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Ki-67 index value and progesterone receptor status can predict prognosis and suitable treatment in node-negative breast cancer patients with estrogen receptor-positive and HER2-negative tumors

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Abstract. Gene profiling has identified at least 4 breast cancer subtypes, including Luminal A, Luminal B, HER2-enriched and basal-like, and immunohistochemistry is used as a guide to determine these subtypes. In the present study, patients with ER-positive, HER2-negative and negative nodes were classified into 4 groups according to the PgR and the Ki-67 status and were retrospectively examined. The analysis was based on the clinicopathological findings, and includes the recurrence score (RS) and disease-free survival (DFS) rates. Patients with invasive breast cancer (n=1866) were classified as LA (high PgR/low Ki-67), LB-1 (high PgR/high Ki-67), LB-2 (low PgR/high Ki-67), and LB-3 (low PgR/low Ki-67). In addition, 41 of the cases underwent a 21-gene expression assay. The data revealed that T1 tumors were more prevalent in the LA group and rare in the LB-2 group. Furthermore, nuclear grade 3 and p53 overexpression was revealed to be significantly correlated with LB-2. In terms of prognosis, LA had a significantly more favorable DFS; however, no differences were observed in the LB-3 group. LB-2 had a significantly worse DFS in all cases, and in the cases administered with endocrine therapy alone. Chemotherapy in combination with endocrine therapy was administered to cases with a higher risk of recurrence. In the LB-2 group, there was no difference in the DFS rates between the cases with endocrine therapy and chemo-endocrine therapy. These findings suggest that chemotherapy could improve the DFS in the LB-2 group. In addition, the majority of cases with LA, LB-3 and LB-1 had a RS of ≤25 and the majority of the LB-2 cases had a RS of >25. The patients with LA and LB-3 had a favorable DFS even in the group that received endocrine therapy alone. LB-2 was significantly correlated with a higher degree of malignancy and benefited from chemotherapy. These data suggest that the PgR and the Ki-67 status are effective in predicting prognosis, and for deciding on the most effective treatment strategy in patients with breast cancer.

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Key words: chemotherapy, early-stage breast cancer, endocrine therapy, estrogen receptor-positive, gene expression assays, Ki-67, breast cancer subtype, progesterone receptor

Introduction

Breast cancer has become a multi-faceted disease with different histopathological and biological features. These biological features are responsible for the distinct behavior often observed in the different kinds of breast cancer and therefore require appropriate therapeutic strategies. Gene profiling has identified 4 breast cancer subtypes [Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2)-enriched and basal-like] and immunohistochemistry (IHC) is used to determine these breast cancer subtypes. In a previous study, Luminal A was defined as estrogen receptor (ER)- and PgR-positive, HER2-negative, Ki-67 'low' and recurrence risk 'low' based on the multi-gene-expression assay (1). A high Ki-67 value (≥20%) and a low PgR value (<20%) are used to separate 'Luminal A-like' and 'Luminal B-like (HER2-negative)' breast cancers. Previous studies (2,3) found that a higher Ki-67 index value at the hotspot significantly correlated with recurrence and that the optimal cut-off value for Luminal/HER2-negative breast cancer was 20% (4). Moreover, it was reported (5,6) that the proposed IHC-based definition of luminal A tumors is a PgR >20% in hormone receptor (HR)-positive/HER2-negative tumors.

The most common subtype among breast cancer is Luminal A type tumors. In the Carolina Breast Cancer Study (7), luminal type tumors represented 64.3% of all patients and 54.3% of the cases were luminal A tumors. The luminal subtypes generally have a good prognosis but luminal B type tumors tend to have a significantly more unfavorable prognosis than the luminal A

subtype (8). Moreover, luminal tumors often respond to endocrine therapy but rarely to conventional chemotherapy (9).

Oncotype DX, also known as the 21-gene assay, evaluates 16 cancer-related genes and 5 normal comparator reference genes and was designed to target ER-positive tumors (10). The purpose of using Oncotype results is to calculate the recurrence score (RS). The higher the RS (scale on a range of 1-100) the worse the prognosis but tumors with a higher RS have a higher probability of responding to chemotherapy (11). Barcenas *et al* (12) evaluated the recurrence-free and overall survival rates of patients with an RS of 11-25 after receiving chemotherapy. They found similar results in patients (RS of 11-25) with or without chemotherapy in HR-positive, HER2-negative, lymph node-negative breast cancer.

In this study, patients with ER-positive, HER2-negative and negative node were classified into 4 groups according to the PgR and the Ki-67 status (cutoff points, 20%) and retrospectively examined. The analysis was based on the clinicopathological findings and include the RS and disease-free survival (DFS) rates.

Patients and methods

Patients. Invasive breast cancer patients (n=1866) between November 2001 and November 2016 were enrolled in this study. Patient backgrounds are shown in Table I. The cases were classified as follows (Fig. 1); LA (high PgR/low Ki-67; 850 cases), LB-1 (high PgR/high Ki-67; 553 cases), LB-2 (low PgR/high Ki-67; 226 cases), and LB-3 (low PgR/low Ki-67; 237 cases). Out of all these cases, 1,510 were treated with endocrine therapy alone. The median observation period was 78.1 months. Moreover, 41 of the cases underwent a 21-gene expression assay and the RS (≤25 and >25) was compared with our above mentioned classification.

Histopathological examination. The factors investigated were the lymph nodal status, tumor size, nuclear grade, ER/PgR and HER2 status, overexpression of p53 protein and the Ki-67 index value. ER, PgR, HER2, p53 and Ki-67 were evaluated using IHC with an autostainer (Benchmark XT; Ventana Medical Systems, Inc., Tucson, USA) in accordance with the procedure previously reported (13). The antibody used was ER (clone SP1; rabbit monoclonal), PgR (clone 1E2; rabbit monoclonal), HER2 (clone 4B5; rabbit monoclonal; all Ventana Medical Systems, Inc.), p53 (clone DO7; mouse monoclonal) and Ki-67 (clone MIB-1; mouse monoclonal; both Dako; Agilent Technologies, Inc., Santa Clara, CA, USA). The ER- and PgR-positive cell rates were calculated using IHC. The ASCO/CAP guidelines recommend a value of ≥1% stained tumor nuclei as being positive.

The percentage of positive nuclei for Ki-67 was calculated based on a count of at least 500 tumor cells in the hot spot. The p53 overexpression was determined in the cases with positive cells ≥50% (8). Positive for HER2 is either a HER2 score of 3 + (strong and diffuse staining) or FISH amplified in equivocal cases (score 2+). The other staining pattern of HER2 was determined as HER2-negative. Fig. 2 shows the subclassified cases of ER-positive and HER2-negative breast cancer according to the PgR and the Ki-67 status.

Statistical analysis. Statistical analysis was performed using SPSS version 21 (IBM Corp., Armonk, NY, USA). Comparisons between groups (Tables II and III) were analyzed using the Chi-square test and Fisher's exact test. Age was determined using the Student's t-test. Cumulative DFS was calculated using the Kaplan-Meier method and evaluated using the log-rank test.

Table I. Patient characteristics (n=1866).

Characteristics	N (%)
Age (years)	58.5+/-0.31 (median+/-SEM)
Menopausal status	
Pre	687 (36.8)
Post	1,179 (63.2)
Tumor size (cm)	
<2	1,400 (75.0)
≥2	462 (24.8)
Unknown	4 cases
Nuclear grade	
1	1,142 (62.6)
2	527 (28.9)
3	154 (8.5)
Unknown	43 cases
ER-positive	
PgR ≥20%	1,404 (75.2)
PgR <20%	462 (24.8)
p53 overexpression	
Without	1,724 (93.2)
With	127 (6.8)
Unknown	15 cases
Ki-67	
<20%	1,087 (58.3)
≥20%	779 (41.7)
Adjuvant therapy	
None	137 (7.3)
Endocrine	1,510 (80.9)
Chemo-endocrine	219 (11.8)

ER, estrogen receptor.

Results

Patient characteristics and biological classification. Table II shows the relationship between the characteristics of the study cohort and the biological classification. The mean age was 58.5 years (range, 25-94). T1 (<2 cm) tumors were often seen in the LA group and rare in the LB-2 group. Nuclear grade 3 and p53 overexpression significantly correlated with LB-2. Endocrine therapy alone was performed in 87.4% (LA), 77.4% (LB-1), 58.8% (LB-2) and 86.9% (LB-3), respectively.

DFS based on the PgR/Ki-67 status in node-negative cases. The Kaplan-Meier curves show the outcomes for DFS according to the PgR/Ki-67 status in the node-negative cases (Fig. 3). There were significant differences in DFS between the LA group (5-year DFS, 98%; 10-year DFS, 95.9%), the LB-2 group

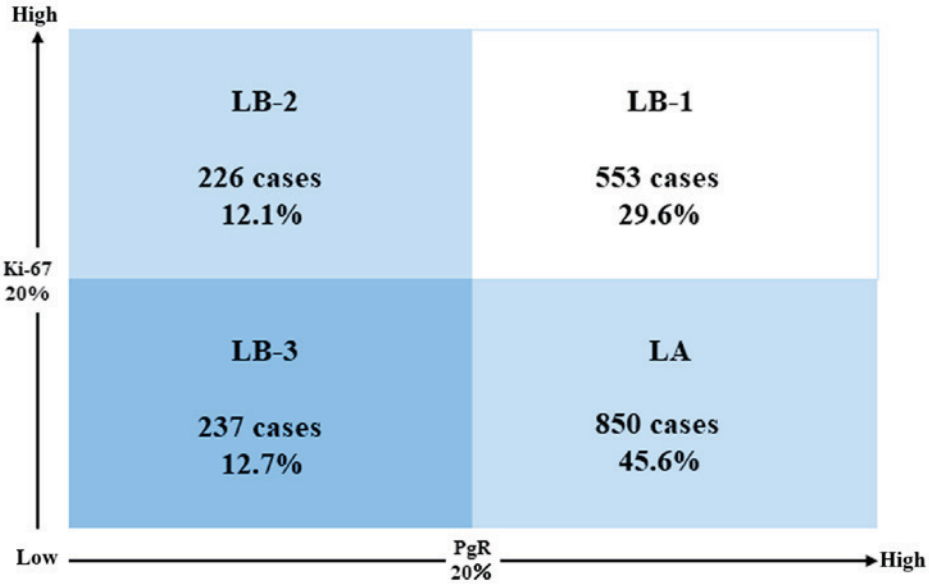

Figure 1. Biological classification using Ki-67 and PgR expressions in ER-positive and HER2-negative breast cancer (n=1866). The cases were classified as follows; LA (high PgR/low Ki-67; 850 cases), LB-1 (high PgR/high Ki-67; 553 cases), LB-2 (low PgR/high Ki-67; 226 cases), and LB-3 (low PgR/low Ki-67; (237 cases). ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

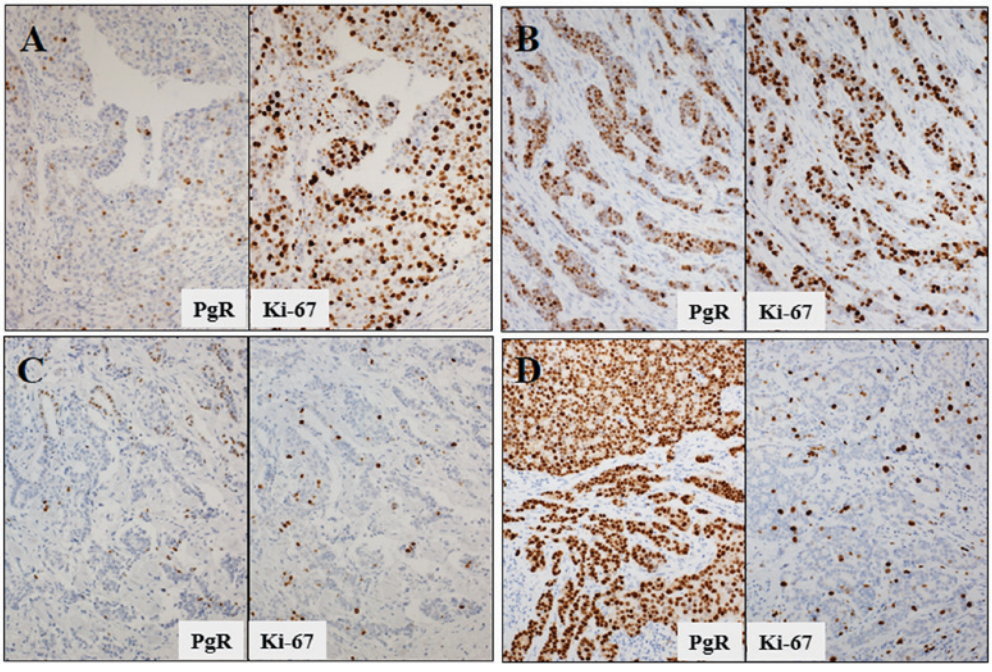

Figure 2. The representative subclassified cases of ER-positive and HER2-negative breast cancer in accordance with the PgR and the Ki-67 status. (A) LB-2 (low PgR/high Ki-67), (B) LB-1 (high PgR/high Ki-67), (C) LB-3 (low PgR/low Ki-67) and (D) LA (high PgR/low Ki-67). All magnifications x200. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

(5 years, 89.9%; 10 years, 83.6%; P<0.0001) and the LB-1 group (5 years, 94.9%; 10 years, 89.5%; P<0.0001), but there was no difference in the LB-3 group (5 years, 98.6%; 10 years, 94.7%; P=0.88). In the cases with endocrine therapy alone (Fig. 4), LA and LB-3 had a similar DFS rate (P=0.25). LB-2 had a significantly worse DFS in all cases and in the cases with endocrine therapy alone. Chemotherapy in combination with endocrine therapy was administered to cases with a higher nuclear grade, a larger tumor and p53 overexpression (Table III). In the LB-2 group (Table IV), no difference in DFS was observed between the cases treated with endocrine therapy and the cases treated with chemo-endocrine therapy. However, in the other groups, the cases treated with endocrine therapy had a significantly more favorable DFS than those treated with chemo-endocrine therapy.

RS using a 21-gene expression assay and biological classification. A relationship was found between the RS derived by using a 21-gene expression assay and biological classification using Ki-67 and PgR expressions (Fig. 5). There were 29 cases with RS ≤25 and 12 cases with RS >25. Moreover, most of the

Table II. The biological classification and clinicopathological characteristics in ER-positive and HER2-negative breast cancer.

	Cancer subtypes					
Characteristics	LA	LB-1	LB-2	LB-3	Total	P-value[a]
Age (years, median+/-SEM)	58.4+/-0.46	55.5+/-0.57	60.5+/-0.89	63.8+/-0.71	58.5	<0.0001
Menopausal status						
Pre	324	283	54	26	687	<0.001
Post	526 (61.9)	270 (48.8)	172 (76.1)	172 (89.0)	1,179	
Tumor size (cm)						
<2	700	390	120	190	1,400	<0.0001
≥2	150 (17.6)	161 (29.2)	104 (46.4)	47 (19.8)	462	
Nuclear grade						
1	649	238	72	182	1,142	<0.0001
2	171	235	78	43	527	
3	11 (1.3)	72 (13.2)	66 (30.6)	5 (2.2)	154	
p53 overexpression						
Without	834	497	165	228	1,724	<0.0001
With	15 (1.8)	54 (9.8)	52 (24.0)	6 (2.6)	127	
Adjuvant therapy						
None	77	20	21	19	137	<0.0001
Endocrine	743 (87.4)	428 (77.4)	133 (58.8)	206 (86.9)	1,510	
Chemo-endocrine	30 (3.5)	105 (19.0)	72 (31.9)	12 (5.1)	219	

[a]by χ^2 test. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LA, high PgR/low Ki-67; LB-1, high PgR/high Ki-67; LB-2, low PgR/high Ki-67; LB-3, low PgR/low Ki-67. No. of cases (%).

Table III. Adjuvant therapy and tumor characteristics in the node-negative cases.

	Adjuvant therapy				
Variables	None	Endocrine	Chemo-endocrine	Total	P-value
Grade					
1	93 (8.1)	1,005 (87.8)	46 (4.0)	1,144	<0.0001[a]
2	28 (5.3)	416 (78.9)	83 (15.7)	527	
3	13 (8.4)	56 (36.1)	86 (55.5)	155	
Total	134	1477	215	1,826	
Tumor size (cm)					
<2	110 (7.8)	1,174 (83.6)	121 (8.6)	1,405	0.04
≥2	30 (6.5)	335 (72.2)	99 (21.3)	464	
Total	140	1,509	220	1,869	
p53 overexpression					
Without	120 (7.0)	1,442 (83.6)	163 (9.4)	1,725	<0.0001[a]
With	17 (13.2)	58 (45.0)	54 (41.9)	129	
Total	137	1,500	217	1,854	

[a]P<0.05 as calculated by χ^2 test. No. of cases (%).

cases with LA (2/2), LB-3 (2/2) and LB-1 (23/27) had a RS of ≤25, and most of the LB-2 (8/10) cases had a RS of >25. There was a significant difference in RS between the LB-1 and LB-2 groups (P=0.00017).

Discussion

The purpose of this study was to investigate the clinical efficacy of a biological classification using the PgR and the Ki-67 status

Table IV. Comparison of DFS between endocrine therapy alone and chemo-endocrine therapy in terms of PgR/Ki-67 status in the node-negative cases.

	DFS (rate)						
	Endocrine therapy			Chemo-endocrine therapy			
Grade	No. of cases	5 years (%)	10 years (%)	No. of cases	5 years (%)	10 years (%)	P-value[a]
LA	745	98.9	97.1	31	83.2	83.2	<0.0001
LB-1	428	96.7	92.3	105	88.1	81.5	<0.0001
LB-2	131	91.7	85.0	72	91.5	81.4	0.24
LB-3	205	98.9	95.7	12	80.9	80.0	0.07

[a]By log-rank test. DFS, disease-free survival; PgR, progesterone receptor. LA, high PgR/low Ki-67; LB-1, high PgR/high Ki-67; LB-2, low PgR/high Ki-67; LB-3, low PgR/low Ki-67.

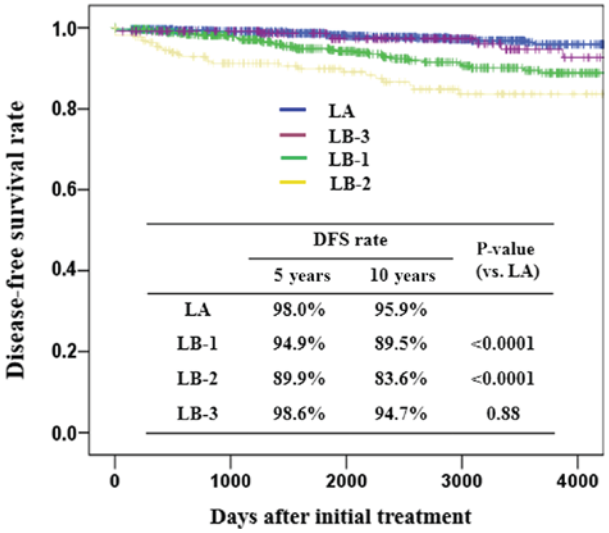

Figure 3. DFS according to PgR/Ki-67 status in the node-negative cases. There were significant differences in DFS between the LA group (5-year DFS, 98%; 10-year DFS, 95.9%), the LB-2 group (5 years, 89.9%; 10 years, 83.6%; P<0.0001) and the LB-1 (5 years, 94.9%; 10 years, 89.5%; P<0.0001), but there was no difference with the LB-3 group (5 years, 98.6%; 10 years, 94.7%; P=0.88). DFS, disease-free survival; LB-1, high PgR/high Ki-67; LB-2, low PgR/high Ki-67; LB-3, low PgR/low Ki-67.

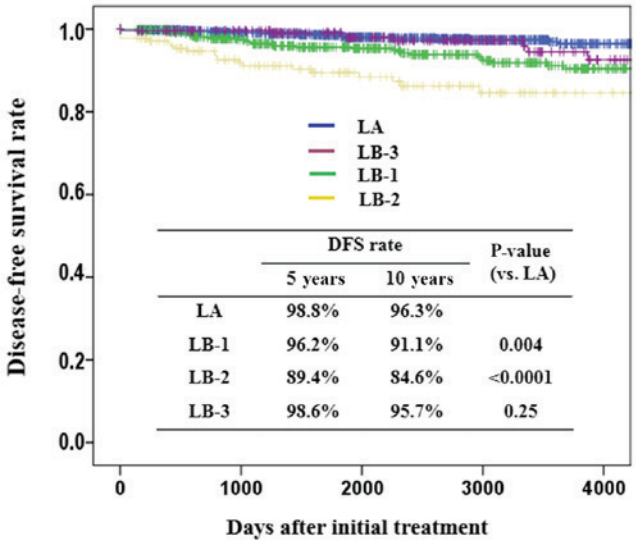

Figure 4. DFS according to PgR/Ki-67 status in the node-negative cases with endocrine therapy alone. In the cases with endocrine therapy alone (Fig. 3), LA showed a similar DFS with LB-3 (P=0.25). LB-2 had a significantly worse DFS in all the cases and in the cases with endocrine therapy alone. DFS, disease-free survival; LB-1, high PgR/high Ki-67; LB-2, low PgR/high Ki-67; LB-3, low PgR/low Ki-67.

(cutoff points: 20%) so that it could be used as an effective prognostic and predictive classification for determining a suitable postoperative treatment for primary breast cancer with luminal/HER2-negative and negative nodes. Moreover, the above mentioned classification was also compared with the RS derived from the Oncotype DX assay. In this study, it was found that only the LB-2 cases (low PgR/high Ki-67) benefited from chemotherapy.

Luminal A type tumors tend to have a better prognosis, a higher survival rate and a lower recurrence rate among all the breast cancer subtypes and luminal B type tumors tend to have a more unfavorable prognosis (14-17). In this study, all of the Luminal type tumors (1,866 cases) were categorized into the following 4 groups; LA and three LB groups. Luminal A occupied the majority and correlated with a smaller tumor, lower nuclear grade and lower p53 overexpression. On the other hand, nuclear grade 3, larger tumor and p53 overexpression significantly correlated with LB-2. In addition, LB-3 correlated with postmenopausal status, smaller tumor and a lower nuclear grade. These findings indicate that there are biological differences among LB tumors.

In terms of prognosis after initial surgery, significant differences in DFS were observed between the LA group, the LB-2 group and the LB-1 group, but there was no difference in the LB-3 group. In the cases with endocrine therapy alone, LA showed a similar DFS as LB-3. LB-2 had a significantly worse DFS in all of the cases and in the cases with endocrine therapy alone. These findings suggest that the tumors with low Ki-67 values (LA and LB-3) had a favorable DFS irrespective of the PgR status. However, the tumors with a high Ki-67 value but a low PgR expression (LB-2) tended to have a worse DFS rate.

Oncotype DX was originally used in ER-positive tumors and is supported by the ASCO (18) and the NCCN Guidelines (19). The RS result predicts the possibility of obtaining a beneficial

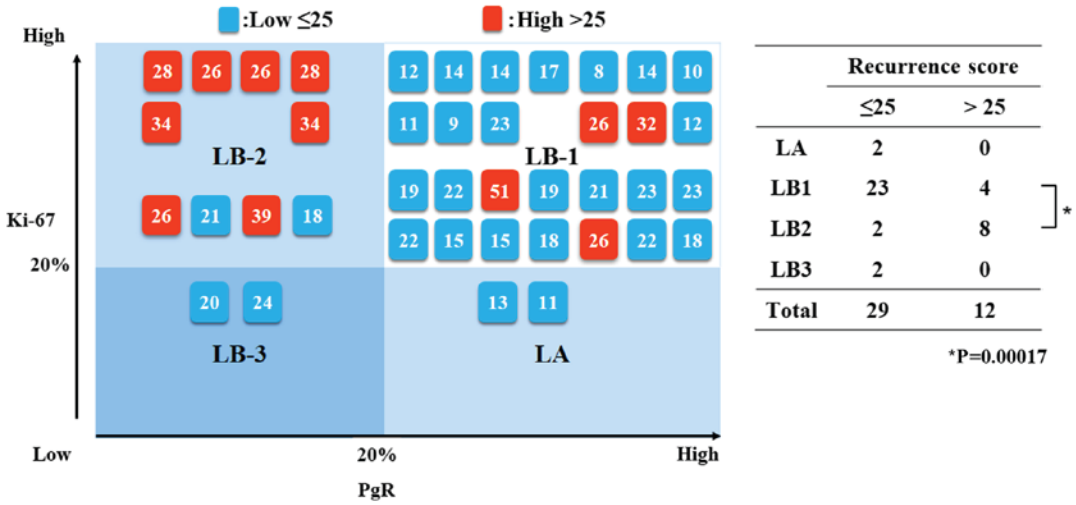

Figure 5. RS using a 21-gene expression assay and biological classification using Ki-67 and PgR expressions in ER-positive and HER2-negative breast cancer. There were 29 cases with RS ≤25 and 12 cases with RS >25. Moreover, most of the cases with LA (2/2), LB-3 (2/2) and LB-1 (23/27) had a RS of ≤25, and most of the LB-2 (8/10) cases had a RS of >25. There was a significant difference in RS between LB-1 and LB-2 (*P=0.00017). ER, estrogen receptor; RS, recurrence score; HER2, human epidermal growth factor receptor 2; LB-1, high PgR/high Ki-67; LB-2, low PgR/high Ki-67; LB-3, low PgR/low Ki-67.

effect from chemotherapy and a higher score increases the potential benefit of chemotherapy (20,21). In this study, RS correlated with the biological classification. RS was lower in the LA, LB-1 and LB-3 groups but higher in the LB-2 group. These data suggest that the LB-2 group has a poorer survival rate but benefits from chemotherapy. There was no difference in DFS between the cases with endocrine therapy alone and the cases treated with chemo-endocrine therapy. On the other hand, the LA, LB-1 and LB-3 groups showed a favorable survival rate in the cases treated with endocrine therapy alone. The cases with a high Ki-67 value and low PgR benefited from chemotherapy and this was also predicted in the RS data.

A multivariate analysis revealed that the Ki-67 index was a significant factor for DFS and that the Ki-67 index value was a significant prognostic factor only in luminal type tumors (3,22). Moreover, according to the data from a large cohort clinical study (23), the Ki-67 index value is frequently evaluated in routine clinical work. The Ki-67 expression is an independent prognostic factor for DFS and OS in addition to common histopathological variables and the Ki-67 index independently raises the possibility of predicting the treatment response and prognosis in a group of breast cancer patients who underwent neoadjuvant treatment (24). The Ki-67 index was also found to be an independent predictive factor for pCR (OR 3.5; 95% CI, 1.4, 10.1). The mean Ki-67 value in patients with pCR was 50.6±23.4%, and the average Ki-67 value in non-pCR patients was 26.7±22.9%. In a previous study it was found (25) that ER-positive and PgR-negative/HER2-negative tumors were associated with poorer survival than cases with ER-positive and PgR-positive tumors and had a comparatively poorer survival rate to that of the triple negative breast cancers. Moreover, Prat *et al* (5) found that the IHC expression of PgR increases the prognostic value within the current IHC-based luminal A definition by improving the identification of favorable breast cancers and the percentage of PgR-positive cells. Moreover, they found that the optimal PgR expression cutoff point to predict outcome was 20%. However, the ER-positive cell rates did not correlate with DFS even after matching for the standard clinicopathologic parameters. A retrospective analysis of three adjuvant clinical trials found that low ER and low PgR expression, and potentially low PgR expression within ER-positive patients were efficacious factors for determining the validity of adding chemotherapy to endocrine therapy (26). These data indicate that the Ki-67 index value and PgR status are important predictors for prognosis and chemotherapy.

In conclusion, the biology, prognosis and suitable treatment for Luminal tumors were evaluated based on the PgR and Ki-67 index value. The patients with a Ki-67 value <20% (LA and LB-3) had a favorable DFS even in the endocrine therapy alone group, whereas those with a Ki-67 value ≥20% (LB-1 and LB-2) had a poorer DFS. Moreover, LB-2 (PgR<20% and Ki-67≥20%) significantly correlated with a higher degree of malignancy but benefited from chemotherapy. The LA and LB-3 cases with low Ki-67 values were considered to be a part of the Luminal A group. These findings suggest that the PgR and Ki-67 status are useful in predicting prognosis and determining the most effective treatment strategy for patients with ER-positive and HER2-negative breast cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RN and YT conceived and designed the experiments. RN, TO, YN, YO, MN and MF performed the experiments and NA, RN, TO, YN, YO, MN and MF analyzed the data. NA and YT contributed reagents/materials/analysis tools. NA and RN wrote the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Kumamoto Shinto General Hospital (no. 30-J01-001). All patients gave a written informed consent to participate in the study.

Patient consent for publication

Written informed consent was provided for the publication of any data/associated images.

Competing interests

The authors have declared that they have no competing interests.

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