

# Clinical, Histopathological, and Immunohistochemical Characteristics of Predictive Biomarkers of Breast Cancer: A Retrospective Study

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**Abstract.** *Background/Aim:* Breast cancer is a complex disease with variability in clinical manifestation, response to current therapy, and biochemical and histological features among various subgroups. *Histologic grading and immunohistochemical evaluation of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and Ki-67 proliferation index play a crucial role in increasing the differential diagnostic value among various types of breast carcinoma. The aim of this study*

*was to determine the histopathological and immunohistochemical characteristics of breast tumors from a University Laboratory of Pathology in Greece. Patients and Methods:* The study included female patients over 18 years of age, whose histopathological and immunohistochemical reports were stored in the archives of the First Department of Pathology of National and Kapodistrian University of Athens. The study involved 197 female patients with a median age of 70 years and median tumor size of 2.6 cm. *Results:* Most tumors were located at the left breast and ductal carcinoma was the most common histologic type (35.5%). Most tumors had histologic grade 2 (106, 53.8%), and were classified as TNM stage IIA (65, 33%). Most grade 1 and 2 tumors exhibited high expression of PR, whereas most grade 3 tumors had no PR expression. Moreover, patients with triple-negative cancer presented with grades 2 and 3 at a lower percentage compared to patients without a triple-negative phenotype ( $p=0.001$ ). *Conclusion:* The study provided valuable insights into the histopathological and immunohistochemical characteristics involved in the development and progression of breast cancer.

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**Key Words:** Breast cancer, immunohistochemistry, pathology, biomarkers, triple-negative cancer, histopathological subtypes, molecular subtypes, histological features, ER, PR, HER-2, Ki-67, e-cadherin, predictive, lobular carcinoma, ductal carcinoma.

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Breast cancer is a complex disease that exhibits variability in clinical manifestation, response to current therapy, and biochemical and histological features among several subgroups. Based on histology, invasive tumors are divided into two categories: histological special types, characterized

by specific diagnostic criteria, with invasive lobular carcinoma (ILC) being the most common among them, and invasive carcinoma of no special type (1). The latter type is also known as invasive ductal carcinoma (IDC) and accounts for 70% of cases. It can be described as a breast invasive epithelial neoplasm that fails to fulfill the criteria for any special type, making it a very heterogeneous group of tumors (2).

The fundamental elements for identifying major prognostic markers are the examination of lesion size, axillary lymph node status, nuclear grade, and histological subtype. The lesion's histopathological features reveal many forms of breast tumor biological behavior (3).

The histological categorization of breast cancer is not without flaws, though. Approximately 85% of cases fall into one of the two main categories of IDC or ILC after using such classification, taking into consideration also the subjectivity of the diagnostic criteria. Determining the histologic grade and the immunohistochemical examination of progesterone receptor (PR), human epidermal growth factor 2 (HER-2) and estrogen receptor (ER), and the Ki-67 proliferation index play a crucial role in increasing the diagnostic accuracy among the various types of breast carcinoma because the system is unable to group tumors with an extensive biological range and clinical behavior in the same categories (4).

A better prognosis is linked to the presence of hormone receptors (HR). As a result, patients with progesterone (PR)-positive tumors live longer and are disease-free longer. In the same vein, tumors that are estrogen (ER)-positive are linked to both a better likelihood of responding to hormone therapy and an increased disease-free survival. In contrast, patients who were negative for both ER and PR had a poorer prognosis than those who were negative for only one of the receptors (5). The HER-2 proto-oncogene, which produces a protein that sends signals for the proliferation of epithelial cells and whose expression is frequently elevated in breast cancer, is another significant tumor marker. A more aggressive clinical behavior of the tumor is caused by HER-2 over-expression, and determining the marker status is crucial for identifying cancer types with a poorer prognosis (6, 7).

High rates of cell proliferation are typically indicative of highly malignant tumors. Therefore, the assessment of mitotic activity is critical for the diagnosis of breast cancer. In order to achieve this, a monoclonal antibody that recognizes the cell proliferation index Ki-67, a nuclear antigen, is employed. Ki-67 is expressed in cells that are entering the cell cycle and measures the percentage of cell growth, making it possible to identify cancers with a poorer prognosis (8).

The aim of the current study was to determine the histopathological and immunohistochemical characteristics of breast tumors from a reference University Laboratory of Pathology in Athens, Greece.

## Patients and Methods

*Study design.* This is a retrospective observational study conducted at the First Department of Pathology of National and Kapodistrian University of Athens, Greece. We examined the medical records of the histopathological and immunohistochemical examinations of consecutive breast tumors conducted in the laboratory from January 2014 to December 2018. The study was conducted in line with the Declaration of Helsinki and obtained approval by the Ethics Committee of Medical School of the National and Kapodistrian University of Athens (protocol no. 062/ 14.01.2019).

*Inclusion criteria.* The study included female patients over 18 years of age, whose medical records regarding both histopathological and immunohistochemical examinations were stored in the archives of the First Department of Pathology of National and Kapodistrian University of Athens, and who agreed to be enrolled in the study by signing of the informed consent form.

*Data collection.* The following data were collected: age, tumor location, parameters related to histopathological examination, and parameters related to immunohistochemical examination. Regarding histopathological aspects, the following were analyzed: tumor size; histologic grade; number of invaded lymph nodes; presence of positive sentinel lymph node; surgical margins; tumor composition; histopathological classification of the tumor (9) and TNM stage (10). As for immunohistochemical features, the following were examined: presence and intensity of expression of cell proliferation antigen Ki-67; expression of HER-2 oncogene; and intensity of expression and presence of ER and PR; expression of E-cadherin, p53, p63, CK14, CK5/6, androgen receptor (AR) and epidermal growth factor receptor (EGFR).

*Immunohistochemical analysis.* Immunohistochemistry was carried out using standard procedures in all tested specimens. First, the sections were stained with the following antibodies on a Dako system (Autostainer Link 48, Agilent Dako Pathology Solutions, Santa Clara, CA, USA), according to the manufacturer's protocol. The sections were stained based on the double-staining protocol of Leica Biosystems in the Bond-III fully automated stainer, with antibodies against estrogen receptor (ER), (clone EP1; Agilent DAKO), progesterone receptor (PR), (clone PgR 636; Agilent DAKO), epidermal growth factor 2 (HER-2) (rabbit polyclonal; Agilent DAKO), cell proliferation index (Ki-67) (clone MIB-1; Agilent DAKO), and E-cadherin, (clone NCH-38; Agilent DAKO). Antigen retrieval was performed at pH 6. The Agilent (Dako) visualization system was used. DAB (3,3-diaminobenzidine) was used as a chromogen and hematoxylin as counterstain.

The histological and immunohistochemical study was performed on tissue pieces fixed in a 10% neutral formalin aqueous solution and embedded in paraffin cubes (Paraplast, Leica Biosystems Inc., Richmond, IL, USA) at 55°C. The indirect immunoperoxidase method was used in the DAKO EnVision FLEX+ detection system (HRP/DAB, Specificity: Anti-Mouse IgG (H+L) – Agilent DAKO, Cat# K8002).

The Sequenza immunohistochemistry device (Thermoshandon, Runcorn, UK) was used using cover-plates technology, which relies on the capillary effect and ensures staining with the minimum amount of reagent and protection of samples from drying.

All the antibodies for immunohistochemical detection were used according to manufacturer's protocol.

The use of indirect immunohistochemical methods with peroxidase contributes to strengthening the signal of the immune reaction. Peroxidase activity was visualized by staining with 3,3', diaminobenzidine tetrahydrochloride chromogen solution (DAB Chromogen, EnVision FLEX, Agilent DAKO), (11).

Histological sections from all samples were observed under a photon microscope. The assessment of immunohistochemical staining was performed independently and blindly by two experienced pathologists.

The staining was evaluated as cytoplasmic and/or membranous, nuclear and/or cytoplasmic, both in the neoplastic tissue and in the adjacent non-neoplastic tissue. Intensity was graded using the following scale: 0 for undetectable staining, 1 for weak intensity, 2 for moderate and 3 for strong. Grading of the percentage of positive cells was performed semi-quantitatively, with the following scale: 0 for immunopositivity in 0-10% of cells, 1 for 10-29%, 2 for 30-59%, and 3 for 60-100%. For each sample, a score was derived from the sum of the intensity and distribution scores, ranging from 0 to 6. Tumors with a final score of 1, 2, 3 were included in the weak expression group, and those with a final score of 4, 5, 6 in the strong expression group.

**Determination of positive expression.** ER and PR were considered positive when >1% of cells showed positive nuclear expression (12). HER-2 categorization was made according to the proposed criteria of the DAKO protocol (13). p53 was considered positive when >10% of cells showed positive nuclear expression (14). Regarding Ki-67 we considered low expression when ≤15%, moderate when 16-30% and high when >30% of cells showed positive nuclear expression (15). CK14 and CK5/6 were scored positive if any (weak or strong) cytoplasmic and/or membranous invasive carcinoma cell staining was observed (16). E-cadherin expression was determined as positive when scores were ≥2 (17). P63 was scored positive when high intensity staining was present on ≥50% of tumor cells (18). A cutoff point of ≥10% cells with at least weak staining intensity was applied to define protein positivity for EGFR (19). AR expression was determined as positive when scores were ≥2 (20).

**Statistical analysis.** The Kolmogorov–Smirnov test was used to determine whether the distribution of the variables was normal. All continuous variables had non-normal distribution and are displayed as median (range); categorical variables are presented as absolute numbers (frequency percent). We utilized the Mann–Whitney *U*-test for non-normally distributed variables with two groups and the Kruskal–Wallis test for those with three groups. Categorical variables were analyzed using Fisher's exact or chi-square tests. The Spearman correlation coefficient was utilized to evaluate associations between continuous variables. *p*-Values under 0.05 were regarded as significant. The IBM SPSS Statistics version 29.0 (IBM, Armonk, NY, USA) was used for the statistical analysis.

## Results

A total of 197 females with a median age of 70 years (range=28-98 years) participated in the study. The median value of maximum tumor size was 2.6 cm (0.4-12 cm). Most tumors were located at the left breast (110, 55.8%). The most frequent histologic type was the ductal carcinoma (70, 35.5%), followed by the lobular carcinoma (49, 24.9%) and the non-specific type (NST) adenocarcinoma (46, 23.4%). Most tumors

were histologic grade 2 (106, 53.8%) and most tumors classified at stage IIA (65, 33%). Twenty-three patients (11.7%) had *in situ* carcinoma and 38 patients (19.3%) had triple-negative breast cancer. Three of the 49 lobular carcinomas were *in situ* carcinomas (6.1%) and 46 of the 49 lobular carcinomas were invasive (93.9%). Nine of the 70 ductal carcinomas were *in situ* (12.9%) and 61 of the 70 ductal carcinomas were invasive (87.1%). Descriptive characteristics of the study population and the analyzed breast tumor types are summarized in Table I. Regarding the age, we observed a statistically significant difference in the median age of patients between those with lobular and non-lobular breast carcinoma [66 (38-92) years in patients with lobular carcinoma *vs.* 72 (28-98) years in patients without, *p*=0.009] (Table II).

Regarding the maximum tumor size, there was a statistically significant difference between the patients with invaded lymph nodes and those without [3.5 (0.9-12) cm *vs.* 2.2 (0.4-10) cm, respectively, *p*=0.001], between the patients with positive sentinel lymph nodes and those without [3.5 (0.9-12) cm *vs.* 2.4 (0.4-12) cm, respectively, *p*=0.001], between the patients with multifocal tumors and those without [2.9 (0.6-10.5) cm *vs.* 2 (0.4-12) cm, *p*=0.007], and between the patients with expression of E-cadherin and those without [2.6 (0.6-7.8) cm *vs.* 1.9 (0.4-10.5) cm, *p*=0.015] (Table III).

Additionally, we observed that all metaplastic-squamous carcinomas had histologic grade 3 (*p*=0.001), and more patients in stage I had histologic grade 1 than in any other stage. Most of the patients with histologic grades 2 and 3 were in stage IIA (*p*=0.001). Most patients with histologic grade 2 exhibited high expression of ER; most patients with grade 3 exhibited no expression of ER; and all the patients with grade 1 had high ER expression (*p*=0.001). Most patients with grades 1 and 2 exhibited high expression of PR, whereas most patients with grade 3 had no PR expression (*p*=0.001). Most patients with grades 1 and 2 had low expression of Ki-67, whereas most patients with grade 3 had high expression of Ki-67 (*p*=0.001). Moreover, patients with triple-negative cancer presented with grades 2 and 3 at a lower percentage compared to patients without a triple-negative phenotype (*p*=0.001) (Figure 1, Figure 2, Table IV).

Most patients of all TNM stages had no positive sentinel lymph node (*p*=0.001), most patients at stages IIA, IIB, and IIIa had multifocal tumors (*p*=0.024), most patients with papillary carcinoma were at stage I (*p*=0.029), most patients with metaplastic-squamous carcinoma were at stage IIIa (*p*=0.003), and most patients at stages I, IIA, IIB, and IIIa had high expression of ER, whereas most patients of stage IIIB had no expression of ER (*p*=0.001). Most patients at stages I, IIA, and IIB had no expression of E-cadherin, and most patients at stages IIIa and IIIB expressed E-cadherin (*p*=0.033). Most patients of all stages did not have the triple-negative phenotype, and most of the triple-negative ones were at stage IIA (*p*=0.016), (Table V).

Table I. Descriptive characteristics of the study population and the analyzed tumor types.

Variable	Median (Range) or N (%)	Variable	Median (Range) or N (%)
Age (years)	70 (28-98)	Histologic grade	
Maximum tumor size (cm)	2.6 (0.4-12)	1	16 (8.1)
Number of invaded lymph nodes	3.5 (1-28)	2	106 (53.8)
Tumor location		3	73 (37.1)
Right breast	87 (44.2)	TNM staging	
Left breast	110 (55.8)	I	40 (20.3)
Invaded lymph nodes		IIA	65 (33)
No	132 (67)	IIB	30 (15.2)
Yes	65 (33)	IIIA	37 (18.8)
Positive sentinel lymph node		IIIB	23 (11.7)
No	160 (81.2)	ER	
Yes	37 (18.8)	No expression	55 (27.9)
Multifocal tumor		Low expression	10 (5.1)
No	85 (43.1)	Intermediate expression	13 (6.6)
Yes	112 (56.9)	High expression	110 (55.8)
Tumor composition		PR	
Solid	102 (51.8)	No expression	66 (33.5)
Subdural	58 (29.4)	Low expression	15 (7.5)
Elastic	16 (8.1)	Intermediate expression	20 (10.2)
Sclerosing	12 (6.1)	High expression	87 (44.2)
Fibrous	7 (3.6)	HER-2	
Cystic-hemorrhagic	1 (0.5)	No expression	148 (75.1)
Positive surgical margins		Intermediate expression	27 (13.7)
No	108 (54.8)	High expression	13 (6.6)
Yes	89 (45.2)	Ki-67	
Lobular carcinoma		No expression	2 (1)
No	148 (75.1)	Low expression (<15%)	133 (67.5)
Yes	49 (24.9)	High expression (30%)	43 (21.8)
Ductal carcinoma		E-cadherin	
No	127 (64.5)	No	45 (22.8)
Yes	70 (35.5)	Yes	55 (27.9)
NST adenocarcinoma		p63	
No	151 (76.6)	No	9 (4.6)
Yes	46 (23.4)	Yes	14 (7.1)
Neuroendocrine carcinoma		p53	
No	196 (99.5)	No	2 (1)
Yes	1 (0.5)	Yes	8 (4.1)
Mucinous carcinoma		CK14	
No	192 (97.5)	No	20 (10.2)
Yes	5 (2.5)	Yes	16 (8.1)
Papillary carcinoma		CK5/6	
No	187 (94.9)	No	13 (6.6)
Yes	10 (5.1)	Yes	6 (3)
NST papillary carcinoma		EGFR	
No	195 (99)	No	15 (7.6)
Yes	2 (1)	Yes	9 (4.6)
NST ethmoid carcinoma		AR	
No	195 (99)	No	7 (3.6)
Yes	2 (1)	Yes	7 (3.6)
Mixed tubular carcinoma		Triple-negative cancer	
No	195 (99)	No	153 (77.7)
Yes	2 (1)	Yes	38 (19.3)
Metaplastic-squamous		<i>In situ</i>	
No	186 (94.4)	No	174 (88.3)
Yes	11 (5.6)	Yes	23 (11.7)
Apocrine carcinoma			
No	196 (99.5)		
Yes	1 (0.5)		

NST: Non-specific type; TNM: the extent of the tumor (T), extent of spread to the lymph nodes (N), and presence of metastasis (M); PR: progesterone receptor, AR: androgen receptor, ER: estrogen receptor; EGFR: epidermal growth factor receptor; HER-2: human epidermal growth factor receptor 2.

Table II. Association of age with various tumor characteristics.

Variable	Age [years, median (range)]	<i>p</i> -Value
Tumor location		0.850
Right breast	72 (28-98)	
Left breast	70 (37-98)	
Invaded lymph nodes		0.978
No	70 (28-98)	
Yes	70.5 (42-93)	
Lobular carcinoma		<b>0.009</b>
No	72 (28-98)	
Yes	66 (38-92)	
Ductal carcinoma		0.573
No	70 (28-98)	
Yes	71 (42-96)	
Metaplastic-squamous		0.084
No	69.5 (28-98)	
Yes	77 (49-98)	
NST adenocarcinoma		0.673
No	71 (38-98)	
Yes	70 (28-94)	
Mucinous carcinoma		0.084
No	70 (28-98)	
Yes	85 (43-98)	

NST: Non-specific type. Statistically significant *p*-values are shown in bold.

Most patients with metaplastic-squamous carcinoma had a triple-negative phenotype ( $p=0.001$ ), and most patients with triple-negative cancer had high expression of Ki-67 ( $p=0.001$ ) and positive expression of p63 and CK14 ( $p=0.010$  and  $p=0.001$ , respectively) (Table VI). In addition, we observed a statistically significant association between the expression of E-cadherin and lymph node involvement ( $p=0.002$ ).

Moreover, we also found a positive weak correlation between the age and the maximum size of the tumor (Spearman's  $\rho=0.253$ ,  $p=0.001$ , data non-shown). At Table VII, we present the 5-year frequency of breast cancer types in the central laboratory of the First Department of Pathology of National and Kapodistrian University of Athens, from 2014-2018, according to histopathological and immunohistochemical classifications. The frequency of the main types of breast cancer in our study is presented in Figure 3.

## Discussion

The median age of the patients included in our study was 70 years (mean age  $69.3\pm 15.19$  years). This result differs from other studies, in which the mean age of the patients has been reported to be 53 years, 53.3 years, 57.5 years, and 48.5 years (21-24). These variations might result from the diverse age range of women examined in these studies.

Regarding the histological classification, the most frequent type found in the current study was ductal carcinoma. This finding is in line with findings from other studies (21, 23,

Table III. Association of maximum tumor size with various tumor characteristics.

Variable	Tumor size (cm) [Median (range)]	<i>p</i> -Value
Invaded lymph nodes		<b>0.001</b>
No	2.2 (0.4-10)	
Yes	3.5 (0.9-12)	
Positive sentinel lymph node		<b>0.001</b>
No	2.4 (0.4-12)	
Yes	3.5 (0.9-12)	
Multifocal tumor		<b>0.007</b>
No	2 (0.4-12)	
Yes	2.9 (0.6-10.5)	
Lobular carcinoma		0.560
No	2.6 (0.6-12)	
Yes	2.4 (0.4-12)	
Ductal carcinoma		0.870
No	2.6 (0.4-12)	
Yes	2.5 (0.6-12)	
Metaplastic-squamous		<b>0.003</b>
No	2.5 (0.4-12)	
Yes	7.2 (1.5-10)	
ER		0.383
No expression	2.3 (0.9-12)	
Low expression	3.4 (1-9)	
Intermediate expression	2.9 (1.3-10.5)	
High expression	2.5 (0.4-7.8)	
PR		0.357
No expression	2.5 (0.6-12)	
Low expression	2.6 (1.5-9)	
Intermediate expression	2.9 (1.9-10.5)	
High expression	2.4 (0.4-7.8)	
HER-2		0.742
No expression	2.5 (0.4-10.5)	
Intermediate expression	1.9 (0.6-6)	
High expression	1.9 (1-5.5)	
Ki-67		0.159
Low expression (<15%)	2.5 (0.4-10.5)	
High expression (30%)	1.8 (1-7.5)	
E-cadherin		<b>0.015</b>
No	1.9 (0.4-10.5)	
Yes	2.6 (0.6-7.8)	
Triple-negative cancer		0.677
No	2.6 (0.4-12)	
Yes	2.4 (0.9-12)	

PR: Progesterone receptor, ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2. Statistically significant *p*-values are presented in bold.

25). In our study, the frequency of lobular carcinoma was 24.9%. Lobular cancer accounts for 15% of breast cancer cases (26). Our study shares similarities with a study from Brazil in which most patients were diagnosed with ductal breast carcinoma, and the second most frequent type of cancer was the lobular one (27).

In our study, most patients with histologic grade 2 exhibited high expression of ER; most patients with grade 3 exhibited no expression of ER; and all the patients with grade 1 had

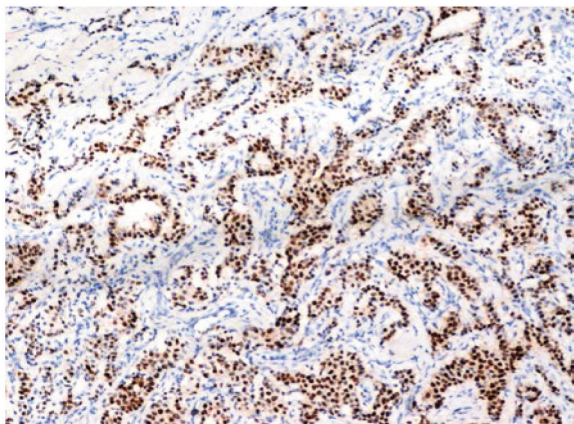


Figure 1. Increased expression of PR detected using immunohistochemistry. PR strong positive expression, magnification  $\times 200$ .

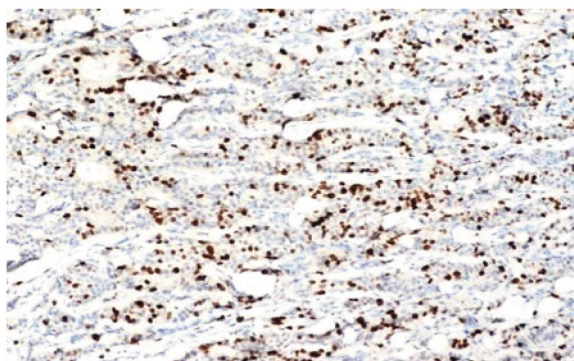


Figure 2. Increased expression of Ki-67 detected using immunohistochemistry. Ki-67 strong positive expression, magnification  $\times 200$ .

high ER expression ( $p=0.001$ ). Most patients in grades 1 and 2 exhibited high expression of PR, while most patients in grade 3 had no PR expression ( $p=0.001$ ). These results indicate an inverse association between the expression of ER and PR. Similar findings have been demonstrated by Soares *et al.* (21). Also, these results are comparable to the findings of Dayal *et al.* (28), who showed that the incidence of histologic grade 3 was greater than 50% in cases where there was no ER expression. However, histologic grade 1 was more common when ER expression was high. A comparable study carried out in Asia (29) found that in 70% of grade 1 carcinomas, 48.2% of grade 2, and 3.5% of grade 3 cases were ER positive ( $p<0.001$ ). Given that the presence of ER and PR in the tumor tissue is associated positively with the response to hormone therapy and chemotherapy (30), we can therefore conclude that better-differentiated tumors (lower grade) are more probable to be ER and PR positive in addition to having a relatively better prognosis.

In addition, in our research, most patients with grades 1 and 2 had low expression of Ki-67, whereas most patients with grade 3 had high expression of Ki-67 ( $p=0.001$ ). This demonstrates that significant cell proliferation, which is a hallmark of tumor progression and a worse prognosis, is mostly seen in carcinomas of higher histologic grade. This conclusion is consistent with that of Narbe *et al.* (31), who likewise observed grade 3 tumors with a mean Ki-67 score of 23.2% and confirmed a substantial positive correlation between Ki-67 and histologic grade ( $p<0.001$ ). Moreover, Soares *et al.* found that the increased expression of Ki-67 was linked to a higher incidence of high histologic grade (21).

Furthermore, Table IV shows that, with regard to the histologic grade, HER-2 showed a similar pattern to Ki-67 while not being statistically significant ( $p=0.051$ ). Arantes Junior observed a similar result; he did not find a statistically significant link but noted that high nuclear grade was associated with HER-2 over-expression (32). Thus, as HER-2 over-expression has no statistically significant correlation with different nuclear grade levels, it appears to be an independent indicator of biological aggressiveness. Its over-expression in individuals with breast cancer indicates a poorer prognosis and a poor response to hormone therapy, such as tamoxifen, which results in lower survival (30).

Concerning tumor size, we did not observe a significant association with ER or PR expression, as shown in Table III. Similarly, Soares *et al.* found that the mean size of tumors of patients with ER-positive was 3.52 cm, whereas that of patients with ER-negative tumors was 3.73 cm. Furthermore, the mean tumor size in patients with PR-positive tumors was 3.51 cm, whereas in patients with PR-negative tumors, it was 3.72 cm. Nevertheless, there was no discernible relationship between tumor size and ER and PR expression (21). Dayal *et al.* (28) and Ariga *et al.* (33) have both reported similar results.

It is well recognized that lymph node status affects breast cancer stage and available treatments. The patient's prognosis in breast cancer is significantly affected by their lymph node status. As the number of positive axillary lymph nodes increases, both the survival rate and the likelihood of recurrence decrease (34). Previous research has shown that HER-2 expression is statistically correlated with lymph node involvement and vascular invasion, while this relationship has not been shown for ER and PR (28, 35, 36). However, this association was observed neither in our study nor in the study by Soares *et al.* (21).

The cell adhesion molecule E-cadherin is expressed in healthy breast tissue and can be used as a phenotypic diagnostic for breast cancer. A shorter disease-free interval, a lower overall survival rate, a larger tumor size, a higher histological grade, the development of distant metastases, and ER receptor-negative cancers are all linked to decreased or impaired e-cadherin expression (37). It has been shown that invasive ductal carcinomas and the associated metastatic lymph nodes express E-cadherin aberrantly. Tumor size and

Table IV. Association of histologic grade with other tumor characteristics.

Variable	Histologic grade			<i>p</i> -Value
	1	2	3	
Tumor location		N (%)		0.438
Right breast	7 (43.8)	42 (39.6)	36 (49.3)	
Left breast	9 (56.3)	64 (60.4)	37 (50.7)	
Lobular carcinoma				0.101
No	12 (75)	74 (69.8)	61 (83.6)	
Yes	4 (25)	32 (30.2)	12 (16.4)	
Ductal carcinoma				0.826
No	10 (62.5)	70 (66)	45 (61.6)	
Yes	6 (37.5)	36 (34)	28 (38.4)	
NST adenocarcinoma				0.329
No	14 (87.5)	83 (78.3)	52 (71.2)	
Yes	2 (12.5)	23 (21.7)	21 (28.8)	
Papillary carcinoma				0.084
No	14 (87.5)	100 (94.3)	72 (98.6)	
Yes	2 (12.5)	6 (5.7)	1 (1.4)	
Mixed tubular carcinoma				0.068
No	15 (93.8)	106 (100)	72 (98.6)	
Yes	1 (6.3)	0 (0)	1 (1.4)	
Metaplastic-squamous				<b>0.001</b>
No	16 (100)	106 (100)	62 (84.9)	
Yes	0 (0)	0 (0)	11 (15.1)	
Apocrine carcinoma				0.999
No	16 (100)	105 (99.1)	73 (100)	
Yes	0 (0)	1 (0.9)	0 (0)	
TNM staging				<b>0.001</b>
I	15 (93.8)	21 (19.8)	4 (5.5)	
IIA	0 (0)	42 (39.6)	23 (31.5)	
IIB	0 (0)	18 (17)	12 (16.4)	
IIIA	1 (6.3)	18 (17)	18 (24.7)	
IIIB	0 (0)	7 (6.6)	16 (21.9)	
ER				<b>0.001</b>
No expression	0 (0)	12 (11.9)	43 (60.6)	
Low expression	0 (0)	7 (6.9)	3 (4.2)	
Intermediate expression	0 (0)	8 (7.9)	5 (7)	
High expression	16 (100)	74 (73.3)	20 (28.2)	
PR				<b>0.001</b>
No expression	0 (0)	21 (20.8)	45 (63.4)	
Low expression	3 (18.8)	9 (8.9)	3 (4.2)	
Intermediate expression	2 (12.5)	10 (9.9)	8 (11.3)	
High expression	11 (68.8)	61 (60.4)	15 (21.1)	
HER-2				0.051
No expression	16 (100)	79 (78.2)	53 (74.6)	
Intermediate expression	0 (0)	18 (17.8)	9 (12.7)	
High expression	0 (0)	4 (4)	9 (12.7)	
Ki-67				<b>0.001</b>
No expression	0 (0)	1 (1)	1 (1.5)	
Low expression (<15%)	14 (93.3)	88 (90.7)	31 (47)	
High expression (30%)	1 (6.7)	8 (8.2)	34 (51.5)	
E-cadherin				0.495
No	5 (62.5)	28 (45.9)	12 (38.7)	
Yes	3 (37.5)	33 (54.1)	19 (61.3)	
Triple-negative cancer				<b>0.001</b>
No	16 (100)	96 (94.1)	40 (55.6)	
Yes	0 (0)	6 (5.9)	32 (44.4)	

NST: Non-specific type; TNM: the extent of the tumor (T), extent of spread to the lymph nodes (N), and presence of metastasis (M); PR: progesterone receptor, ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2. Statistically significant *p*-values are presented in bold.

Table V. Association of TNM staging with other tumor characteristics.

Variable	TNM staging					p-Value
	I	IIA	IIB	IIIA	IIIB	
Tumor location			N (%)			0.117
Left breast	25 (62.5)	35 (53.8)	16 (53.3)	26 (70.3)	8 (36.4)	
Right breast	15 (37.5)	30 (46.2)	14 (46.7)	11 (29.7)	14 (63.6)	
Positive sentinel lymph node						<b>0.001</b>
No	40 (100)	59 (90.8)	23 (76.7)	20 (54.1)	16 (69.6)	
Yes	0 (0)	6 (9.2)	7 (23.3)	17 (45.9)	7 (30.4)	
Multifocal tumor						<b>0.024</b>
No	20 (50)	27 (41.5)	9 (30)	12 (32.4)	16 (69.4)	
Yes	20 (50)	38 (58.5)	21 (70)	25 (67.6)	7 (30.4)	
Lobular carcinoma						0.729
No	28 (70)	49 (75.4)	24 (80)	30 (81.1)	16 (69.6)	
Yes	12 (30)	16 (24.6)	6 (20)	7 (18.9)	7 (30.4)	
Ductal carcinoma						0.934
No	25 (62.5)	43 (66.2)	20 (66.7)	24 (64.9)	13 (56.5)	
Yes	15 (37.5)	22 (33.8)	10 (33.3)	13 (35.1)	10 (43.5)	
Papillary carcinoma						<b>0.029</b>
No	35 (87.5)	65 (100)	29 (96.7)	35 (94.6)	22 (95.7)	
Yes	5 (12.5)	0 (0)	1 (3.3)	2 (5.4)	1 (4.3)	
Metaplastic-squamous						<b>0.003</b>
No	40 (40)	64 (98.5)	29 (96.7)	31 (83.8)	20 (87)	
Yes	0 (0)	1 (1.5)	1 (3.3)	6 (16.2)	3 (13)	
Apocrine carcinoma						0.999
No	40 (100)	64 (98.5)	30 (100)	37 (100)	23 (100)	
Yes	0 (0)	1 (1.5)	0 (0)	0 (0)	0 (0)	
Histologic grade						<b>0.001</b>
1	15 (37.5)	0 (0)	0 (0)	1 (2.7)	0 (0)	
2	21 (52.5)	42 (64.6)	18 (60)	18 (48.6)	7 (30.4)	
3	4 (10)	23 (35.4)	12 (40)	18 (48.6)	16 (69.6)	
ER						<b>0.001</b>
No expression	5 (12.8)	19 (30.2)	5 (17.2)	15 (41.7)	11 (52.4)	
Low expression	2 (5.1)	2 (3.2)	3 (10.3)	2 (5.6)	1 (4.8)	
Intermediate expression	0 (0)	6 (9.5)	3 (10.3)	3 (8.3)	1 (4.8)	
High expression	32 (82.1)	36 (57.1)	18 (62.1)	16 (44.4)	8 (38.1)	
PR						0.077
No expression	6 (15.4)	23 (36.5)	8 (27.6)	17 (47.2)	12 (57.1)	
Low expression	5 (12.8)	3(4.8)	4 (13.8)	2 (5.6)	1 (4.8)	
Intermediate expression	5 (12.8)	7 (11.1)	4 (13.8)	1 (2.8)	3 (14.3)	
High expression	23 (59)	30 (47.6)	13 (44.8)	16 (44.4)	5 (23.8)	
HER-2						0.689
No expression	35 (89.7)	48 (76.2)	21 (72.4)	28 (77.8)	16 (76.2)	
Intermediate expression	2 (5.1)	11 (17.5)	5 (17.2)	5 (13.9)	4 (19)	
High expression	2 (5.1)	4 (6.3)	3 (10.3)	3 (8.3)	1 (4.8)	
Ki-67						0.833
No expression	0 (0)	2 (3.3)	0 (0)	0 (0)	0 (0)	
Low expression (<15%)	31 (81.6)	45 (73.8)	20 (74.1)	23 (67.6)	14 (77.8)	
High expression (30%)	7 (18.4)	14 (23)	7 (25.9)	11 (32.4)	4 (22.2)	
E-cadherin						<b>0.033</b>
No	14 (60.9)	17 (53.1)	6 (46.2)	2 (12.5)	6 (37.5)	
Yes	9 (39.1)	15 (46.9)	7 (53.8)	14 (87.5)	10 (62.5)	
Triple-negative cancer						<b>0.016</b>
No	36 (92.3)	52 (80)	26 (89.7)	25 (69.4)	13 (61.9)	
Yes	3 (7.7)	13 (20)	3 (10.3)	11 (30.6)	8 (38.1)	
<i>In situ</i>						0.882
No	34 (85)	59 (90.8)	27 (90)	32 (86.5)	21 (91.3)	
Yes	6 (15)	6 (9.2)	3 (10)	5 (13.5)	2 (8.7)	

PR: Progesterone receptor, ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2. Statistically significant p-values are shown in bold.

Table VI. Association of triple-negative phenotype with other tumor characteristics.

Variable	Triple-negative breast cancer		<i>p</i> -Value	Variable	Triple-negative breast cancer		<i>p</i> -Value
	No	Yes			No	Yes	
Tumor location	N (%)	N (%)	0.908	ER			<b>0.001</b>
Left breast	87 (56.9)	22 (57.9)		No expression	17 (11.3)	38 (100)	
Right breast	66 (43.1)	16 (42.1)		Low expression	10 (6.7)	0 (0)	
Invaded lymph nodes			0.554	Intermediate expression	13 (8.7)	0 (0)	
No	101 (66)	27 (71.1)		High expression	110 (73.3)	0 (0)	
Yes	52 (34)	11 (28.9)		PR			<b>0.001</b>
Positive sentinel lymph node			0.999	No expression	28 (18.7)	38 (100)	
No	123 (80.4)	31 (81.6)		Low expression	15 (10)	0 (0)	
Yes	30 (19.6)	7 (18.4)		Intermediate expression	20 (13.3)	0 (0)	
Lobular carcinoma			0.057	High expression	87 (58)	0 (0)	
No	110 (71.9)	33 (86.8)		HER-2			<b>0.001</b>
Yes	43 (28.1)	5 (13.2)		No expression	110 (73.3)	38 (100)	
Ductal carcinoma			0.256	Intermediate expression	27 (18)	0 (0)	
No	102 (66.7)	21 (55.3)		High expression	13 (8.7)	0 (0)	
Yes	51 (33.3)	17 (44.7)		Ki-67			<b>0.001</b>
NST papillary carcinoma			0.999	No expression	2 (1.4)	0 (0)	
No	151 (98.7)	38 (100)		Low expression (<15%)	122 (85.3)	11 (31.4)	
Yes	2 (1.3)	0 (0)		High expression (30%)	19 (13.3)	24 (68.6)	
Metaplastic-squamous			<b>0.001</b>	E-cadherin			0.216
No	151 (98.7)	29 (76.3)		No	42 (47.7)	3 (25)	
Yes	2 (1.3)	9 (23.7)		Yes	46 (52.3)	9 (75)	
Histologic grade			<b>0.001</b>	p63			<b>0.010</b>
1	16 (10.5)	0 (0)		No	4 (100)	4 (22.2)	
2	96 (63.2)	6 (15.8)		Yes	0 (0)	14 (77.8)	
3	40 (26.3)	32 (84.2)		CK14			<b>0.001</b>
TNM staging			<b>0.019</b>	No	12 (100)	8 (33.3)	
I	36 (23.7)	3 (7.9)		Yes	0 (0)	16 (66.7)	
IIA	52 (34.2)	13 (34.2)					
IIIB	26 (17.1)	3 (7.9)					
IIIA	25 (16.4)	11 (28.9)					
IIIB	13 (8.6)	8 (21.1)					

NST: Non-specific type; TNM: the extent of the tumor (T), extent of spread to the lymph nodes (N), and presence of metastasis (M); PR: progesterone receptor; ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2. Statistically significant *p*-values are shown in bold.

the number of metastasized lymph nodes are strongly correlated with E-cadherin expression in the metastasized lymph node (38). Notably, in specimens in which the expression of E-cadherin was examined, we observed a positive correlation between its expression and lymph node involvement. This finding is in line with a recent study indicating that E-cadherin promotes, rather than suppresses, the development of metastasis and invasiveness (39).

Of note, a positive correlation between the age and the maximum size of the tumor was observed. It has been reported in the literature that younger females tend to have more aggressive breast tumors (40, 41). It has been shown that a lower age at diagnosis raises the chance of death, and this effect is most noticeable in women under 35 years of age (42). Also, it has been reported that the proportion of tumors with lymphatic invasion decreases progressively with increasing age (43). However, studies from elderly females

with breast cancer suggest that there is an association between old age and increased tumor size. These findings indicate that breast cancer might be detected at more advanced stages in the ageing population (44).

Moreover, we would like to note that neoadjuvant anti-HER2 therapy with trastuzumab is highly effective and could be administered to all HER2-positive early breast cancer patients who do not have contraindications. One year of trastuzumab therapy is standard for the vast majority of HER2-positive patients and thus we can realize the clinical significance of immunohistochemical prognostic biomarkers in breast cancer (45).

For the first time, we demonstrate the distribution of molecular breast cancer subtypes and their relation to some clinicopathological characteristics in a large cohort of Greek females. However, our study has some limitations. It is a single-center retrospective study. Also, we do not provide

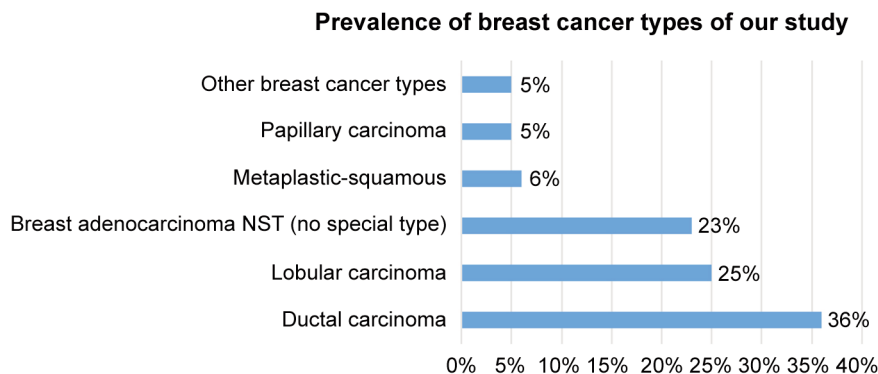


Figure 3. Prevalence of the main breast cancer types of our study as a percentage.

Table VII. 5-year frequency of breast cancer types in the central laboratory of the First Department of Pathology, National and Kapodistrian University of Athens, from 2014-2018, according to histopathological and immunohistochemical classifications.

Breast tumor subtypes	Frequency	
	N Number of incidents	% Percent
<b>Histopathological subtypes</b>		
Lobular carcinoma	49	24.9
Ductal carcinoma	70	35.5
NST adenocarcinoma (no special type)	46	23.4
Breast carcinoma <i>in situ</i>	23	11.7
Invasive breast carcinomas	174	88.3
Metaplastic-squamous	11	5.6
Papillary carcinoma	10	5.1
Mucinous carcinoma	5	2.5
Mixed tubular carcinoma	2	1
Apocrine carcinoma	1	0.5
Neuroendocrine carcinoma	1	0.5
<b>Molecular subtypes</b>		
Luminal A (ER+ and/or PR+ HER2- and Ki-67 <14%)	88	44.7
Luminal B (ER+ and/or PR+ HER2- and Ki-67 ≥14%)	6	3.05
HER2+ positive	40	20.3
Basal-like (Triple-negative cancer)	38	19.3
Luminal hybrid (ER+ and/or PR+ HER2+)	21	10.7
ER+/PR+ positive (hormonal receptor positive cancers)	119	60.4

NST: Non-specific type; PR: progesterone receptor; AR: androgen receptor, ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2.

data on the outcome of these patients, but this was out of the scope of the research study.

**Conclusion**

Our findings highlight the significance of tumor analysis carried out using immunohistochemistry and histopathological analysis. Moreover, our findings indicate that molecular and immunohistochemical analyses can be helpful in advancing biological understanding and enhance the treatment of breast cancer patients.

**Conflicts of Interest**

The Authors declare that they have no competing interests in relation to this study.

**Authors' Contributions**

PP collected all the data, conceived, and designed the study. VEG statistically analyzed the data. PP and VEG prepared the manuscript. DK, RV, CT contributed to the bibliographic support. FAA, AN, ACL, GCZ, NK, GET interpreted all relevant clinical and laboratory data and

provided critical revisions. PP, AN, ACL and GET confirm the authenticity of all the raw data. All Authors contributed to manuscript revision and have read and approved the final version of the manuscript.

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