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Original article

CORRELATION OF CLINICAL-IMMUNOHISTOCHEMICAL WITH MOLECULAR CHARACTERISTICS IN EARLY BREAST CANCER

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Abstract

Introduction: In modern oncology, immunohistochemistry and genomic tests are two complementary approaches in the characterization of early breast cancer. Their combination better reflects the biological potential for growth and metastasis.

Material and methods: A total of 88 patients with early breast cancer were analyzed, classified according to immunohistochemistry and MammaPrint/BluePrint, after which a clinical correlation was made.

Results: Molecular typing with MammaPrint/BluePrint presented 42 patients (47.73%) with a high risk of recurrence. There was no statistically significant association with age (p=0.655), nodal status pN (p=0.113), or histological type of cancer (p=1.0). Patients with high and low risk differed significantly in terms of tumor size, i.e. by pT classification (p=0.0036), and G classification (p=0.0044).

Conclusion: Genomic profiling should complement, not replace, immunohistochemistry, as the combined approach allows for the most accurate assessment of risk, biology, and optimal therapeutic strategy for each patient with early breast cancer.

Keywords: BluePrint, early breast cancer, immunohistochemistry, MammaPrint, recurrence

Introduction

Improved understanding of molecular phenotypes, elucidating the heterogeneity of breast cancer, supports prognostic classification and individualized treatment management approaches in early stages. The most widely accepted is pathology-based immunohistochemistry (IHC), which is the basis for subtype classification and therapeutic decision-making. However, there are limitations in predicting individual risk and response to therapy, especially in patients with borderline or heterogeneous tumors. Therefore, the approach is being expanded with molecular classification and genomic profiling[1,2]. The MammaPrint/BluePrint combination identifies subgroups with different prognostic characteristics and provides guidance for adjuvant therapy.

Studying their correlation allows for the validation of traditional IHC parameters and their agreement with molecular classification. It identifies cases where IHC is not sufficiently precise to decide on systemic therapy.

IHC is a standard, widely available and routine method for the initial assessment of the biological characteristics of the tumor. It allows the determination of estrogen (ER) and progesterone receptor (PR) status, expression of Human Epidermal Growth Factor Receptor 2 (HER2) and proliferation index (Ki-67). Based on these markers, tumors are divided into subtypes: Luminal A, Luminal B, HER2-positive and triple-negative (basal) type.

IHC methodology is limiting, as it is semi-quantitative and depends on the subjective interpretation of the pathologist, the type of antibody, the method of fixation and the defined thresholds of positivity. In addition, there are "borderline" categories in which Ki-67 or HER2 status are not clearly defined, which makes the biological classification uncertain.

MammaPrint is a genomic test that analyzes the expression of 70 genes in isolated tumor RNA from patients with early-stage disease, categorizing them into "low risk" or "high risk" groups of disease recurrence and/or progression to a higher stage within the next 10 years[3]. It is a clinically validated test for assessing clinical risk based on: early stage (I and II), regardless of ER or HER2 status, tumor size ≤5.0 cm, negative or 1-3 positive lymph nodes (N0-1)[3-6]. High risk suggests the need for a more aggressive treatment plan with hormone therapy and chemotherapy[4], while for low-risk patients, hormone therapy alone is standard of care, meaning chemotherapy can be omitted[7].

The 80-gene Molecular Subtyping Profile (BluePrint) was developed to provide a molecular classification technique based on gene expression of three functional and target pathways, distinguishing: luminal type, HER2 enriched type, and basal type. By exploiting the prognostic accuracy and clinical utility of each test in a specific manner, it enables more precise and personalized therapy for each patient[8].

The correlation between IHC (ER, PR, HER2, Ki-67) and MammaPrint/BluePrint in early breast cancer aims to improve the accuracy of subtyping, prognostic assessment, and personalized therapy selection.

Material and methods

The study is a retrospective analysis of a total of 88 patients with early breast cancer, treated in the period from 07.2024-05.2025, at the Oncology Clinic, University Clinical Center in Pristina, Kosovo. Data were obtained from the patient documentation, microscopic slides, and corresponding paraffin blocks were downloaded from the archive of the Institute of Pathology at the University Clinical Center-Pristina and the Laboratory for Pathological Diagnostics and Research in Pristina, Kosovo.

Patients were aged 32-75 years, with a mean age of 50.4±11.2 years; 46 (52.27 %) patients were over 50 years of age. According to pTNM classification parameters, the distribution of patients showed that the most common tumor size was pT2, observed in- 46 patients (52.27 %), and most of them had N0 nodal status - 59(67.04 %). The M classification presented distant metastases in 1(1.14 %) patient. According to the differentiation of the cells, the majority of patients 53 (60.23 %) had intermediately differentiated (G2) carcinoma. Ductal carcinoma dominated as a histopathological type in 77 patients (87.5 %).

Immunohistochemical methodology

Evaluation of IHC results for ER and PR receptors, Her-2/neu receptors and Ki-67 receptors was performed on slides stained with clones EP1, 636, Herceptest and MIB-1 (DAKO), as well as SP-1, 1E2, 4B4 and 30-9 (Ventana). ER and PR expression was assessed using a scoring system of 0-50 % for weak expression and 51-100 % for strong expression.

Her-2/neu status was assessed using a scoring system in which cases of 0-1+ were considered negative and cases with a score of 2-3+ were considered positive, using the chromogenic *in situ* hybridization method.

The proliferative index was ranked as absent (0 %), low (1–19 %), or high (20–100 %), tested with clone MIB-1 and 30-9.

MammaPrint methodology

The MammaPrint analysis is based on RNA isolated from the tumor to assess the expression of 70 genes. It classifies patients as low or high risk for disease recurrence in the next 10 years.

BluePrint methodology

It is an 80-gene molecular subtyping profile that classifies tumors as Luminal-like, HER2-like, or Basal-like[9,10]. When combined with MammaPrint, it refines Luminal subtypes into Luminal A (low-risk) and Luminal B (high-risk) and confirms the primary molecular driver (HER2 *vs*. basal-like).

Results

Molecular typing using MammaPrint/BluePrint identified 42 patients (47.73 %) as high risk, 46 patients (52.27 %) as low risk, and 5 (5.68 %) as very low risk for recurrence.

IHH analyses showed that majority of patients had strong estrogen expression - 78 (88.64%), more than half of patients had strong progesterone expression - 48(54.54 %), the majority of patients were with negative HER2 status - 79(89.77 %), and more than half had a low proliferative index - 48(54.54 %).

Patients' age was not significantly associated with the risk of recurrence, according to the result of MammaPrint/BluePrint (p=0.655). There was also no statistically significant dependence in the comparison with the nodal pN status, the histological type of the cancer (p=1.0). Patients with high and low risk significantly differed in terms of tumor size, i.e. by pT classification (p=0.0036), and G classification (p=0.0044) (Table 1).

Table 1. Distribution of low/high risk patients

variable		n	MammaPrint/BluePrint low risk n (%)	MammaPrint/BluePrint high risk n (%)	p-value
Age groups	≤50	46	23(50)	23(50)	X^2=0.19
	>50	42	23(54.76)	19(45.24)	p=0.655
Age	mean ± SD		49.3±10.9	51.4±11.4	t=0.911
	min- max		32-72	35-75	p=0.365
pT	T1	40	28(70)	12(30)	Fisher's exact
	T2	47	18(38.30)	29(61.70)	**p=0.0036
	T3	1	0	1(100)	
pN	N0	59	27(45.76)	32(54.24)	Fisher's exact
	N1	21	15(71.43)	6(28.57)	p=0.113
	N2	6	3(50)	3(50)	
	Nx	2	1(50)	1(50)	
G	G1	10	9(90)	1(10)	Fisher's exact
	G2	53	28(52.83)	25(47.17)	**p=0.0044
	G3	23	7(30.43)	16(69.57)	
Histological type	lobular type	6	3(50)	3(50)	Fisher's exact
	NST (ductal)	77	40(51.95)	37(48.08)	p=1.0
	mixed NST (ductal) and lobular type	5	3(60)	2(40)	

X^2(Chi-square test); t(Student t-test)

The expression of ER receptors did not show significant correlation with: patient age (p=0.437), tumor size (p=0.41), pN status (p=0.87), tumor grade (p=0.775) and histological type (p=0.757) (Table 2).

Table 2. Distribution of patients according to ER%

variable		n	ER% 1 – 50% n (%)	51 – 100% n (%)	p-value
Age groups	≤50	46	8(17.39)	38(82.61)	YatesX^2=2.34
	>50	42	2(4.76)	40(95.24)	p=0.126
Age	mean ± SD		47.8±12.6	50.7±11.0	t=0.78
	min- max		35-72	32-75	p=0.437
pT	T1	40	3(7.5)	37(92.5)	Fisher's exact
	T2	47	7(14.89)	40(85.11)	p=0.41
	T3	1	0	1(100)	
pN	N0	59	7(11.86)	52(88.14)	Fisher's exact
	N1	21	3(14.29)	18(85.71)	p=0.87
	N2	6	0	6(100)	
	Nx	2	0	2(100)	
G	G1	10	0	10(100)	Fisher's exact
	G2	53	7(13.21)	46(86.79)	p=0.775
	G3	23	3(13.04)	20(86.96)	
Histological type	lobular type	6	1(16.67)	5(83.33)	Fisher's exact
	NST (ductal)	77	9(11.69)	68(88.31)	p=0.757
	mixed NST (ductal) and lobular type	5	0	5(100)	

X^2(Chi-square test); t(Student t-test)

PR expression did not show a statistically significant difference in correlation with age (p=0.1), tumor size (p=0.544), pN status (p=0.29), tumor differentiation (p=0.25), and histological type (p=0.72) (Table 3).

Table 3. Distribution of patients according to PR%

variable		n	PR% 0% n (%)	1 – 50% n (%)	51 – 100% n (%)	p-value
Age groups	≤50	46	2(4.35)	14(30.43)	30(65.22)	Fisher's exact
	>50	42	7(16.67)	17(40.48)	18(42.86)	p=0.052
Age			57.0±11.1	51.2±11.1	48.6±10.9	F=2.33
			36-72	35-72	32-75	p=0.1
pT	T1	40	5(12.5)	15(37.5)	20(50)	Fisher's exact
	T2	47	4(8.51)	15(31.91)	28(59.57)	p=0.544
	T3	1	0	1(100)	0	
pN	N0	59	8(13.56)	23(38.98)	28(47.46)	
	N1	21	1(4.76)	5(23.81)	15(71.43)	Fisher's exact
	N2	6	0	1(16.67)	5(83.33)	p=0.29
	Nx	2	0	2(100)	0	
Grade	G1	10	0	3(30)	7(70)	Fisher's exact
	G2	53	4(7.55)	18(33.96)	31(58.49)	p=0.25
	G3	23	5(21.74)	9(39.13)	9(39.13)	
Histological type	lobular type	6	1(16.67)	2(33.33)	3(50)	
	NST (ductal)	77	8(10.39)	26(33.77)	43(55.84)	Fisher's exact
	mixed NST (ductal) and lobular type	5	0	3(60)	2(40)	p=0.72

X^2 (Chi-square test); F (Analisys of Variance)

The correlation between the proliferative index Ki67% did not show statistical significance with: age (p=0.35), N status (p=0.29), tumor differentiation G (p=0.19), histological type of carcinoma (p=0.398). Tumor size had a significant impact on Ki67 (p=0.034) (Table 4).

Table 4. Distribution of patients according to Ki67%

variable		n	Ki67% 1 – 19% n (%)	Ki67% 20 – 100% n (%)	p-value
Age groups	≤50	45	27(60)	18(40)	X^2=0.09
	>50	37	21(56.76)	16(43.24)	p=0.67
Age			48.8±9.8	51.2±12.6	t=0.94
			35-70	32-75	p=0.35
pT	T1	38	27(71.05)	11(28.95)	Fisher's exact
	T2	43	20(46.51)	23(53.49)	*p=0.034
	T3	1	1(100)	0	
pN	N0	56	29(51.79)	27(48.21)	Fisher's exact
	N1	20	14(70)	6(30)	p=0.29
	N2	5	4(80)	1(20)	
	Nx	1	1(100)	0	
Grade	G1	9	7(77.78)	2(22.22)	Fisher's exact
	G2	52	31(59.62)	21(40.38)	p=0.19
	G3	19	8(42.11)	11(57.89)	
Histological type	lobular type	5	2(40)	3(60)	Fisher's exact
	NST (ductal)	72	44(61.11)	28(38.89)	p=0.398
	mixed NST (ductal) and lobular type	5	2(40)	3(60)	

X^2 (Chi-square test); t (Student t-test); *sig p<0.05

Discussion

Correlation of IHH and molecular typing with MammaPrint/BluePrint in invasive early breast cancer identifies potential synergies between genomic and protein expression profiles that guide treatment decisions and contribute to the development of integrated diagnostic approaches and the improvement of personalized medicine. The study analysis showed that patients with larger tumors and poor tumor differentiation were more likely to be classified as high risk according to the MammaPrint/BluePrint test. This means that adjuvant chemotherapy is warranted. The results of this study are in line with those reported in several international studies.

The MINDACT study[5,9] re-stratified 54 % of patients with pathological Luminal-B subtype obtained by IHC with molecular typing using MammaPrint into a low-risk Luminal A-type group, with comparable outcomes. Molecular classification helped to identify a larger group of patients with a low risk of disease recurrence, which led to treatment adjustments by avoiding adjuvant chemotherapy. Cardoso *et al*.[3] reported a 5-year progression-free survival rate among patients with early breast cancer who were at high clinical risk but low genomic risk of recurrence and therefore did not receive adjuvant chemotherapy. Glück *et al*.[10], using molecular subtyping, identified 90 of 435 (21 %) patients as Luminal A-type with BluePrint and low risk according to MammaPrint, who had a good prognosis, excellent survival, and no benefit from chemotherapy. Nguyen *et al*.[11] compared subtyping from three assays (BluePrint, MammaPrint, and TargetPrint) with clinical IHH subtyping. They noted a high degree of discordance between Luminal A and B substratification based on MammaPrint *versus* IHH-assessed Ki-67 or differentiation grade. Pooled results from multiple studies[12-14] have established definitive results for adjuvant chemotherapy planning. Low-risk patients had a 1.3 % risk of recurrence; the MINDACT study[3] showed no benefit from chemotherapy in this

group of patients. High-risk patients have an 11.7 % risk of recurrence[3] and these patients have been shown to have significantly better outcomes with chemotherapy[14]. Almost half (46 %) of the clinically high-risk patients were reclassified as MammaPrint low-risk and were able to forgo chemotherapy without compromising their outcomes[13].

By systematically correlating IHC parameters with MammaPrint/BluePrint results in early breast cancer, important insights are gained into the frequency which genomic tests significantly change clinical risk assessments and whether these changes lead to revised treatment decisions. If the observed rates of reclassification and treatment adjustments are significant, this supports the routine integration of genomic analyses, especially for borderline or intermediate-risk cases.

The findings of this study support further expansion of this research, with the possibility of tailored treatment strategies, by integrating genomic and IHC data in the management of early breast cancer.

Conflict of interest statement. None declared.

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