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Treatment-related morbidity and long-term sequelae related to chemotherapy-induced amenorrhea are assuming greater importance as the number of breast cancer survivors increases.

Chemotherapy-Induced Amenorrhea and Fertility in Women Undergoing Adjuvant Treatment for Breast Cancer

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Background: *The majority of women diagnosed with early-stage breast cancer have an excellent long-term prognosis, but many will undergo temporary or permanent chemotherapy-induced amenorrhea.*

Methods: *While breast cancer is more common in older women, about 1 in 200 women under the age of 40 is at risk to develop breast cancer. Many of these women benefit from chemotherapy but are afraid to risk the opportunity to bear children. The authors review the current studies on the impact of adjuvant chemotherapy on amenorrhea and fertility in women with breast cancer.*

Results: *The likelihood of amenorrhea is based on the specific adjuvant chemotherapy regimen administered and the age of the patient. Future childbirth is a viable option for women treated for breast cancer at an early stage. While the use of tamoxifen as a hormonal therapy in premenopausal breast cancer is now the standard of care, no conclusive data confirm the benefit of GnRH agonists in adjuvant therapy after treatment with chemotherapy followed by tamoxifen.*

Conclusions: *As more women over the age of 35 consider pregnancy, fertility issues are becoming important areas of investigation for the adjuvant treatment of breast cancer. Whether chemotherapy-induced amenorrhea has a prognostic effect remains unclear, and further studies are warranted.*

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Introduction

Approximately one third of new cases of invasive breast cancer predicted to occur in the United States in 2003 will occur in women under 50 years of age.¹ With improved education and increased screening, it is likely that more women will be diagnosed with early-stage breast cancer at younger ages than ever before. Fortunately, most breast cancers are diagnosed at an early stage. Most national guidelines of early-stage inva-

sive breast cancer recommend treatment with adjuvant cytotoxic chemotherapy and hormone therapy with estrogen receptor-positive (ER+) tumors. The exception to these guidelines refers to cases in which the tumors are small. Thus, the majority of young women diagnosed with early-stage breast cancer will undergo adjuvant chemotherapy. Long-term survival is likely when breast cancer is diagnosed at an early stage, especially after adjuvant therapy. Temporary or permanent menopause is a consequence that specifically affects young women diagnosed with breast cancer and treated with adjuvant chemotherapy. In addition, premature ovarian failure has been associated with increased morbidity and mortality.²

According to the US Census Bureau of Statistics, the average age of onset of menopause in American women is between 50 and 52 years. The median age of women who develop amenorrhea following adjuvant chemotherapy varies from 38 to 46 years.³ The likelihood of permanent chemotherapy-induced menopause is directly related to age. Women older than age 40 have a higher incidence of amenorrhea than women younger than age 40. The incidence rate of amenorrhea varies from 21% to 71% in younger women, whereas in women older than 40, the rate ranges from 49% to 100%.³ Thus, as more women with breast cancer survive the disease, treatment-related morbidity and long-term sequelae related to chemotherapy-induced amenorrhea will assume greater importance. Therefore, future trials will require more attention to reporting the incidence of chemotherapy-induced amenorrhea and will consider prevention and treatment strategies for the symptoms and long-term side effects of menopause.

Table 1. — Change of Fertility by Age: US Census Bureau

	Number (× 1000)	Fecund (%)	Surgical Contraception (%)	Impaired Fecundity (%)
All women	60,201	62.5	24.2	10.2
15-24 yrs	18,002	92.2	1.6	6.1
25-34 yrs	20,758	65.6	22.0	11.2
35-44 yrs	21,440	34.6	45.3	12.8
Parity 0				
15-24 yrs	14,113	94.3	0.2	5.5
25-34 yrs	7,139	82.5	2.9	13.9
35-44 yrs	3,991	54.3	11.9	25.7
Parity 1 or more				
15-24 yrs	3,889	84.6	6.7	8.4
25-34 yrs	13,620	56.7	32.1	9.8
35-44 yrs	17,449	30.1	52.9	9.8

Percentages may not equal 100% as some women are surgically sterile for noncontraceptive reasons.

Table 2. — Fertility Rates in the United States (per 1,000 Women)*

Age of Mother	1980	1999
15-24 yrs	168.1	169.6
25-34 yrs	174.8	207.4
35-44 yrs	23.7	45.7

* Data from the US Census Bureau suggest that while the fertility is strongly influenced by age, this is more prominent in women who are nulliparous. This may be in partly explained by an increase in selection bias found in the older women with desire to conceive. The decrease in the number of children born in multiparous women may to a larger degree due to the significant increase in surgical contraception rather than truly impaired fecundity.

Another important aspect of therapy decisions in the young premenopausal woman undergoing adjuvant chemotherapy is the preservation of fertility (Table 1). At present, there are no conclusive data suggesting that deleterious effects from subsequent pregnancy will occur in women with a prior history of breast cancer.⁴ Therefore, in a population with a high likelihood of long-term survival, interventions to preserve fertility should be considered. In addition, in the absence of clearly documented benefits of premature ovarian failure with regard to disease-free survival (DFS), the preservation of ovarian function may improve all-cause survival by decreasing the risks of heart disease and osteoporosis. Over the last 2 decades, the median age at first live birth has steadily increased (Table 2). The number of children born to women over 30 years of age has doubled and now comprises 30% of all live births. In 1999, in the United States alone, more than 500,000 babies were born to women over 35 years of age and many to women over age 40. According to the US Census Bureau, the birth rate in women over age 40 is projected to increase by 7% over the next 10 years. The likelihood of successful conception decreases over age 34 in nulliparous women, and this may be due to selection bias rather than biologic reasons. The infertility rate of parous women remains at 5% or less up to the age of 44. These findings suggest that the preservation of fertility even in women over the age of 35 years is important. Major strides have been made in reproductive medicine that allow many women to become pregnant even when subfertile or when fertility chances are waning due to older age. However, most reproductive interventions are either not possible or exceedingly difficult after menopause. The rate of premature ovarian failure (ie, menopause under the age of 40) has been estimated at 1% and is often familial.⁵ Early menopause (ie, menopause between 41-44 years) is estimated to occur in approximately 5% of women. While breast cancer is more common in older women, about 1 in 200 women under the age of 40 is at risk to develop breast cancer. Many of these women benefit from chemotherapy but are afraid to risk the opportunity to bear children.

Incidence of Chemotherapy-Induced Amenorrhea

The most commonly used chemotherapies for the adjuvant treatment of breast cancer are presented in Table 3. The cytotoxic agent that has been most intensely studied and known to induce amenorrhea is cyclophosphamide.^{6,9} Cyclophosphamide is an integral part of most of the commonly used regimens for the adjuvant treatment of breast cancer. Table 3 depicts amenorrhea rates associated with various regimens used in the adjuvant setting. Two thirds of premenopausal women experience amenorrhea with the adjuvant regimen of cyclophosphamide, methotrexate, and 5-fluorouracil (5FU) (CMF).³

The antimetabolites methotrexate and 5FU in the CMF regimen have not been not associated with an increased rate of amenorrhea. Bines et al³ investigated methotrexate and 5FU in the adjuvant setting and reported an amenorrhea rate of 9%, whereas standard CMF regimens utilizing oral cyclophosphamide are associated with a 69% amenorrhea rate in an age-matched population. Furthermore, the higher the cumulative dose of cyclophosphamide, the higher the observed incidence of menopause. Goldhirsch et al¹⁰ published rates of menopause based on cumulative doses of cyclophosphamide. One perioperative dose of CMF was associated with a 10% incidence of amenorrhea; rates increased to 33% and 61% in a younger population of women treated with 6 and 12 months of CMF, respectively.

Amenorrhea associated with anthracycline therapy is less well understood and shows significant variations among studies. The most recent meta-analysis has shown that regimens containing prolonged oral use of

cyclophosphamide, such as cyclophosphamide, doxorubicin, and 5FU (CAF) and cyclophosphamide, epirubicin, and 5FU (CEF), are superior to CMF in terms of DFS and overall survival (OS),¹¹ but data on the incidence of amenorrhea associated with these regimens is limited. However, the Canadian NCIC adjuvant trial comparing CMF with CEF indicated that the incidence of amenorrhea was slightly higher in the CEF arm (51%) vs the CMF arm (42.6%).¹² Most anthracycline-based regimens have a lower incidence of amenorrhea, most likely due to the lower cumulative cyclophosphamide doses used in comparison to the classic CMF regimen. The doxorubicin and cyclophosphamide (AC) regimen has been reported by Bines et al³ to result in amenorrhea at a rate of 34%. The incidence of amenorrhea after adjuvant taxanes is not yet clearly established. The incidence of amenorrhea reported from the recently presented BCIRG 01 trial comparing TAC and FAC in early-stage breast cancer was 51.4% and 32.8%, respectively. However, this trial was presented at an early stage of follow-up, and the method of assessment was not reported¹³ (Table 3). A small retrospective trial evaluated the addition of paclitaxel after AC and did not find a significant increase in amenorrhea.¹⁴ Larger prospective studies are needed to confirm these data. Further data will be derived from the larger trials such as CALGB 9344 and NSABP B27 and B28 that are evaluating paclitaxel and docetaxel in the adjuvant and neoadjuvant setting. These studies have completed case accrual and are currently awaiting maturity.

Variation in Reporting Time Points of Amenorrhea

Inconsistencies exist in the manner of reporting the incidence of amenorrhea with various adjuvant chemotherapy regimens. Some report the incidence of amenorrhea upon completion of chemotherapy, while others report continued amenorrhea at various time points after the start of chemotherapy. The time to development of amenorrhea in women under the age of 40 undergoing adjuvant chemotherapy ranges from 6 to 16 months.³ The time point most commonly used for the reporting of amenorrhea related to adjuvant chemotherapy is after 12 months. Padmanabhan et al¹⁵ reported the incidence of amenorrhea from the beginning of treatment with CMF chemotherapy at 3, 6, and 12 months later as 50%, 70%, and 80%, respectively. Twelve months follow-up may be a reasonable time point to report the incidence of amenorrhea. However, other reports¹⁶ indicate a chemotherapy-induced amenorrhea rate of 66% within 18 months of surgery that increases to 76% after a median follow-up of 36 months.

Table 3. — Selected Trials of Chemotherapy-Induced Amenorrhea

Author	Adjuvant Chemotherapy	Incidence of Amenorrhea
Goldhirsch et al ¹⁰	Classic CMF	61% (<40 yrs) 95% (≥40 yrs)
Bines et al ³	AC	34%
Nabholtz et al ¹³	FAC	32.8%
	TAC	51.4%
Hortobagyi et al ¹⁷	Doxorubicin-based	59%
Levine et al ¹²	CEF	51%

CMF = cyclophosphamide, methotrexate, 5FU
 AC = doxorubicin, cyclophosphamide
 FAC = 5FU, doxorubicin, cyclophosphamide
 TAC = docetaxel, doxorubicin, cyclophosphamide
 CEF = cyclophosphamide, epirubicin, 5FU

The Effect of Age on Chemotherapy-Induced Amenorrhea

The rate of chemotherapy-induced amenorrhea varies according to patient age. The Milan regimen consisting of CMF with and without doxorubicin reported an amenorrhea rate of 4% for women under 30 years of age, 50% in women 36 to 40 years of age, 86% in women aged 41 to 45 years, and 100% in women over the age of 45.¹⁶ Women older than age 40 have a much higher risk of developing amenorrhea compared with those 40 years of age and younger.³ The rates of chemotherapy-induced amenorrhea vary from 21% to 71% in women under age 40 compared to 49% to 100% in those at least age 40 or older. Amenorrhea occurred for at least 3 months in approximately 40% of young women and in 76% of older women after a CMF-based chemotherapy regimen.³ In women between 40 and 49 years of age, doxorubicin-containing regimens were associated with a chemotherapy-induced amenorrhea rate of 96%.¹⁷ Similar results were seen with epirubicin.¹⁸

The exact mechanism of chemotherapy-induced amenorrhea is poorly understood. Preclinical studies have suggested that chemotherapy induces apoptotic changes in pregranulosa cells that subsequently lead to follicle loss. However, these findings have not been confirmed in human studies.¹⁹ The significant increase in amenorrhea seen in older women treated with chemotherapy may be due to the relatively lower number of existing oocytes. Approximately 2 million oocytes are present at birth; they have decreased to 200,000 by puberty and to 400 at menopause.²⁰ Following treatment with chemotherapy, the ovaries have a decreased number of oocytes available for follicular recruitment, along with evidence of fibrosis.^{8,9,21} These changes are similar to those observed in natural postmenopausal ovaries. However, the chemotherapy induces further reduction and insult to a diminished population of oocytes, which may manifest in overt ovarian failure. The cytotoxic damage appears to be progressive and irreversible in the ovary, as germ cells are limited in number and cannot be regenerated.^{22,23}

Survival and Chemotherapy-Induced Amenorrhea

Is short-term or permanent menopause important to breast cancer survival in premenopausal women? Several reported trials suggest that women under the age of 35 have a poorer prognosis and that age appears to be an independent risk factor. Poikonen et al²⁴ reported on the prognostic effect of amenorrhea and elevated gonadotropin levels induced by the adjuvant chemotherapy regimen of six courses of CMF every 3

weeks. In multivariate analysis, amenorrhea after chemotherapy was associated with improved DFS. In univariate analysis, improved OS was associated with chemotherapy-induced menopause. However, in multivariate analysis, statistical significance was not observed for this observation. Chemotherapy-induced amenorrhea was associated with improved DFS in women with estrogen receptor-positive (ER+) breast cancers, and no association was observed in those with ER- tumors. However, these data are based on a small number of young women (n = 126). In this trial, tamoxifen was not given as an adjuvant treatment for ER+ tumors. Also, the serum FSH level did not correlate with prognosis and was not a reliable indicator of amenorrhea.

The International Breast Cancer Research Study Group (IBCSG) has reported on its prognostic findings in 3,000 premenopausal and perimenopausal women who participated in trials I, II, V, and VI.²⁵⁻³⁰ These trials were CMF-based adjuvant treatment studies conducted by the IBCSG from 1978 to 1993. The major findings were that women under the age of 35 had a significantly higher risk of relapse and death than older women. The actuarial 10-year DFS for the younger and older women was 35% and 47%, respectively ($P < .001$). OS was 49% and 62%, respectively ($P < .001$). Furthermore, the younger women with ER+ tumors had a significantly worse prognosis than the younger women with ER- tumors. The 10-year DFS was 25% for the ER-premature ovarian failure positive group vs 47% for the ER- group ($P = .002$). However, none of these trials had incorporated adjuvant tamoxifen.

To further explore the interaction between age and ER status, the US Cooperative Groups including the National Surgical Adjuvant Breast and Bowel Project (NSABP), the Eastern Cooperative Oncology Group (ECOG), and the Southwest Oncology Group (SWOG) analyzed outcomes in premenopausal women who had participated in adjuvant chemotherapy-alone trials. The groups also added the IBCSG results of premenopausal women from the above-mentioned trials, including only the subset with known ER status.¹⁴ Cohorts in the IBCSG, NSABP, and SWOG trials revealed a statistically significant poorer outcome for younger patients (age <35) with ER+ tumors compared to their older counterparts. In contrast, among patients with ER- tumors, no substantial differences in survival were observed in the younger vs older population. Tamoxifen as an adjuvant therapy was not used in these trials.

US Cooperative Groups have subsequently analyzed trials investigating the combination of chemotherapy and tamoxifen in the adjuvant setting. Interestingly, they have observed no differences in DFS in the older vs younger populations. Furthermore, there is no interac-

tion observed between age and ER status.¹⁴ The effectiveness of adjuvant ovarian suppression in addition to chemotherapy and tamoxifen in premenopausal women remains unclear. Data are limited regarding the combination of tamoxifen and an ovarian ablative therapy after adjuvant chemotherapy in a young population of patients with ER+ tumors. In the metastatic setting, one trial demonstrated a 1-year survival benefit from buserelin, the gonadotropin-releasing hormone (GnRH) agonist, combined with tamoxifen compared to either treatment alone in premenopausal women with ER+ breast cancers.³¹ Houghton et al³² presented their findings from the “Zoladex in Premenopausal Patients” (ZIPP) trial in 2000. Women with early-stage breast cancer (n = 2,631) were randomized to receive either tamoxifen for 2 years, goserelin for 26 months, tamoxifen plus goserelin, or no adjuvant treatment. Some of the women received adjuvant chemotherapy in addition to the hormonal treatments. While this trial has not reached maturity, early analysis suggested that at a median follow-up of 4.3 years, event-free survival was significantly better in the groups who received goserelin compared to those who did not (261 vs 330 events, *P*=.001). However, confounding factors such as the additional benefits of chemotherapy or tamoxifen alone have not yet been determined. The OS appeared to be similar in both groups. In 1999, Davidson et al³³ reported that 5-year DFS and OS were similar in premenopausal women who had received CAF alone vs CAF plus goserelin (Zoladex) (CAFZ) vs CAF plus goserelin (Zoladex) plus tamoxifen (CAFZT). The CAFZT group had a better relapse-free survival compared to the CAF group or the CAFZ group, and the CAF vs CAFZ group had a similar relapse-free survival. The OS at 5 years was similar in all three groups. However, there was no arm consisting of CAF plus tamoxifen to determine the potential benefits of goserelin either compared with or in addition to tamoxifen. Estradiol levels were measured, and the benefit of the addition of goserelin and tamoxifen was more evident in the premenopausal women less than 40 years of age who had elevated serum estradiol levels. It is noteworthy that serum estradiol levels vary among women who develop chemotherapy-induced menopause.³⁴ Serum estradiol levels may remain elevated for several months to years after the onset of amenorrhea.

These data further complicate the issue of what serologic marker or endpoint to evaluate in future studies investigating the effects of goserelin in premenopausal women on adjuvant chemotherapy for breast cancer. Overall, is it more important to measure the incidence of amenorrhea or to measure serum estradiol levels, or should both parameters be used? All trials to date have included only a small proportion of premenopausal patients significantly younger than age 40 who remain premenopausal after adjuvant

chemotherapy. The majority of the patients in many of these trials are over age 40; in the 5th decade, the incidence of naturally occurring menopause will also increase from 5% to close to 100%. International trials are being developed to address this issue.

To date, there is no clear evidence that chemotherapy-induced amenorrhea provides significant additional benefits for young women beyond standard adjuvant treatment with combination chemotherapy and tamoxifen. Further studies are needed to investigate optimal adjuvant hormonal interventions in the premenopausal population.

Preservation of Chemotherapy-Related Amenorrhea and Fertility

In addition to the adjuvant use of GnRH agonists as a primary treatment, these agents have also been investigated as a supportive treatment to preserve fertility while undergoing chemotherapy. Preservation of ovarian function has been evaluated in a limited number studies with few patients. Although the data are encouraging, this approach may need to be evaluated with regard to disease and specific therapy. A study of buserelin given to 8 women treated for advanced Hodgkin’s disease reported that at 3 years of follow-up, 50% of the women were amenorrheic vs 75% in the control group.³⁵ A prospective study²⁰ evaluated the use of GnRH agonist in 44 women who were 15 to 40 years of age with various malignancies treated with chemotherapy. All but 1 of the patients in the GnRH-agonist group resumed spontaneous ovulation as evidenced by menses within 6 months, while less than half of the patients in the control arm resumed ovarian function and regular cyclic activity. Another trial evaluating chemotherapy-induced amenorrhea in patients with lymphomas showed a protective effect of co-treatment with a GnRH agonist (Table 4).²³ In a recently reported trial investi-

Table 4. — Clinical Data and the Rate of Premature Ovarian Failure in Two Groups of Young Women Undergoing Chemotherapy With or Without GnRH-Agonist Co-Treatment

	GnRH/Chemotherapy	Chemotherapy
No. of patients (total)	55	55
Age range	15-40	14-40
Hodgkin’s disease	33/55 (60%)	33/55 (60%)
Non-Hodgkin’s disease	22/55 (40%)	22/55 (40%)
Pregnancies	18 in 12 women	13 in 8 women
Age range of pregnant women at chemotherapy	18-33 years	16-24 years
Cyclic ovarian function	47/50 (94%)	22/50 (44%)
Premature ovarian failure	3/50 (6%)	28/50 (56%)
Data from Blumenfeld et al. ²³		

gating the protective effects of goserelin on ovarian function,³⁶ premenopausal women received goserelin combined with various adjuvant chemotherapy regimens including CMF, CEF, and high-dose chemotherapy followed by bone marrow transplant as adjuvant treatment of breast cancer. The median age of the 64 women accrued to the trial was 42. They were given 3.6 mg of goserelin every 28 days for 1 year. After a median follow-up of 54 months, 86% had resumed regular menses within 12 months of completing the adjuvant chemotherapy. The DFS was 84% and the OS was 94%. Given the median age of 42 and the previous data on permanent amenorrhea rates associated with the above-mentioned adjuvant chemotherapies, it was assumed that the majority of women would remain in menopause long-term. Therefore, it was surprising and encouraging to observe an 86% ovarian preservation rate after the use of goserelin. Further investigation of GnRH analogues for this indication is warranted.³⁶

While a potential benefit has been suggested by these findings, benefit has not been confirmed in a larger trial with stratification for disease, age and type of therapy. In addition, the risk of chemotherapy-induced amenorrhea has not been clearly defined. Although GnRH analog treatment parallel with chemotherapy may be advantageous during adjuvant therapy for young women, it is not the only option for fertility preservation. Several studies report on cryopreservation of mature metaphase II oocytes after hMG/hCG ovarian stimulation. To date, this approach has been successful only in rodents.^{37,38} A future possible alternative may be the retrieval of human immature oocytes for cryopreservation and in vitro maturation after thawing. Cryopreservation of fertilized ova, after IVF before chemotherapy, is clinically available but rarely is feasible in single young women. Moreover, the ovarian stimulation with hMG/hCG before in vitro fertilization egg retrieval would postpone the initiation of chemotherapy. Most oncologists would hesitate to recommend hMG/hCG ovarian stimulation with an increase in estradiol in a patient diagnosed with breast cancer. A further possibility, which has recently been the focus of intense investigations, is the transplantation of ovarian tissue.^{39,40} However, despite promising preclinical results in ovarian cortex transplantation, these techniques have not been optimized. The success of these procedures will depend on several factors such as the number of follicles that survive the transplant and the ability of these follicles to develop and ovulate.⁴¹

Conclusions

Studies to date have not used uniform definitions of menopause and have not required clarification

between temporary and permanent menopause. Most studies report outcomes related to induction of chemotherapy-induced amenorrhea in a premenopausal population using nonhormonal adjuvant trials. While the use of tamoxifen as a hormonal therapy in premenopausal breast cancer has now become the standard of care, there are no conclusive data to confirm the benefit of GnRH agonists in adjuvant therapy after treatment with chemotherapy and then tamoxifen; however, trials are in development. At present, no sufficient persuasive data are available on DFS or OS to warrant prescription of GnRH agonists as standard therapy in the adjuvant setting for women who do not develop permanent amenorrhea. Ironically, the investigation of treatment with GnRH agonists during adjuvant chemotherapy for the purpose of preserving fertility may find additional outcome benefits through the induction of temporary amenorrhea. Such trials addressing the impact of amenorrhea on survival are vital to further elucidate the role of preservation of fertility in young women undergoing adjuvant treatment for breast cancer.

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