Molecular Subtypes and Tumor Response to Neoadjuvant Chemotherapy in Patients with Locally Advanced Breast Cancer

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Key Words

Breast cancer • Molecular subtype • Neoadjuvant chemotherapy • Pathologic complete response

Abstract

Objective: Pathologic complete response (pCR) is the most predictive factor for patients with neoadjuvant chemotherapy and we investigated the rate of pCR according to molecular subtypes defined by immunohistochemical staining.

Methods: Our subjects comprised 257 breast cancer patients who received 3 cycles of anthracycline/taxane-based neoadjuvant chemotherapy. The patients were classified into 4 subtypes: luminal A, luminal B, HER2 and triple negative. We analyzed the pCR rate and treatment outcome according to these subtypes. **Results:** Of a total of 257 patients, the pCR rate of luminal A, luminal B, HER2 and triple negative was 3.9, 5.0, 10.5 and 21.1%, respectively ($p = 0.001$). The 5-year disease-free survival of the pCR group (88.4%) was higher than that of the non-pCR group (65.6%), but it was not significant ($p = 0.228$). Among patients who have residual disease, the 5-year disease-free survival of luminal A, luminal B, HER2 and triple negative was 64.0, 65.7, 75.2 and 66.5%, respectively ($p = 0.243$). Triple negative and HER2 subtypes

are more sensitive to neoadjuvant chemotherapy. **Conclusion:** To increase the pCR rate, type-specific approaches according to subtypes, such as an addition of trastuzumab, increasing the number of cycles or a novel regimen, should be considered.

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Introduction

Neoadjuvant chemotherapy (NCT) has become a primary option for patients with locally advanced breast cancer [1]. The rationale for NCT is to improve surgical options [2, 3] and to gain information on drug response by in-breast assessment. Furthermore, NCT provides the opportunity to discover predictive markers of chemotherapy.

The pathologic complete response (pCR) after NCT is probably most predictive with respect to long-term treatment outcomes [4–6]. The results of early-generation clinical trials revealed that patients with locally advanced breast cancer who achieved a pCR have a good prognosis, while those who had a residual disease showed worse treatment outcomes [4]. Another interesting observation

from NCT trials was that some tumor clinicopathologic characteristics can be used as a predictor of the likelihood of pCR. The prediction of the possibility of pCR before starting NCT is an important research goal in order to maximize the treatment effect and minimize unnecessary toxicity [7].

Breast cancer has recently been shown to be divided into distinct molecular subtypes (luminal A, luminal B, HER2 and triple negative) by gene expression profiling with prognostic significance [8–10]. For practical issue in hospital setting, immunohistochemical surrogate panels of estrogen receptor (ER), progesterone receptor (PR) and HER2 have been proposed to potentially discriminate the subtypes as a substitution of gene expression profiling [11–15]. We hypothesized that the distinct molecular subtype defined by ER, PR and HER2 might have a different response to NCT. To investigate the relationship between the provability of pCR and molecular subtypes, we designed this study.

Patients and Methods

Study Population

The patient cohort of the present study was histologically confirmed with primary breast cancer and received neoadjuvant adriamycin (50 mg/m², day 1) plus docetaxel (75 mg/m², day 1) chemotherapy (AT) every 3 weeks for 3 cycles from January 2004 to December 2008 at Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. After NCT, all patients underwent definitive surgery including an axillary lymph node dissection followed by a subsequent 3 cycles of adjuvant AT chemotherapy and radiation therapy. All but 1 patient with luminal subtypes also received adjuvant endocrine therapy followed by 3 cycles of adjuvant AT chemotherapy. Because trastuzumab for the HER2-positive breast cancer was not covered by health insurance until 2009, herceptin was not utilized.

262 consecutive locally advanced breast cancer patients met the above criteria. The surgical specimens were entirely submitted for routine pathologic evaluation. Among them, 257 (98.1%) cases were available for all information on ER, PR and HER2 status. Five (1.9%) cases were excluded primarily because of incomplete information on ER, PR and HER2 status for classification of molecular subtypes.

We retrospectively reviewed the clinicopathological data including age, clinical T stage, clinical N stage, histologic type, pCR rate to NCT, and follow-up data. All data were extracted from the Severance Hospital Breast Cancer Registry which is a prospectively maintained database that includes clinical information, pathologic information, treatment modality and details of outcome.

Classification of Molecular Subtypes

We used an immunohistochemistry and/or fluorescence in situ hybridization (FISH) assay for 3 surrogate markers of ER, PR and HER2. Immunohistochemistry was performed for ER (SP1, Thermo Scientific, Fremont, Calif., USA), PR (PgR636, Dako,

Table 1. Classification and distribution of breast cancer subtype by ER, PR and HER2 status

Subtype	ER and/or PR	HER2	n	%
Luminal A	positive	negative	103	40.1
Luminal B	positive	positive	40	15.6
HER2	negative	positive	38	14.8
Triple negative	negative	negative	76	29.6
Total			257	100

Glostrup, Denmark) and HER2 (Dako) as a routine clinical diagnostic procedure. The cutoff values for ER and PR were 10% positive cells, irrespective of intensity. HER2 staining was scored according to the American Society of Clinical Oncology/College of American Pathologists guideline [16]. FISH analysis (Vysis Pathvision c-erbB2 probe and Dako FISH histology accessory kit) was performed manually, and the evaluation of signals was carried out according to the Vysis manual.

The study population was grouped into 4 subtypes: luminal A (ER and/or PR positive, HER2 negative), luminal B (ER and/or PR positive, HER2 positive), HER2 (ER and PR negative, HER2 positive) and triple negative (ER negative, PR negative and HER2 negative).

Study Endpoint and Statistics

The primary endpoint of this study was the pCR rate according to molecular subtypes. The pCR was defined as disappearance of all invasive cancer in the breast and axilla after NCT [17]. Patients with residual ductal carcinoma in situ were also considered as pCR [18]. The pCR rates were calculated for each molecular subtype and the Fisher exact test was used to evaluate the relationship between subtypes and the pCR rate.

The secondary endpoint was disease-free survival (DFS) among patients who had a residual disease. First, we compared the 5-year DFS rate of the pCR and non-pCR groups. Second, we compared the difference of the 5-year DFS among patients with residual disease. DFS was calculated as the time from the date of surgery to the date of the development of local, regional and distant metastases, and the date of death before recurrence. DFS estimation was performed according to the Kaplan-Meier method, and the comparison of the groups was based on a log-rank test. All reported p values are 2-sided, and p values <0.05 were considered significant.

Results

Patient Characteristics and Distribution of Molecular Subtypes

Patient characteristics and distribution of molecular subtypes are summarized in tables 1 and 2. The luminal A, luminal B, HER2 and triple negative subtype accounted for 103 (40.1%), 40 (15.6%), 38 (14.8%) and 76 cases

Table 2. Patient characteristics

		Luminal A	Luminal B	HER2	Triple negative	p
Age (n = 257)	≤50 years	73 (70.9)	28 (70.0)	16 (42.1)	49 (64.5)	0.014
	>50 years	30 (29.1)	12 (30.0)	22 (57.9)	27 (35.5)	
Clinical T (n = 248)	1	15 (15.3)	5 (12.5)	3 (8.1)	11 (15.1)	>0.05
	2	69 (70.4)	32 (80.0)	31 (83.8)	47 (64.4)	
	>3	14 (14.3)	3 (7.5)	3 (8.1)	15 (20.5)	
Clinical N (n = 257)	negative	3 (2.9)	1 (2.5)	3 (7.9)	7 (9.2)	>0.05
	positive	100 (97.1)	39 (97.5)	35 (92.1)	69 (90.8)	
Histologic type (n = 252)	ductal	94 (94.0)	35 (87.5)	33 (89.2)	67 (89.3)	>0.05
	lobular	3 (3.0)	0 (0.0)	1 (2.7)	0 (0.0)	
	other	3 (3.0)	5 (12.5)	3 (8.1)	8 (10.7)	
Type of surgery (n = 257)	BCT	10 (9.7)	7 (17.5)	3 (7.9)	19 (25.0)	0.02
	mastectomy	93 (90.3)	33 (82.5)	35 (92.1)	57 (75.0)	

Figures in parentheses are percentages. BCT = Breast conservation therapy.

Table 3. pCR rate according to clinicopathologic characteristics

Subtype	Total	No pCR	pCR	p
Luminal A	103	99 (96.1)	4 (3.9)	0.001
Luminal B	40	38 (95.0)	2 (5.0)	
HER2	38	34 (89.5)	4 (10.5)	
Triple negative	76	60 (78.9)	16 (21.1)	
Total	257	231 (89.9)	26 (10.1)	

Figures in parentheses are percentages.

(29.6%), respectively (table 1). The 4 molecular subtypes did not differ significantly by clinical T stage, clinical axillary node status and histologic type (all $p > 0.05$), while patients with a HER2 type were older than other subtypes ($p = 0.014$) (table 2).

pCR Rate according to Breast Cancer Subtypes

Of the 257 patients tested, 26 patients achieved pCR; thus, the pCR rate was 10.1% (table 3). The rates of pCR differed significantly among the 4 molecular subtypes with 3.9% (4/103) for luminal A, 5.0% (2/40) for luminal B, 10.5% (4/38) for HER2 and 21.1% (16/76) for triple negative ($p = 0.001$) (table 3). The greatest difference of the pCR rate was observed between the triple negative and luminal A/B subtype. Breast conservation therapy was performed more frequently in patients with the triple

negative than in those with other subtypes ($p = 0.02$) (table 2).

We also tested the association of the pCR rate and other clinicopathological variables such as age (≤ 50 vs. > 50 years), clinical T stage (T1 vs. T2 vs. $> T3$), clinical N stage (negative vs. positive) and histologic type (ductal vs. luminal vs. other). There were no clinicopathological variables that were significantly correlated with pCR (data not shown).

Survival Analysis

The mean follow-up time for the present study cohort was 23.2 months (median 21.3), and 41 patients (16.0%) relapsed during follow-up. The Kaplan-Meier survival estimation showed that the 5-year DFS of patients who achieved pCR was better than of those who had a residual invasive disease (88.4 vs. 65.6%; absolute difference 22.8%; $p = 0.228$), but the difference did not reach statistical significance, probably due to the small study population and the short follow-up period (fig. 1).

To compare the DFS between subtypes within the patients who had residual disease, we performed a survival analysis after excluding the patients who achieved pCR. The estimated 5-year DFS of luminal A, luminal B, HER2 and the triple negative subtype was 64.0, 65.7, 75.2 and 66.5%, respectively ($p = 0.243$) (fig. 2). These results suggest that the DFS of patients who have a residual disease is poor regardless of subtypes.

Fig. 1. Kaplan-Meier 5-year DFS estimation in patients with pCR and in those who had residual invasive disease.

Fig. 2. Kaplan-Meier 5-year DFS estimation according to breast cancer subtype among patients who had residual invasive disease.

Discussion

The results of the clinical trials revealed that pCR is related to good treatment outcomes and can be used as a surrogate marker of better survival [4–6]. Patients with a low chance of pCR might be spared unnecessary toxicity and allowed a chance of alternative treatment [7].

Gene expression studies have identified distinct molecular subtypes of breast cancer with a different prognosis [8–10]. It is believed that various subtypes of breast cancer not only have different prognoses but also show different sensitivities to systemic chemotherapy. Rouzier et al. [19] evaluated the gene expression profiles of patients treated with NCT and reported that the pCR rate was 45% for the basal-like and HER2-positive subtypes and 6% for the luminal tumors.

Although the original method to define the breast cancer molecular subtype is microarrays for gene expression analysis, subsequent work has shown that an immunohistochemical panel composed of 3–5 markers could serve as a proxy for breast cancer subtyping [12, 13, 15, 20], and there is increasing evidence that distinct subtypes defined by immunohistochemical markers differ in their response to chemotherapy. Recent reports using these markers revealed significantly higher pCR to NCT among the triple negative and HER2-positive/ER-negative subtypes compared with the luminal subtype [15, 20].

In the present study, we used similar molecular classifications that were previously reported [15, 20]. The pri-

mary subdivision of molecular subtypes was by the ER status. The ER-positive tumors were subdivided into luminal A and luminal B according to the HER2 status. The ER-negative tumors were subdivided into the HER2 and triple negative subtypes. The pCR rate of the present study was slightly lower compared with other adriamycin-taxane-based neoadjuvant trials [21, 22]. This difference is likely explained by split NCT with only 3 cycles before surgery. Nevertheless, we have also found that patients with the triple negative and HER2 subtype of breast cancer have a higher rate of pCR to NCT than the luminal subtype. As a consequence, breast conservation surgery was performed more often in patients with the triple negative subtype ($p = 0.02$) (table 2).

There are multiple potential reasons that the response to chemotherapy differed by subtype. The triple negative and HER2-positive/ER-negative breast cancers are characterized by the high expression of the proliferation cluster of genes [8, 9]. A prognostic index that is heavily influenced by proliferation genes was recently shown to predict pCR to doxorubicin/docetaxel primary chemotherapy [23]. Furthermore, the triple negative tumor is basically ER/PR negative, and evidence suggests that the negative hormone receptor status is one of the strongest predictive markers associated with the higher likelihood of pCR to NCT [24–26].

In the present study, we confirmed previous observations that patients who had a pCR to NCT had a good prognosis [27, 28]. Although the DFS of patients with pCR

and non-pCR was not significantly different because of the short follow-up period, patients who achieved pCR had a better 5-year DFS with 22.8% of absolute difference. We also tested the survival difference between patients with residual invasive disease according to molecular subtypes. Carey et al. [15] reported that triple negative tumors tend to have a worse prognosis compared with those with ER-positive tumors among patients with residual disease. However, our data showed that the DFS of patients who have a residual disease is poor across all molecular subtypes. If pCR is not achieved, the luminal subtype is also more likely to relapse. This result suggests that failure to respond to NCT is indicative of a particularly poor outcome regardless of subtype.

To increase the pCR rate, it is reasonable to use different strategies according to different molecular subtypes. As shown in recent data of a 43–65% pCR rate by trastuzumab-containing NCT [29–31], incorporation of trastuzumab with neoadjuvant chemotherapeutics is a promising alternative for the HER2 subtype. These trials demonstrated significant improvement of DFS in patients who received neoadjuvant trastuzumab and suggest that the addition of trastuzumab to NCT should be considered for women with a HER2-positive tumor [29–31]. In the present study, HER2-positive tumors consisted of the luminal B (ER/PR positive and HER2 positive) and HER2 (ER/PR negative and HER2 positive) subtypes. Although influence of the ER status on responsiveness to neoadjuvant trastuzumab is unclear [27], adding trastuzumab to the neoadjuvant regimen can be considered in patients with both an ER- and HER2-positive tumor.

For the triple negative subtype, we still lack a target for this particularly poor prognosis group. Therefore, to maximize the pCR, extension of neoadjuvant cycles and new agents should be considered. Recent reports demonstrated that tumors from women with BRCA1 mutations were highly responsive to neoadjuvant cisplatin use [32, 33]. The triple negative subtype shares many histopathologic characteristics with BRCA-associated tumors, and a preclinical study demonstrated that a BRCA mutation carrying a tumor defective in DNA double-strand break repair was sensitive to cisplatin [34, 35]. Beside a new chemotherapeutic, a clinical trial using a novel regimen based on the synthetic lethality strategy such as poly (ADP-ribose) polymerase inhibitors is warranted [36, 37].

It is even less likely that patients with luminal A will achieve a pCR. Clinicians often wonder whether additional NCT should be given to increase pCR. However, trials have shown that a tumor which was resistant to initial NCT had consistent resistance to extended cycles or

a changed regimen with non-cross-resistant chemotherapeutics [38, 39]. In addition, a delay in surgery and hormonal therapy resulting from non-effective NCT is problematic. We believe that if the intent is to achieve pCR, the luminal A subtype should not be considered for NCT, and offering initial treatment with surgery is optimal. If preoperative treatment is chosen to change local treatment options, the concurrent chemoendocrine regimen can be considered. Within the context of a clinical trial, a variety of approaches could be tested, including the use of a combination of new chemotherapeutics/endocrine agent and target agents such as angiogenesis inhibitors.

There are several limitations of this study. This is a retrospective study, and unrecognized biases might have influenced our results. Although our data suggested a strong association of pCR with breast cancer subtypes, the molecular subtype may not be independently associated with pCR because of the high correlation between the molecular subtype and the ER and HER2 status. In addition, the tumor grades and molecular subtypes are closely interlinked [27]. Because of this association, molecular subtypes might not replace the conventional clinicopathologic predictors such as ER, HER2 or tumor histologic grade with improving the prediction accuracy. To solve this issue, we need a large patient cohort that makes it possible to perform multivariate analysis with sufficient statistical power.

There is increasing evidence that ER-positive tumors consist of 2 biologically different subtypes and the proliferation index is the strongest parameter to distinguish luminal B from luminal A subtypes [11, 19, 20, 40]. Recent studies suggest that luminal A and B breast cancers appear to be distinguished by application of Ki-67 expression [11, 40], and Ki-67 expression identifies a subset of patients who could be sensitive to taxane-based treatment in an adjuvant setting [11, 41]. In this retrospective study, we tried to separate 2 different luminal subtypes by Ki-67 expression with a cutoff point of 13.25% established by Cheang et al. [40], but only 27 tumors of a total of 143 luminal subtypes were able to evaluate Ki-67 expression. We separated luminal B tumors from luminal A by their expression of HER2 without incorporation of Ki-67 expression.

In conclusion, we reported additional evidence that the triple negative and HER2 subtypes of breast cancer are more sensitive to adriamycin/taxane-based NCT than the luminal subtype. Those patients who had a residual invasive disease showed a worse prognosis regardless of subtypes. To increase the pCR rate, it is reasonable to consider new approaches according to each subtype. The addition of trastuzumab for the HER2 or luminal B

subtypes is a promising alternative strategy. For the triple negative subtype, extension of neoadjuvant cycles and neoadjuvant trial with a new chemotherapeutic such as cisplatin or other novel regimens should be considered. For the luminal A subtype, the conventional approach of early surgical intervention followed by adjuvant chemoendocrine therapy is optimal. To improve the surgical option, a neoadjuvant trial with concurrent chemoendocrine therapy can be considered.

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