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Original Research

Chemotherapy-induced ovarian failure in young women with early breast cancer: Prospective analysis of four randomised neoadjuvant/adjuvant breast cancer trials

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Abstract ***Background:*** Young women receiving chemotherapy for early breast cancer (EBC) have a high probability for ovarian failure, defined by chemotherapy-induced amenorrhea (CIA) as a surrogate. CIA is insufficiently reliable and reproducible. We analysed

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induced ovarian failure; Chemotherapy-induced amenorrhea; Ovarian reserve; Early breast cancer; Young women

chemotherapy-induced ovarian failure (CIOF) by assessing hormone parameters, CIA, and antral follicle count (AFC).

Methods: Blood samples of women aged ≤45 years treated with anthracycline/taxane-based chemotherapy for EBC from four neoadjuvant/adjuvant trials were collected at baseline, at the end of treatment (EOT), and at 6, 12, 18, and 24 months after EOT. Centrally assessed oestradiol (cutoff <52.2 ng/L) and follicle-stimulating hormone (cutoff >12.4IU/L) were used to define CIOF for patients with baseline premenopausal hormone levels, anti-Müllerian hormone (AMH), and AFC to assess ovarian reserve. Further analyses included CIA, regain of premenopausal hormone levels, and disease-free survival (DFS) also in subgroups.

Results: Six hundred ninety-six patients aged ≤45 years had premenopausal hormone levels at baseline. Overall, 85.1% (592/696) experienced CIOF at EOT, and 147 of 592 had further hormone measurements after EOT. Of those, 32.7% (48/147) regained premenopausal hormone levels after 6 months, 57.9% (66/114) regained premenopausal hormone levels after 12 months, 83.0% (73/88) regained premenopausal hormone levels after 18 months, and 89.2% (74/83) regained premenopausal hormone levels after 24 months. After 24 months, 72.4% (21/29) of patients without CIOF and 100% (14/14) with CIOF had low AMH levels. Four-year DFS without CIOF versus CIOF was 65.9% versus 84.6% (hazard ratio [HR] = 2.09, 95% confidence interval [CI]: 1.37–3.19; $P < 0.001$); in hormone receptor positive 61.8% versus 87.5% (HR = 2.69, 95% CI: 1.57–4.60; $P < 0.001$); in <30 years 68.3% versus 92.6% (HR = 4.87, 95% CI: 1.05–22.63; $P = 0.026$).

Conclusion: Most premenopausal women experienced CIOF after chemotherapy for EBC. After 2 years, nearly all regain premenopausal hormone levels. CIOF was associated with better DFS, especially in patients with hormone receptor–positive EBC or aged <30 years.

1. Introduction

Approximately 20% of all new breast cancer diagnoses occur below the age of 45 years [1]. Those patients are prone to experience chemotherapy-associated ovarian damage. Several mechanisms lead to chemotherapy-induced ovarian injury and reduction of the ovarian reserve, directly affecting the growing follicle, damage to blood vessels, and induction of local fibrosis [2,3]. Premenopausal women will experience menopausal symptoms and may face a reduction of their fertility. Indirect parameters as menstrual status have been used as a surrogate for the impact of chemotherapy on ovarian function. Chemotherapy-induced amenorrhea (CIA) is difficult to distinguish from physiologically induced amenorrhea, especially in patients whose age makes it plausible. Amenorrhea is neither a good marker for infertility nor a reliable indicator of menopause, particularly if women are pre/perimenopausal at the beginning of chemotherapy [4]. Almost all available data describe CIA. Most premenopausal patients experienced CIA for at least 6 months after anthracycline/taxane-based chemotherapy. Resumption of menses often occurs within 2 years [2,5,6]. Higher incidence and longer duration are observed in patients aged >40 years [7]. Reported rates range between 10% and 93% [8], reflecting the difference in the definitions used and in follow-up time and patient characteristics. Interstudy comparison is therefore difficult. Because of the CIA-intrinsic limitations, we focused on hormone parameters to define chemotherapy-induced ovarian failure (CIOF). Follicle-stimulating hormone (FSH) and oestradiol are objective and might be more accurate in defining the ovarian damage [9]. Anti-Müllerian hormone (AMH) and antral follicle count (AFC) are used to define the ovarian reserve [10]. AMH is stable across the menstrual cycle and on tamoxifen [11] and seems to provide similar information to AFC [9].

We analysed oestradiol, FSH, AMH, AFC, and menstrual status to define CIOF and reduction of the ovarian reserve with modern chemotherapy for early breast cancer (EBC). The impact of CIOF and CIA on long-term outcome has been investigated.

2. Material and methods

2.1. Patients

In the neoadjuvant/adjuvant GeparSixto [12], GeparSepto [13], GAIN2 [14], and Genevieve [15] studies, blood samples were prospectively collected within the ovarian substudy in women aged ≤45 years at predefined time points: before treatment start, at the end of treatment (EOT), and at 6, 12, 18, and 24 months after EOT (Appendix S1). Samples collected at baseline and at EOT within the main studies for women aged ≤45 years were considered. Oestradiol, FSH, and AMH were centrally measured. AFC was locally evaluated through

transvaginal ultrasound, and menstrual status was documented.

All patients provided their written informed consent for data/biomaterial collection. All studies have been approved by the responsible ethics commission.

2.2. Objectives

The main objective was the rate of patients experiencing CIOF after anthracycline/taxane-based therapy (overall; taxane monotherapy) at the predefined time points. Factors influencing CIOF at EOT were investigated. The rate and time to regain of premenopausal hormone levels were analysed for patients with CIOF at EOT and with assessment of FSH and oestradiol available at the later time points. An exploratory analysis on CIA was conducted. Further objectives were the change in hormone levels, ovarian reserve defined by AMH and AFC, the correlation between AMH and AFC, and the effect of CIOF and CIA on outcome (overall, in subgroups by age and hormone receptor status).

2.3. Endpoints

CIOF was defined as postmenopausal levels of FSH (>12.4 IU/L) and oestradiol (<52.2 ng/L) after treatment, CIA as the absence of menstrual periods, and low AMH level as <0.22 ng/mL (Appendix S1). Central laboratory cutoffs were used. Disease-free survival (DFS) and overall survival (OS) were previously defined [16].

2.4. Statistical analysis

Two-sided X^2 and exact Fisher test *P* values were used to compare CIOF and CIA rates at EOT in subgroups according to age (<30, 30–34, 35–39, and ≥40 years), body mass index (<30 and ≥30 kg/m^2), hormone receptor status and HER2 status (negative and positive), chemotherapy arm (paclitaxel/doxorubicin, paclitaxel-epirubicin/cyclophosphamide [EC], nab-paclitaxel [nP]-EC, cabazitaxel, paclitaxel, E-nP-C, and dose-tailored EC docetaxel), and duration (12, 16–18, and 24 weeks). The rates and time to regain premenopausal hormone levels were investigated using the Kaplan–Meier product-limit method (actual time); patients with no regain were censored at the date of the last hormone assessment. The crude rates at nominal times were reported. There was no competing risk event. For the continuous parameters, the median and interquartile range (IQR) were provided overall at the different time points and in subgroups at EOT. The median and IQR of AMH and AFC were analysed according to CIOF and CIA at the different time points. The percentage of patients with low AMH level after treatment among patients with pre-/post-menopausal hormones was analysed. The correlation between AMH and AFC was assessed using the Spearman's correlation coefficient.

DFS and OS were analysed using the 6-month landmark analysis and compared between patients according to CIOF using the log-rank test overall, according to hormone receptor status and age. Four-year DFS and OS rates were estimated. Cox proportional hazard model was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for CIOF versus no-CIOF and by FSH (continuous and quartiles). DFS was analysed in the overall cohort according to CIA. Multivariate cox regression analysis (MVA) is described in Appendix 1. Alpha was set to 0.05 (two sided). No adjustment for multiple comparisons was performed.

3. Results

3.1. CIOF and menstrual status

A total of 696 of 740 patients aged ≤45 years with premenopausal hormone levels were eligible (Fig. 1 and Fig. S1, Appendix S1). Baseline characteristics are shown in Table 1.

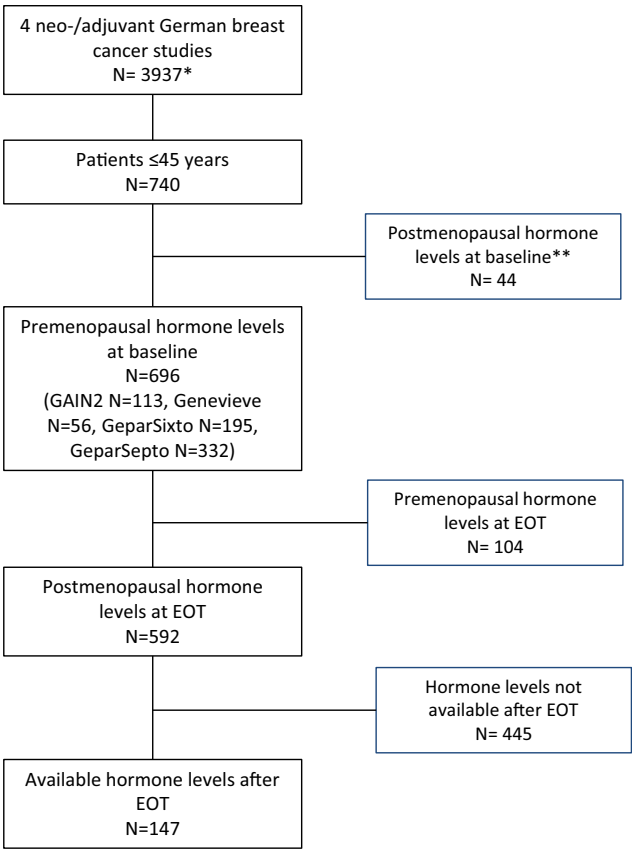


Fig. 1. Flowchart. AFC, antral follicle count; BC, breast cancer; EOT, end of treatment. *Patients started treatment as of 31st January 2016 (before samples selection). **Excluded from the analysis.

Table 1
Baseline characteristics and treatment.

Characteristics		<30 years		30–34 years		35–39 years		≥40 years		Overall	
		N = 60		N = 99		N = 200		N = 337		N = 696	
		N	valid %	N	valid %	N	valid %	N	valid %	N	valid %
Age	(median [range])	28 [21–29]		33 [30–34]		37 [35–39]		43 [40–45]		39 [21–45]	
Body mass index	<30 kg/m^2	53	88.3	90	91.9	169	84.5	279	82.8	591	84.9
	≥30 kg/m^2	7	11.7	9	9.1	31	15.5	58	17.2	105	15.1
T stage	1–2	52	88.1	90	91.9	173	86.9	306	90.8	621	89.6
	3–4	7	11.9	8	8.1	26	13.1	31	9.2	72	10.4
N stage	N0	39	66.1	58	60.4	114	57.9	193	58.5	404	59.2
	N+	20	33.9	38	39.6	83	42.1	137	41.5	278	40.8
Histological tumour type	Ductal/ductal lobular invasive	58	96.7	86	86.9	169	84.5	303	89.9	616	88.5
	Lobular invasive	0	0.0	2	2.0	7	3.5	9	2.7	18	2.6
	Other	2	3.3	11	11.1	24	12.0	25	7.4	62	8.9
Grading	G1-2	17	28.3	37	37.3	75	37.5	144	42.8	273	39.2
	G3	43	71.7	62	62.6	125	62.5	193	57.3	423	60.8
Biological subtype	HR+/HER2+	16	26.7	24	24.2	41	20.5	94	27.9	175	25.1
	HR+/HER2-/G1-2	2	3.3	13	13.1	33	16.5	71	21.1	119	17.1
	HR+/HER2-/G3	11	18.3	11	11.1	36	18.0	43	12.8	101	14.5
	HR-/HER2+	5	8.3	14	14.1	24	12.0	32	9.5	75	10.8
	TNBC	26	43.3	37	37.4	66	33.0	97	28.8	226	32.5
Chemotherapy regimen	PM	9	15.0	16	16.2	23	11.5	49	14.5	97	13.9
	PMCb	9	15.0	24	24.2	23	11.5	42	12.5	98	14.1
	P-EC	16	26.7	22	22.2	55	27.5	86	25.5	179	25.7
	nP-EC	14	23.3	21	21.2	39	19.5	79	23.4	153	22.0
	Cabazitaxel	1	1.7	3	3.0	9	4.5	15	4.5	28	4.0
	Paclitaxel	3	5.0	2	2.0	14	7.0	9	2.7	28	4.0
	iddEnPC	3	5.0	7	7.1	16	8.0	24	7.1	50	7.2
	dtEC-dtD	5	8.3	4	4.0	21	10.5	33	9.8	63	9.1
Chemotherapy duration	12 weeks[a]	4	6.7	5	5.1	23	11.5	24	7.1	56	8.0
	16–18 weeks	26	43.3	51	51.5	83	41.5	148	43.9	308	44.3
	24 weeks	30	50.0	43	43.4	94	47.0	165	49.0	332	47.7
Menstrual status	No amenorrhea	13	76.5	23	88.5	67	95.7	100	97.1	203	94.0
	Amenorrhea	4	23.5	3	1.5	3	4.3	3	2.9	13	6.0
Hormone levels (median [IQR])	FSH IU/I	4.75 [2.35–6.85]		5.60 [3.70–7.20]		5.50 [3.70–8.40]		5.90 [4.20–8.50]		5.7 [3.80–8.10]	
	E2 ng/mL	108.50 [46.50–163.50]		88.00[42.00–161.00]		88.00 [53.00–157.50]		97.00 [62.00–166.00]		94.0 [54.0–161.0]	
	AMH ng/mL	2.15 [1.125–3.49]		2.29 [1.30–4.16]		1.31 [0.56–2.47]		0.60 [0.25–1.27]		1.05 [0.42–2.14]	
	AFC N[a]	9.50 [5.00–12.50]		8.00 [5.00–11.0]		4.50 [1.50–8.50]		4.50 [2.00–8.00]		6.00 [2.00–9.00]	

AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; C, cyclophosphamide; Cb, carboplatin; D, docetaxel; dt, dose-tailored; E, epirubicin; FSH, follicle-stimulating hormone; G, grading; HR, hormone receptor; idd, intense dose-dense; IQR, interquartile range; M, doxorubicin; P, paclitaxel; nP, nab-paclitaxel; TNBC, triple-negative breast cancer.

[a] Paclitaxel/cabazitaxel monotherapy.

Table 2
CIOF and amenorrhea rate at EOT in the analysed subgroups.

Subgroups		CIOF					Menstrual status*				
		No CIOF		CIOF		*P* value	No CIA		CIA		*P* value
		n	valid %	n	valid %		n	valid %	n	valid %	
Age (years)	<30	30	50.0	30	50.0	<0.001	5	31.3	11	68.8	0.001
	30–34	28	28.3	71	71.7		3	12.0	22	88.0	
	35–39	27	13.5	173	86.5		5	7.4	63	92.6	
	≥40	19	5.6	318	94.4		4	3.6	107	96.4	
BMI	<30 kg/m^2	91	15.4	500	84.6	0.552	14	7.8	166	92.2	1.00
	≥30 kg/m^2	13	84.6	92	87.6		3	7.5	37	92.5	
HR status	Negative	39	13.0	262	87.0	0.238	3	4.2	69	95.8	0.281
	Positive	65	16.5	330	83.5		14	9.5	134	90.5	
HER-2 status	Negative	71	15.9	375	84.1	0.376	12	7.9	140	92.1	1.00
	Positive	33	13.2	217	86.8		5	7.4	63	92.6	
CT duration	12 weeks°	24	42.9	32	57.1	<0.001	2	11.8	15	88.2	0.036
	16–18 weeks	18	5.8	290	94.2		2	2.2	89	97.8	
	24 weeks	62	18.7	270	81.3		13	11.6	99	88.4	

*Data on menopausal status are not available for PM and for PMCb arm; °paclitaxel/cabazitaxel monotherapy.
C, cyclophosphamide; Cb, carboplatin; CIA, chemotherapy-induced amenorrhea; CIOF, chemotherapy-induced ovarian failure; CT, chemotherapy; D, docetaxel; dt, dose-tailored; E, epirubicin; HR, hormone receptor; idd, intense dose-dense; M, doxorubicin; P, paclitaxel; nP, nab-paclitaxel.

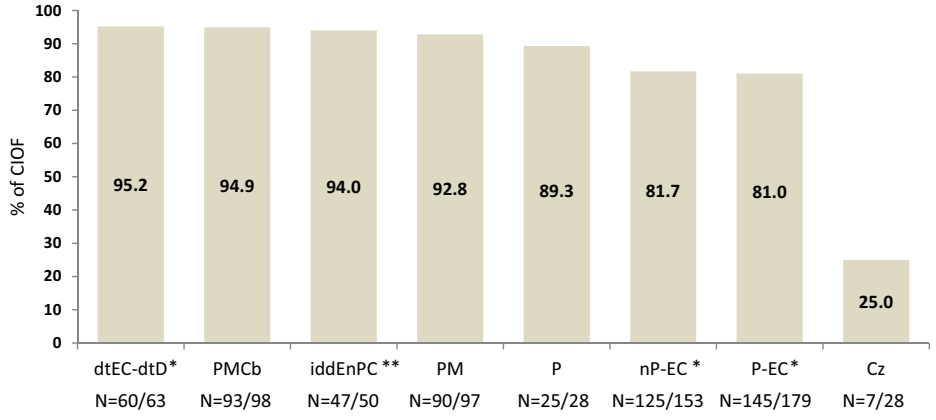


Fig. 2. Chemotherapy-induced ovarian failure at EOT according to treatment. *The cumulative dose of cyclophosphamide was 2400 mg/m^2; ** the cumulative dose of cyclophosphamide was 6000 mg/m^2. C, cyclophosphamide; Cb, carboplatin; CIOF, chemotherapy-induced ovarian failure; Cz, cabazitaxel; D, docetaxel; dt, dose-tailored; E, epirubicin; EOT, end of treatment; idd, intense dose-dense; M, doxorubicin; P, paclitaxel; nP, nab-paclitaxel.

CIOF rate was 85.1% (592/696) at EOT, 60.8% (101/166) at 6 months, 50.9% (54/106) at 12 months, 39.1% (25/64) at 18 months, and 32.6% (14/43) at 24 months after EOT. After taxane monotherapy, the CIOF rate was 57.1% (32/56) at EOT, 55.6% (5/9) at 6 months, 33.3% (2/6) at 12 months, 25.0% (1/4) at 18 months, and 25.0% (1/4) at 24 months after EOT. Older patients, dose-dense/dose-intensified regimen, and longer treatment were associated with a higher rate of CIOF (Table 2, Fig. 2). Overall, 24.8% (147/592) of patients with CIOF at EOT had hormone measurements at subsequent time points. Of those, 32.7% (48/147) regained premenopausal hormone levels at 6 months, 57.9% (66/114) regained premenopausal hormone levels at 12 months, 83.0% (73/88) regained premenopausal hormone levels at 18 months, and 89.2% (74/83) regained premenopausal hormone levels at 24 months after EOT. Actual time of regain and estimated regain rates are presented in Fig. S2.

CIA was reported by 92.3% (203/220) of patients at EOT, 78.4% (80/102) at 6 months, 68.1% (47/69) at 12 months, 60.5% (26/43) at 18 months, and 62.5% (15/24) at 24 months after EOT. With taxane monotherapy, CIA was reported by 88.2% (15/17) of patients at EOT, 85.7% (6/7) at 6 months, 66.7% (4/6) at 12 months, 25.0% (1/4) at 18 months, and 33.3% (1/3) at 24 months after EOT. Age, chemotherapy, and treatment duration influenced the menstrual status at EOT (Table 2, Fig. S3).

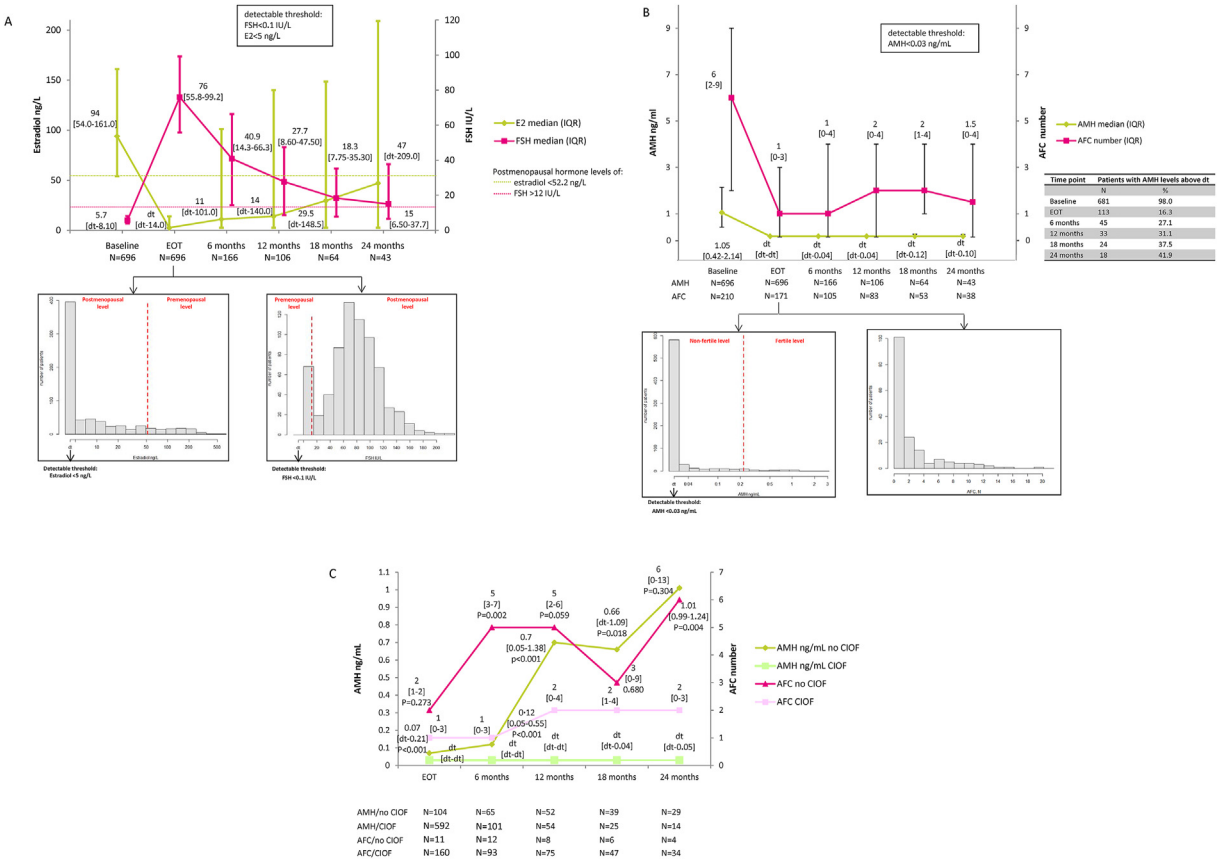


Fig. 3. Changes in median hormone levels of FSH and E2 (A) and of AMH and AFC (B) and correlation of AMH values and AFC with chemotherapy-induced ovarian failure (C) per timepoint. AFC, antral follicle count; AMH, anti-Müllerian hormone; dt, detectable threshold; E2, oestradiol; EOT, end of treatment; FSH, follicle-stimulating hormone. In figure C, *P* values are given for the comparison of AMH values in patients with and without CIOF and for the comparison of AFC in patients with and without CIOF at the different time points.

3.2. Changes in oestradiol, FSH, and ovarian reserve according to AMH and AFC after treatment

At EOT, the median levels of FSH and oestradiol reached the postmenopausal range (Fig. 3A, Table S1). The median values of AMH at EOT decreased. Only 12.4% (113/696) of patients showed values above the detectable threshold (dt < 0.03 ng/mL; Fig. 3B, Table S1). From 6 months after EOT until 2 years after EOT, the median AMH levels remained under the dt. The percentage of women with detectable levels increased over time. The median AFC at EOT was very low and did not recover significantly thereafter (Fig. 3B, Table S1).

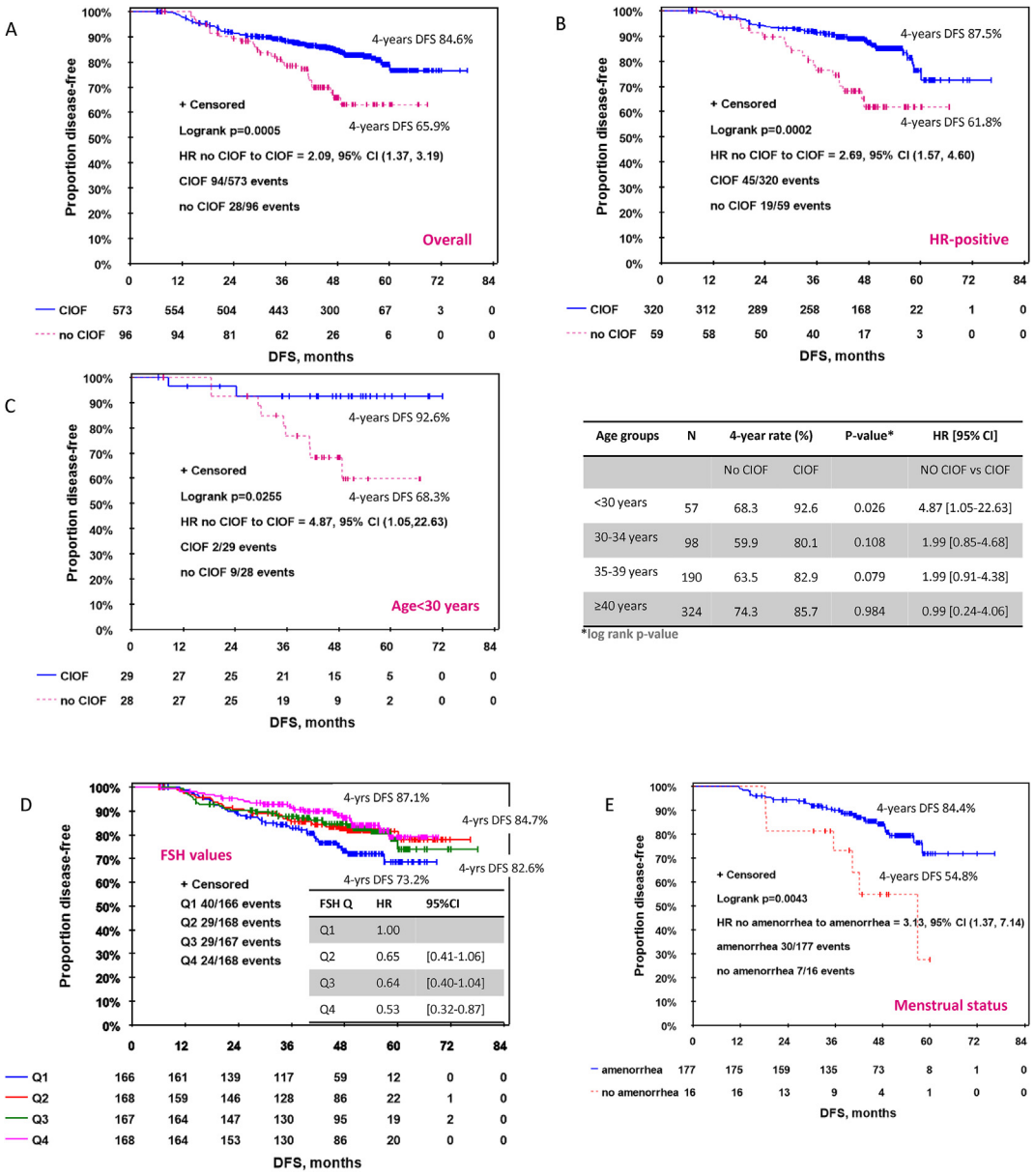
At EOT, the median levels of AMH were low in patients with and without CIOF (Fig. 3C). Low AMH levels were present in 99.8% (591/592) of patients with CIOF and in 76.9% (80/104) of patients without CIOF (Fig. S4). At later time points, the median values of AMH were higher in patients without CIOF (Fig. 3C). After 2 years, low AMH levels were detected in 72.4% (21/29) of patients with CIOF compared with all (14/14) patients without CIOF (Fig. S4). The results were similar for CIA (Fig. S5). A similar trend was found for AFC (Fig. 3C, Fig. S4, S5).

The correlation between AMH and AFC was moderate at baseline ($P = 0.356$; 95% CI: 0.234–0.478) and weak/none at EOT ($P = 0.168$; 95% CI: 0.048–0.289).

3.3. Outcome

The median follow-up was 49.6 (IQR 48.8–50.3) months. Patients with CIOF had a better DFS compared with patients without CIOF (4-year DFS no-CIOF versus CIOF: 65.9% versus 84.6%; HR = 2.09, 95% CI: 1.37–3.19, $P = 0.0005$; Fig. 4A; MVA HR = 2.75, 95% CI: 1.66–4.56, $P < 0.0001$). The benefit was statistically significant in patients with hormone receptor–positive disease (Fig. 4B) but not with hormone receptor–negative disease (4-year DFS no-CIOF versus CIOF: 73.6% versus 80.9%; HR = 1.45, 95% CI: 0.71–2.95; $P = 0.309$; Fig. 4B) and in patients aged <30 years (Fig. 4C).

For better quantification of prognosis, continuous values of FSH and oestradiol at EOT were considered as



Age groups	N	4-year rate (%)		P-value*	HR [95% CI]
		No CIOF	CIOF		NO CIOF vs CIOF
<30 years	57	68.3	92.6	0.026	4.87 [1.05-22.63]
30-34 years	98	59.9	80.1	0.108	1.99 [0.85-4.68]
35-39 years	190	63.5	82.9	0.079	1.99 [0.91-4.38]
≥40 years	324	74.3	85.7	0.984	0.99 [0.24-4.06]

*log rank p-value

Fig. 4. DFS according to no CIOF versus CIOF at EOT overall (A), in patients with hormone receptor positive status (B) according to age (C), FSH levels at EOT (D), and (E) menstrual status. CIOF, chemotherapy-induced ovarian failure; EOT, end of treatment; HR, hazard ratio; Q, quartile; Q1, lower quartile; Q4, upper quartile.

predictors for DFS; only analysis according to FSH was feasible because oestradiol values at EOT mostly fell under the dt. Each 10 IU/L increase in FSH values was associated with a reduction in the risk for a DFS event of 9% ($HR_{10unit} = 0.91$, 95% CI: 0.87–0.96, $P < 0.001$). Patients in the highest FSH quartiles derived the greatest benefit (Fig. 4D). Patients with CIA at EOT had a better DFS compared with patients without CIA (Fig. 4E; MVA HR = 3.2, 95% CI: 1.22–8.59, $P < 0.0187$). OS was significantly improved only in patients with hormone receptor–positive disease (88.4% versus 95.9%; HR = 2.49, 95% CI: 1.04–5.99, $P = 0.035$; Table S2).

4. Discussion

The study showed that the majority of women aged ≤45 years receiving (neo)adjuvant chemotherapy for EBC rendered postmenopausal after chemotherapy, and almost all had amenorrhea. After 2 years, almost three-quarters regained premenopausal hormone levels of FSH and oestradiol. However, only one-third maintained the ovarian reserve as defined by AMH.

The rates of CIOF and CIA observed are consistent with published data reporting rates between 53% and 89% with polychemotherapy [8,17]. Discrepancies between CIOF and CIA relate to the fact that cessation of

menses does not always imply true ovarian failure, as oestrogen levels can remain in a premenopausal range despite more than one year of CIA [18].

After taxane monotherapy [15], 57% of patients had CIOF, but only 25% still experienced CIOF after 2 years. Cabazitaxel was associated with the lowest CIOF rate, which complies with the low pathologic complete response rate in Genevieve. In contrast to previous reports [19], paclitaxel monotherapy showed a higher CIOF rate compared with the taxane and EC combination. The results might have been confounded by the older age of the patients in Genevieve (25% of the patients versus 36% in GeparSepto [13] were aged between 40 and 50). The subgroup is too small to derive any conclusions. Although the majority of patients experienced CIA right after treatment, only approximately 30% were still affected after 2 years. The median time to restoration of menstruation was about 7 months, as previously reported [5]. The concomitant administration of anthracycline/taxane/cyclophosphamide demonstrated the highest rate of CIOF and CIA, monochemotherapy the lowest [17,20]. Dose-dense/dose-intense regimen including cyclophosphamide and longer treatment caused the highest rate of CIOF and CIA compared with conventional regimen [20] and shorter therapy duration [8]. Discordant results with other trials might be because of the different regimens and doses used [19,21]. Cyclophosphamide has a high impact on CIOF [22]. Older patients showed a higher rate of CIOF and CIA, likely because of fewer primordial ovarian follicles with increasing age [23].

Approximately 70% of patients regain premenopausal levels of oestradiol and FSH 2 years after treatment. Previous observations showed the rates of menstruation regain between 39% and 55% for women aged <40 years [24]. Besides the used definition, differences in age and length of follow-up might be additional confounders [25]. GnRH analogues for ovarian function preservation may increase the chance of regaining menstrual function [5,6,25].

As previously reported [4,24], the median AFC declined after therapy to very low levels [26], without subsequent recovery. AMH and AFC reduction after chemotherapy indicates a direct therapy–induced damage to the granulosa cells of the growing follicles. The drop in AMH might have accelerated the recruitment of primordial follicles, which became more vulnerable to chemotherapy, leading to depletion in the primordial follicle pool [2]. AMH might also preserve the ovarian reserve [27]. Although most patients regained premenopausal hormone levels 2 years after treatment, only one-third maintained their ovarian reserve estimated by AMH. AMH is a sensitive marker for the prediction of ovarian function after chemotherapy, whereas menopausal status, oestradiol, and FSH are less reliable, as patients with low AMH might have premenopausal hormone levels.

CIOF after treatment was associated with a better DFS, especially in patients aged <30 years or with hormone receptor–positive disease. Amenorrhea after treatment was associated with improved outcome. Each 10 unit increase in FSH values was associated with a progressive improvement in DFS. Not only postmenopausal FSH levels are fundamental for a better prognosis, but also the degree of ovarian function suppression is important.

Several studies have described the association of CIA with improved outcome regardless of hormone receptor status [28] and patients with hormone receptor–positive tumours only [17,29]. The largest prospective data set from the NSABP-B30 demonstrated a survival advantage independent of the hormone receptor status [28]. The 12-month landmark analysis, which might be more accurate by minimising the guarantee-time bias, showed a survival benefit confined to the hormone receptor–positive cohort [30]. Accordingly, we observed an improved OS only for patients with hormone receptor–positive tumours.

The mechanism leading to an improved outcome in patients achieving CIA is under debate. Because of the benefit seen in some studies irrespective of the hormone receptor status, a direct cytotoxic effect of chemotherapy on the follicle pool and an indirect endocrine effect have been postulated [20,29]. The latter might be enhanced by endocrine therapy, but the results are discordant. In the IBCSG 13-93 trial, CIA led to an improved DFS independent from tamoxifen [29]. This was confirmed by a meta-analysis [31]. Young BC patients have a worse survival [32] partly because of more aggressive tumours but seem to derive a greater benefit from chemotherapy compared with older women, especially if luminal like [33,34]. This may in part be due to an endocrine effect by inducing CIOF, as also observed in the RxPonder trial [35]. Ovarian function suppression induced by luteinising hormone–releasing hormone (LHRH) analogues as in SOFT/TEXT [36] and the EBCTCG study [37] is highly effective in addition to tamoxifen and aromatase inhibitors. Older trials demonstrated LHRH analogue alone to be as effective as cyclophosphamide-methotrexate-fluorouracil, the latter by inducing CIOF [37]. Patients regaining ovarian function after CIOF benefit also from starting LHRH thereafter [38]. Endocrine management is important in young patients to obtain a better prognosis.

The strength of this analysis is the prospective collection of samples across clinical trials using different chemotherapy. Hormones were centrally assessed. Because of data collection at prespecified time points, it was not possible to identify the exact onset of amenorrhea, and assumptions were made. The heterogeneous definition of CIOF and CIA used in other studies is a known bias of analyses on ovarian suppression. Despite the prospective nature of the study,

blood collection decreased with time mainly because of follow-up not performed at the study centre and samples selection. The cohort of patients with further samples after EOT was still representative for the entire cohort. The low adherence to measuring AFC might be explained by the need to transfer patients to a gynaecologist. Chemotherapy received was heterogeneous. Data on adjuvant endocrine therapy and LHRH analogue were not available, but all study protocols recommended treatment per current guidelines. Most patients with hormone receptor–positive EBC might have received tamoxifen alone, and one-third escalated hormone therapy [39,40].

In conclusion, most premenopausal patients aged ≤45 years experience CIOF after chemotherapy for EBC. After 2 years, more than 70% regained premenopausal hormone levels, but in only one-third, the ovarian reserve was not diminished. CIOF was associated with better DFS, especially in patients with hormone receptor–positive EBC or aged <30 years. The degree of ovarian suppression was linked to prognosis. The presented data provide new information and additional insight useful for clinicians to counsel premenopausal patients about the risk of ovarian suppression and diminishing of ovarian reserve after chemotherapy for EBC.

Authors' contribution

The study was designed by J.F., V.N., and S.L. Statistical data analysis was performed by V.N. with contributions by J.F., and S.L., J.F., S.L., and V.N. had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors interpreted the data. The first draft of the article was written by J.F. The decision to submit the article for publication was made by all the authors. All authors contributed to the review of the article.

Role of funding sources

The work was supported by the Walter Schultz Foundation. The Walter Schultz Foundation had no role in the collection, analysis, or interpretation of the data, and had no access to the study data.

Conflict of interest statement

M.F. reports personal fees from Roche, AstraZeneca, Pfizer, Tesaro, Novartis, Amgen, PharmaMar, GenomicHealth, CureVac, EISAI, Celgene, Clovis, Janssen-Cilag, Immunomedics, GSK, MSD, Lilly, outside the submitted work. U.M. reports personal fees and non-financial support from Abbvie, Amgen GmbH, Astra Zeneca, BMS, Celgene GmbH, Daiji Sankyo, Eisai GmbH, Lilly Deutschland, Lilly Int., MSD Merck, Mundipharma, Myriad Genetics, Odonate, and Pfizer GmbH; personal fees from PUMA Biotechnology; personal fees and non-financial support from Roche Pharma AG, Sanofi Aventis Deutschland GmbH, TEVA Pharmaceuticals Ind Ltd, Novartis; personal fees from Pierre Fabre; and personal fees and non-financial support from Clovis Oncology, outside the submitted work. S.A. reports grants from Celgene, Roche, and AbbVie; personal fees from Roche, AstraZeneca, Celgene, Pfizer, Novartis, MSD, Tesaro, Lilly; and others from Roche, outside the submitted work. F.P.A. reports personal fees from Novartis; grants from BioNTech; personal fees from Roche, Pfizer, Daiichi-Sankyo, Astra Zeneca, Eisai, and Merck Sharp & Dohme; grants from Cepheid; personal fees from Lilly, Pierre Fabre, and Seattle Genetics, during the conduct of the study. D.C. reports personal fees from Novartis, Roche, MSD Oncology, and Daiichi Sankyo; grants from Myriad Genetics; and others from Sividon Diagnostics/Myriad, outside the submitted work. In addition, D.C. has a patent EP18209672 pending, a patent EP20150702464 pending, and a patent software (VMscope digital pathology) pending. V.M.M. reports personal fees from AstraZeneca, Amgen, Novartis, Genomic Health, Lilly, Pfizer, and Roche, outside the submitted work. M.V. reports grants and personal fees from Roche, during the conduct of the study; personal fees from Amgen, Astra Zeneca, Daiichi-Sankyo, Eisai, Pfizer, MSD, Novartis, Roche, Teva, and Seattle Genetics; consultancy honoraria from Genomic Health, Hexal, Roche, Pierre Fabre, Amgen, ClinSol, Novartis, MSD, Daiichi-Sankyo, Eisai, Lilly, Tesaro, and Nektar; personal fees from Genomic Health, Hexal, Roche, Pierre Fabre, Amgen, ClinSol, Novartis, MSD, Daiichi-Sankyo, Eisai, Lilly, Tesaro, and Nektar; and others from Novartis, Roche, Seattle Genetics, Genentech, outside the submitted work. L.S. reports grants and others from Abbvie, Amgen, Celgene, Novartis, and Roche; others from Seattle Genetics, PriME/Medscape; personal fees from Chugai; grants and others from Daiichi-Sankyo; others from Lilly, Samsung, BMS, and Puma; others from MSD; grants from Immunomedics; grants and others from AstraZeneca and Pfizer; others from Pierre Fabre; and others from Merck outside the submitted work. In addition, L.S. has a patent EP14153692.0 pending. All other authors declare no competing interests.

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Appendix A. Supplementary data

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