Resumption or persistence of menstruation after cytotoxic chemotherapy is a prognostic factor for poor disease-free survival in premenopausal patients with early breast cancer

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Background: We investigated the relationship between resumption or persistence of menstruation after cytotoxic chemotherapy (RM) and disease-free survival (DFS) in premenopausal patients with early breast cancer.

Methods: Medical records from 872 patients who received cytotoxic chemotherapy for stage I to III breast cancer were retrospectively reviewed.

Results: The median patient age was 41 years (range, 21–54) and the median follow-up duration was 6.2 years (range, 0.7–10.4). Six hundred ninety-two patients (79.4%) were hormone receptor (HR) positive and the majority of these received tamoxifen therapy after completing chemotherapy. The chemotherapy-induced amenorrhea (CIA) rate was 76.7% (n = 669), and 51.8% (n = 452) experienced RM during the follow-up period. One hundred twenty-one (13.9%) patients had persistent menstruation without CIA. DFS was significantly affected by younger age at diagnosis (≤35 years) (P = 0.013), tumor size > 2 cm (P < 0.001), node positivity (P < 0.001), HR negativity (P < 0.001), HER2 positivity (P = 0.010), and RM (P < 0.001). HR negativity [hazard ratio 1.7, 95% confidence interval (CI) 1.2–2.4, P = 0.006], tumor size > 2 cm (hazard ratio 2.1, 95% CI 1.4–3.0, P < 0.001), node positivity (hazard ratio 3.0, 95% CI 2.0–4.7, P < 0.001), and RM (hazard ratio 1.8, 95% CI 1.2–2.7, P = 0.004) remained significant factors for DFS on multivariate analysis.

Conclusions: A considerable proportion of premenopausal patients treated with chemotherapy experienced RM after CIA. RM was a poor prognostic factor for DFS in premenopausal patients with early breast cancer.

Key words: adjuvant chemotherapy, breast cancer, chemotherapy-induced amenorrhea, premenopause

introduction

Globally, approximately one-third of newly diagnosed invasive breast cancers occur in women < 50 years of age [1]. Chemotherapy has been the mainstay of adjuvant therapy for premenopausal women with node-positive breast cancer, even when patients have a hormone receptor (HR)-positive tumor [2, 3]. It has been argued that cytotoxic chemotherapy is beneficial for premenopausal women because it causes premature menopause. Chemotherapy-induced amenorrhea (CIA) caused by treatment-related ovarian failure is a well-characterized adverse event in premenopausal patients with breast cancer. The incidence of CIA differs with respect to patient age, chemotherapeutic regimens used, and tamoxifen use [4–7]. Although CIA has been evaluated as a prognostic factor in relation to disease-free survival (DFS) or overall survival (OS) in some studies, the results have been inconsistent [5, 6, 8–10]. These studies were retrospective and included heterogeneous populations with heterogeneous chemotherapeutic regimens. Most of these prior studies were conducted based on the cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) regimen, without incorporating adjuvant endocrine therapy. In addition, CIA was not consistently defined across the studies and temporary amenorrhea was not differentiated from permanent CIA.

CIA is expected to be beneficial in patients with hormone-sensitive tumors because of the indirect antitumor effect of estrogen deprivation, which is supported by the results of prior studies [8, 11, 12]. Given that CIA is considered a surrogate marker of the effective cytotoxicity of a particular chemotherapy, resumption or persistence of menstruation after cytotoxic chemotherapy (RM) may be a prognostic factor for poor survival in premenopausal breast
patients and methods

study population

Between March 2001 and December 2006, a total of 2523 patients underwent surgery for breast cancer at the National Cancer Center Hospital in Korea. Of all patients, 1245 premenopausal patients were included in this retrospective study (CONSORT diagram). Patients who lacked information on the menstrual history or had a hysterectomy after chemotherapy were excluded from the analysis. We also excluded patients who received GnRH analogue as part of the adjuvant endocrine treatment. Premenopausal status was defined as having regular menstrual cycles within 3 months, regardless of each patient’s age.

The chemotherapy regimens used were as follows: (i) anthracycline-based: doxorubicin 60 plus cyclophosphamide 600 mg/m² on day 1 every 3 weeks for four cycles (AC) and 5-FU 500, doxorubicin 50, and cyclophosphamide 500 mg/m² on day 1 every 3 weeks for six cycles (FAC); (ii) taxane-containing: doxorubicin 50 plus taxotere 75 mg/m² on day 1 every 3 weeks for six cycles (AT) and doxorubicin 60 plus cyclophosphamide 600 mg/m² on day 1 every 3 weeks for four cycles, followed by taxotere 75 mg/m² on day 1 and/or capectabine 2000 mg/m² on day 1 to 14 every 3 weeks for four cycles (AC-T[X]); (iii) cyclophosphamide 600 mg/m² on day 1, methotrexate 40 mg/m² on day 1 and 8, and 5-FU 600 mg/m² on day 1 and 8 every 3 weeks for six cycles (CMF). Patients with HR-positive tumors received tamoxifen upon completion of chemotherapy. Estrogen receptor (ER) or progesteron receptor (PgR) expression status was determined by immunohistochemistry (IHC) and a cut-off value of ≥ 10% of the positively stained nuclei was used to define ER and PgR positivity. The IHC of ER and PgR was carried out using paraffin-embedded tissues and antibody for ER is SP1 (Ventana, Vancouver, Canada) and for PgR, 1E2 (Ventana).

After local and adjuvant treatment (including radiotherapy if needed), patients visited the outpatient clinics every 3–4 months for the first 3 years of follow-up, every 6 months for the next 2 years, and annually thereafter. At each follow-up visit, patients’ menstrual status was recorded in addition to physical examination and other surveillance studies.

CIA was defined as the cessation of menstruation for > 6 consecutive months. Resumption of menstruation was defined as regular cyclic bleeding after CIA for > 3 months without pathologic etiology. In this study, we limited the definition of resumption of menstruation to a clinical phenomenon and did not take into account chemical findings, such as elevated serum estradiol (E2) accompanied by follicle-stimulating hormone levels ≤20 mIU/ml.

results

patient characteristics

We assessed 872 patients out of a total of 1245 premenopausal patients for the study, after excluding patients who did not receive chemotherapy, were missing data, or were lost to follow-up (for CONSORT diagram see Figure 1). The median age at diagnosis was 41 years (range, 21–54) and the median follow-up duration was 6.2 years (range, 0.7–10.4). Six hundred and ninety-two patients (79.4%) were ER positive or PgR positive, and 671 patients received tamoxifen after cytotoxic chemotherapy (Table 1). The number of patients with tumor size ≤2 cm was 392 (45.0%), and 361 patients (41.4%) were node negative. All patients received cytotoxic chemotherapy before or after breast surgery and 434 (49.8%) patients received taxane-containing regimens.

effects of RM on survival

RM was significantly affected by the following factors: age ≤35 years (hazard ratio 10.1, 95% CI 6.5–15.7, \( P < 0.001 \)); tamoxifen use (hazard ratio 0.3, 95% CI 0.2–0.5, \( P < 0.001 \)); statistical methods

The primary end point of this study was the effect of RM on DFS in premenopausal breast cancer patients. DFS was defined as the length of time from the date of diagnosis to any invasive breast cancer relapse or death or was censored at the last follow-up date. OS was defined as the length of time from the date of diagnosis to death from any cause or last follow-up date. DFS and OS were estimated using the Kaplan–Meier method and compared using the log-rank test. Cox’s logistic regression analyses were used to control for various clinical factors and to estimate hazard ratio and 95% confidence interval (CI) for each factor. Survival rates were calculated using the life table method, and 5-year DFS rates were compared using Gehan’s method. Proportions were compared using two-way tables and the chi-square test. Forest plots were used to summarize the results of various subgroup analyses. All \( P \) values were two-tailed, with 5% significance levels. All statistical analyses were carried out using STATA software version 10.0.

Figure 1. Consort diagram.
and body mass index ≥ 23 kg/m² (hazard ratio 0.7, 95% CI 0.5–0.9, P = 0.013). Multivariate analysis showed that lack of tamoxifen use (hazard ratio 0.4, 95% CI 0.3–0.5, P < 0.001) and age ≤ 35 years (hazard ratio 9.5, 95% CI 6.1–14.7, P < 0.001) were the most important factors for persistent menstrual cycles (supplemental Table S1 is available at Annals of Oncology online). In fact, 85% of patients aged ≤ 35 years experienced RM and the rate of menstruation was much higher in the HR-negative patient group (supplemental Figure S1 is available at Annals of Oncology online).

DFS was significantly lower among patients with RM than among those without RM (P < 0.001) (Figure 2A–C). While the 5-year DFS rate was 90.0% (95% CI 86.1% to 93.9%) in patients without RM, it was significantly lower in patients with RM (5-year DFS = 84%, 95% CI 80.1% to 87.9%, P < 0.001). Although there was no evidence of interaction between RM and either HR status or age with respect to DFS (P = 0.941 and P = 0.132, respectively), the effect of RM could not be estimated in younger patients because of the high rate of resumed menstruation. Therefore, we conducted a subgroup analysis in patients > 35 years (n = 665). In this subset, the effect of RM on DFS was significant in both HR-negative and HR-positive patients (Figure 2D–F). There was no difference in DFS between patients with RM after CIA and those with RM without CIA (data was not shown).

Several clinical factors including RM were associated with DFS in univariate analysis (Table 2). In multivariate analysis, tumor size > 2 cm (hazard ratio 2.6, 95% CI 1.8–3.8, P < 0.001), nodal involvement (hazard ratio 3.4, 95% CI 2.2–5.3, P < 0.001), HR negativity (hazard ratio 2.2, 95% CI 1.5–3.1, P < 0.001), and RM (hazard ratio 2.0, 95% CI 1.4–2.8, P < 0.001) remained important factors associated with shorter DFS. In a subgroup analysis, RM appeared to be a prognostic factor for shorter DFS in all subgroups tested with the exception of patients aged ≤ 35 years (Figure 3). In particular, the effect of RM was more prominent in patients with node positive and larger tumors and was greater among patients who received taxane-containing regimens than nontaxane regimens.

**effects of CIA on survival**

During cytotoxic chemotherapy, 669 (76.7%) patients experienced CIA and 121 (13.9%) patients had persistent menstruation. While 420 (48.2%) patients never menstruated after CIA, 452 (51.8%) patients reported that regular menstruation reappeared or was persistent during the follow-up period.

In univariate analysis, the incidence of CIA was significantly higher when patients were older (≥ 35 years), received taxane-containing chemotherapy, or received tamoxifen treatment (supplemental Table S3 is available at Annals of Oncology online). All of these factors demonstrated a significant influence on the appearance of CIA in multivariate analysis.
DFS and OS were estimated according to the presence of CIA by the Kaplan–Meier method. The differences were not statistically significant ($P = 0.452$, $P = 0.488$, respectively), even when the subgroup analyses were conducted for patients > 35 years and patients with HR-positive or -negative tumors (data not shown). Five-year DFS was 90.7% (95% CI 82.8% to 95.0%) in patients without CIA and 91.1% (95% CI 88.6% to 93.1%) in patients with CIA.

**Figure 2.** Disease-free survival according to the presence of menstruation after cytotoxic chemotherapy. (A) All patients ($P < 0.001$); (B) HR+ patients ($P = 0.041$); (C) HR− patients ($P = 0.118$); (D) all patients > 35 years ($P < 0.001$); (E) HR+ patients > 35 years ($P = 0.047$); (F) HR− patients > 35 years ($P = 0.033$). HR, hormone receptor; RM, resumption/persistence of menstruation after cytotoxic chemotherapy.
Table 2 Analyses for DFS investigating the impact of resumption of menstruation after cytotoxic chemotherapy (RM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age ≤ 35 years</td>
<td>1.6</td>
<td>1.1 to 2.2</td>
</tr>
<tr>
<td>RM</td>
<td>2.0</td>
<td>1.4 to 2.8</td>
</tr>
<tr>
<td>T &gt; 2 cm versus T ≤ 2 cm</td>
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<td>1.8 to 3.8</td>
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<tr>
<td>N positive versus negative</td>
<td>3.4</td>
<td>2.2 to 5.3</td>
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<tr>
<td>HR negativity</td>
<td>2.2</td>
<td>1.5 to 3.1</td>
</tr>
<tr>
<td>HER2 positivity</td>
<td>1.7</td>
<td>1.1 to 2.5</td>
</tr>
<tr>
<td>BMI &lt; 18.5 kg/m²</td>
<td>0.7</td>
<td>0.2 to 1.8</td>
</tr>
<tr>
<td>BMI ≥ 23 kg/m²</td>
<td>1.1</td>
<td>0.8 to 1.5</td>
</tr>
</tbody>
</table>

BMI, body mass index; CI, confidence interval; DFS, disease-free survival; HR, hormone receptor; N, node; RM, resumed/persistent menstruation after cytotoxic chemotherapy; T, tumor diameter.

Figure 3. Hazard ratios for various subgroups, according to the presence of menstruation. CI, confidence interval; HR, hormone receptor.

discussion

According to our data, a considerable proportion of premenopausal patients treated with chemotherapy experienced RM with or without CIA. The incidence of RM was significantly associated with age and tamoxifen use. In our study population, RM not CIA was a poor prognostic factor for DFS in premenopausal patients with early breast cancer.

The incidence of CIA is related to patient age, the type of chemotherapeutic agents used, dose intensity of chemotherapy, and tamoxifen use [1, 4, 7]. Some studies have demonstrated improved survival with amenorrhea, while others have shown no impact [5, 6, 8, 15]. Different chemotherapeutic regimens, inconsistency in the definition of CIA, and lack of standardized data collection (including bleeding history) in heterogeneous populations may explain these discrepancies [1].

A recent analysis of the National Surgical Adjuvant Breast and Bowel Project Protocol B-30 (NSABP B-30) trial revealed that amenorrhea after chemotherapy was associated with substantial survival benefit [15]. In the NSABP B-30 trial, all patients received taxane-containing regimens and both OS and DFS were significantly increased among patients who experienced CIA, regardless of their ER status. In that study, CIA was defined as lack of menstruation for ≥ 6 months during 24 months of follow-up. When the authors carried out a 12-month landmark analysis to escape bias due to the misclassification of women without CIA who experienced relapse or death before reaching 24 months of follow-up, the clinical significance of CIA disappeared in HR-negative patients [16]. According to our previous study [4], the proportion of menstrual resumption was increasing by 2 years after chemotherapy and then reached a plateau. Therefore, the
status of CIA can be changed at each time point until at least 2 years of follow-up duration, which would significantly influence various studies’ results.

In the present study, we focused on RM rather than CIA because CIA is a heterogeneous status comprises cases of true menopause as well as cases of transient amenorrhea. There are patients whose estrogen levels remain in a premenopausal range despite experiencing a cessation of menstruation for \( \geq 1 \) year [4]. According to our results, RM was significantly associated with shorter DFS in both HR-positive and HR-negative patients. The rate of menstrual bleeding differed with patient age and HR status. HR-negative patients more frequently experienced menstrual bleeding than did HR-positive patients, possibly due to tamoxifen treatment in HR-positive patients. Therefore, we conducted subgroup analysis according to HR status and patient age. In each subgroup, DFS decreased significantly in patients with RM, although CIA itself was not correlated with DFS or OS.

Contrary to previous reports that the endocrine effect of chemotherapy was observed only in HR-positive patients [6, 9, 10, 17–19], the absence of menstruation resulted in a beneficial effect on DFS in both HR-negative and HR-positive patients in our study. We assumed that different results could be drawn by the different proportion of younger patients among a total of study population. Compared with other studies, our study included a significantly higher proportion of patients who were \(< 40\) years of age (\(> 45\%\)), which resulted in much higher incidence of RM and relatively lower incidence of permanent CIA. Thus, CIA may not adequately represent an endocrine effect of chemotherapy in this population. Although we could hypothesize that amenorrhea is a surrogate marker for the effectiveness of chemotherapy in HR-negative patients, the current data suggested that RM was more important marker rather than CIA that often represented a certain duration of amenorrhea. However, RM was only meaningful in patients \(> 35\) years because of very high incidence of RM in very young patients in our series.

Interestingly, the effect of RM on DFS was observed among high-risk patients with \(T > 2\) cm or node positive. Most of these patients received taxane-containing chemotherapy. Provided that RM was a marker of the effect of chemotherapy including its indirect endocrine effect, the effect of RM consequently might be more prominent in high-risk patients because the effect of chemotherapy itself would not be so great in low-risk patients.

Limitations of the current study are that it was a retrospective analysis, which had a risk of bias and the duration of CIA was not included as a clinical parameter. Most patients in the present study reported that menstruation resumed within 2 years after cytotoxic chemotherapy; however, the dates of vaginal bleeding after CIA were missed in most patients because the data collection process was not standardized. Parulekar et al. [6] demonstrated that 6-month CIA rates were not associated with prognosis, although CIA at 12 months was significantly associated with relapse-free survival and OS in patients with HR-positive breast cancer [6]. Therefore, further analysis will be needed to investigate the clinical significance of the duration of CIA combined with menstrual resumption.

Although more patients who received taxane-containing regimens experienced CIA, we did not find any association between RM and chemotherapeutic regimens. We did not include the dose intensity of chemotherapy due to heterogeneous chemotherapeutic regimens in this study.

In conclusion, RM after cytotoxic chemotherapy is likely to influence DFS in premenopausal women with early breast cancer. These results suggest that RM may be a potential indicator of the hormonal effect of chemotherapy as well as a predictive marker of the effectiveness of chemotherapy in premenopausal women. It is necessary to address the clinical effect of RM in large prospective studies for tailored therapy for premenopausal patients with early breast cancer.

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**disclosure**

The authors declare no conflicts of interest.

**references**

Calpain system protein expression in basal-like and triple-negative invasive breast cancer

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Background: Basal-like and triple-negative breast tumours encompass an important clinical subgroup and biomarkers that can prognostically stratify these patients are required.

Materials and methods: We investigated two breast cancer tissue microarrays for the expression of calpain-1, calpain-2 and calpastatin using immunohistochemistry. The first microarray was comprised of invasive tumours from 1371 unselected patients, and the verification microarray was comprised of invasive tumours from 387 oestrogen receptor (ER)-negative patients.

Results: The calpain system contains a number of proteases and an endogenous inhibitor, calpastatin. Calpain activity is implicated in important cellular processes including cytoskeletal remodelling, apoptosis and survival. Our results show that the expression of calpastatin and calpain-1 are significantly associated with various clinicopathological criteria including tumour grade and ER expression. High expression of calpain-2 in basal-like or triple-negative disease was associated with adverse breast cancer-specific survival (P = 0.003 and <0.001, respectively) and was verified in an independent cohort of patients. Interestingly, those patients with basal-like or triple-negative disease with a low level of calpain-2 expression had similar breast cancer-specific survival to non-basal- or receptor- (oestrogen, progesterone or human epidermal growth factor receptor 2 (HER2)) positive disease.

Conclusions: Expression of the large catalytic subunit of m-calpain (calpain-2) is significantly associated with clinical outcome of patients with triple-negative and basal-like disease.

Key words: basal, breast cancer, calpain, calpastatin, triple negative

Introduction

Breast cancer is a heterogeneous disease that displays a range of phenotypes with different clinical characteristics including altered clinical outcome, varying prognostic characteristics and differential response to treatment. The triple-negative and basal-like subgroups of breast cancer are of considerable clinical interest as they exhibit an aggressive phenotype and a higher metastatic potential. Triple-negative breast cancer lacks expression of both oestrogen receptor (ER) and progesterone receptor (PgR) and do not overexpress human epidermal growth factor receptor 2...