Anti-Müllerian hormone: determination of ovarian reserve in early breast cancer patients

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Abstract

Breast cancer is the most common invasive cancer in women of reproductive age. In young women, chemotherapy may induce amenorrhea: it is still uncertain how to assess menopausal status in these patients despite the importance of its definition for choosing appropriate endocrine treatment. In the development of sensitive biomarkers for fertility and ovarian reserve, anti-Müllerian hormone (AMH) is considered a promising marker of ovarian reserve. The clearest data regarding a clinical use of AMH are related to the measurement of the ovarian pool in women who undergo IVF: the available data, also in breast cancer patients, seem to suggest that AMH measurement, before gonadotropin administration, can be a useful marker for the prediction of women at risk for poor-response or no response to ovarian stimulation. The utility of AMH as a potential marker of chemotherapy-induced ovarian follicular depletion and an early plasma marker of chemotherapy-induced gonadal damage has been evaluated both in young women after treatment for cancer in childhood and in young survivors of hematological malignancies and solid tumors. Several studies have demonstrated a potential utility of AMH, inhibin, or follicle-stimulating factor as biomarkers predicting infertility risk in breast cancer patients, but the studies conducted so far are not conclusive. Further studies are needed in order to define the regimen-specific action of chemotherapy on AMH levels, the percentage of post-treatment recovery of plasma levels of the hormone, and the relationship between menopausal status and AMH.

Key Words
► breast
► growth factor
► hormone action
► oncology

Introduction

During the last 50 years, we have witnessed a rise in the mean age at which women deliver their first child (Johnson *et al*. 2012). Female fertility begins to decline from the early 1920s due to decreasing ovarian reserve and for this reason, the infertility problem is now more common (Gougeon *et al*. 1994, te Velde & Pearson 2002).

Breast cancer is the most common invasive cancer seen in women of reproductive age (Smigal *et al*. 2006):

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~6% of women with breast carcinoma are diagnosed before the age of 40 (Surveillance, Epidemiology and End Results (SEER) web site, available online: http://www.seer.cancer.gov) and recent data have shown that the incidence of breast cancer diagnosed in young women is increasing (Merlo et al. 2012). The increasing number of earlier diagnosis and adjuvant therapy are expected to increase the number of breast cancer survivors (Jemal et al. 2003, Ganz & Hahn 2008). In young women, chemotherapy may induce amenorrhea and it is still uncertain how to assess menopausal status in these patients despite the fact that the definition of the menopausal status is relevant for choosing appropriate antihormonal treatment (Amir et al. 2009, 2010).

As greater emphasis is placed on the quality of life of breast cancer survivors due to higher survival rates, fertility preservation has become a key component of cancer care (Gracia & Jeruss 2013, Rodriguez et al. 2013, Salama et al. 2013). In the development of sensitive biomarkers for fertility and ovarian reserve, anti-Müllerian hormone (AMH) is considered a promising marker of ovarian reserve (Anderson et al. 2012, Garcia et al. 2012).

The assessment of ovarian function and reserve, the chance of fertility preservation, and the role of AMH are emerging topics in premenopausal women with breast cancer who undergo chemotherapy and hormonal treatment.

Determination of menopausal status in patients treated for early breast cancer

Menopause is a cornerstone both in breast cancer pathophysiology and in clinical management (Clemons & Simmons 2007). For example, late menopause is traditionally considered as a risk factor for breast cancer development and is normally defined as the absence of menstrual periods for 12 consecutive months with no cause (Randolph et al. 2006).

National Comprehensive Cancer Network (NCCN) criteria consider the following to determine menopause: age ≥ 60 or a prior bilateral oophorectomy, or age < 60 years and amenorrhea for ≥ 12 months in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression and follicle-stimulating hormone (FSH) and estradiol (E_2) levels in postmenopausal range (NCCN Guidelines, available at: http://www.nccn.org/professional/physician_gls/f_guidelines.asp).

Adjuvant chemotherapy in premenopausal patients can cause amenorrhea and decrease ovarian reserve (Wallace et al. 2005). The incidence of a return of menses after adjuvant chemotherapy is a function of age and regimen type (Amir et al. 2009, 2010).

If premenopausal status is present at the beginning of chemotherapy, amenorrhea is not a reliable indicator of menopause (Smith et al. 2006). Therefore, in breast cancer management, menopausal status can be defined as the permanent and profound decrease in ovarian estrogen synthesis.

Biochemical tests can help in the measurement of hormonal levels, but these tests are limited because serial determinations over time are needed and there are no cutoff points for FSH, luteinizing hormone (LH), and E_2.

Furthermore, the use of tamoxifen after chemotherapy could be associated with a marked fall in FSH levels in patients with chemotherapy-induced amenorrhea. However, the effect of tamoxifen on hormone levels is not clear. E_2 levels can be elevated as result of the cross-reactivity of tamoxifen and its metabolites in the E_2 assay (Lum et al. 1997). Generally, hormone levels may not reliably reflect menopausal status in breast cancer patients on tamoxifen (Hadji et al. 2012).

AMH and fertility

AMH is a glycoprotein of the transforming growth factor β family that seems to reflect the continuous non-cyclical growth of small follicles (Massagué 1990) and can be considered an indirect index of the size of the resting primordial follicle pool. Accordingly, AMH represents a marker of ovarian reserve. In females, AMH is produced by granulosa cells and released into the circulation from the time of birth until menopause (Rajpert-De Meyts et al. 1999).

The major production is seen at the preantral and small antral follicle stages, and its expression decreases as follicles reach antral and preovulatory stages (Weenen et al. 2004).

An important role of AMH is to protect the number of primordial follicles by inhibiting their rapid recruitment, sparing the follicle pool exhaustion (Durlinger et al. 1999). In addition, AMH prevents premature depletion of the follicle pool by decreasing follicular sensitivity to FSH. By this negative influence on initial recruitment, AMH controls the number of preantral and small antral follicles that do undergo atresia and continue to grow into the preovulatory stage (Durlinger et al. 2001).

It is difficult to measure ovarian reserve directly, but hormonal markers (FSH, E_2, and inhibin B) and ultrasonographic tests (antral follicle count (AFC) and measurement

of ovarian volume) can help to determine, indirectly or directly, the size of the antral follicle pool.

FSH, E_2, and inhibin B indirectly reflect ovarian reserve, but their cyclical fluctuations tend to make the determination of ovarian reserve difficult. In contrast, AMH better reflects the longitudinal decline of oocyte/follicle pool over time (La Marca et al. 2006). Indeed, AMH levels are stable across the menstrual cycle and are not affected by pregnancy or treatment with gonadotropin-releasing hormone agonists. In addition, although an effect of combined oral contraceptive pill on ovarian reserve has been described (Bentzen et al. 2012, Deb et al. 2012, Kallio et al. 2013), its use seems to be associated with minimal suppression of the later FSH-dependent stages of follicle development (Arbo et al. 2007, Streuli et al. 2008, Sowers et al. 2010, Li et al. 2011, Shaw et al. 2011). It is still debated if clinical and behavioral variables, such as BMI and smoking habit, may influence AMH plasma levels.

AMH and assisted reproductive technology

Currently, much of the value of AMH lies in its relation to the declining ovarian pool with age, and thus its potential ability to predict future reproductive outcomes. The clearest data regarding a clinical use of AMH are related to the measurement of the ovarian pool in women who undergo IVF. In recent years, extremely interesting data have been published showing the possible clinical application of AMH measurement in the prediction of quantitative and qualitative ovarian response in assisted reproductive technologies (ART; La Marca et al. 2010). Several retrospective and prospective studies have found a strong positive correlation between the number of retrieved oocytes and basal AMH serum levels in women undergoing ovarian stimulation (La Marca et al. 2010).

Elevated plasma AMH levels are associated with better fertility rates (Lee et al. 2009). Seifer et al. (2002), the first authors reporting an association between serum AMH levels and ovarian response to gonadotrophin, showed that AMH levels were 2.5-fold higher in patients with at least 11 oocytes compared with those with six oocytes or fewer retrieved. These results were successively confirmed by many authors in several retrospective and prospective studies.

AMH seems to reflect not only quantitative but also qualitative ovarian responsiveness in ART. Several studies found a significant positive correlation between AMH levels and the quality of oocytes (Hazout et al. 2004, Ebner et al. 2006, Silberstein et al. 2006, Cupisti et al. 2007, Fanchin et al. 2007, Lekamge et al. 2007) and embryo

morphology (Silberstein et al. 2006). However, this relationship has not been confirmed by other authors (Smeenk et al. 2007, Lie Fong et al. 2008a), and the predictive value of serum AMH on oocyte competence and embryo quality remains controversial (La Marca et al. 2010).

The possible correlation between AMH levels and embryo/oocyte cryopreservation cycle (ECC) outcomes has also been evaluated in breast cancer patients undergoing stimulation with letrozole and FSH for fertility preservation (Lee et al. 2011). Lee et al. retrospectively analyzed the correlation between AMH levels during the early-follicular phase of the menstrual cycles and AFC and ECC outcomes in 41 women with breast cancer before adjuvant treatment. AMH and AFC showed a stronger correlation with the total number of oocytes and the number of mature oocytes retrieved than age, FSH, and inhibin B levels. Patients with an ovarian response to stimulation classified as low (less than four mature oocytes retrieved) had AMH levels ≤ 1.2 ng/ml; in contrast, AMH level >1.2 ng/ml before chemotherapy was associated with a high possibility of obtaining more than four mature oocytes (Lee et al. 2011). The authors concluded that together with AFC as a biophysical marker in breast cancer patients, AMH is the most reliable serum marker of ECC outcomes. Therefore, AMH and AFC appear to be useful in counseling breast cancer patients who want to undergo ovarian stimulation for fertility preservation (Lee et al. 2011).

In conclusion, the available data seem to suggest that AMH measurement before gonadotropin administration can be a useful marker for the prediction of women at risk for poor-response or no response to ovarian stimulation. Although few data are available in cancer patients undergoing cryopreservation procedures for fertility preservation before cancer treatments, AMH could help physicians to better counsel these women.

AMH and chemo-induced gonadal damage in cancer patients

Many of the chemotherapeutic regimens used in the treatment of common cancers are gonadotoxic and increase the risk of premature ovarian failure in young women (Lee et al. 2006).

The utility of hormone and ultrasound measurements to assess ovarian reserve in cancer survivors is well known as even in the presence of normal ovarian function (regular menstrual cycles), ovarian reserve of these patients may be altered due to the treatment received.

Hormone level analysis (FSH, E_2, and inhibin B) and ultrasound measurements in these patients show a diminished ovarian reserve, indicating that adult survivors with spontaneous cycles may have a shortened reproductive span and an early menopause (Larsen et al. 2003a,b). The utility of AMH as a potential marker of chemotherapy-induced ovarian follicular depletion has been evaluated both in young women after treatment for cancer in childhood and in young survivors of hematological malignancies and solid tumors (Table 1).

Three studies investigated ovarian reserve in adults after treatment for childhood cancer (Bath et al. 2003, van Beek et al. 2007, Lie Fong et al. 2009): all studies showed that cancer survivors who had been treated with chemotherapy during childhood had significantly lower ovarian reserve as determined by lower AMH levels than healthy women.

Gracia et al. (2012) carried on a cross-sectional analysis to compare measures of ovarian reserve (E_2, FSH, inhibin B, AMH, and AFC) in young cancer survivors with those in unexposed females of similar age and with a cohort of late-reproductive-age women. A total of 71 cancer survivors (47 patients with hematological malignancy and 24 with solid tumors), 67 similarly aged unexposed controls, and 69 regularly menstruating women of late reproductive age were included. In adjusted models, cancer survivors had significantly higher FSH and lower AMH (0.81 vs 2.85 ng/ml; $P < 0.001$) and AFC levels compared with unexposed women of similar age. Increased dose of alkylating agent was associated with increased levels of FSH and decreased levels of AMH. AMH levels were not significantly different between women previously exposed to high-dose cancer therapy and 40–42-year-old controls (Gracia et al. 2012).

Recently, Behringer et al. (2013) have provided important data on the impact of currently used chemotherapy in Hodgkin lymphoma on gonadal function. The authors evaluated hormone parameters, menstrual cycle, symptoms of hypogonadism, and offspring in women younger than 40 years of age and men younger than 50 years of age at diagnosis in ongoing remission at least 1 year after therapy within two clinical German studies for early- and advanced-Hodgkin lymphoma. Out of 1323 patients enrolled, 562 were women. The authors showed that hormone levels correlated with the intensity of chemotherapy, but also, age was a relevant factor for reduced ovarian reserve. Specifically, AMH levels were significantly worse after more intensive alkylating agent-containing chemotherapy and with older age. However, pregnancies in women with low or undetectable AMH levels were reported, underlining the need to further analyze the relevance of AMH assessment in female survivors of cancer. The authors concluded that 'AMH is obviously not suited to predict fertility in individual patients; however, low AMH levels might indicate a reduced ovarian reserve and thus an increased risk of future premature ovarian failure' (Behringer et al. 2013).

AMH seems to be an early and sensitive plasma marker of gonadal damage after chemotherapeutic treatment, as AMH falls very rapidly after the initiation of chemotherapy. There are available data both for patients with hematological malignancies and solid nonbreast tumors and for children treated for cancer (Table 2).

Lie Fong et al. (2008b) measured serum AMH levels in 25 patients with hematological malignancies before and after cancer treatments and compared them with normo-ovulatory controls to assess subclinical ovarian damage in patients treated with gonadotoxic agents. AMH concentrations in all patients before treatment were lower than in controls, and although menstrual cyclicity was restored in most patients treated with chemotherapy, median serum AMH levels were lower in patients than in controls. All 12 patients who underwent stem cell transplantation after total body irradiation developed premature ovarian failure and undetectable serum AMH concentrations (Lie Fong et al. 2008b).

Decanter et al. (2010) published a study in which AMH was measured to characterize the evolution of follicular depletion in 30 young patients treated for lymphoma (mean age 24), assigned to an ABVD (adriamicyn, bleomycin, vinblastine, dacarbazine) protocol or to a non-ABVD protocol (a regimen including cyclophosphamide). Plasma levels of AMH were measured before and during chemotherapy until a period of 1 year after the end of treatment. The hormone levels quickly decreased in all patients, but in patients treated with ABVD, returned to normal 1 year after the end of treatment. This analysis clearly demonstrates the toxicity of alkylating agent-based therapy (Decanter et al. 2010).

Rosendahl et al. (2010) evaluated AMH and hormonal markers in 17 patients with a hematological malignancy or breast cancer before, during, and after chemotherapy. They showed that AMH and inhibin B levels immediately declined after chemotherapy administration, indicating direct chemotherapy-induced damage to the granulosa cells of the growing follicles. Furthermore, they showed that high pretreatment AMH levels were predictive of higher AMH levels during recovery of ovarian function after chemotherapy (Rosendahl et al. 2010).

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Table 1 AMH as a potential marker of chemotherapy-induced ovarian follicular depletion in young women after treatment for cancer in childhood, and in young survivors for hematological malignancies and solid tumors, previously treated with anticancer-treatments

References	Type of study	Type of cancer	Treatment received by cancer patients	Number of patients (mean/median age)	Objective of the study	Results from analysis of AMH
Bath et al. (2003)	Case–control study	Cancer during childhood (ALL, NHL, HL, sarcomas, Wilm's tumor, medulloblastoma)	CT ± RT or CRT	Ten cancer survivors (24 years) 11 controls (23 years) Ten cancer survivors taking COCP (20 years) Ten controls (23 years)	To investigate basal and stimulated hormone production by the ovary to detect and assess the degree of loss of ovarian reserve	AMH levels were lower (13.0±3.0 vs 21.0±3.4 pmol/l; $P<0.05$) in cancer survivors with regular menstrual cycles than in controls. AMH levels were unchanged in the COCP groups before and after stimulation with FSH
van Beek et al. (2007)	Case–control study	Cancer during childhood (HL)	ABVD, EBVD, COOP, MOPP	32 cancer survivors (25 years) NR	To evaluate the long-term effects of combination CT by treatment on gonadal function in women after treatment for childhood HL	AMH levels were lower in patients treated with six or more cycles of MOPP than in healthy women (0.39 vs 2.10 µg/l; $P<0.01$); AMH was also significantly lower in women treated with MOPP compared with women treated without MOPP (0.39 vs 1.40 µg/l; $P=0.01$).
Lie Fong et al. (2009)	Case–control study	Cancer during childhood (ALL, NHL, HL, AML, sarcomas, Wilm's tumor, neuroblastoma, others solid tumors)	CT (MOPP; EBVD; CT containing alkylating agents or without alkylating agents) ±RT or TBI	185 cancer survivors (25.5 years) 42 control women (age NR)	To assess a possible treatment-induced gonadal damage in a cohort of adult female childhood cancer survivors using AMH	Mean AMH levels were not different in the analyzed cohort compared with controls (1.7 vs 2.1 µg/l; $P=0.57$); significantly lower AMH levels than controls were found in survivors treated with three or more procarbazine-containing CT (0.5 µg/l; $P=0.004$) and in survivors treated with TBI (<0.1 µg/l; $P<0.001$)
Gracia et al. (2012)	Case–control study	Cancer during childhood (NHL, HL, AML, sarcomas, Wilm's tumor, others solid tumors)	CT (with or without alkylating agents) ±RT or TBI	71 cancer survivors (age 25.67 years) 67 healthy controls (27.26 years) 69 late reproductive-age controls (45.4 years)	To determine if measures of ovarian reserve differ between females exposed to cancer therapies in a dose-dependent manner compared with healthy controls of similar age and late reproductive age	AMH levels differed between exposed and unexposed subjects (0.81 vs 2.85 ng/ml). Alkylating agent dose score was associated with decreased levels of AMH; exposure to pelvic RT was associated with impairment in AMH. AMH levels were similar in survivors exposed to high-dose CT and in late-reproductive-age controls
Behringer et al. (2013)	NR	Adult cancer patients with HL	CT (ABVD, BEACOPP)	562 women (32 years)	To provide data on the impact of currently used CT in HL on gonadal function	AMH levels correlated significantly with therapy intensity ($P<0.001$). AMH levels >2.0 µg/l were only observed in women younger than 30 years after ABVD therapy

AMH, anti-Müllerian hormone; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; CT, chemotherapy; RT, radiotherapy; CRT, cranial RT; COCP, combined oral contraceptive pill; FSH, follicle-stimulating hormone; ABVD, adriamicyn, bleomycin, vinblastine, dacarbazine; EBVD, epirubicin, bleomycin, vinblastine, dacarbazine; COPP, cyclophosphamide, vincristine, procarbazine, prednisone; MOPP, mechlorethamine, vincristine, procarbazine, prednisone; NR, not reported; AML, acute myeloid leukemia; TBI, total body irradiation; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone.

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Table 2 AMH values modifications before and after chemotherapy in childhood cancer patients and in young patients with hematological malignancies and solid tumors

References	Type of cancer	Treatment received by cancer patients	Number of patients (mean/median age)	Objective of the study	Results from analysis of AMH
Lie Fong et al. (2008b)	Patients with hemato-logical malignancies	CT or STC after TBI and high-dose CT	Group A: 13 patients treated with CT (29.4 years) Group B: 12 treated with STC after TBI and high-dose CT (25.3 years) Group C: 42 controls (29.9 years)	To assess subclinical ovarian damage in women treated with gonadotoxic agents, measuring AMH levels before and after cancer treatment	AMH levels were lower in cancer patients than in controls before treatment (1.0 μg/l in group A, 0.9 μg/l in group B, and 2.1 μg/l in group C). After treatment, AMH levels were lower in cancer patients than in controls despite the restoration of menstrual cyclicity (0.3 μg/l in group A, 0.0 μg/l in group B, and 1.3 μg/l in group C). All patients that underwent SCT developed POF
Decanter et al. (2010)	Patients with hemato-logical malignancies (NHL and HL)	ABVD and non-ABVD (R-CHOP, ACVBP, BEAM, BEACOPP, MINE)	30 women (24 years)	To assess the acute and long-term effects of different CT regimens on the ovarian reserve, measuring AMH prior to, during and after treatment	In all patients AMH concentrations fell drastically just after the start of CT and were close to the detection limit at the end of the treatment. In the ABVD group AMH levels increased from the third month after the end of CT and returned to pretreatment levels 12 month after the end of CT. Conversely, no significant change was observed in the non-ABVD group throughout the follow-up period
Rosendahl et al. (2010)	Patients with hemato-logical malignancies (NHL and HL) and solid tumors (BC and Ewing sarcoma)	BEACOPP, MBVP, CHOEP, VAI CEF, CE, Tax	17 patients (30 years)	To study ovarian follicular dynamics during CT to understand the mechanisms behind CT-induced ovarian follicular depletion and to evaluate if pretreatment levels of ovarian reserve markers were predictive of the post-treatment levels	AMH levels dropped from 2.7 to 1.1 and to 0.4 ng/ml immediately after one and two series of CT respectively. High pretreatment AMH levels predicted higher post-treatment AMH levels
Brougham et al. (2012)	Childhood cancer patients (HL, sarcomas, T-cell lymphoma, neuroblastoma, Wilm's tumor, retino-blastoma, germ cell tumor, hepatoblastoma, Langerhans cell histiocy-tosis)	CT (cisplatin, cyclophosphamide, melphalan, ifosfamide, procarbazine, chlorambucil, vinblastine, dacarbazine)	22 children (4.4 years)	To evaluate AMH measure-ment in children as a marker of ovarian toxicity during cancer treatments	AMH decreased progressively during CT (P<0.0001) in both prepubertal and pubertal girls, becoming undetectable in 50% patients. A recovery was seen for patients treated with CT with low/-medium risk of gonadotoxicity, while in the high-risk group AMH became undetectable in all patients and showed no recovery
Dillon et al. (2013)	Patients with different cancers (breast cancer, leukemia, lymphoma, sarcoma)	CT (different regimen)	46 patients (mean age 26.1 years)	To identify factors associated with ovarian reserve impair-ment during and immedi-ately after CT	Pretreatment AMH levels were associated with the rate of recovery of AMH after treatment. Patients with a pretreatment AMH level >2 ng/ml recov-ered at a rate of 11.9% per month after CT, whereas patients with pretreatment AMH levels ≤2 ng/ml recovered at a rate of 2.6% per month after therapy

AMH, anti-Müllerian hormone; CT, chemotherapy; SCT, stem cell transplantation; TBI, total body irradiation; POF, premature ovarian failure; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; ABVD, adriamycin, bleomycin, vinblastine, dacarbazine; R-CHOP, rituximab, adriamicyn, cyclophosphamide, vincristine, prednisone; ACVBP, adriamicyn, cyclophosphamide, bleomycin, vindesin; BEAM, BCNU, etoposide, cytarabine, melphalan, procarbazine, vincristine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; MINE, mitoguazon, etoposide, ifosfamide, vinorelbine; BC, breast cancer; MBVP, methotrexate, teniposide, carmustine, prednisone; CHOEP, cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone; VAI, vincristine, dactinomycin, ifosfamide; CEF (or CE), cyclophosphamide, epirubicin, 5-fluorouracil; Tax, docetaxel.

Brougham *et al.* (2012) prospectively evaluated AMH levels in children as a marker of ovarian reserve during cancer treatment. A total of 22 patients with a median age of 4.4 years were enrolled before initiation of cancer treatment. Serum AMH levels were measured at diagnosis, after each chemotherapy course and during follow-up. Risk of gonadotoxicity was classified as low/medium or high based on chemotherapy agent, cumulative dose, and radiotherapy involving the ovaries. The authors demonstrated that AMH was detectable in girls of all ages but decreased progressively during chemotherapy in both pre- and post-pubertal girls, eventually becoming undetectable in 50% of patients. AMH levels showed no recovery in the high-risk group, while there was a recovery in the low/medium risk group after the completion of chemotherapy (Brougham *et al.* 2012). Interestingly, authors observed that AMH, due to the physiological quiescence of the hypothalamic–pituitary–gonadal axis at this age, was the only reliable biomarker for ovarian damage evaluation in prepubertal children (Brougham *et al.* 2012).

Recently, Dillon *et al.* (2013) have evaluated factors (FSH, LH, E_2, inhibin B, and AMH) associated with ovarian reserve impairment during and immediately after chemotherapy. All measures of ovarian reserve demonstrated statistically significant changes during chemotherapy: alkylating agent exposure and baseline ovarian reserve were acutely associated with the magnitude of impairment. Particularly, pretreatment AMH levels were associated with the rate of recovery of AMH after treatment (Dillon *et al.* 2013).

In conclusion, a fall in AMH levels during chemotherapy with recovery dependent on the toxicity of the regimen used was detected not only in young cancer women but also in prepubertal girls, and, generally, AMH concentrations are reduced in women following treatment of cancer, either in childhood or adulthood, with low AMH concentrations most consistently seen following total body radiation.

AMH, chemo-induced gonadal damage, and adjuvant therapy for breast cancer

In the last few years, some studies have tried to better define the association between ovarian function and AMH in breast cancer patients (Table 3). The impact of cancer treatments on gonodal function of young breast cancer patients is well known, but it is still not known if the malignancy itself impacts ovarian reserve. Before counseling patients for fertility preservation, it is important to consider whether ovarian reserve is impacted by cancer.

To explore this issue by determining if AMH, FSH, and inhibin B levels differ in young women with and without breast cancer, Su *et al.* (2013) performed a cross-sectional study involving 207 participants, 108 women with breast cancer and 99 healthy women. The authors showed that mean AMH levels were not significantly different between breast cancer participants and healthy controls (0.85 vs 0.76 ng/ml, $P = 0.60$), although AMH may be lower with breast cancer status in women older than 37 years (Su *et al.* 2013).

Data are available regarding the use of AMH as a marker to better assess ovarian reserve, even in the presence of regular menstrual cycles, in breast cancer patients who undergo anticancer treatments.

Anderson & Cameron (2011) recruited 56 premenopausal women with early-stage breast cancer in a 5-year prospective study of ovarian function and bone mass, in which 42 patients received adjuvant chemotherapy. Hormonal and biophysical markers of ovarian function at 4–5 years of follow-up were analyzed in relation to menstrual activity. Higher levels of AMH, inhibin B, and E_2 and lower levels of FSH were observed in women with ongoing menses vs women that became amenorrheic after chemotherapy, confirming the validity of menstrual diary data. The authors' conclusion was that the AMH level before adjuvant chemotherapy predicts long-term ovarian function (Anderson & Cameron 2011).

Partridge *et al.* (2010) compared markers of ovarian reserve between 20 survivors of breast cancer exposed to cytotoxic chemotherapy (50% received tamoxifen) and 20 matched controls in a cross-sectional evaluation. This study demonstrated that premenopausal breast cancer survivors have diminished ovarian reserve compared with controls. Interestingly, survivors receiving tamoxifen had lower AFC, AMH, inhibin B levels and higher E_2 levels than non-tamoxifen-treated survivors (Partridge *et al.* 2010).

The same group has recently published an interim analysis of an ongoing prospective multi-center cohort study, in which breast cancer patients younger than 41 years of age had blood drawn and surveys about their menstrual cycle collected 1 year after diagnosis (Ruddy *et al.* 2012). A total of 199 women with a median age of 37 years were eligible in the analysis. The majority of the women received chemotherapy (74%) and 56% received tamoxifen. Median AMH and E_2 levels were significantly lower and FSH levels were significantly higher in women with amenorrhea (defined as more than 6 months between blood drawn and last menstrual period). The authors concluded that AMH, E_2, and FSH are promising

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Table 3 AMH evaluation in breast cancer patients

References	Type of study	Number of patients (mean/median age)	Objective of the study	Results from analysis of AMH
Su et al. (2013)	Cross-sectional study	108 BC women (40.2 years) 99 healthy women (33.0 years)	To determine if ovarian reserve is impacted by cancer, before any therapeutic intervention	Adjusting for age and others confounding factors, mean AMH levels were not significantly different between BC patients and controls (0.85 vs 0.76 ng/ml, $P=0.60$). AMH may be lower with BC status in women older than 37 years
Anderson & Cameron (2011)	Prospective longitudinal study	42 BC women treated with CT (age not reported) 14 BC women who did not receive CT (age not reported)	To assess markers of ovarian reserve and age as long-term predictors of ovarian function after chemotherapy	Pretreatment serum AMH levels predicted late ovarian activity by univariate analysis. Moreover, AMH was predictive in a multivariate logistic regression (OR=13; 95% CI=2.5–66.7): 0.71 ng/ml gave peak likelihood ratio of 7.0 with 54% sensitivity and 92% specificity
Partridge et al. (2010)	Cross-sectional study	20 BC women (36.8 years) 20 controls (36.9 years)	To compare markers of ovarian reserve between women exposed to cytotoxic chemotherapy for early-stage BC and matched controls	There were significant differences in mean AMH levels between BC women and controls (0.6 vs 1.8 ng/ml; $P=0.0004$), indicating better ovarian reserve in controls. Survivors on tamoxifen had lower AMH levels than non-tamoxifen-treated survivors
Ruddy et al. (2012)	Prospective cohort study	199 women (37 years)	To improve understanding of ovarian reserve in women with a history of BC by comparing AMH, E_2 and FSH levels in survivors with and without amenorrhea	Median AMH was lower in women with amenorrhea (0.01 vs 0.22 ng/ml; $P<0.0001$). This difference remained significant when BC patients on and off tamoxifen were analyzed separately
Su et al. (2010)	Case–control study	127 BC women (45.3 years) 110 controls (46.1 years)	To test whether AMH and inhibin B were impacted by BC treatments by comparing cancer survivors and age-matched controls, and to determine the association between these hormones and post-CT menstrual pattern	Cancer women had significantly lower AMH levels than controls (53.1 vs 99.5 pg/ml; $P=0.004$). AMH was found to be significantly associated with risk of CT-related amenorrhea. AMH was significantly lower ($P=0.03$) in menstruating subjects who developed subsequent CT-related amenorrhea
Anderson et al. (2006)	Prospective observational study	50 BC women (41 years)	To investigate and to compare the effects of CT and long-term gonadotrophin withdrawal on ovarian function, measuring the markers of ovarian reserve	AMH levels showed a rapid and marked fall during CT, with undetectable concentrations in many women ($P<0.0001$). Regimens containing taxanes in addition to cyclophosphamide showed increased gonadotoxicity. Gonadotrophin suppression resulted in a delayed fall in AMH level after 6 months ($P<0.0001$)
Yu et al. (2010)	Prospective cohort study	26 BC women (37 years)	To assess levels of AMH, E_2, FSH and menstrual status in women undergoing CT	Serum AMH decrease significantly at 6 weeks after CT and remained suppressed for 52 weeks. Amenorrheic and menstruating women were found to have similar AMH levels at baseline and follow-up
Anders et al. (2008)	Prospective cohort study	44 BC women (40 years)	The evaluation of AMH, FSH, inhibin A and B pre- and post-treatment, to assess if they could represent predictive markers of CT-related amenorrhea	Pre-CT median AMH levels were lower among women with CT-related amenorrhea (0.16 vs 1.09 ng/ml; $P=0.02$). The risk of CT-related amenorrhea was increased among women with lower pretreatment AMH (RR, 1.83; $P=0.05$)

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Table 3 Continued

References	Type of study	Number of patients (mean/median age)	Objective of the study	Results from analysis of AMH
Anderson et al. (2013)	Prospective study	59 BC women (42.6 years)	To test the hypothesis that AMH, measured at the time of diagnosis would be a clinically useful predictor of amenorrhoea after chemotherapy for early breast cancer in comparison to age or other biochemical markers of ovarian reserve (FSH, inhibin B)	Pretreatment AMH was significantly lower in women with amenorrhoea at 2 years ($P < 0.0001$), but FSH and inhibin B did not differ between groups. By logistic regression, pretreatment AMH, but not age, FSH, or inhibin B, showed to be an independent predictor of ovarian status at 2 years ($P = 0.005$; OR, 0.013)
Lutchman Singh et al. (2007)	Case-controls study	22 BC women (age 22–42 years) 24 age-matched controls	To evaluate ovarian function in young women treated by cyclophosphamide-based CT for BC using clinical, biochemical, and biophysical parameters	Significant differences were revealed for basal AMH when compared with the controls (0.95 vs 7.89 ng/ml; $P < 0.001$). In the six evaluated patients, pre-CT AMH levels were lower than immediately following completion of CT (4.40 vs 0.07 ng/ml)

AMH, anti-Müllerian hormone; BC, breast cancer; CT, chemotherapy; OR, odds ratio; E_2, estradiol; FSH, follicle-stimulating hormone; RR, relative risk.

biomarkers to define amenorrhea and residual ovarian function in breast cancer survivors (Ruddy et al. 2012).

Su et al. (2010) analyzed 127 breast cancer patients post chemotherapy that were premenopausal at diagnosis. The primary endpoint was chemotherapy-related amenorrhea (CRA). Compared with age-matched controls, AMH levels were significantly lower ($P < 0.03$) and FSH levels were significantly higher ($P < 0.04$) in menstruating cancer patients who developed CRA (Su et al. 2010).

As in hematological and pediatric cancer patients, in breast cancer patients AMH seems to be an early and sensitive plasma marker of gonadal damage after chemotherapeutic treatment.

Anderson et al. (2006) conducted a study in which markers of ovarian reserve (AMH, E_2, FSH, inhibin B, and AFC) were measured in 50 patients with early breast cancer who received adjuvant treatment. Of these patients, 42 received chemotherapy and eight received hormonal treatment. Patients were reevaluated 3, 6, 9, and 12 months after starting chemotherapy. AMH demonstrated its role as an early indicator of chemotherapy-induced ovarian follicle loss, and the authors suggested that FSH and AMH concentration measurements would be useful for the comparison of ovarian toxicity of different chemotherapy regimens (Anderson et al. 2006). Furthermore, they found that the addition of a taxane to a regimen containing cyclophosphamide seems to be more gonadotoxic, resulting in lower plasma AMH levels. In patients treated with analogs of LH-releasing hormone (LHRHa), AMH concentrations showed a delayed fall (Anderson et al. 2006).

Yu et al. (2010) demonstrated that serum AMH decreased significantly at 6 weeks after chemotherapy and remained suppressed for 52 weeks in 26 women treated for early breast cancer. Amenorrheic and menstruating women were found to have similar AMH values at baseline and at follow-up.

Anders et al. (2008) analyzed 44 early-stage breast cancer patients at risk of CRA. FSH, E_2, inhibin A and B, and AMH levels were prospectively evaluated prechemotherapy, post-chemotherapy, 6 months, and 1 year after the end of chemotherapy. Pre-chemotherapy median inhibin B and AMH values were lower among women experiencing CRA ($P = 0.03$ and $P = 0.02$ respectively), thus indicating that these markers might be predictive of CRA among premenopausal women undergoing chemotherapy for breast cancer (Anders et al. 2008).

Recently, Anderson et al. (2013) have shown that pretreatment AMH was a useful predictor of long-term

post-chemotherapy loss of ovarian function after chemotherapy in women with early breast cancer. Pretreatment AMH showed a significant positive correlation with menses: pretreatment AMH was significantly lower in women with amenorrhoea at 2 years ($P<0.0001$); by logistic regression, pretreatment AMH was shown to be an independent predictor of ovarian status at 2 years ($P=0.005$) (Anderson et al. 2013).

Lutchman Singh et al. (2007) evaluated ovarian function in 22 young breast cancer women treated with chemotherapy and in 24 age-matched controls. The patients were recruited before and after the completion of chemotherapy and serum samples were analyzed for FSH, LH, E_2, inhibin A and B, and AMH. Significantly higher basal FSH values ($P<0.001$) and lower AMH ($P<0.001$) and inhibin B ($P<0.001$) levels were found in breast cancer patients than in the control group, indicating their potential use as markers of ovarian reserve in young women with breast cancer (Lutchman Singh et al. 2007).

In conclusion, several studies have demonstrated a potential utility of AMH, inhibin, or FSH as biomarkers predicting infertility risk in breast cancer patients. These studies demonstrated that breast cancer itself does not seem to have a negative impact on ovarian function before starting anticancer treatments, and that increased rates of CRA have been associated with low pretreatment AMH and inhibin levels, and high FSH levels. BRCA1-positive patients could be considered an exception

due to their pre-chemotherapy that reduced AMH levels (Oktay et al. 2010): reduced ovarian reserve and premature ovarian aging in these patients could be related to an impaired repair of DNA double stand breaks as a consequence of BRCA1 mutation (Titus et al. 2013). Furthermore, AMH was shown to have a potential role as an early indicator of chemotherapy-induced follicle loss; finally, decreased AMH and inhibin levels and increased FSH levels compared with age-matched healthy controls seem to correlate with decreased ovarian reserve in patients previously treated with chemotherapy.

AMH and ovarian suppression with the LHRHa in breast cancer patients

The administration of LHRHa is one of the available fertility preservation techniques for young breast cancer patients (Lambertini et al. 2013) although it remains controversial whether this should be considered a standard or still an experimental strategy (Loren et al. 2013).

Data on hormone levels are available from several trials that evaluated the efficacy of LHRHa as a fertility preservation strategy in breast cancer patients; however, data specifically relating to the impact of LHRHa treatment on AMH levels are very limited (Turner et al. 2013). In studies that included AMH assessment, its evaluation was carried out only in a minority of patients and was reported only as exploratory or in abstract form (Table 4).

Table 4 AMH evaluation in clinical trials that evaluated LHRHa for the prevention of CT-induced early menopause

References	Number of patients with AMH evaluation (total of patients enrolled in the clinical study)	Comparison by treatment arms	Results from analysis of AMH
Gerber et al. (2011)	17 (60)	No	AMH strongly correlated with age, with lower AMH levels in older patients. An AMH level >0.2 μg/l was seen in 3 of 9 (33%) patients in the group without goserelin vs 4 of 8 (50%) patients in the goserelin group
Elgindy et al. (2013)	NR (100)	Yes	AMH decreased significantly at 1 year after the end of CT in all groups regardless of treatment arms (2–2.3 vs 0.2–0.4 ng/ml for pre- and post- CT respectively). No differences in AMH levels between control arms and LHRHa arms at 12 months after the end of CT
Leonard et al. (2012)	117 (227)	No	AMH was lower following CT than before CT initiation (0.40 ± 0.65 vs 1.38 ± 1.82 ng/ml; $P<0.001$). Pre-CT AMH was a significant predictor of post-treatment amenorrhea ($P=0.001$)
Giraudi et al. (2009)	26 (281)	No	AMH levels were significantly lower in old patients (≥41 years) as compared to younger ones. After CT, mean value of AMH significantly decreased from 2.04 to 0.59 ng/ml ($P=0.0003$). The mean decrease of AMH levels was not affected by the type of CT regimens

LHRHa, analogs of luteinizing hormone; CT, chemotherapy; AMH, anti-Müllerian hormone; NR, not reported.

In the GBG 37 ZORO study, 60 hormone-insensitive breast cancer patients younger than 46 years of age were allocated to receive chemotherapy with or without goserelin (Gerber et al. 2011). Out of 60 patients, 17 were accessible for hormone assessment (inhibin B, AMH, E_2, and FSH) and follicle count by ultrasound during follow-up to estimate ovarian function. Out of these 17 patients, eight received goserelin and nine were treated without LHRHa. AMH correlated strongly with age, with lower AMH levels in older patients. An AMH level >0.2 μg/l was seen in 3 of 9 (33%) patients in the group without LHRHa vs 4 of 8 (50%) patients in the goseriln group respectively. A direct comparison between treatment arms was not possible due to limited patient numbers. The AFC was available in ten patients. Two patients had a high follicle count (≥ 4) and AMH level >0.2 μg/l (Gerber et al. 2011).

Elgindy et al. (2013) randomly assigned 100 hormone-insensitive breast cancer patients younger than 40 years of age to receive triptorelin during cyclophosphamide-based chemotherapy (with the addition of an LHRH antagonist for a few days in patients who started chemotherapy earlier than 10 days before study inclusion). AMH decreased significantly at 1 year post chemotherapy in all groups regardless of treatment arms (2–2.3 vs 0.2–0.4 ng/ml for pre- and post-chemotherapy respectively). No difference in AMH levels was seen between control arms and LHRHa arms at 12 months after the end of chemotherapy (Elgindy et al. 2013).

In the OPTION trial, 227 premenopausal breast cancer patients were randomly assigned to receive (neo)-adjuvant chemotherapy with or without goserelin (Leonard et al. 2010). AMH was measured in 117 patients pretreatment and 1 year following initiation of chemotherapy (Leonard et al. 2012). No differences in pretreatment AMH levels were seen between control and goserelin-treated groups. AMH levels were lower following chemotherapy than before the initiation of chemotherapy (0.40 ± 0.65 vs 1.38 ± 1.82 ng/ml: $P < 0.001$) and pretreatment AMH level was a significant predictor of post-treatment amenor-rhea ($P = 0.001$). No data are available on the differences between treatment arms (Leonard et al. 2012).

In the PROMISE-GIM6 phase III trial, 281 premenopausal breast cancer patients who were candidates for (neo)-adjuvant chemotherapy were randomly assigned to receive chemotherapy alone or combined with triptorelin for the prevention of chemotherapy-induced early menopause (Del Mastro et al. 2011). In a total of 26 patients, AMH, FSH, and E_2 levels and menstrual activity were evaluated before chemotherapy, and every 3 months after chemotherapy (Giraudi et al. 2009). Levels of AMH were significantly lower (1.26 ng/ml) in women 40 years or older than in younger women (2.97 and 3.63 ng/ml in patients aged 25–36 and 37–40 years, respectively; $P = 0.018$). After the administration of chemotherapy, the mean value of AMH significantly decreased from 2.04 to 0.59 ng/ml ($P = 0.0003$). The mean decrease in AMH levels was not affected by the type of chemotherapy regimens ($P = 0.97$). Among ten patients with both early (0–5 months) and delayed (6–11 months) post-chemotherapy evaluations, no changes in AMH values were observed with longer follow-up: mean values were 1.49, 0.35, and 0.36 ng/ml, at baseline, after 0–5 months, and after 6–11 months respectively. Menstrual activity resumption occurred in 48% of patients. In this preliminary analysis, baseline and post-chemotherapy values of AMH were not significantly associated with menstrual resumption (Giraudi et al. 2009).

Discussion

The studies conducted thus far are inconclusive regarding the role of AMH in patients treated for early breast cancer. Even if the function of this hormone as a marker of ovarian reserve seems to be well defined, the clinical impact for this group of patients is still unclear. Several questions remain in determining if we can consider AMH a useful clinical marker.

First of all, data regarding different regimens of chemotherapy are few, the number of patients involved in the studies is probably not adequate, and the trial designs are not comparable. For example, in Anderson's study, 42 patients received six different regimens of chemotherapy and eight different hormonal treatments (Anderson & Cameron 2011). The chemotherapy containing cyclophosphamide and taxanes seemed to be more gonadotoxic, but because of the small number of participants, this cannot be regarded as conclusive (Anderson et al. 2011). It would be interesting to determine if all types of alkylating regimens decrease plasma levels of AMH in the same way and if the potential recovery can be influenced by the regimen received.

A major point to be clarified is the biological explanation of recovery of AMH post-chemotherapy. Post-chemotherapy increase in AMH levels is related to the renewal of pre-antral and small antral follicles pool, which are the main source of this hormone. Obviously, this recovery is observed only in patient with a partially reversible chemotherapy-induced ovarian damage. This recovery is probably influenced not only by the age of patients but also by other factors, such as the

chemotherapy regimen used and other biological characteristics. Further studies are required in order to better clarify this topic and the specific toxicity of the different chemotherapy protocols.

AMH seems to be uninfluenced by the hormonal status of patients, but, the Partridge et al. (2010) and Anderson & Cameron (2011) studies suggest a possible role of hormonal treatment in changing levels of AMH compared with baseline. Wider clinical records could confirm or refute these data.

An interesting perspective is the association between AMH and other markers of ovarian reserve (FSH, E_2, and inhibin B). This association would be useful in determining why in patients in whom we detect premenopausal levels of FSH, AMH levels can differ from normal to undetectable. Is there a biological reason which we cannot explain? AMH could be useful only in the determination of ovarian reserve or could help the clinician to assess hormonal status post chemotherapy. Undetectable levels of AMH and elevated levels of FSH and LH can further confirm the menopausal status after chemotherapy.

As previously stated, determination of menopausal status in patients who have received chemotherapy is a pathophysiologic and clinical cornerstone especially important in choosing hormonal treatment, for example in switching from tamoxifene to an aromatase inhibitor (Guerrero et al. 2013).

Moreover, menopausal status cannot be defined in a considerable number of patients (Amir et al. 2009, 2010). One study suggests that AMH levels decrease to undetectable levels 5 years before the onset of the menopausal status (date of last menses), so undetectable plasma levels of AMH suggest a pending menopausal status (Sowers et al. 2008). However, longitudinal studies are required in order to establish if different or undetectable levels of AMH could predict timing of onset of postmenopausal status: this knowledge could be useful in the determination of menopausal status in chemotherapy-treated patients.

Conclusion

In the past few years, the issue of female fertility determination and preservation has assumed greater importance. AMH acts as an endocrine marker of follicle depletion, reflecting ovarian reserve and possibly the onset of the late stage of menopause transition. Nevertheless, the clinical role of AMH in patients with early breast cancer needs to be defined as well as its role in predicting treatment-induced infertility.

Further studies are awaited in order to define the regimen-specific action of chemotherapy on AMH levels, the percentage of post-treatment recovery of plasma levels of the hormone, and the relationship between menopausal status, infertility, and levels of AMH.

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