Original Article

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Prognostic Importance of PTEN and P53 in Aggressive Luminal A Subtype Breast Cancers

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ABSTRACT

Objective: While prognostic and predictive factors in breast cancer are well established, data on aggressive behavior in luminal A subtype breast cancers are limited. The aim of this study was to investigate histomorphological and clinicopathological parameters that may predict treatment resistance and aggressive behavior in luminal A subtype, as well as the expression of two key proteins, PTEN and p53, involved in breast carcinoma development.

Materials and Methods: We included breast carcinoma cases diagnosed at a Turkish University Hospital between 2016 and 2017. Tumor tissue with internal control was available for all cases. PTEN and p53 expression were evaluated immunohistochemically, based on staining strength and percentage.

Results: Of the 114 cases diagnosed in the study period, 18 (%) were recurrent and 5 (%) were Luminal A subtype. We observed significantly lower overall and disease-free survival in patients with ≤50% tumor infiltrating lymphocytes density, which was present in all recurrent cases. PTEN immunoreactivity scores were <6 in all recurrent luminal A cases, but no significant difference was found between recurrent and non-recurrent cases (p>0.05). The p53 H-score for luminal A was significantly lower than in luminal B, triple negative, and human epidermal growth factor receptor 2+ groups (p<0.05). Furthermore, p53 H-scores <50 were more common in grade 2 tumors than in grade 3 (p<0.05).

Conclusion: PTEN loss, observed in all recurrent luminal A cases and 77.1% of all cases, supports its role as a tumor suppressor. The findings suggest that PTEN expression loss may be a prognostic marker, and immune-modulating treatments should be considered for breast cancer patients.

Keywords: Breast cancer subtypes; luminal A; molecular subtypes; p53; PTEN

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Key Points

- Predictive factors for aggressive behavior in luminal A breast cancer remain limited.
- Patients with tumor-infiltrating lymphocyte density ≤50% had significantly lower overall and disease-free survival.
- All recurrent luminal A cases had PTEN immunoreactivity scores <6, suggesting PTEN loss as a potential prognostic marker.
- p53 H-score was significantly lower in luminal A compared to other subtypes.
- Findings support the prognostic significance of PTEN loss and suggest that immune-modulating therapies should be considered for this patient group.

Introduction

Breast cancer is one of the most common cancers worldwide and the leading cause of cancer-related deaths in women (1). Molecular classification of invasive breast carcinoma, based on estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and ki-67 immunohistochemical markers, are used to delineate differences in survival, prognosis, and treatment responses among subtypes.

Endocrine therapy (ET) targeting the ER is the primary treatment for luminal A (LumA) subtype breast cancer, with numerous agents improving survival outcomes. While LumA patients generally have better prognoses, up to 27% develop resistance to ET, resulting in metastases and fatal outcomes (2).

PTEN, a lipid phosphatase that suppresses the PI3K pathway, is lost in 15–50% of breast cancers, shortening progression-free survival (3-5). A study found a significant correlation between PTEN expression and

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smaller tumor size, lower tumor grade, ER/PR positivity and lower ki-67 (a marker of cellular proliferation) levels (6). *TP53*, mutated in 30–35% of invasive breast cancers, varies by molecular subtype, with 17% in LumA, 41% in luminal B (LumB), 69% in apocrine, 88% in basal-like, and 50% in HER2-amplified tumors (7). Increased p53 expression was associated with larger tumor size, higher grade, nodal metastasis, reduced ER/PR levels and overexpression of HER2 (8). These mutations make p53 a potential biomarker and therapeutic target (9).

The aim of this study was to investigate the relationship between histomorphological and clinical features with prognosis in aggressive LumA carcinoma, defined as metastasis or local recurrence within five years, compare it with other subtypes, explore the independent and combined roles of PTEN and p53 in prognosis, and identify potential new therapeutic targets for this patient group.

Materials and Methods

Case Selection and Clinicopathological Features

In this study, breast cancer patients whose samples were sent to the Medical Pathology Clinic of University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital between 2016 and 2017 were retrospectively analyzed. Patients who received neoadjuvant treatment, had metastatic or microinvasive carcinoma at diagnosis, or had insufficient tumor tissue for immunohistochemical staining were excluded. Histopathological parameters (subtype, grade, size, location) were determined by re-evaluating the preparations alongside pathology reports. Clinical data (gender, age, menopausal status, surgical procedure, recurrence, metastasis, treatments, and survival times) were collected from electronic records and physicians in general surgery and oncology. Ethics committee approval from the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (approval number: 2021-13-03) was granted on 05.07.2021. The antibodies and kits were funded by the hospital's fund.

Histomorphological Evaluation

Haematoxylin and eosin (H&E) and immunohistochemically stained slides of the cases were evaluated independently by two pathologists. Slides were stained for H&E and immunohistochemically for ER, PR, cellular erythroblastic oncogene B2 (CERBB2), ki-67, and E-Cadherin. Key features, such as histological type, grade [using the Nottingham system (10)], molecular subtype (based on ER, PR, CERBB2, and ki-67 status), presence of ductal carcinoma *in situ* (DCIS), lobular carcinoma *in situ* (LCIS), extensive intraductal component [extensive intraductal component (EIC): ≥25% DCIS], perineural invasion (PNI), lymphovascular invasion (LVI), microcalcification, surgical margin status, tumor-infiltrating lymphocyte (TIL) percentage (<50% defined as low, ≥50% as high) (11), and non-tumor breast tissue were assessed. Pathological staging was performed using the American Joint Committee on Cancer 8th edition TNM staging system (12).

Immunohistochemical Method and Evaluation

ER, PR, CERBB2, and ki-67 mitotic index were assessed by re-evaluating immunohistochemical slides. Paraffin-embedded tissue blocks containing internal control tissue, lacking necrosis, and with adequate tumor tissue for immunohistochemical analysis were selected. External controls used were normal brain tissue and malignant melanoma for PTEN, and serous ovarian carcinoma for p53. Staining was performed on an automated immunohistochemistry device (Ventana Benchmark XT: Roche Diagnostics Corporation, Indianapolis, IN, USA) using p53 (Clone DO-7, Dako Omnis: Agilent Technologies, Inc., Santa Clara, CA, USA) and anti-PTEN [RM265] primary antibodies (RevMAb Biosciences, Burlingame, CA, USA).

Positive immunoreactivity for PTEN was defined as cytoplasmic and nuclear staining of tumor cells. Staining was graded by strength (0: no expression, 1: weak, 2: moderate, 3: strong) and by the percentage of reactive cells (0: <1%, 1: 1–10%, 2: 11–50%, 3: 51–80%, 4: >80%) (Figures 1, 2). The immunoreactivity score (IRS) was calculated by multiplying these values and categorized as 0, 1–6, or 7–12, with IRS ≤6 considered PTEN loss (13).

Nuclear staining of tumor cells with p53 was considered immunoreactive. Staining was evaluated by strength (0: no expression, 1: weak, 2: moderate, 3: strong) and by the percentage of reactive tumor cells (1: ≤10%, 2: 11–50%, 3: 51–70%, 4: >71%) (Figures 3, 4). The H-score, obtained by multiplying strength and percentage scores, was grouped as <50 or ≥50 (14).

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics, version 22 (IBM Inc., Armonk, NY, USA). Normality was assessed with the Shapiro-Wilks test. Descriptive statistics (mean, standard deviation,

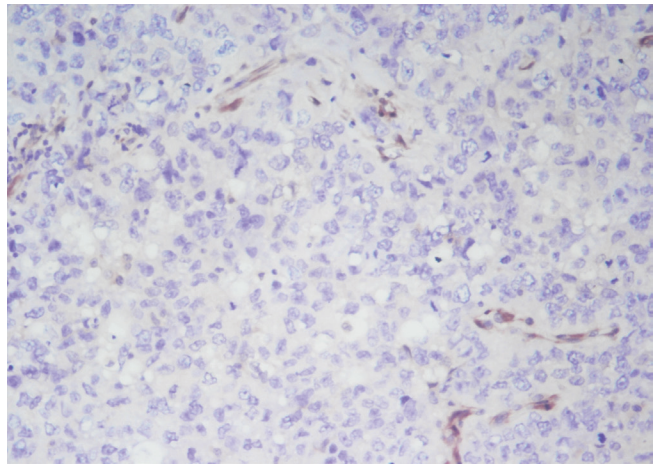

Figure 1. PTEN IRS 0x0 (strength group x % group; x100)

IRS: Immunoreactivity score

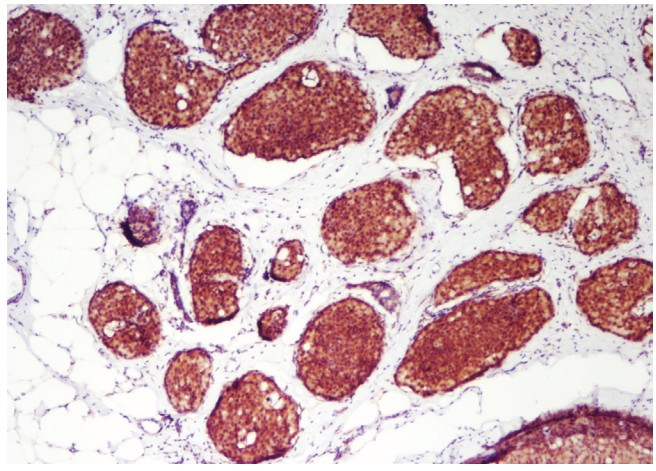

Figure 2. PTEN IRS 3x4 (strength group x % group; x100)

IRS: Immunoreactivity score

frequency) were calculated. Non-normally distributed data were analyzed using the Kruskal-Wallis test (with Dunn's test) and the Mann-Whitney U test for multi-group and two-group comparisons, respectively. Qualitative data comparisons utilized chi-square, Fisher's exact, Fisher-Freeman-Halton, and Yates' Correction tests. Significance was set at $p<0.05$.

Results

The study included 114 patients aged 27–80 years, (mean age 53.3±13.6 years). Most were female (98.2%, *n* = 112), with 2 males (1.8%). Of the patients, 44.6% (*n* = 50) were premenopausal, and 55.4% (*n* = 62) were postmenopausal. Tumor location was right breast in 41.2% (*n* = 47), left breast in 57% (*n* = 65), and bilateral in 1.8% (*n* = 2). Localization included upper outer (8.8%), upper inner (30.1%), lower outer (13.3%), lower inner (3.5%), retroareolar (15.9%), and multiple quadrants (28.3%).

Unifocal tumors were observed in 78.1% (*n* = 89), and multifocal tumors in 21.9% (*n* = 25). Surgical interventions included modified radical mastectomy (58.8%, *n* = 67), breast-conserving surgery (32.5%, *n* = 37), and simple mastectomy (8.8%, *n* = 10). Tumors at surgical margins were found in 3.5% (*n* = 4), while 42.5% (*n* = 48) were within 1 cm, and 54% (*n* = 61) were >1 cm distant from the margin.

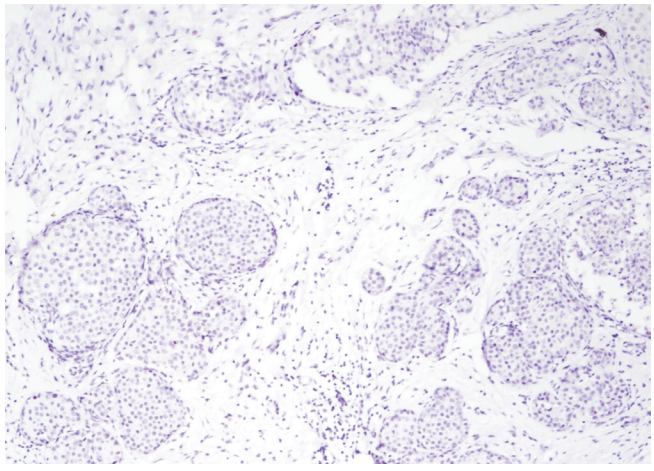

Figure 3. p53 H-score 0 (strength group x %; x100)

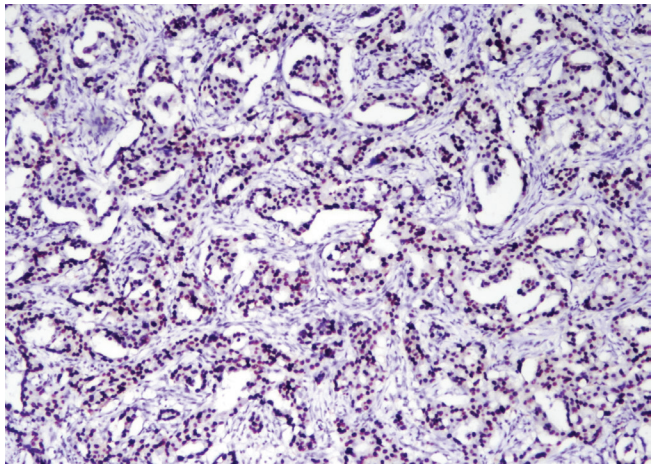

Figure 4. p53 H-score 3x95% = 285 (strength group x %; x100)

The histological subtypes of the cases were as: 91 (79.8%) invasive ductal carcinoma (IDC), 9 (7.9%) invasive lobular carcinoma (ILC), 9 (7.9%) mixed carcinoma, 2 (1.8%) mucinous carcinoma, 1 (0.9%) solid papillary carcinoma, 1 (0.9%) cribriform carcinoma and 1 (0.9%) metaplastic carcinoma.

Tumor size, grade, TIL status, DCIS, LCIS, PNI, microcalcifications, and non-tumor findings are detailed in Table 1.

Immunohistochemical study results and molecular subtype distribution are shown in Table 2.

Of the 114 patients, 59.6% had no LVI, while 40.4% did. EIC was absent in 77.2% and present in 22.8%. Tumor stages were distributed as follows: 1.8% pT1a, 4.4% pT1b, 21.2% pT1c, 61.1% pT2, 7.1% pT3, and 4.4% pT4. Regarding pN stages, 54.4% were pN0, 28.1% pN1a, 1.8% pN1mi, 9.6% pN2a, and 6.1% pN3a.

Overall survival (OS) ranged from 7 to 74 months (52.0±15.6), while disease free survival (DFS) ranged from 3 to 74 months (49.1±17.7). During follow-up, 15.8% of patients experienced recurrence/metastasis.

Table 1. Distribution of the parameters of tumor size, histological grade, TIL, non-tumor breast tissue status, DCIS, LCIS, PNI and presence of microcalcification

		Min-Max	Mean (SD)
Tumor size (cm)		0.5-10	2.9 (1.5)
		n	%
Histological grade	**G1**	18	15.8
	G2	73	64
	G3	23	20.2
TIL	**≤50%**	106	93
	>50%	8	7
Non-tumor breast tissue	**No features**	55	48.2
	Fibrocystic changes	40	35.1
	Atypical ductal hyperplasia	14	12.3
	Flat epithelial atypia	1	0.9
	Columnar cell change	4	3.5
DCIS	**Absent**	42	36.8
	Present	72	63.2
LCIS	**Absent**	101	88.6
	Present	13	11.4
PNI	**Absent**	101	88.6
	Present	13	11.4
Microcalcification	**Absent**	64	56.1
	Present	50	43.9

DCIS: Ductal carcinoma *in situ*; LCIS: Lobular carcinoma *in situ*; PNI: Perineural invasion; SD: Standard deviation; TIL: Tumor infiltrating lymphocyte; Min: Minimum; Max: Maximum

Adjuvant therapy was administered as follows: 71.1% received chemotherapy (CT) (various regimens), 19.3% did not receive CT, and 9.6% had less common regimens. Radiotherapy was given to 71.1% of patients, and 86.8% underwent ET (55.2% letrozole, 31.6% tamoxifen).

The p53 H-score ranged between 0 and 285, with 82.5% scoring <50 and 17.5% scoring ≥50. The PTEN IRS ranged between 0 and 12, with 15.8% scoring 0, 62.3% scoring 1–6, and 21.9% scoring 7–12 (Table 2).

Grade 2 group had a statistically significantly higher rate of p53 H-scores below 50 (89%) compared to grade 3 (56.5%) (p = 0.001), with no significant differences among other grades (p>0.05).

PTEN IRS distribution varied significantly between histological subtypes (p = 0.010). Mixed carcinoma had higher PTEN IRS of 7–12 (55.6%) compared to IDC (20.9%) and ILC (0%) (p_1 = 0.004 and p_2 = 0.003, respectively).

Table 2. Distribution of ki-67, ER, PR and CERBB2 status, luminal A and recurrence status, molecular subtype, p53 H-score, PTEN IRS parameters

		n	%
Ki-67	<14%	59	52.2
	14-19%	14	12.4
	≥20%	40	35.4
ER	0	18	15.8
	1-80%	32	28.1
	>80%	64	56.1
PR	0	41	36
	1-80%	56	49.1
	>80%	17	14.9
CERBB2	Score 0	83	72.8
	Score 1	11	9.6
	Score 2	4	3.5
	Score 3	16	14
Luminal A with recurrence status	No recurrence	52	91.2
	Recurrence	5	8.8
Molecular subtype	Luminal A	57	50
	Luminal B	38	33.3
	TN	10	8.8
	HER2/NEU	9	7.9
p53 H-score	<50	94	82.5
	≥50	20	17.5
PTEN IRS	0	18	15.8
	1-6	71	62.3
	7-12	25	21.9

ER: Estrogen receptor; PR: Progestrone receptor; TN: Triple negative; CERBB2: Cellular erythroblastic oncogene B2; IRS: Immunoreactivity score; HER2: Human epidermal growth factor receptor 2

OS values for the TN group were lower than LumA and LumB (p_1 = 0.013, p_2 = 0.001, respectively).

LumA group had a higher rate of p53 H-scores below 50 (94.7%) compared to LumB (78.9%), TN (60%), and HER2/NEU (44.4%) (p_1 = 0.022, p_2 = 0.008 and p_3 = 0.001, respectively), with no significant differences among other subtypes (Table 3).

OS and DFS values were significantly lower in patients not receiving ET compared to those receiving Letrozole or Tamoxifen (p = 0.042 and p = 0.031, respectively), with no significant difference between Letrozole and Tamoxifen groups. A significant difference was found in p53 H-score distribution among ET groups (p = 0.005). Patients not receiving ET had fewer H-scores below 50 (53.3%), with no difference between Letrozole and Tamoxifen groups (p>0.05).

OS values were significantly higher in patients with upper inner tumor location compared to those with lower inner, lower outer, retroareolar, and multiple quadrant locations (p_1 = 0.021, p_2 = 0.007, p_3 = 0.049, and p_4 = 0.004, respectively). No significant differences were found between other tumor location groups (p>0.05).

DFS values were significantly lower in patients with multiple quadrant tumors compared to upper inner and upper outer locations (p_1 = 0.008 and p_2 = 0.022, respectively) and in lower outer tumors compared to the upper inner group (p = 0.034).

OS and DFS values were significantly higher in patients with EIC compared to those without EIC (p = 0.008 and p = 0.049, respectively).

Recurrence rates were significantly higher in patients with LCIS (38.5%) compared to those without LCIS (12.9%) (p = 0.032).

LumA with recurrence rates were significantly higher in patients with PNI (50%) compared to those without PNI (3.9%) (p = 0.006).

Recurrence rates were significantly higher in patients with LVI (30.4%) compared to those without LVI (5.9%) (p = 0.001).

DFS values in the pT3 group were significantly lower than those in the pT1b and pT1c groups (p_1 = 0.022 and p_2 = 0.018, respectively). No significant differences were found among other pT groups. Recurrence rates were lower in the pN0 (8.1%) and pN1a (6.3%) groups compared to pN2a (63.6%) and pN3a (42.9%) groups (p_1<0.001 and p_2<0.001, respectively).

OS and DFS values were lower in patients with TIL ≤50% compared to TIL>50% (p = 0.026 and p = 0.012, respectively).

DFS values were lower in cases without ER staining compared to 1–80% and >80% groups (p_1 = 0.013, and p_2 = 0.038), with no difference between the 1–80% and >80% groups (p>0.05).

A significant difference was identified between ki-67 groups in the distribution rates of p53 H-score groups (p = 0.009). The proportion of cases with ki-67 <14% (93.2%) was significantly higher than those with ki-67>20% (70%) (p = 0.005).

Comparisons could not be made between grade groups, histological subtypes, ET groups, tumor locations, or the presence of DCIS, EIC, LCIS, PNI, LVI, as well as pT stage, pN stage, surgical margin groups, TIL groups, CERBB2 groups, and ki-67 in the LumA sub-group with recurrence because of small sample size.

Table 3. Evaluation of OS, DFS, presence of recurrence, p53 H-score and PTEN IRS parameters according to molecular subtype groups

Luminal A (Min-Max)-Mean (SD)		Molecular subtype				*p*
		Luminal B	TN	HER2/NEU		
		(Min-Max)-Mean (SD)	(Min-Max)-Mean (SD)	(Min-Max)-Mean (SD)		
OS (months)		(8–71)–52.0 (15.1)	(7–74)–55.3 (15.7)	(24–62)–38.3 (13.6)	(22–69)–52.6 (14.1)	[1]**0.017***
DFS (months)		(4–71)–50.9 (16.3)	(3–74)–50.7 (18.1)	(10–62)–34.1 (17.6)	(6–69)–47.5 (19.5)	[1]0.057
		n (%)	n (%)	n (%)	n (%)	
Recurrence	**Absent**	52 (91.2)	30 (78.9)	7 (70)	7 (77.8)	[2]0.122
	Present	5 (8.8)	8 (21.1)	3 (30)	2 (22.2)	
p53 H-score	**<50**	54 (94.7)	30 (78.9)	6 (60)	4 (44.4)	[2]**<0.001***
	≥50	3 (5.3)	8 (21.1)	4 (40)	5 (55.6)	
PTEN IRS	**0**	8 (14)	7 (18.4)	3 (30)	0 (0)	[3]0.279
	1–6	36 (63.2)	23 (60.5)	7 (70)	5 (55.6)	
	7–12	13 (22.8)	8 (21.1)	0 (0)	4 (44.4)	

[1]: Kruskal-Wallis test

[2]: Fisher-Freeman-Halton test

[3]: Chi-squared test

DFS: Disease free survival; OS: Overall survival; SD: Standard deviation; TN: Triple negative; IRS: Immunoreactivity score; Min: Minimum; Max: Maximum; SD: Standard deviation

Similarly, comparisons for TIL in terms of recurrence were not possible due to insufficient data.

DFS values were significantly lower in cases with LumA with recurrence compared to those without (p = 0.010).

No comparison could be made between those with and without LumA and recurrence in p53 H-score groups due to insufficient numbers.

There was no significant difference between those with and without LumA and recurrence in terms of OS duration and PTEN IRS distribution rates.

Except for the significant differences mentioned above and statistical comparisons that could not be made due to insufficient numbers, no significant differences were observed in statistical comparisons between each of histological grade, histological subtype, molecular subtype, CT/RT/ET status, presence of DCIS/EIC/LCIS, presence of LVI, pT/pN stages, TIL groups, ki-67 groups and each of OS/DFS durations, LumA with recurrence status, recurrence rates, p53 H-score/PTEN IRS.

Similarly, no significant differences were observed in the statistical comparisons between each of menopausal status, laterality, focality, tumor location, presence of microcalcification, presence of PNI, ER/PR staining percentages, CERBB2 scores and each of OS/DFS durations, LumA with recurrence status, recurrence rates.

In addition to the statistical findings, we share a detailed analysis of recurrent cases with tumors of the LumA subtype, which was the focus of our study (Table 4).

Discussion and Conclusion

The risk of local recurrence and metastasis, key indicators of aggressive breast cancer prognosis, is influenced by tumor stage and molecular characteristics. Saphner et al. (15) reported a 30% recurrence rate in patients undergoing appropriate treatment, while a meta-analysis of trials published in the Lancet (16) found a recurrence rate of 20–30% in early breast cancer. In the present study, involving both early and non-early-stage cases, 15% of patients developed distant metastasis with local recurrence during a 5-year follow-up, a relatively low rate.

Sørlie et al. (17) highlighted longer OS and DFS durations in LumA subtypes, with *TP53* mutations found in 13% of LumA, 71% of HER2+, and 82% of Basal-like subtypes. In the present study, OS values were significantly lower in TN compared to LumA and LumB groups. ER-negative cases also showed significantly lower DFS values than those with ER staining percentages of 1–80% and >80%. This finding may be due to the unique biology of molecular subtypes as discussed by Bosch et al. (18) on the molecular characteristics and pathogenesis of TNs. We also hypothesize that this may be explained by the scoring we used to show the presence of p53 overexpression, which is associated with poor prognosis in breast cancers (19), showed that the proportion with an H-score above 50 in the LumA group (5.3%) was significantly smaller than in the LumB (21.1%), TN (40%) and HER2+ (55.6%) groups.

Ki-67 is important for classifying luminal subtypes, but the optimal threshold value remains unclear. Following the Saint Gallen Consensus (20, 21), we used 14% as a threshold, analyzing cases as <14%, 14%–<20%, and ≥20%. No significant differences were found in OS, DFS, or recurrence rates across ki-67 groups. However, p53 overexpression (H-score >50) was significantly more common in ki-67 >20% than in the <14% group, suggesting a 20% threshold may better predict poor prognosis and guide management.

Although the breast cancer tumor microenvironment harbors diverse cells, TILs are a key group. Studies have shown that TILs play critical roles in cancer progression (22). Korkaya et al. (23) demonstrated that interleukins secreted by certain TILs may promote tumor development. Another study found that higher TIL concentrations predicted response to neoadjuvant CT across all molecular subtypes and improved survival in HER2-positive and TN breast cancers, but were a negative prognostic factor for survival in luminal-HER2-negative cancers (24). In the present study, OS and DFS values were

Table 4. Study parameters in recurrent luminal A subtype cases

	Case 1	Case 2	Case 3	Case 4	Case 5
Age	46	80	79	55	44
Gender	Female	Female	Female	Female	Female
Menopausal status	Pre	Post	Post	Post	Pre
Procedure	MRM	MRM	MRM	MRM	MRM
Laterality	Right	Left	Right	Right	Left
Focality	Uni	Uni	Multi	Multi	Uni
Tumor site	*	Multi quadrant	Lower outer	Upper outer	RA
Tumor size (cm)	*****	**10**	**4**	**2**	**2.2**
Histological subtype	IDC	IDC	IDC	ILC	IDC
Histological grade	G2	G2	G2	G2	G2
LVI	Present	Present	Present	Absent	Present
PNI	Present	Absent	Present	Absent	Present
DCIS	Present, HG	Absent	Present, IG	Absent	Present, HG
EIC	Absent	Absent	Absent	Absent	**Present**
LCIS	Absent	Absent	Absent	**Present**	Absent
Microcalcification	Absent	Absent	Present	Present	Present
TIL (%)	15	5	5	5	10
Surgical margins-tumor distance	*	<1 cm	**Positive**	<1 cm	>1 cm
pTN	T*N2a	T3N0	**T4N2a**	T1cN0	**T2N3a**
ER (%)	20	90	90	80	90
PR (%)	20	70	20	0	0
Ki-67 (%)	10	12	10	10	5
p53 H-score	0 (null)	<10	<10	<10	<10
PTEN IRS	**2**	**0**	**0**	**3**	**2**
Chemotherapy	STD	**Refused**	STD	STD	STD
Radiotherapy	Received	Received	Received	Received	Received
Endocrine therapy	Letrozole	Letrozole	Letrozole	Letrozole	Letrozole
OS (month)	29	36	8	65	59
DFS (month)	21	12	4	60	35
Location of metastasis	Bone	Bone, LN, liver	Bone, liver	Bone, liver	Bone, LN
Molecular subtype of metastasis biopsy	Luminal A	Luminal A	*	Luminal A	No biopsy performed

DCIS: Ductal carcinoma *in situ*; DFS: Disease free survival; EIC: Extensive intraductal component; ER: Estrogen receptor; HG: High grade; IDC: Invasive ductal carcinoma; IG: Intermediate grade; ILC: Invasive lobular carcinoma; LCIS: Lobular carcinoma *in situ*; LN: Lymph node; LVI: Lymphovascular invasion; MRM: Modified radical mastectomy; OS: Overall survival; PNI: Perineural invasion; PR: Progestrone receptor; RA: Retroareolar; STD: Standard treatment; Adriamycin+Cyclophosphamide+Docetaxel; TIL: Tumor infiltrating lymphocyte

*: Non-avaiable data

significantly poorer in cases with TIL values ≤50% compared to those >50%. Furthermore, all 18 recurrence cases had TIL values ≤50%, supporting the inclusion of immune-modulating therapies in breast cancer treatment.

We believe the lower recurrence rate in the non-luminal group, despite its worse prognosis compared to luminal subtypes, is due to the significantly smaller number of cases in this group. Additionally, a detailed discussion of our five aggressive recurrent LumA cases, described in the findings section, will support the main aim of our study.

Case 1: The right mastectomy material of a patient, whose operation and initial pathology were conducted at another center, revealed axillary lymph node metastases (pN2a) at diagnosis with notable LVI. Axillary lymph node metastasis is a critical prognostic factor in early-stage breast cancer. Weigelt et al. (25) reported that 70–80% of lymph node-positive patients develop distant metastasis, compared to 20–30% of node-negative patients.

In the current study, the recurrence rate in the pN0 group (8.1%) was significantly lower than in the pN2a (63.6%) and pN3a (42.9%) groups. Similarly, the pN1a group (6.3%) had a significantly lower recurrence rate than the pN2a (63.6%) and pN3a (42.9%) groups.

Lymph node metastasis, influenced by patient clinical features and tumor biology, may explain the aggressive course in this case. Notably, this is the only patient among the five LumA cases with recurrence that had p53 H-score: 0 (null) and PTEN IRS: 2 (IRS<6), indicative of mutations associated with poor prognosis.

Case 2: The left mastectomy material of an 80-year-old patient revealed a tumor <1 cm from the posterior surgical margin, with prominent LVI, and a tumor size of 10 cm-the largest in our study. LVI is a poor prognostic indicator in breast cancer. Kuhn et al. (26) identified LVI as an independent prognostic factor linked to local recurrence, distant metastasis, and worse DFS and OS outcomes, even in lymph node-negative patients. It also influences radiotherapy decisions.

In our cohort, recurrence rates were significantly higher in patients with LVI (30.4%) than those without LVI (5.9%). The large tumor size and potentially inadequate surgical margins may explain the patient's aggressive disease course. Moreover, the patient had comorbidities due to advanced age, and she also declined CT. Fisusi and Akala (27) emphasized that tailored therapeutic strategies minimize toxicity and recurrence risk in breast cancer patients. Refusal of CT likely contributed to local recurrence and distant metastasis, seen in this patient.

The absence of PTEN immunoreactivity further supports the aggressive prognosis in this case.

Case 3: In this 79-year-old patient, examination of the right mastectomy material revealed tumor cells at the posterior surgical margin, with the tumor stage classified as T4 due to breast skin ulceration caused by two separate tumor foci. This case also exhibited diffuse columnar cell changes in non-tumor tissue, a unique finding among LumA cases with recurrence. The present study found a significantly higher recurrence rate (50%) in cases with positive tumor margins compared to those with a tumor-to-margin distance of <1 cm (6.3%).

In addition to the positive surgical margin, the presence of multifocal tumors, skin ulceration, axillary lymph node metastases (pN2a), and LVI likely contributed to local recurrence. Lymph node metastasis, as previously discussed, is a poor prognostic indicator. The patient's complete loss of PTEN expression is another factor that may have facilitated tumor recurrence.

Case 4: In this 55-year-old patient, the right mastectomy material revealed multifocal tumor foci with an ILC histological type, unlike the other LumA cases with recurrence. ILC is associated with a worse prognosis compared to IDC in luminal subtypes, as noted by Adachi et al. (28), although another study has shown better OS for hormone receptor-positive HER2-negative ILC compared to IDC (29).

This patient also had LCIS at the superior and posterior surgical margins, with tumor cells <1 cm from the superior, posterior, and anterior margins. Our study found a significantly higher recurrence rate (38.5%) in cases with LCIS compared to those without (12.9%). The presence of LCIS in the surgical margins and a PTEN score of 3 (IRS <6) likely contributed to the increased risk of recurrence, highlighting an aggressive prognosis in this case.

Case 5: In the left mastectomy specimen of a 44-year-old patient, the most striking finding was the presence of EIC, which was the only EIC among our patients having LumA with recurrence. EIC is known to complicate preoperative imaging assessments of tumor size and location and is linked to higher surgical margin positivity. A study by Chagpar et al. (30) demonstrated an increased risk of local recurrence in breast cancers with EIC. However, while Corsi et al. (31) found that EIC was not associated with local recurrence-free survival or distant metastasis, it was linked to improved 5-year OS in pT1-stage cancers, but not in pT2-stage cases.

In the current study, patients with EIC had statistically higher OS values than those without. Although EIC may have contributed to local recurrence in this patient, the development of distant metastasis at 35 months could be attributed to the advanced pathological stage at diagnosis (pT2N3a). Furthermore, the patient's PTEN score of 2 (IRS <6) suggested a poor prognosis.

Among LumA cases with recurrence, 4/5 had H-scores <10, and 1 had a score of 0. The LumA group had a significantly higher rate of H-scores <50 (94.7%) compared to LumB (78.9%), TN (60%), and HER2+ (44.4%) subtypes.

TP53 mutations in LumA were detected at a rate of 21%, comprising 15.7% with p53 loss (null type) and 5.2% with p53 overexpression. Deletions leading to p53 protein loss are more common in Apocrine and Basal-like subtypes (9).

Tumors with an H-score below 50 were significantly more frequent in grade 2 (89%) than grade 3 (56.5%). A previous study showed that p53 overexpression correlated with higher grades and reduced ET/CT response (32). However, immunohistochemical methods may miss non-missense mutations, potentially causing false negatives (33). Advanced methods, such as Next Generation Sequencing will improve detection accuracy.

A study showed that PTEN loss was associated with adverse clinicopathological features (6) while our study did not demonstrate a statistical relationship between PTEN loss and recurrence in LumA patients. However, the presence of PTEN loss in all recurrent cases and its overall rate of 77.1% (88/114) highlights its tumor suppressor role.

These findings may support the use of PTEN as a prognostic marker in breast cancer.

Study Limitations

The study's main limitation was the small number of recurrent LumA cases, restricting some analyses. However, it is the first to focus on the relationship between p53 and PTEN status with aggressive prognosis in LumA tumors.

Understanding the biological variations within LumA subtype breast cancers is important for developing targeted treatment strategies. Larger studies, advanced sequencing techniques, and identifying pathways beyond PTEN and p53 mutations could enhance early diagnosis and improve survival outcomes for patients with aggressive LumA tumors.

Ethics

Ethics Committee Approval: Ethics committee approval from the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (approval number: 2021-13-03) was granted on 05.07.2021.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.D.G., S.A., İ.G., D.T., M.K.; Concept: Ö.D.G., S.A., D.N.S.; Design: Ö.D.G., S.A., İ.G., M.K.; Data Collection or Processing: Ö.D.G., İ.G., M.K.; Analysis or Interpretation: Ö.D.G., S.A., D.N.S.; Literature Search: Ö.D.G., İ.G., Writing: Ö.D.G.

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