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GnRH analogs do not protect ovaries from chemotherapy-induced ultrastructural injury in Hodgkin's lymphoma patients

Markus Nitzschke · Juliane Raddatz ·
Michael K. Bohlmann · Petra Stute ·
Thomas Strowitzki · Michael von Wolff

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Abstract

Purpose To determine the protective effect of gonadotropin-releasing hormone analogs (GnRHa) using several ultrasound and endocrine markers to detect ultrastructural ovarian damage in Hodgkin's lymphoma patients.

Methods Ten patients who had been treated for Hodgkin's lymphoma and had received GnRHa to protect ovarian function were matched with patients at similar age, who had received the same chemotherapy regimens without GnRHa. In addition, ten healthy women at the same age were matched to the study groups to compare ovarian markers. Blood samples were drawn to determine anti-Müllerian hormone, Inhibin B, follicle-stimulating hormone and transvaginal ultrasound scans were performed to determine antral follicle count and ovarian volume. All women were asked about their menstrual cycle pattern.

Results No difference was found when comparing the ovarian parameters of both study groups. Compared with

healthy women, all ovarian parameters in the Hodgkin's lymphoma patients were significantly different.

Conclusions The results of this study demonstrate ultrastructural ovarian damage in Hodgkin's lymphoma patients irrespective of GnRHa co-treatment. These findings do not support previous studies, showing GnRHa to protect ovarian function.

Keywords GnRH analogs · Chemotherapy · Ovarian reserve · Fertility preservation · Hodgkin's lymphoma · Cancer

Introduction

Ovarian failure followed by amenorrhea and infertility is a common side effect of cytotoxic treatments for malignant disease in women [1]. These treatments cause depletion of the primordial follicle pool in a drug- and dose-dependent manner [2]. To sustain fertility in young women undergoing cytotoxic therapies, fertility preservation strategies have been placed into the focus of interest of health professionals and patients.

Among the currently discussed fertility preserving strategies, the concept of administering a non-invasive medical co-treatment such as gonadotropin-releasing hormone analogs (GnRHa) seems very attractive [3]. Glode et al. [4] using a murine model, tested this hypothesis for the first time almost three decades ago. They concluded that GnRHa appeared to protect male mice from the gonadal damage normally caused by cyclophosphamide, whereas several investigators have demonstrated that GnRHa can inhibit chemotherapy-induced ovarian follicular depletion in non-human species, uncertainty remains regarding human application [5].

M. Nitzschke and J. Raddatz contributed equally to this work.

M. Nitzschke · P. Stute · M. von Wolff (✉)
Department of Gynecological Endocrinology
and Reproductive Medicine, Woman's University
Hospital of Bern, Effingerstrasse 102, 3010 Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

J. Raddatz · T. Strowitzki
Department of Gynecological Endocrinology
and Reproductive Medicine, Woman's University
Hospital of Heidelberg, Heidelberg, Germany

M. K. Bohlmann
Department of Obstetrics and Gynecology,
University Hospital of Schleswig-Holstein,
Campus Luebeck, Luebeck, Germany

Several studies have already tested the efficacy of GnRHa in humans. As described in three recent meta-analyses [6–8], the published clinical studies only provide evidence but still do not prove statistically that GnRHa co-treatment may reduce gonadal toxicity. This is due to the retrospective and non-randomized nature of most of the studies. A recent prospective randomized controlled trial found that the administration of GnRHa before and during chemotherapy for breast cancer could preserve post treatment ovarian function in young women [9].

However, more studies are needed. One reason is that a biological plausibility for gonadal protection by GnRHa in humans is still lacking [5] and another reason is that most studies only describe the prevalence of amenorrhea, even though depletion of primordial follicles may occur despite maintenance of regular menstrual cycles [10, 11]. The amount of surviving primordial follicles after cytotoxic treatments can only be quantified in vivo using several distinct hormonal and ultrasound-based surrogates [12].

Therefore we set up this small, but carefully designed trial with patients who were exactly matched for age and treatment. A broad set of ultrasound parameters (antral follicle count (AFC), ovarian volume) and endocrine markers (follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH), Inhibin B) were used to measure ovarian reserve more precisely than simple prevalence of amenorrhea could do.

Materials and methods

This case-control study was performed at the Department of Oncology together with the Department of Endocrinology and Reproductive Medicine at the University Hospital of Heidelberg (Germany) in cooperation with the German Hodgkin Study Group (<http://www.ghsg.org>). The local ethics review committee approved the study protocol and a written informed consent was obtained from all patients.

Patient population

Ten patients who had received chemotherapy and radiotherapy for Hodgkin's lymphoma between 2001 and 2007 had been co-treated with GnRHa (leuprorelin 3.57 mg s.c or goserelin 3.6 mg s.c. monthly before and during chemotherapy) to protect ovarian function (Group A).

In cooperation with the German Hodgkin Study Group, we localized a total of 31 patients who had also been treated for Hodgkin's lymphoma in nearby University Hospitals during the same period of time, but had not received GnRHa. These patients were carefully matched

to our study group for age and a similar chemotherapy regimen (Group B). Ten additional healthy women, who were treated in our department of reproductive medicine for severe male factor infertility, were matched to our study groups by age to compare the ovarian markers (Table 1).

The following chemotherapies were used: Four patients received two cycles of ABVD (Adriamycin, Bleomycin, Vinblastin, Dacarbacin), eight patients received four cycles of ABVD, six patients received two cycles of OPPA (Oncovin, Procarbacin, Prednison, Adriamycin) and two cycles of COPP (Cyclophosphamid, Oncovin, Procarbacin, Prednison) and two patients received eight cycles of BEA-COPP-14 (Bleomycin, Etoposid, Adriamycin, Cyclophosphamid, Oncovin, Procarbacin, Prednison). All radiotherapies were supradiaphragmal. At the time the study was performed, all patients had completed their treatment cycles at least 6 months before.

Study protocol

All participating women were seen twice within a 1- to 3-month period. Except the two patients with amenorrhea, all women were seen at the second to fifth day of the cycle. No woman was taking oral contraception (OC) or hormone replacement therapy (HRT) during the study period. Blood samples were drawn to determine AMH, Inhibin B and FSH in the serum and transvaginal ultrasound scans were performed to determine AFC and ovarian volume. All women were asked about their menstrual cycle pattern.

Treatment of the samples

Sera were obtained after centrifugation and stored at -80°C in five aliquots of 2 mL in standard Eppendorf tubes. The samples were centrifuged for 10 min at 3,500 rpm. Concentrations of FSH were analyzed using commercially available immunoradiometric kits (Boehringer, Mannheim, Germany) and analyzed on the Elecsys immunoassay analyzer (Roche Molecular Biochemicals, Mannheim, Germany).

AMH was assayed using an ultra sensitive ELISA (AMH/MIS active coated well ELISA, DSL-10-14400, Diagnostic System Laboratories, DSL, TX, USA). The assay range was 0.025–15 ng/mL, and the functional sensitivity 0.017 ng/mL. Intra-assay and inter-assay coefficients of variance were 3.4 and 6.5%, respectively (conversion factor to pmol/L = ng/mL \times 7.143). Inhibin B was quantified with the Inhibin B active coated well ELISA, DSL-10-84100 (Diagnostic System Laboratories, DSL, TX, USA). The assay range was 10–531 pg/mL, and the functional sensitivity 7 pg/mL. Intra-assay and inter-assay coefficients of variance

Table 1 Comparison between Hodgkin's lymphoma patients with (+) GnRHa and without (–) GnRHa co-treatment and healthy women

	Group	Pat. ID	Age	Therapy (chemo-/IF-RT)	Mens	FSH (U/L)	AMH (ng/mL)	Inhibin B (pg/mL)	AFC	Ov-vol (cm ³)
1	Healthy	U.S	28	–	reg.	9.2	3.2	73.3	11	9.3
	Hod + GnRHa	C.G	26	2× ABVD/s	reg.	6.4	4.3	23.2	12	13.6
	Hod – GnRHa	J.F	30	2× ABVD/s	reg.	6.7	2.4	7.8	11	11.8
2	Healthy	D.H	20	–	reg.	5.3	5.4	79.5	19	12.6
	Hod + GnRHa	I.G	20	2× ABVD/s	reg.	8.1	1.9	23.6	0	3.1
	Hod – GnRHa	I.H	23	2× ABVD/s	reg.	6.3	1.1	90.9	6	4.7
3	Healthy	J.S	21	–	reg.	3.3	5.9	76.5	10	9.3
	Hod + GnRHa	M.Mz	20	4× ABVD/s	reg.	5.9	1.5	11.5	5	7.1
	Hod – GnRHa	D.W	23	4× ABVD/s	reg.	5.5	3.0	8.5	16	8.0
4	Healthy	N.B.	25	–	reg.	6.9	4.1	79.5	12	7.5
	Hod + GnRHa	S.B.	25	4× ABVD/s	reg.	4.4	4.1	41.3	18	13.6
	Hod – GnRHa	S.O.	26	4× ABVD/s	reg.	3.7	1.5	5.4	9	5.9
5	Healthy	J.R.	28	–	reg.	5.6	3.7	74.2	14	6.6
	Hod + GnRHa	K.H	29	4× ABVD/s	reg.	2.4	1.6	2.1	0	6.7
	Hod – GnRHa	C.S.	27	4× ABVD/s	reg.	5.3	1.3	1.3	6	4.8
6	Healthy	S.R.	30	–	reg.	6.9	3.3	94.6	15	15.3
	Hod + GnRHa	N.K.	30	4× ABVD/s	reg.	6.0	1.5	18.6	3	5.7
	Hod – GnRHa	S.M.	33	4× ABVD/s	reg.	6.3	3.4	74.1	10	5.1
7	Healthy	F.C.	18	–	reg.	5.7	2.4	61.8	8	12.7
	Hod + GnRHa	L.K.	17	2× OPPA + 2× COPP/s	reg.	5.6	0.5	11.3	6	13.3
	Hod – GnRHa	C.B.	17	2× OPPA + 2× COPP/s	reg.	6.5	0.2	95.4	0	2.4
8	Healthy	T.B.	19	–	reg.	4.3	5.5	38.0	11	7.6
	Hod + GnRHa	E.H.	19	2× OPPA + 2× COPP/s	reg.	10.0	0.0	0.0	4	1.6
	Hod – GnRHa	S.H.	27	2× OPPA + 2× COPP/s	reg.	7.0	0.3	8.7	3	3.9
9	Healthy	J.J.	21	–	reg.	4.1	6.7	82.5	13	16.3
	Hod + GnRHa	K.F.	20	2× OPPA + 2× COPP/s	reg.	26.2	0.2	11.2	8	8.2
	Hod – GnRHa	A.SI.	22	2× OPPA + 2× COPP/s	reg.	6.2	0.7	40.0	6	4.6
10	Healthy	M.E.	34	–	reg.	2.8	4.0	96.4	14	7.5
	Hod + GnRHa	N.S.	33	8× BEACOPP-14/s	am.	71.7	0.0	0.0	0	1.6
	Hod – GnRHa	E.P.	35	8× BEACOPP-14/s	am.	91.7	0.0	0.0	0	1.9

Age age when study was performed, RT radio therapy, s supradiaphragmal radio therapy, IF involved field radio therapy, Mens menstrual cycle, reg. regular, am. amenorrhea, AMH anti-Muellerian hormone, AFC antral follicle count, Ov-vol ovarian volume

were 4.6 and 6.7%, respectively. The manufacturer's protocol was followed in both cases. All assays were done in duplicate.

Ultrasound scans

The ultrasound scans were performed via transvaginal probe by the same investigator. The examining person was blinded concerning hormonal status and medical history of the patient. Antral follicles (2–12 mm) were counted on both ovaries [13] and video monitored to allow analysis by a second examiner. Ovarian volume was calculated using the prolate ellipsoid formula $L \times H \times W \times 0.523$ (L = length in mid sagittal, H = height in mid

sagittal, W = width in axial) [14]. The volume of both ovaries was added.

Outcome measures

The main outcome measures to compare the three study groups were: secondary amenorrhea, FSH (U/L), AMH (ng/mL), Inhibin B (pg/mL), AFC and ovarian volume (cm³).

Statistical analysis

Mann–Whitney U test was used as a non-parametric test to compare the parameters of ovarian reserve between the

study groups. Statistical significance was presumed to be achieved for values of $P \leq 0.05$.

Results

Characteristics of trial participants

The mean age of patients treated for Hodgkin's lymphoma (Group A + B) was 25.1 [SD 5.2] years when the study was performed (range 17–35 years). There was no significant difference in the mean age between Groups A and B (23.9 [SD 5.1] and 26.3 [SD 5.1] years). Mean age of the group of healthy controls was 26.3 [SD 5.1] years (range 18–35 years). Again no significant difference was seen in the mean age between the study groups (Group A + B) and the healthy women. Both patients who had received BEACOPP-14 (one in each study group) experienced secondary amenorrhea.

The time interval between the end of the chemotherapy and the analysis of the ovarian parameters was 30.6 months [SD 7.2] for Group A (range 19–45 months) and 32.2 months [SD 20.4] for Group B (range 6–58 months). Patients were not matched for this time interval for the following reasons:

As the development of AMH producing secondary and tertiary follicles takes only around 3 months, ovarian function is sufficiently restored not later than 6 months after the end of chemotherapy. Thereafter, AMH concentrations remain stable in the same subject. The intra-individual variation of AMH is only 8% (coefficient of variation) if AMH is measured in the same subjects initially and again after 2 months according to an own unpublished study in 21 patients. For this reason and for the fact that other parameters such as age and the type of chemotherapy are far more important parameters to be matched, we accepted a limited variation in the time interval between the end of the chemotherapy and the AMH measurement.

Outcome of study groups

No difference was found when comparing the ovarian parameters in Hodgkin patients with (Group A) and without (Group B) GnRH_a co-treatment. For FSH, the mean concentration in Group A was 14.7 U/L [SD 6.6] and in Group B 14.5 U/L [SD 0.9]. The mean concentration of AMH in Group A was 1.6 ng/mL [SD 1.4] and in Group B 1.4 ng/mL [SD 1.1]. For Inhibin B, the mean concentration in Group A was 14.3 pg/mL [SD 8.7] and in Group B 33.2 pg/mL [SD 12.5]. Moreover, no significant difference was seen for the mean AFC (Group A: 5.6 follicles [SD 5.5] and Group B: 6.7 follicles [SD 4.7]) or the mean ovarian volume (Group A: 7.5 cm³ [SD 4.5] and Group B: 5.3 cm³ [SD 2.6]) in both groups (Fig. 1).

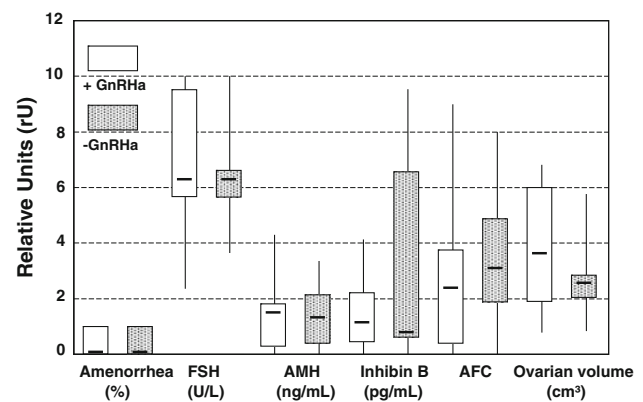


Fig. 1 Comparison of ovarian parameters in Hodgkin's lymphoma patients with (+) and without (-) GnRH_a co-treatment

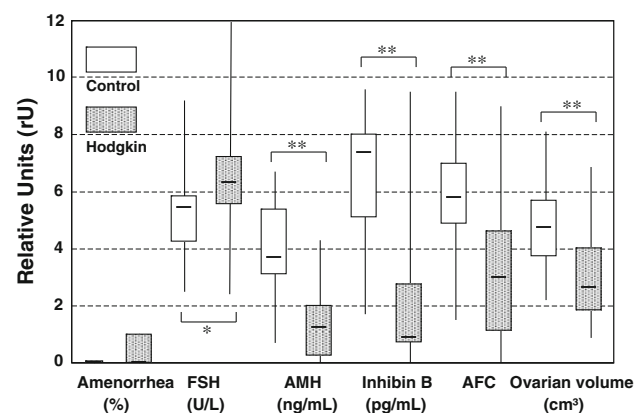


Fig. 2 Ovarian parameters in healthy women compared with those in Hodgkin's lymphoma patients of the same age (* $P < 0.05$; ** $P < 0.01$)

Compared with the healthy women of the control group, all ovarian parameters in the Hodgkin's lymphoma patients (Group A + B) were significantly different. The mean concentration of FSH was significantly higher in patients after chemotherapy (14.6 U/L [SD 4.8] vs. 5.3 U/L [SD 1.8]). The mean concentration of AMH and Inhibin B was significantly lower in those women (1.5 ng/mL [SD 1.3] vs. 3.9 ng/mL [SD 1.2] for AMH and 23.7 pg/mL [SD 18.6] vs. 64.9 pg/mL [SD 15.7] for Inhibin B). The AFC and the ovarian volume were significantly reduced in the Hodgkin's groups as well (6.2 follicles [SD 4.5] vs. 11.6 follicles [SD 2.9] for AFC and 6.4 cm³ [SD 3.8] versus 9.7 cm³ [SD 3.3] for ovarian volume). The P value for FSH was $P < 0.05$ and for all other parameters $P < 0.01$ (Fig. 2).

Discussion

The concept of using GnRH_a to prevent chemo- or radiotherapy induced gonadal damage was introduced by Glode et al. [4]. Since then, several animal studies suggested a

protective role of GnRHa co-treatment against follicle loss during cytotoxic treatments. However, no benefit could be demonstrated against radiotherapy induced gonadal damage [15, 16]. It has been highly debated whether human application of GnRHa would be safe and useful to preserve fertility and ovarian function in women treated for cancer [5–7].

In a recent prospective randomized study including 80 women receiving chemotherapy for breast cancer, GnRHa co-treatment was demonstrated to be effective in preserving ovarian function [9]. Patients were randomly assigned to receive chemotherapy combined with monthly injections of goserelin or chemotherapy alone. After 8 months of follow-up, 89.6% of the women in the study group (with GnRHa) resumed menses and 69.2% had spontaneous ovulation. In contrast only 33.3% of the patients in the control group (without GnRHa) resumed menses and 25.6% had spontaneous ovulation. These results were highly significant ($P < 0.001$). However, this study suffers from an important shortcoming: The control group had significantly higher mean serum FSH and Estradiol (E_2) levels even before start of the treatment. As both are important endocrine markers of ovarian reserve, a difference between the study groups could be, at least in a part, due to a lower initial ovarian reserve in the control group.

A second published prospective randomized study, including 30 men and 18 women receiving chemotherapy for Hodgkin's disease, revealed that GnRHa treatment seemed to be ineffective in preserving fertility as judged by sperm count and menstrual function [17]. Patients were randomly assigned to receive buserelin (20 men and 8 women) prior to and for the duration of cytotoxic chemotherapy. After 3 years of follow-up, all men in both, the study and control, groups became oligo/azoospermic, while four of the eight women treated with a GnRHa and six of the nine female controls became amenorrheic.

In a third randomized study, Sverrisdottir et al. [18] examined if goserelin as adjuvant treatment protects the ovaries from premature failure in premenopausal breast cancer patients. A total of 285 patients received CMF (cyclophosphamide, methotrexate and 5-fluorouracil). All patients were randomly assigned to receive 2 years of goserelin, goserelin plus tamoxifen, tamoxifen alone or no endocrine treatment. Endocrine treatment was given concomitant to chemotherapy. One year after completed CMF and endocrine therapy, 36% of the women in the goserelin group reported menses, compared to 7% in the goserelin plus tamoxifen group, 13% in the tamoxifen group and 10% of the controls.

Several other non-randomized studies in the past provided mixed results [6]. In addition to the lack of consistent support from clinical studies, there is currently no biological explanation for how GnRHa can protect ovarian

reserve. Primordial follicles do not express FSH or GnRH receptors [5]. GnRHa, in essence, returns the hormonal milieu to prepubertal state. A clinical example for why gonadal suppression may not protect ovaries is the fact that prepubertal children receiving high-dose chemotherapy given before hematopoietic stem cell transplantation still suffer from ovarian failure [19]. Nevertheless, because younger patients have a larger ovarian reserve, they might tolerate some loss in the follicular pool, and immediate ovarian function might not be affected in the short term. All patients who receive high-dose gonadotoxic chemotherapy will eventually suffer from premature ovarian failure [20, 21].

In our study, a broad spectrum of five sensitive parameters was used to analyze ovarian damage after cytotoxic treatment more precisely than the simple determination of secondary amenorrhea could do.

Only the two patients who had received the BEACOPP-14 regimen experienced secondary amenorrhea, whereas all other patients were still regularly menstruating. However, using sensitive parameters to compare ovarian reserve in Hodgkin's lymphoma patients and healthy women, we were able to demonstrate highly significant differences. This was also the case in those patients who had received the ABVD regimen, which was thought not to affect ovarian reserve. According to our results, chemotherapy for Hodgkin's disease can, therefore, be considered to potentially accelerate ovarian aging.

Furthermore, we could demonstrate ultrastructural ovarian damage in Hodgkin's lymphoma patients who were co-treated with GnRHa. The ovarian damage was, according to our parameters, identical to those patients who had not received GnRHa. We thus conclude that GnRHa did not seem to protect the ovaries from the cytotoxic effect of the chemotherapies in our setting.

Our findings are in concordance with the results of the only other study using sensitive ovarian parameters to measure the ovarian reserve after chemotherapy and GnRHa co-treatment. Giuseppe et al. [22] evaluated methods to assess the ovarian reserve in 29 women with Hodgkin's disease treated with chemotherapy. Furthermore, they examined the effect of GnRHa co-treatment on ovarian function. Ovarian reserve was studied by measuring the serum levels of FSH, LH, Inhibin B, AMH and AFC following treatment with or without GnRHa. They did not find a significant difference of all parameters measured following GnRHa treatment in comparison to the untreated control group.

Obviously the power of this study as well as of our study is limited due to the low number of analyzed patients. However, analysing previous studies in detail reveals that a huge number of patients are needed to draw distinct conclusions concerning the effect of GnRHa. The high number of different and heterogeneous chemotherapy regimes, the age

dependency of individual ovarian reserve as well as the application of the rough parameter “amenorrhea” also limits the power of the previously published prospective randomized studies. Accurate matching of patients and controls in relation to chemotherapy and age as well as the application of several sensitive parameters to determine ovarian reserve does allow drawing a conclusion irrespective of the small number of treated patients in our study. It thereby contributes to the discussion about the efficiency of GnRHa regardless of its design.

We could also demonstrate that only the application of a broad set of sensitive parameters may properly reflect ovarian reserve. Therefore, these markers may be used to better quantify the damage of the ovaries following exposition to chemotherapies in future studies. Such analyses may not only require a prospective randomised design, but also the determination of several sensitive ovarian markers to measure the supposed protective effect of GnRHa on ultrastructural changes of ovarian function during cytotoxic treatment. Such studies will hopefully finally solve the still open question concerning the efficacy of GnRHa as a fertility preserving technique.

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Conflict of interest statement None.

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