Best Practice & Research Clinical Obstetrics and Gynaecology 26 (2012) 379–390

Contents lists available at SciVerse ScienceDirect

Best Practice & Research Clinical Obstetrics and Gynaecology

journal homepage: www.elsevier.com/locate/bpobgyn

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Chemotherapy and fertility

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Keywords:
fertility preservation
gonadotoxicity
GnRH-agonist
POF (premature ovarian failure)
chemotherapy

The overall increase in cancer prevalence and the significant increase in long-term survival have generated worldwide interest in preserving fertility in young women exposed to gonadotoxic chemo- and radiotherapy. Infertility represents one of the main long-term consequences of combination chemotherapy given for lymphoma, leukaemia and other malignancies in young women. The gonadotoxic effect of various chemotherapeutic agents is diverse, may involve a variety of pathophysiologic mechanisms, and is not unequivocally understood. Proliferating cells, such as in tissues with high turnover (i.e. bone marrow, gastrointestinal tract and growing ovarian follicles) are more vulnerable to the toxic effect of alkylating agents. These agents may also be cytotoxic to cells at rest, as they are not cell-cycle specific. Alkylating agents, the most gonadotoxic chemotherapeutic medications, cause dose-dependent, direct destruction of oocytes and follicular depletion, and may bring about cortical fibrosis and ovarian blood-vessel damage. The reported rate of premature ovarian failure after various diseases and chemotherapeutic protocols differ enormously, and depend mainly on the chemotherapeutic protocol used and age range of the woman. Several options have been proposed for preserving female fertility, despite gonadotoxic chemotherapy: ovarian transposition, cryopreservation of embryos, unfertilised metaphase-II oocytes and ovarian tissue, and administration of gonadotropin-releasing hormone agonistic analogs in an attempt to decrease the gonadotoxic effects of chemotherapy by simulating a prepubertal hormonal milieu. None of these methods is ideal and none guarantees future fertility in all survivors; therefore, a combination of methods is recommended for maximising women's chances of future fertility.

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doi:10.1016/j.bpobgyn.2011.11.008

Introduction

The overall increase in cancer prevalence and the significant increase in long-term survival have generated worldwide interest in preserving fertility in young women exposed to gonadotoxic chemo- and radiotherapy. In the past 2 decades, the survival rates for many of the malignancies that affect young women have significantly improved. For many of these malignancies, survival rates exceed 80–90%.[1–11] Therefore, the remote effects of cancer treatment have recently gained worldwide interest, and the protection against iatrogenic infertility caused by chemotherapy assumes high priority. Indeed, malignancy is estimated to occur in one out of 49 women under the age of 40 years in the Western world.[1,2] This is significantly different to previous estimations: at present, one in every 250–715 people in the adult population will be a cancer survivor.[3,4] Therefore, the late effects of cancer treatment is of interest to reproductive endocrinologists, haematologists, oncologists, gynaecologists, endocrinologists, rheumatologists, general practitioners, and all healthcare providers,[5] and the protection against iatrogenic infertility caused by chemotherapy assumes a high priority.

Critchley et al.[12] estimated that one in a 1000 adults, younger than 35 years, are survivors who have suffered and been treated for malignancy in their childhood. Although chemotherapy, radiotherapy, or both, can permanently impair reproductive functions,[13] preserving fertility in women is crucial, as a high percentage will develop premature ovarian failure (POF) as a result of follicular damage.[14] About 50% of women over 25 years of age and 20% of women under the age of 25 years treated with mechloroethamine, vincristine, procarbazine and prednisone (MOPP) will develop POF.[15] The most common significant long-term toxicity in premenopausal women receiving chemotherapy is POF. The effect of POF after chemotherapy and its associated infertility is of great importance to the individual woman and her family.

Prolonged survival of over 80–90% is now expected for a high proportion of young women treated with cytotoxic chemotherapy for Hodgkin's lymphoma.[3–11] The goal of complete cure without serious late secondary effects has, therefore, been positioned as a possible achievable goal. Although cytotoxic-induced damage may be reversible in other tissues of rapidly dividing cells (i.e. bone marrow, gastrointestinal tract and thymus), it is usually irreversible in the ovary, where the number of germ cells is believed to be fixed from fetal life.[4–11]

Infertility is one of the main long-term consequences of combination chemotherapy given for lymphoma, leukaemia and other malignancies in young women.[3–11]

Gonadotoxicity of chemotherapy

The gonadotoxic effect of various chemotherapeutic agents is diverse, may involve a variety of pathophysiologic mechanisms, and is not unequivocally understood. Furthermore, it is not an all or none phenomenon, and depends on the woman's age, chemotherapeutic regimen, and existing number of primordial follicles (ovarian reserve). The gonadotoxic effect may consist of interruption of basic cellular processes and interference with cell proliferation.[16] The alkylating agents can create covalent bonds between deoxyribonucleic acid (DNA) strands, interfere with cleavage during DNA replication, and bring about disruption of cell division. Although proliferating cells (i.e. tissues with high turnover), such as bone marrow, gastrointestinal tract and growing ovarian follicles, are more vulnerable to the toxic effect of alkylating agents, these agents may also be cytotoxic to cells at rest, as they are not cell–cycle specific.[16–20] Alkylating agents and cyclophosphamide therapy have been shown to cause dose-dependent, direct destruction of oocytes and follicular depletion.[16,20–22] In addition to follicular depletion, alkylating agents may cause cortical fibrosis and blood-vessel damage.[4,16]

Other chemotherapeutic agents include antibiotics, such as anthracyclines. Doxorubicin is the most known and commonly used in this group. Platinum agents (i.e. carboplatin, platinol, paclitaxel and cisplatin) are female-specific mutagens.[4,16,23] These agents may cause chromosomal aberrations leading to dyskarriosis, such as deletions, ring formations and DNA rearrangements, leading to embryotoxicity and embryonic demise.[16,21] Chemotherapy combinations, especially those containing alkylating agents, cause cortical fibrosis, ovarian atrophy and injury to blood vessels, leading to follicular depletion.[4,16,23–25] When follicular depletion is so significant, as in natural menopause, the woman experiences POF. In 8% of young women, survivals of prepubertal chemotherapy, who resume

normal cyclic ovarian function, even in women who experience spontaneous pregnancies and deliveries, POF may occur before the age of 40 years.[4,5,10,26–29]

Various chemotherapeutic agents have been categorised according to their gonadotoxicity into three risk categories[4,16]: high-risk, medium-risk and low-risk chemotherapeutic agents.

High-risk chemotherapeutic agents include the following alkylating agents: cyclophosphamide, busulphan, chlorambucil, procarbazine, melphalan, ifosfamide, chlormethamine. Medium-risk chemotherapeutic agents include platinum agents (cisplatin, carboplatin); anthracycline antibiotics (adriamycin [doxorubicin]); and taxoids (docetaxel and paclitaxel). Low-risk agents include vinca plant alkaloids (vincristine and vinblastine); anthracycline antibiotics (bleomycin) and antimetabolites (methotrexate, 5-fluorouracil, 6-MP [mercaptopurine]).

In an attempt to quantify the relative age-adjusted damage potential, it has been calculated that alkylating agents induce the most severe damage, with an odds ratio of almost 4.[4,30] Platinol derivatives increase ovarian failure, with an odd ratio of 1.77, whereas the other drug families do not cause a significant increase in ovarian failure rates.[4,30] Clinical data suggest, however that even chemotherapeutic agents considered less gonadotoxic may decrease ovarian reserve and even induce POF, especially when combination protocols are given, as is usually the case in many, if not most, malignant diseases.[7–10]

Effect on pregnancy

Pregnancies achieved in survivors of chemotherapy several years after the gonadotoxic effect do not have an increased rate of fetal malformations or pregnancy wastage; however, the exposure of a pregnant women to chemotherapy, especially in the first trimester during embryonic and fetal organogenesis, is associated with an increased risk of fetal anomalies, miscarriage and intrauterine fetal demise.[4,10,16] Therefore, preventing conception is recommended in the first 2 years after chemotherapeutic insult. The purpose of this recommendation is to prevent fertilisation of ova that might have been exposed to chemotherapy during the vulnerable period of folliculogenesis and growth, as the growth of ovarian follicles, in humans, from the primordial to metaphase II stage may take 3–9 months.[31,32] Another reason for abstaining from pregnancy in the first 2 years after completing chemotherapy is that most recurrences are detected within the first 2 years. Furthermore, diagnostic interventions, such as positron emission tomography and computed tomography, which consist of radioactive materials, are commonly experienced in the first year or two after completing chemo- and radiotherapy. Indeed, Meirow et al.[4,33] have extrapolated rodents' data to humans, speculating that, within 1 year after chemotherapy, the rate of fetal malformations and pregnancy wastage may be significantly increased. It may, therefore, be concluded that chemotherapy and radiotherapy can induce genetic defects in oocytes, depending on the agent used and the stage of gamete maturation.[4,33] In fact, in pregnancies occurring within several months after treatment, increased fetal demise and malformation rates have been reported.[4,33] On the contrary, it is reassuring that no increases in miscarriage or congenital anomalies were detected in neonates born more than 2 years after the gonadotoxic insult.[4,33]

The premature ovarian failure rate after various diseases

The reported POF rate resulting from the use of chemotherapeutic protocols to treat various diseases differ enormously but depend mainly on the chemotherapeutic protocol selected and age range.[4,14] Even for similar protocols and age groups, significant differences have been observed between different studies. Overall, the rate of POF ranges from 10% to almost 100%, although it is usually around 30–70% for premenopausal women exposed to gonadotoxic regimens, and 90–100% for adult, premenopausal women undergoing haematopoietic stem-cell transplantation (usually referred to as bone marrow transplantation [BMT]), combined with preceding aggressive chemotherapy-conditioning protocols.[4,6–11,16]

Hodgkin's and non-Hodgkin's lymphoma

Currently 80–90% of young women with lymphoma may enjoy many years of survival; therefore, in this population, the issue of long-term preservation of ovarian function and fertility has clinical importance. The reported POF rate after Hodgkin's lymphoma ranges from zero to 50%, depending on the

woman's age and chemotherapeutic protocol.[4,6–11,16,20] For example, the older protocol of chlormethamine, oncovin, procarbazine and prednisone combined with adriamycin, bleomycin, vinblastine, dacarbazine (MOPP-ABV[D]) was associated with a high rate of POF in about 50% of treated women, whereas the newer, softer protocol of ABVD induces POF in less than 10% of young women of reproductive age.[4,8,19,20,34,35] On the other hand, the more modern aggressive protocol of eight cycles of escalated bleomycin, etoposide, adriamycin, cyclophosphamide, oncovin, procarbazine, and prednisone (BEACOPP) endorsed a high rate of gonadotoxicity, resulting in POF in almost all young women older than 30 years and 51% in women younger than 30 years[4,20,34,35]; however, a much lower rate of POF is seen when only two cycles of escalated BEACOPP are combined with ABVD or conventional BEACOPP, as the experience of our group and others has shown.[19,20,35] In the experience of the German Hodgkin Lymphoma Study Group,[34] almost 20% of over 400 women with Hodgkins's lymphoma, contacted on follow up, reported amenorrhea. On multivariate analysis, escalated BEACOPP, age older than 30 years, advanced stage of Hodgkin's lymphoma, and absence of oral birth control pills were detrimental factors associated with higher risk of POF. The cumulative cyclophosphamide dosage leading to POF is 5 g at 40 years, 9 g at 30 years, and 20 g at 20 years. The age association is clearly demonstrated by the findings that two cycles of the cyclophosphamide, oncovin, procarbazine and prednisolone (COPP) and ABVD combination protocol will induce POF in less than 4% of young women below the age of 30 years compared with more than three times as much (12.2%) in women older than 30 years. Four cycles of the same protocol, however, will cause POF in 23.5% of women younger than 30 years and in 53.3% of those older than 30 years.[20,34–36] Similarly, eight cycles of BEACOPP will induce hypergonadotropic amenorrhea in almost 12% of young women younger than 30 years compared with over 42% of women older than 30 years. Eight cycles of the escalated-BEACOPP protocol will cause POF in 40.4% of women younger than 30 years compared with over 70% of women older than 30 years.[20,34–36]

The level of anti-Müllerian hormone (which best represents the ovarian reserve and remaining pool of small follicles) 1 year after treatment with ABVD returned to normal after decreased levels during chemotherapy; however, anti-Müllerian hormone levels remained significantly lower in the more aggressive protocols.[35]

In contrast to previously reported findings, a recently published Cochrane review compared two different chemotherapy regimens: chemotherapy with escalated BEACOPP, and chemotherapy with ABVD for women with early unfavourable or advanced-stage Hodgkin's lymphoma. The reviewers found no significant differences between both regimens for secondary malignancies, treatment-related mortality or infertility.[36]

The ovarian toxicity of the cyclophosphamide, adriamycin, oncovin, and prednisone (CHOP) protocol, or mega-CHOP, usually used in women with non-Hodgkin's lymphoma, is much lower. Most young women (90%) resume cyclic ovarian function, and only 10% suffer POF.[9,16,19,35,37]

The risk of POF after irradiation depends on the irradiated field and dose. Mantle-field radiotherapy, exerted superior to the diaphragm, exerts a negligible scattered radiation dose to the ovaries of about 0.1%.[35] Irradiation gonadotoxicity is affected by a dose as low as 1.5 Gy in women older than 40 years. After an irradiation dose of 2.5–5 Gy, about one-third of women between 15 and 40 years, and over 90% of women older than 40 years, will suffer POF.[35] On the other hand, total body and sub-diaphragmatic irradiation will sterilise most women with a direct-age association.

Leukaemia

High rates of cyclic ovarian function (COF) have been reported after treatment for prepubertal acute lymphoblastic leukaemia, with only a 10% POF rate[16,38–40]; however, poor fertility and the lowest rate of pregnancy were found in survivors of leukaemia; high rates of POF were most recently reported among adolescents and adults with cancer.[41] The lowest chance of fertility and highest POF rate is experienced by women with leukaemia undergoing BMT.[11]

Breast cancer

As with other malignant diseases, the rates of reported POF after treating women of reproductive age for breast cancer differs significantly, and ranges between zero to almost 70%, depending on the

woman's age and chemotherapeutic protocol used.[4,16] A total of 45–68% of premenopausal women experienced POF after cyclophosphamide, methotrexate, 5-fluorouracyl (CMF) treatment, and less than 15% of those treated with adriamycin and cyclophosphamide (AC) suffered long-term POF.[4,16,20] For similar chemotherapeutic protocols or even the same CMF protocol, 45–68% of premenopausal women aged over 35 years experienced POF, whereas only 0–28% of young women aged younger than 35 years suffered POF after either CMF of cyclophosphamide, adriamycin, 5-fluorouracyl (CAF) protocols. More specifically, for premenopausal women over the age of 40 years, six cycles of the chemotherapeutic protocols CMF, CAF or cyclophosphamide, epirubicin, 5-fluorouracyl (CEF), will cause hypergonadotropic amenorrhea and ovarian failure in more than 80% of the women treated; however, the same protocols will induce POF in 20–80% of women between the ages of 30 and 39 years, and less than 20% in women younger than 30 years.[20] Four cycles of the AC protocol will induce POF in less than 20% of the women treated aged under 40 years, whereas 20–80% of premenopausal women older than 40 years will experience permanent hypergonadotropic amenorrhea and menopause after treatment.[20]

Systemic lupus erythematosus and autoimmune diseases

Cyclophosphamide is an effective therapy in severe life-threatening rheumatic conditions. Repeated cyclophosphamide pulsatile therapy (CPT) is a standard treatment regimen in lupus nephritis, severe mixed connective tissue disease, systemic sclerosis with lung involvement, and vasculitic syndromes, such as Wegener's granulomatosis and polyarteritis nodosa.[8,10,42–44] Although cyclophosphamide pulsatile therapy may improve survival and reduce end-organ damage in women with severe connective tissue diseases, it may bring about an unacceptable high incidence of POF, ranging from 30–60% among various studies.[8,10,42–44]

Preservation of ovarian function despite chemotherapy

Several options have been forwarded for preserving female fertility: ovarian transposition, cryopreservation of embryos, unfertilised metaphase–II oocytes and ovarian tissue, and administration of gonadotropin-releasing hormone agonistic (GnRH-a) analogs in an attempt to decrease the gonadotoxic effects of chemotherapy by simulating a prepubertal hormonal milieu.[7–10,20] Indeed, in the past decade, the number of studies on fertility preservation has dramatically increased. Unfortunately, none of the suggested methods is ideal and none guarantees future fertility in survivors. In-vitro fertilisation and embryo cryopreservation, the only non-investigational, ubiquitously agreed upon and clinically established method, necessitates postponing chemotherapy for at least 2 weeks, and is frequently not applicable to young and single women. In the past 2 decades, cryopreservation of unfertilised ova has gained popularity and increased success owing to the vitrification method; however, it does not guarantee future fertility.

Women who have undergone cryopreservation and transplantation of ovarian tissue, and have been cured from their malignancy, have gone on to give birth successfully several years after the chemotherapeutic insult (18 reported cases).[45–50] It cannot be assumed, however, that these techniques are unequivocally safe and successful.[45–50]

More specifically, Dolmans et al.,[45] who reported on the first ever delivery after thawed ovarian tissue transplantation, recently reported low in-vitro fertilisation success rates in women with orthotopically grafted ovarian tissue owing to a significantly higher risk of empty follicles (29%), abnormal and immature oocytes (38%), and low rate of embryo transfer (24%). The same group has also highlighted the risk of ovarian tissue harboring malignant cells, which could induce disease recurrence.[46] This suggests that auto-implantation of the cryopreserved ovarian tissue from women with leukaemia may induce disease recurrence because of ovarian contamination by malignant cells. Similarly, Rosendahl et al.,[47] the most experienced group in ovarian cryopreservation and transplantation, have also detected potentially malignant cells in most cases of cryopreserved ovaries from women with leukaemia. They concluded that, at present, reimplantating the ovarian cortex in women with leukaemia cannot be recommended.[47] Similarly, ovarian involvement in Ewing's sarcoma or Hodgkin's lymphoma has been reported recently by other groups carrying out ovarian cryopreservation.[48,49] Furthermore, Kyono et al.[50] examined the potential indications for ovarian autotransplantation based on the analysis of 5571 autopsy findings in young Japanese women, under the age of 40 years. They found ovarian involvement

with metastatic malignant cells in 8–55% of overall autopsies and 4–13% ovarian involvement in lymphoma, concluding that no reliable method is available to rule out minimal residual disease in the cryopreserved ovarian tissue. This highlights the significant risk of ovarian auto-reimplantation in young women undergoing ovarian tissue cryopreservation in an attempt to preserve future fertility.[50] The in-vitro maturation of primordial follicles to fertilisable metaphase–II oocytes is a promising endeavour with enormous potential; however, many technological obstacles need to be overcome and it is not yet clinically available. Therefore, GnRH-a has been used by many clinicians to decrease the gonadotoxic effects of chemotherapy[7–11] by simulating a prepubertal hormonal milieu, with the rationale and philosophy that preventing POF in survivors is preferable to treating it, following the dictum: 'an ounce of prevention is worth a pound of cure'.

To date, 16 studies (13 retrospective and three prospective randomised-controlled trials [RCTs]) have reported on more than 1000 women treated with GnRH-a in parallel to chemotherapy. They showed a significant decrease in the rate of hypergonadotropic amenorrhea and menopause in survivors[7–10,51,52] compared with seven studies that included 409 women, which reported no beneficial effect of the GnRH-a. Overall, women exposed to gonadotoxic chemotherapy and concurrent GnRH-a preserved their COF in over 90% of cases compared with 41% of control participants, with a pregnancy rate of 19% in women who had received treatment.[53]

Four recently published meta-analyses have concluded that GnRH-a are beneficial and decrease the risk of POF in survivors.[53–56] The first meta-analylsis[53] found a significant beneficial role for the agonists on both preservation of ovarian function and conception, and 68% increase in the rate of preserved ovarian function (RR 1.68; 95% CI 1.34 to 2.1). Among women treated with GnRH-a, 22% achieved pregnancy compared with 14% who were not treated with GnRH-a (RR 1.65; 95% CI 1.03 to 2.6). This meta-analysis concluded that GnRH-a cotreatment seems to improve ovarian function and the ability to achieve pregnancy, and that premenopausal women exposed to gonadotoxic chemotherapy should be counselled about ovarian preservation options, including the use of GnRH-a co-treatment. This is in keeping with the recently published recommendation by the multicenter Fertiprotekt network of more than 70 centres in Germany, Switzerland and Austria, involved in fertility preservation.[20]

The second meta-analysis concluded that GnRH-agonists are effective in reducing amenorrhea (RR 0.26, 95% CI 0.14 to 0.49), and that pregnancy rate was higher in women who have received GnRH-a in addition to chemotherapy.[54] A similar conclusion was reached in another meta-analysis,[55] which suggested that GnRH-a co-treatment during chemotherapy can protect ovarian function and decrease gonadotoxicity. The recently published fourth meta-analysis of RCTs[56] similarly concluded that the incidence of POF or resumption of ovulation both showed a statistically significant difference in favour of GnRH-a co-treatment.

The only prospective study with histological assessment of the remaining follicles, which obviously cannot be conducted in humans, has been carried out in Rhesus monkeys. Follicular loss was examined after exposure to alkylating agents alone or in parallel to GnRH-a cotreatment.[57] During the gonadotoxic exposure, $64.6 \pm 2.8\%$ of the total primordial follicles were lost in the cyclophosphamide group compared with only $28.9 \pm 9.1\%$ in the GnRH-a plus cyclophosphamide group ($P < 0.05$). The daily follicular decline was $0.12 \pm 0.012\%$ for the cyclophosphamide group compared with $0.057 \pm 0.019\%$ ($P < 0.05$) for the GnRH-a plus cyclophosphamide group.[57] These investigators concluded that GnRH-a co-treatment can protect the ovary against cyclophosphamide-induced gonadotoxicity in Rhesus monkeys. Similarly, two recently published RCTs in women have also found that GnRH-a co-treatment significantly decreased the gonadotoxic effects of chemotherapy and decreased the POF rate in survivors.[58,59]

Furthermore, a study by the American Society of Clinical Oncology also found rates of POF to be 0–6% with GnRH-a compared with 32–47% without GnRH-a.[60] Additionally, the most recently published and convincing prospective RCT[61] is a multicentre, open-label, randomised, phase III trial of 281 women with breast cancer, median age 39 years, treated with chemotherapy alone or chemotherapy and GnRH-a. One year after treatment with chemotherapy, POF was observed in 32.3% in the group who received chemotherapy alone compared with only 13.5% in women who had been treated with chemotherapy and GnRH-a ($P = 0.0002$), with a 19% absolute reduction (95% CI 8 to 29). Cyclic menstrual activity and premenopausal oestradiol levels were observed in 58% of women in the group receiving chemotherapy alone compared with 77% in the group receiving chemotherapy and GnRH-a ($P = 0.006$).[61] Logistic regression analysis showed that GnRH-a was independently associated with

a higher probability of COF preservation ($P = 0.001$).[61] In summary, four prospective, peer-reviewed studies,[57–59,61] in addition to 13 non-RCTs, and four meta-analyses have concluded that the addition of GnRH-a to chemotherapy can significantly preserve ovarian function and fertility in premenopausal women facing gonadotoxic chemotherapy.

Seven studies have claimed, however, that GnRH-a is not efficient for preserving ovarian function.[62–68] Opponents of this preservation method have used these data as evidence against its use as a co-treatment. Waxman et al.[62] found that four out of eight women in the GnRH-a group suffered POF (50%) compared with six out of nine women in the control group (66.7%). This study, however, was underpowered. Moreover, as the investigators themselves point out,[62] the pituitary ovarian suppression in the women receiving treatment was not complete, possibly leading to the results.

Another study with negative results,[66] published as a meeting abstract, has not been subsequently published in a peer-reviewed journal, as expected. The study was not completed as scheduled, and 88% (chemotherapy regimen) and 90% (chemotherapy and GnRH-a) of the women resumed menses regardless of whether or not they received GnRH-a in addition to chemotherapy. For such a low rate of gonadotoxicity, the overall number of 49 women in both groups lacks the power to detect any effect of the GnRH-a. Thus, when the gonadotoxicity of the chemotherapeutic protocol is either low,[66,64] or when it is high,[63] the power needed to detect a difference between the two treatment arms is hundreds of patients, much more than the range of patients (17–60) used in these negative studies.[62–67]

In another study,[68] 227 women were randomised to receive chemotherapy and GnRH-a compared with chemotherapy alone. Preliminary results did not show a difference in ovarian protection between the two arms. A possible explanation for the different and contradictory reported results may be the different timing of ovarian function assessment in the various studies. As resumption of ovarian function may occur as late as a year or more after ending chemotherapy, an early assessment of the outcome at 6 months[67,68] may underestimate the true effect of GnRH-a treatment.[51,52] Indeed, in the study by Gerber et al.,[67] the number of women resuming cyclic menstruation at 2 years after chemotherapy is significantly higher than those with 'two menses at 6 months' as chosen by the investigators for defining the resumption of ovarian function. Furthermore, the gonadotropin levels of women in the control group were significantly higher ($P = 0.015$) in the menopausal range compared with women receiving goserelin in addition to chemotherapy.[67] This highlights a serious discrepancy between the conclusion and the results of this study.[69]

Although no randomised trials have assessed the role of embryo or oocyte cryopreservation strategies for fertility preservation, many opponents to the use of GnRH-a refer to cryopreservation of ovarian tissue as an established method of fertility preservation. The quality of evidence for recommending such strategies should be considered low, because it is based on non-randomised, case-control or observational studies. On the contrary, the role of GnRH-a therapy in preserving ovarian function has been assessed in randomised and in non-randomised, case-control studies.[7–10,53–61]

Opponents of GnRH-a, such as Nitzschke et al.,[65] claim that: 'A clinical example for why gonadal suppression may not protect ovaries is the fact that prepubertal children receiving high-dose chemotherapy given before haematopoietic stem-cell transplantation still suffer from ovarian failure'. Recently, Remerand et al.[70] described four spontaneous pregnancies and successful deliveries in a pre-pubertal girl who had high-dose busulphan and cyclophosphamide (Bu-Cy) conditioning and BMT, demonstrating that successful pregnancies are possible in women who have undergone prepubertal BMT. Similarly, the only published case of repeated spontaneous pregnancies and two successful deliveries after repeated autologous BMTs combined with GnRH-agonist treatment during the gonadotoxic chemotherapy has been recently described in a postpubertal girl with lymphoma. This suggests that the prepubertal milieu induced by the GnRH-a cotreatment might have contributed to preserved fertility despite repeated BMT.[11] Only 0.6% of women conceive after one autologous or allogeneic BMT, according to an extensive survey involving 37,362 women.[71] Thus, the estimated odds for pregnancy after two BMTs are negligible (theoretically: $0.006 \times 0.006 = 0.000036$).[11] Similarly, in another study[72] of 619 women, only 3% conceived after one BMT. Thus, theoretically, according to the findings of this study, the estimated odds for conceiving after two SCTs are $0.03 \times 0.03 = 0.0009$, about 1 out of 1000.[72] The administration of GnRH-a before and in parallel to the gonadotoxic conditioning chemotherapy simulated the prepubertal hormonal milieu, and might have minimised the gonadotoxic effect and augmented the odds of ovulations, conceptions, and deliveries.[11]

Opponents to GnRH-a also argue that 8% of prepubertal children exposed to gonadotoxic chemotherapy may suffer POF by the age of 40 years, and therefore the rationale for creating a prepubertal milieu is illogical. Indeed, in three recent publications,[27–29] survivors of childhood cancer have been found to have an 8% risk of suffering POF before the age of 40 years, compared with less than 1% in the general population. This is in keeping with the results of published trials showing that women of reproductive age receiving GnRH-a in addition to chemotherapy suffer POF in about 7–11% of cases (simulating prepubertal exposure). Those treated without the agonist, however, have about a 30–60% risk of POF.[7–10,53–56] Therefore, it does not only contradict, but strongly supports the rationale of generating a pre-pubertal milieu during the gonadotoxic insult of chemotherapy.

Another concern is that the agonist may reduce the efficiency of chemotherapy; however, a meta-analysis,[73] based on 11,906 young women with breast cancer randomised in 16 trials, found that the addition of GnRH-a to tamoxifen, chemotherapy, or both, reduced recurrence by 12.7% ($P < 0.02$) and death after recurrence by 15% ($P < 0.03$). This contradicts the raised hypothetical speculation. Furthermore, the publications of Del-Mastro et al.[61] and Recchia et al.[74] show improved 5- and 10-year survival rates with GnRH-a compared with the published survival rates in women treated with similar protocols without the GnRH agonist. More than 250 women have been treated, in the past 2 decades in our medical centre with GnRH-a in parallel to chemotherapy for different indications, and the survival rate was not lower than the survival of those who did not receive the agonist.[7–9]

Level I evidence is based on prospective RCTs. Notwithstanding all the above, two studies[75,76] have reported that good observational studies give results similar to those of RCTs. Many unknown and equivocal parameters exist in the important issue of fertility preservation. Therefore, these data seem to suggest that clinical medicine is still far from having a ubiquitous solution for all these young survivors. None of the suggested avenues for fertility preservation guarantees unequivocal success in future fertility preservation. Even in-vitro fertilisation and cryopreservation of a few embrya cannot guarantee future pregnancy. Therefore, several modalities should be combined and considered.[7–10,20] Maximising women's future chances of fertility may necessitate the combination of ovarian cryopreservation, GnRH-a administration, and follicular aspiration, and all these modalities should be offered to all women. Furthermore, GnRH-a can effectively prevent the thrombocytopenia-associated menorrhagia in these women.[77]

Finally, it may seem ethically incorrect to deprive women from information on possible fertility preservation. Because most of the methods involving ovarian or egg cryopreservation are not yet clinically established and 100% successful, these young women deserve to be informed of all the various modalities to minimise gonadal damage and to preserve ovarian function and future fertility.[7–10,20] Furthermore, combining the various modalities for a specific individual may increase the odds of preservation for future fertility. Ovarian biopsy for cryopreservation, combined with GnRH-a administration and follicular aspiration, has no contraindication, as recently reported.[7–10,20] In cases in which chemotherapy has caused POF, as is frequently the case in total body irradiation and BMT, the woman has cryopreserved ova, embryos, or primordial follicles to fall back on. In cases in which conventional chemotherapy regimens, such as those commonly used for young women with lymphoma, are applied, GnRH-a cotreatment may preserve ovarian function and prevent POF without necessitating the use of cryopreserved ova, embryos or ovarian tissue.

Conclusion

The gonadotoxic effect of various chemotherapeutic agents is diverse, involves different pathophysiologic mechanisms, and is not unequivocally understood. The reported rates of POF after treatment of various diseases with chemotherapeutic protocols differ enormously, and depend mainly on the chemotherapeutic protocols used and age ranges of the women. None of the options suggested for preserving female fertility despite gonadotoxic chemotherapy methods is ideal and none guarantees future fertility in all survivors; therefore, a combination of methods is recommended for maximising chances of future fertility. There is no contraindication to ovarian biopsy for cryopreservation combined with GnRH-a administration and follicular aspiration. If chemotherapy causes POF (e.g. after total body irradiation and BMT), the patient has cryopreserved ova, embryos, or primordial follicles to fall back on. Preserving ovarian function by using GnRH-a is preferable to treating infertility after POF, as it is less invasive, inexpensive, and prevents menopausal syndrome and osteoporosis.

Practice points

- The gonadotoxic effect of various chemotherapeutic agents is diverse, involves different pathophysiologic mechanisms, and is not unequivocally understood.
- The reported rates of POF after treatment of various diseases with chemotherapeutic protocols differ enormously, and depend mainly on the chemotherapeutic protocols used and age ranges of the women.
- Several options have been forwarded for preserving female fertility despite gonadotoxic chemotherapy: ovarian transposition; cryopreservation of embryos; unfertilised ova; ovarian tissue; and administration of GnRH-a in an attempt to decrease the gonadotoxic effects of chemotherapy.
- None of these methods is ideal and none guarantees future fertility in all survivors; therefore, a combination of methods is recommended for maximising chances of future fertility.
- There is no contraindication to ovarian biopsy for cryopreservation combined with GnRH-a administration and follicular aspiration.
- If chemotherapy causes POF (e.g. after total body irradiation and BMT), the woman has cryopreserved ova, embryos, or primordial follicles to fall back on.
- Preserving ovarian function by using GnRH-a is preferable to treating infertility after POF, as it is less invasive, inexpensive, and prevents menopausal syndrome and osteoporosis.

Research agenda

- Validate the effect of GnRH-a in preserving long-term fertility, in addition to ovarian function.
- The results of additional good, prospective, large, RCTs using GnRH-a co-treatment are awaited for assessment of accurate role of this method for fertility preservation.
- Minimal or non-invasive methods of using anti-apoptotic molecules such as Sphingosine-1-Phosphate, AS101, or others for fertility preservation despite gonadotoxic chemo- and radiotherapy are awaited.
- Validate the accurate pregnancy rate of success of ovarian tissue transplantation after cryopreservation, by a worldwide summary of all cases.
- Assess the accurate risk of ovarian tissue transplantation after cryopreservation to prevent reimplantation of malignant cells.
- Future endeavors are awaited for developing the technology of in-vitro maturation of primordial follicles to mature fertilisable M-II oocytes.

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