Fertility in women survivors of hematological malignancies: what is the real role of GnRH analogue treatment?

L. Driul[1], A.P. Londero[1], C. Papadakis[1], A. Candoni[2], S. Bertozzi[3], R. Fanin[2], D. Marchesoni[1]

[1]Clinic of Obstetrics and Gynecology, [2]Clinic of Hematology and Bone Marrow Transplantation, [3]Department of Surgery
Teaching Hospital "SM della Misericordia", Udine (Italy)

Summary

Purpose of investigation: The aim of this study was to evaluate the ovarian function in women who received or not gonadotropin-releasing hormone (GnRH) analogue co-treatment compared to the control group that did not receive it. *Materials and Methods:* This study analyzed 124 patients affected by hematological diseases between 1998 and 2007. The data were analyzed using R (v 2.9.1). *Results:* In the women treated with GnRH analogue, the authors found 33% post-treatment secondary amenorrhea and 6% had a pregnancy post-treatment, while in the other group the prevalence were respectively 49% and 4% (p n.s.). Moreover, in multivariate analysis the authors found bone marrow transplantation to be a risk factor for secondary amenorrhea, while the association of chemotherapy with radiotherapy was a protective factor ($p < 0.05$). Finally, none of the considered factors were predictive of pregnancy achievement post-treatment. *Conclusions:* The authors found no statistical evidence to support that Gn-RH analogue treatment preserves ovarian follicular reserve during hematologic cancer treatment, but more evidence must be obtained.

Key words: Fertility; Hematologic disease; GnRH analogue treatment.

Introduction

Cancer is not rare in reproductive age young women [1]. The improved long-term survival of adolescents and young women treated for cancer has resulted in an increased focus on the effects of chemotherapy on ovarian function and its preservation. One of the major quality issues for young cancer survivors is preserving gonadal function and fertility [2].

It is known that the alkylating agents are associated with the highest risk of infertility. Then the most common significant long-term toxicity of chemotherapy in women is premature ovarian failure. Early loss of ovarian function not only jeopardizes the patients with a premature menopause and the related complications, but it is also associated with loss of fertility. Furthermore, women in Italy, like in the Western world, have been delaying initiation of childbearing to later in life. As cancer survivors, they face the risk of developing premature ovarian failure (POF) before they even consider having children. The prevalence of POF as a late medical sequel is, however, not as well-documented. Patients whose ovarian function recovers immediately after treatment or who maintain ovarian function, may still face risk of developing POF several years after therapy [3].

For the variations in type and dose of chemotherapy, the type of cancer, the time available before onset of treatment, the patient's age and the partner status, renders each case unique and requires a different strategy of fertility preservation. The number of options is growing continuously: the use of gonadotropin-releasing hormone analogues (GnRH-a) as a co-treatment during chemotherapeutic regimens is an experimental one. Whereas several investigators have demonstrated that GnRH-a inhibit chemotherapy induced ovarian follicular depletion in the rat [4], uncertainty remains regarding application in humans.

The aim of this study was to evaluate the ovarian function in women who received GnRH-a co-treatment and in women who were treated with the same chemotherapy for hematologic diseases, without the agonist or only with estroprogestinic treatment.

Materials and Methods

This prospective non-randomized study analyzed 124 patients affected by hematological diseases treated in the Hematological Clinic and in the Obstetrics and Gynecological Clinic at the University Hospital of Udine between 1998 and 2007. The patients were affected mainly by: Hodgkin lymphoma, non-Hodgkin lymphoma, acute lymphoblastic leukemia, and acute myeloid leukemia (Table 1). The authors considered women with available clinical follow-up data in the five years after treatment. The authors categorized the patients into two groups: the first group treated with monthly depot injection of 3.75 mg GnRH-a (D-TRP6-GnRH-a; Decapeptyl C.R.; Ferring, Germany) for all the duration of chemotherapy, and the second group without GnRH-a treatment. The inclusion in the treatment or control group was a woman's personal choice.

In this study the authors considered the following outcomes: the pregnancy rate after chemotherapy, amenorrhea after chemotherapy, and the climacteric syndrome after chemotherapy. The authors took into account the following factors: characteristics of menstrual periods before and after chemotherapy, chemotherapeutic agents, associated radiotherapy, hematological pathology, bone marrow transplantation, physiologic, and gynecological history.

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The data were analyzed using R (v 2.9.1). The authors used bi-variate analysis (chi-square or Fisher exact test, Wilcoxon test or t-test, and monovariate logistic regression) and multivariate analysis (multivariate logistic regression). The authors considered statistically significant a $p < 0.05$.

The GnRH-a treatment protocol was approved by the human ethical committee at the University of Udine. After informed consent, the GnRH-a administration was timed as early as possible, usually within ten to 14 days before starting chemotherapy.

In seven cases, where the hematologists indicated urgency to the initiation of chemotherapy, the interval was shorter. POF was defined as persistent hypergonadotropic amenorrhea (FSH > 40 U/l in two occasions) and low E2 levels. During the study period, these protocols were used: 1) for Hodgkin lymphoma (HL) ABVD: adriamycin 25 mg/m^2, bleomycin 10 mg/m^2, vinblastin 6 mg/m^2, dacarbazine 375 mg/m^2 (all the drugs were given on day 1 and 15); 2) for non-Hodgkin lymphoma CHOP: cyclophosphamide 750 mg/mg on day 1, doxorubycina 50 mg/mq on day 1, prednisone 100 mg/die on day 1, vincristine 1.4 mg/m^2 on day 1, rituximab 375 mg/mq on day 1 and 7 of chemotherapy.

Results

The authors analyzed 124 patients treated of which 98 were still in follow-up or had completed the five years follow-up. The mean age of the women included in this study was 27.84 (± 8.82) years.

Group 1 included 33 women treated with GnRH-a, and about 24% of these (8/33) were co-treated with oestroprogestinic treatment. These 33 women were treated for an average of 7.17 months (± 6.43). Group 2 included 45 women without treatment, or treated only with estroprogestinic treatment in 40% of the cases (18/45).

In Table 1 the authors show the population characteristics and observed no statistically significant difference, except for the lower prevalence of nulliparous women in the control group ($p < 0.05$). Before the start of chemotherapy, regular menstruation was presented in 91% (30/33) of the patients treated with GnRh-a and in 90% (25/39) of the patients in the control group (p = 0.550).

There was no statistical difference (p = 0.490) in the incidence of gynecological diseases between the group treated with GnRH-a (9%) and the control group (5%).

In Figure 1, the hematological diseases are divided into two study groups: Acute Lymphoid Leukemia (ALL); Acute Myeloid Leukemia (AML); Acute non Lymphoid Leukemia (ANLL); Hodgkin Lymphoma (HL); Chronic Myeloid Leukemia (CML); Non-Hodgkin Lymphoma (NHL); Castleman disease; Multiple Mieloma (MM); Idiopatic Thrombocytopenic Purpura (ITP); Essential Trombocytemia (ETC); Werlhof disease.

In the two groups, Hodgkin disease, non-Hodgkin lymphoma, acute myeloid leukemia, and acute lymphoid leukemia were uniformly represented. The overall mortality prevalence was 14% (18/124) without considering the specific pathology.

In Table 2 the authors analyzed the chemotherapeutic regimens divided into risk categories considering the previous published data. The high-risk chemotherapeutic regimens were more prevalent in the group not treated with GnRHa, and this group included all bone marrow transplantations. While the low-risk regimens were higher in the group treated with GnRH-a-Cyclophosphamide was used in about 30% of cases. In addition, cyclophosphamide was used in 33% (11/33) of women treated with GnRH-a and 29% (13/45) of non-treated women (p 0.674).

In Table 1 the authors also considered the different prevalence of radiotherapy and bone marrow transplantation between the two groups and there was no difference that achieved statistical significance. Furthermore, the authors performed a logistic bi-variate analysis without finding statistical significance of GnRH-a treatment for the following outcomes: protection for amenorrhea post-therapy OR 0.52 (CI 0.95 0.21-1.33, p 0.172); achievement of pregnancy post-therapy OR 1.39 (CI 0.19-10.39, p 0.750); and protection against climacteric syndrome OR 0.42 (CI 0.08-2.22, p 0.307).

Among the considered outcomes, the authors analyzed two more in detail: pregnancy achievement after therapy and secondary amenorrhea after therapy. As shown before and in Table 1, there was no significant difference in pregnancy prevalence between treated and non-treated women with GnRH-a. Also the other possible predictors (histological type, treatment options, and age) were not significantly correlated to pregnancy achievement. Considering amenorrhea post-therapy at a maximum of five years follow-up, the authors confirmed GnRH-a not to be statistical significant protective (Table 3).

While radiotherapy was protective (OR 0.3 CI.95 0.1 - 0.9) $p < 0.05$) and the bone marrow transplantation was a risk factor for amenorrhea post-therapy (OR 7.2 CI.95 2.4 - 21.5 $p < 0.05$) in univariate and multivariate analyses after correction for age, parity, histological type and other treatment options.

Discussion

Modern chemotherapy and radiation therapy regimens have enabled many girls and reproductive age women to survive their cancers, but at the cost of rendering them sterile due to ovarian failure. The GnRh-a as fertility-preserving agents is highly debated in hematology and most of the studies are criticized for their lack of randomization and the different and shorter follow-up periods for treatment and control groups. The most numerous studies were not randomized and resulted in favour of GnRh-a efficacy to spare fertility [5, 6].

The incidence of chemotherapy-induced amenorrhea is related to patient age, the specific agents used, and the total dose administered. Therefore, the authors carefully compared the study and control group for each of these parameters. Neither the age nor the dosages of the various cytotoxic drugs were significantly different between the two groups. Moreover, there was no significant difference in the incidence of POF between the two groups (33% vs 49% p = 0.170).

The cumulative dose of the alkylating agent, which is

Table 1. — *Patients' characteristic in GnRH-a treated and control groups*.

	GnRH-a treatment (n = 33)	Control group (n = 45)	*p*
Characteristics before chemotherapy			
Mean age at treatment (years)	27.25 (± 7.25)	28.58 (± 10.53)	0.588
BMI (kg/m²)	22.68 (± 3.77)	22.4 (± 3.84)	0.791
Mean age at menarche (years range)	12.44 (± 1.69)	12.46 (± 1.06)	0.964
Miscarriages	9% (3/33)	13% (6/45)	0.560
Nulliparous women	82% (27/33)	58% (26/45)	< 0.05
E2 (pg/ml)	23 (19-26.25)	14 (12-21)	0.400
FSH	64 (62-67)	60 (58-80)	1.000
Regular periods (eumenorrhea)	91% (30/33)	90% (35/39)	0.550
Oligomenorrhea	3% (1/33)	10% (4/39)	0.260
Polymenorrhea	6% (2/33)	0% (0/39)	0.110
Amenorrhea	0% (0/33)	0.00% (0/45)	1.000
Haematological diagnosis			
Hodgkin's lymphoma	52% (17/33)	35% (16/45)	0.160
Non-Hodgkin's lymphoma	30% (10/33)	27% (12/45)	0.720
Acute myeloid leukemia	15% (5/33)	9% (4/45)	0.390
Chronic myeloid leukemia	0% (0/33)	11% (5/45)	0.070
Other malignancies	3% (1/33)	18% (8/45)	0.070
Oncological treatment			
Chemotherapy	100% (33/33)	100% (45/45)	1.000
Number of cycle of chemotherapy	6 (4-6)	6 (4-6)	0.457
Radiotherapy	49% (16/33)	32% (14/44)	0.140
Bone marrow transplant	27% (9/33)	44% (20/45)	0.120
Outcomes			
Pregnancy after therapy (5 years follow-up)	6% (2/33)	4% (2/45)	0.750
Amenorrhea	33% (11/33)	49% (22/45)	0.170
Polymenorrhea	3% (1/33)	0% (0/45)	0.240
Oligomenorrhea	0% (0/33)	0% (0/45)	1.000
Dyspareunia	3% (1/33)	2% (1/45)	0.820
Climacteric syndrome	6% (2/33)	13% (6/45)	0.300

Table 2. — *Type of chemotherapy in the different risk category for POF*.

	GnRH-a treatment	Control group	*p*
High-risk	27% (9/33)	47% (21/45)	0.082
Low-risk	67% (22/33)	44% (20/45)	0.052
Unknown	6% (2/33)	9% (4/45)	0.643

Table 3. — *Amenorrhea post-therapy (multivariate logistic regression analysis correction for women age, parity, histological type and other treatment options)*.

	OR (CI 95%)	*p*
Radiotherapy	0.3 (0.1 - 0.9)	< 0.05
Bone marrow transplantation	7.2 (2.4 - 21.5)	< 0.05
GnRH-therapy	0.8 (0.3 - 2.4)	0.713

among one the most important parameters determining the risk of ovarian damage in this study, was the same in the group with Gn-Rh-a (33.3% ciclophosphamide) and in the control group (28.8%).

Older women have a higher risk of ovarian failure and permanent infertility in comparison with younger women, since primordial follicle reserve declines with age [7]. For this reason in this protocol, the authors treated with GnRH-a only patients with an age between 15 and 36 years. The incidence of ovarian dysfunction after chemotherapy is strictly dependent on the doses of alkylating agents, and it has been calculated that the total dose of cyclophosphamide induced amenorrhea in a 40-year-old woman is four times less than the equivalent dose in a 20-year-old girl. Alkylation agents are gonadotoxic, producing damage to the ovarian reserve (primordial follicles) because they are not cell cycle-specific drugs [8]. If GnRH-a are given during the follicular phase of the cycle, they may actually cause a flare effect and create the opposite of the desired impact [9]. For this reason the authors, after informed consent, suggested the GnRH-a administration as early as possible, usually within ten to 14 days before starting chemotherapy. Future studies should examine GnRH antagonists instead of agonists for the achievement of a faster hypogonadotropic milieu, eliminating the waiting period of 7-14 days [10, 11]. However, in a recent study Danforth *et al*. [12] demonstrated in rodents that GnRH antagonists did not protect the ovary from the damaging effects of cyclophosphamide. Whereas the GnRH agonist significantly minimized the follicular depletion caused by cyclophosphamide, the GnRH antagonists did not prevent the gonadotoxic effect. This observation, although preliminary, raises concerns regarding the ability of GnRH-antagonists to substitute the agonists.

It has been well-established that chemotherapy with total body irradiation followed by allogeneic or autologous bone marrow transplantation causes permanent elevation of gonadotrophin levels and amenorrhoea in 92-100% of female patients [13]. The authors confirmed this data, but the association of chemotherapy with radiotherapy and no bone marrow transplantation resulted to be protective.

The possibility of administering an adjuvant treatment that may decrease the gonadal damage caused by an otherwise successful treatment is attractive [14-16]. It has been suggested that inhibition of the pituitary-gonadal axis may reduce the rate of folliculogenesis and consequently render the germinal epithelium less susceptible to the gonadotoxic effects [17].

In hematologic malignancies, different studies [5, 6, 18-20] suggested a statistical significant improvement in the preservation of ovarian function by the use of GnRH-a in keeping with this study. The authors found no statistical significance but GnRH-a seems to be protective for amenorrhea post-therapy (OR 0.5 CI.95 0.2 - 1.3, p 0.172), a promoting factor for a pregnancy post-therapy OR 1.4 (CI.95 0.2 - 10.4, p = 0.75), and protective for the climacteric syndrome OR 0.42 (IC 0.1 - 2.2, p = 0.307).

There are some important factors that differ between studies. First, the time of follow-up is important because POF is related to the age of women and menopause is a

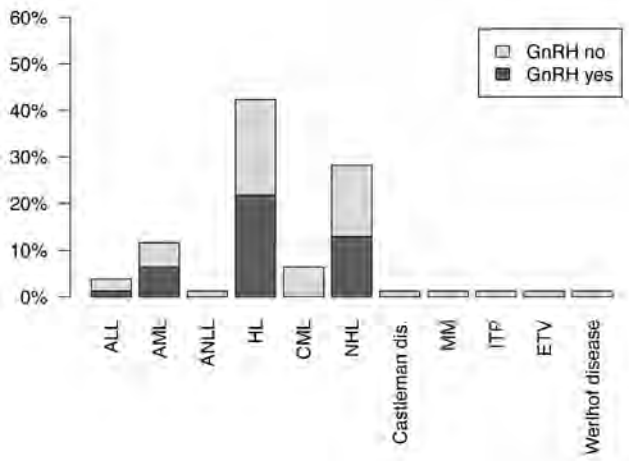

Figure 1. — Hematological diseases between the two studied groups. ALL: Acute Lymphoid Leukemia; AML: Acute Myeloid Leukemia; ANLL: Acute non Lymphoid Leukemia; HL: Hodgkin Lymphoma; CML: Chronic Myeloid Leukemia; NHL: Non-Hodgkin Lymphoma; Castelmann disease; MM: Multiple Mieloma; ITP: Idiopatic Thrombocytopenic Purpura; ETC: Essential Trombocytemia; Wehrlof disease.

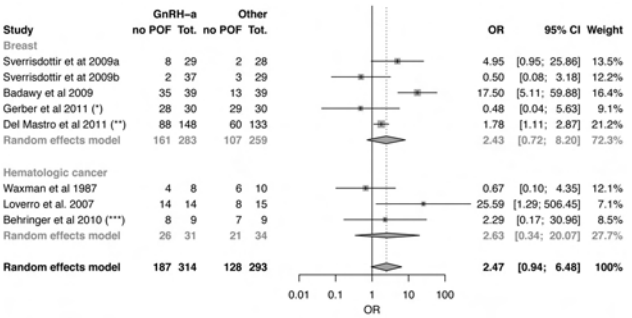

Figure 2. — This plot presents the incidence of not having premature ovarian failure with or without GnRH-a co-treatment among randomized studies and separately considering breast and hematologic pathology. (*) The authors considered the outcome at 24 months. (**) New randomized study. (***) The authors excluded one patient from GnRH–a group because the menstrual status was unknown.

physiologic event. In this study the authors considered a follow-up of five years while the majority of studies took in consideration a short follow-up period. Second, POF and depletion of ovarian reserve are two different endpoints: 1) secondary amenorrhea associated with hormonal dosages will determine the actual diagnosis of POF but will not estimate the ovarian reserve depletion in the cohort of women with resumption of menstruation [21]; 2) ovarian reserve depletion could be estimated in two ways longer follow-up times to establish time to menopause and hormonal dosages.

Different hormonal dosages have been proposed for the estimation of ovarian reserve depletion. The FSH measurements on the second or third day of the menstrual period was found to be reliable and if it exceeded 12 mIU/ml (20 mIU/ml by radioimmunoassay), the probability of pregnancies was very low [22]. Likewise, elevation of estradiol levels above 75 pg/ml on the second or third day of the menstrual period is also associated with compromised fertility [23]. However recent studies found anti-Müllerian hormone (AMH) to be more reliable. AMH is expressed by granulosa cells [24] and its expression is initiated in the smallest growing follicles and declines in the early antral stages as one follicle is selected for dominance and the rest of them become atretic. In a recent study, compared to estradiol and FSH, AMH showed a more rapid and sustained change after chemotherapy [25]. However all these hormonal methods are limited as any other test while the time to menopause seams a more reliable method to establish the ovarian reserve damage because the aim should be to say to a 30 years-old woman the average risk to develop menopause during the next ten years in taking or not GnRH-a, in comparison to the normal population. For example the answer should be the menopause in the average of population is at x years; if you take GnRH-a it will be 1 year before; if you do not it will be 2 years before.

In a recent review, Badawy *et al*. took in consideration all randomized studies with GnRHa for preservation of ovarian function during gonadotoxic chemotherapy [26-30]. However they considered different tumors other than hematologic and two extremely recent randomized studies were missing, therefore in Figure 2 the authors analyzed the GnRH-a value in breast and hematologic cancer including the two new randomized studies. Furthermore, in case of the ZORO study the authors considered the 24 months outcome instead of the six months (*) [27]. In Figure 2 the two new studies are one for breast cancer (**) and one for hematological cancer (***) [31, 32]. The first point that can be observed is that no significance is achieved among breast or hematological cancers but the majority of studies are in favour of GnRH-a efficacy. The second point is that in the whole randomized studies of hematological pathologies have enrolled only 65 women, while Blumenfeld alone in a non-randomized study have evaluated 157 women [6]. The authors agree with Blumenfeld when he says that some studies are giving conclusion without enough evidence [33] and all need randomized studies with longer follow-ups that are not only assessing hormonal levels, but also the prevalence of evident diagnosis of POF during longer follow-ups.

Moreover, patients undergoing myelosuppressive therapy are at high-risk of menorrhagia during thrombocytopenia. In patients with cancer receiving aggressive chemotherapy, the authors used GnRH-a treatment to prevent thrombocytopenia-associated menorrhagia [34].

In conclusion in these patients, the authors suggested before the beginning of conventional chemotherapy regimens, the GnRH-a co-treatment because it prevents menorrhagia and could preserve ovarian function, but other further evidence is required to confirm the effect of treatment of GnRH-a in preservation of fertility in young patients exposed to chemotherapy.

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Address reprint requests to:
A.P. LONDERO, M.D.
Clinic of Obstetrics and Gynecology
Piazzale S.M. della Misericordia, 15
33100 Udine (Italy)
e-mail: ambrogio.londero@gmail.com