Reproductive Factors, Steroid Receptor Status, and Tumour Markers of HER2-Positive Breast Cancer in Northern China

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Key Words

Invasive ductal breast cancer · HER2 · Reproductive factors · Steroid receptors · Tumour markers

Summary

Background: Although China has a breast cancer incidence that surpasses all other cancer registries, there have been few reports to evaluate the relationships of reproductive history, steroid receptor status, and tumour markers with HER2 status of breast cancer. **Patients and Methods:** This project included 274 primary invasive ductal breast cancer patients. Information concerning the reproductive factors and tumour characteristics of the patients had previously been collected. HER2 and steroid receptor status were detected in tumour tissues. Serum CEA, CA15-3, and CA125 levels were analyzed for all patients. **Results:** Younger age at menarche was observed in patients with HER2-positive than in those with HER2-negative status ($p = 0.03$). Statistically significant differences were found between the HER2-positive group and HER2-negative group for estrogen receptor (ER) and progesterone receptor (PR) status ($p < 0.01$). Age of onset, other reproductive factors, tumour characteristics, and serum tumour marker level were not significantly different between those patients with HER2-positive and those with HER2-negative status. **Conclusion:** We confirm that age at menarche may plausibly be differentially correlated with the risk of HER2-positive invasive ductal breast cancer because it is presumed to impact exposure to endogenous sex hormones. HER2 is inversely related to ER and PR in invasive ductal breast cancer patients of Northern China.

Schlüsselwörter

Invasiv duktales Brustkrebs · HER2 · Reproduktive Faktoren · Steroidrezeptoren · Tumormarker

Zusammenfassung

Hintergrund: Obwohl China eine Brustkrebsrate aufweist, die sämtliche anderen Krebsregister übersteigt, gab es bislang nur wenige Arbeiten, die die Beziehung zwischen vorangegangenen Schwangerschaften, Steroidrezeptor-Status und Tumormarkern mit dem HER2-Status des Brustkrebses evaluieren. **Patienten und Methoden:** Dieses Projekt umfasste 274 Patienten mit primär invasivem duktalem Brustkrebs. Informationen zu den Reproduktionsfaktoren und den Tumorcharakteristika der Patienten wurden im Vorfeld gesammelt. Der HER2- und der Steroidrezeptor-Status wurde im Tumorgewebe erfasst. Bei allen Patienten wurden die Werte von CEA, CA15-3 und CA125 im Serum analysiert. **Ergebnisse:** Ein jüngeres Alter bei der Menarche wurde bei Patienten mit HER2-positivem im Vergleich zu Patienten mit HER2-negativem Status festgestellt ($p = 0.03$). Statistisch signifikante Differenzen wurden zwischen der HER2-positiven und der HER2-negativen Gruppe für den Östrogenrezeptoren(ÖR)- und den Progesteronrezeptoren (PR)-Status festgestellt ($p < 0.01$). Alter beim Krankheitsausbruch, weitere reproduktive Faktoren, Tumorcharakteristika und der Wert der Tumormarker im Serum unterschieden sich nicht signifikant zwischen den Patienten mit HER2-positivem und denen mit HER2-negativem Status. **Schlussfolgerung:** Wir bestätigen, dass das Alter der Menarche glaubhaft differenziell mit dem Risiko des HER2-positiven invasiven duktales Brustkrebses korrelieren mag, da angenommen wird, dass es die Einwirkung von endogenen Sexualhormonen beeinflusst. HER2 steht invers mit ÖR und PR bei nordchinesischen Patienten mit invasivem duktalem Brustkrebs in Beziehung.

Introduction

Although numerous studies have evaluated the relationship between reproductive factors and breast cancer risk [1, 2], little is known about how reproductive factors exactly affect the risk of breast cancers. To date, the majority of risk factors for breast cancer are related to reproductive hormones. Epidemiologic data, animal models, and in vitro studies have shown that reproductive hormones, particularly estrogen, play a critical role in breast cancer etiology via steroid receptors, mainly estrogen receptors (ER) and progesterone receptors (PR) [3].

As clinical and molecular biology findings have led to the identification of tumour overexpression of the human epidermal growth factor receptor 2 (HER2) as a distinct subset of breast cancer, HER2 has generated numerous studies on its predictive value for survival, on the response to adjuvant therapy in breast cancer, and on the suggestion that overexpression of HER2 has been associated with a more aggressive phenotype with decreased survival [4, 5]. For breast cancer, some serum tumour markers also play important roles in determining prognosis, predicting response to specific therapies, and the early detection of recurrence after curative surgery in patients [6, 7]. Therefore, it is possible that some tumour markers correlate to HER2 amplification for breast cancer.

As a country with rapid social and economic development over the past 3 decades, China has a breast cancer incidence that surpasses all other cancer registries [8]. However, to date there have been few reports to evaluate the relationships of reproductive history, ER and PR status, and tumour markers with HER2 status of breast cancer in northern China. We hypothesised that these factors would be associated with HER2 status in breast cancer in this region. To study these hypotheses, we observed age at menarche, first full-term pregnancy, menopause, breast feeding, tumour characteristics, ER and PR expression, and serum CEA, CA15-3, and CA125 levels in HER2-positive invasive ductal breast cancer patients registered in our institution, which is located in a typical city in Northern China.

Patients and Methods

Patient Selection

Archival primary invasive ductal breast cancer patients which followed primary surgical treatments were identified retrospectively within a 5-year period (June 2004–June 2009) in our institution. The following selection criteria were known: female; primary invasive breast cancer with no adjuvant or neoadjuvant chemotherapy and Trastuzumab prescribed; and sufficient tumour material for assays of biological factors. Thus, data from 274 patients were available for analysis.

For all patients, information concerning the reproductive factors had previously been collected, including age at menarche, first live birth and menopause, and breast feeding. Data describing clinical characteristics of the patients (including tumour size, lymph node status, and TNM classification) were obtained from our institution.

HER2 Gene Amplification and HER2 Gene Expression Detection in Tumour Tissues

Tissue blocks were initially fixated in 10% neutral buffered formalin within 4–8 h for 6–48 h (minimum 6 h). HER2 gene amplification was determined by fluorescent in situ hybridisation (FISH) according to the manufacturer's instruction of the kit (GP Medical Technologies, Ltd, Beijing, China) [9]. The GP HER2 FISH kit contains a HER2 probe (Spectrum Orange) and a chromosome enumeration probe (CEP17; Spectrum Green) for the centromeric region of chromosome 17. Briefly, unstained, formalin-fixed, paraffin-embedded tumour sections were deparaffinised, pre-treated, and denatured with the hybridisation probe before incubation overnight. After hybridisation, the slides were washed and counterstained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI). The sections were evaluated for HER2 gene copy number using a fluorescence microscope at a magnification of $\times 1,000$ with a triple bandpass filter for simultaneous detection of Spectrum Orange, Spectrum Green, and DAPI. In each slide, at least 30 non-overlapping tumour cells within an area of invasive carcinoma were scored for both HER2 and CEP17. FISH results were evaluated according to ASCO/CAP guidelines [10], and HER2 gene amplification was defined as positive with HER2/CEP17 ratios >2.2 , and negative with ratios <1.8 . For these cases with HER2/CEP17 ratios from 1.8 to 2.2, a second review was undertaken by counting 60 tumour cells. A breast tumour with previously identified HER2 amplification and overexpression was used as a positive control for HER2 FISH. Infiltrating lymphocytes and normal breast epitheliums accompanying the tumour cells served as internal normal controls for probe hybridisation. Scoring was performed by 2 cytogenetic technologists with at least 1 year experience in clinical testing.

Expression of HER2 in tumour tissues was determined by immunohistochemistry (IHC), using HercepTest kits with a prediluted antibody (Dako, Carpinteria, CA, USA). Tumour tissues were evaluated semi-quantitatively according to a standard, written protocol (HercepTest) [11]. All patients with amplified HER2 genes and a 3+ result of HER2 IHC in tumours where FISH analysis could not be evaluated were considered HER2-positive.

ER and PR Immunohistochemistry in Tumour Tissues

After common preparation, ER and PR in tumour tissues were detected according to the manufacturer's instruction (Dako, Carpinteria, CA, USA). Positive controls of known positive breast tissues and negative controls with primary antibody replaced with TBS were run with the patient/study slides. Tumours with more than 10% positively stained nuclei were considered positive.

Serum Collection and CEA, CA15-3, and CA125 Measurement

Blood samples were obtained from patients before operation. Serum CEA, CA15-3, and CA125 levels were measured by electrochemiluminescence (Elecsys 2010; Roche Diagnostics, Indianapolis, IN, USA).

Statistical Analyses

The statistical calculations were performed using SPSS version 13.0 (SPSS, Chicago, IL, USA). Comparisons of age of onset, reproductive factors (age at menarche, first full-term pregnancy, and menopause), tumour size, and tumour marker levels between the 2 groups (HER2-positive vs. HER2-negative) were made by Mann-Whitney U test. Differences in breast feeding, tumour characteristics (grade and lymph node status), and ER and PR status between the 2 groups were compared using Chi-square test. All p-values corresponded to two-sided tests, and values less than 0.05 were considered significant.

Results

HER2 status was determined in 274 patients from FISH data available in 185 patients and IHC data in 274 patients. There

Table 1. Relationships between reproductive factors and HER2 status in breast cancer patients

Variables	HER2-positive	HER2-negative	p-value
Age of onset, years	52.1 ± 11.3	51.6 ± 11.8	0.42
Age at menarche, years	14.2 ± 1.5	15.9 ± 1.2	0.03
Age at first live birth, years	25.2 ± 1.6	24.2 ± 1.9	0.38
Age at menopause, years	51.8 ± 3.0	51.5 ± 1.6	0.96
Ever breast feeding, % (n)	85.7 (66/77)	87.8 (173/197)	0.64

Table 2. Relationships between tumour characteristics and HER2 status in breast cancer patients

Variables	HER2-positive	HER2-negative	p-value
Tumour size, mm	23.3 ± 12.5	25.1 ± 13.6	0.78
TNM classification, % (n)			0.84
I	24.6 (19/77)	24.4 (48/197)	
II	53.2 (41/77)	56.9 (112/197)	
III	22.1 (17/77)	18.8 (37/197)	
Lymph node status positive, % (n)	46.8 (36/77)	50.2 (99/197)	0.60

Table 3. Relationships between hormone receptor status and HER2 status in breast cancer patients

Steroid receptor status	HER2-positive	HER2-negative	p-value
ER-positive, % (n)	41.6 (32/77)	73.6 (145/197)	<0.01
PR-positive, % (n)	49.4 (38/77)	68.5 (135/197)	0.02

Table 4. Relationships between serum tumour markers level and HER2 status in breast cancer patients

Tumour marker	HER2-positive	HER2-negative	p-value
CEA, ng/ml	2.2 ± 1.6	1.9 ± 2.1	0.12
CA15-3, U/ml	12.4 ± 7.6	13.3 ± 7.2	0.49
CA125, U/ml	12.1 ± 5.4	13.2 ± 13.4	0.54

was a strong correlation between HER2 3+ by IHC and HER2 gene amplification by FISH. Of the 274 analysed patients, 77 patients (28.1%) were defined as HER2-positive by the combination of gene amplification and/or HER2 3+.

A younger age at menarche was observed in patients with HER2-positive tumours than in those patients with HER2-negative tumours ($p = 0.03$), but not age of onset, age at first live birth, and age at menopause ($p > 0.05$). A detailed description of the data is given in table 1. The relationships between HER2 and tumour characteristics are presented in table 2. There were no effects by HER2 status in breast feeding, tumour size, grade, or pathological nodal status.

When analysing the association between HER2 status and steroid receptor status, we found statistically significant differences between the HER2-positive group and the HER2-negative group for ER and PR status ($p < 0.01$). HER2-negative tumours were more likely to be ER-positive than HER2-positive tumours (73.6 vs. 41.6%). Similarly, HER2-negative tumours were more likely to be PR-positive than HER2-positive tumours (68.5 vs. 49.4%) (table 3). However, analysing

the relationship between the serum tumour marker level and HER2 status, we found no statistically significant differences between the HER2-positive group and the HER2-negative group for tumour markers, including CEA, CA15-3, and CA125 (table 4).

Discussion

HER2 status by validated methods using HercepTest and FISH at centralised laboratories is today included in the routine analysis of breast cancer and used as a prognostic marker [12]. In the present study, we analysed HER2 status by FISH and IHC in a cohort of invasive ductal breast cancer patients in our institute which is located in a city in Northern China. 28.1% of primary invasive breast cancer patients were defined as HER2-positive. Similar reports have been made in the literature [13, 14] that either amplification of the HER2 proto-oncogene or overexpression of HER2 has been observed in 25–30% of primary breast cancers.

It is well known that the reproductive factors, including age at menarche, age at first full-term pregnancy, and number of live births, are associated with breast cancer risk [1, 2]. Certain reproductive factors may have a greater impact on the risk of HER2-positive subtypes of breast cancer compared with others. In our study, we defined that age at menarche is younger in HER2-positive invasive ductal breast cancer patients than in HER2-negative patients. Age at menarche is a marker of puberty, a time during which the breast is undergoing rapid

development, and our study suggests that younger age is more strongly associated with risk of HER2-positive than with risk of HER2-negative invasive ductal breast cancer. Although the result may not have direct clinical implications, it indirectly supports the notion of an important role of hormonal factors regarding the down-regulation of HER2 in the evolution of invasive ductal breast cancer.

A previous report [15] had suggested a direct interaction between ER and HER2 signalling pathways. We observed a strong correlation between HER2 positivity and steroid receptor negativity, as generally described in the literature with a variety of techniques [16, 17], that HER2-negative tumours were more likely to be ER-positive or PR-positive than HER-positive tumours of invasive ductal breast cancer.

The significant prognostic importance of the levels of tumour markers CEA, CA15-3, and CA125 for prognosis in breast cancer of pre-menopausal women was proven [18, 19], but little research involved the relationship between serum CEA, CA15-3, and CA125 levels and HER2 status in breast cancer. Data of our study indicate that increased concentrations of these 3 tumour markers, including CEA, CA15-3, and CA125, did not relate to HER2-positive status in invasive ductal breast cancer.

In conclusion, we confirm that age at menarche may plausibly be differentially correlated with the risk of HER2-positive invasive ductal breast cancer because it is presumed to impact exposure to endogenous sex hormones. For invasive ductal breast cancer patients, HER2 status was not correlated to tumour size, grade, or pathological nodal status. HER2 is inversely related to steroid receptors (ER and PR) in invasive ductal breast cancer patients of Northern China, similarly to other regions, whereas no significant correlation exists between tumour markers (CEA, CA15-3, and CA125) and HER2 status in invasive ductal breast cancer patients.

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Conflict of Interest

There are no conflicts of interest in the study.

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