

No protection of the ovarian follicle pool with the use of GnRH-analogues or oral contraceptives in young women treated with escalated BEACOPP for advanced-stage Hodgkin lymphoma. Final results of a phase II trial from the German Hodgkin Study Group

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Background: The reduction of treatment-related toxic effects is the main goal in the current trials of the German Hodgkin Study Group (GHSg). In this regard, the protection of the ovarian reserve in young women is very important. Therefore, the GHSg investigated the use of gonadotropin-releasing hormone-analogues (GnRH-a) and oral contraceptives (OC) in young women with advanced-stage Hodgkin lymphoma (HL).

Patients and methods: Women (18–40 years) were randomly assigned either to receive daily OC or monthly GnRH-a during escalated combination therapy with bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPPesc). Hormonal levels were determined at baseline, during therapy, and at follow-up.

Results: The study was closed prematurely after an interim analysis of 12 patients in arm A (OC) and 11 in arm B (GnRH-a), 9 and 10 are assessable for the primary end point. Women's median age was 25 years in both arms. The anti-Müllerian hormone level after at least 12 months was reduced in all patients. For the entire study cohort, the respective ovarian follicle preservation rate was 0% (95% confidence interval 0% to 12%).

Conclusion: We observed no protection of the ovarian reserve with hormonal co-treatment during BEACOPPesc. This result supports efforts of ongoing trials to reduce chemotherapy intensity and toxicity. Alternative strategies for the protection of fertility must be offered to young female HL patients before the start of BEACOPPesc therapy.

Key words: anti-Müllerian hormone, fertility, GnRH-a, Hodgkin lymphoma, ovarian reserve

Introduction

Hodgkin lymphoma (HL) has become a curable disease in the past decades. Even in advanced stages, long-term progression free and overall survival can be reached by using combined modality treatment, including polychemotherapy and radiotherapy. The highest cure rates have been reported for the escalated combination therapy with bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone regimen (BEACOPPesc), which is therefore regarded as standard of care in the German Hodgkin Study Group (GHSg) [1]. Though the tumor control with this

regimen is excellent, the acute and long-term side-effects cause major concerns. Among these toxic effects, chemotherapy-associated gonadal dysfunction is of particular importance to young HL survivors. Since most patients are diagnosed in the third decade of life, many young female patients in childbearing age are affected [2].

The ovarian damage is mainly caused by alkylating agents, which form the backbone of the BEACOPP regimen [3–8]. Many small studies had shown the detrimental effect of alkylators on fertility using combination chemotherapy with mechlorethamine, vincristine, procarbazine, and prednisone-like therapies in HL patients. Therefore, the GHSg analyzed the menstrual status after HL treatment of 405 patients <40 years at diagnosis. After a median follow-up of 3.2 years, 51.4% of the women who had received eight cycles of escalated BEACOPP

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reported permanent amenorrhea [9]. This retrospective analysis showed a beneficial effect of oral contraceptives (OC) on the preservation of a regular cycle. Therefore, OC is regarded as standard of care for young female patients within the GHSG protocols, though this recommendation cannot be supported by prospectively controlled trials.

Pharmacological methods to prevent chemotherapy-induced gonadal toxicity in young female patients aim at suppressing the pituitary gonadotropin secretion and cyclic ovarian function. Resting follicles are thought to be more resistant to chemotherapy. According to this hypothesis, besides OC also gonadotropin-releasing hormone-analogues (GnRH-a) might be suited to protect the ovaries from cytotoxic damage [10]. Unfortunately, in HL patients, until now only contradictory [10–13], small randomized [14, 15], mostly retrospective case-controlled [16–18], or non-controlled [19] results on these protective effects have been published. Thus, we initiated a prospective randomized study of hormonal co-treatment with OC or GnRH-a during intensive polychemotherapy consisting of eight cycles of BEACOPPesc to protect the ovarian reserve in young HL patients.

patients and methods

patient selection

Patients between the age of 18 and 40 years had to have biopsy-proven HL at first diagnosis to be eligible for randomization. Eligibility criteria before study enrollment included adequate organ function as described elsewhere [1]. Patients had to have a history of a spontaneous menstrual cycle, no primary ovarian failure, and follicle-stimulating hormone (FSH) levels ≤ 30 U/l at baseline. Written informed consent on the basis of the institutional review board guidelines had to be given for trial participation. This study was carried out in accordance with the declaration of Helsinki and the International Conference on Harmonization-guidelines for Good Clinical Practice (ICH-GCP). The present analysis of the GHSG trial database is on the basis of the analysis of September 2009.

study design

Patients in advanced stages [clinical stage (CS) IIB with risk factor extranodal involvement or large mediastinal mass, all CS III + IV] were treated with BEACOPPesc chemotherapy as published elsewhere [1]. Cumulative dose of alkylating agents in this regimen are 10.0 g/m^2 for cyclophosphamide and 5.6 g/m^2 for procarbazine. Patients were randomly assigned either to receive daily OC (levonorgestrel 0.15 mg + ethinyl estradio 0.03 mg) or the GnRH-a goserelin acetate 3.8 mg (the medication was supplied by AstraZeneca) administered monthly subcutaneously during eight cycles of BEACOPPesc (Figure 1A). The first GnRH-a dose had to be administered 1 week before the onset of chemotherapy. Primary end point was the protection of the ovarian reserve as determined by FSH levels. However, in the last years, anti-Müllerian hormone (AMH) has been established as a cycle independent and much more valid indicator for the ovarian follicle reserve [20–29]. Thus, AMH levels (threshold 0.46 µg/l) after at least 12 months were used for this final analysis to determine the primary objective.

Blood samples were drawn for determination of hormonal levels at baseline; monthly during therapy; and 6, 12, and 18 months after therapy. Hormonal profile included FSH, luteinizing hormone (LH), estradiol, AMH, testosterone, dehydroepiandrosteronsulfat (DHEAS), inhibin B, and progesterone.

hormonal analysis

Blood samples were processed and stored at -20°C until analysis. Endocrine screening included serum assays for FSH, LH (both solid-phase two-side chemiluminescent immunometric assays, Immulite 2000; Siemens

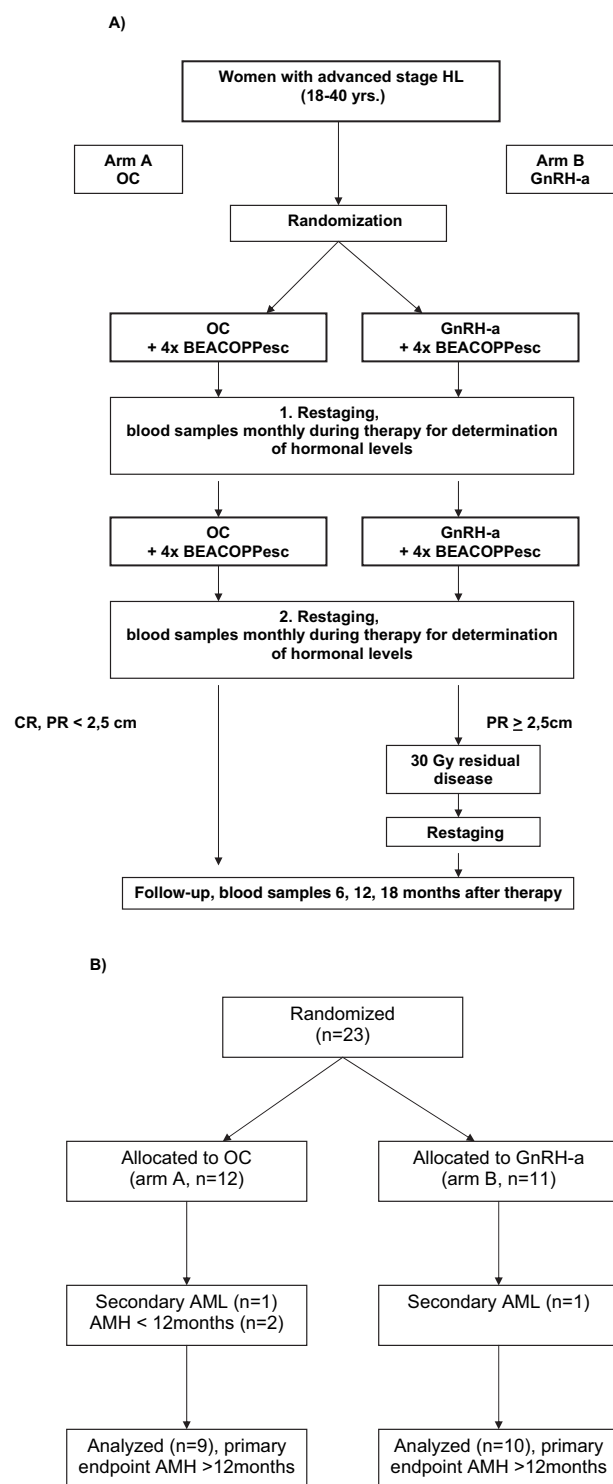


Figure 1. (A) Trial design and (B) Consort flow diagram. AMH, anti-Müllerian hormone; BEACOPPesc, escalated combination therapy with bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone; GnRH-a, gonadotropin-releasing hormone-analogues; HL, Hodgkin's lymphoma; OC, oral contraceptives; CR, complete remission; PR, partial remission; AML, acute myeloid leukemia.

Healthcare Diagnostics GmbH, Eschborn, Germany), estradiol (elektrochemiluminescent immunometric assay, Cobas-e; Roche Diagnostics GmbH, Mannheim, Germany), inhibin B (active inhibin B ELISA DSL-10-84100), AMH (active MIS/AMH ELISA DSL-10-14400), progesterone

(solid-phase competitive chemiluminescent enzyme immunoassay, Immulite 2000; Siemens), testosterone (Coat-A-Count Total Testosterone solid-phase ¹²⁵I radioimmunoassay; Siemens), and DHEAS (solid-phase competitive chemiluminescent enzyme immunoassay, Immulite 2000; Siemens). Normal laboratory ranges are as follows—FSH: follicular phase: 3.0–11.0 U/l, mid-cycle: 8.0–20.0 U/l, and luteal phase: 2.0–10.0 U/l; LH: follicular phase: 1.5–14.0 U/l, mid-cycle: 16.0–80.0 U/l, and luteal phase: 0.7–14.0 U/l; estradiol: 8–518 ng/l; progesterone: 0.1–28.0 µg/l; testosterone: <0.4 µg/l; DHEAS: 0.5–3.8 mg/l; AMH: 0.46–7.6 µg/l; and inhibin B: <164 ng/l. When women used OC in the follow-up period, blood samples were drawn on the last day of the pill-free interval.

statistics

The hypothesis that GnRH-a provide an at least 20% higher ovarian protection rate than OC should be tested in a two-stage sequential design with a global alpha error level of 0.05. After 2 × 30 patients, an interim analysis with Fisher’s exact test on FSH-based ovarian protection rates was planned. The interim analysis should test whether H₀ can be already rejected, whether the aims of the study can be reached with reasonable effort, and it should allow appropriate modifications of a priori assumptions. The final analysis with Fisher’s exact test on ovarian protection rates should be conducted after 2 × 100 patients maximally. For reasons detailed below, we closed the study prematurely after enrollment of 23 patients. Therefore, we describe in this paper the endocrine parameters before, during, and after therapy for each patient individually and summarize results with appropriate descriptive statistics and 95% confidence intervals (CIs).

results

study design

Due to slow enrollment and upcoming concerns about our a priori assumptions (i.e. 50% protection rate for OC and 70% for GnRH-a), we carried out an unplanned early interim analysis after recruitment of 23 patients. The AMH levels of this interim analysis showed that the observed ovarian follicle protection rate was significantly lower than assumed. Thus, the study had to be

closed by the principal investigator in accordance with the protocol. The final analysis reported here was carried out after a minimum follow-up period of 12 months.

patients characteristics

Overall, 23 patients were enrolled into the trial from 2004 to 2007. Twelve patients were randomly assigned to receive OC (arm A) and 11 to GnRH-a co-treatment (arm B, Figure 1B). Patients median age at the time of HL diagnosis was 25.95 years in arm A and 25.26 years in arm B (Table 1). Of the entire cohort, two patients were lost to follow-up and two patients went off study due to secondary acute myeloid leukemia (arm A: 6 months after the end of therapy and arm B: 1 month after the end of therapy). All remaining patients are in continuous first complete remission so far. Overall, 19 women, 9 in arm A and 10 in Arm B, are assessable for the primary end point (Figure 1B). The median observation time was 25.4 months after randomization and 18.2 months after end of therapy (range 12.5–33.3 months).

menstrual status/childbearing after therapy

In arm A, a total of three women experienced amenorrhea at last follow-up. Menstrual cycle after therapy was irregular in two patients and regular in three patients. After co-treatment with GnRH-a (arm B), a regular cycle after therapy was more often reported (7 of 10). No woman gave birth to a child after HL treatment in both arms (Table 1).

hormonal profiles before, during, and after therapy for patients in arm A and arm B

Table 2 shows the individual endocrine parameters for FSH, estradiol, and AMH at baseline, during chemotherapy, 6 months and minimally 12 months after the end of HL therapy. Table 3

Table 1. Patients characteristics

Arm	Patient	Age (years)	Stage	B	LMM	E	≥3 LN	ESR	OC baseline	OC follow-up	Menstrual cycle after therapy
A	27 856	24.37	III	No	No	No	Yes	No	Yes	No	Irregular
	28 499	22.71	III	No	No	No	Yes	No	No	No	Regular
	29 229	31.36	III	No	No	No	No	No	No	Unknown	Irregular
	29 594	26.51	III	No	No	No	Yes	Yes	No	Unknown	Regular
	29 799	38.14	III	Yes	Yes	No	Yes	Yes	No	No	Amenorrhea
	30 005	25.40	III	Yes	No	No	Yes	Yes	Yes	Yes	Regular
	30 159	35.00	IV	No	No	No	Yes	No	Yes	No	Amenorrhea
	30 526	19.68	IV	Yes	Yes	No	Yes	Yes	No	Unknown	Unknown
	30 800	23.33	II	Yes	No	Yes	Yes	Yes	Yes	No	Amenorrhea
	27 922	26.43	III	Yes	No	No	Yes	Yes	Yes	No ^a	Regular
B	27 995	21.36	IV	No	No	No	Yes	No	Yes	Yes	Regular
	28 625	24.46	IV	Yes	No	No	Yes	Yes	Yes	Yes	Regular
	29 033	28.16	III	No	No	No	Yes	No	No	No	Amenorrhea
	29 549	25.26	II	Yes	No	Yes	Yes	Unknown	No	Unknown	Irregular
	29 775	23.08	III	No	No	No	Yes	No	Yes	Yes	Unknown
	29 902	25.68	IV	No	No	Yes	Yes	Unknown	Yes	Yes	Regular
	30 131	30.51	II	Yes	Yes	Yes	Yes	Unknown	Yes	No	Regular
	30 273	23.21	III	Yes	No	Yes	Yes	Yes	No	No	Regular
	30 454	23.21	IV	No	No	Yes	Yes	Yes	Unknown	No	Regular

^aNo OC but depot gestagene injection every 3 months.
B, B-symptoms; LMM, large mediastinal mass; E, extranodal disease; ≥3 LN, 3 or more lymph node areas involved; ESR, high ESR; OC, oral contraceptives.

Table 2. MD, range, and individual hormonal levels of FSH, estradiol, and AMH

Arm	Patient	FSH (U/l)				estradiol (ng/l)				AMH (µg/l)			
		T ₀	T _C	T ₆	T ₁₂	T ₀	T _C	T ₆	T ₁₂	T ₀	T _C	T ₆	T ₁₂
A	27 856	4.2	<0.1	123.0	116.0	154	<12	<12	<12	2.353	<0.017	<0.017	< 0.017
	28 499	5.2	0.3	84.2	19.1	96	<12	<12	60	0.465	<0.017	<0.017	0.032
	29 229	3.7	1.4	62.0	7.2	126	<12	20	189	2.260	<0.017	<0.017	<0.017
	29 594	6.1	<0.1	162.0	68.1	38	<12	<12	85	0.507	<0.017	<0.017	<0.017
	29 799	5.4	9.7	57.1	84.1	103	<12	<12	<12	0.282	<0.017	<0.017	<0.017
	30 005	1.9	0.2	87.5	78.4		<12	<12	37	4.482	<0.017	<0.017	<0.017
	30 159	1.6	1.2	61.2	80.0	<12	<12	13	18	0.381	<0.017	<0.017	<0.017
	30 526	6.0	<0.1	76.5	28.2	53	<12	<12	18	1.789	<0.017	<0.017	0.019
	30 800	0.8	<0.1	66.5	93.1	<12	<12	<12	16	5.849	<0.017	<0.017	<0.017
	MD	4.2	0.2	76.5	78.4	74.5	<12	<12	18	1.789	<0.017	<0.017	<0.017
B	Normal	78%	11%	0%	22%	67%	0%	22%	78%	78%	0%	0%	0%
	27 922	0.1	6.5	62.3	88.0	16	<12	<12	<12	0.254	<0.017	<0.017	<0.017
	27 995	5.0	2.5	75.5	77.1	22	<12	<12	<12	0.831	<0.017	<0.017	<0.017
	28 625	3.4	19.0	7.7	7.9	49	<12	106	86	0.259	<0.017	0.231	0.681 ^a
	29 033	19.3	6.0	160.0	185.0	49	<12	<12	<12	0.431	<0.017	<0.017	<0.017
	29 549	5.2	10.4	131.0	54.4	64	<12	<12	60	0.989	<0.017	<0.017	<0.017
	29 775	3.1	5.5	67.9	64.1	169	<12	<12	<12	0.478	<0.017	<0.017	<0.017
	29 902	4.7	2.2	32.0	18.9	179	<12	<12	335	4.46	<0.017	<0.017	0.063
	30 131	9.0	6.0	95.9	43.0	327	<12	<12	13	3.83	<0.017	<0.017	<0.017
	30 273	2.9	5.8	18.9	8.4	21	<12	335	133	1.988	<0.017	0.063	<0.017
Total	30 454	1.3	5.7	156.0	62.8	45	<12	24	206	4.942	0.052	<0.017	0.073
	MD	4.7	5.9	71.7	58.6	49	<12	<12	36.5	0.989	<0.017	<0.017	<0.017
	Normal	80%	100%	20%	30%	100%	0%	30%	60%	70%	0%	0%	10%
Total	MD	4.2	2.5	75.5	64.1	51	<12	<12	18	.989	<0.017	<0.017	<0.017
	Normal	79%	58%	11%	26%	84%	0%	26%	68%	74%	0%	0%	5%

^aIn this woman, AMH recovered transiently 12 months after the end of therapy to a low standard value with 0.68 µg/l but fell again below the normal range (0.24 µg/l) 18 months after therapy.

MD, median; FSH, follicle-stimulating hormone; AMH, anti-Mullerian hormone; T₀, baseline; T_C, during chemotherapy; T₆, 6 months after the end of therapy; T₁₂, minimally 12 months after the end of therapy.

shows the individual parameters for LH, progesterone, DHEAS, testosterone, and inhibin B at baseline and minimally 12 months after the end of HL therapy.

follicle-stimulating hormone

arm A. Most women had normal baseline FSH levels. During therapy, FSH levels remained normal or suppressed reflecting the negative feed back effect of OC on the FSH secretion. Six months after the end of therapy, FSH levels were increased in all women. Levels returned to normal values in a total of two women 12 months after the end of therapy (Table 2; Figure 2A).

arm B. Most women had normal baseline FSH levels. During chemotherapy, FSH levels remained normal or reached a low standard value. After an initial increase, FSH returned to normal values 12 months after the end of therapy in three women (Table 2; Figure 2B).

estradiol

arm A. In three patients, estradiol levels were suppressed at baseline. During therapy, suppressed estradiol levels were seen in all patients. A total of seven women had normal estradiol levels 12 months after the end of therapy (Table 2; Figure 2E).

arm B. Estradiol levels were within the normal range in all patients at baseline and they were suppressed during therapy. A total of six women showed estradiol levels at least close to the lower normal bound during follow-up (Table 2; Figure 2F).

anti-Mullerian hormone

arm A. AMH levels were reduced already before treatment in two women. During the whole follow-up period, levels remained reduced below the normal range in all patients (Table 2; Figure 2C). The ovarian reserve preservation rate was 0% (95% CI 0% to 22%).

arm B. Three women had reduced AMH levels before therapy. During therapy and in the follow-up period, reduced AMH levels were measured in all women. In one woman, AMH recovered transiently 12 months after the end of therapy to a low standard value with 0.68 µg/l but fell again below the normal range (0.24 µg/l) 18 months after therapy (Table 2; Figure 2D). The ovarian reserve preservation rate was 0% (95% CI 0% to 24%).

Taken together, AMH levels remained at very low levels in all women in the follow-up period at least 12 months after therapy. Fourteen patients had no detectable AMH concentration.

Table 3. MD, range, and individual hormonal levels of LH, progesterone, DHEAS, testosterone, and inhibin B

Arm	Patient	LH (U/l)		Progesterone (µg/l)		DHEAS (mg/l)		Testosterone (µg/l)		Inhibin B (ng/l)	
		T ₀	T ₁₂	T ₀	T ₁₂	T ₀	T ₁₂	T ₀	T ₁₂	T ₀	T ₁₂
A	27 856	4.8	44.5	15.5	<0.5	1.55	0.48	<0.20	<0.20	<7	<7
	28 499	9.1	12.3	3.7	6.5	0.79	1.24	<0.20	0.49	<7	<7
	29 229	4.5	4.9	7.2	<0.5	0.47	0.60	0.35	<0.20	<7	<7
	29 594	3.8	31.8	0.6	<0.5	2.48	0.64	0.41	<0.20	39	<7
	29 799	5.4	58.0	1.9	<0.5	0.34	1.86	0.21	<0.20	22	<7
	30 005	0.2	28.2	<0.5	<0.5	<0.30	<0.30	<0.20	<0.20	<7	<7
	30 159	1.1	46.5	<0.5	<0.5	1.44	1.25	<0.20	0.42	<7	<7
	30 526	11.3	23.7	<0.5	<0.5	1.34	0.48	0.41	<0.20	22	<7
	30 800	0.7	54.6	<0.5	<0.5	1.85	1.65	0.38	0.49	<7	<7
	MD	4.5	31.8	0.6	<0.5	1.34	0.64	0.21	<0.20	7	7
	Normal	89%	100%	56%	11%	67%	67%	78%	67%	33%	0%
B	27 922	0.0	58.4	<0.5	<0.5	0.92	1.76	0.25	0.24	<7	<7
	27 995	6.3	24.0	<0.5	<0.5	0.57	1.20	0.26	0.23	<7	<7
	28 625	2.6	8.5	<0.5	15.9	0.41	1.07	<0.20	0.24	40	<7
	29 033	11.5	50.0	5.2	<0.5	1.47	0.48	0.54	0.21	<7	<7
	29 549	8.4	21.9	5.2	<0.5	0.43	1.24	<0.20		<7	<7
	29 775	3.7	18.8	1.9	<0.5	3.83	2.86	0.51	0.37	22	<7
	29 902	27.5	29.1	7.1	1.0	0.92	0.84	<0.20	<0.20	<7	<7
	30 131	24.0	10.3	1.7	<0.5	1.38	1.69	0.42	<0.20	74	<7
	30 273	6.3	24.2	<0.5	3.0	1.47	2.08	0.36	0.81	<7	<7
	30 454	0.7	61.1	0.9	1.8	0.47	1.46	<0.20	0.28	<7	37
	MD	6.3	24.1	1.3	<0.5	0.92	1.35	0.26	0.24	<7	<7
	Normal	90%	100%	60%	40%	70%	90%	70%	90%	30%	10%
Total	MD	4.8	28.2	0.9	<0.5	0.92	1.24	0.25	0.22	<7	<7
	Normal	89%	100%	58%	26%	79%	95%	47%	37%	32%	5%

MD, median; LH, luteinizing hormone; DHEAS, dehydroepiandrosteronsulfate; T₀, baseline; T₁₂, minimally 12 months after the end of chemotherapy.

ovarian follicle preservation rate of entire cohort

Combining both treatment arms, the respective ovarian follicle preservation rate was 0% (95% CI 0% to 12%) after minimally 12 months and thus significantly lower than assumed (50%).

discussion

Though the prognosis of patients with advanced-stage HL could be substantially improved by the use of BEACOPPesc therapy during the past decade, many patients suffer from long-term sequelae due to this aggressive treatment. A major concern is the preservation of the gonadal function, especially in young female patients. This is the first prospectively randomized trial using the AMH to determine the ovarian reserve after prophylactic co-treatment with either an OC or a GnRH-a during BEACOPPesc chemotherapy. The most important finding from this study is that neither OC nor GnRH-a co-treatment is able to ensure a meaningful protection of the ovarian reserve. In contrast, none of 19 patients returned to stable normal AMH levels 1 year after the end of therapy. This finding has major implications for patients and physicians using the BEACOPPesc regimen:

- 1 Young female patients should be informed about the significantly decreased ovarian follicle pool regardless of the use of hormonal prophylaxis.

- 2 The impaired ovarian reserve not necessarily means infertility but certainly will result in a premature onset of ovarian failure (POF) and menopause. Thus, women with low AMH levels and desire for children after BEACOPPesc therapy should be informed about their significantly shortened reproductive life span and decreased chance of spontaneous conception.
- 3 Alternative strategies to preserve the chance of motherhood should be offered to the patients including cryopreservation of oocytes or ovarian tissue before chemotherapy.
- 4 With regard to the ovarian reserve, the GnRH-a showed no efficacy questioning its off-label use as prophylaxis, at least during eight cycles of BEACOPPesc.
- 5 It must be stated though that this trial had to be stopped early and the numbers are too small to draw firm conclusions on gonadal functions other than the ovarian follicle pool.

A very important question for many young women who receive chemotherapy is, whether fertility will be maintained thereafter. The increased likelihood of infertility with the use of BEACOPPesc for HL patients is a major concern for patients and physicians. Unfortunately, the inability to conceive or give birth to a child might be difficult to diagnose at a given time point since it is a time-dependent parameter. Thus, infertility is hard to assess as a primary end point in clinical studies. Still, the exact incidence of gonadal damage in women after cancer

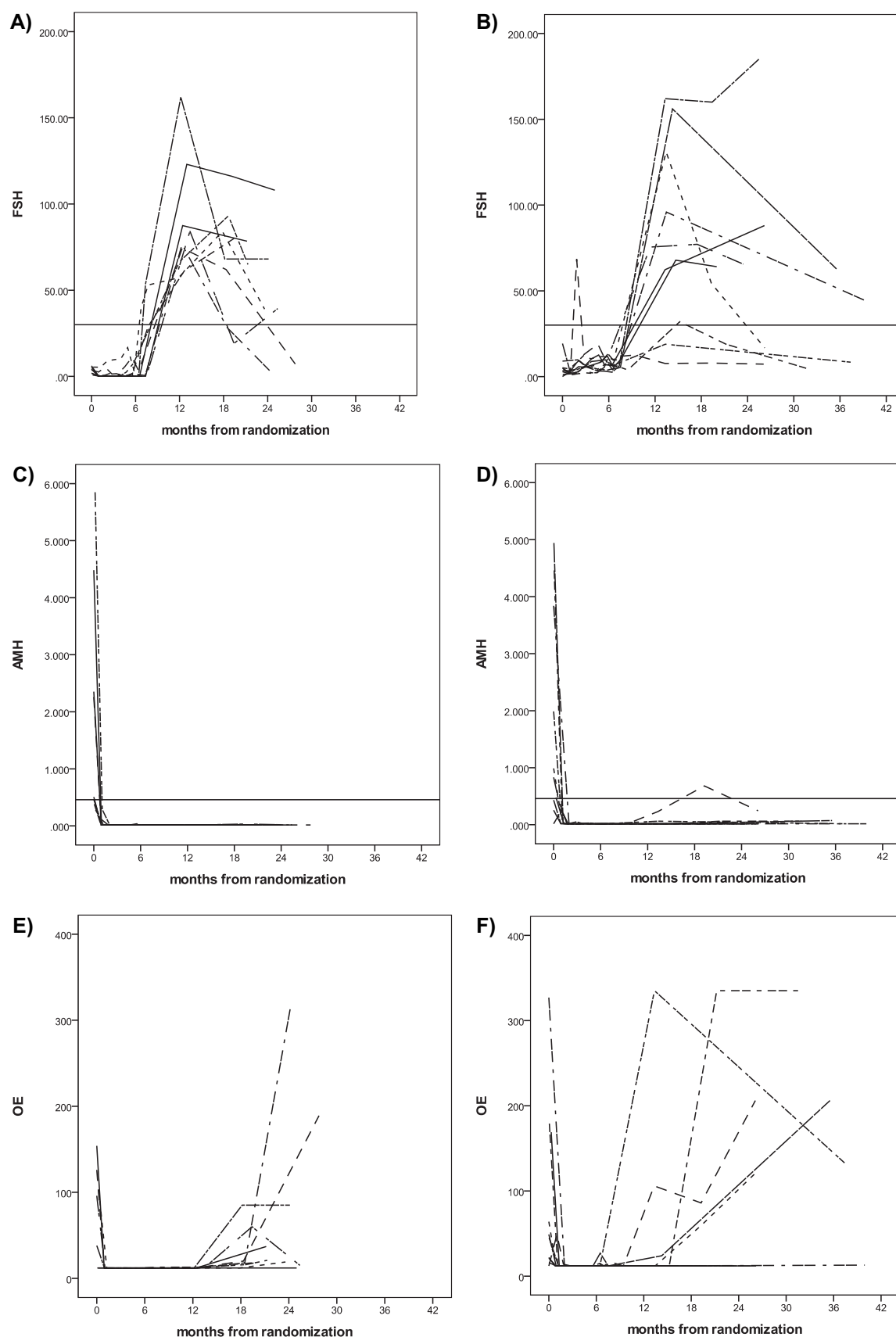


Figure 2. (A) Follicle-stimulating hormone (FSH) in arm A, (B) FSH in arm B, (C) anti-Müllerian hormone (AMH) in arm A, (D) AMH in arm B, (E) estradiol (OE) in arm A, and (F) OE in arm B.

therapy is difficult to predict as methods for measuring the ovarian reserve and defining the follow-up time vary in the literature [30]. Some authors report on chemotherapy-induced amenorrhea and others define gonadal toxicity as persistent amenorrhea and elevated FSH level after therapy [8, 9, 31, 32]. Other studies further include inhibin B, AMH, and ultrasound scans to assess ovarian reserve [30, 33, 34]. Certainly, menstrual status alone is no reliable indicator for the ovarian reserve [35].

However, a surrogate parameter for the prospective infertility is the ovarian reserve or follicle pool. When this study was planned, the FSH level was thought to best reflect the ovarian reserve. However, FSH levels in our cohort showed considerable variance. This might be due to the following limitations: first, FSH levels exhibit interindividual variations even in young normo-ovulatory women [36]. Second, it must be measured on a distinct menstrual cycle day (i. e. early follicular phase FSH concentrations on day 3) to be comparable at all. Third, FSH levels are obviously affected by the use of OC, which are commonly used.

Recent research indicates that the AMH level represents the most sensitive marker for the prediction of the ovarian follicle pool and is therefore an indicator of ovarian aging [20–23, 29]. AMH is more strongly related to ovarian follicular status than inhibin B, estradiol, FSH, and LH on day 3 [24]. Furthermore, AMH levels are not influenced by the stage of the menstrual cycle [25–27]. Interestingly, AMH shows a rapid and sustained change after chemotherapy, which might reflect primordial and preantral follicles as the primary site of toxicity [21, 28]. Sowers et al. demonstrated that AMH levels decline 5 years before the final menstrual period, thus this hormone is a useful parameter to predict age at menopause [22, 23]. Due to the clear correlation between AMH levels and the ovarian reserve, providing the opportunity to predict age at menopause, and the observed limitations for FSH, we finally used AMH levels as more conclusive primary end point for the analysis of the required protection of the ovarian follicle pool.

Unexpectedly, undetectable or very low AMH levels in all patients were documented in our cohort at the interim analysis. These results indicate that all of our patients will experience a very early onset of POF and premature menopause. This results in a considerably shortened reproductive life span. Furthermore, the onset of a premature menopause implies that women may suffer from hormone deficiency symptoms, such as hot flushes, osteoporosis, night sweats, headaches, and mood changes many years earlier than the general population. Accordingly, ovarian insufficiency has an impact on patient self-esteem and quality of life [37]. The surprisingly high incidence of a significantly reduced ovarian reserve in our study indicates the importance of prospectively controlled trials and the need for a more standardized and adequate ovarian reserve testing, including AMH in adult cancer survivors [30, 38–40].

Though we did not observe any meaningful effect of the prophylactic hormonal intervention, we cannot exclude a possible protective effect of OC or GnRH-a when less toxic regimens are applied. Huser et al. [17] could demonstrate a modest protective effect with GnRH-a when treatment consisted of $4 \times$ adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) or $2 \times$ escalated BEACOPP + $2 \times$ ABVD. Recently, preliminary data from a randomized trial in 80 breast cancer patients undergoing

treatment with fluorouracil, adriamycin, and cyclophosphamide chemotherapy have been published. After 8 months, menstruation was documented in 89.6% of the GnRH-a co-treatment patients compared with 33.3% in the control group. However, the cumulative dose of cyclophosphamide in this regimen was only 3 g/m^2 [34]. Unfortunately, AMH was not determined in both studies. However, a potential protective effect of OC or GnRH-a in less aggressive regimens might be possible and should be evaluated in clinical trials.

Also, due to limited number of patients in our trial, the CI per treatment arm is large and minor protective effects of each single intervention cannot be excluded. Nonetheless, these effects are obviously smaller than expected. Both treatment arms were combined to evaluate the effect of hormonal prophylaxis in general since both approaches (OC and GnRH-a) aim at suppressing cyclic ovarian function. Thus, their putative protective effect is mediated by the same mechanism of action. The result indicates that hormonal intervention is not active when aggressive chemotherapy is administered. This is in accordance with the hypothesis that the primordial follicle growth is an FSH-independent process and alkylating agents are not cell cycle specific; thus; they might damage even resting primordial follicles [12].

In young female patients with advanced-stage HL, the risk of gonadal damage has to be balanced against superior cure rates with BEACOPPesc. Obviously, this is a highly individual decision for which patients have to be very well informed. Our results close a gap of knowledge and can serve as a valid basis for an informed consent. For the GHSG, these results strongly support the ongoing efforts to reduce the therapy-induced toxicity. In the HD15 trial that was running in parallel to this study, the reduction from eight to six cycles of BEACOPPesc or to eight cycles of BEACOPP-14 has been investigated. Eight cycles of BEACOPP-14 contain only half of the cumulative dose of cyclophosphamide (5.2 g/m^2) [41]. Therefore, as well physicians as patients preferred treatment within the HD15 trial in many cases, what hindered enrollment to the PROFE study (K. Behringer and B. van den Hoonaard personal communication). The current HD18 study even further reduces the chemotherapy intensity by using a positron emission tomography (PET)-guided early response adapted design. By this personalized approach, the GHSG aims at a reduction to only four cycles of chemotherapy for good responders what would also halve the procarbazine dose. Whether this strategy will be sufficient in terms of preservation of the ovarian function is under investigation in this trial. Until then, invasive methods such as the cryopreservation of oocytes or ovarian tissue before therapy should be used to maintain the chance of motherhood [42–44].

To summarize, the results of this study show the detrimental effect of an aggressive polychemotherapy regimen as BEACOPPesc for the ovarian reserve in young women, which underscores the need for the development of a less toxic and more personalized treatment strategy and standardized approaches to maintain gonadal functions and fertility.

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disclosure

None of the authors declare conflicts of interest.

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appendix

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Secretary:	P. Borchmann
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PET panel:	M. Dietlein
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