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Heterogeneity of genomic risk in low progesterone receptor–expressing ER-positive breast cancer assessed by a 21-gene assay

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Abstract

Background:

Low progesterone receptor (PgR) expression is associated with poor prognosis in estrogen receptor (ER)-positive/human epidermal growth factor receptor 2 (HER2)-negative breast cancer. However, the relationship between PgR expression assessed by immunohistochemistry (IHC) and genomic risk remains incompletely understood.

Methods:

We retrospectively analyzed 201 patients with ER-positive/HER2-negative breast cancer who underwent curative surgery and the 21-gene assay (Oncotype DX®). PgR IHC expression was categorized into three groups (0%, 1–20%, and > 20%). Associations between PgR expression, PGR mRNA levels, and recurrence score (RS) were evaluated.

Results:

Low PgR expression (≤ 20%) was observed in 40 patients (19.9%) and was associated with significantly higher RS compared with high PgR expression (> 20%) (median, 23.5 vs. 15; $P < 0.001$). However, RS ranged widely within the low PgR group (5–70), indicating substantial heterogeneity. In particular, tumors with low-level PgR expression (1–20%) exhibited a broad distribution of RS values. PGR mRNA expression showed a clear stepwise increase across PgR IHC categories and was more strongly correlated with RS than IHC-based PgR expression (Spearman's $\rho = -0.61$ vs. -0.46).

Conclusions:

Although low PgR expression has been associated with high genomic risk, our findings demonstrate that tumors within this subgroup exhibit substantial variability in RS. PgR assessment by IHC alone may be insufficient to reliably stratify genomic risk, particularly in tumors with low-level PgR expression.

Introduction

Among hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative breast cancer (BC), absent or low progesterone receptor (PgR) expression has been associated with poorer prognosis [1–4]. Loss of PgR expression has been associated with reduced responsiveness to endocrine therapy and may reflect impaired estrogen receptor (ER) signaling [5].

In Japan, the 21-gene assay (Oncotype DX®) has been covered by national insurance since 2023 and is increasingly used for risk stratification and treatment decision-making. High recurrence score (RS)

tumors are more frequently observed in cases with low PgR expression [6] PgR expression is routinely assessed by immunohistochemistry (IHC); however, evaluation in the low-expression range is subject to variability and interpretative challenges. In contrast, mRNA-based assessment may better reflect the underlying tumor biology. However, the relationship between IHC-based PgR assessment and PGR mRNA expression, particularly in tumors with low PgR expression, and how these relate to RS remains insufficiently understood.

The aim of this study was to evaluate whether PgR assessment based on IHC adequately reflects tumor biology and genomic risk, as assessed by PGR mRNA expression and the 21-gene recurrence score, in ER-positive/HER2-negative BC.

Patients and Methods

We retrospectively reviewed patients with ER-positive/HER2-negative BC who underwent curative breast surgery and Oncotype DX® testing at our institution between September 2023 and January 2026. Oncotype DX® was performed at the discretion of the treating physicians in accordance with Japanese clinical guidelines. All eligible patients during the study period were included without predefined exclusion criteria to reflect real-world clinical practice.

Pathological findings

ER positivity was defined as ≥ 1% nuclear staining by IHC in surgical specimens. HER2 negativity was defined as an IHC score of 0 or 1+, or an IHC score of 2 + with a HER2/CEP17 amplification ratio of < 2.0, as assessed by dual-color in situ hybridization.

Biomarker assessment

Low PgR expression assessed by IHC was defined as 0–20%. PgR IHC expression was dichotomized at 20% for baseline comparisons and further categorized (0%, 1–20%, and > 20%) for detailed analyses. mRNA expression levels of ESR1 (estrogen receptor) and PGR (progesterone receptor) were assessed using Oncotype DX®. mRNA expression levels of ESR1 and PGR were dichotomized according to predefined cut-off values provided in the Oncotype DX® assay report (ESR1 < 6.5 and PGR < 5.5 were defined as negative).

Statistical Analysis

mRNA expression levels were visualized using scatter plots according to IHC expression levels to assess concordance between IHC- and mRNA-based assessments. PgR IHC expression was categorized into three groups (0%, 1–20%, and > 20%) for group comparisons. Values below the lower limit of quantification were set to the corresponding lower limit values (ESR1: 3.7; PGR: 3.2), and values

exceeding predefined upper thresholds were capped at those threshold values (ESR1: 12.5; PGR: 10.0) for descriptive purposes.

Continuous variables are presented as medians with interquartile ranges, and categorical variables as frequencies and percentages. Correlations between IHC expression levels and corresponding mRNA values were assessed using Spearman's rank correlation coefficient. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. Statistical analyses were performed using EZR (version 1.68; Saitama Medical Center, Jichi Medical University).

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital (Approval No. 3625). Because this was a retrospective study using existing clinical data, written informed consent was not required. Instead, information about the study was disclosed on the hospital website, and patients were given the opportunity to opt out.

Results

A total of 201 patients with ER-positive/HER2-negative breast cancer (BC) who underwent curative breast surgery and 21-gene assay (Oncotype DX®) testing was included in the final analysis. Of these, 40 patients (19.9%) had low PgR tumors. Baseline clinicopathological characteristics stratified by PgR expression are summarized in Table 1. Among patients with low PgR tumors, the median age was 57.5 years (range, 31–75). The recurrence score (RS) was significantly higher in the low PgR group than in the high PgR group (median, 23.5 [range, 5–70] vs. 15 [range, 0–33]; $P < 0.001$).

Table 1
Patient characteristics according to PgR expression

Variable	**PgR ≤ 20% (N = 40)**		**PgR > 20% (N = 161)**		**P**
Age, years, median (range)	57.5	(31–75)	50	(30–78)	< 0.001
Menopausal status					
Premenopausal	11	(27.5%)	96	(59.6%)	< 0.001
Postmenopausal	29	(72.5%)	65	(40.4%)	
Histological type					
IDC	38	(95.0%)	154	(95.7%)	0.86
Other special types	2	(5.0%)	7	(4.3%)	
Histological grade					
Grade1 or 2	29	(72.5%)	136	(84.5%)	0.08
Grade3	11	(27.5%)	25	(15.5%)	
Ki-67					
<30%	23	(57.5%)	70	(43.5%)	0.11
≥30%	17	(42.5%)	91	(56.5%)	
mRNA PGR					
<5.5	28	(70%)	1	(0.6%)	< 0.001
≥5.5	12	(30%)	160	(99.4%)	
pT stage					
T1	26	(65%)	99	(61.5%)	0.74
T2	13	(32.5%)	60	(37.3%)	
T3	1	(2.5%)	2	(1.2%)	
pN stage					
N0	25	(62.5%)	96	(59.6%)	0.74
N1	15	(37.5%)	65	(40.4%)	
RS, median (range)	23.5	(5–70)	15	(0–33)	< 0.001

IDC, invasive ductal carcinoma; ER, estrogen receptor; PgR, progesterone receptor; RS, recurrence score

Variable	PgR ≤ 20% (N = 40)		PgR > 20% (N = 161)		P
RS					
<26	25	(62.5%)	152	(94.4%)	< 0.001
≥26	15	(37.5%)	9	(5.6%)	

IDC, invasive ductal carcinoma; ER, estrogen receptor; PgR, progesterone receptor; RS, recurrence score

Correlation between PgR IHC expression and mRNA levels of PGR

When stratified into three groups according to PgR IHC expression (0%, 1–20%, and > 20%), RS differed significantly among the groups (Kruskal–Wallis test, $p < 0.001$. Figure 1). PGR mRNA expression levels were inversely correlated with RS (Spearman's $\rho = -0.61$, $p < 0.001$, Fig. 2). PgR IHC expression levels showed an inverse correlation with RS (Spearman's $\rho = -0.46$, $p < 0.001$, Fig. 3).

Discussion

In the present study, tumors with low PgR expression (≤ 20%) showed higher RS values; however, RS ranged widely from 0 to 70, indicating substantial heterogeneity within this group. Notably, tumors with low-level PgR expression (1–20%) exhibited a particularly broad distribution of RS values. In contrast, PGR mRNA expression demonstrated a more consistent stepwise increase across PgR IHC categories, suggesting that mRNA-based assessment may better reflect the underlying tumor biology than IHC alone.

Previous studies have reported that discordance between IHC- and mRNA-based PgR assessments occurs more frequently in tumors with low PgR expression [7–14]. Cheang et al. reported that tumors with low or borderline PgR expression constitute a biologically heterogeneous group [15]. PgR expression is considered a downstream marker of functional ER signaling [16], and low PgR expression may therefore reflect reduced activity of the ER pathway. In addition to biological factors, methodological aspects should be considered when interpreting discordance between IHC- and mRNA-based PgR assessments. IHC results can be influenced by pre-analytical variables such as fixation conditions and antibody selection [17], whereas mRNA quantification may be affected by tumor cellularity and RNA quality [18], potentially contributing to discrepancies between protein expression and mRNA levels.

These findings suggest that tumors with weakly positive PgR expression cannot be uniformly regarded as low-risk based on IHC alone, and additional genomic assessment may be warranted in this subgroup. Further characterization of tumors within the low PgR subgroup, including clinicopathological and

molecular features associated with high genomic risk, may help to refine risk stratification and warrants further investigation.

This study has several limitations. First, it was a single-center retrospective observational analysis. Second, the study population was limited to patients who underwent Oncotype DX®, rather than including all luminal BC cases, introducing potential selection bias. Therefore, the findings may not be fully generalizable to the entire population of ER–positive BC patients. Third, the cut-off values used for PgR IHC and PGR mRNA expression were predefined, and alternative thresholds may yield different results. Finally, survival outcomes were not evaluated in this study, and the prognostic impact of low PgR requires further investigation with longer follow-up.

Conclusion

Although low PgR expression has been associated with high genomic risk, our findings demonstrate that tumors within this subgroup exhibit substantial variability in RS. This indicates that PgR IHC alone may not reliably stratify genomic risk, particularly in tumors with low or weakly positive PgR expression.

Declarations

The datasets generated and/or analyzed during the current study are not publicly available due to institutional restrictions and patient privacy concerns, but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Figures

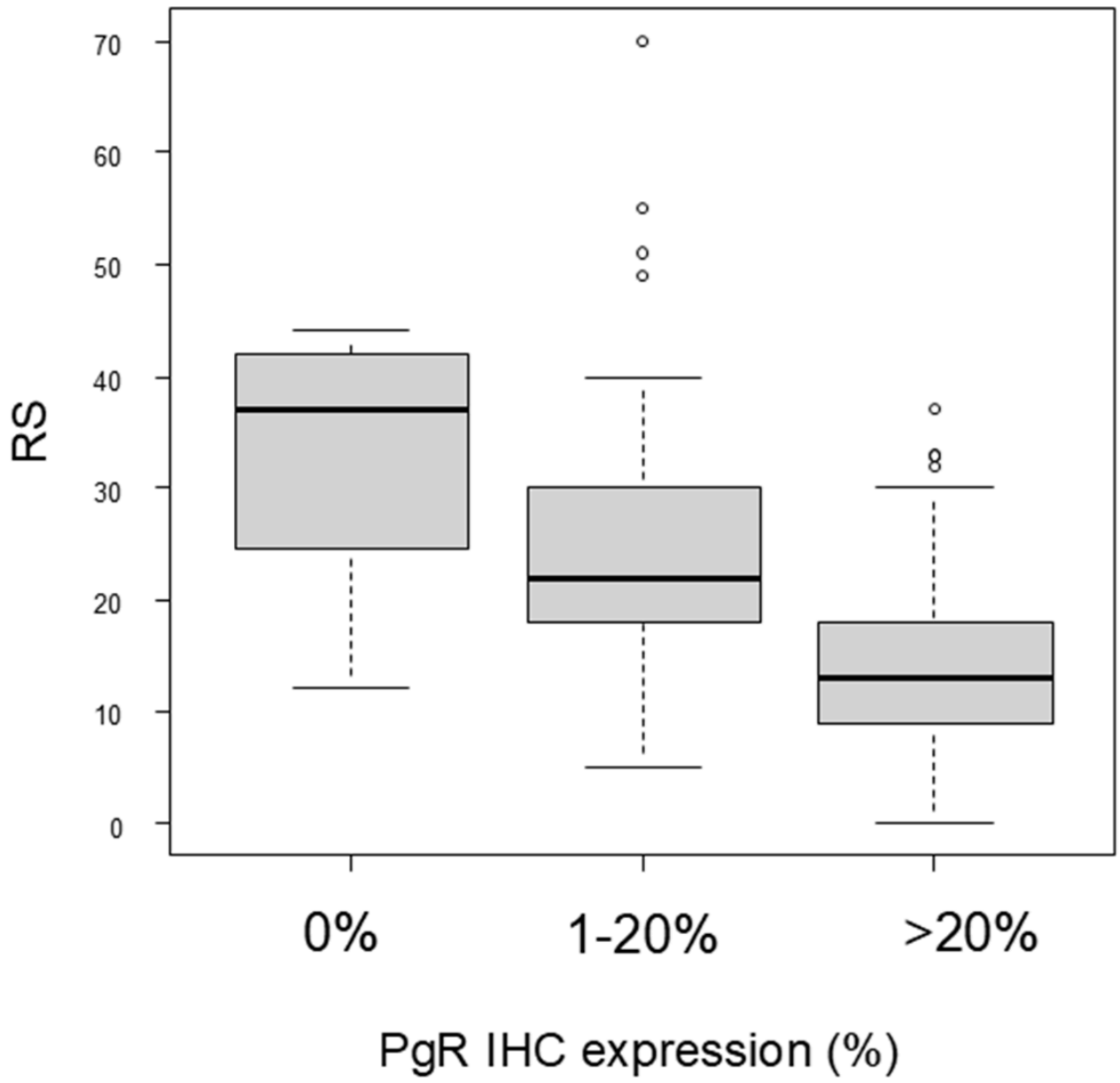


Figure 1

Association between PgR IHC expression and recurrence score (RS)
RS was compared among three groups stratified by PgR IHC expression (0%, 1–20%, and >20%). Differences among the three groups were assessed using the Kruskal–Wallis test ($p < 0.001$).

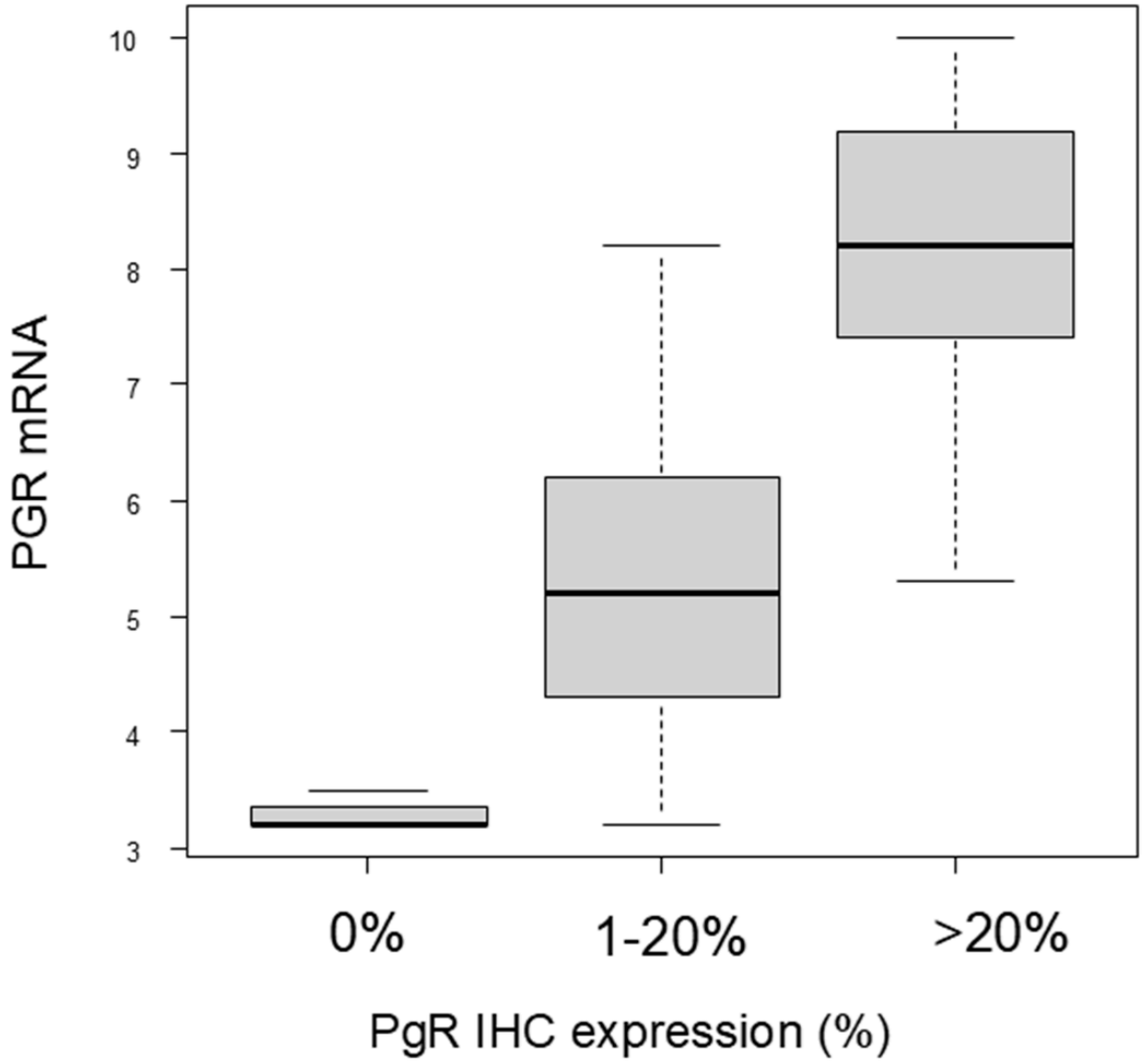


Figure 2

Distribution of PGR mRNA expression according to PgR IHC categories

PGR mRNA expression levels increased stepwise across PgR IHC categories (0%, 1–20%, and >20%) (Kruskal–Wallis test, $P < 0.001$).

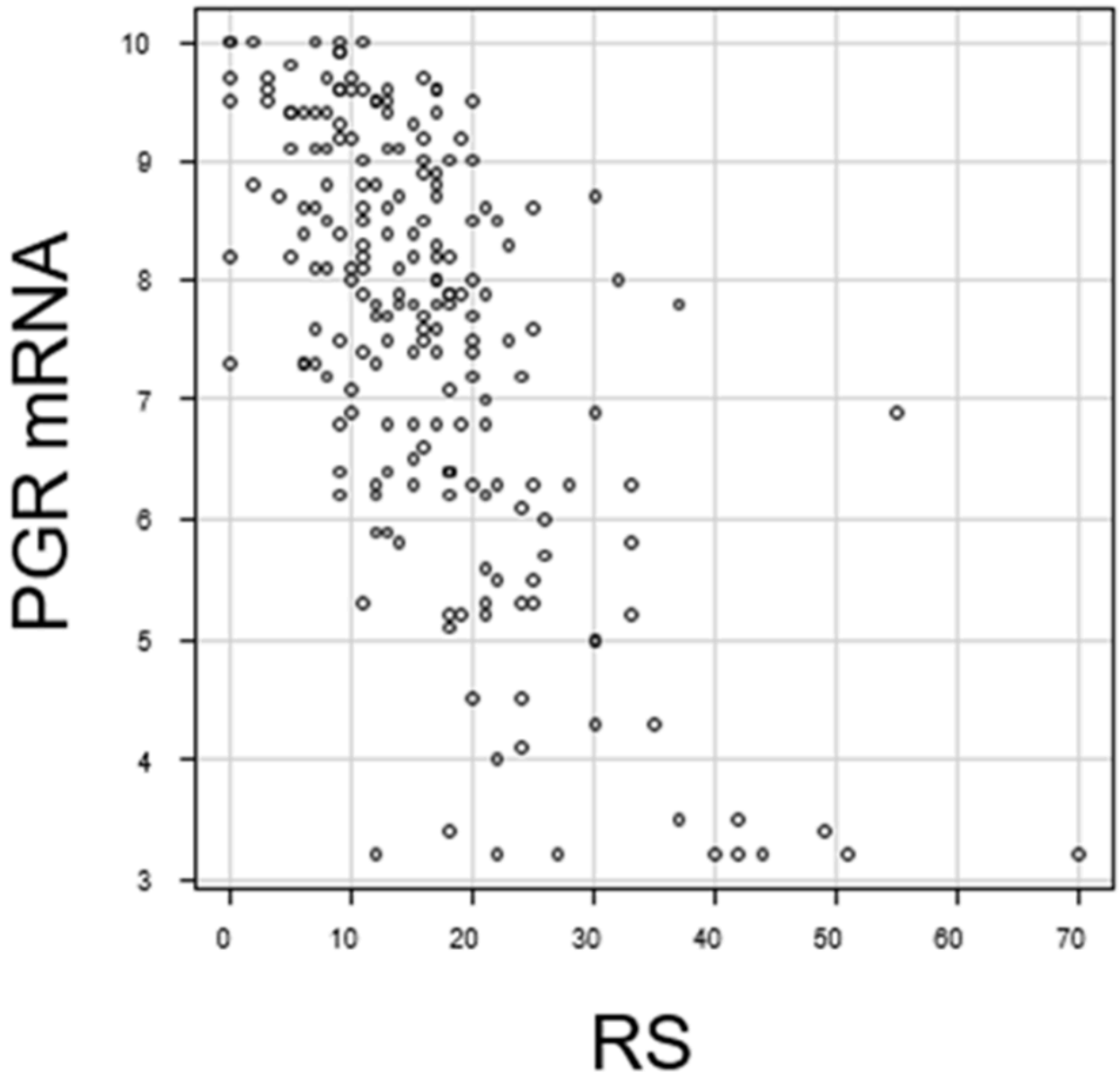


Figure 3

Correlation between PGR mRNA expression and recurrence score (RS)
PGR mRNA expression levels showed an inverse correlation with RS (Spearman's $\rho = -0.61$, $p < 0.001$).

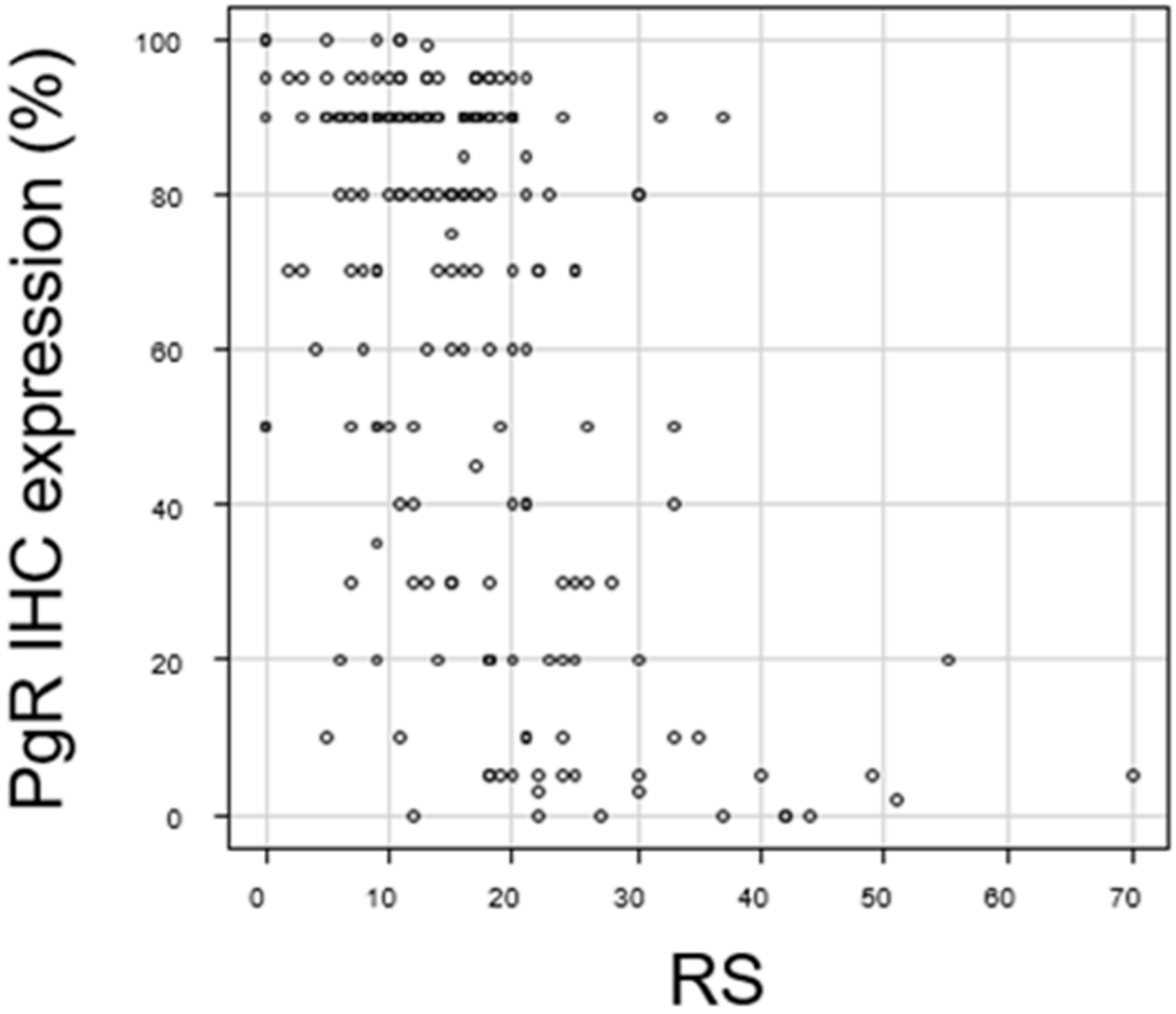


Figure 4

Correlation between PgR IHC expression and recurrence score (RS)
PgR IHC expression levels showed an inverse correlation with RS (Spearman's ρ = −0.46, p < 0.001).