinical Center of University of Sarajevo, Clinic for Anestesiology and Reanimatology, , Sarajevo, Bosnia and Herzegovina

Contact: Sadat Pušina;

Bolnička 25, 71000 Sarajevo

E-mail address: sadatpusina1975@gmail.com

Breast cancer, the most common malignancy in women, represents a significant health issue, and biomarkers such as Ki-67 index and uPA/PAI complex can provide insight into treatment outcomes and therapeutic response (1-5). The aim of our retrospective cohort study was to investigate the prognostic and predictive significance of these biomarkers in 166 patients with early invasive breast cancer, surgically treated at the Department of General and Abdominal Surgery, Clinical Center of the University of Sarajevo, in order to contribute to improving the efficacy of their treatment. The main outcome of the study was the assessment of five-year disease-free survival (DFS), defined as the postoperative period until the occurrence of locoregional or distant metastases and death from any cause. Univariate regression analysis identified an increased probability of DFS shorter than five years in patients with negative hormone receptors, positive HER-2 receptor, with ≥8 positive lymph nodes, and Ki-67 index ≥14% ($p<0.05$). Multivariate regression analysis revealed that T2 stage, tumor size of 20-50 mm, and Ki-67 index ≥14% were associated with a higher probability of DFS shorter than five years ($p<0.05$). The five-year DFS rate was higher in patients with Ki-67 index <14% compared to those with ≥14% ($p=0.011$), while there was no difference in five-year DFS among patients with different levels of uPA/PAI-1 complex ($p=0.636$). Our study highlights the importance of Ki-67 proliferative index as a strong prognostic predictive factor for DFS in patients operated for early invasive breast cancer. Additional monitoring and tailored therapeutic strategies may be beneficial in patients with elevated Ki-67 index values, T2 stage, and tumor size of 20-50 mm.

Keywords: biomarkers, general surgery, treatment outcome, women's health

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Prognostičko-prediktivni značaj Ki-67 proliferativnog indeksa i preoperativnih vrijednosti uPA/PAI-1 kompleksa u serumu kod pacijentica sa ranim invazivnim karcinomom dojke

Sadat Pušina[1], Edin Hodžić[1], Mirhan Salibašić[1], Emir Bičakčić[1], Naida Herenda Pušina[2], Emsad Halilović[1]

[1]Klinički centar Univerziteta u Sarajevu, Klinika za opštu i abdominalnu hirurgiju, Sarajevo, Bosna i Hercegovina

[2]Klinički centar Univerziteta u Sarajevu, Klinika za anesteziju i reanimaciju, Sarajevo, Bosna i Hercegovina

Kontakt: Sadat Pušina

Bolnička 25, 71000 Sarajevo

E-mail adresa: sadatpusina1975@gmail.com

Karcinom dojke, najčešći malignitet kod žena, predstavlja značajan zdravstveni problem, a biomarkeri poput Ki-67 indeksa i uPA/PAI kompleksa mogu pružiti uvid u ishode lečenja i terapijski odgovor (1-5). Cilj naše retrospektivne kohortne studije studije bio je istražiti prognostički i prediktivni značaj ovih biomarkera kod 166 pacijentica sa ranim invazivnim karcinomom dojke, hirurški tretiranim na Klinici za opštu i abdominalnu hirurgiju Kliničkog centra Univerziteta u Sarajevu, kako bismo doprinijeli unaprjeđenju efikasnosti njihovog lečenja.
Glavni ishod studije bila je procena petogodišnjeg preživljavanja bez bolesti (DFS), definiranog kao postoperativno razdoblje do pojave loko-regionalnih ili udaljenih metastaza i smrti od bilo kojeg uzroka. Univarijantnom regresionom analizom utvrđena je povećana verovatnoća za DFS kraći od pet godina kod pacijentica sa negativnim hormonskim receptorima, pozitivnim HER-2 receptorom, sa ≥8 pozitivnih limfnih čvorova i Ki-67 indeksom ≥14% ($p<0.05$). Multivarijantnom regresionom analizom utvrđeno je da su T2 stadij, veličina tumora od 20-50 mm i Ki-67 indeks ≥14% povezani sa većom verovatnoćom za DFS kraći od pet godina ($p<0.05$). Petogodišnja stopa DFS-a bila je veća kod pacijenata sa Ki-67 indeksom <14% u odnosu na one sa ≥14% ($p=0.011$), dok nije bilo razlike u petogodišnjem DFS-u među pacijenticama sa različitim nivoima uPA/PAI-1 kompleksa ($p=0.636$). Naša studija ističe važnost Ki-67 proliferativnog indeksa kao snažnog prognostičko prediktivnog faktora za DFS kod pacijentica operisanih zbog ranog invazivnog karcinoma dojke. Dodatni nadzor i prilagođene terapijske strategije mogu biti korisni kod pacijentica sa povišenim vrijednostima Ki-67 indeksa, T2 stadijem i veličinom tumora od 20-50 mm.

Ključne reči: biomarkeri, opšta hirurgija, ishodi liječenja, zdravlje žena

Introduction

Breast cancer is the most common cancer in women worldwide and represents a significant public health issue, being the fifth leading cause of cancer death in the developed world (1, 2).

Early invasive breast cancer, which includes stages T1T2, N0N1, and T3N0, can be genetically analyzed to classify into four main molecular subtypes: Luminal A, Luminal B, HER2-positive, and "basal-like" or "triple-negative" (3,4). Ki-67 antigen proliferative index, a marker of cell proliferation in breast cancer, shows positive protein expression in all phases of the cell cycle (except the G0 phase), with its elevated expression being associated with an increased risk of disease recurrence and a reduced response to systemic therapy (5).

However, due to the lack of standardized laboratory analysis methodology and clear cut-off values for the application of systemic therapy, the Ki-67 proliferative index has not yet been accepted as a universal biomarker for breast cancer prognosis (6).

The occurrence of metastases is the main cause of mortality in breast cancer, with extracellular matrix degradation playing a key role in this process, facilitated by the urokinase plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) complex (7, 8).

Due to its clinical relevance, the uPA/PAI-1 complex determined in tumor tissue or cytosol has been recognized as a prognostic and predictive biomarker for breast cancer, as confirmed by the recommendation of the American Society of Clinical Oncology (9).

In today's medical practice, increasing emphasis is placed on the prognostic-predictive value of genetic panels that play a key role in the individualization of treatment and improvement of treatment outcomes in various diseases, including breast cancer (10). However, in transitional countries, these genetic markers are not yet widely accepted and available in practice (11-13).

Existing shortcomings and controversies underscore the need to investigate the role of Ki-67 proliferative index and uPA/PAI-1 complex in serum and their integration into the

existing concept of prognosis and prediction in patients with early invasive breast cancer.

Aim

The aim of our study was to investigate the prognostic and predictive significance of Ki-67 proliferative index values and preoperative levels of uPA/PAI-1 complex in serum in patients operated for early invasive breast cancer to contribute to the improvement of their treatment efficacy.

Patients and Methods

Our prospective-retrospective cohort study included 166 patients older than 18 years with pathologically verified early invasive breast cancer, surgically treated at the Department of General and Abdominal Surgery, Clinical Center of the University of Sarajevo (CCUS) from September 2015 to February 2017. Patients without cutaneous manifestations of the disease and those without previous premalignant or malignant breast diseases were included. Additionally, patients with a negative history of immune, chemo, radio, and hormonal therapies, as well as those without previous breast or axillary lymph node surgeries, were included.

Patients with advanced forms of cancer, including infiltration and inflammation of the breast skin region, and those with multiple breast cancers were excluded from the study. Patients with systemic liver, kidney, or cardiovascular diseases, as well as those who didn't provide informed consent to participate in the study, were also excluded.

Surgical treatment involved radical modified mastectomies or breast-conserving surgeries. Furthermore, complete dissection of the first and second layers of ipsilateral axillary lymph nodes or sentinel lymph node biopsy was performed (14).

Laboratory tests were conducted at the Clinical Biochemistry with Immunology Department of CCUS. Preoperative concentration of uPA/PAI-1 complex in serum ranged from 0.1 to 100 ng/ml according to the manufacturer's instructions (15).

Pathohistological analysis was performed at the Clinical Pathology, Cytology, and Human Genetics Department of CCUS. The threshold value to distinguish "high" and "low" Ki-67 proliferation index was set at 14% (16).

Disease-free survival (DFS) was defined as the postoperative time until the occurrence of locoregional or distant metastases and death from any cause, expressed in months. Patient follow-up included five-year monitoring through annual mammographic and clinical examinations, following the standard protocol for early invasive breast cancer (17).

IBM SPSS Statistics version 22.0 for Windows was used for statistical analysis. The X2 test was used to examine the association between variables. Univariate and multivariate regression analysis were applied to assess the independent and adjusted effects of the predictors of DFS, respectively. Receiver operating characteristic (ROC) analysis was used to evaluate the discriminative power of uPA/PAI-1 markers in predicting DFS. Kaplan-Meier analysis was used to evaluate the assessment of five-year DFS according to Ki-67 index and uPA/PAI-1 complex values. The most important results were presented in the form of tables and figures.

Results

Univariate regression analysis revealed that patients with negative estrogen receptors (OR=2.89; p=0.040; 95% CI: 1.050, 7.975), negative progesterone receptors (OR=2.91; p=0.022; 95% CI: 1.170, 7.261), patients with positive human epidermal growth factor receptor 2 (HER-2) receptor (OR=0.349; p=0.029; 95% CI: 0.136, 0.897), with eight or more positive lymph nodes (OR=0.148; p=0.004; 95% CI=0.041, 0.537), those without Luminal A tumor (OR=3.67; p=0.008; 95% CI=1.410, 9.599), and with Ki-67 index ≥14% (OR=3.301; p=0.014; 95% CI: 0.117, 0.787) had a higher likelihood of DFS shorter than five years. No statistically significant association was found between other predictors and five-year DFS, as shown in Table 1.

Table 1. Representation of predictors of five-year disease-free survival and the results of univariate regression analysis

Variables		N (%)	5Y-DFS		P*	95% CI*
			Achieved 144 (86.7)	Not Achieved 22 (13.3)		
			N (%)	N (%)		
Age	< 45 years	19 (11.4)	16 (84.2)	3 (15.8)	0.729	(0.336; 4.747
	45 years and above	147 (88.6)	128 (87.1)	19 (12.9)		
Menstrual status	Premenopause	25 (15.2)	22 (88.0)	3 (12.0)	0.832	(0.237; 3.185)
	Postmenopause	140 (84.8)	121 (86.4)	19 (13.6)		
Tumor stage	T1 stage	74 (44.6)	65 (87.8)	9 (12.2)	0.710	(0.338; 2.209)
	T2 stage	92 (55.4)	79 (85.9)	13 (14.1)		
Tumor size (mm)	0.1-19.9 mm	78 (47.0)	71 (91.0)	7 (9.0)	0.132	(0.185; 1.247)
	20-50 mm	88 (53.0)	73 (83.0)	15 (17.0)		
Estrogen receptor	Negative	27 (16-3)	20 (74.1)	7 (25.9)	0.040	(1.050; 7.975)
	Positive	139 (83.7)	124 (89.2)	15 (10.8)		
Progesteron receptor	Negative	54 (32.5)	42 (77.8)	12 (22.2)	0.022	(1.170; 7.261)
	Positive	112 (65.7)	102 (91.1)	10 (8.9)		
HER-2 receptor	Negative	129 (77.7)	116 (89.9)	13 (10.1)	0.129	(0.136; 0.897)
	Positive	37 (22.3)	28 (75.7)	9 (24.3)		
Lymph nodes	Negative	83 (50.0)	76 (91.6)	7 (8.4)	0.073	(0.161; 1.085)
	1-3 positive lymph nodes	51 (30.7)	43 (84.3)	8 (15.7)	0.539	(0.291; 1.906)
	4-7 positive lymph nodes	21 (12.7)	19 (90.5)	2 (9.5)	0.592	(0.329; 7.031)
	8 or more positive lymph nodes	11 (6.6)	6 (54.5)	5 (45.5)	0.004	(0.441; 0.537)
Molecular subtypes	Luminal A	98 (59.0)	91 (92.9)	7 (7.1)	0.008	(1.410; 9.599)
	Luminal B, HER-2 positive	16 (9.6)	14 (87.5)	2 (12.5)	0.926	(0.228; 5.097)
	Luminal B, HER-2 negative	25 (15.1)	19 (76.0)	6 (24.0)	0.093	(0.141; 1.164)
	HER-2 positive	9 (5.4)	6 (66.7)	3 (33.3)	0.085	(0.064; 1.193)
	Triple negative	18 (10.8)	14 (77.8)	4 (22.2)	0.243	(0.485; 1.634)
uPA/PAI-1 complex levels	0-0.99 ng/ml	35 (21.1)	29 (82.9)	6 (17.1)	0.203	(0.653; 1.258)
	1-1.99 ng/ml	93 (56.0)	80 (86.0)	13 (14.0)		
	2-2.99 ng/ml	33 (19.9)	30 (90.9)	3 (9.1)		
	3 ng/ml or above	5 (3.0)	5 (100)	0 (0.0)		
Ki-67 index	< 14%	144 (86.7)	119 (90.2)	13 (9.8)	0.014	(0.117; 0.787)

	14 % or above	34 (13.3)	25 (73.5)	9 (26.5)		

*Univariate regression analysis for 5Y-DFS

5Y-DFS, Five-year disease-free survival; CI, Confidence interval; HER-2, Human epidermal growth factor receptor 2; Ki-67, Antigen Kiel 67; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

In multivariate regression analysis, patients with T2 stage tumor (OR: -0.302; p=0.009; 95%CI: 1.991, 2.622), tumor size of 20-50 mm (OR: -0.304; p=0.005; 95%CI: 0.007, 0.413), or Ki-67 index ≥ 14% (HR: -0.292; p=0.031; 95%CI: 0.097, 1.152) had a significantly higher likelihood of DFS shorter than five years. Multivariate regression analysis did not demonstrate statistically significant predictive roles of other variables for five-year DFS, as shown in Table 2.

Table 2. Multivariate regression analysis of predictors of five-year disease-free survival

		5Y-DFS*	
		P	95% CI
Age	< 45 years	0.564	(0.220; 6.053)
	45 years and above		
Menstrual status	Premenopause	0.585	(0.081; 4.128)
	Postmenopause		
Tumor stage	T1 stage	0.009	(1.991; 2.622)
	T2 stage		
Tumor size (mm)	0.1-19.9 mm	0.005	(0.007; 0.413)
	20-50 mm		
Estrogen receptor	Negative	0.994	(0.080; 2.773)
	Positive		
Progesteron receptor	Negative	0.961	(0.229; 4.057)
	Positive		
HER-2 receptor	Negative	0.508	(0.195; 2.244)
	Positive		
Lymph nodes	Negative	0.070	(0.957; 3.044)
	1-3 positive lymph nodes		
	4-7 positive lymph nodes		
	8 or more positive lymph nodes		
Molecular subtypes	Luminal A	0.366	(0.638; 3.387)
	Luminal B, HER-2 positive		
	Luminal B, HER-2 negative		
	HER-2 positive		
	Triple negative		
uPA-PAI-1 complex levels (ng/ml)	0-0.99 ng/ml	0.178	(0.239; 1.303)
	1-1.99 ng/ml		
	2-2.99 ng/ml		
	3 ng/ml or above		
Ki-67 index (%)	< 14%	0.031	(0.097; 1.152)
	14 % or above		

*Multivariate regression analysis for 5Y-DFS

5Y-DFS, Five-year disease-free survival; CI, Confidence interval; HER-2 receptor, Human epidermal growth factor receptor 2; Ki-67, Antigen Kiel 67; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

ROC analysis revealed a low discriminatory power of uPA/PAI-1 markers for predicting five-year DFS (AUC=0.472, p=0.675, 95% CI: 0.340, 0.605), as depicted in Figure 1. For patients with Ki-67 index <14%, the estimated DFS was 48.08 months, while for those with Ki-67 index ≥14%, it was 44.03 months, with a statistically significant difference demonstrated by the Log Rank test (X2=7.08; p=0.008). The five-year DFS rate for patients with Ki-67 index <14% was 90.2%, while for those with Ki-67 index ≥14%, it was 73.5%, with a statistically significant difference (p=0.011), as shown in Figure 2A.

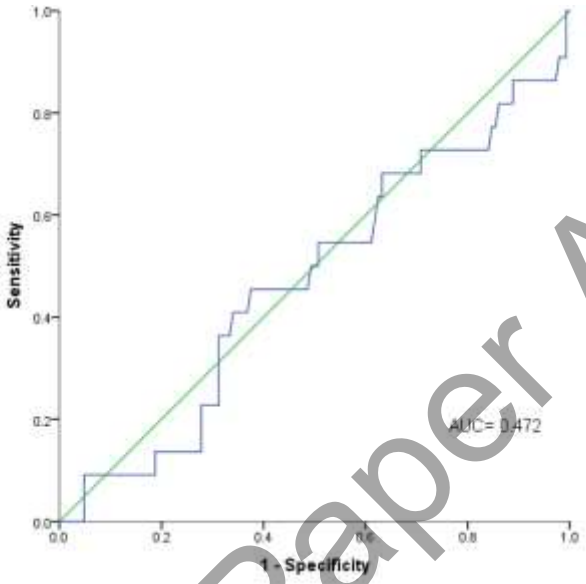

Figure 1. ROC Curve for uPA-PAI-1 complex in predicting five-year disease-free survival

ROC, Receiver operating characteristic; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

No statistically significant difference in estimated DFS was found among patients with different levels of uPA/PAI-1 markers by the Log-rank test (X2=1.706; p=0.636). The five-year DFS rates for different marker levels were as follows: 0-0.99 ng/ml (82.9%), 1-1.99 ng/ml (86.0%), 2-2.99 ng/ml (90.9%), 3 ng/ml and above (100.0%), with no statistically significant difference (p=0.623), as shown in Figure 2B.

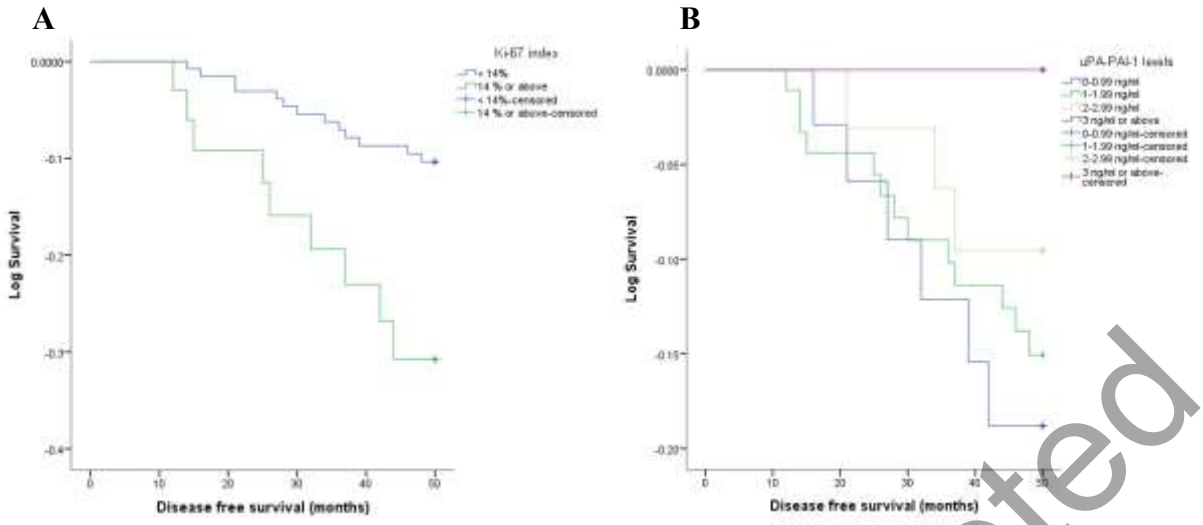

Figure 2. Five-year disease-free survival based on Ki-67 index (A) and uPA-PAI-1 (B) marker levels

Ki-67, Antigen Kiel 67; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

Discussion

Our study analyzed the prognostic and predictive significance of the Ki-67 proliferation index and preoperative values of the uPA/PAI-1 complex in serum in patients with early invasive breast cancer. Unlike the uPA/PAI-1 complex in serum, the Ki-67 proliferation index proved to be a significant prognostic-predictive factor for DFS in these patients. Patients with negative estrogen receptors have a statistically significant higher risk for a shorter DFS, likely due to biologically more aggressive tumors and less benefit from hormonal therapy (18-20). Negative progesterone receptors are also associated with increased risk of shorter DFS, emphasizing the importance of hormonal signaling (21). The HER-2 hormonal expression system plays a crucial role in therapy selection and response intensity but carries a higher risk of unfavorable outcomes (22, 23). Axillary lymph node analysis is crucial for accurately determining disease stage and adjusting therapy, especially in patients with multiple positive lymph nodes (24-27). The Luminal A tumor subtype of breast cancer typically responds positively to hormonal therapy, which may contribute to longer DFS, particularly in the first five years (28, 29). Various studies have confirmed that patients with high Ki-67 indices have a greater risk of shorter DFS (30, 31). The Ki-67 index, which measures the rate of tumor cell

proliferation, is associated with accelerated cell division and faster tumor growth (32). Reduced sensitivity of these tumors to certain therapeutic protocols can also contribute to an increased likelihood of shorter DFS, as noted in our study (33).

Multivariate regression has demonstrated the association of tumor stage, tumor size, and Ki-67 index with DFS, supporting previous findings regarding their prognostic-predictive significance (34, 35). These factors together reflect the complexity of the disease and its potential impact on outcomes. The Ki-67 index, as a proliferation marker, further contributes to understanding the disease dynamics (32, 36-39).

The study conducted by Mahmood et al. (40) investigated serum uPA-PAI-1 in the context of early invasive breast cancer, highlighting the need to consider systemic factors in interpreting serum biomarkers and emphasizing the importance of considering potential influences of cytokines and other tumor markers on uPA-PAI-1 complex expression. Additionally, the values of this complex measured in serum don't represent a reliable prognostic and predictive parameter, unlike its values in the cytosol or tumor tissue (41-44).

Limitations of our study include the lack of analysis of the interaction between the Ki-67 index and the uPA/PAI-1 complex both mutually and with other standard clinicopathological characteristics. Such analysis would enable better identification of patient subsets that could benefit from combined analysis of these markers. Furthermore, other genetic or molecular characteristics that could affect the prognostic and predictive significance of these biomarkers were not included (11-13). The lack of long-term follow-up, as the follow-up only covered the first five postoperative years, is considered a limitation of the study (45).

Our results indicate a statistically significant effect of elevated Ki-67 values on shortening DFS, with its consistency, regardless of the presence of uPA/PAI-1 and other previously documented prognostic factors considered in our study.

Conclusion

Our study emphasizes the strong and consistent prognostic and predictive ability of the Ki-67 index in assessing DFS in patients operated on for early invasive breast cancer. In contrast, preoperative values of the uPA/PAI complex in serum, whether alone or considering other predictors, didn't show significant prognostic and predictive potential in assessing DFS in these patients. Additional monitoring and tailored therapeutic strategies may be useful in patients with elevated Ki-67 values, T2 stage, and tumor size of 20-50 mm.

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