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Independent Predictors of Pathological Complete Response in Breast Cancer: A Multivariable Analysis

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ABSTRACT

Neoadjuvant chemotherapy (NAC) is widely used in the treatment of breast cancer, though response significantly varies across patients (Wang et al., 2017; Abidi et al., 2023). Biomarkers such as HER2 status, hormone receptor (HR) status, and MammaPrint risk score are commonly used to predict treatment outcomes, but their independent predictive value remains unclear.

This study aimed to determine which of these biomarkers independently predict pathological complete response (pCR) when analyzed simultaneously. Publicly available data from 654 patients in the I-SPY2 trial (GSE194040) were analyzed using chi-square tests and multivariable logistic regression.

HER2-positive status (OR = 4.62, 95% CI: 3.04–7.11, $p < 0.001$) and ultra-high MammaPrint risk (OR = 3.10, 95% CI: 1.95–4.98, $p < 0.001$) were identified as significant independent predictors of pCR, while HR status was not significant after adjustment (OR = 0.67, 95% CI: 0.43–1.03, $p = 0.068$). The model demonstrated moderate predictive performance (AUC = 0.70).

These findings suggest that evaluating biomarkers in isolation may overestimate their predictive importance, and that multivariable analysis provides a more accurate approach for interpreting treatment response, supporting better informed clinical decision-making.

INTRODUCTION

Neoadjuvant chemotherapy (NAC) is a commonly used treatment, particularly for patients diagnosed with locally advanced and high-risk breast cancer (Wang et al., 2017). It is applied before surgery to reduce tumor size and improve surgical results, while simultaneously allowing clinicians to assess how tumors respond to treatment in real time. One key measure of response is pathological complete response (pCR), defined as the absence of detectable invasive cancer at the time of surgery. Although achieving pCR is strongly associated with improved health outcomes in the long-term, including lower recurrence rates and increased survival, response to NAC varies widely, with only 20–30% of patients achieving full pCR (Abidi et al., 2023). As a result, many patients undergo intensive chemotherapy without clear clinical benefit, exposing them to unnecessary side effects, healthcare costs, and burden from treatment.

To guide treatment decisions, clinicians rely on biomarkers such as human epidermal growth factor receptor 2 (HER2) status, hormone receptor (HR) status, and genomic risk assessments like the MammaPrint score. These biomarkers reflecting tumor biology, including growth signaling pathways, hormone sensitivity, and proliferative activity, are routinely used to estimate the likelihood of treatment response. Previous studies have shown that these biomarkers are associated with chemotherapy outcomes (Hayes et al., 2007; Pusztai et al., 2021). More recent research has suggested that combining multiple biomarkers may improve the accuracy of prediction, and that certain gene-based tumor classifications can still predict response even after accounting for HR status (Blumencranz et al., 2023; Mazo et al., 2020).

Despite these advances, it remains unclear which biomarkers provide independent predictive value when considered together. Many existing studies evaluate biomarkers individually, comparing response rates across groups based on a single variable at a time. While this approach identifies associations, it does not determine whether a biomarker is truly predictive on its own or simply reflects its relationship with other tumor characteristics or factors. This distinction is important, as overestimating the importance of certain biomarkers could lead to less accurate treatment decisions and suboptimal patient selection.

Therefore, the objective of this study is to determine which biomarkers independently predict pCR when HER2 status, HR status, and MammaPrint risk scores are analyzed simultaneously using a multivariable model.

METHODS

Data Source

Publicly available data from the I-SPY2 clinical trial were analyzed using the Gene Expression Omnibus database (GSE194040). The I-SPY2 trial is a randomized study evaluating neoadjuvant chemotherapy in patients with high-risk, early-stage breast cancer. The full dataset included 987 patients, however for this analysis, 654 patients from the Agilent GPL20078 platform with complete data for all variables of interest were included. Patients with missing data or from other platforms were excluded to ensure consistency and reliability of the analysis and results.

Study Variables

The primary outcome variable was pathological complete response (pCR), coded as a binary variable (1 = pCR; 0 = no pCR). Independent variables included baseline tumor characteristics measured prior to treatment: HER2 status (1 = positive, 0 = negative), hormone receptor (HR) status (1 = positive, 0 = negative), and MammaPrint risk score (0 = high risk [MP1], 1 = ultra-high risk [MP2]). These variables were selected because they are commonly used in clinical decision-making and reflect key aspects of tumor biology, including growth signaling.

Hypotheses

The research hypothesis (H_1) was that HER2-positive status, HR-negative status, and ultra-high risk MammaPrint scores are independently associated with a higher likelihood of achieving pCR when analyzed simultaneously. The null hypothesis (H_0) stated that no significant association exists between these biomarkers and pCR when controlling for other variables. A significance level of $\alpha = 0.05$ was used.

Statistical Analysis

All analyses were conducted using R (version 4.5.2). Descriptive statistics, including frequencies and percentages, were first used to summarize the study population (Table 1). Following the descriptive statistics, chi-square tests of independence were performed to assess the relationship between each predictor variable and pathological complete response, allowing for the identification of initial associations.

A multivariable binary logistic regression test was then used to identify independent predictors of pCR by evaluating all variables simultaneously. This approach allows for the effect of each biomarker to be assessed while controlling for potential confounding between variables. Results are reported as odds ratios (OR) with 95% confidence intervals (CI), where $OR > 1$ indicates an increased likelihood of pCR and $OR < 1$ indicates a decreased likelihood. Statistical significance was defined as $p < 0.05$ (Figure 2).

The model performance of the multivariable test was evaluated using receiver operating characteristic (ROC) analysis to assess how well the model distinguishes between patients who achieved pCR and those who did not. The area under the curve (AUC) was used to summarize this performance, where 0.5 indicates no discrimination and 1.0 indicates perfect prediction.

RESULTS

Variable	***Category***	***N***	***%***
Treatment Outcome	*Complete Response*	*214*	*32.7*
	No Response	*440*	*67.3*
HER2 Status	*Positive*	*147*	*22.5*
	Negative	*507*	*77.5*
Hormone Receptor Status	*Positive*	*360*	*55.0*
	Negative	*294*	*45.0*

MammaPrint Risk	*High Risk (MP1)*	*293*	*44.8*
	Ultra-High Risk (MP2)	*361*	*55.2*

Table 1: Patient Characteristics (N = 654). Values represent the number of patients (N) and percentage of the total sample.

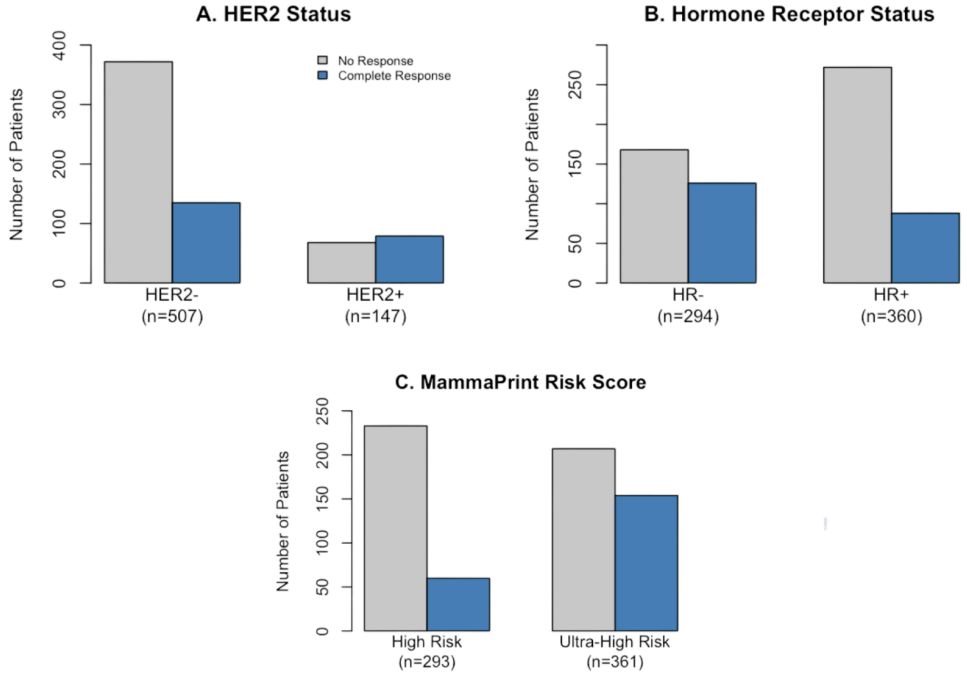

Figure 1: Chi-square tests comparing pathological complete response across patient biomarker subgroups (N=654). **(A)** HER2 status: HER2- vs HER2+; $p < 0.001$. **(B)** Hormone receptor status: HR- vs HR+; $p < 0.001$. **(C)** MammaPrint risk score: High risk vs Ultra-high risk ; $p < 0.001$.

Chi-square tests showed that all 3 factors were linked with pcR (Figure 1). HER2-positive patients had higher response rates than HER2-negatives (53.7% versus 26.6%). Hormone receptor-negative patients responded better than HR-positive patients (42.9% versus 24.4%). Ultra-high risk tumors responded better than high-risk tumors (42.7% versus 20.5%).

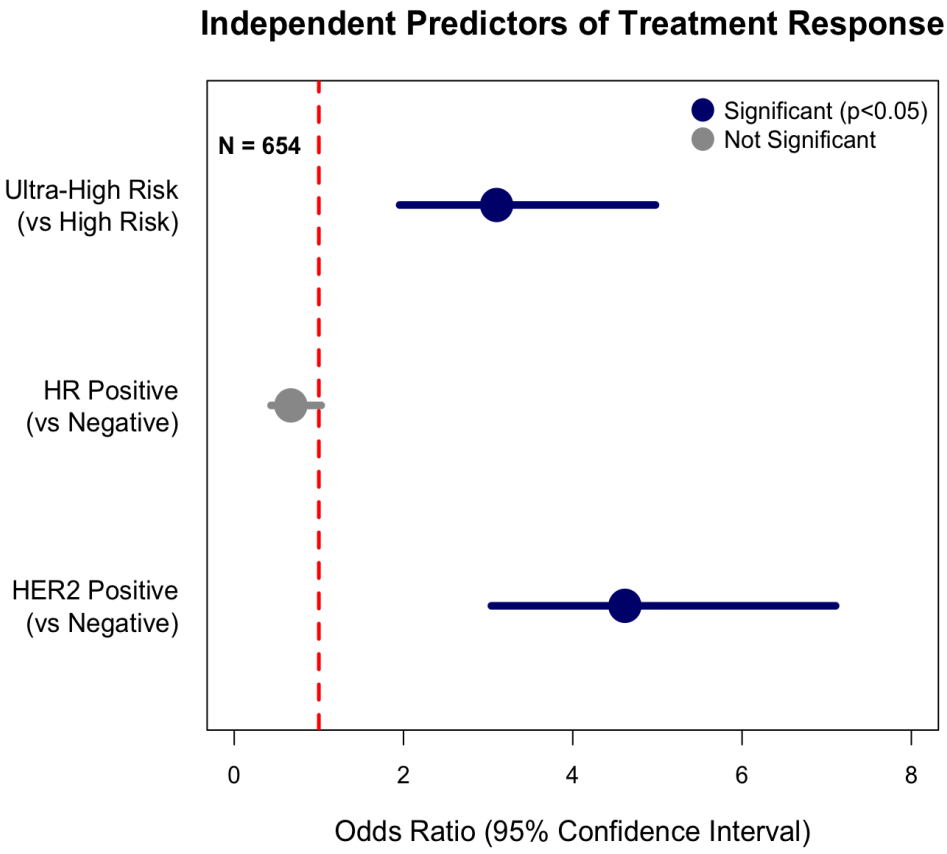

Figure 2: Independent predictors of pathological complete response. Forest plot from logistic regression (N=654) showing odds ratios for patient characteristics predicting treatment response. Navy=significant ($p<0.05$); grey=not significant. Red line=no effect (OR=1). Horizontal lines=95% confidence intervals.

Logistic regression identified which factors remained significant when analyzed together (Figure 2). HER2-positive status was the strongest independent factor, with patients approximately 4.6 times more likely to achieve pCR compared to HER2-negative (OR = 4.62, 95% CI: 3.04–7.11, $p < 0.001$). Ultra-high risk tumors also showed a strong association (OR = 3.10, 95% CI: 1.95–4.98, $p < 0.001$). As illustrated in Figure 2, both HER2-positive status and ultra-high risk tumors have confidence intervals that do not cross 1, confirming their statistical significance. In contrast, hormone receptor status has a confidence interval that crosses 1 (OR = 0.67, 95% CI: 0.43–1.03, $p = 0.068$), indicating that it is not a statistically significant independent predictor when other biomarkers are included in the model.

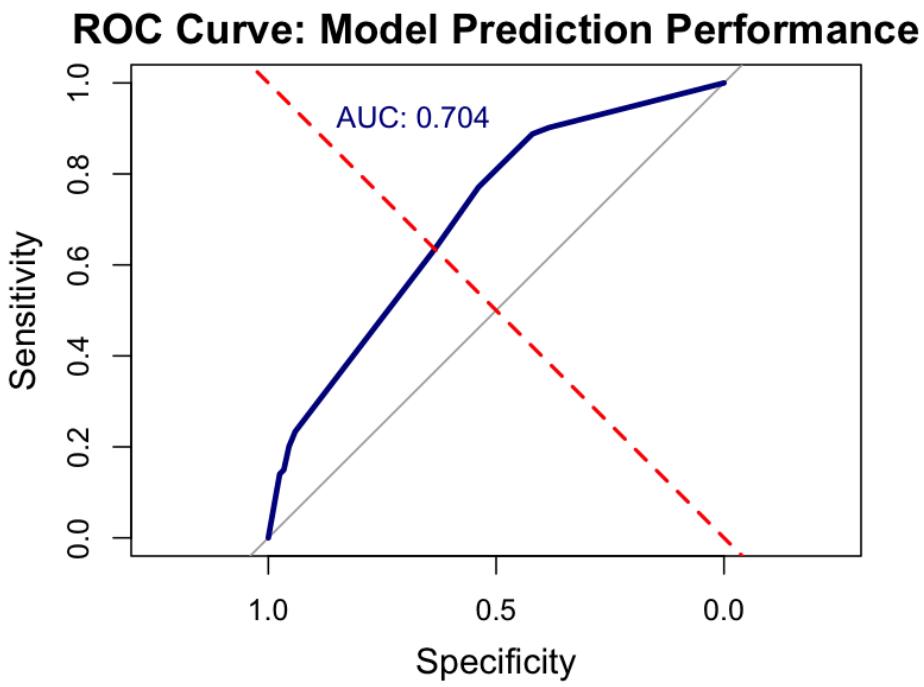

Figure 3: Independent predictors of pathological complete response. ROC curve evaluating the regression model's ability to predict pCR (N = 654). Model includes HER2 status, HR status, and MammaPrint risk score as predictors. Area under the curve (AUC) = 0.70 (95% CI: 0.66–0.75). Blue curve = model performance; red dashed diagonal line = random chance (AUC = 0.5).

The performance of the multivariable model was evaluated using ROC analysis (Figure 3). The model achieved an AUC of 0.70 (95% CI: 0.66–0.75), indicating a moderate ability to distinguish between patients who achieved pCR and those who did not. As shown in Figure 3, the ROC curve lies above the diagonal line representing random classification, highlighting that the model performs better than chance. However, the curve does not approach the upper-left corner, indicating that the model's predictive performance could be improved with the inclusion of additional variables.

DISCUSSION

The results of this study show that while several biomarkers are associated with chemotherapy response, not all retain independent predictive value when being analyzed together. HER2 status and MammaPrint risk remained strong independent predictors of pathological complete response, consistent with their biological roles in tumor proliferation. HER2-positive tumors are characterized by an overexpression of a growth factor receptor that is associated with increased cell proliferation, while MammaPrint ultra–high-risk tumors reflect gene expression patterns associated with high cellular proliferation. Because

chemotherapy primarily targets rapidly dividing cells, these tumor types may be more responsive to treatment.

In contrast, hormone receptor (HR) status, although commonly used in clinical decision-making (Sasanpour, et al, 2018) , did not remain significant in the multivariable model. While HR-negative tumors showed higher response rates in the chi-square analysis, this association disappeared once HER2 and MammaPrint were controlled for. These findings suggest that HR status primarily represents its association with other tumor characteristics rather than providing independent predictive value. This is a critical distinction that is often overlooked in studies that evaluate biomarkers individually.

The predictive model demonstrated a moderate performance (AUC = 0.70), indicating a fair discrimination between responders and non-responders. This reinforces the idea that even when combining commonly used biomarkers, prediction of treatment response can be further improved.

Furthermore, these findings both align with and extend previous research. For instance, Hayes et al. (2007) demonstrated that HER2-positive patients have higher response rates to chemotherapy, which is supported by our results. Similarly, Pusztai et al. (2021) reported that ultra-high MammaPrint risk is associated with improved response in the I-SPY2 trial. However, this study extends existing evidence by demonstrating that these biomarkers remain independently predictive when analyzed simultaneously, rather than appearing significant only in isolation. In contrast, while Sasanpour, et al. (2018) reported higher response rates in HR-negative tumors, our findings refine this understanding by showing that this relationship does not remain significant when other biomarkers are considered simultaneously. Together, these findings clarify relationships between frequently used biomarkers that are often overlooked in single-marker studies, offering a more precise interpretation of existing clinical tests to better inform treatment decision-making.

The implications of these findings are relevant and applicable to clinical treatment decision-making. Interpreting biomarkers such as HR status in isolation may lead to overestimating a patient's likelihood of benefiting from chemotherapy. In contrast, distinguishing between biomarkers that are independently predictive and those that are not could improve patient stratification. For example, patients with HER2-positive or ultra-high risk tumors, both of which remain independently associated with pCR, may be more appropriate candidates for neoadjuvant chemotherapy, while patients whose profiles are driven primarily by HR status may require more careful evaluation before treatment regimen is selected. This distinction could support the proper usage of existing clinical data, which may assist in reducing unnecessary exposure to chemotherapy in patients that are less likely to benefit, while prioritizing treatment for those with a higher probability of responding. Importantly, this does not diminish the role of HR status in guiding hormone therapy, but rather refines its role in predicting chemotherapy response.

However, several limitations should also be acknowledged. For the study, the outcome measure was binary and does not capture partial responses. Additionally, the I-SPY2 population consists of only high-risk, early-stage breast cancer patients, which may limit generalizability to other populations.

Furthermore, while HER2 status, hormone receptor status, and MammaPrint risk score are clinically relevant and widely used, breast cancer response is also influenced by potential confounding factors that were not included in this analysis. These include differences in treatment regimens within the I-SPY2 trial, as well as patient demographic and tumor-related variables such as age, tumor stage, and Ki-67 proliferation index. As a result, the model represents a simplified framework of treatment response rather than a comprehensive clinical prediction tool. Despite these limitations, the study benefits from high-quality trial data and a statistical approach that isolates independent effects, allowing for a more accurate interpretation of relationships between biomarkers.

Overall, this study highlights an important gap in how biomarkers are commonly evaluated. By analyzing multiple predictors simultaneously, it demonstrates that evaluating biomarkers in isolation can overestimate their importance and obscure underlying relationships between characteristics. This provides a more refined understanding of how existing clinical tests should be interpreted in practice.

Future research should focus on validating these findings in independent datasets and exploring why HR status appears predictive in isolation but loses significance when analyzed alongside other factors. Longitudinal studies could also assess how adjusting the role of HR status in treatment decisions impacts patient outcomes over time. Lastly, predictive performance may be improved by incorporating a broader set of clinical and molecular variables in future research. By distinguishing between associated and independently predictive biomarkers, this study provides a more precise structure for interpreting clinical data and supports informed chemotherapy decision-making.

CONCLUSION

This study demonstrates that while several biomarkers are associated with chemotherapy response in breast cancer, not all retain independent predictive value when analyzed together. HER2 status and MammaPrint risk remained independent predictors of pathological complete response, while hormone receptor status did not remain significant after adjustment. These findings suggest that commonly used biomarkers may be overinterpreted when evaluated in isolation. By distinguishing between associated and independently predictive factors, this study provides a more refined approach for interpreting clinical biomarker data. This approach may support more informed decisions regarding treatment regimens, by improving the alignment between patients and therapies most likely to be effective.

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Ayra Dhillon is a high school sophomore at Abbotsford Senior Secondary School in Abbotsford, B.C. She takes interest in biology and precision medicine, hoping to explore how these areas intersect to improve patient outcomes. She plans to study a STEM-related field in college, with a focus on statistical data analysis and labwork.