The Value of the Combination of Progesterone Receptor and Ki-67 in Prognostic Assessment of Hormone Receptor-Positive Moderately Differentiated Early-Stage Breast Cancer Postprint

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Date: 2018-10-26T00:00:00+00:00

Abstract

Objective To investigate the value of the combination of progesterone receptor (PR) and Ki-67 index in prognostic assessment of hormone receptor-positive, axillary lymph node-negative, moderately differentiated breast cancer. Methods Clinical and pathological data were collected from 389 patients with moderately differentiated early-stage breast cancer who underwent 21-gene assay. Patients were stratified into low-risk (247 cases), intermediate-risk (115 cases), and high-risk (27 cases) groups based on the 21-gene recurrence score (RS). The correlation between the combination of PR and Ki-67 index and the 21-gene RS was analyzed, and the prognostic value of the two approaches was compared. Results Based on PR expression and Ki-67 index, the 389 patients were categorized into PK low-risk group (PR 10% and Ki-67 20%) with 248 cases (63.8%), PK intermediate-risk group (neither high-risk nor low-risk) with 125 cases (32.1%), and PK high-risk group (PR<10% and Ki-67>20%) with 16 cases (4.1%). The predictive sensitivity of the PK combination for RS low-risk and high-risk groups was 75.3% and 37.0%, respectively, with positive predictive values of 75.0% and 62.5%, respectively, and discordance rates of 0.4% and 6.3%, respectively. Results from a median follow-up of 40 months showed that the overall event rates for PK low-risk, intermediate-risk, and high-risk groups were 3.6%, 7.2%, and 12.5%, respectively, while those for RS low-risk, intermediate-risk, and high-risk groups were 3.2%, 8.7%, and 7.4%, respectively, demonstrating comparability between the two approaches. Conclusion The combination of PR and Ki-67 demonstrates prognostic assessment value comparable to that of the 21-gene RS for hormone receptor-positive, axillary lymph node-negative, moderately differentiated breast cancer.

Full Text

Preamble

Prognostic Value of Progesterone Receptor and Ki-67 Combination in Hormone Receptor-Positive Grade 2 Early-Stage Breast Cancer

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Abstract

Objective: To evaluate the prognostic value of the combination of progesterone receptor (PR) and Ki-67 index in hormone receptor (HR)-positive, axillary lymph node-negative, grade 2 breast cancer.

Methods: Clinicopathologic data from 389 patients with grade 2 early-stage breast cancer who underwent 21-gene recurrence score (RS) testing were retrospectively analyzed. Patients were stratified into low-risk (247 cases), intermediate-risk (115 cases), and high-risk (27 cases) groups based on the 21-gene RS. The correlation between the PR and Ki-67 index combination and the 21-gene RS was analyzed, and the prognostic value of these two approaches was compared.

Results: Based on PR expression and Ki-67 index, the 389 patients were divided into PK low-risk group (PR 10% and Ki-67 20%, 248 cases [63.8%]), PK intermediate-risk group (neither high- nor low-risk, 125 cases [32.1%]), and PK high-risk group (PR $<$ 10% and Ki-67 $>$ 20%, 16 cases [4.1%]). The predictive sensitivity of the PK combination for RS low-risk and high-risk groups was 75.3% and 37.0%, respectively, with positive predictive values of 75.0% and 62.5%, and discordance rates of 0.4% and 6.3%, respectively.

After a median follow-up of 40 months, the overall event rates for PK low-, intermediate-, and high-risk groups were 3.6%, 7.2%, and 12.5%, respectively. The corresponding event rates for RS low-, intermediate-, and high-risk groups were 3.2%, 8.7%, and 7.4%, respectively, demonstrating comparability between the two classification systems.

Conclusions: The combination of PR and Ki-67 for HR-positive, axillary lymph node-negative, grade 2 breast cancer provides prognostic value comparable to that of the 21-gene RS.

Subject Words: Breast neoplasms; Prognosis; Progesterone receptor; Ki-67; 21-gene assay

Introduction

Breast cancer is the most common malignancy among women, with its incidence continuing to rise in recent years. For patients with early-stage breast cancer expressing hormone receptors (HR) and lacking human epidermal growth factor receptor 2 (HER-2) overexpression, the benefits of chemotherapy and prognostic assessment remain key clinical concerns. Since 2011, the National Comprehensive Cancer Network (NCCN) breast cancer clinical guidelines have recommended the use of 21-gene testing in HR-positive, HER-2-negative, axillary lymph node-negative early breast cancer [1] to inform prognosis and chemotherapy decisions. However, given the high cost of 21-gene testing (8,000–10,000 RMB per case), few medical institutions in China can routinely perform this assay. Consequently, treatment decisions in many smaller medical centers rely primarily on pathological staging and immunohistochemistry results.

In this study, we analyzed clinicopathologic and survival data from 389 patients with HR-positive, HER-2-negative, axillary lymph node-negative, grade 2 breast cancer who underwent 21-gene testing. We investigated the feasibility of using a combination of progesterone receptor (PR) and Ki-67 index (PK combination) to estimate the 21-gene recurrence score (RS) and examined its correlation with breast cancer prognosis in grade 2 early-stage disease.

Materials and Methods

From May 2012 to May 2017, 593 patients with HR-positive, HER-2-negative early invasive breast cancer without lymph node metastasis underwent surgical treatment and 21-gene testing in the Department of Breast Surgery at Peking Union Medical College Hospital. Among these, 389 patients with histological grade 2 tumors were selected for this study. All 389 patients were female, aged 26–69 years with a median age of 48 years.

All histological slides were reviewed by two experienced pathologists who confirmed diagnoses and assessed pathological parameters, including histological grade, estrogen receptor (ER), PR, HER-2, and Ki-67 expression. Pathological diagnosis followed the WHO 2012 classification of breast tumors. HR positivity was defined as 1% of tumor cells showing nuclear staining for ER or PR. HER-2 negativity was defined as immunohistochemistry (IHC) score of (-) or (+); IHC (+++) was considered positive, while IHC (++) cases underwent fluorescence in situ hybridization (FISH) testing, with no gene amplification classified as negative (indeterminate results were treated as negative) and amplification as positive.

21-Gene Testing Method and Scoring

The Geneseeq® branched DNA-liquid chip technology was used to detect mRNA expression levels of the 21 genes in surgical specimens, and RS was calculated.

RS < 18 indicated low recurrence risk, 18 RS < 31 indicated intermediate risk, and RS 31 indicated high recurrence risk.

Based on our research group' s previous clinical findings [2], patients with grade 2 tumors were stratified according to PR expression and Ki-67 index into PK low-risk group (PR 10% and Ki-67 20%), PK high-risk group (PR < 10% and Ki-67 > 20%), and PK intermediate-risk group (neither high- nor low-risk).

Follow-up

Follow-up was conducted through electronic medical record review, outpatient visits, and telephone interviews. The last follow-up date was January 2018, with follow-up duration ranging from 8 to 68 months and a median follow-up time of 40 months. Follow-up content included postoperative adjuvant therapy, recurrence and metastasis, imaging examinations, and survival status.

Statistical Methods

Statistical analysis was performed using SPSS 24.0 software. Continuous variables were expressed as mean ± standard deviation, and comparisons among three groups were performed using one-way ANOVA. Categorical data were compared using chi-square test and Fisher' s exact test. P < 0.05 was considered statistically significant.

Results

Relationship Between Clinicopathologic Features and 21-Gene RS

After excluding 20 patients with unknown histological grade from the 593 patients, the remaining 573 were stratified by histological grade and 21-gene RS . Among the 389 grade 2 (histological grade II) breast cancers, 247 cases (63.5%) were classified as low RS risk, 115 cases (29.6%) as intermediate risk, and 27 cases (6.9%) as high risk. Age distributions were comparable across groups (P = 0.213). However, ER expression, PR expression, and Ki-67 index differed significantly among the three groups (all P 0.001,). No significant differences were observed among groups regarding surgical approach (P = 0.651) or axillary lymph node management (P = 0.122). The high-risk group had a significantly higher proportion of patients receiving postoperative adjuvant chemotherapy compared with intermediate- and low-risk groups (P < 0.001,), while no significant difference was found in postoperative endocrine therapy (P = 0.489).

Relationship Between PK Combination and 21-Gene RS

Based on PR expression and Ki-67 index, the 389 patients were divided into PK low-risk group (248 cases [63.8%]), PK intermediate-risk group (125 cases [32.1%]), and PK high-risk group (16 cases [4.1%]). Among the 247 RS low-risk patients, 186 (75.3%) were correctly classified as PK low-risk, 60 were assigned to PK intermediate-risk, and 1 was overestimated as PK high-risk. Among the

27 RS high-risk patients, 10 (37.0%) were classified as PK high-risk, 16 were assigned to PK intermediate-risk, and 1 was underestimated as PK low-risk .

Accuracy of PK Combination in Predicting RS Groups

We compared sensitivity, positive predictive value (PPV), and discordance rate (RS low-risk misclassified as PK high-risk or RS high-risk misclassified as PK low-risk), using high sensitivity, high PPV, and low discordance as selection criteria. Using PK combination 2 (with PR cutoff increased from 10% to 20%) as a comparative example , PK combination 1 showed significantly higher sensitivity for predicting RS low-risk group (75.3% vs. 55.8%), with similar PPV and discordance rates. For RS high-risk prediction, PK combination 1 demonstrated higher PPV (62.5% vs. 35.7%) and lower discordance (6.3% vs. 17.9%) compared with PK combination 2, despite similar sensitivity. Based on these results, PK combination 1 was selected as the recommended model.

PK combination 1 (recommended): Low-risk defined as PR 10% and Ki-67 20%; high-risk defined as PR $<$ 10% and Ki-67 $>$ 20%.
PK combination 2: Low-risk defined as PR 20% and Ki-67 20%; high-risk defined as PR $<$ 20% and Ki-67 $>$ 20%.

Relationship Between PK/RS Stratification and Recurrence/Metastasis

At the end of follow-up, 14 locoregional recurrence events had occurred (7 chest wall recurrences, 1 ipsilateral breast recurrence, and 6 ipsilateral axillary or supraclavicular lymph node metastases), including 5, 7, and 2 cases in RS low-, intermediate-, and high-risk groups, respectively, and 6, 6, and 2 cases in PK low-, intermediate-, and high-risk groups, respectively. Six distant metastasis events were documented (4 bone metastases, 1 lung metastasis, and 1 case of mediastinal/abdominal lymph node metastasis with brain metastasis), with 3, 3, and 0 cases in RS low-, intermediate-, and high-risk groups, respectively, showing identical distribution in PK groups. Overall event rates for RS low-, intermediate-, and high-risk groups were 8 (3.2%), 10 (8.7%), and 2 (7.4%), respectively, with significantly higher rates in intermediate- and high-risk groups compared with low-risk. PK low-, intermediate-, and high-risk groups showed overall event rates of 9 (3.6%), 9 (7.2%), and 2 (12.5%), respectively, demonstrating comparability with RS groups and a clear trend of increasing event rates with higher PK risk category .

Discussion

With advances in genetic testing technology, prognostic assessment and treatment selection for HR-positive, HER-2-negative early breast cancer no longer rely solely on clinical-pathological staging. Since the 2011 NCCN breast cancer guidelines recommended 21-gene testing in this patient population [1], numerous domestic and international studies have confirmed the value of the 21-gene RS in guiding adjuvant chemotherapy decisions and prognosis [2-7]. However,

given China' s large population, high disease incidence, and the limited availability and high cost of genetic testing, widespread implementation of 21-gene testing in Chinese medical institutions will require time. Our research group previously demonstrated that histological grade, ER/PR expression, and Ki-67 index are the primary immunohistochemical parameters influencing 21-gene RS [2]. In settings where RS is unavailable, estimating RS based on existing immunohistochemical results represents a worthwhile research direction.

Analysis of the 573 patients with known histological grade revealed that among 136 well-differentiated tumors, 86% were low RS risk and only 0.7% were high risk. In contrast, poorly differentiated tumors (48 cases, 8.4% of 573) showed approximately equal distribution across low-, intermediate-, and high-risk groups. Thus, most well-differentiated tumors represent low recurrence risk, while poorly differentiated tumors, often associated with rapid proliferation and poor prognosis, are less frequently referred for 21-gene testing before chemotherapy decisions. Within grade 2 tumors, ER/PR expression and Ki-67 index become the predominant factors influencing 21-gene RS.

Among our 389 grade 2 breast cancers, only 1 case lacked ER expression, while 29 lacked PR expression and 41 showed PR $< 10\%$. The high-risk group exhibited PR negativity and low PR expression rates of 26% and 37%, respectively, significantly higher than intermediate- and low-risk groups. Studies have shown that ER-positive, PR-negative breast cancer represents an aggressive subtype with tamoxifen resistance and relatively poor prognosis [8]. Prat et al. proposed using 20% PR expression as a cutoff to distinguish luminal A-like from luminal B-like breast cancer [9], a criterion adopted by the 2013 St. Gallen consensus [10]. Kurozumi et al. [11] found that PR expression level was an independent prognostic factor in ER-positive, HER-2-negative breast cancer, particularly significant for patients with Ki-67 index between 10% and 30%, where PR $< 20\%$ carried substantial prognostic weight. In our study, the proportion of patients with PR $< 20\%$ increased progressively across RS low-, intermediate-, and high-risk groups (8.1% [20/247], 20.9% [24/115], and 40.7% [11/27]), consistent with these findings. However, whether 20% represents the optimal PR cutoff requires validation. We tested cutoffs of 0%, 10%, and 20%, finding that lower PR cutoffs yielded higher sensitivity for low-risk prediction but lower sensitivity for high-risk prediction. As the cutoff decreased, PPV for RS low-risk group remained stable with slight decline, while PPV for RS high-risk group increased substantially (detailed data not shown). Therefore, PR 10% was selected as the low-risk criterion.

Ki-67, a cell proliferation-associated antigen reflecting cell cycle activity, is considered an ideal marker for assessing proliferative activity [12]. Studies have demonstrated Ki-67 as a predictive and prognostic factor for chemotherapy in HR-positive, HER-2-negative early breast cancer [13,14]. The 2017 St. Gallen consensus [15] continued to recognize Ki-67 as a reference factor for distinguishing luminal A-like from luminal B-like disease, though without specifying a definitive cutoff. Given variability in Ki-67 testing methods (manual vs. auto-

mated counting), tumor heterogeneity, and resulting inter- and intra-observer reproducibility issues, cutoff determination must incorporate institutional clinical experience, particularly for grade 2 breast cancer where reproducibility appears poorer [16]. In this study, we combined PR and Ki-67 and, based on our previous research, used Ki-67 20% as the cutoff for low proliferation to maximize sensitivity and PPV for RS group prediction while minimizing discordance.

With a median follow-up of 40 months, PK low-, intermediate-, and high-risk groups showed overall event rates of 3.6%, 7.2%, and 12.5%, respectively, comparable to RS group rates of 3.2%, 8.7%, and 7.4%. Given limited follow-up duration and few distant metastasis events, the trend of increasing locoregional recurrence rates with higher PK risk category was more pronounced , providing preliminary validation of the PK combination' s predictive value.

Literature review reveals numerous efforts to estimate RS using immunohistochemical results, from the Magee equation [17] to IHC4 score [18], Breast Cancer Prognostic Score [19], and modified Magee equation [20]. Our recommended PK combination for grade 2 breast cancer is simpler and more practical than these scores and formulas, particularly for institutions without access to 21-gene testing, enabling more accurate risk assessment.

In summary, using tumor immunohistochemical markers to estimate 21-gene RS in HR-positive, lymph node-negative early breast cancer is feasible. Our PR and Ki-67 combination for grade 2 breast cancer provides prognostic value comparable to RS. However, as a single-center retrospective analysis, selection bias is unavoidable, and limited follow-up duration precludes definitive assessment of PK combination' s predictive value for distant metastasis. We recommend that additional researchers apply the PK combination to estimate RS and further refine the model using institutional data.

Conflict of Interest: None

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Note: Figure translations are in progress. See original paper for figures.