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Chemotherapy-Related Amenorrhea in Breast Cancer: Review of the Main Published Studies, Biomarkers of Ovarian Function and Mechanisms Involved in Ovarian Toxicity

M. Berliere, F.P. Duhoux, Ch. Galant, F. Dalenc, J.F. Baurain, I. Leconte, L. Fellah, L. Dellvigne, P. Piette and J.P. Machiels
Catholic University of Louvain
Belgium

1. Introduction

Breast carcinoma is the most common cancer in women of reproductive age. In Europe and in the United States, approximately 30% of all breast cancers occur before menopause and 15% of women are diagnosed in the reproductive age (age of 45 or younger). Adjuvant chemotherapy prolongs disease-free survival (DFS) and overall survival (OS), especially in young women, but also induces long-term and severe side effects such as temporary or definitive ovarian function suppression which results in potential loss of fertility and premature exposure to the risks of menopause including cardiovascular diseases, osteoporosis, hot flashes and genitourinary dysfunctions Bines et al. (1996). The probability of menopause with chemotherapy depends on the type of regimen used and on the age of the patient. In the literature, the estimated risk of amenorrhea varies between 0% and 60% in women younger than 40 years and between 40% and 100% in women older than 40 years. Beyond age and the type of regimen used, important variations reflect different durations of follow-up and variable definitions of menopause and of chemotherapy-related amenorrhea Bines et al. (1996).

2. Defining menopause status

The average age of menopause in women of Caucasian/European origin is around 51 years Bines et al. (1996); Burger et al. (2007); Gracia et al. (2005); Welt et al. (2006). Pituitary gonadotrophins stimulate ovarian steroid hormone production (estrogens, progesterone and androgens). Estradiol [E2] Welt et al. (2006) and progesterone act via a negative feedback loop to inhibit pituitary gonadotrophin release. Hormone release occurs on a cyclical basis so that concentrations of follicle-stimulating hormone (FSH) peak in the mid-follicular phase and decrease during the luteal phase, rising again shortly before menstruation Randolph et al. (2006); Tanay & Fenton (2010). As E2 levels rise in the late follicular phase, concentrations of luteinizing hormone (LH) surge (via positive feedback) in mid-cycle and then fall again during the luteal phase (negative feedback) under the influence of progesterone and E2. Both

E2 and inhibin-B are under gonadotrophin influence. Both hormones are stimulated by FSH. This allows the preservation of E2 secretion in the late reproductive phase Randolph et al. (2006); Tanay & Fenton (2010); Welt et al. (2006). The decline in quality and quantity of ovarian follicles (ovarian aging process) Broekmans et al. (2009); Burger et al. (2008) triggers the hormonal and symptomatic changes of menopausal transition. This decline accelerates around age 35 when the number of oocytes drops to approximately 25,000 (having been approximately 300,000 at puberty). Anti-Müllerian hormone (AMH) Sowers et al. (2008); Yang et al. (2011) produced by small preantral follicles is one of the earliest markers of ovarian aging since it reflects the remaining pool of follicles. Compared with changes in FSH, LH, progesterone, AMH and inhibin-B secretions, the fall in E2 levels occurs relatively close to the menopause Welt et al. (2006). Before menopause, climacteric symptoms and bleeding irregularities can occur as a consequence of changes in E2 and progesterone secretion. Menopause is commonly defined as the last menstrual bleed or final menstrual period (commonly named FMP). This can only be recognized retrospectively after 12 consecutive months of amenorrhea. The FMP is typically preceded by a period of hormonal instability and irregularity in the menstrual cycle lasting up to several years. In the literature, there is no uniform definition of when this transitional period also called "perimenopause" begins.
However, different criteria (such as the first occurrence of more than 7 days difference in cycle length) have been proposed. Perimenopause extends until the 12 month-period of amenorrhea has elapsed. Prior to FMP, bleeding patterns are highly unpredictable, vasomotor symptoms are common but not present in all cases. There is no hormonal marker which infallibly signals permanent cessation of menstruation. In 2001, the stages of reproductive aging workshop proposed FSH Burger et al. (2007); Randolph et al. (2006) as the best marker available but did not establish levels that defined the menopause. FSH secretion itself is variable. Elevations may occur up to 10 years before the menopausal transition. Furthermore, there is a lack of agreement between assays, and body size and age have effects independent of menstrual status. Based on changes in menstrual cycles and levels of FSH, the first standardized classification of stages or reproductive aging workshop (STRAW) was proposed in 2001Soules et al. (2001). This classification includes 7 stages. These stages take into account not only the changes in bleeding patterns but also the changes in hormone levels. AMH could be of potential use in better defining the stages of menopausal transition. However, its widespread use has been precluded by cost and the lack of sensitivity and reproducibility of available assaysLedger (2010). According to the most recent National Comprehensive Cancer Network (NCCN) guidelines Guidelines (2010) on the management of breast cancer, a woman can reasonably be considered postmenopausal if any of the following conditions have been fulfilled :

- She has had prior bilateral oophorectomy.
- She is age 60 years or older.
- If less than 60, she has had amenorrhea of 1 year or longer in the absence of chemotherapy, tamoxifen, toremifene or ovarian suppression, and FSH and E2 levels are in the postmenopausal range.
- If she is taking tamoxifen or toremifene and is under 60 years of age, FSH and E2 levels are in the postmenopausal range.

The guidelines mention that it is not possible to determine menopausal status when a woman is taking a GnRH agonist or antagonist. If none of these conditions are fulfilled and yet the patient has infrequent or no menses, she should be considered pre- or perimenopausal.

3. Defining chemotherapy-related amenorrhea

In premenopausal patients, chemotherapy can induce temporary or permanent ovarian dysfunction Meirow (2000); Valagussa et al. (1993); Walshe et al. (2006).
The definition of CRA is not consistent across the literature and this helps explain the wide range in reported rates among chemotherapy trials Bines et al. (1996); Walshe et al. (2006). According to the American College of Obstetricans and Gynecologists, chemotherapy-related amenorrhea is defined as cessation of menses for 6 months. However other authors have defined CRA as the cessation of menses lasting 3 to 6 months or longer, or used the criterion of menstrual cessation lasting at least 12 months. Other difficulties are explained by the fact that inconsistencies exist in the way amenorrhea is reported. Some authors report the incidence of amenorrhea immediately upon completion of chemotherapy, while others select various time points after the start and end of chemotherapy Meirow (2000); Valagussa et al. (1993); Vegetti et al. (2000). The time point most commonly encountered in the literature is 12 months after the end of chemotherapy. Chemotherapy-related amenorrhea is generally linked to the patient's age as well as treatment protocol (types of chemotherapeutic agents used, doses and schedules) Bonadonna et al. (2005); Goldhirsch et al. (1990); Padmanabhan et al. (1986); Valagussa et al. (1993); Warne et al. (1973). Data on ovarian function are widely available for certain regimens, such as cyclophosphamide, methotrexate and 5-fluorouracil (CMF) polychemotherapy Bonadonna et al. (2005); Goldhirsch et al. (1990); Warne et al. (1973) and anthracycline-based treatments Hortobagyi et al. (1986); Levine et al. (1998); Pritchard et al. (2005); Roche et al. (2006), but fewer studies have been conducted on taxane-based regimens Abusief et al. (2006); Berlière et al. (2008); Clemons & Simmons (2007); Davis et al. (2005); Fornier et al. (2005); Martin et al. (2003; 2005); Tham et al. (2007), and they unfortunately show contradictory results. Other problems can be summarized by the lack of prospective studies and by the limited duration of follow-up.

4. Rates of chemotherapy-related amenorrhea with main cytotoxic agents

4.1 Cyclophosphamide-based regimens Bonadonna et al. (2005); Goldhirsch et al. (1990); Warne et al. (1973)

As previously mentioned, the incidence of ovarian dysfunction is related to patient age, the specific agents used and the total dose administered, especially the dose of alkyaling agents such as cyclophosphamide. Amenorrhea rates following combination chemotherapy consisting of CMF regimens range from 21% to 71% in women aged 40 years and younger, and from 40% to 100% in older ones Bonadonna et al. (2005); Goldhirsch et al. (1990); Padmanabhan et al. (1986); Warne et al. (1973). In the interpretation of the data with CMF regimens, many difficulties exist due to a lack of homogeneity of CMF regimens: variations in the doses and type of administration of cyclophosphamide (oral vs. intravenous) and variations in the total number of courses: 3 to 12 courses. In a manuscript dedicated to "30 years follow-up of randomized studies of adjuvant CMF in operable breast cancer", Bonadonna at al. Bonadonna et al. (2005) defined drug-induced amenorrhea as "the irreversible cessation of menstrual periods during chemotherapy treatment or in the first years

of follow-up, in the absence of disease relapse". Amenorrhea rates were mentioned for 12 versus 6 cycles of CMF. CMF regimen consisted of cyclophosphamide (100 mg/m^2)/orally from day 1 to day 14, methotrexate (40 mg/m^2) intravenously on day 1 and 8, and 5-fluorouracil (600 mg/m^2) every 4 weeks. The incidence of iatrogenic amenorrhea was reported in the two regimens (6 cycles versus 12 cycles) by age group. Overall, drug-induced amenorrhea was reported more often in the longer regimen (75% versus 62%) than in the shorter one. In women younger than 45 years, the incidence of amenorrhea was 52.3% in the longer regimen vs. 31% in the shorter regimen. However, in women aged 45 or older, the incidence of amenorrhea was unrelated to the duration of treatment (97% vs. 96%). Unfortunately, only few investigators have considered the fact that chemotherapy may cause incomplete ovarian damage resulting in premature menopause months or years after completion of treatment.

4.2 Doxorubicin-based regimens Hortobagyi et al. (1986); Levine et al. (1998); Roche et al. (2006)

The association of doxorubicin with amenorrhea and infertility was initially debatableHortobagyi et al. (1986); Levine et al. (1998). Bines et al. Bines et al. (1996) reported an amenorrhea rate of 34% after therapy with adriamycin and cyclophosphamide (AC). However, these authors did not differentiate between younger and older women. A Canadian adjuvant trial (NCIC CTG MA5) Pritchard et al. (2005) that compared 6 courses of CMF (Standard Bonadonna regimen) (cyclophosphamide 100 mg/m^{2}orally days 1 through 15, methotrexate 40 mg/m^{2}Gracia et al. (2005) intraveinously days 1 and 8 and fluorouracil 60 mg/m^{2}Gracia et al. (2005) intraveinously days 1 and 8) with 6 courses of intensive CEF (cyclophosphamide 75 mg/m^{2}Gracia et al. (2005) orally days 1 through 14, epiadriamycin 60 mg/m^{2}Gracia et al. (2005) intraveinously days 1 and 8 and fluorouracil 500 mg/m^{2}intraveinously day 1 and 8) reported that the incidence of CRA was slightly higher in the CEF arm (51%) than in the CMF arm (42.6%). This difference was observed at 6 months but no difference was observed at 12 months. An interesting conclusion of this study is that late chemotherapy-induced amenorrhea (amenorrhea at 12 months) seems to be associated with improved outcome in premenopausal patients with receptor-positive breast cancer.

4.3 TaxanesAbusief et al. (2006); Berlière et al. (2008); Clemons & Simmons (2007); Davis et al. (2005); Fornier et al. (2005); Martin et al. (2003; 2005); Tham et al. (2007)

Taxanes, including paclitaxel and docetaxel, have recently been introduced in the adjuvant setting of breast carcinoma, based on Phase III data with adjuvant anthracycline and taxane combinations or sequences demonstrating significant benefits compared with non taxane-containing regimensClemons & Simmons (2007); Davis et al. (2005); Fornier et al. (2005); Martin et al. (2003); Roche et al. (2006); Tham et al. (2007). The rates of chemotherapy-induced amenorrhea associated with taxane-based regimens reported by different studies are discordant. Since taxanes are administered either sequentially or concurrently with anthracyclines and cyclophosphamide, it is difficult to evaluate the true impact of taxanes on the development of amenorrhea.

Breast Cancer International Research Group (BCIRG) Trial 001 Martin et al. (2003; 2005) reported an incidence of amenorrhea after adjuvant docetaxel 75 mg/m 2, doxorubicin 50 mg/m^{2}and cyclophosphamide 500 mg/m^2(TAC) 6 courses every 3 weeks or 5-fluorouracil 500 mg/m 2, doxorubicin 50 mg/m^{2}and cyclophosphamide 500 mg/m^2(FAC) of 51.4%

and 32.8%, respectively and the latest update of this trial presented at the San Antonio Breast Cancer Symposium in 2010 with a longer follow-up confirmed these results: 47% of amenorrhea in the TAC group versus 30% in the FAC group (median follow-up 10 years).

In the study by Fornier Fornier et al. (2005), 166 very young patients were reviewed. All patients were treated with AC (doxorubicin at a dose of 60 mg/m 2 + cyclophosphamide at a dose of 600 mg/m^{2}for 4 cycles followed by a taxane). The majority of patients were given AC followed by paclitaxel at a dose of 175 mg/m 2 for 4 cycles administered every 2 - 3 weeks. Only 7 patients received docetaxel (100 mg/m^2). In this cohort, long-term amenorrhea was defined as the absence of menstruations $>$ 12 months after the completion of all chemotherapy. No hormone values were available and the conclusions of this study were that addition of a taxane did not appear to produce a higher rate of chemotherapy-related amenorrhea compared to historical controls.

In the study by Davis et al. Davis et al. (2005), 159 premenopausal patients were reviewed. As initial chemotherapy, 102 women received AC (doxorubicin - cyclophosphamide), 39 received CMF (cyclophosphamide – methotrexate-fluorouracil) and 18 received CAF (cyclophosphamide - doxorubicin - 5-fluorouracil). Following the initial regimen, 51 patients received additional adjuvant chemotherapy, generally with a taxane for 12 weeks (paclitaxel in 32 patients and docetaxel in 19 patients). The conclusions of this study were similar to those of Fornier et al. Fornier et al. (2005): sequential addition of taxanes did not appear to increase the risk of chemotherapy-induced amenorrhea, when added to a non-taxane regimen. Moreover, authors did not find any impact of the type of initial chemotherapy administered. Unfortunately, in this study, no hormone values were available.

More recently, Tham et al. Tham et al. (2007) published a study involving 191 patients (including 158 patients $<$ 40 years of age at the start of chemotherapy). The patients received 4 cycles of AC alone or followed by a taxane. There was no stratification between paclitaxel and docetaxel. The definition of CRA was a little different in this study. Indeed, it was defined as cessation of menses within 1 year of starting chemotherapy and lasting $>$ 6 months.

In a subgroup of young patients ($<$ 40 years), addition of a taxane resulted in a higher incidence of CRA (61% versus 44%). In women over 40 years of age, amenorrhea rates were high in both the group of AC alone and the group of AC followed by a taxane (81% versus 85%). No statistically significant difference was observed between the two groups.

Our team and the team of Toulouse Berlière et al. (2008); Roche et al. (2006) conducted a substudy with patients included in the PACS 01 study. The main objective of our retrospective study was to evaluate the incidence of reversible chemotherapy-related amenorrhea in patients treated with 6FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^{2}and cyclophosphamide 500 mg/m^2) and 3FEC/3D (3 FEC followed by 3 docetaxel 100 mg/m 2), and the impact of sequential docetaxel on the rate of CRA. The incidence of CRA at the end of chemotherapy was similar in the 2 groups: 93% in the 6FEC arm and 92.8% in the 3FEC/3D arm. However, in the year following the end of chemotherapy, more patients recovered menses in the 3FEC/3D arm than in the 6FEC arm: 35% versus 23.7% (p=0.019).

Among the patients for whom hormone values were available, 43% in the 3FEC/3D arm and 29% in the 6FEC arm showed premenopausal levels one year after the end of chemotherapy ($p<0.01$).

5. Prognostic impact of chemotherapy-related amenorrhea

Whether or not induction of amenorrhea by cytotoxic chemotherapy (34-44) is a prognostic factor in the treatment of premenopausal women is still controversial. A positive impact on DFS was found by some Aebi et al. (2000); Borde et al. (2003); Brincker et al. (1987); Del Mastro et al. (1997); Parulekar et al. (2005); Poikonen et al. (2000); Powles (1998); Swain et al. (2010) but not confirmed by others Ferretti et al. (2006); Vanhuyse et al. (2005). Del Mastro et al Del Mastro et al. (1997) conducted a review of 13 studies involving 3929 patients undergoing CMF-based regimens, with follow- up ranging from 3 to 20 years. A statistically significant association was found between the development of chemotherapy-related amenorrhea and DFS. In the majority of cases, OS was found to be associated with amenorrhea (in 3 out of 5 studies reviewed). In a study recently published by Parulekar et al. Parulekar et al. (2005), similar results were observed with intensive CEF (cyclophosphamide, epirubicin, 5-fluorouracil therapy), which induced a higher rate of amenorrhea than the classic CMF protocol, but OS was also better in the CEF arm than in the CMF arm.

In the Trial VI study by the International Breast Cancer Study Group (IBCSG)Goldhirsch et al. (1990) cessation of menses, even for a limited time period, appeared to be beneficial, especially in patients with ER-positive breast tumors. In this study, however, the greatest effect was observed in patients receiving suboptimal treatment with only 3 initial CMF courses. Bonadonna et al.Pagani et al. (1998) exhibits a different point of view. Their analysis of the influence of drug-induced amenorrhea on the therapeutic outcome after CMF treatment refutes the hypothesis that adjuvant chemotherapy acts merely as chemical castration. As reported in many individual trials and the worldwide overview, adjuvant chemotherapy benefits hormone-responsive and hormone-unresponsive tumors, whereas endocrine therapy has no worthwhile benefit in E2 receptor-negative subpopulations. Bonadonna et al. Bonadonna et al. (2005) estimates that adjuvant chemotherapy has cytotoxic effects regardless of the putative hormone dependency of the tumor cells.

In the PACS 01 trial Roche et al. (2006), a survival advantage in favor of the 3FEC/3D arm was observed only for women aged over 50 years, but not for the younger population. The reason for this is unclear but the impact of reversible amenorrhea needs to be investigated further, since our small retrospective analysis suggests that amenorrhea Berlière et al. (2008) was correlated with DFS in the 3FEC/3D group. In the NSABP B30 trialGanz et al. (2011), 5531 breast cancer patients were randomly assigned to sequential doxorubicin (A) 20 mg/m 2 + cyclophosphamide (C) 600 mg/m 2 4 courses every 3 weeks followed by docetaxel (T) 100 mg/m^{2}4 courses every 3 weeks, or concurrent TAC (docetaxel 75 mg/m^2, doxorubicin 50 mg/m^{2}and cyclophosphamide 500 mg/m^{2}4 courses every 3 weeks) or 4 cycles of AT (doxocuribin 50 mg/m 2 + docetaxel 75 mg/m 2 every 3 weeks). Tamoxifen was administered in all patients with E2 hormone receptor positive tumors. The results indicated that patients with more than 6 months amenorrhea had a better prognosis than patients with a shorter period of amenorrhea.

6. Assessing post-chemotherapy ovarian function

Assessing post-chemotherapy ovarian function in breast cancer survivors of late reproductive age is important to clinical decision making on a range of issues such as choice of endocrine therapy Berlière et al. (2010); Ganz et al. (2011); Su (2010). It is therefore important to analyze the different available tools.

Currently, the primary tool for assessing post-chemotherapy ovarian function is menstrual pattern. However, in patients who received chemotherapy and endocrine therapy, lack of menses does not always represent ovarian failure.

In an abstract presented in poster form at the San Antonio Breast Cancer Symposium in 2010 Berlière et al. (2010), we reported the results of a prospective study conducted in our Breast Clinic between 1999 and 2003, comparing ovarian function between premenopausal breast cancer patients receiving tamoxifen alone (group I) and those receiving tamoxifen following chemotherapy (group II). 138 premenopausal patients, treated for early breast cancer were included: 68 patients in the group of tamoxifen alone and 70 patients in the group of tamoxifen administered after chemotherapy (6 cycles of FEC 100 on day 1 every 3 weeks – 5- fluorouracil 500 mg/m^2, epirubicin 100 mg/m^{2}and cyclophosphamide 500 mg/m 2 – or 4 cycles of EC on day 1 every 3 weeks – epiadriamycin 75 mg/m 2 and cyclophosphamide 500 mg/m^2). All patients had a confirmed premenopausal status (biological data) at the entry of the study or 3 months later. Three patients were out of study in groups I and 2 were out of study in group II. The results of this prospective study were analyzed at the end of 2009. We identified 4 different ovarian patterns of response to tamoxifen in the 2 groups:

1. regular menses ($>$ 10 cycles/year)
2. oligomenorrhea (5 to 9 cycles/year)
3. severe oligomenorrhea(1 to 4 cycles/year)
4. complete amenorrhea.

The number of patients in each subgroup was respectively

- for group I (65 patients): 3 (4%), 19 (29%), 38 (58%) and 5 (8%)
- for group II (68 patients): 2 (3%), 21 (30%), 38 (55%) and 7 (10%).

We confirmed that amenorrhea is an insufficient parameter to define menopausal status in patients receiving tamoxifen. The most common biomarkers used are FSH and E2. Low E2 levels are also insufficient to define menopause because while on tamoxifen therapy patients can exhibit low E2 levels, low FSH levels and oligomenorrhea. These data are very important in the choice of endocrine therapy.

Measurement of ovarian reserve in breast cancer survivors may increase understanding of a woman's reproductive potential after cancer chemotherapy Partridge et al. (2008; 2010); Su (2010). Reproductive potential is generally related both to the quantity and quality of ovarian primordial follicles. Several other markers of ovarian reserve have been evaluated including early follicular phase serum E2, FSH, AMH and inhibin-B as well as measurements of antral follicle count (AFC) and ovarian volume. The interesting study of Partridge et al. Partridge et al. (2010) confirms that young breast cancer survivors who have undergone cytotoxic chemotherapy and remain premenopausal have diminished AFC levels when compared with healthy controls. This study also reveals that a lower level of AMH appears to be the best serum predictor of diminished AFC Partridge et al. (2008; 2010), which is thought to reflect reduced likelihood of future pregnancy. AMH, the anti-Müllerian hormone, is a member of the transforming growth factor β (TGFβ) family and is produced by FSH-sensitive early antral follicles. In this way, it may be a more sensitive predictor of ovarian reserve than other markers, such as AFC and inhibin-B, which detect more mature primordial follicles. Some studies exhibit interesting results Anderson & Cameron (2011); Domingues et al. (2010); Knauff et al. (2009); Rosendahl et al. (2010); Yu et al. (2010). In the study of

Partridge Domingues et al. (2010); Partridge et al. (2010), AFC and AMH seem to be the best markers of ovarian reserve. These two markers are highly correlated and for breast cancer patients receiving tamoxifen, lower AFC, AMH and inhibin-B were observed than for non-tamoxifen-treated survivors.
However, it is important to insist on the fact that breast cancer survivors can become pregnant with undetectable levels of AMH Anderson & Cameron (2011). In our institution, this year and the year before, 4 patients (30, 39, 36 and 30 years respectively) became pregnant with undetectable levels of AMH, but we have no values before the start of chemotherapy. Some variations of AMH while on metformin, tamoxifen and aromatase inhibitors have been described Cordes et al. (2010); Dieudonné et al. (2011); Panidis et al. (2010).
In conclusion, AMH seems to be the most interesting biological marker but further prospective studies are needed to evaluate the exact value of the different markers (AFC, AMH and inhibin-B). In the future, women interested in post-treatment fertility may be able to undergo ovarian reserve testing before and upon completion of systemic therapy. Prospective studies are needed to determine the predictive values of these tests for pregnancy after chemotherapy as well as the potential value in predicting premature menopause in young cancer survivors. Quality of life outcomes also need to be investigated prospectively Knobfm (2006).

7. Mechanisms of ovarian injury

The follicular reserve within the ovaries consists mainly of quiescent primordial follicles developed during fetal life. A tremendous number of primordial follicles will be annihilated before or shortly after birth and throughout postnatal life by a physiological programmed cell death process named apoptosis Faddy & Gosden (1995). This physiological cellular machinery may predispose the follicles to apoptosis induced by exogenous signals, such as chemotherapeutic agents. The first histological study performed on human ovaries after chemotherapy Browne et al. (2011); Meirow et al. (2007; 1999) demonstrated that the end result of chemotherapy was ovarian atrophy and global loss of primordial follicles. But the effect of chemotherapy on the ovary is not an “all or nothing phenomenon”. The mechanisms involved in the loss of primordial follicles in response to anticancer therapy are not well understood. A few human and animal studies Faddy & Gosden (1995); Meirow et al. (1999) demonstrated that chemotherapy-induced damage to ovarian pregranulosa cells and that apoptosis occurred during oocyte and follicle loss.
The results of a study conducted by Meirow et al. Meirow et al. (2007) indicate that injury to blood vessels and focal fibrosis of the ovarian cortex are present in ovaries of patients previously exposed to chemotherapy. These modes of injury were present in non-atrophic ovaries of patients that were not sterilized by chemotherapy.
Ben Aharon and his coworkers Ben-Aharon et al. (2010) evaluated the effects of doxocuribin (injected intraperitoneally) on mice ovaries. A single injection of doxorubicin resulted in a major reduction in both ovarian size and weight that lasted even one month post-treatment. A dramatic reduction in ovulation rate was also observed one week after treatment, followed by a partial recovery at one month. In an attempt to characterize the apoptotic effect of doxorubicin on the ovary, the authors were able to detect apoptosis in histological sections of mice ovaries by depicting caspase-3 activity and TUNEL staining. The authors observed in the doxorubicin-treated mice a loss of premature follicles as well as perivascular changes already

described in human ovaries following administration of other chemotherapeutic agents such a cyclophosphamide and cisplatin Browne et al. (2011); Meirow et al. (1999).
In a study conducted in our laboratory, mice were injected intraperitoneally with a single dose of cyclophosphamide (200 mg/kg). These experiments did not allow us to identify an increase of apoptosis in mice ovaries treated with cyclophosphamide.
Other previous studies also showed that a single dose of cyclophosphamide was not associated with an increased rate of apoptosis. This is why in our laboratory, we will repeat the injections of cyclophosphamide in new experiments to try to observe apoptosis in mice ovaries.
The studies of Meirow et al. Meirow et al. (2007) and Aharon Ben-Aharon et al. (2010) hypothesized a combined mechanism of neovascularization and ovarian tissue scarring with a direct toxic effect on the primordial follicles.
Personally, we think that additional processes that lead to ovarian damage and follicles loss after chemotherapy may be involved such as vascular complications and ischemic mechanisms. We have also planned to investigate these mechanisms on mice ovaries. Other drugs such as taxanes need also to be studied to elucidate ovarian toxicity of modern drugs and to give accurate information to young breast cancer patients.

8. Conclusion

This chapter was written after review of the literature (Pubmed research) and after analysis of personal data (PACS 01 substudy, evaluation of ovarian function while on tamoxifen, personal laboratory experiments). Our review highlights important difficulties:

- The definition of chemotherapy-related amenorrhea suffers from a lack of uniformity in the literature.
- Many studies are retrospective and evaluate old chemotherapy regimens. In the prospective studies, the duration of follow-up is too short and very often limited to anamnestic data.
- We thus recommend a prospective evaluation of endocrine function before chemotherapy and after treatment completion, and in this context we have initiated a multicentric prospective study. Follow-up has to last for a very long time (10 years minimum).
- The impact of taxanes on ovarian function requires further studies and laboratory studies.

Correct estimation of the risk of menopause and possibilities for preserving fertility according to age and treatment will facilitate the decision-making process regarding adjuvant therapy in breast cancer. This process requires precise information and will enable each patient to balance the potential benefits of treatment against the potential adverse effects and future risk.

9. References

Abusief, M., Missmer, S., Ginsburg, Weeks, J., Wiener, E. & Partridge, A. (2006). The effect of paclitaxel, dose density and trastuzumab in chemotherapy-related amenorrhea (cra) in premenopausal women with breast cancer, *San Antonio* p. Abstract 2079.

Aebi, S., Gelbert, S., Castiglione-Guertsh, M., Gelbert, R., Collins, J., Thürlimann, B., Rudenstam, C., Lindtner, J., Crivellari, D., Cortes-Funes, H., Simoncini, E., Werner, I., Coates, A. & Goldhirsch, A. (2000). Is chemotherapy alone adequate for young women with estrogen receptor-positive breast cancer?, *Lancet* 355: 1869–1874.

Anderson, R. & Cameron, D. (2011). Pretreatment serum Anti-müllerian hormone predicts long- term ovarian function and bone mass after chemotherapy for early breast cancer, *J Clin Endocrinol Metab* 96(5): 1333–43.

Ben-Aharon, I., Bar-Joseph, H., Tzarfaty, G., Kuchinsky, L., Eizel, S., Stemmer, S. & Shalbi, R. (2010). Doxorubicin-induced ovarian toxicity, *Reprod Biol Endocrinol* 8: 20.

Berlière, M., Dalenc, F., Malingret, N., Vindevogel, A., Pierre, P., Roche, H., Donnez, J., Symann, M., Kerger, J. & Machiels, J. (2008). Incidence of reversible amenorrhea in women with breast cancer undergoing adjuvant anthracyclin-based chemotherapy with or without docetaxel, *BMC Cancer* 8: 56.

Berlière, M., Dalenc, F., Piquard, N., Leconte, I., Fellah, L., Baurain, J., Galant, C., Duhoux, F. & Machiels, J. (2010). Tamoxifen and ovarian function, *San Antonio,* Abstract n°9544-13.

Bines, J., Oleske, D. & Cobleigh, M. (1996). Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer., *J Clin Oncol* 14: 1718–1729.

Bonadonna, G., Moliterni, A., Zambetti, M., Daidone, M., Pilotti, S., Gianni, L. & Valagussa, P. (2005). 30 years' follow-up of randomised studies of adjuvant CMF in operable breast cancer, *BMJ* p. BMJ 10.1136/BMJ 38314. 6220958F.

Borde, F., Chapelle-Marcilac, I., Fumoleau, P., Hery, M. & Roché, H. (2003). Role of chemotherapy- induced amenorrhea in premenopausal node-positive operable breast cancer patients: 9-year follow-up results of french adjuvant study group, *FASG data base. Breast Cancer Res Treat* 1982, S30.

Brincker, H., Rose, C., Rank, F., Mouridsen, H., Jacobsen, A., Dombernowsky, P., Panduro, J. & Andersen, K. (1987). Evidence of a castration-mediated effect of adjuvant cytotoxic chemotherapy in premenopausal breast cancer, *J Clin Oncol* 5: 1771–1778.

Broekmans, L., Soules, M. & Fauser, B. (2009). Ovarian aging: mechanisms and clinical consequences, *Endoc Rev* 30(5): 463–93.

Browne, H., Moon, K., Mumford, S., Schisterman, E., Decherney, A., Segars, G. & Armstrong, A. (2011). Anti-müllerian marker of acute cyclophosphamide-induced ovarian follicular destruction in mice pretreated with cetrorelix, *Fertil Steril* .

Burger, H., Hale, G., Dennerstein, L. & Robertson, D. (2008). Cycle and hormone changes during perimenopause: the key role of ovarian function, *Menopause* 15(4): 603–12.

Burger, H., Hale, G., Robertson, D. & Dennerstein, L. (2007). A review of hormonal changes during the menopausal transition: focus on findings from the melbourne women's midlife health project., *Hum Reprod Update* 13: 559–65.

Clemons, M. & Simmons, C. (2007). Identifying menopause in breast cancer patients: considerations and implications, *Breast Cancer Res. Treat.* 104: 115–120.

Cordes, T., Schultz-Mosgau, A., Diedrich, K. & Griesinger, G. (2010). Ongoing pregnancy after human menopausal gonadotrophin stimulation and timed intercourse in 40 years old women with undetectable Anti-müllerian hormone levels, *Fertil Steril* 94(4): e71–2.

Davis, L., Klitus, M. & Mintzer, D. (2005). Chemotherapy-induced amenorrhea from adjuvant breast cancer treatment: the effect of the addition of taxanes, *Clin. Breast Cancer* 6(5): 421–424.

Del Mastro, L., Venturini, M., Sertoli, M. & Rosso, R. (1997). Amenorrhea induced by adjuvant chemotherapy in early breast cancer patients: prognostic role and clinical implications, *Breast Cancer Res Treat* 43: 189–190.

Dieudonné, A., Vandenberghe, J., Geerts, I., Billen, J., Paridaens, R., Wildiers, H. & Neven, P. (2011). Undectable Anti-müllerian hormone levels and recovery of

chemotherapy-induced ovarian failure in women with breast cancer on an oral aromatase inhibitor, *Menopause* p. 12.

Domingues, T., Rocha, A. & Serafini, P. (2010). Test for ovarian reserve: reliability and utility, *Curr Opin Obstet Gynecol* 22(4): 271–6 (review).

Faddy, M. & Gosden, R. (1995). A mathematical model of follicle dynamics in the human ovary, *Hum Reprod* 10: 770–5.

Ferretti, G., Carlini, P., Bria, E., Felici, A., Giannarelli, D., Ciccarese, M., Papaldo, P., Fabi, A. & Cognetti, F. (2006). Chemotherapy-induced amenorrhea in early breast cancer, *Ann Oncol* 17(2): 352.

Fornier, M., Modi, S., Panageas, K., Norton, L. & Hudis, C. (2005). Incidence of chemotherapy-induced, long-term amenorrhea in patients with breast carcinoma aged 40 years and younger after adjuvant anthracycline and taxane, *Cancer* 104(8): 1575–1579.

Ganz, P., Land, S., Geyer, C. J., Scecchini, R., Costantino, J., Pajon, E., Fehrenbacher, L., Atkins, J., Polikoff, J., Vogel, V., Erban, J., Livingston, R., Perez, E., Mamounas, E., Wolmark, N. & Swain, M. (2011). Menstrual history and quality of life outcomes in women with node positive breast cancer treated with adjuvant therapy in the NSABP B30 trial, *J Clin Oncol* 29(9): 1110–16.

Goldhirsch, A., Gelber, R. & Castiglione, M. (1990). The magnitude of endocrine effects of adjuvant chemotherapy for premenopausal breast cancer patients: the international breast cancer study group, *Ann Onco* 1: 183–188.

Gracia, C., Sammel, M., Freeman, E., Lin, H., Langan, E., Kapoor, S. & al (2005). Defining menopause status: creation of a new definition to identify the early changes of the menopausal transition., *Menopause* 12: 128–35.

Guidelines (2010). Breast cancer NCC and practice guidelines in oncology.

Hortobagyi, G., Buzdar, A., Marcus, C. & Smith, T. (1986). Immediate and long-term toxicity of adjuvant chemotherapy regimens containing doxorubicin in trials at M.D Anderson hospital and tumor institute, *J Natl Cancer Inst Monogr* 1: 105–109.

Knauff, E., Eijkemans, N., Lambalk, C., ten Kate-Booij, N., Hoek, A., Beerendonk, C. & Fauser, B. e. a. (2009). Anti-müllerian hormone, inhibin-b and antral follicles count in young women with ovarian failure, *Dutch Premature Ovarian Failure Consortium. J Clin Endocrinol Metab* 94(3): 786–92.

Knobfm, T. (2006). The influence of endocrine effects of adjuvant therapy on quality of life outcomes in younger breast cancer survivors, *The Oncologist* 11: 96–110.

Ledger, W. (2010). Clinical utility of measurement of Anti-müllerian hormone in reproductive endocrinology, *J Clin Endocrinol Metab* 95(12): 5144–54.

Levine, M., Brawell, V., Pritchard, K., Norris, B., Shepherd, L., Abu-Zahra, H., Findlay, B., Warr, D., Bowman, D., Myles, J., Arnold, A., Vandenberg, T., Mackenzie, R., Robert, J., Ottaway, J., Burnell, M., Williams, C. & Tu, D. (1998). Randomized trial of intensive cyclophosphamide, epirubicin and fluorouracil chemotherapy compared with cyclophosphamide, methotrexate and fluorouracil in premenopausal women with node- positive breast cancer, *National Cancer Institute of Canada. Clinical Trial School. J Clin Oncol* 16: 2651–2658.

Martin, M., Pienkowski, T., Mackey, J., Pawlicki, M., Guastalla, J., Weaver, C., Tomiak, E., Al-Tweigeri, T., Chap, L., Juhos, E., Guevin, R., Owell, A., Fornander, T., Hainsworth, J., Coleman, R., Vinholes, J., Modiano, M., Pinter, T., Tang, S., Colwell, B., Prady, C., Provencher, L., Walde, D., Rodriguez-Lescure, A., Hugh, J., Loret, C., Rupin, M., Blitz,

S., Jacobs, P., Murawsky, M., Riva, A. & Vogel, C. (2003). TAC improves disease-free survival and overall survival over FAC in node-positive early breast cancer patients, BCIRG 001, *SABCS* .

Martin, M., Pienkowski, T., Mackey, J., Pawlicki, M., Guastalla, J., Weaver, C., Tomiak, E., Al-Tweigeri, T., Chap, L., Juhos, E., Guevin, R., Owell, A., Fornander, T., Hainsworth, J., Coleman, R., Vinholes, J., Modiano, M., Pinter, T., Tang, S., Colwell, B., Prady, C., Provencher, L., Walde, D., Rodriguez-Lescure, A., Hugh, J., Loret, C., Rupin, M., Blitz, S., Jacobs, P., Murawsky, M., Riva, A. & Vogel, C. (2005). Adjuvant docetaxel for node-positive breast cancer, *N Engl J Med* 352(22): 2302–12.

Meirow, D. (2000). Reproduction post-chemotherapy in young cancer patients, *Mol Cel Endocrinol* 169: 123–131.

Meirow, D., Kaufman, B., Shrim, A., Rabinovici, J., Schiff, L., Raanani, H., Lebron, J. & Fridman, E. (2007). Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy: potential mechanisms of ovarian injury, *Hum Reprod* 22: 1626–33.

Meirow, D., Lewis, H., Nugent, D. & Epstein, M. (1999). Subclinical depletion of primordial follicular reserve in mice treated with cyclophosphamide: clinical importance and proposed accurate investigative tool, *Hum Reprod* 14: 1903–07.

Padmanabhan, N., Howell, A. & Rubens, R. (1986). Mechanism of action of adjuvant chemotherapy in early breast cancer, *Lancet* 2: 411–414.

Pagani, O., O'Neill, A., Castiglione, M., Gelber, R., Goldhirsch, A., Rudenstam, C., Lindtner, J., Collins, J., Crivellari, D., Coates, A., Cavalli, F., Thürlimann, B., Simoncini, E., Fey, M., Price, K. & Senn, H. (1998). Prognostic impact of amenorrhea after adjuvant chemotherapy in premenopausal breast cancer patients with axillary node involvement: results of the international breast cancer study group (IBCSG) trial VI, *Eur J Cancer* 34(5): 632–640.

Panidis, D., Geopoulos, N., Piouka, A., Katsikis, I., Saltamavros, A., Devalas, G. & Diamanti-Kandarakis, E. (2010). The impact of oral contraceptives and metformine on Anti-müllerian hormone serum levels in women with polycystic ovari syndrome and biochemical hyperandrogenemia, *Gynecol Endocrinol* p. 14.

Partridge, A., Gelber, S., Peppercorn, J., Ginsburg, E., Sampson, E., Rosenberg, Przypyszny, M. & Winger, E. (2008). Fertility and menopausal outcomes in young breast cancer survivors, *Clin Breast Cancer* 8: 65–9.

Partridge, A., Ruddy, K., Gelber, S., Schapira, L., Abusief, M., Meyer, M. & Ginsburg, E. (2010). Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer, *Fertil Steril* 94(2): 638–44.

Parulekar, W., Day, A., Ottaway, J., Shepherd, L., Trudeaun, M., Bramwell, V., Levine, M. & Pritchard, K. (2005). Incidence and prognostic impact of amenorrhea during adjuvant therapy in high-risk premenopausal breast cancer: analysis of a national cancer institute of canada clinical trials group study / NCIC CTG MA.5, *J Clin Oncol* 23: 6002–6008.

Poikonen, P., Saarto, T., Elomaa, I., Joensuu, H. & Blomqvist, C. (2000). Prognostic effect of amenorrhea and elevated serum gonadotrophin levels induced by adjuvant chemotherapy in premenopausal node-positive breast cancer patients, *Eur J Cancer* 36: 43–48.

Powles, T. (1998). Prognostic impact of amenorrhea after adjuvant chemotherapy, *Eur J Cancer* 34: 603–605.

Pritchard, K., Levine, M., Bramwell, V., Shepherd, L., Tu, D. & Paul, N. (2005). A randomised trial comparing CEF to CMF in premenopausal women with node positive breast cancer. update of NCIC CGT MA5 (national cancer institute of canada clinical trials group trial MA5), *J. Clin. Oncol* 23(22): 5166–70.

Randolph, J., Crawford, S., Dennerstein, L., Cain, K., Harlow, S., Little, R., Mithcell, E., B, N., Taffe, J. & Yosef, M. (2006). The value of follicle-stimulating hormone concentration and clinical findings as markers of the late menopausal transition., *J Clin Endocrin Metab* 91: 3034–40.

Roche, H., Fumoleau, P., Spielmann, M., Canon, J., Delozier, T., Serin, D., Symann, M., Kerbrat, P., Soulié, P., Eichler, F., Viens, P., Monnier, A., Vindevoghel, A., Campone, M., Goudier, M., Bonneterre, J., Ferrero, J., Martin, A., Geneve, J. & Asselain, B. (2006). Sequential adjuvant epirubicin-based and docetaxel chemotherapy for node-positive breast cancer patients: the FNLCC PACS 01 trial, *J Clin Oncol* 24: 5664–5671.

Rosendahl, M., Andersen, C., La Cour Freiesleben, N., Juul, A., Loss, K. & Andersen, A. (2010). Dynamics and mechanisms of chemotherapy-induced ovarian follicular depletion in women of fertile age, *Fertil Steril* 94(1): 156–66.

Soules, M., Sherman, S., Parrott, E., Rebar, R., Santoro, N., Utian, W. & Woods, N. (2001). Stages of reproductive aging workshop (STRAW), *J Women's Health Gend Based Med* 10: 843–8.

Sowers, M., Eyvazzadeh, A., Mc Connell, D., Yosef, M., Jannausch, M., Zhang, D., Harlow, S. & Randolph, J. (2008). Anti-müllerian hormone and inhibin-b in the definition of ovarian aging and the menopause transition, *J Clin Endocrinol Metab* 93: 3478–83.

Su, H. (2010). Measuring ovarian function in young cancer survivors, *Minerva Endocrinol* 35(4): 259–70 (review).

Swain, S., Jeong, J. & Wolmark, N. (2010). Amenorrhea from breast cancer therapy – not a matter of dose, *N Engl J Med* 363(23): 2268–70.

Tanay, N. & Fenton, A. (2010). Can we predict the age of menopause transition?, *Climacteric* 13(6): 507–8.

Tham, Y., Sixton, K., Weiss, H., Elledge, R., Friedman, L. & Krame, R. (2007). The rates of chemotherapy-induced amenorrhea in patients treatd with adjuvant doxorubicin and cyclophosphamide followed by taxane, *Am. J. Clin. Oncol* 30: 126–132.

Valagussa, P., Moliterni, A., Zambetti, M. & Bonadonna, G. (1993). Long-term sequelae from adjuvant chemotherapy: recent results, *Cancer Res* 127: 247–255.

Vanhuyse, M., Fournier, C. & Bonneterre, J. (2005). Chemotherapy-induced amenorrhea influence on disease-free survival and overall survival in receptor-positive premenopausal early breast cancer patients, *Ann Oncol* pp. 1283–1288.

Vegetti, W., Marozzi, A., Manfredini, E., Testa, G., Alagna, F., Nicolosi, A., Caliari, L., Taborelli, M., Tibiletti, M., Dalpra, L. & Crosignani, P. (2000). Premature ovarian failure, *Mol Cell Endocrinol* 161: 53–57.

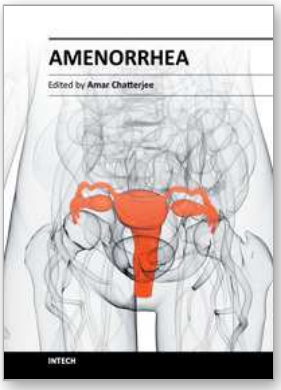
Walshe, J., Denduluri, N. & Swain, S. (2006). Amenorrhea in premenopausal women after chemotherapy, *J Clin Oncol* 24: 5769–5779.

Warne, G., Feirley, K., Hobbs, J. & Martin, F. (1973). Cyclophosphamide-induced ovarian failure, *New Engl J Med* 289: 1159–1162.

Welt, C., Jimenez, Y., Sluss, P., Smith, P. & Hall, J. (2006). Control of estradiol secretion in reproductive aging., *Hum Reprod* 21(8): 2189–93.

Yang, Y., Hur, N., Kim, S. & Yong, K. (2011). Correlation between sonographic and endocrine markers of ovarian aging as predictors for late menopausal transition, *Menopause* 18: 238–45.

Yu, B., Douglas, N., Ferin, M., Nakhuda, G., Crew, K., Lobo, R. & Hershman, D. (2010). Changes in markers of ovarian reserve and endocrine function in young women with breast cancer undergoing adjuvant chemotherapy, *Cancer* 116(9): 2099–105.



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This book on "Amenorrhea" is a wonderful collection of updated reviews dealing mostly with the aphysiological aspects of secondary amenorrhea. The book represents a collection of eight chapters, each chapter in the book is written by the international experts with extensive experience in the areas covered. We hope that readers will find this book interesting, helpful and inspiring.

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Phone: +385 (51) 770 447
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821