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Progesterone Receptor Expression Level Within Immunohistochemically Determined Early Stage Node Negative Luminal-B Breast Cancer: Forecasting Implication, Outcome and Concordance to Oncotype-DX Recurrence Scores

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Abstract

Purpose: To depict Progesterone Receptor (PR) expression exerted modulations on Oncotype-DX Recurrence Scores (RS) in immune-histochemically determined node-negative luminal-B-like breast cancers with Ki67 between ≥ 14% and <30% alongside their potentials in forecasting the outcome.

Methods: The impact of PR variations on Oncotype-Dx RS alongside their implications to the different prognosticators, including adjuvant chemotherapy, local and distant Recurrence-Free Survival (RFS) were scrutinized. Additionally, the concordance of the Hormone Receptor (HR) quantifying approaches devising immune staining and Reverse Transcriptase-Polymerase Reaction (RT-PCR) were statistically particularized.

Results: We selected 250 surgically treated node-negative Luminal-B1 (Ki67 ≥ 14%-<20%) and Luminal-B2(Ki67 ≥20%-<30%) breast cancers who had Oncotype-DX RS analyzed. The PR ≤ 20% was linked to high-grades tumors (P=0.013, 0.012) and accentuated Oncotype-DX RS (P=0.003, 0.001) in both Luminal-B1and B2. Multivariate regression revealed that PR ≤ 20% was a substantial forecaster of the enhanced RS and the adjuvant chemotherapy use (P<0.0001, 0.002), respectively. The Cox regression divulged that the accentuated Oncotype-Dx RS alongside the PR ≤ 20% were independently attributed to lower RFS, with a hazard ratio of 1.84 (95% confidence interval [CI], 3.67-8.14) and 2.53 (95% CI, 2.62-6.12), respectively. Furthermore, ER and PR characterized by immune staining and RT-PCR were concordant in 98.2% and 86.7% of cases.

Conclusion: In node-negative luminal-B with Ki67 between ≥14% and <30% breast cancers, PR ≤ 20% was a robust prognosticator of enhanced RS and adjuvant chemotherapy. The accentuated RS and PR ≤ 20% were independently attributed to reduced recurrence-free survival. Furthermore, a substantial concordance was attained between HR status defined by immune staining and RT-PCR that mounted up to 98.2% and 86.7% for ER and PR, respectively.

Keywords: Progesterone receptors expression • Oncotype-DX recurrence scores • Immunohistochemically

Introduction

The refined breast cancer prognostication centered on gene profiling concluded four basic molecular categories [Luminal-A, Luminal-B, Human epidermal-growth factor-receptor-2-positive (Her2-neu) and Basal-like] that portended peculiar outcomes for each genotype [1,2]. However, these sophisticated gene profiling analyses are expensive, time-consuming and necessitate multi-layered quality assurance processes that restrict their global availability. Accordingly, a comprehensive prognostication of the fundamental breast cancer subtypes based on immune-histochemical staining of tissues was devised as an efficient, convenient substitute [3,4]. The Immune-Histochemical (IHC) staining accurately elaborated the Hormone Receptor (HR) status, Her2-neu expression and the proliferation-index Ki67. Consequently, ER and PR-positive tumors were assorted as Luminal-A-like or Luminal-B-like, based on conventional IHC staining. Luminal-A-like tumors are stereotypically low grade, exhibiting both ER/PR consistent, intense positivity, while they are entirely lacking the Her2-neu receptors and revealing reduced proliferation indices. In contrast, luminal-B-like tumors are HR-positive, but they divulge oscillating levels of ER and PR expressions; moreover, they possess an augmented proliferation index and higher tumor grades [4].

The St. Gallen Panel validated the IHC calcification of breast cancer and emphasized its role in implementing adjuvant treatment. The panel also substantiated devising tumor differentiation or Ki-67 to discriminate between the Luminal-A and B-like tumors [5]. At least 1% of cells in the examined tissue specimen should express either ER or PR- positive IHC staining to label it as HR-positive, as affirmed by the College of American Pathologists (CAP) [6]. Interestingly, ER-positive status is the most prevalent phenotype in primary breast cancers. It approximately constitutes seventy-five percent of cases and more than half simultaneously express PR-positivity [7]. Several studies have concluded that the lack of PR expression is an autonomous forecaster of limited benefit from hormonal treatment, accentuated incidence of relapses and worsened survival [7-9]. Prat A, et al. [10] formulated a quantitative scoring of PR expression to enhance furthers the IHC depiction of luminal-A and B-like

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tumors. They concluded a substantial survival improvement among luminal-A-like tumors directly linked to PR expression >20% within tumor cells [10].

Moreover, Ki67 is an authorized cellular proliferation index alongside its robust competence in extricating luminal-A from B-breast cancers. However, Ki67 optimal threshold values created a significant contention attributed to the inconsistency of cut values devised in different studies that ranged between 10-20%, whereas others authorized the mean or median value as standard cut-points [11-14]. Fortunately, Cheang MCU, et al. [15] was the front-runners implementing measurable Ki67 optimum IHC cut-points to assort breast cancers centered on the 50-gene PAM50 classifier. This technique's foremost superiority resided in the optimal threshold value of Ki67 (14%) specified depending on individual breast cancer biological parameters rather than the clinical results or the calculated Ki67 mean and median. Accordingly, this validated Ki67 threshold (14%) can be extrapolated to all other studied breast cancer populations regardless of the identified prognosticators or the adopted management protocols [15].

Because of the debatable quantitative and qualitative authenticity of Ki67 Immunohistochemistry (IHC), in breast cancer, it suffered a restricted effectiveness in therapeutic intervention. The International Ki67 Working Group (IKWG) recently confirmed that developing an optimized, consistent optical counting method is critical to maximizing its quantitative relevance. The IKWG also established the indisputable Ki67 efficacy in predicting outcome exclusively for stage I or II estrogen receptor-positive and HER2-negative patients to refine the need for adjuvant chemotherapy, as patients with Ki67 of 5% or less do not require chemotherapy. Those with Ki67 levels of 30% or higher, on the other hand, are treated with chemotherapy [16]. The St. Gallen Panel agreed with the IKWG recommendation that chemotherapy to be given to tumors with a Ki67 of 30% or more as opposed to tumours with a Ki67 of 5% or less who do not benefit from adjuvant chemotherapy. The majority of early-stage, ER-positive tumours, however, reside in the middle of these two ends of the spectrum. Upon surveying the Panel, it was found that there was no unified Ki67 threshold between 10%-25% to advise chemotherapy for ER-positive, node-negative breast cancer and a considerable number of the Panel members held the view that there was no such threshold [17]. The surrogacy of genomic assays in tailoring adjuvant therapy has significantly reduced the use of adjuvant chemotherapy in early-stage, node-positive as well as limited 1-3 node positive, ER-positive, HER2-negative breast cancers, without jeopardizing survival rates [18].

Correspondingly, proper risk assessment is the ultimate element in implementing the adjuvant treatment strategy for the surgically treated early-stage node-negative Luminal-A and B-breast cancers expressing variable ER and PR levels while lacking Her2-neu receptors as they possess a broad spectrum of diverse prognosticators that result in dissimilar outcomes. The 21-gene recurrence-score assay (Oncotype-DX, Genomic Health) is one of several commercially existing gene profiling assays that identify indispensable prognostic characteristics in ER and PR-positive, node-negative early breast cancers [8,19]. The Oncotype-DX-Recurrence Score (RS) is numerical between 0 and 100 that provides a consistent calculation of the probability of disseminated relapse in early-stage node-negative Luminal A and B-breast cancers. For statistical purposes, three risk groups have been defined: Low, Mid-range and High-recurrence risk groups of the following values (0-10, 11-25 and 26-100), respectively [8]. The RS assay also forecasts the absolute benefit driven by adjuvant chemotherapy [20,21]. Correspondingly,

Luminal B-tumors were linked to worsened outcomes compared to luminal-A tumors. They, however, encompassed an extremely heterogeneous spectrum of tumors exhibiting Ki67 ≥14%, diverse ER, PR expressions and wide range of

Recurrence scores as assessed by the Oncotype-DX gene assay and 70-gene prognostic signatures [9,21-23].

Subsequently, the current work's fundamental primary objectives were to depict the modulations exerted by PR expression cut-off variations on Oncotype-DX-RS in IHC-determined node-negative Luminal-B-like breast cancers with Ki67index ranging between ≥14% and <30% to emphasize the need of adjuvant chemotherapy in this group of patients. The implications of Oncotype-DX (RS) and PR expression on local and distant recurrence-free survival as well as other prognostic indicators in the patient population under study will be analyzed. Second to scrutinize the concordance of ER and PR status quantified by conventional IHC approaches to those verified by the Oncotype-DX assay, employing an RT-PCR assay.

Methodology

The study comprised patients between 18 and 75 years with IHC-determined, pathologically staged T1b-T3, node-negative Luminal-B-like breast cancer with Ki67index between ≥14% and <30%. The patients were further classified into Luminal-B1 with Ki67 ≥14% and <20% and Luminal-B2 with Ki67 ≥20% and <30%, respectively, to emphasize the diversity in clinicopathological features and outcomes among the studied Luminal-B cohort. They were also assessed for Oncotype-DX RS and treated at King Fahad Specialist Hospital, Saudi Arabia, between January 2017 and December 2021. The eligible patients must have completed surgical management (mastectomy or local excision with a negative margin of at least 1mm width and axillary dissection or sentinel node sampling) 84 days prior to adjuvant systemic therapy. Patients with pathologically positive axillary nodes, a history of ipsilateral or contralateral invasive breast cancer or ductal carcinoma in situ, or bilateral synchronous malignancies, were excluded.

We investigated the modulation in Oncotype-DX RS prompted by fluctuations in PR expression among Luminal-B1and 2like breast tumors which were further classified according to the PR optimum cut-point of 20%. We adapted St. Gallen's criteria for Luminal-A and B-like tumors based on conventional immune staining in selecting our studied patient populations [4,5].

The recurrence gene assay was conducted at Genomic Health Laboratory in the United States. Following Institutional Ethical Committees' acceptance, the designated patients' clinical and pathological information (age, menstrual status, tumor type, size, differentiation, Ki67 scores and hormonal receptors status) were retrospectively collected. Furthermore, all management aspects, such as surgery and adjunctive treatment (radiotherapy, chemotherapy, or hormonal therapy), were scrutinized.

Immune-staining of the primary tumor

The anti-Estrogen Receptor (ER) (SP1) and the anti-Progesterone Receptors (PR) (SP2) (Ventana Medical Systems. Inc.) are rabbit monoclonal antibodies (IgG) designated for the qualitative assessment of ER and PR-antigens in tissue sections that were fixed in formalin and embedded in paraffin. The positive-immune staining is confirmed if over 1% of malignant cells exhibited nuclear staining for ER or PR.

Oncotype-DX assay

The Oncotype-DX RS was initially devised to dichotomize the nine-year likelihood of distant relapse in breast cancer patients receiving five years of Tamoxifen [8,21]. The studied patients were classified into three groups based on the recurrence-risk scores devised in the TAILORX prospective study: Low (0-10), Mid-range (11-25) and High (≥26-100), respectively [8,9]. Additionally, the assay adopted single quantitative scores to particularize the gene expression status for hormone and Her2-neu receptors: ER scores-positive/negative cut-off is 6.5 units and PR score-positive/negative cut-off is 5.5. In contrast, the Her2-neu-positivity cut-off is ≥11.5 units. Low risk (0-10) patients were offered hormonal therapy, while High-risk scores (≥26-100) were provided chemotherapy followed by hormonal therapy. For the Mid-range RS patients (11-25), the adjuvant systemic treatment was guided by clinical risk factors (age, tumor size, and grade and PR level). Adjuvant chemotherapy followed by hormonal therapy was given to young patients<50 years with high-grade tumors and low PR ≤ 20%. Hormonal therapy alone was offered to old patients with low clinical risk factors and low to Mid-ranged RS tumors with PR> 20%.

Statistical analysis

The student T-test and Wilcoxon rank-test were used to comparing

numerical and categorical data. A linear regression test specified the substantial prognosticators in forecasting the Oncotype-DX assay parameters.

The Chi-square test evaluated the concordance of ER and PR-status quantified by conventional immune staining (IHC) approaches to those verified by the Oncotype DX assay, which employed an (RT-PCR) assay.

The statistical significance was set at P<0.05. The local and distant recurrence-free survival was computed using the Kaplan Meier analysis. Log-rank and Cox regression tests were premeditated to link the various clinical and pathological parameters to recurrence-free survival. All tests were accomplished using SPSS 16.0 package program (SPSS, Chicago, IL, USA).

Results

The prevailing study embodied two hundred and fifty node-negative Luminal-B like breast cancer who had Oncotype-Dx assay for the recurrence risk stratification. The luminal-B1-like disease was encountered in 196(78.4%) patients, while 54(21.6%) patients had Luminal-B2-like tumors by IHC. The preponderance of cases was postmenopausal (69.6%). The invasive mammary carcinoma was the prevalent pathological subtype (81.6%). Pathological PT2 and Grade2 tumors were encountered in 57.6% and 70.4% of patients. All clinical features were itemized in (Table 1). Luminal-B1-like tumors expressed significantly higher ER and PR than Luminal-B2-like tumors (P= 0.001, 0.003). In contrast, Luminal-B2-like tumors showed significantly higher RS compared to Luminal-B1-like tumors (<0.0001) (Table 2). Moreover, Luminal-B2-like tumors were of higher grade and were treated mainly using a chemotherapy regimen than Luminal-B1-like tumors (P= 0.003, 0.002), respectively (Table 3). Both Luminal-B1 and B2-like tumors were divided into two groups depending on the cut-off levels, with a high PR>20% and a low group PR ≤ 20% to elaborate on the impact of the PR cut-off value of 20% on different clinical-pathological parameters. Our results revealed that tumors exhibiting PR ≤ 20% were essentially invasive ductal carcinoma (P= 0.025, 0.031) and unveiled a greater T stage (P= 0.024, 0.011). Moreover, they were expressing high grade (G3) tumors (P=0.013, 0.012) and possessing high Oncotype-DX-RS (P=0.003, 0.001) in both Luminal-B1and 2-like tumors respectively (Table 1-4).

How is oncotype-dx rs linked to clinical-pathological co-variates?

High-grade (G3) tumors were significantly linked to enhance Oncotype-DX RS when interrelated with moderately-differentiated (G2) tumors (P<0.003) (Table 5). The tumors expressing PR ≤ 20% were considerably conjoined to enhanced RS, forecasting an accentuated 10-year probability of distant recurrence compared with tumors possessing PR>20% in both Luminal-B1 and B2-like intrinsic subtypes (P=0.001,0.003), respectively. Furthermore, a Low Oncotype-DX RS was encountered in (68.9%) Luminal-B1-like tumors with PR >20%. In comparison, Mid-range RS were dominantly unveiled in (44.4%) Luminal-B2-like tumors with PR>20%, respectively. The High RS was mainly linked to tumors exhibiting PR ≤ 20% in both Luminal-B-like groups (Table 5). It is also worth mentioning that the need for adjuvant chemotherapy was considerably encountered in patients with PR ≤ 20% in both Luminal-B1 and B2 groups (P=0.003, 0.002), respectively, compared to patients with PR >20% (Tables 4 and 5).

Linear regression analysis revealed that Luminal-B1 and B2-like breast cancers expressing PR ≤ 20% were substantial forecasters of the enhanced RS and the need for adjuvant chemotherapy (P<0.0001, 0.002), respectively. Age, menstrual status, tumour histology, grade and size were not robust prognosticators of RS.

We opted to scrutinize the influence of Oncotype-DX RS and PR expression levels on the studied patient population's local and distant recurrence-free survival (RFS). A high recurrence score contributed to the worst 5-years RFS (89.9%) compared to that achieved by the Mid-range (96.3%) and Low Scores (100%), respectively (P=0.016) (Figure 1). Additionally, PR ≤ 20% contributed to a reduced five years RFS (85.6%) compared to PR>20% (97.9%) (P=0.017) (Figure 2). Conversely, the remaining clinical-pathological variables did not significantly influence recurrence-free survival. Cox regression analysis was implemented to scrutinize the clinical-pathological variables' impact on 5-years RFS. It is worth mentioning that high RS alongside the PR ≤ 20% were independently interrelated to lower RFS, with hazard ratios of 1.84 (95% [CI], 3.67-8.14) and 2.53 (95% CI, 2.62-6.12), respectively (Figures 1 and 2).

Table 1. Patients characteristics at baseline (N=250).

		No of Patients	Percentage %
	Age Mean ± SD	56.2 ± 9.1	-
Menstrual Status	Premenopausal	76	30.4%
	Postmenopausal	174	69.6%
Tumor Size	T1b	36	14.4%
	T1c	64	25.6%
	T2	144	57.6%
	T3	6	2.4%
Tumor Grade	G2	176	70.4%
	G3	74	29.6%
Histology	Invasive ductal carcinoma	204	81.6%
	Invasive Lobular carcinoma	46	18.4%
Intrinsic Subtype	Luminal-B1(Ki67 ≥ 14 %-<20%)	196	78.4%
	PR ≤ 20%	16	8.2%
	PR>20%	180	91.8%
	Luminal-B2 (Ki67≥20%-<30%)	54	21.6%
	PR≤20%	12	22.2%
	PR>20%	42	77.8%
Oncotype Dx	Low RS(0-10)	134	53.6%
	Mid-range RS(11-25)	54	21.6%
	High RS(≥ 26-100)	62	24.8%
Surgery	Lumpectomy	216	86.4%
	Mastectomy	34	13.6%
	Chemotherapy	86	34.4%
	Hormonal Treatment	164	65.6%
	Aromatase Inhibitors (AI)	70	42.7%
	Tamoxifen (TAM)	76	46.3%
	Switch AI and Tam	18	11%

Table 2. The mean expression levels of estrogen, progesterone receptors and oncotype dx recurrence score of luminal b1 and b2 like breast cancer patients at baseline.

	Luminal-B1 (Ki67 ≥ 14 %-<20%)	Luminal-B2 (Ki67 ≥ 20%-<30%)	P value
ER % Range	80%-100%	1%-100%	0.001*
Mean ± SD	94% ± 6%	60% ± 12%	
PR % Range	1%-100%	1%-75%	0.003*
Mean ± SD	85% ± 10%	50% ± 8%	
Oncotype Dx recurrence score Mean ± SD	17.4 ± 7.43	24.6 ± 8.6	<0.001*

Concordance between IHC and RT-PCR in determining hormone receptor status

Discordance was unveiled during matching hormone receptor status identified by IHC-staining and RT-PCR. Ten (4.3%) of the 230 ER and PR-positive cases detected by IHC were ER-positive, PR-negative and four (1.7%) were ER and PR-negative by RT-PCR (P 0.002) (Table 6), (Figure 3). Concordance between IHC and RT-PCR for ER and PR status was 98.2% and 86.7%, respectively (Table 6 and Figure 3).

Discussion

An enormous leap in breast cancer management was realized by introducing molecular clustering of intrinsic tumor subtypes, which unveiled substantial

Table 3. Clinicopathologic characteristics of luminal-b1and b2 like breast cancer patients at baseline.

		Luminal-B1 (Ki67 ≥14 %-<20%) (196Pt)	Luminal-B2 (Ki67≥20%-<30%) (54Pt)	P value
Age		56(27-75)	55(28-75)	-
Menstrual Status	Premenopausal	40(20.4%)	36(66.7%)	0.964
	Postmenopausal	156(79.6%)	18(33.3%)	
Tumor Size	T1b	36(18.4%)	-	0.026*
	T1c	64(32.7%)	-	
	T2	96(48.9%)	48(88.9%)	
	T3	-	6(11.1%)	
Tumor Grade	G2	158(80.6%)	18(33.3%)	0.003*
	G3	38(19.4%)	36(66.7%)	
Histology	Invasive ductal carcinoma	158(80.6%)	46(85.2%)	0.624
	Invasive Lobular carcinoma	38(19.4%)	8(14.8%)	
Progesterone Receptors (PR)	PR≤20%	16(8.2%)	12(22.2%)	0.742
	PR>20%	180(91.8%)	42 (77.8%)	
Oncotype Dx	Low RS(0-10)	128(65.3%)	6(11.1%)	<0.001*
	Mid-range RS(11-25)	46(23.5%)	8(14.8%)	
	High RS(≥26-100)	22(11.2%)	40(74.1%)	
Adjuvant Treatment	Chemotherapy	50(25.6%)	36(66.7%)	0.002*
	Hormonal treatment	146(74.4%)	18(33.3%)	

Table 4. Characteristics of the patients at baseline relative to progesterone receptor (pr) cut points in both luminal b1 and b2 like breast cancers.

			Luminal-B1 (Ki67 ≥ 14 %-<20%) (196Pt)			Luminal-B2 (Ki67 ≥ 20%-<30%) (54Pt)		
		No of Patients	PR ≤ 20% 16	PR>20% 180	P value	PR ≤ 20% 36	PR>20% 18	P value
Age		56.2 ± 9.1	56(28-75)	57(29-75)	-	54(27-75)	55(26-75)	-
Menstrual Status	Premenopausal	76	8(50%)	50(27.8%)	0.894	8(22.2%)	10(55.6%)	0.782
	Postmenopausal	174	8(50%)	130(72.2%)		28(77.8%)	8 (44.4%)	
Tumor Size	T1b	36	-	36(20%)	0.024*		-	0.011*
	T1c	64	4(25%)	60(33.3%)			-	
	T2	144	12(75%)	84(46.7%)		30(83.3%)	18(100%)	
	T3	6	-	-		6(16.7%)	-	
Tumor Grade	G2	176	0	158(87.7%)	0.013*	-	18(100%)	0.012*
	G3	74	16(100%)	22(12.2%)		36(100%)	-	
Histology	Invasive ductal carcinoma	204	16(100%)	142(78.8%)	0.025*	36(100%)	10(55.5%)	0.031*
	Invasive Lobular carcinoma	46	-	38(21.2%)		-	8(44.5%)	
Oncotype Dx Recurrence Score(RS)	Low RS (0-10)	134	-	124(69%)	0.003*	-	10(55.6%)	0.001*
	Mid-range RS (11-25)	54	0	46(25.5%)		-	8(44.4%)	
	High RS(≥ 26-100)	62	16 (100%)	10(5.5%)		36(100%)	-	
Surgery	Lumpectomy		12(75%)	180 (100%)	0.351*	6(16.7%)	18(100%)	0.641
	Mastectomy	34	4(25%)	-		30(83.3%)	-	
	Chemotherapy	86	16(100%)	34(18.9%)		36(100%)	-	
	Hormonal treatment	164	-	146(81.1%)	0.003*	-	18(100%)	0.002*

forecasting potentials [21-25]. Correspondingly, the current study confirmed that the node-negative Luminal-B-like breast cancers with Ki67 indices ranging between ≥14% and <30% encompassed a highly heterogeneous group of patients who exhibited substantially variable prognosticators and outcomes were significantly interrelated to progesterone receptors' expression levels.

It is also worth mentioning that few studies have evaluated the association among variations in ER/PR level of expression, different clinical-pathological parameters and prognostic outcomes [15-18]. Our results revealed that PR ≤ 20% tumors were essentially invasive ductal carcinoma (P= 0.025, 0.031) and exhibited grander tumors (P= 0.024, 0.011). Moreover, they were expressing high grade (G3) tumors (P=0.013, 0.012) and possessing high RS (P=0.003, 0.001) in both Luminal-B1 and B2-like tumors, respectively. Similarly, Yao et al. reported that low PR expressing tumors (PR ≤ 25%) had a larger tumor size (P=0.014), worse clinical and biologic characteristics (P<0.001) and were mainly invasive ductal carcinomas (P=0.030).

The Oncotype-DX assay established by Genomic Health provides valuable outcome forecasting data in early-stage lymph node-negative Luminal breast cancer patients [9,10]. The implication of Oncotype-DX in implementing treatment strategies in those patients was ascertained in several studies Prat A, et al. [10], Clahsen PC, et al. [11], Kwa M, et al.

Table 5. Characteristics of the patients at baseline, according to oncotype dx recurrence-score.

		Total Patients	Low RS (0-10)	Mid-Range RS (11-25)	High RS (≥ 26-100)	P value
		250	134	54	62	-
	Age	-	58(28-75)	55(27-75)	56(27-75)	-
Menstrual Status	Premenopausal	76	34 (44.7%)	14(18.5%)	28(36.8%)	0.783
	Postmenopausal	174	100 (57.5%)	40 (23%)	34(19.5%)	
Tumor Size	T1b	36	36(100%)	-	-	0.231
	T1c	64	12(18.8%)	24(37.5%)	28(43.7%)	
	T2	144	86(59.7%)	30(20.8%)	28(19.5%)	
	T3	6	-	-	6(100%)	
Tumor Grade	G2	176	134(76.1%)	30(17%)	12(6.9%)	0.003*
	G3	74	-	24(32.4%)	50(67.6%)	
Histology	Invasive Ductal Carcinoma	204	98(48%)	44(21.6%)	62(30.4%)	0.146
	Invasive Lobular Carcinoma	46	36(78.3%)	10(21.7%)	-	
Intrinsic Subtype	Luminal-B1 (Ki67 ≥ 14 %-<20%)	196	-	-	-	0.001*
	PR≤20%	16	-	-	16(100%)	
	PR>20%	180	124(68.9%)	46(25.6%)	10(5.5%)	
	Luminal-B2 (Ki67 ≥ 20%-<30%)	54				0.003*
	PR≤20%	36	0	0	36(100%)	
	PR>20%	18	10(55.6%)	8(44.4 %)	0	
Surgery	Lumpectomy	216	134(62%)	50(23%)	32 (15%)	0.314
	Mastectomy	34	-	4(11.7%)	30(88.3%)	
	Chemotherapy	86	-	24(28%)	62(72%)	
	Hormonal Treatment	164	-	-	-	0.012*
	Aromatase Inhibitors (AI)	70	70(100%)	-	-	
	Tamoxifen (TAM)	76	52(68.4%)	24(31.6%)	-	
	Switch AI and Tam	-	-	-	-	
	-	18	12(66.6%)	6(33.3%)	-	

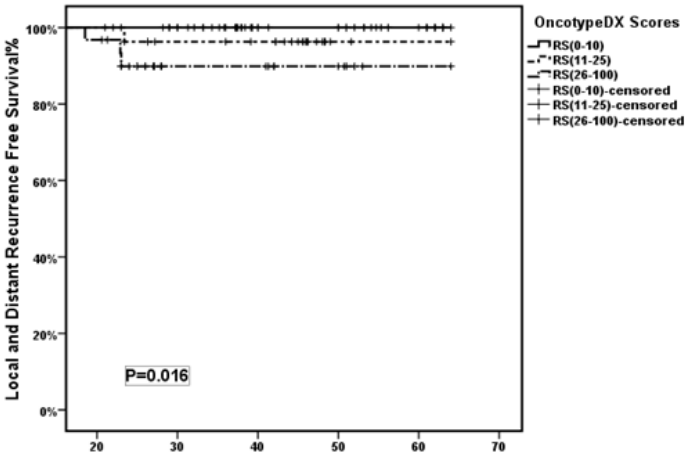

Figure 1. The local and distant recurrence free suvival moduations by oncotypeDX recuurence scoring.

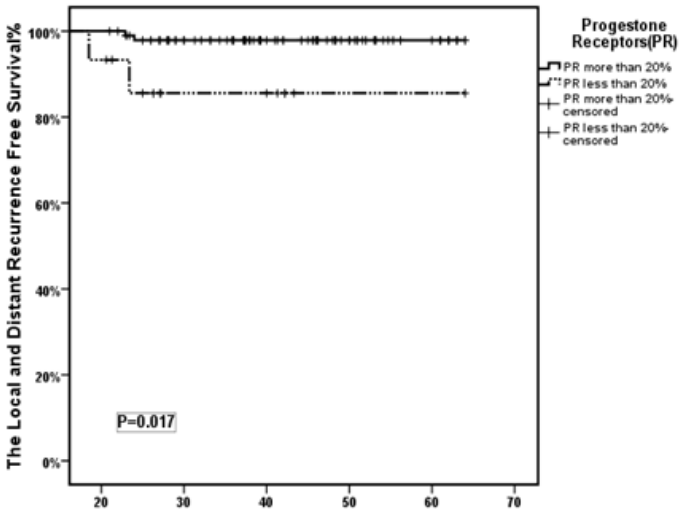

Figure 2. The local and distant recurrence free suvival modualtions by progesterone receptor expression levels in months.

[20], Soonmyung P, et al. [21], Sparano JA, et al. [22], Cardoso F, et al. [23]. Conversely, limited research has scrutinized the link between conventional clinical covariates and Oncotype-DX-RS. Clark et al. concluded that an enhanced PR expression was interrelated to a lower RS [26]. Auerbach J, et al. [27] reported that the lack of PR expression and enhanced mitotic index >1 remained the consistent independent predictors of Mid-range or High-RS in regression analysis. Furthermore, the current study emphasized the converse correlation between PR receptor expression and RS among Luminal-B breast cancer. As accentuated, RS was considerably interrelated to tumors expressing PR ≤ 20% in both Luminal-B1 and B2-like intrinsic subtypes (P=0.001, 0.003), respectively. Moreover, the need for adjuvant chemotherapy was considerably encountered in patients with PR ≤ 20% in both Luminal-B1 and B2 groups (P=0.003, 0.002), respectively, compared to patients with PR >20%. Consequently, the accentuated RS predicted a greater 10-year risk of distant recurrence. Moreover, (68.9%) of Luminal-B1-like tumors with PR>20% had Low RS, while (44.4%) of luminal-B2-like tumors with PR>20% possessed Mid-range RS, respectively. Additionally, linear regression analysis confirmed that reduced PR ≤ 20% in both luminal-B1 and B2 cancers were substantial prognosticators of accentuated RS (P<0.0001, 0.002), respectively. Correspondingly, Chaudary et al. concluded that PR status

Table 6. Concordance between IHC and oncotype DX hormone receptor results.

	RTPCR			Total	P value
	ER+/PR+	ER+/ PR_	ER-/ PR_		
IHC ER+/PR+Count	216	10	4	230	<0.001*
% within IHC	93.9%	4.3%	1.7%	100%	
% within RTPCR	98.2%	38.5%	100.0%	92.0%	
IHC ER+/ PR_Count	4	16	0	20	
% within IHC	20%	80%	0	100%	
% within RTPCR	1.8%	61.5%	0%	8.0%	
Total Count	220	26	4	250	
% of Total	88.0%	10.4%	1.6%	100.0%	

ER= Estrogen Receptor; IHC= Immunohistochemistry; PR= Progesterone Receptor; RT-PCR= Reverse Transcriptase Polymerase Chain Reaction
*Chi-square test

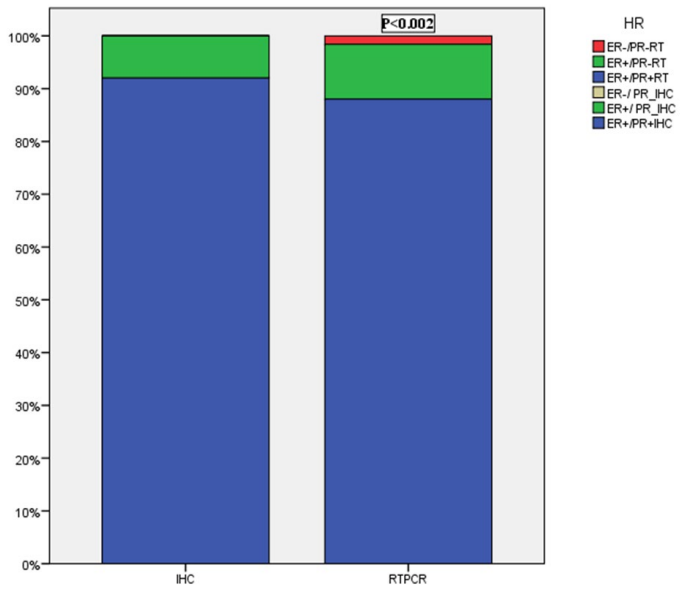

Figure 3. The concordance level between hormone receptor status identified by Immune Histochemical staining (IHC) and Reverse Transcriptase Chain Reaction (RT-PCR).

alongside high-grade tumors were robust predictors of RS (P< 0.0001, 0.002) [28]. Additionally, our conclusion conquered with other researchers that age, menopausal condition, pathological subtype and tumor size were not potential forecasters of RS [28]. It is noteworthy to mention that the 5-years freedom from local and distant recurrences for Low (100%), Mid-range (96.3%) and High-RS (89.9%) were comparable to those reported by Sparano JA, et al. [22] in their study Soonmyung P, et al. [21]. Correspondingly, Prat et al. reported that low positive PR ≤ 20% exhibited significantly worsened survival compared with tumors with PR> 20%, which concordantly conquer with our results denoting that PR ≤ 20% contributed to reduced five-years of recurrence-free survival (85.6%) compared to PR >20% (97.9%) (P=0.017) [10].

More interestingly, the concordances between ER and PR status determined by IHC staining and RT-PCR were 98.2% and 86.7%, respectively. Many sentinel studies further validated our results [29-31] as Badve SS, et al. [29] reported that the two methods yielded 93% and 88% concordance for ER and PR successively [29]. Moreover, Park et al. reported statistical concordance, which mounted up to 98.9% and 91.3% for ER and PR when IHC staining and RT-PCR were correlated [30]. Interestingly, the reduced level of concordance for PR achieved by comparing the two approaches at 86.7% can be induced by immune histochemical staining criteria at our institution.

The current study's earned strength is credited to its emphasis on the clinical implications of PR diverse expressions and their influence in forecasting RS in node-negative Luminal-B1, B2-like breast cancer. The robust association we confirmed between PR ≤ 20%, the need for adjuvant chemotherapy and enhanced Oncotype-DX RS may help settle the debate over the modulation of adjuvant systemic treatment in the node-negative Luminal-B1, B2-cohorts with Ki67 between ≥14% and <30%. Additionally, we further validated the concordance of the HR status verified by conventional immune staining and RT-PCR reported by Oncotype-DX analysis. However, the main restrictions of the current work are the comparatively succinct number of patients included and the absence of prospective design. Nevertheless, the achieved conclusions merit further authorization by a properly designed prospective randomized trial which would establish the robustness of PR expressions in forecasting the recurrence probability and optimizing the treatment of early-stage Luminal breast cancer [31].

Conclusion

In node-negative luminal-B with Ki67 between ≥14% and <30% breast cancers, PR ≤ 20% was a robust prognosticator of enhanced RS and adjuvant chemotherapy. The accentuated RS and PR ≤ 20% were independently attributed to reduced recurrence-free survival. Furthermore, a substantial concordance was attained between HR status defined by immune staining and RT-PCR that mounted up to 98.2% and 86.7% for ER and PR, respectively.

Declarations

Ethics approval and consent to participate

This study protocol and patients' informed consent to participate their clinical information in this retrospective review were approved by the Institute Review Board (IRB) at King Fahad Specialist Hospital, Al Dammam, KSA. The Institute Review Board (IRB) at King Fahad Specialist Hospital is R00231.

Consent for publication

The authors declare that written informed consents from patients were

Obtained to publish their data anonymously.

Availability of data and materials

The datasets used and analyzed during the current study are collected from medical records of patients treated at King Fahad Hospital and are available with the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Hala Zaghloul (HZ): Designed the study, developed the protocol,

checked the patient's eligibility, performed the statistical analysis and wrote the Manuscript.

Miral Mashhour (MM): Developed the protocol, conducted the data collection, reviewed the literature and revised the final version of the manuscript.

Lulwah Abduljabbar (LA): Assisted with the data collection, checking the patient eligibility and reviewing the literature.

Karim Abdel Halim (KA): Assisted with the data collection, checking the patient eligibility and reviewing the literature.

All authors have read and approved the final manuscript.

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Conflict of Interest

The authors affirmed that there was no conflict of interest no receipt of any financial support for their study.

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