Original article

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Incidence of chemotherapy-induced amenorrhea depending on the timing of treatment by menstrual cycle phase in women with early breast cancer

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Background: The aim of this study was to characterize the factors associated with chemotherapy-induced amenorrhea (CIA) and to examine whether the phase of the menstrual cycle at chemotherapy start could affect the rate of CIA in premenopausal women with early breast cancer.

Methods: CIA was defined as the cessation of menses for at least 3 months during or after chemotherapy. Menstrual phase was defined as days 1–6, follicular phase as days 7–14, luteal phase as days 15–20 and premenstrual phase as days 21–28. Univariate and multivariate predictors of CIA were examined.

Results: Among 111 premenopausal women, univariate analysis showed a higher incidence of CIA in patients treated in the follicular phase rather than in other menstrual cycle phases (67.6% compared with 45.5%; $P = 0.03$). The rate of CIA increased with age: 65.2% and 45.8% in patients aged >42 and ≤42 years, respectively ($P = 0.05$). Upon multivariate analysis these differences remained statistically significant and duration of chemotherapy of more than six cycles correlated significantly with the incidence of CIA ($P = 0.03$).

Conclusions: The major implication of this analysis is that the timing of treatment within the menstrual cycle may potentially modulate the onset of CIA. This work and its future confirmation using prospective randomized trials would be useful in predicting the likelihood of CIA and in counseling breast cancer patients, especially those with a good prognosis who benefit less from chemical castration.

Key words: adjuvant chemotherapy, amenorrhea, early breast cancer, menstrual cycle phase

Introduction

With the widespread use of adjuvant chemotherapy in early breast cancer patients, the long-term effects of treatment are becoming increasingly important. Chemotherapy-induced amenorrhea (CIA), with resulting infertility and prolonged exposure to risks and symptoms of menopause, has been receiving increasing attention in recent years.

Age and type of adjuvant chemotherapy appear to be the primary determinants of ovarian failure. The incidence of CIA increases with age. Women older than 40 years have a much higher risk of developing amenorrhea compared with younger women, whether receiving cyclophosphamide, methotrexate and 5-fluorouracil (5-FU) (CMF) or anthracycline-based chemotherapy (reviewed in [1]). Median time to onset of ovarian failure is shorter in older than in younger women (2–4 months compared with 6–16 months), and ovarian failure is less likely to be reversible in older women (in ~10% compared with up to 50% of cases) [1]. The risk of CIA with polyagent adjuvant chemotherapy has been reported to range from 53% to 89%. Two-thirds of premenopausal women experience amenorrhea with the adjuvant regimen of CMF [1]. However, the Canadian NCIC adjuvant trial, comparing CMF with CEF, indicated that the incidence of amenorrhea was slightly higher in the CEF arm (51%) compared with the CMF arm (42.6%) [2]. Most anthracycline-based regimens have a lower incidence of amenorrhea, most likely due to the lower cumulative cyclophosphamide doses used in comparison with the classic CMF regimen [3]. The incidence of amenorrhea after adjuvant taxanes is not yet clearly established [4]. In addition, the cumulative dose of chemotherapy has been associated with the risk of menopause [1]. It is not clear

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whether duration of treatment or dose intensity exerts effects that are independent of cumulative dose. One cycle of perioperative CMF has been reported to result in menopause in 31% of women [5], a rate that is substantially lower than that reported with multiple cycles.

Previous work has focused on whether cellular immune function [6], breast cancer metastatic potential [7] and breast cancer surgical cure frequency [8] could be modulated by fertility cycle as a source of predictable variability relevant to host-cancer biology. Very little information exists about pharmacodynamic differences between young, menstrually functioning women and older women [9]. Even less relevant information is available for young women at different phases of their menstrual cycle. Conversely, several reports have focused on CIA as a prognostic factor, showing that much of the gain of adjuvant chemotherapy in premenopausal patients occurs in those who developed amenorrhea on chemotherapy with estrogen receptor (ER)-positive primary cancers [10]. In addition, the cessation of menses does not have to be permanent in order to achieve a benefit for outcome, as shown by Pagani et al. [11]. Thus, an enhanced ability to predict the likelihood of CIA could facilitate decision-making by patients and physicians, facing these trade-offs between beneficial and detrimental effects, and leading to a more informed choice of treatment.

In this historical context, we began a retrospective review from an initial cohort of 200 premenopausal women with newly diagnosed breast cancer receiving adjuvant chemotherapy. The aim of the present study was to characterize the factors associated with CIA and, particularly, to examine whether the phase of the menstrual cycle at chemotherapy start could affect the rate of CIA in premenopausal women with early breast cancer.

Patients and methods

Patients

The medical records of 200 consecutive premenopausal women with operable breast cancer [pT1-3 pN0-2 M0 according to the 6th tumor–node–metastasis (TNM) staging system classification], as reported by the AJCC Cancer Staging Manual observed at our institution (Regina Elena Cancer Institute, Rome, Italy) between December 1993 and September 2003 were reviewed. Patients who used any kind of hormonal therapy (i.e. birth control pills, progestins) at the time of evaluation and initiation of chemotherapy were excluded from the study. Forty patients had not been asked to provide information on their menopausal status during follow-up and 49 had an incomplete timing history of CIA. As a consequence, 111 women were finally found to be available for analysis. All patients had undergone surgical resection of their primary breast tumor (mastectomy or quadrantectomy with margins clear of invasive disease) with axillary node dissection. Data were retrospectively collected from medical charts available starting from surgery and during administration of systemic adjuvant therapy and follow-up.

Data collection

All women provided oral consent for their clinical data to be reviewed by investigators. Information was collected on age, tumor stage and size, nodal stage, number of nodes involved and removed, hormone receptor status (estrogen, progesterone) and other biological variables (HER2, p53, bcl2), surgical (mastectomy, quadrantectomy) and radiation treatment, adjuvant chemotherapy (type and duration in months) and eventual endocrine therapy. The chemotherapy regimens used were as follows: (i) cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, 5-FU 600 mg/m^2 (CMF) days 1 and 8 every 28 days for six cycles; (ii) epirubicin 120 mg/m^2 day 1 every 28 days for four cycles followed by four cycles of CMF cycles (E → CMF); (iii) 5-FU 600 mg/m^2, epirubicin 75 mg/m^2, cyclophosphamide 600 mg/m^2 (FEC) day 1 every 28 days for six cycles; (iv) epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2 (EC) day 1 every 28 days for four cycles; (v) epirubicin 120 mg/m^2 day 1 every 28 days for four cycles followed by docetaxel 100 mg/m^2 day 1 every 28 days for four cycles followed by four cycles of CMF cycles (E → D → CMF); and (vi) epirubicin 120 mg/m^2 day 1 every 28 days for four cycles. When prescribed, adjuvant endocrine therapy consisted of tamoxifen 20 mg/day for 5 years ± luteinizing hormone-releasing hormone analog for at least 2 years.

According to previous work in the field [5, 7], women with active menstrual cycles or with the occurrence of last regular menses within the 6 weeks preceding initiation of adjuvant chemotherapy were considered premenopausal, and CIA was defined as the cessation of menses for at least 3 months during or soon after chemotherapy [5, 10]. Each patient was interviewed about her last menstrual period and length of menstrual cycle, both before surgery and at the time of the chemotherapy cycle. The date of the first day of the last menstrual period was used to estimate the phase of the menstrual cycle at the start of chemotherapy. By setting the 14th day after onset of menses as the putative day of ovulation, we divided the menstrual cycle as follows: menstrual phase, days 0–6; follicular phase, days 7–14; luteal phase, days 15–20; and premenstrual phase, days 21–28 [12].

After treatment, patients were followed up for at least 12 months, at 4-monthly intervals for the first year and at 6-monthly intervals thereafter. During follow-up, patients were interviewed regarding menopausal status at each visit.

Statistical analysis

Median, percentiles and range were analyzed for each continuous variable, which was then divided into classes according to these values. For categorical variables, frequency tables were generated. The candidate explanatory variables in the univariate analyses of CIA onset were: age at diagnosis, type of surgery (mastectomy or quadrantectomy), tumor stage (T1 or T2/3), nodal status (N0 or N1), timing of chemotherapy start in relation to menstrual cycle phase, type and duration of adjuvant chemotherapy, and adjuvant tamoxifen (yes/no). For the univariate analysis, Pearson's χ^2, Mann–Whitney and Kruskal–Wallis tests were performed for the categorical variables considered.

A multivariate logistic regression analysis was then performed using onset of amenorrhea as a binary outcome variable. The candidate explanatory variables in the initial model were all those that were univariately significant at the 0.10 level. The purpose of this analysis was to identify those clinical factors that jointly provided the best prediction of the onset of amenorrhea. In a stepwise fashion, the explanatory variables with the lowest P values entered the model after it was confirmed that they were not involved in a significant two-way interaction with any one of the remaining variables. Significance was tested using χ^2 tests based on the change in deviance. This was repeated until no variable could be removed at a threshold P value of 0.15.

Results

Patient characteristics

Median age was 42 years (range 27–51). The population characteristics are listed in Table 1. The majority of women (59.5%) had undergone quadrantectomy with postoperative

Table 1. Patient characteristics

	No. of patients ($n = 111$)	%
Type of surgery		
Quadrantectomy	66	59.5
Mastectomy	45	40.5
Tumor stage		
T1	65	58.6
T2	40	36
T3	3	2.7
Tx	3	2.7
Nodal stage		
Node-negative	42	37.8
Node-positive (1–3)	55	49.6
Node-positive (>3)	12	10.8
Nx	2	1.8
Receptor status		
ER+PgR−	4	3.6
ER−PgR+	3	2.7
ER+PgR+	42	37.8
ER−PgR−	15	13.5
Unknown	47	42.4
Adjuvant radiation therapy		
Yes	87	78.4
No	24	21.6
Adjuvant chemotherapy		
CMF	19	17.1
EC	26	23.4
E→CMF	38	34.3
E → D → CMF	10	9.0
FEC	17	15.3
E	1	0.9
Adjuvant endocrine therapy		
Tamoxifen	24	21.6
Tamoxifen+LHRH analog	13	11.7

ER+/−, estrogen receptor-positive/-negative; PgR+/−, progesterone receptor-positive/-negative; CMF, cyclophosphamide, methotrexate, 5-fluorouracil (5-FU) (see Patients and methods); EC, epirubicin, cyclophosphamide; E→CMF, epirubicin, CMF; E → D → CMF, epirubicin, docetaxel, CMF; FEC, 5-FU, epirubicin, cyclophosphamide; E, epirubicin; LHRH, luteinizing hormone-releasing hormone.

radiation. Sixty-five (58.6%) had T1 and 40 (36%) had T2 disease, 42 (37.8%) had no axillary node involvement, and 100 (90%) had an Eastern Cooperative Oncology Group performance status of 0 or 1. Nineteen patients (17.1%) received CMF as adjuvant chemotherapy, 26 (23.4%) EC, 17 (15.3%) FEC, 38 (34.3%) EPI → CMF, 10 (9%) EPI → D → CMF and one (0.9%) epirubicin only. After chemotherapy, 24 (21.6%) patients received tamoxifen and 13 (11.7%) received tamoxifen plus LHRH analog.

Table 2. CIA and distribution according to menstrual cycle phase (111 patients)

MCP (days) at chemotherapy start	No CIA [No. of patients (%)]	CIA [No. of patients (%)]
1–6	15 (53.6)	13 (46.4)
7–14	11 (32.4)	23 (67.6)
15–20	11 (64.7)	6 (35.3)
21–28	16 (50)	16 (50)

CIA, chemotherapy-induced amenorrhea; MCP, menstrual cycle phase.

Univariate predictors of onset of CIA

CIA occurred in 58 out of 111 (52.2%) patients (Table 2); eight (7.2%) women resumed menstrual activity between 3 and 24 months after the cessation of menses. At univariate analysis, the incidence of CIA was significantly higher when chemotherapy started in the follicular phase (7–14 days) rather than in the next of the menstrual cycle. In fact, 67.6% of patients who began chemotherapy between days 7 and 14 of the menstrual cycle developed CIA compared with 45.5% of women who started treatment in one of the other menstrual phases (0–6, 15–20 and 21–28 days). Although, 35% of patients in the luteal phase developed CIA compared with 55% of those treated in the other phases, the difference was not statistically significant. Intriguingly, the same analysis showed that only 23% of patients treated between days 15 and 18 developed CIA, as compared with 56.1% of those treated in the rest of menstrual cycle ($P = 0.03$). Prognostic factors (i.e. tumor size, nodal status, stage of disease, receptor status, type and duration of chemotherapy) were well balanced among the two groups of women collected with respect to menstrual cycle phase at treatment start [follicular phase (days 7–14) compared with all other phases (0–6, 15–20, 21–28), early luteal phases phase (days 15–28) compared with all other phases (0–14 plus 19–28)].

We next analyzed the impact of age on CIA incidence at treatment start. When median age (42 years in our series) was chosen as cut-off, age was a significant univariate predictor of CIA occurrence, with the rate of CIA being 65.2% and 45.8% in women aged >42 and ≤42 years, respectively ($P = 0.05$). When the analysis was conducted for ranges between the 33rd and 66th percentiles, CIA occurred in 53.8%, 44.7% and 67.9% of patients aged 27–39, 40–44 and ≥45 years, respectively ($P = 0.18$; Kruskal–Wallis test). Among the 58 women with CIA, no significant correlation was found between patient age and time elapsed from chemotherapy start to menses cessation during or soon after chemotherapy. In fact, menses disappeared after a median of three courses (range one to eight) from chemotherapy start in both ≤42- and >42-year-old women ($P = 0.95$; Mann–Whitney test). When age ranges were fixed between the 33rd and 66th percentile, menses cessation occurred after a median of three, two and three courses (range one to eight) from chemotherapy start in women aged 27–39, 40–44 and ≥45 years, respectively ($P = 0.18$; Kruskal–Wallis test).

Among patients with CIA, time to menses cessation ranged between 21 and 341 days from the beginning of chemotherapy (median 73 days). In women who received CMF, median time to menses cessation was 82 days (range 42–140), while in patients treated with FEC/EC it was 56 days (range 21–84) and in those treated with E→CMF it was 42 days (range 21–217); these differences were not statistically significant. The use of tamoxifen in addition to either type of chemotherapy did not result in a statistically significant increase in the incidence of CIA.

Upon univariate analysis, no difference was observed in terms of incidence of CIA and time to menses cessation with respect to chemotherapy duration (six or fewer cycles versus more than six cycles). In fact, 28 out of 62 (45.1%) patients treated with four to six chemotherapy cycles experienced CIA compared with 29 out of 49 (59.1%) treated with eight to 12 cycles ($P = 0.10$). Women treated with four to six cycles presented a median number of courses to menses cessation of two (range one to five), while patients treated with eight to 12 cycles presented a median of three courses (range one to eight) ($P = 0.3$).

No significant correlation was found with respect to the type of regimen used, with the rate of CIA being 58%, 47.4% and 45.2% in patients treated with CMF-containing, CMF only and FEC/EC regimens, respectively (CMF versus FEC/EC regimens, $P = 0.3$; CMF-containing versus FEC/EC regimens, $P = 0.21$) (Table 3). In order to eliminate any possible influence of age on this result (94.7% of women treated with CMF and 54% of those treated with a CMF-containing regimen were aged >42 years compared with 30% of coeval patients treated with FEC/EC/E; $P = 0.0001$ and $P = 0.02$, respectively), we compared the incidence of CIA with respect to treatment administered in patients younger and older than 42 years. Even in this case, we found no statistical significant difference among type of chemotherapy and incidence of CIA. However, the numbers presented are too small to draw any definitive conclusion on the relationship between the incidence of CIA or time to menses cessation and the type of chemotherapy regimen.

No significant correlation was found between CIA and tumor size, number of involved axillary nodes, stage of disease and receptor status. At the same time, the expression of the biological variables p53, bcl-2, Ki-67 and HER2 was not significantly correlated with CIA. Among nine HER2-overexpressing (HercepTest 3+, Dako Corporation, Glostrup, Denmark, http://www.dakocytomation.dk) breast cancer patients, seven developed CIA, whereas among the 61 HER2-negative (DAKO-HercepTest 0 or 1+) breast cancer patients, 34 developed CIA ($P = 0.14$). These results should be interpreted with caution due to the extremely limited number of HER2-positive cases.

Multivariate analysis of onset of menopause

Multivariate analysis with a linear logistic model was adopted. Chemotherapy initiation by menstrual cycle phase, age at diagnosis (≤42 versus >42 years) and duration of chemotherapy (six or less versus more than six cycles) were each significantly and independently associated with CIA onset. The chemotherapy start during the follicular phase of the menstrual cycle was significantly correlated with CIA ($P = 0.05$). The incidence of CIA was significantly increased when chemotherapy was administered to women over the age of 42 years ($P = 0.02$). Duration of chemotherapy of more than six cycles significantly correlated with incidence of CIA ($P = 0.03$).

Table 3. Univariate and multivariate analysis: incidence of CIA

Variable	*P* value
Univariate	
Follicular phase versus other phases	0.03
Age (≤42 versus >42 years)	0.05
Duration (≤6 versus >6 cycles)	NS
CMF versus FEC/EC or CMF-containing versus FEC/EC	NS
Cycles to menses cessation (≤42 versus >42 years)	NS
Time to menses cessation (CMF versus FEC/EC, CMF versus E→CMF)	NS
Multivariate	
Age (≤42 versus >42 years)	0.02
Follicular phase versus other phases	0.05
Duration (≤6 versus >6 cycles)	0.03

CIA, chemotherapy-induced amenorrhea; NS, not significant; CMF, cyclophosphamide, methotrexate, 5-fluorouracil (5-FU) (see Patients and methods); FEC, 5-FU, epirubicin, cyclophosphamide; EC, epirubicin, cyclophosphamide; E→CMF, epirubicin, CMF.

Discussion

Early menopause may have important physiological and psychosocial consequences. For women who wish to consider becoming pregnant after breast cancer, risk of infertility following chemotherapy is a major concern. Other problems include accelerated bone mineral density loss, increased cardiovascular morbidity, hot flashes onset, genitourinary symptoms, and both psychological and psychosexual difficulties (reviewed in [13]).

Furthermore, questions remain open about the utility and the duration of chemo-castration in breast cancer patients receiving adjuvant chemotherapy. If part of the benefit of adjuvant therapy is due to early menopause, then there should be little added benefit in also causing ovarian ablation by other means. Several studies have addressed the therapeutic role of LHRH analogs in premenopausal breast cancer patients. The addition of 2 years of LHRH analog to chemotherapy in premenopausal women with endocrine-responsive (ER-positive), node-negative breast cancer has recently been evaluated in the International Breast Cancer Study Group trial VIII, showing no significant difference in 5-year disease-free survival (DFS) between CMF followed by goserelin or either modality alone [14]. In a smaller report on 92 premenopausal women with ER-positive node-positive disease, the addition of the LHRH analog after epirubicin

provided no statistically significant benefit in terms of overall survival or DFS [15]. Recently, the results of combining tamoxifen and LHRH analog after anthracycline-based chemotherapy have been published in abstract form [16], showing an increase in DFS after a median follow-up of 6 years. However, similar results have been found with regard to overall survival among patients treated with chemotherapy alone or with chemotherapy and LHRH analog with or without tamoxifen. Therefore, the therapeutic significance of ovarian suppression in premenopausal breast cancer women after cytotoxic chemotherapy remains controversial.

Traditionally, determining the impact of anticancer treatment on gonadal function has involved clinical assessment of puberal development, menstrual history in females and seminal analysis in males. There is no single 'gold standard' to assess and monitor menstrual status in breast cancer patients receiving adjuvant chemotherapy. In this report, we have focused on women who were premenopausal with regular menses at diagnosis. As endocrine profile was not available for our retrospective analysis, we necessarily used, like others in the English medical literature [1, 3, 5], an indirect clinical measurement to examine the effects of chemotherapy on the ovary and we defined the outcome in dichotomous terms: whether or not menses cessation occurred. Furthermore, according to others [8, 17] who demonstrated the impact of timing of surgery within the menstrual cycle, we defined the menstrual phase at chemotherapy start for each woman on the basis of reported date of last menstrual cycle. However, some women may have ovarian function activity and estradiol secretion without evidence of menses [18] and, based only on reported menstrual cycle history, at least 16% of premenopausal women are misclassified following chemotherapy [19]. On the other hand, continuation of regular menses post-treatment does not necessarily imply that the ovaries have escaped damage, and patients who continue to ovulate after chemotherapy remain at risk of undergoing premature menopause a number of years after treatment [20]. Thus, the effects of chemotherapy on the ovary can be concealed and require more research beyond the limitations of this and preceding clinical studies.

Taking into account data from the literature, the vast majority of women who remain amenorrheic 1 year after treatment will not regain ovarian function [21]. In our series, the percentage of women resuming menstrual activity after chemotherapy was lower than that of previous studies, which reported that between 12% and 15% of younger women experienced a return of menses after a period of amenorrhea [1]. However, as stated above, ovarian injury does not appear to be an 'all or nothing phenomenon', and partial loss of primordial follicle reserve might result either in CIA recovery or in premature menopause as a delayed reaction to treatment. Further investigations are needed to address this issue. The risk of premature menopause appears to be related to patient age and to the specific chemotherapeutic agents used, whereas the role of treatment duration and total dose administered remains uncertain. In our cohort, age was a significant predictor of risk of menopause. In fact, 65.2% of women aged >42 years developed CIA compared with 45.8% of patients ≤42 years ($P = 0.05$). Unfortunately we did not include a control group of women not receiving chemotherapy in order to evaluate the risk of menopause onset in women in their 40s, regardless of whether tamoxifen was used.

Preceding reports have identified a higher risk of amenorrhea associated with larger cumulative dose or longer duration of treatment [1]. In our cohort of patients, a duration of chemotherapy of more than six cycles was significantly correlated with incidence of CIA ($P = 0.03$), as shown by multivariate analysis. However, a recent study in mice has demonstrated that ovarian damage occurs regardless of the dose of drug received [22], and it is noteworthy that in the report by Pagani et al. [11], most benefit occurred in those patients with CIA who received 'suboptimal' chemotherapy. Furthermore, clinical studies [23, 24] have shown that estrogen, progesterone and luteinizing hormone levels are affected early on in breast cancer patients, within the first chemotherapy courses. These findings suggest that alterations in pituitary and/or ovarian hormone levels, driven by cytotoxics drugs' action on ovarian function, although considered dose-dependent, could be a sharp and immediate phenomenon. As a consequence, we ruled out the possibility of considering those patients who continued to have menses after the first chemotherapy course as still 'regularly' menstruating, and we limited the evaluation of CIA with respect to timing of chemotherapy at treatment start.

Our data on CIA in women with early breast cancer are similar to those in the published literature; however, we have extended previous observations by providing estimates of the incidence of CIA according to the timing of chemotherapy start by menstrual cycle phase. To our knowledge, no previous extensive work has adequately investigated this field. The work of Mehta et al. [24] could prove to be the exception; however, they limited their analysis to 70 patients receiving CMF chemotherapy and considered only pre- and postovulatory phases, without finding any statistical difference. Herein, we showed that starting treatment during the follicular phase (7–14 days) of the menstrual cycle might be an important predictor of CIA onset in women with newly diagnosed breast cancer. This result suggests a shift in attention away from the type or duration of adjuvant chemotherapy towards when chemotherapy is begun with respect to menstrual cycle phase. Furthermore, the impact of systemic adjuvant therapies may vary according to initiation at different times in the menstrual cycle. With regard to endocrine therapy, in Love's study [7], women who received a mastectomy and surgical oophorectomy and tamoxifen during the luteal phase had better outcomes than women who received surgery during the follicular phase. It could be hypothesized that, during specific times of the menstrual cycle, upregulation of hormonally controlled genes might influence tumor cell shedding and survival. The rapid lowering of hormonal levels by surgical oophorectomy during the luteal phase may exert a cytocidal effect through several mechanisms, such as differences in the regulation or

levels of angiogenic factors and proteases between luteal and follicular phases [25].

The direct mechanisms of chemotherapy-induced ovarian failure are poorly understood. Each female fertility cycle represents a tight coordination of cell division events within the ovary. Ovarian follicular development (follicular phase) is totally dependent upon the division of cells tending the ripening ovum. In response to precisely timed surges of pituitary hormones, the mature follicle ruptures and the egg becomes available for fertilization, supported hormonally by the quiescent non-dividing follicle that is now called the corpum luteum (luteal phase). In mouse experiments, the effect of cyclophosphamide on female gametes appears to be influenced by the stage of follicle development at the time of exposure. Oocytes exposed to chemotherapy as late pre-antral follicles seem particularly vulnerable to cyclophosphamide-induced lethal damage, whereas oocytes, which began the maturation process during chemotherapy treatment, are most susceptible to non-lethal damage [26]. The hormonal coordination of ovarian follicle cell DNA synthesis might be important with respect to the toxicity of anticancer agents that may affect DNA synthesis and RNA processing. The human menstrual and the rodent estrous cycles differ substantially in length; however, it is noteworthy that 5-FU administration during estrus (the murine phase associated with follicular cell division) determines the lowest successful pregnancy rate [27]. In contrast, 5-FU given in the progesterone-rich pro-estrus stage results in the highest rate of animals with the most undisturbed reproductive capacity. Based upon the protective role of progesterone rising in the mouse model, it has been suggested that chemotherapy may be best tolerated following ovulation [27]. Although limited to a small number of patients, the results presented herein regarding the low incidence of CIA among patients treated in the luteal phase and, particularly, in the days 15–18 period of the menstrual cycle, characterized by progesterone rising, appear to be compatible with those of the preclinical models [26, 27]. An attempt to explain the effects of chemotherapy on gonadal function and the major risk of CIA in patients treated during the days 7–14 phase can be made, taking into account the role played by follicle-stimulating hormone (FSH) in folliculogenesis. The large majority of human oocytes are destined to undergo atresia, and only follicles able to respond to stimulation by FSH enter the final stage of development and ovulate. Follicles destined to become dominant gain sensitivity to FSH, whereas follicles destined to become atretic lose their sensitivity to FSH (reviewed in [28]). Recent studies have suggested that follicle atresia in mammals is associated with apoptosis, which is an active, intrinsic, genetically governed process of selective cell deletion [28]. Antral follicular atresia is increased in a dose-dependent fashion in response to cyclophosphamide administration [29] and *in vitro* evidence suggests that the incidence of oocyte apoptosis is dramatically increased to >70% by the addition of doxorubicin [30]. Therefore, chemotherapy administered within the follicular phase could be responsible for the follicular maturation impairment, primordial follicle depletion and amplification of physiological apoptotic mechanisms occurring in this phase.

The immediate practical implication of the current study is that gonadotoxicity resulting from the administration of chemotherapy may be potentially diminished by optimally timing it within the menstrual cycle. In addition, estimates of the risk of menopause according to age and treatment may facilitate the decision-making process regarding adjuvant therapy in breast cancer. This process requires that each woman balance the potential benefits of treatment against the potential adverse effects and future risks. When absolute benefits are small, as is the case in low-risk, node-negative breast cancer, risk of menopause and its sequelae may tip the balance toward or away from specific treatments. When absolute benefits are larger, as is the case in node-positive and high-risk node-negative disease, effects of adjuvant therapy on disease recurrence and survival will usually outweigh menopausal considerations. Clearly, further investigations are needed both to confirm our findings, to establish the possible biological basis underlying CIA with respect to the menstrual cycle phase, and to overcome the selection bias and the power limitation of this retrospective analysis. It is our opinion that this work and its future confirmation using prospective randomized trials would be very helpful in counseling young breast cancer patients, especially those with a good prognosis who benefit less from chemical castration.

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