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ORIGINAL ARTICLE

The clinical value of progesterone receptor expression in luminal breast cancer: A study of a large cohort with long-term follow-up

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Abstract

Background: The routine assessment of progesterone receptor (PR) expression in breast cancer (BC) remains controversial. This study aimed to evaluate the role of PR expression in luminal BC, with emphasis on the definition of positivity and its prognostic significance as compared to Ki67 expression.

Methods: A large cohort (n = 1924) of estrogen receptor (ER)-positive/HER2-negative BC was included. PR was immunohistochemically (IHC) stained on full face sections and core needle biopsies (CNB) where the optimal scoring cutoff was evaluated. In addition, the association of PR with other clinicopathological factors, cellular proliferation, disease outcome, and response to adjuvant therapy were analyzed.

Results: Although several cutoffs showed prognostic significance, the optimal cutoff to categorize PR expression into two clinically distinct prognostic groups on CNB was 10%. PR negativity showed a significant association with features of aggressive tumor behavior and poor outcome. Multivariate analyses indicated that the association between PR negativity and poor outcome was independent of tumor grade, size, node stage, and Ki67. PR negativity showed independent association with shorter survival in patients who received endocrine therapy whereas Ki67did not.

Conclusion: PR IHC expression provides independent prognostic value superior to Ki67. Routine assessment of PR expression in BC using 10% cutoff in the clinical setting is recommended.

Plain Language Summary

- In this study, we have established an optimal approach to determine the prognostic value of progesterone receptor expression in estrogen receptor-positive breast cancer patients.

- To do this, the levels of progesterone receptor were measured in a large cohort of estrogen receptor-positive breast cancer patients.
- We have refined the definition of progesterone receptor positivity in estrogen receptor-positive breast cancer.
- We show that progesterone receptor expression adds prognostic and predictive value of endocrine therapy in estrogen receptor-positive breast cancer patients, and our results show that the absence of progesterone receptor is associated with poorer outcomes independent of tumor grade, size, node stage, and Ki67 expression.

KEYWORDS
assessment, breast cancer, endocrine therapy, Ki67, PR

INTRODUCTION

Progesterone receptor (PR) is an upregulated target gene of estrogen receptor (ER) and its expression is dependent on estrogen levels. There is a mechanistic mutual regulatory interaction between PR and ER expression, where ER regulates PR expression and, in turn, PR modulates ER expression.[1,2] Presence of PR indicates that the ERα pathway is intact and functionally active.[3] Additionally, PR plays a role in control of several important normal cellular functions, including cell integrity, growth, and proliferation.[4] Although ER and PR are prognostic markers in invasive breast cancer (BC) and key markers for intrinsic subtype classification,[5] only ER is used in the clinical setting as a well-established predictive marker of endocrine therapy (ET) and its assessment is mandated in all BC.[6]

Despite its prognostic significance,[7–10] and the recommendation for its routine assessment in BC by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP)[6] and the requirement of its availability by the American Joint Committee on Cancer Staging Manual (8th edition) for prognostic staging,[11] the assessment of PR status in the clinical settings remains controversial and some laboratories do not assess it routinely. The United Kingdom Royal College of Pathologists (RCPath) breast data set regards PR testing in BC as optional.[12] This is mainly related to its uncertain predictive value in the adjuvant setting.[13,14] This lack of predictive significance compared to ER expression seems understandable because almost all PR-positive tumors are ER-positive, and ER-negative PR positive tumors are rare.[15] In addition, different scoring cutoffs to define PR positivity have been published,[16–18] and the discordance between core biopsy and surgical excision specimens vary widely compared to ER.[19,20]

In the current era of precision medicine, the routine use of PR should be reviewed, and evidence should be interpreted in the correct context and in comparison, with other prognostic and predictive markers in BC that have attracted much attention, including Ki67. In this study, we aimed to use a large and well characterized cohort of ER-positive, HER2-negative (luminal) BC to (1) assess the prognostic significance PR expression in the various subgroups and in comparison, to Ki67, (2) evaluate the optimal cutoff for PR categorization), and 3) compare the expression of PR in core biopsy and surgical excision specimens.

MATERIALS AND METHODS

Study cohort

This study was conducted on a large cohort ($n = 1924$) of ER-positive and HER2-negative BC from patients presented to Nottingham University Hospitals NHS Trust between 1999 and 2006 with available clinicopathological, treatment, and outcome data. Data included patient age at diagnosis, menopausal status, tumor size, tumor grade, histological tumor type, lymph node status, lymph vascular invasion (LVI), and Nottingham prognostic index (NPI) (Table S1). Original clinical PR score, which was performed on the diagnostic core biopsies, was available in a subset of cases ($n = 727$), where 90% (651/727) were positive using the 1% cutoff to define PR positivity. BC-specific survival (BCSS), defined as the time (in months) from the date of the primary surgery to the time of death from BC, and distant metastasis-free survival (DMFS), defined as the time (in months) from the primary surgery until the first event of distant metastasis, were calculated. The median time of follow-up is 125 months (range, 2–257 months).

Ki67 expression levels, as assessed in full face sections, using the standard methodology as previously published,[21,22] were available and were used for comparative analysis with PR. Adjuvant endocrine therapy was offered to 90% ($n = 1715$ of 1904) patients included in the study, whereas 189 patients (10%) did not receive endocrine therapy. Those patients were diagnosed before 2000 and at that time, endocrine therapy was offered according to local hospital protocols.

PR IHC staining on full face sections

All archived hematoxylin–eosin slides were retrieved and histologically reviewed to select the representative formalin-fixed, paraffin-

embedded (FFPE) block suitable for the study containing the highest tumor burden. The IHC staining was performed automatically using DAKO Cytomation EnVision + detection system using the same protocol used for PR staining in the clinical setting. Briefly, full face FFPE sections were prepared from each case. Unstained tissue sections were heated for 10 minutes on a hot plate at 60°C and allowed to cool. Citrate buffer (pH 6.0) was used for antigen retrieval. Primary antibody (anti-progesterone receptor [1E2] rabbit monoclonal primary antibody) was applied to tissue sections and incubated for 30 minutes. Positive control tissue from a known PR-positive BC case, was included for each staining run, whereas a negative control was included by omitting the primary antibody.

For exploring the effect of core biopsy versus excisional section on PR scoring, the correlation between PR status in this study using full face sections and the PR status originated from core biopsies was tested to assess if there was any discrepancy in PR scores between the core biopsies and full-face sections.

Visual estimation of PR expression

The percentage of PR expression was visually assessed by estimation of the proportion of invasive tumor cells that showed nuclear staining for PR through scanning of the whole tumor at low power magnification using 4× or 10× objectives. Staining in normal terminal duct lobular units or associated in situ carcinoma was not considered in the final score. Histo score (H-score) was estimated, which included the percentage of PR positivity as well as the staining intensity. Staining intensity is categorized into 0, 1, 2, or 3 (for negative, weak, moderate, and strong, respectively) PR H-score was dichotomized into two groups based on the most common cutoff used in the literature for ER (H-score = 50).

Investigating the optimal cutoff to categorize PR expression in BC

To assess the optimal cutoff for PR on a prognostic prospective, the PR cutoff was assessed initially based on association with BCSS using X-tile bioinformatics software version 3.6.1 (School of Medicine, Yale University, New Haven, Connecticut).[23] The previously recommended cutoffs including 1%, 10%, and 20%, which are usually applied in either the clinical practice or in previous publications, were also explored.[16–18] The optimal cutoff was chosen based on the results of the univariate analysis using the most significant p values associated with the highest hazard ratio (HR) in CNB and full-face sections and taking the consideration of concordance between both biopsies. The sensitivity and specificity of the refined cutoff for PR expression in core biopsies was evaluated based on PR expression in full face sections, which was considered as the benchmark. Two observers with experience in histopathology analyzed the expression of PR in this study. One pathologist scored the whole cohort, whereas the second observer scored (randomly selected) 20% of the cohort. Using the continuous score, the interclass correlation coefficient was good (ICC = 0.8). When different PR cutoffs were applied, the κ values were 0.4, 0.9, and 0.7 at 1%, 10%, and 20% cutoffs, respectively. In addition, the interobserver concordance was calculated between the PR status using the original (clinical setting) scores obtained from core biopsies and the full-face sections scores generated in this study, and the κ value was 0.9.

Statistical analysis

Statistical Package for the Social Sciences software v.26.0 (SPSS, Chicago, Illinois) was used for statistical analysis. To assess the expression of PR, either bimodal or linear, the cohort was categorized into three groups, low ≤20%, intermediate (21%–70%), and high expression >70% and a distribution curve was performed.[24] The χ^2 test was used for analysis of categorical data, whereas Pearson correlation coefficient test was used for the continuous data. The percentage of Ki67 positivity was categorized into low versus high proliferative groups based on the median, which was 12%,[22] and for further evaluation of the role of PR, several cutoffs of Ki67 were used for comparison with PR (5%, 10%, 14%, 20%, and 30%). Ki67 continuous scores were also analyzed against PR in multivariate analysis.[21,25] For analyses including multiple correlations, Bonferroni corrections were performed, and the adjusted p value was used. In addition, univariate and multivariate analyses were used to assess the correlation of PR expression and patients' outcome. The association of PR expression with the response of adjuvant therapy was also evaluated. Post hoc tests, which are an integral part of ANOVA, were used to test the equality of mean of PR expression and other prognostic factors. Outcome analysis was assessed using Kaplan–Meier curves and the log-rank test. Cox regression models were used for the multivariate analysis. Estimated HR and 95% confidence interval (95% CI) were calculated from univariate and multivariate regression models. Considering the censoring of some events in BCSS, we calculated the cumulative incidence function (CIFs), which means the predicted adjusted cumulative risk of death using competing risk-regression analysis. A correlation matrix was conducted for all the variables included in the multivariate analysis to assess their association with each other. Furthermore, linear regression models with interaction test were used to study the effect size and interaction between PR and endocrine therapy. Sub distribution hazard ratios (sHR) for Fine–Gray models were calculated with 95% CIs. STATA software (17 Base Reference Manual; College Station, Texas: Stata Press, Stata Corp. 2019) was used to calculate competing risk and interaction.[26,27] For all tests, $p < .05$ (two-tailed) were deemed statistically significant.

This study was approved by the Yorkshire and the Humber-Leeds East Research Ethics Committee (REC reference: 19/YH/0293) under the Integrated Research Application System Project (ID 266925). Data collected were fully anonymized.

RESULTS

Evaluation of the best cutoff for PR in BC

The mean ± SD percentage of PR expression was 64 ± 38% and the median was 80%, (range, 0%–100%). The mean ± SD H-score of PR was 145 ± 104, median was 150 (range, 0–300).

Using X-tile software, a very high cutoff was identified as the best prognostic stratification of PR expression (75% and 25% in full face sections and in CNB, respectively). However, considering the distribution of cases in each group and the discordance between full face sections and CNB, other existing and previously used cutoffs were also tested, including 1%, 10%, and 20%. Table 1 and Tables S2 and S3 show the prognostic significance of the various cutoffs, the specificity and sensitivity of each when assessed in CNB and the concordance between CNB and full-face sections. These analyses indicated that a 10% (negative PR ≤10 and positive PR >10) cutoff achieved best association with patient outcome including those who received endocrine therapy, significance in both platforms, concordance between CNB and excision specimens and distribution of positive and negative cases in addition to being consistent with revised ER cutoffs of actual positivity.[6] For further validation of PR cutoff, PR levels of expression of the whole cohort were categorized into three groups PR <1%, PR (1%–10%), and PR >10%. There was a significant difference in patient outcome between both PR <1% and PR <10% groups compared to PR >10% group ($p = .015$) whereas no significant difference was found between the PR <1 and PR <10% group ($p = .9$). Similar results were observed in endocrine therapy treated patients (Figure S1).

Using a 10% cutoff, the sensitivity and specificity of CNB to predict the positive expression on full face sections was of 91% and 86%, respectively. Although strong association was found between PR scores using full face sections and their corresponding scores using CNB ($p < .0001$), 2% (16 of 727) of cases changed from PR-negative in CNB to be PR-positive in full face sections (>10%) that is considered a low percentage of discrepancy and confirms that PR scoring using core biopsies is valid and represents the actual expressions of PR. In full face sections, using the 10% cutoff, 80% of cases (1523 of 1904) showed PR positivity whereas 20% of cases (381 of 1904) were negative (Figure 1). In endocrine therapy-naive patients, PR positivity was detected in 64% (158 of 189), whereas in the endocrine therapy-treated patients, PR positivity was observed in 80% (1365 of 1715).

PR percentage showed bimodality of expression as 20% of PR were negative (≤10%) and 69% were highly expressed (>70%). (Figure S2).

TABLE 1 Comparison between different cutoffs of PR expression in full face sections

	Negative versus positive, No. (%)		BCSS in the whole cohort			DMFS in the whole cohort			BCSS in patients who received endocrine therapy			DMFS in patients who received endocrine therapy		
PR cutoffs	Negative	Positive	HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*
1%	226 (12)	1678 (88)	0.60	0.41–0.88	.010	0.64	0.45– 0.91	.0140	0.61	0.41–0.91	.015	0.67	0.47–0.97	.030
10%	381 (20)	1523 (80)	0.62	0.45–0.86	.004	0.58	0.44–0.78	<.0001	0.60	0.45–0.90	.011	0.61	0.45–0.82	.001
20%	452 (24)	1452 (76)	0.60	0.44–0.83	.001	0.60	0.46–0.79	<.0001	0.60	0.45–0.87	.005	0.60	0.46–0.82	.001
75%	865 (45)	1039 (55)	0.48	0.36–0.65	<.0001	0.51	0.39–0.64	<.0001	0.49	0.36–0.69	<.0001	0.52	0.39–0.68	<.0001

Abbreviations: BCSS, breast cancer–specific cancer; CI, confidence interval; DMFS, distant metastasis free survival; HR, hazard ratio; PR, progesterone receptor.

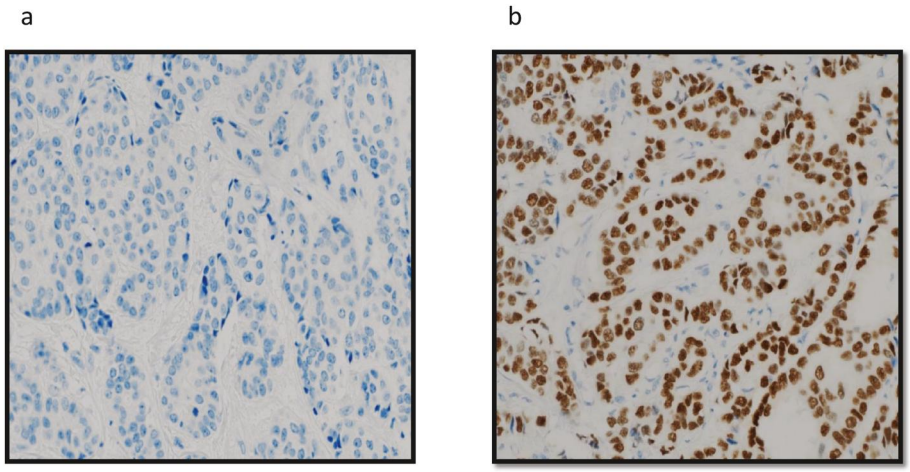

FIGURE 1 (A) Negative expression of progesterone receptor. (B) Moderate to strong progesterone receptor expression (×20).

Correlation between PR expression and clinicopathological parameters

In the whole cohort, there was strong association between PR negativity (<10%) and post-menopausal status and grade 3 tumors (adjusted *p* value < .0001) (Table 2). Notably, PR positivity showed a significant association with grade components (prominent tubule formation and low mitotic count), however; no association was observed between PR status and the degree of pleomorphism, tumor size, lymph node status, or LVI. Similarly, in endocrine treated cohort, negative PR status showed association with parameters characteristic of aggressive tumor behavior (Table S4). There was an association between PR negativity and high Ki67 expression. Similarly, low PR H-score showed significant associations with other parameters

TABLE 2 Relationship between progesterone receptor status and clinicopathological parameters using full-face sections

	PR status, No. (%)			
Variables	**Negative (≤10%)**	**Positive (>10)**	**χ^2 *p* value**	**Adjusted *p* value***
Age at diagnosis (years)				
<50	50 (11.5)	383 (88.5)	25.1	<.0001
≥50	331(22.5)	1140 (77.5)	5.5×10^{-7}	
Menopausal state				
Premenopausal	58 (11.2)	462 (88.8)	35.1	<.0001
Post-menopausal	323 (23.3)	1061 (76.7)	3.2×10^{-9}	
Tumor size (cm)				
≤2	258 (19.6)	1059 (80.4)	047	.49
>2	123 (21.0)	464 (79.0)	0.49	
Histologic tumor grade				
1	75 (16.6)	377 (83.4)	18.8	.0005
2	185 (18.4)	820 (81.6)	0.00008	
3	121 (27.1)	326 (72.9)		
Histologic tumor types				
NST	209 (20.5)	809 (79.5)	15.4	
Lobular	64 (27.4)	170 (72.6)	0.002	.01
Other special types	28 (21.5)	102 (78.5)		
NST mixed	80 (15.3)	442 (84.7)		
Lymph node invasion				
Absent	282 (20.4)	1098 (79.6)	0.56	.45
Present	99 (18.9)	425 (81.1)	0.45	
Lymphovascular invasion				
Absent	315 (20.3)	1236 (79.7)	0.47	.49
Present	66 (18.7)	287 (81.3)	0.49	
Nottingham prognostic index				
Good prognostic group	183 (18.2)	821 (81.8)	4.9	.2
Moderate prognostic group	172 (21.6)	625 (78.4)	0.08	
Poor prognostic group	26 (25.2)	77 (74.8)		
PR status (original core biopsy)				
Negative	60 (87.9)	16 (21.1)	188.7	<.0001
Positive	83 (12.7)	568 (87.3)	1.5×10^{-69}	

Abbreviations: NST, no special type; PR, progesterone receptor.

*Adjusted *p* value was calculated using Bonferroni correction.

characteristic of aggressive tumor behavior as shown in Table S5. Using continuous expression levels of ER, there was a significant positive correlation between ER and PR scores ($p = .006$).

Outcome analysis

In luminal BC, PR negativity showed a significant association with poor outcome in terms of shorter BCSS and DMFS, ($p = .02$ and <.0001, respectively) (Figure 2 and Figure S3). When the cohort was stratified based on tumor grade, PR status was able to stratify grade 2 tumors into two distinct prognostic subgroups ($p = .03$) (Figure S4).

When adjuvant system therapy was considered, PR negativity showed strong association with poor outcome in patients who received endocrine therapy in terms of DMFS (HR, 0.61; 95% CI, 0.45–0.82; $p = .001$) and BCSS (HR, 0.60; 95% CI, 0.45–0.90; $p = .01$). (Figure 3). Similarly, Ki67 showed strong association with worse outcome in terms of shorter BCSS and DMFS in patients who received endocrine therapy (both $p < .0001$) (Table 3). There was no statistical difference in outcome between PR-negative and PR-positive groups in patients who received both endocrine and chemotherapy ($p = .38$).

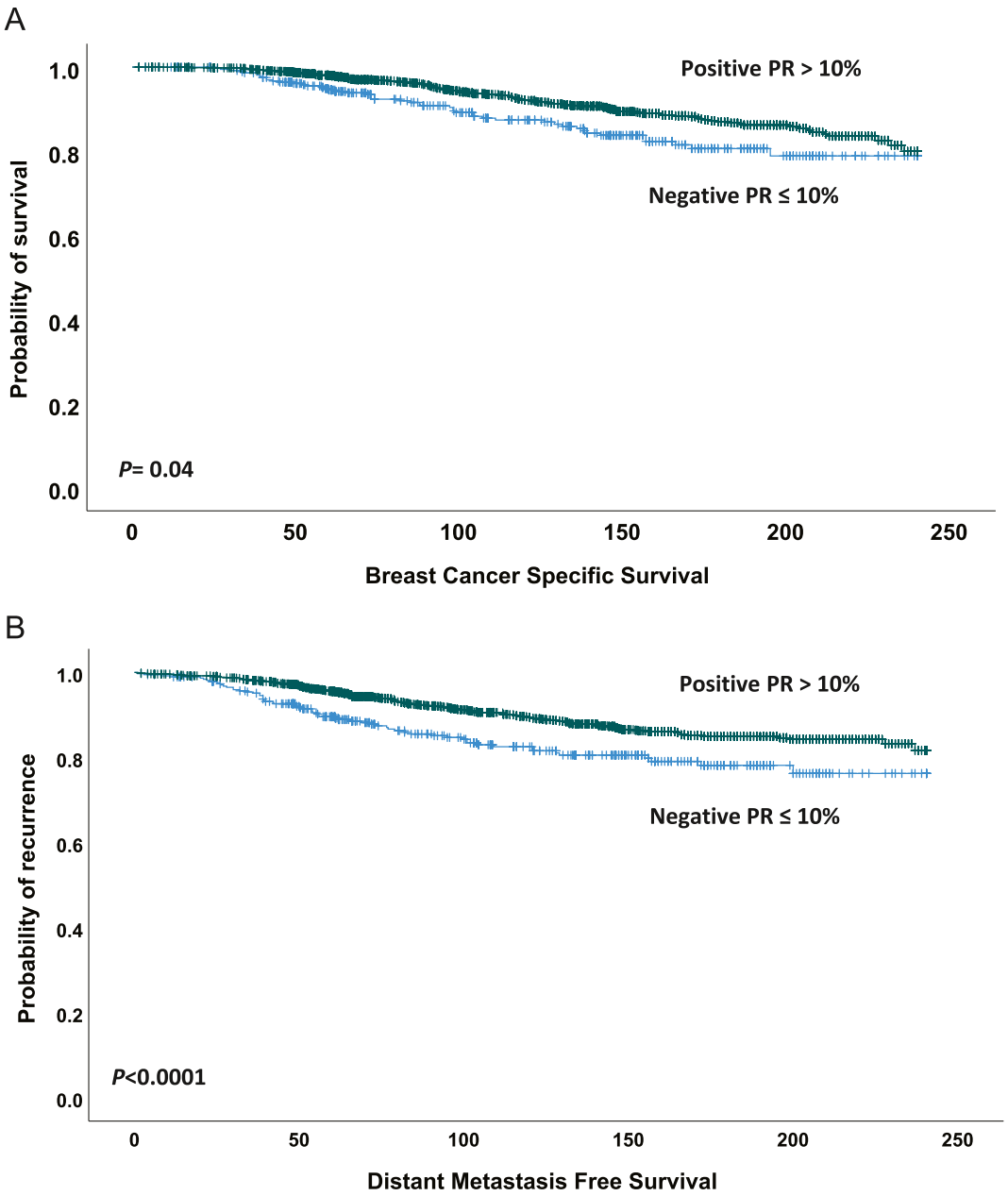

FIGURE 2 (A) Kaplan–Meier association of progesterone receptor status with breast cancer–specific survival and (B) with distant metastasis-free survival.

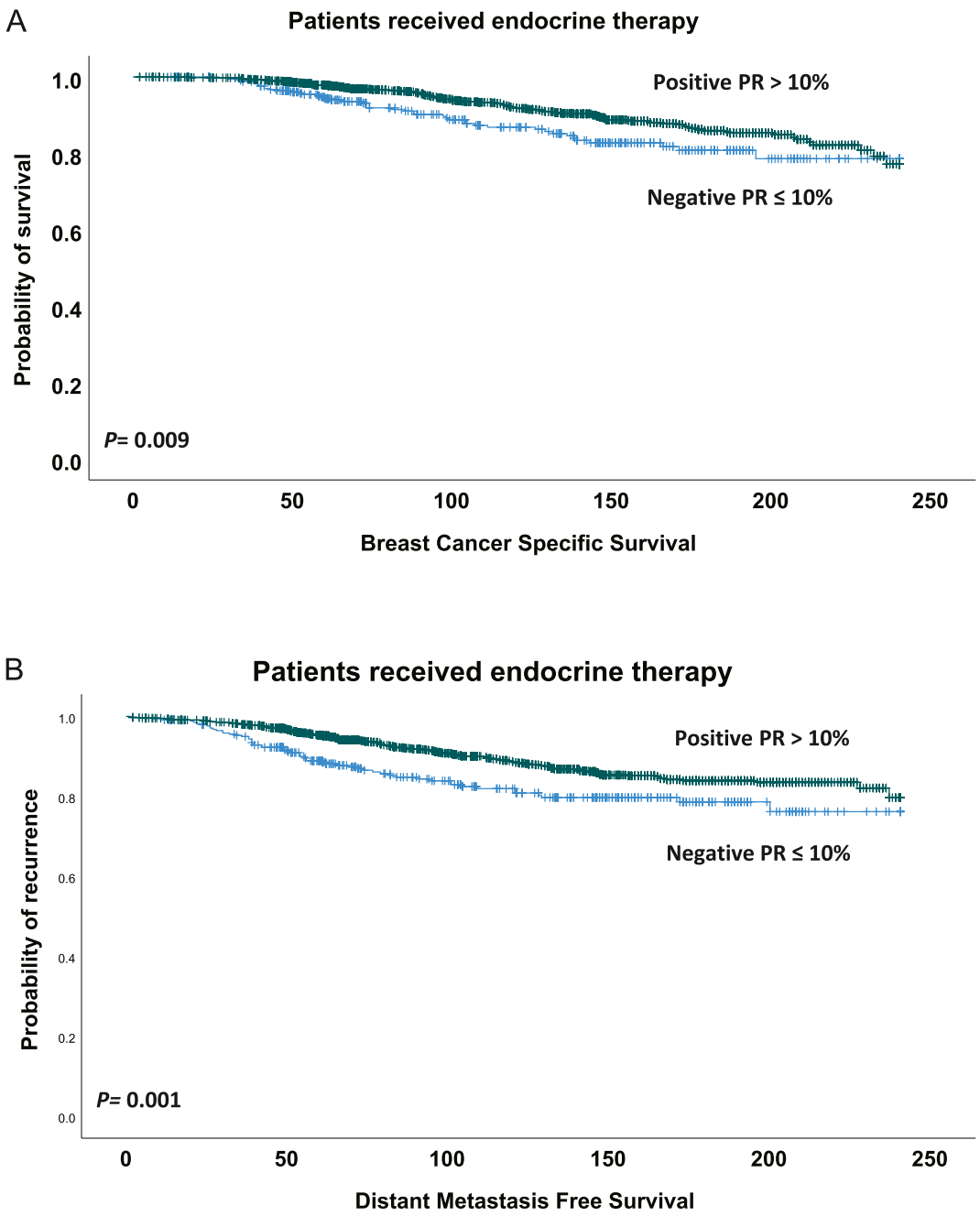

FIGURE 3 Kaplan–Meier plots showing significant association between negativity of progesterone receptor and poor breast cancer–specific survival (A) and distant metastasis-free survival (B) in patients who received endocrine therapy.

Similar results were obtained with Ki67 ($p = .8$). In patients who did not receive endocrine therapy, no significant difference in patient outcome was observed between PR-negative and PR-positive groups. However, Ki67 showed a significant association with outcome in those patients.

Before multivariate analysis, a correlation matrix was performed for all potential prognostic parameters (Figure S5). Each of these variables was tested separately and in different combinations with PR in multivariate Cox regression analysis models. A model was performed including all the variables that were associated with patient outcome, and PR was independently associated with outcome (Table S6). Moreover, PR was a prognostic factor independent of histologic grade, tumor size, nodal stage, and Ki67 in another model. Importantly, in patients who received endocrine therapy, where multivariate analysis included tumor size, grade, and lymph node status, PR remained as an independent prognostic factor ($p = .008$) whereas Ki67 lost its significance (Table 4). In addition, PR status maintained the significant association with patient outcome in all the subsets of the analysis.

For further confirmation of the superiority of PR over Ki67 in endocrine-treated patients, PR status was compared with Ki67 at different Ki67 cutoffs. It was observed that PR was an independent

TABLE 3 Comparison between the prognostic and predictive values of progesterone receptor and Ki67 in univariate analysis

		PR (negative/positive)		Ki67 (low/high)	
Survival data	**No. of patients**	**Log-rank**	***p***	**Log-rank**	***p***
BCSS in the whole cohort	1904	10	.004	43.29	<.0001
DMFS in the whole cohort		12.9	<.0001	36.7	<.0001
BCSS in patients received endocrine therapy only	1477	5.1	.020	30.6	<.0001
DMFS in patients received endocrine therapy only		8.1	.005	29.86	<.0001
BCSS in endocrine therapy-naive patients	189	1.2	.27	10.45	.001
DMFS in endocrine-naive therapy patients		2.7	.1	5.69	.01
BCSS in patients received both endocrine and chemotherapy	238	0.79	.38	0.05	.8
DMFS in patients received both endocrine and chemotherapy		1.6	.20	0.1	.75

Note: A 10% cutoff was used for determination of PR status.

Abbreviations: BCSS, breast cancer–specific cancer; DMFS, distant metastasis free survival; PR, progesterone receptor.

TABLE 4 Multivariate analysis of progesterone receptor and Ki67 in breast cancer using full face sections

	BCSS in the whole cohort (*n* = 1904)			**BCSS in endocrine-treated patients (*n* = 1715)**		
Parameters	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
PR	0.62	0.45–0.86	.005	0.64	0.44–0.90	.009
Ki67	1.50	1.10–2.10	.027	1.40	0.90–2.01	.093
Grade	1.90	1.01–2.00	<.0001	2.10	1.60–2.92	<.0001
Stage	2.00	1.50–2.70	<.0001	2.09	1.60–2.83	<.0001
Tumor size	1.50	1.10–2.10	.006	1.50	1.12–2.10	.01

Note: A 10% cutoff was used for determination of PR status.

Abbreviations: BCSS, breast cancer–specific cancer; CI, confidence interval; DMFS, distant metastasis free survival; HR, hazard ratio; PR, progesterone receptor.

TABLE 5 Multivariate analysis of Ki67 and progesterone receptor in breast cancer using core needle biopsies

	BCSS in endocrine-treated patients (*n* = 727)		
Parameters	**HR**	**95% CI**	***p***
PR	0.38	0.15–0.95	.039
Ki67	0.67	0.24–1.96	.44
Grade	2.4	1.1–5.60	.038
Stage	2.1	0.81–5.34	.13
Tumor size	2.2	0.79–6.34	.12

Note: A 10% cutoff was used for determination of PR status.

Abbreviations: BCSS, breast cancer–specific cancer; CI, confidence interval; HR, hazard ratio; PR, progesterone receptor.

prognostic factor, whereas Ki67 lost its prognostic significance at the various cutoffs in Cox regression analysis (Table S7). Similarly, using Ki67 expression levels as a continuous score and compared against PR in multivariate analysis, PR status maintained the significance with patient outcome, whereas Ki67 lost it in the whole cohort as well as in endocrine-treated patients. The independent prognostic factor of PR in BC was confirmed with competing risk-regression analysis as shown in Table S8. The interaction between PR and endocrine therapy was also established using linear regression model ($p = .03$; 95% CI, 0.2–0.9). Moreover, when we compared PR expression and Ki67 in CNB, PR status showed independent prognostic significance in the group of patients who received endocrine therapy as well as in the whole cohort, whereas Ki67 lost its significance in the multivariate analysis (Table 5).

When the cohort was stratified based on Ki67 expression, negative PR expression showed association with DMFS in both the high and low proliferative groups (Ki67) ($p = .02$ and $p = .007$, correspondingly) further highlighting the value of PR assessment alone or in combination with Ki67 status. Similar results were observed in terms of DMFS (Figure 4).

DISCUSSION

Adjuvant endocrine therapy is the main systemic treatment for the majority of hormone receptor–positive early-stage BC. For this reason, a comprehensive individual assessment for BC includes ER for prognostic stratification other markers are used including PR, HER2, and Ki67.[28,29] PR positivity is seen in 65%–75% of BC, and in approximately 80%–90% of ER-positive cases, and this proportion varies depending on the cutoff of positivity used.[30] In addition, there has been equivocal data about the predictive role of PR expression in early BC.[13,14] Ki67 has attracted much attention as a prognostic factor in luminal BC. However, its assessment in routine practice is still limited.[31] In this study, using full face sections of a large cohort of luminal BC from one institution, we show that PR can categorize luminal BC into low and high groups comparable to or even superior

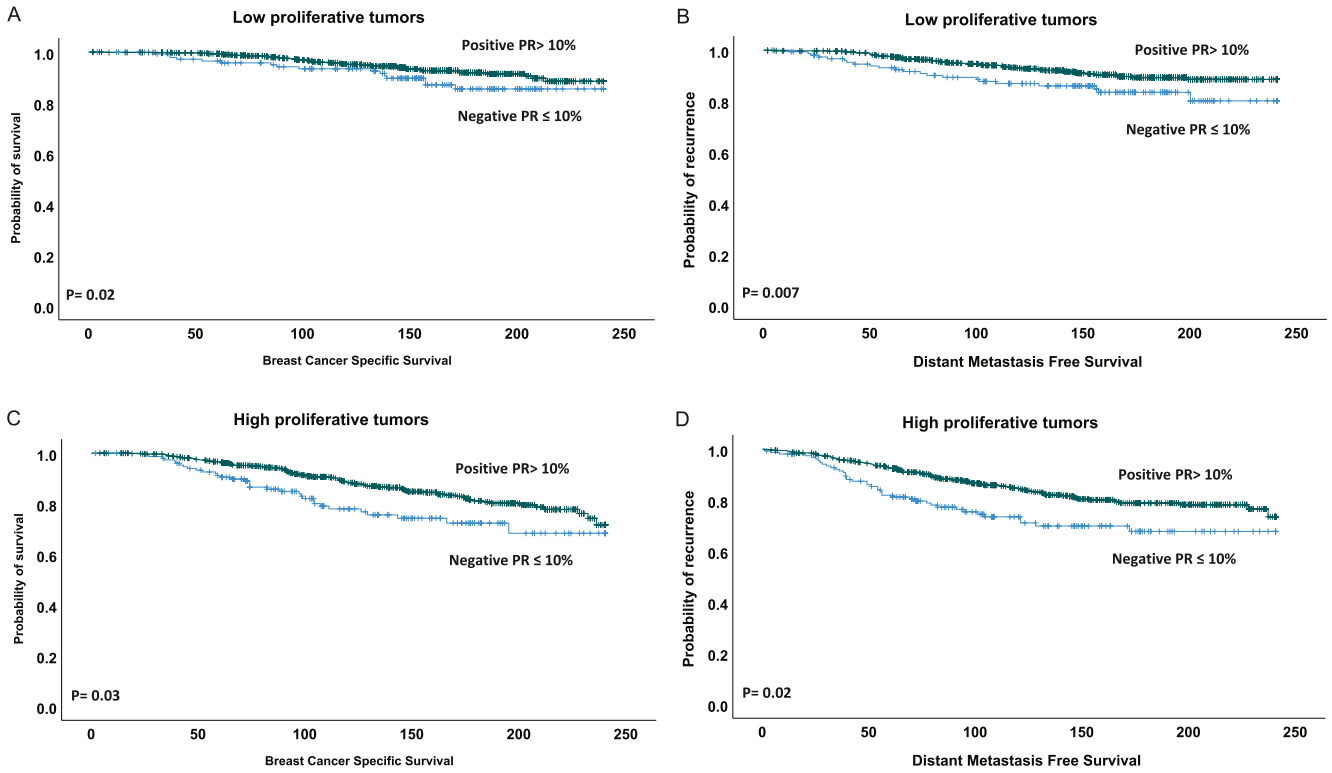

FIGURE 4 (A and B) Kaplan–Meier plots show a significant association between negative progesterone receptor expression with both breast cancer–specific survival and distant metastasis-free survival in tumors with low Ki67 expression as well as in high Ki67 tumors (C and D).

to the Ki67 index. PR expression, similar to ER, is usually assessed using IHC following the same standard and quality assurance measurements applied for ER staining, which ensures the validity, reliability, and consistency of the results. Using PR can provide an additional quality assurance method for the accuracy of ER status as PR-positive/ER-negative cases are extremely rare.[32]

Although ASCO/CAP guidelines recommended the utility of PR testing with similar principles to ER, 1% expression was used to define positivity, 1%–10% was used to define ER-low positive, and >10% was used to define ER-positive tumors.[6] Further clinical consideration is recommended for ER-low positive patients. However, these guidelines were not applied to PR implying that patients with 1%–100% PR expression will be treated equally. However, our study showed that cases with PR-positive in the range of 1%–10% behave similar to cases with <1% PR expression, consistent with the updated ASCO/CAP guidelines of ER-low positive patients.[6] In addition, we found that a 1% cutoff for PR failed to show a prognostic significance in CNB, which is the main tissue type used in the clinical setting for its assessment. Several cutoffs for PR expression have also been reported.[16,33] In this study, using the X-tile tool to identify the optimal PR cutoff for predicting patient outcome, 75% was identified using full face sections, whereas it was 25% for CNB. Because using such threshold as cutoff results in classifying a large number of cases as PR-negative, and to be consistent with ER cutoffs, the three commonly used PR cutoffs (1%, 10%, and 20%) were also tested. This indicated 10% cutoff as the optimal to achieve association with patients' outcome in both biopsies and provided high concordance between CNB and full-face sections. Similar results using a 10% cutoff were reported.[33] Furthermore a 10% cutoff is consistent with the ASCO/CAP guidelines of ER expression categorization into ER-low and ER- positive groups.[6]

In this study, PR showed bimodal expression in BC, which suggests that PR positivity in BC is an "all-or-none" phenomenon. Similarly, many studies have reported bimodal expression of both PR and ER.[24,34] Indeed, low ER expression <10% does not show survival difference from ER-negative tumors.[35] However, other studies claimed that some of the bimodality of expression (of ER) is likely attributable to increasingly sensitive IHC assays artifactually rendering lower positive tumors to stain more diffusely and more intensely.[36,37]

Negative PR expression was significantly associated with post-menopausal status and many previous studies have shown that post-menopausal patients had low PR expression[18,38,39] suggesting that PR expression is affected by the menopausal status. In this study, negative PR expression showed strong association with features characteristic of aggressive tumor behavior. It also showed that ER-positive/PR-negative BC has a similar prognosis to hormone receptor–negative BC and that some ER-positive/PR-negative tumors, as defined by gene expression profiles, share gene expression patterns with ER-negative/PR tumors.[15] In addition, PR negativity was associated with poor outcome in the whole cohort, and there are several supporting studies that confirmed the prognostic value of PR in BC[18,39–41]

In this study, high Ki67 showed a significant association with poor outcome in BC patients. However, negative PR expression was an independent poor prognostic factor and inclusion, or exclusion of Nottingham grade had an impact on the association of Ki67 with outcome but not affecting the prognostic significance of PR. Additionally, when using different Ki67 cutoffs[21] or its continuous scores, the PR status conserved its significance, whereas Ki67 lost association in multivariate analysis. This may indicate that Ki67, but not PR, works as complementary of grade. The PR status in proliferating cancer cells reflects the biology of luminal-type BC.[42] Although the number of patients in the endocrine therapy-naive group was limited, PR did not show significant association with outcome in this group. However, in endocrine therapy-treated patients, PR positivity showed improvement in patient outcome reflecting the predictive value of PR.

Our results indicate that loss of PR predicts endocrine resistance, and this suggests that PR is a key marker of prediction of endocrine response and absence of PR expression in ER-positive tumors can be considered as an indication for additional systemic therapy.[7,34] In this study, negative PR expression in patients who received chemotherapy showed improved outcome similar to patients who had positive PR. Similar findings were shown regarding high Ki67 expression. High Ki67 is indicative of a greater benefit from chemotherapy when added to endocrine therapy in luminal BC.[43] Our study provides evidence that adding chemotherapy to PR negative luminal BC as well as cases with high Ki67 can improve the outcome.[44,45]

Although it was reported that luminal BC with low Ki67 expression has good outcome regardless of PR status,[46] we, and others,[7] have demonstrated that PR can categorize the low proliferating BC group (low Ki67 expression cohort) into two distinct prognostic groups. Therefore, PR can be used as an alternative to Ki67, especially in centers that do not assess Ki67 in their laboratories.

Intratumoral heterogeneity of PR expression is a significant feature that has been reported in the literature,[47,48] and only 80% concordance per tumor was found between full face sections of resected tumor and CNB.[19,49] In our study, there was a significant association between both tissue types, and the concordance rate was high with only 2% of negative PR tumors in CNB PR-positive in full face sections. This low percentage of discrepant cases provides further evidence that PR assessment in CNB is reliable and reflects the actual PR status.

In conclusion, this study provides further evidence that assessment of PR status in the luminal BC can provide valuable prognostic and predictive information independent of other clinicopathological variables comparable to that provided by Ki67 and can outperform it in certain situations. Using 10% rather than 1% cutoff for PR expression provides the optimal prognostic significance.

AUTHOR CONTRIBUTIONS

Ayat G. Lashen: Scoring of the cases, data analysis, interpretation, writing–original draft, agrees with manuscript results and conclusions, and critically edited and reviewed the article. **Michael S. Toss:** Data analysis interpretation, agrees with manuscript results and conclusions, and critically edited and reviewed the article. **Nigel P. Mongan:** Agrees with manuscript results and conclusions and critically edited and reviewed the article. **Andrew R. Green:** Agrees with manuscript results and conclusions and critically edited and reviewed the article. **Emad A. Rakha:** Conceived and planned the study, supervised the project, provided the data and contributed to data interpretation, made critical revisions, and approved the final version.

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CONFLICTS OF INTEREST

The authors made no disclosures.

DATA AVAILABILITY STATEMENT

All data used in this study are archived and could be available on a reasonable request.

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