Chemotherapy-induced ovarian toxicity in patients affected by endocrine-responsive early breast cancer

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Abstract

Cytotoxic chemotherapy may variably affect ovarian function depending on age and ovarian reserve at diagnosis, type of chemotherapy and use of tamoxifen.

Ascertaining whether a premenopausal patient with endocrine-responsive early breast cancer and chemotherapy-induced amenorrhea has reached menopause is essential not only in order to provide accurate information on residual fertility, but also to appropriately prescribe

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endocrine therapy. Indeed, aromatase inhibitors are contraindicated in women with residual ovarian reserve. However, the diagnosis of menopause in patients with chemotherapy-induced amenorrhea is challenging, since clinical features, follicle-stimulating hormone and estradiol levels may be inaccurate to this aim. Recent studies demonstrated that the anti-müllerian hormone may improve the assessment of ovarian reserve residual to chemotherapy in women with early breast cancer.

Herein, we review the incidence of amenorrhea and menopause induced by cytotoxic chemotherapy in women affected by early breast cancer and the suggested mechanisms that sustain these side-effects. Furthermore, it has been scrutinized the potential of new markers of ovarian reserve that may facilitate the selection of appropriate endocrine treatment for premenopausal women who develop amenorrhea following adjuvant chemotherapy for early breast cancer.

1. Introduction

Breast cancer (BC) is the second most common cancer in Western Countries and the most common invasive malignancy in reproductive-aged women [1,2]. Approximately 30% of patients are premenopausal and 10% are aged 35–44 at diagnosis [3]. Despite the fact that BC in women under the age of 40 is infrequent (5.5% in the United States) [4], a dramatic increase in the number of BC diagnosed in premenopausal women has been reported in several countries [5]. BC, even in early stages (EBC), presents more aggressive features, and prognosis is poorer in younger compared with older women [6–8]. Therefore, most of these patients receive adjuvant cytotoxic treatments (CT) followed by endocrine therapy (ET) in the case of endocrine-responsive (ER+) disease [9,10].

CT prolongs survival of patients affected by EBC, even in the case of endocrine-responsive disease, particularly in women under 50 [11]. However, in a variable percentage of pre-/perimenopausal patients CT may cause amenorrhea (chemotherapy-related amenorrhea, CRA) or menopause (chemotherapy-related menopause, CRM). These side-effects are predictive of improved clinical outcomes in most clinical trials [12,13], but expose women to a number of physical and socio-psychological distresses regarding residual fertility, sexual dysfunction, bone loss and menopausal symptoms, with a markedly negative effect on quality of life [14].

A number of factors influence the onset of CRA/CRM, including age, type of CT received and use of tamoxifen (Table 1). Estimation of the individual risk to develop CRA or CRM, however, remains approximate.

Nowadays, to ascertain whether a patient with endocrine-responsive EBC and CRA is actually menopausal is of utmost importance not only in order to provide accurate information on residual fertility, but also for prescribing the most suitable ET (i.e., tamoxifen or aromatase inhibitors, AI). Indeed, only in postmenopausal patients are AI recommended as up-front treatment, or sequentially after tamoxifen, since they reduce the risk of recurrence [16]. Instead, AI as single agents are contraindicated in premenopausal patients and in those presenting residual ovarian function [16]. In these patients, the inappropriate use of AI induces a temporary inhibition of estrogen production, leading to a feedback increase in gonadotropin levels, which, in turn, stimulate follicular growth, aromatase activity and restoration of pre-CT estradiol levels [17]. These changes in hormonal levels would be expected to reduce or abolish the efficacy of the anticancer treatment received and expose patients to further unjustified side-effects, including pain from ovarian hyperstimulation and increased risk of unplanned pregnancy [18]. Confirmation of the menopausal status is, therefore, mandatory before prescribing AI [19].

In current clinical practice, menopause in a patient with CRA may be only presumed based on some clinical features, such as age, the likelihood of gonadal toxicity from chemotherapy, use of tamoxifen, menstrual history, vasomotory symptoms, together with serum levels of FSH and estradiol. Information obtained by these parameters, however, may be misleading. In particular, serum levels of FSH and estradiol, widely used to confirm menopausal status, may provide erroneous information in 25–35% of cases [18,20].

This article reviews the incidence and the pathophysiology of gonadal damage induced by CT in women affected by endocrine-responsive EBC. Moreover, practical approaches integrating new markers of ovarian reserve are proposed to distinguish truly menopausal women who may receive an AI as adjuvant ET from those with CRA.

2. Ovarian insufficiency induced by cytotoxic agents

2.1. Definitions

The progressive loss of oocytes from fetal life through menopause is physiological [21]. At approximately the age of 37, however, an accelerated atresia of the oocytes is responsible for the average age of menopause being about 51 years (range 40–60 years) in Western women [14,21,22]. Cessation of menses that occurs before the age of 45 is defined as "early" menopause. When menses cease even earlier (before the age of 40), it is called "premature" menopause or premature ovarian failure (POF). The latter is an arbitrary cut-off point designated by the World Health Organization (WHO) corresponding to menopause occurring at an age two standard deviations below the population mean [23]. Instead, there is no WHO proposed definition for early menopause, which is, however, largely used in several studies.

Table 1
Rate of chemotherapy-related amenorrhea and resumption of menses in selected trials of cytotoxic chemotherapy including only women affected by early breast cancer.

Authors	CT regimen	No. patients	CRA definition (duration)	Median age (range; yrs)	Median follow-up (range; mos)	CRA (%)		Resumption of menses (%)
						<40 yrs	≥40 yrs	
Goldhirsch et al. [56]	CMF (400 mg/mq,1)	541				10	33	
	Classic CMF × 6	387	<9 mos	NA	NA	33	81	NA
	Classic CMF × 12	399				61	95	
Venturini et al. [58]	FEC21	250					64	
	FEC14	253	>3 mos	NA	123 (48–144)		64	NA
Jung et al. [70]	CMF	188			109.8		43	12.5
	FAC	53	≥6 mos	40 (25–53)	(16.6–193)		65.2 p = ns	3.3
Parulekar et al. [79]	CMF	236					71	NA
	CEF	224	≥3 mos	43.8 (23–55)	114 (3–145)	76 p = ns		
Di Cosimo et al. [60]	CMF	19					47.4	
	CMF-ED	10					58	
	FEC/EC	43	≥3 mos	42 (27–51)	NA		45.2	Overall 7.2
	E/E-CMF	39					p = ns	
Martin et al. [47]	FAC	403					52.4	
	TAC	420	>3 mos	49 (23–70)	55		61.7 p = .0007	NA
Fornier et al. [49]	AC-T	166	>12 mos	36 (27–40)	37.9 (12.2–77.8)		15	85
Tham et al. [45]	AC	75					55	12
	AC-T	116	≥3 mos	NA	NA		64 p = ns	28 p = ns
Berliere et al. [51]	FEC	84		43.5			92.5	23.7
	FEC-D	70	<12 mos	44	79		93 p = ns	36.5 p = .019
Pérez-Fidalgo et al. [53]	A-based	212		44			75.5	
	TXs-based	93	≥12 mos	43 (29–53)	NA		82.7 p = ns	Overall 6.3
Ganz et al. [55]	AT	752		50			47.1	
	TAC	705	≥6 mos	51	NA (6–24+)		67.2	NA
	AC-T	692		51			83.3	
Petrek et al. [61]	AC	120					53	
	ACT	168					42	
	CMF	83	NA	39.5 (20–45)	NA (1–60+)		82	NA
	FAC	38					NA	
	FACT	34					NA	
	TAC	19					45	
Sukumvanich et al. [62]	CMF	76					34	23
	(F)AC	146					37	68
	(F)ACT	188	≥6 mos	39 (20–45)	>24 (6–60+)		45	57 p = .002
Najafi et al. [48]	CMF	40					52.5	19.1
	AC/CAF	111	≥6 mos	40.9 ± 6.8	36 (12–120)		66.7	27.1
	ACT/AC-T	75					78.7	49.1

Table 1 (*Continued*)

Authors	CT regimen	No. patients	CRA definition (duration)	Median age (range; yrs)	Median follow-up (range; mos)	CRA (%) <40 yrs	CRA (%) ≥40 yrs	Resumption of menses (%)
Lee et al. [52]	AC/FAC	154					57	
	FAC-T/ACT	148	≥6 mos	42 (22–50)	37 (12–80)		58	Overall 14
	CMF	24					56	
							p = ns	
Abusief et al. [59]	AC	228					54.8	
	AC-T	183	≥6 mos	43 (25–55)	33 (6–114)		56.4	NA
	AC-T (DD)	120					58.7	
	AC-T+H	39					46.1	
Han et al. [46]	TX vs AC	122					90.2	33.9
	AC-T	34	>3 mos	40 (21–50)	40 (18.8–69.3)		73.5	23.2
	FAC	129					72.1	p = ns
Zhou et al. [50]	FEC	78					44.9	
	TE	66	≥12 mos	42.5 (23–53) Mean	38.5 (17–83)		30.3	NA
	NE	26					23.1	
Gerber et al. [64]	AC ± T	30		38.5 (29–47)			93.3	56.7
	AC ± T + Goserelin	30	6 mos	35 (26–44)	24+		83.3	70
				p = .092				p = .284
Wong et al. [88]	AC/EC ± TXs/FEC + Goserelin	132	NA	35 (20–45)	58 (4–119)			91 (<35 yrs)
								76 (>35 yrs)
Badawy et al. [89]	FAC	39		30 ± 3.51			76.7	33.3
	FAC + Goserelin	39	NA	29.2 ± 2.93	8 (all patients)		11.4	89.6
				p = .76				p = .001
Munster et al. [65]	CT (AC ± T/FEC/FAC)	21		38 (26–44)			9.5	90.5
	CT + Triptorelin	26	NA	39 (21–44)	18 (5–43)		11.6	88.4
							p = ns	p = .36
Del Mastro et al. [63]	CT alone	133		39 (25–45)			CRM	49.6
	CT + Triptorelin	148		39 (24–45)			25.9	63.3
			CRM (§)		24+		8.9	p = .03
							p < .001	
Leonard et al. [90]	A ± C ± TXs + Goserelin	<40 yrs: 87					No resumption of menses	
		>40 yrs: 53	12	NA	12+ (all patients)		34.7	
							15.8	
							p ≤ .05	

Definition of chemotherapy-related amenorrhea (CRA): absence of menses lasting for the indicated period of time; A = adriamycin; AC: adriamycin, cyclophosphamide; AT: adriamycin, docetaxel; CMF: cyclophosphamide, methotrexate, 5-fluorouracil; C = cyclophosphamide; CRM: chemotherapy-related menopause; CEF: cyclophosphamide, epirubicin, 5-fluorouracil; CT: cytotoxic chemotherapy; D = docetaxel; DD = dose dense regimen; EC = epirubicin, cyclophosphamide; FAC: 5-fluorouracil, adriamycin, cyclophosphamide; FACT: 5-fluorouracil, adriamycin, cyclophosphamide, paclitaxel; FEC: 5-fluorouracil, epirubicin, cyclophosphamide; mos = months; N = navelbine; NA = not available; TAC: docetaxel, adriamycin, cyclophosphamide; T = paclitaxel; TXs = taxanes; X = capecitabine; vs: versus; yrs = years. (§) CRM: no resumption of menstrual activity and postmenopausal levels of follicle-stimulating hormone and estradiol 1 year after the last cycle of chemotherapy.

Table 2
Estimated risk of permanent amenorrhea resulting from single agent chemotherapy and combination regimens used as adjuvant treatment for early breast cancer.

	Single drug	Adjuvant regimens
High risk (>80%)	Cyclophosphamide	
	Ifosfamide	
	Chlorambucil	
	Melphalan, busulfan	• CMF, FEC, FAC × 6 cycles in women aged ≥40 years
	Nitrogen mustard	
	Procarbazine	
	thiotepa	
Intermediate risk	Cisplatin	• CMF, FEC, FAC × 6 cycles in women aged 30–39 years
	Carboplatin	• AC, EC × 4 in women aged ≥40 years
	Adriamycin	
	Taxanes	• Taxane-containing combinations
Low risk (<20%) or no risk	Bleomycin	
	Dactinomycin	
	Vincristine	• CMF, FEC, FAC × 6 cycles in women aged <30 years
	Vinblastine	
	Methotrexate	
	Mercaptopurine	• AC, EC × 4 in women aged <40 years
	5-Fluorouracil	
To be determined	Trastuzumab, bevacizumab	
	Lapatinib	

Modified from Ref. [107].

POF refers to primary ovarian insufficiency, in which the cause of ovarian dysfunction is inherent in the ovary [24]. There is debate within the literature as to whether POF should refer only to spontaneous cessation of ovarian function and exclude induced ovarian failure.

2.2. Pathogenesis of POF induced by CT

POF caused by CT can arise through impairment of follicular maturation or primordial follicle depletion, or both [3,25]. Ovaries are variably sensitive to most cytotoxic drugs (Table 2) [26]. Alkylating agents are most commonly associated with permanent and irreversible gonadal damage, presumably because these drugs are not cell cycle-specific. Therefore, both resting and growing primordial follicles can be damaged [25,26]. Cycle-specific agents, such as methotrexate, 5-fluorouracil, bleomycin and vinca alkaloids are associated with mild or no gonadotoxicity. Cisplatin and adriamycin modestly affect ovarian function.

Chemotherapy-induced ovarian damage resembles accelerated ovarian aging [3,24,27]. Pathologic features associated with this type of POF mainly include reduction in the follicle pool, with follicular apoptosis, premature atresia, harmed blood vasculature and cortical fibrosis, eventually leading to ovarian atrophy [27,28].

Currently, several mechanisms have been proposed to explain the damage induced by cytotoxic agents to the ovaries, thoroughly reviewed in [29,30] and summarized in Table 3. Presumably, different classes of anticancer drugs act through a range of mechanisms, possibly targeting different ovarian cells [29]. Oocyte apoptotic death could be the consequence of the direct damage to the oocyte itself, to somatic cells, or both. This, in turn, stimulates primordial follicles to grow to replace growing follicles, but reduces the primordial pool of resting follicles (the "burn-out" hypothesis) [27]. Recently, a correlation between acute vascular damage and alteration in ovarian blood flow, size, and function, associated with an abnormal hormonal profile and clinical symptoms, was demonstrated in a small cohort of 20 premenopausal patients with EBC who were treated with neoadjuvant/adjuvant CT [31]. Notably, the observed pattern of acute ovarian failure was similar and homogeneous in all patients, despite age and variability in CT-regimens.

2.3. Clinical aspects of POF

The clinical degree of ovarian dysfunction (i.e., oligomenorrhea, transient/prolonged amenorrhea, and true menopause) is essentially related to the extent of injury caused by CT. Oligomenorrhea or temporary amenorrhea are the consequence of damage to both steroid-producing cells (granulosa cells and theca cells) and the oocytes of growing follicles. When exposure to CT induces near complete follicular depletion or few follicles remain viable (approximately <1000), periods may cease and menopause will occur [14,32]. Despite the fact that many patients > 40 years of age develop CRA, ovarian failure may be temporary in a considerable number of women. It is not yet known what percentage of women with CRA/oligomenorrhea will later develop CRM. The remaining follicles, however, may still be recruited from the primordial pool. Accordingly, gonadotropin levels may return to normal, menstrual cycles may resume and/or fertility may recover months to years after withdrawal/end of CT [26]. Menses are more likely to return in younger women, in those receiving less gonadotoxic regimens and, in any case, in those with a higher basal number of follicles [12,14].

Women who continue to menstruate following CT-regimen may have a reduced likelihood of pregnancy, even if menses are regular [33–35]. Women with temporary CRA present an increased risk of CRM compared to those who continue to menstruate throughout treatment [36].

2.4. Factors predicting the onset of POF and resumption of menses in EBC patients

The loss or impairment of ovarian function as side effects of adjuvant CT have been extensively studied in patients affected by EBC. In these patients, a few variables mainly influence the risk of POF induced by CT: the age of a patient when she commences CT and the type and the doses of the prescribed CT [24,37,38]. Furthermore, tamoxifen may variably affect the onset of POF in premenopausal patients with endocrine-responsive EBC. Recently, the

Table 3
Overview of preclinical pathogenic evidence on ovarian toxicity induced by anticancer drugs available for the treatment of patients affected by breast cancer.

Drugs	Types of ovary cells targeted	Mechanism(s) of ovarian toxicity/features of the damage	References
Cyclophosphamide (alkylating agenst?)	Oocytes/PMF	In human fetal ovary exposed to CTX, apoptosis was shown before in oocytes (PMF), than GCs	[121]
		CTX metabolites induce H2AX, a marker of double strand DNA breaks predominantly in mouse oocytes (but also in GCs) cultured in vitro	[122]
	GCs	In the GCs of rat ovaries CTX damage to mitochondria induce the release of the cytocrome C into cytoplasm leading to cell apoptosis mediated by caspase family proteins. Damage in human GCs nuclei and follicular basement membranes	[32,33,122–129]
		Oxidative stress has been associated with CTX toxicity in GCs of mature follicles. CTX induces depletion in glutathione and rise in reactive oxygen species mediating apoptosis in GCs	
	Stromal cells/vessels	In human ovaries severe signs of stromal fibrosis and capillary changes or cortical fibrosis and blood vessel damage were found following regimens including alkylating agents	[34,35,130,131]
Platinum compounds	Oocytes/PMF	Experimental studies on platinum-induced ovarian toxicity limited to cisplatin. Pre-granulosa cell cytoplasmic and nuclear swelling followed by PMF architecture disruption with disappearance of the lumen and its oocyte. In rats cisplatin induces reduction in ovulation rate and a decline in AMH and inhibin-A	[27,126]
	GCs/TCs	After cisplatin exposure, decline in the level of hyperpolarization-activated cation channels in rat oocytes, GCs and TCs may contribute to decreased ovarian function	[127,128]
		Another proposed mechanism for cisplatin-induced ovarian toxicity is the accumulation of or activation of p63, a member of p53 family, eventually leading to oocyte death	[129]
Doxorubicin (anthracyclines)	Oocytes/PMF	Doxorubicin induces apoptosis in mammalian oocytes and significantly decreases their survival in vitro. Several molecular pathways are involved in apoptosis of oocytes, including damage to chromosomes, activation of mitochondria and elevation of superoxides that induces endoplasmic reticulum stress with the increase in intracellular Ca^{++}. This cascade in turn leads to apoptosis via calpain and caspase 12	[130–133]
	GCs	DNA damage and apoptotic cell death induced by doxorubicin has been demonstrated both in human primordial follicles and GCs in vitro	[134]
	Stromal cells/vessels	Acute reduction in ovarian blood flow and disintegration of the vessel wall following in vivo administration of doxorubicin	[30,135]
Taxanes	Mature follicles	Only preclinical studies on taxane-induced ovarian toxicity are available, limiting to paclitaxel. The drug induces apoptosis mainly in mature follicles and to a lesser extent in less mature populations; also estradiol serum levels were reduced by the drug	[136]
5-Fluorouracil	Unknown	Ovarian function in mice may be affected (reduced fertility) when the drug is administered during the immediate postovulatory phase, but not during the other phases of fertility cycle	[137]

AMH, anti-müllerian hormone; ECs, endothelial cells; GCs, granulosa cells; PMF, primordial follicles; TCs, theca cells.

potential role of genetic marker as a predictive factor of ovarian toxicity has emerged [28]. Notably, comparisons of POF rates between studies are hampered by substantial variability in treatment used, median age of patients, prevalence of endocrine-responsive disease, and follow-up duration. Inconsistencies also exist in the manner of reporting the incidence of CRA, and even in the definition of CRA. Some authors report the incidence of amenorrhea upon completion of chemotherapy, while others report continued amenorrhea at various points of time after the start of chemotherapy. In studies on adjuvant treatment for BC, the definition of CRA may vary from the absence of menses for at least 3 months to more than 1 year (Table 1).

2.4.1. Age

Age is currently the most relevant biological factor influencing the risk of CRM/CRA [12]. The occurrence of CRM in patients with EBC has been specifically evaluated in very few studies [39,40]. It has been reported in 22–61% of women aged < 40 and 61–97% in those > 40 years [3,39,41]. In this group of patients, the incidence of CRA has been more extensively evaluated (Table 1). By maintaining the arbitrary cut-off value of 40 years of age, rates of CRA may range from 49 to 100% in women > 40 years, and from 10 to 71% in younger women [3,12].

Resumption of menses (ReMen) in patients affected by EBC and CRA has been reported in 11% of those aged > 40 years and 22–56% of those aged < 40 years, respectively [3]. Analysis of ReMen in the IBCSG study VI population (in which patients received 3–9 cycles of CMF) indicated a recovery rate of 43% in patients under 40, and of 9% in those aged 40 years and over [42]. In the NSABP B30 study (708 patients receiving 4 cycles of AC and 4 cycles of docetaxel) ReMen occurred within 24 months in 45.3% of patients aged under 40, 10.9% of those aged 40–50 and in 3.2% of patients aged over 50 [43].

2.4.2. Cytotoxic regimens

Other than age, the incidence of CRM/CRA mainly depends on the type of CT and the schedule of administration used (Table 1) [39,44]. However, further factors related to CT delivery may influence the onset of POF, including the combination of more than one drug (poly-CT), the cumulative dose of alkylating agents, and the phase of the menstrual cycle when CT is delivered. In women candidate to adjuvant CT for EBC, the most commonly used CT-regimens incorporate multiple agents that are generally responsible for a more negative impact on ovarian activity, compared with monochemotherapy [3,26,39]. Higher cumulative doses of alkylating agents are associated with the highest risk of CRM, particularly in older premenopausal patients [32,45,46]. Similarly, patients who received higher cumulative doses of cyclophosphamide, such as with CMF and CEF/CAF-type regimens, showed a higher rate of transient and prolonged amenorrhea, compared to AC [3]. The influence of taxanes on the risk of CRA has been evaluated in several studies,

but remains controversial. When taxanes are added to standard regimens, the risk of CRA increases in the majority of [45–50], but not in all, trials [51–54]. However, the extra-risk of CRA associated with these drugs is transient, being limited to 12 months following the end of CT. When CRA rates were evaluated in a comparison between AC followed by docetaxel, TAC or AT, the latter regimen led to the lowest CRA rate (69.8%, 57.7%, 37.9%, respectively) [55]. Higher doses and longer duration of CT-regimens may result in higher rates of CRA [56,57]. In two studies evaluating dose-dense FEC and AC-T, respectively, the incidence of CRA was identical in both the control and intensified arms (Table 1) [58,59]. However, definitive data regarding the effect of dose-dense therapy on rates of CRA are lacking. The specific impact of taxanes on the development of CRM is still unclear. Finally, when CT-regimens are administered during the follicular phase of the menstrual cycle they may increase the risk of ovarian toxicity [3,12,60].

According to CT-regimen, ReMen may occur early or may be delayed even after 2–3 years from the onset of CRA. In a large prospective study investigating factors affecting the maintenance of menstrual bleeding (595 patients affected by EBC; CT-regimens: FAC, AC ± taxanes or CMF), women receiving CMF were more likely to bleed during the first month after chemotherapy than women on AC-containing regimens, but, by one year and thereafter, these women were less likely to bleed than those who received AC-regimens. Adding taxanes resulted in a further reduction of monthly bleeding [61]. Recently, the same group reported results in a cohort of 466 premenopausal patients (20–45 years) receiving CT (AC/ACT or CMF) for EBC. Of these, 41% and 29% experienced CRA, respectively, after 6 and 12 months. In both groups, approximately 50% of patients resumed bleeding within 3 years. ReMen differed significantly according to treatment after 6 months of CRA (AC 68%, ACT 57%, CMF 23%), but not after one year. After 2-year CRA, none of the patients who had received CMF experienced ReMen, compared with almost 25% of those treated with AC-based regimens [62]. In the study of Abusief et al. [59], which included 431 patients treated with doxorubicin and cyclophosphamide, with or without paclitaxel, ReMen between 6 and 12 months following completion of chemotherapy was reported in 14% and after > 12 months in an additional 3% of cases.

Recently, a number of Phase III Trials evaluated the protective role of gonadothropin-releasing hormone (GnRH) agonists in patients affected by EBC (see below). Since in these studies the primary end-point was the ovarian function after CT, the rates of CRA/CRM in the control group may represent a more reliable estimation of CT-induced ovarian toxicity. In a study by Del Mastro et al. [63] on 281 patients (age ≤ 45 years; CT-regimens were anthracycline-based, anthracycline plus taxane-based, or CMF-based ± triptorelin), CRA and CRM rates were 75.5% and 25.9%, respectively. ReMen was reported by 49.6% of patients enrolled in the control group [63]. In the study

by Gerber et al. [64] on 60 patients under the age of 46 (CT-regimens: AC with or without taxane ± goserelin), at 6 months after CT the rate of temporary CRA was 83.3% with ReMen in 56.7% of patients in the control group. Lower rates of CRA/CRM were reported by Munster et al. [65]. In this study, patients (44 years old or younger) were treated with AC-T or FEC ± triptorelin. After 49 patients were enrolled, the accrual was halted early due to futility. However, in the control group the rates of CRA, CRM and ReMen were 24%, 10% and 90%, respectively (median follow-up of 18 months from completion of CT). The differences in terms of ovarian toxicity showed in these trials may be explained by the prevalence of younger patients, the cumulative dose of alkylating agents delivered and the use of tamoxifen, but also by the different definition of amenorrhea, stratification criteria and duration of the follow-up period [66].

2.4.3. Endocrine treatment

Tamoxifen is the cornerstone of endocrine treatment of premenopausal patients affected by endocrine-responsive EBC. Premenopausal women receiving tamoxifen showed a reduced probability to have monthly bleeding within one year after chemotherapy [79]. In large prospective trials, the use of this drug has been significantly associated with an increased rate and/or duration of CRA [39,42,43,66–69]. In one of these studies, the effect of adjuvant tamoxifen on CRA appeared to be limited to women > 40 years of age [68]. Adjuvant tamoxifen following CT for EBC appeared to result in a slightly, but statistically significant, increase in the risk of CRM [3]. Still, how tamoxifen influences ovarian toxicity remains unclear. The suggested mechanisms of interference include the alteration of the hypothalamic-ovarian feedback loop regulating estrogen synthesis and higher plasma estradiol levels, due to the selective estrogen receptor modulation induced by tamoxifen [35,70].

2.4.4. Genetic factors

It has been proposed that specific genes have a role not only in determining the age of natural menopause [71] but also in CRA/CRM [28]. In a prospective study on a cohort of 127 young BC survivors, a common variant of a cyclophosphamide metabolizing enzyme (CYP3A4*1B) was found to be associated with higher risk of ovarian failure after cyclophosphamide-based chemotherapy compared with the wild-type genotype [72]. These data show that genetic markers, other than age and characteristics of CT-regimens, may have a role in predicting ovarian toxicity induced by CT. Further prospective correlative trials will clarify the clinical impact of these new predictive biomarkers of toxicity.

3. The prognostic significance of CRA, CRM and RM in patients with EBC

A positive influence of CRA and CRM on the survival of patients affected by EBC has been reported in the majority of studies [12]. The mechanisms underlying the benefits of chemotherapy in endocrine-responsive EBC patients, however, remain uncertain. Clinical evidence suggests that CT may exert a dual mechanism: direct cytotoxic activity and an indirect suppressive hormonal effect mediated by damage to ovarian estrogen-producing cells [12,32]. This may explain the clinical evidence that chemotherapy is more effective in younger, rather than in older, patients.

In the largest prospective trial evaluating amenorrhea as a consequence of adjuvant CT-regimens (AT/ACT/AC-T) in premenopausal EBC patients, it was initially shown that patients with CRA (defined as lack of menstruation for >6 months during 24 months of follow-up) had significantly better overall and disease-free survival than those without amenorrhea, regardless of treatment received and estrogen-receptor status [13,43]. In a subsequent 12-month landmark analysis, the clinical significance of CRA was found to be restricted to patients with ER + disease [73]. These data are consistent with results from IBCSG Trial 13–93 and IBCSG Trial VIII, where ovarian function suppression obtained by CT (CRA) was associated with improved outcome only in the subgroup of patients with ER + BC [74,75].

Whether prolonged duration of transient CRA results in survival advantages is uncertain. In some studies, patients treated with anthracyclines/CMF showed an improved survival benefit if they experienced amenorrhea, irrespective of the time of onset and duration [76,77]. In another study, women who received CMF/CEF showed a better survival rate if they had achieved amenorrhea at 12 months, but not at 6 months [78]. Interestingly, in a recent retrospective analysis on 872 premenopausal patients (median age at diagnosis: 41 years, range, 21–54) who received various CT-regimens for stage I–III BC, RM was significantly associated with shorter DFS (not OS) in both hormone receptor-positive and -negative patients at multivariate analysis [87]. These results suggest that ReMen, more adequately than CRA, might reflect the endocrine-mediated anticancer effect of chemotherapy, at least in patients > 35 years [79].

4. Preserving fertility in breast cancer patients

Infertility is among the major consequences of POF induced by CT [15]. Issues related to residual fertility following exposure to CT-regimens are of mounting importance due to the rising tendency by an increasing proportion of women to delay pregnancy to later in life [15]. It has been reported that more than 50% of young women have fertility concerns upon being diagnosed with BC, and CT-induced infertility may influence treatment decisions in almost 30% of younger BC patients [80]. Moreover, whether subsequent pregnancy could increase the risk of recurrence remains a great concern in BC survivors, particularly in those with endocrine-responsive disease. This is despite a number of observational studies that showed no detrimental effects from pregnancy after BC [13,42,73,78].

A number of effective methods are available for younger BC patients wishing to preserve their fertility potential from damage of CT, including cryopreservation techniques and/or the administration of GnRH-agonists [15,44]. However, each of these methods presents various *pros* and *contras*. Methods based on cryopreservation are costly, complex, and have a significant failure rate [81,82]. Both embryo and oocyte preservation techniques require ovarian stimulation before harvest. This exposes patients to high estrogen levels, a potential risk in patients with ER+BC. In addition, laparoscopy and general anesthesia are required, inducing a delay of 2–6 weeks in starting CT. Importantly, the efficacy of these treatments in patients > 40 years of age seems to be uncertain [83].

The use of GnRH analogs has some advantages. The treatment is simple to administer, is low in cost, and CT need not be delayed. Moreover, a male partner is not required.

However, GnRH agonists must be administered one week to six months before CT and continue until the end of CT. Also the safety of the use of GnRH agonists in cancer patients has been questioned. Since GnRH receptors are expressed by a variety of cancers and mediate several effects (e.g., inhibition of proliferation, induction of cell-cycle arrest, and inhibition of apoptosis), it has been suggested that the concomitant administration of GnRH agonist therapy might antagonize CT, reducing its efficacy [84,85]. Therefore, ovarian suppression with GnRH agonists might be safer in women with estrogen receptor-negative BC [86]. Detrimental effects of GnRH agonists on patients under CT, however, were not shown in clinical trials.

Moreover, results from clinical trials evaluating the efficacy of GnRH agonists in preventing CT-induced ovarian toxicity are conflicting. A recent meta-analysis including six randomized trials that evaluated ovarian function after co-treatment with a GnRH agonist and various CT-regimens concluded that this approach may be beneficial for protection of menstrual function [87]. However, the treatment with a GnRH agonist combined with CT was not associated with a statistically significant difference in the rate of spontaneous pregnancy following CT. Limitations of these findings included the small size of most of the trials evaluated, the lack of adequate analyzable data, the generally short period of follow-up, methodological weakness of one of the trials, and the use of different treatment protocols [87].

More recent studies have not clarified the issue. The positive role of GnRH agonists in preserving ovarian function during chemotherapy for EBC patients is supported by two trials, the Italian PROMISE-GIM6 trial [63], and a trial from the Royal Marsden Hospital, UK [88]. Similar results were obtained by Badawy et al. [89] in a smaller study. Conversely, other smaller trials failed to demonstrate any benefit of the GnRH agonists in preserving fertility in patients with EBC, including the Zoladex Rescue of Ovarian Function (ZORO) trial [64], the Ovarian Protection Trial in Estrogen Negative (OPTION) [90] and the study by Munster et al. [65]. Results from ongoing studies aimed at evaluating the role of ovarian suppression in patients affected by EBC under CT (for example, the Prevention of Early Menopause Study – POEMS; South West Oncology Group-SWOG study 0230) are awaited.

Finally, it has been suggested that the concomitant use of GnRH agonists for ovarian suppression and cryopreservation increases the chances of preserving fertility in young women [44,88].

According to an update of American Society of Clinical Oncology practical guidelines on fertility preservation in cancer patients, sperm and embryo cryopreservation, as well as oocyte cryopreservation, are considered standard practice. Other fertility preservation methods should be considered investigational and should be performed by providers with the necessary expertise [91].

5. Measuring ovarian reserve in healthy women and in those with cancer

Ovarian reserve (OR) indicates the number and quality of follicles that, at any given age, are available to produce a dominant follicle late in the follicular phase of the menstrual cycle. Several causes impair OR, including ovarian surgery, exposure to viral or environmental toxic agents such as smoking, with anticancer CT or radiotherapy being the most common cause of acquired loss of OR [24]. However, OR may be reduced in cancer patients at diagnosis [24]. Similarly, ovarian response of patients who undergo controlled ovarian hyperstimulation before CT is diminished even before treatment [92]. In a large cohort of 40-year-old women not undergoing systemic treatment, an unusual earlier median age of menopause onset was found [93–95]. Conversely, other authors did not find any impact of BC on OR [96]. Therefore, the real impact of cancer (particularly of solid malignancies) on OR, and the potential mechanisms of impairment of ovarian function induced by cancer itself remain to be clarified.

In the fertility and assisted reproduction setting, OR is measured by a number of procedures, such as ultrasound evaluation of the antral follicle count (AFC) and assessment of serum levels of FSH, estradiol, inhibin-B and anti-Müllerian hormone (AMH) [97]. AMH is emerging as the most promising measure of OR. It is produced by the small growing follicles and exerts a paracrine effect on the primordial follicle. AMH inhibits the recruitment of primordial follicles into the growing follicle pool by reducing sensitivity to FSH. Conversely, inhibin-B positively influences folliculogenesis in the ovary (Fig. 1) [98]. AMH serum levels in normal cycling women decline with age and become undetectable in menopausal women [99]. In several studies, AMH accurately reflects the ovarian follicle reserve and represents the most sensitive marker for the age-related decline in the number of primordial follicles. In addition, AMH and AFC offer the most reliable assessment of the reproductive lifespan of the ovaries, as well as of the risk of premature ovarian
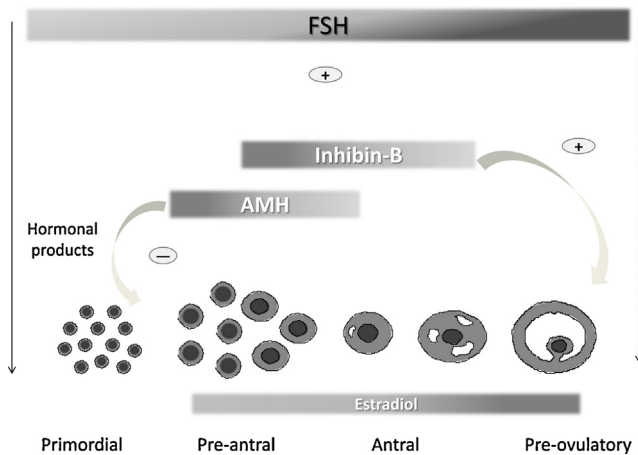
Fig. 1. Changing patterns of hormone production by ovarian follicles from resting to pre-ovulatory stages (darker shading in hormone labels corresponds to greater hormone production). Legend: The rise in FSH serum levels at the end of a previous menstrual cycle drives the initial follicle recruitment. Growing follicles produce estradiol and other peptide hormones, such as AMH and inhibin-B, which are important in feedback regulation of FSH. AMH is secreted by pre-antral and antral follicles and appeared to play an inhibiting role in initial recruitment of primary follicles from the resting primordial follicle pool and in the selection of the dominant follicle, by reducing the sensitivity of antral follicles for FSH. Inhibin-B may have paracrine functions positively influencing folliculogenesis.

failure. Moreover, they are the best predictors of the success of in vitro fertilization cycles [97].

In women treated with chemotherapy for Hodgkin's lymphoma, and in young women who received chemotherapy/radiotherapy for childhood cancer, [100–102] AMH was the most sensitive predictor of OR, compared with FSH and inhibin-B.

A limited number of studies evaluated OR in patients affected by EBC receiving adjuvant CT/ET. Basal measurements of AMH, FSH, inhibin-B, estrogen levels and AFC have been found to be sensitive predictors of OR in patients with EBC undergoing CT [33,35,103]. Women still menstruating after the end of CT present, however, higher FSH levels, lower AMH and inhibin-B levels, as well as lower AFC, compared to controls [35,103]. Lower levels of AMH were strictly related to a low AFC, thus predicting a reduced likelihood of future pregnancy [35]. High pretreatment levels of AMH were associated with high levels of AMH one year after completing treatment [104]. Furthermore, patients with pretreatment levels of AMH above the median of the study group showed more favorable post-treatment ovarian function, including those with EBC [104].

AMH levels decrease after the first courses of chemotherapy and progressively thereafter during treatment [34,104]. In a cohort of 50 premenopausal women with EBC, it was demonstrated that CT induced rapid decreases in AMH, inhibin-B, and AFC, without modifying estradiol levels [33]. Moreover, AMH levels prior to chemotherapy were lower in women who became amenorrheic within 6 months, compared to those who resumed menses. No significant difference

in pretreatment inhibin-B levels was found between groups [33]. On the other hand, it has been observed that pre-chemotherapy inhibin-B and AMH were lower in women with EBC who experienced CRA, both predicting CRA [105]. Likewise, in a cohort of 127 women who received adjuvant CT for EBC, AMH and inhibin-B were significantly associated with the risk of CRA, compared with age-matched controls [102]. AMH was significantly lower and FSH significantly higher in menstruating women who developed subsequent CRA [106]. Conflicting results have been reported regarding the influence of tamoxifen on AMH and inhibin-B [33,35,106].

6. When are patients with chemotherapy-related amenorrhea actually menopausal?

In healthy women, a few well defined clinical aspects (age, menstrual history and menopausal symptoms) are generally indicative of menopausal status. Nevertheless, natural menopause can only be retrospectively established after 12 consecutive months of spontaneous amenorrhea, with biochemical confirmation of FSH and estradiol serum levels within the menopausal range.

In studies assessing ovarian function following CT, the definition of menopause is heterogeneous [107]. In most of these reports, the cessation of menses was the only surrogate marker of menopause, although the follow-up period was limited. According to the National Cancer Comprehensive Network (NCCN) the diagnosis of menopause in BC patients should include any of the following criteria: prior bilateral oophorectomy; age older than 60 years; age younger than 60 years and amenorrhea for ≥12 months in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression and FSH and estradiol in the postmenopausal range. If the patient is taking tamoxifen or toremifene, and is under 60, then FSH and plasma estradiol levels should be in postmenopausal ranges [10]. In these guidelines, it was emphasized that for premenopausal women starting adjuvant ET, CRA is not a reliable indicator of menopausal status, as ovarian function may still be intact or resume despite chemotherapy-induced anovulation/amenorrhea. If treatment with AI is foreseen, serial measurements of FSH/estradiol are recommended in patients with CRA [10]. However, these factors are not completely reliable to confirm menopause in these patients [108].

In particular, biochemical tests present a number of limits. Elevated FSH and reduced estradiol levels generally confirm the clinical diagnosis of menopause. The duration of transition toward menopause, however, is highly variable, as it is a dynamic *continuum*, with FSH/estradiol values subject to wide fluctuation. Therefore, a diagnostic cut-off for these biomarkers is difficult to define. Additionally, testing for FSH/estradiol only at single points in time is not sufficient to confirm menopause. In this respect, serial assessments of these biomarkers would be more reliable, but the number and

intervals of time points of assessment remain arbitrary. Moreover, when CT is used, the prevision of the definitive loss of ovarian function may be more erratic. In addition, some technical aspects may negatively affect reliability of biochemical tests. Current plasma estradiol radioimmunoassay is not sufficiently sensitive to detect the low postmenopause estradiol levels [109]. Tandem mass spectrometry, a validated method dosing steroids in the pg/ml range, may not be widely available [18]. Furthermore, tamoxifen has been reported to increase circulating estrogens and decrease FSH levels [110]. Increased levels of estradiol may result from cross-reactivity of tamoxifen and its metabolites in the estradiol assay. Likewise, exemestane, a steroidal AI, may interfere with serum estradiol measurement [111]. AI provoke a strong decrease in estrogen levels and increase FSH levels in postmenopausal patients [110]. In premenopause, though, the inhibition of estrogen production is only temporary and the subsequent FSH increase may, in turn, stimulate follicular growth, aromatase production and restore pre-CT estradiol levels [17].

Interestingly, AMH and inhibin-B have shown a potential predictive role of ovarian function recovery in studies evaluating OR in limited cohorts of cancer patients, including those with BC. AMH levels, in particular, appear to provide a practical tool useful in defining the individual risk of POF induced by CT [104]. Conversely, in a retrospective study on a very young population of women who received CT/ET for EBC (median age 37 years, range 27–40), compared with age-matched controls, neither the baseline nor changing levels in AMH appeared to be predictive markers for subsequent menstrual status following CT [112].

Unfortunately, due to some limitations in these studies, it is not possible to define the role of these new markers of OR as predictive factors of CRM, especially in BC patients. In fact, none of these studies has been specifically designed to test AMH/inhibin-B as predictive factors of CRM; cohorts of patients are limited, the age distribution among the cohorts appears inhomogeneous, as does the treatment received, the duration of follow-up and the sample collection time.

Very recently, in a prospective study with a 5-year median follow-up, basal serum AMH was reported to be strongly predictive of long-term ovarian function in a cohort of patients undergoing CT (±ET) for EBC [113]. In particular, AMH remained the only significant predictive factor of OR in a multivariate analysis including age and FSH.

7. Ovarian reserve markers and the choice of endocrine therapy in EBC patients

In clinical practice, ascertaining residual ovarian activity in a premenopausal patient with endocrine-responsive EBC and CRA is of key importance not only to define the fertility potential following completion of CT, but also to select the appropriate adjuvant ET (tamoxifen versus AI) [114]. To this aim, AMH may be used as suggested in Fig. 2.
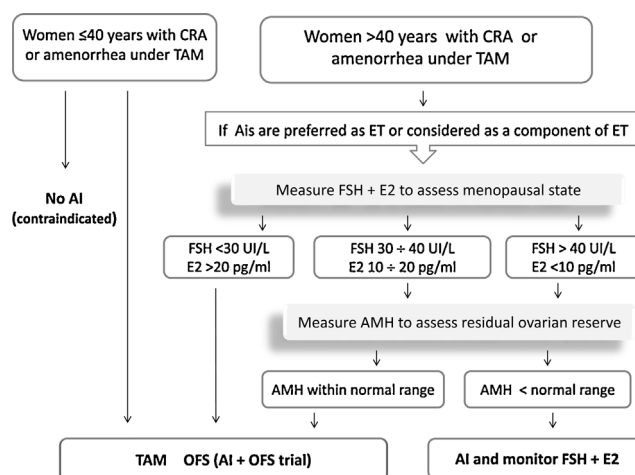

Fig. 2. Practical approaches suggested when aromatase inhibitors are the preferred endocrine strategy for patients with endocrine-responsive EBC and CRA or amenorrhea under tamoxifen. *Abbreviations*: AI, aromatase inhibitor; AMH, anti-Müllerian hormone; CRA, chemotherapy induced amenorrhea; E2, estradiol; EBC, early breast cancer; ET, endocrine therapy; FSH, follicle-stimulating hormone; OFS, ovarian function suppression (oophorectomy or chemical suppression with a gonadotropin-releasing hormone agonist); TAM, tamoxifen

Women under the age of 40 generally maintain residual OR following CT, despite persistent CRA, and, therefore, they should not receive an AI as the only adjuvant ET. If estrogen depletion is the desired endocrine strategy, this should include ovarian function suppression (OFS; oophorectomy or chemical suppression with a gonadotropin-releasing hormone agonist) in combination with tamoxifen [9,10]. Alternatively, entering a clinical trial is an advisable option.

Women over the age of 40 who have developed CRA or amenorrhea during tamoxifen should measure FSH and estradiol, preferably with a high-quality estradiol assay (i.e., mass spectrometry), which allows a high sensitivity (in the order of pg/ml) together with a high-specificity (no cross reaction with other steroidal molecules) [115]. Women with levels within the premenopausal range (i.e., FSH < 30 UI/L and estradiol > 20 pmol/L), should receive tamoxifen alone or tamoxifen + OFS.

If intermediate hormone levels (FSH: 30–40 UI/L and estradiol:10–20 pg/ml) appear to be indicative of menopausal transition/perimenopause, measuring AMH levels may be useful in detecting residual ovarian activity [113]. If FSH/estradiol levels are consistent with menopause (FSH > 40 UI/L and estradiol < 10 pmol/L), AI may be prescribed. However, basal and serial hormone monitoring should be performed (e.g., every 4 months), and AMH levels should be prudentially measured even in this group.

Since CRA may be a reversible condition especially in younger women, patients under AI who experience the occurrence of certain symptoms, such as the onset of vaginal bleeding or the sudden cessation of hot flashes, should be rapidly referred to the clinician [18]. Similarly, sexually active women with CRA need to be counseled for the

maintenance of birth control, given that they may ovulate and, therefore, become pregnant, despite the absence of menses.

8. Conclusions

Ovarian toxicity is a major concern of young cancer patients who need CT for a cure. The reduction in fertility potential induced by CT-regimens should be taken into consideration in family planning decisions throughout diagnosis and follow-up, and effective approaches aimed at preserving ovarian function should be regularly offered to women who need to receive CT and ET [5,34,63,91,116]. However, since cryopreservation of embryos or oocytes requires controlled ovarian stimulation, the risk of increased peak estradiol levels with this strategy in patients with endocrine-responsive EBC should be discussed before the procedure. The controlled ovarian stimulation using tamoxifen or letrozole in conjunction with gonadotropin may be safer for women with endocrine-responsive BC [116].

Evaluation of OR in cancer patients may be challenging, and, therefore, availability of predictors providing more reliable assessments of ovarian function is of major clinical importance. At present, AFC, AMH and inhibin-B are reliable predictive factors of fertility, even in cancer patients. Ongoing large prospective trials will define which values of the available parameters assessing OR are indicative of irreversible loss of ovarian function (i.e., the true menopausal status) in women with endocrine-responsive EBC and CRA. However, in a multidisciplinary evaluation with endocrinologists, a careful use of AMH values may improve the assessment of residual OR and, therefore, of CRM, particularly in a specific subset of women, such as those with endocrine-responsive EBC, CRA and uncertain values of FSH/estradiol.

Further investigations aimed at establishing the effects on ovarian function of newer cytotoxic regimens would offer effective alternative treatments with less ovarian toxicity. A deeper knowledge of potential mechanisms sustaining ovarian toxicity triggered by specific drugs remains the rational approach to select the most suitable strategy to prevent ovarian toxicity.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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