Management of fertility in patients treated for Hodgkin's lymphoma

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ABSTRACT

The risk of developing premature ovarian failure and azoospermia is a major concern in long-term survivors treated for Hodgkin's lymphoma. Alkylating chemotherapy containing procarbazine and/or cyclophosphamide causes prolonged azoospermia in 90-100% of men and premature ovarian failure in 5-25% of women under the age of 30. The risk of infertility increases with the cumulative dose of alkylating agents and the risk is high after salvage therapy including conditioning and autologous or allogeneic transplantation. The doxorubicin-bleomycin-vinblastine-dacarbazine regimen is associated with a lower risk of gonadal damage; the rate of infertility is less than 10%. The risk of premature ovarian failure is limited after the doxorubicin-bleomycin-vinblastine-dacarbazine regimen. However, age is an important factor; women over 30 years of age are at a much higher risk of ovarian failure. Semen cryopreservation should be routinely offered, especially before initial treatment with bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone or salvage therapy with high-dose chemotherapy and autologous transplantation. For women with a stable partner, *in vitro* fertilization for embryo cryopreservation is a routine procedure but can only be offered to a small number of patients and requires a delay in treatment initiation for at least four weeks. Cryopreservation of mature or immature oocytes remains experimental. Ovarian tissue cryopreservation is promising but has so far resulted in only a small number of pregnancies and births. This method, usually involving the removal of an entire ovary, is only proposed before treatment leading to a high risk of infertility. Analogs of LHRH were investigated in order to preserve fertility in women but are not recommended in the absence of studies demonstrating their effectiveness. The risk of secondary infertility should be discussed with patients from the time of the diagnosis and requires multidisciplinary collaboration between hematologists and Assisted Reproductive Techniques (ART) teams.

Key words: Hodgkin's lymphoma, gonadal toxicity, fertility preservation, female, male.

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Introduction

The treatments currently administered can cure about 80% of patients with Hodgkin's lymphoma, all stages combined, but progress is still required in order to reduce the potential side effects of treatment and particularly long-term adverse effects. The evolution of treatment has resulted in the maintenance of chemotherapy and radiotherapy in localized supradiaphragmatic stages, chemotherapy alone in advanced stages and the abandonment of extensive radiotherapy. However, refractory disease and relapses require the use of more gonadotoxic chemotherapy.

Care from the diagnosis, given the risk of secondary infertility, is an important issue for patients. Concerted multidisciplinary discussions between hematologists, Assisted Reproductive Techniques (ART) teams and surgeons are required to improve the quality of patient information,[1] propose measures to preserve fertility adapted to each patient and to assess the consequences of treatment.[2]

Normal gonadal structure and function

The ovaries contain a pool of primordial follicles which attains a maximum level during the 7th month of intrauterine life. The stock of primordial follicles decreases exponentially during life, from 1 to 2 million follicles at birth to 400,000 during early puberty. After the age of 37 onwards, the decline in stocks accelerates down to fewer than an average 1,000 follicles at menopause (50.4 years).[3]

The ovarian reserve, namely the number of primordial follicles present at a given age, evolves in line with fertility in women of childbearing capacity. The ovarian cycle is regulated by gonadotropin hormones.

Premature ovarian failure (POF) is defined as the premature (age 40 years) termination of ovarian function of peripheral origin.[4] The destruction of part or of the entire stock of primordial follicles is the cause of hypergonadotropic amenorrhea defined as the loss of negative feedback. The main consequence of this syndrome is infertility, but women also exhibit symptoms of estrogen deprivation with hot flushes, vaginal dryness, and dyspareunia. There are several causes for POF but the most common is exposure of the ovaries to a toxic substance, particularly a cytotoxic agent.

Therefore, chemotherapy is responsible for DNA damage that directly affects the ovary. Alkylating agents produce

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covalent bonds between DNA strands, rendering cleavage impossible during replication and thus blocking division. These lesions induce apoptosis that not only affects the follicle but also the growing population of primordial follicles. The interruption of this signaling pathway leading to apoptosis is currently an interesting way to avert damage to the ovary.[5,6]

Spermatogenesis begins in the testes in males during early puberty. It encompasses the entire development process, from spermatogonia (primordial differentiated germ cells) to sperm. This process occurs in the seminiferous tubules and consists of the different stages of differentiation of germ cells and Sertoli cells, the supporting cells which are essential for spermatogenesis. A complete cycle of spermatogenesis takes about 74 days. The eventual restoration of spermatogenesis after treatment depends on the integrity of spermatogonia and their ability to differentiate.

Infertility risk factors

Patients' characteristics

Among women treated for cancer, age, and thus the status of the ovarian reserve before treatment initiation, exert a major impact on the subsequent risk of infertility. For the same treatment, the percentage of POF and the resulting percentage of infertility increases with age. This risk is greatest after 30 years of age.[7,8] All studies on Hodgkin's lymphoma report an impact of age on fertility. A Norwegian study in which the median follow up (20 years) is higher than in other studies, showed that women under 25 years age run the same risk of developing POF as women aged over 30 but after a much longer interval: 15 years *versus* two years.[9]

Unlike women, in men the spermatogonia stock is constant throughout life. This explains why age is not a predictor of infertility in men treated with chemotherapy.

Studies evaluating the quality of semen at the diagnosis of Hodgkin's lymphoma, showed a high percentage of abnormal sperm analyses before treatment. This percentage varies from one study to another. Some authors described up to 80% of abnormal sperm analyses.[10] The most recent study is a retrospective study conducted by the EORTC (European Organization for Research and Treatment of Cancer Lymphoma Group) and the GELA (*Groupe d'Etude des Lymphomes de l'Adulte*). They reported that among 474 patients analyzed, 41% had a normal semen analysis, 49% an intermediate-grade analysis, 7% a poor analysis and 3% of patients were azoospermic, including 2 who recovered normal sperm parameters after chemotherapy.[11] Risk factors are the presence of general symptoms at the time of the diagnosis, especially fever and night sweats and an increased erythrocyte sedimentation rate (ESR). The exact mechanisms are unknown but proinflammatory cytokines are assumed to be implicated. The stage of the disease has no impact.

Treatment-related risks in first-line regimens

Table 1 presents an overview of the treatment-related risk of infertility.[12-21] In women, the type and dose of chemotherapy and age are the most important risk factors for infertility.[7] This risk is greatest after exposure to alkylating agents, with a relative risk of 3.98 compared to an unexposed population. It increases with the cumulative dose.

The two protocols currently used in Hodgkin's lymphoma during first-line therapy are the ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) regimen and the escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone) regimen, which differ essentially due to the presence of alkylating agents in the BEACOPP regimen.[22] For example, the total dose of cyclophosphamide known to lead to the highest risk of ovarian failure at the end of treatment is 20 g at 20 years, 9 g at 30 and 5 g at 40 years.

Several studies have investigated the impact of these two chemotherapy regimens on fertility. They are all retrospective studies with different objectives (children, the study of hormone assays in the 2 sexes and the study of menstrual cycles and ultrasound in women). Thus the German Hodgkin Study Group published a paper in 2005 on the recovery of normal menstrual cycles (which is one aspect of ovarian function) after BEACOPP, COPP/ ABVD or ABVD.[12] After a median of 3.2 years, 19.3% of the 405 women contacted had amenorrhea. The main risk factors identified in the multivariate analysis were the type of treatment, with a higher risk for amenorrhea during treatment with the escalated BEACOPP regimen than with the baseline BEACOPP, ABVD or COPP/ ABVD, patient age (under or over 30 years) disease stage, and surprisingly, the absence of oral contraception. Decanter *et al.* compared the anti-Müllerian hormone (AMH) assay in patients treated with ABVD and in patients treated with chemotherapy containing alkylating agents. The rate of AMH decreased significantly in the two groups but at one year, the ABVD group recovered a normal AMH rate whereas it remained depleted in the other group.[13]

A Norwegian study published in 2007 and a French study published in 2003, investigating the number of pregnancies *per* woman, confirmed that the type of treatment, and in particular the dose of alkylating agents, and age were the two main risk factors for infertility.[14,15] Pregnancies after chemotherapy did not give rise to more complications than in the general population.[23] Finally, two other studies confirmed the safety of ABVD with a birth rate comparable to that of the general population regardless of patient age or the number of treatment courses.[16,17]

Among men, the risk of infertility depends on the treatment used. The risk is low after radiotherapy alone if pelvic radiation is avoided or chemotherapy without alkylating agents. It increases significantly when alkylating agents are used. The incidence of azoospermia is lower after ABVD compared to ABVD/COPP or MOPP.[18] Van der Kaaij *et al.* analyzed follicle-stimulating hormone (FSH) levels and reported that after a median time of 19 months, recovery of fertility occurred in 82% of patients treated without alkylating chemotherapy, compared to only 30% of those treated with alkylating agents, with a median time to recovery of 27 months ($P<0.001$).[19]

After BEACOPP, abnormal sperm or an increase in serum FSH levels were observed in 90-100% of cases. There is no difference between baseline BEACOPP and escalated BEACOPP.[24] After COPP/ ABVD, the infertility rate is between 60 and 91% and varies depending on the number of treatment courses. After ABVD, the infertility rate is 0-8%, and after chemotherapy and pelvic radiotherapy an infertility rate of 2-11% has been reported.[20,21] Alkylating agents are the main risk factor for infertility and that risk is proportional to dose. The recovery of normal exocrine testicular function mainly depends on the dose of

alkylating agents and may take several years.

A Norwegian study reported 269 men treated for Hodgkin's lymphoma between 1971 and 1998.[14] From 1985 to 1990, primary chemotherapy with MVPP/ChlVPP (mechlorethamine, vinblastine, procarbazine, prednisone/chlorambucil, vinblastine, procarbazine, prednisone) was gradually replaced by ABOD (doxorubicin, bleomycin, vincristine, dacarbazine) or EBVP (epirubicin, vinblastine, procarbazine, prednisone) for limited disease. At relapse, patients were treated with non-cross resistant chemotherapy, or from 1990 with high-dose chemotherapy with autologous stem cell support (HDT). Fractionated total body irradiation (TBI) with high-dose cyclophosphamide was used as a conditioning regimen for HDT until 1995, and was subsequently changed to chemotherapy alone (BEAC/BEAM). Among 120 males

Table 1. Treatment-related risk of infertility for patients with Hodgkin's lymphoma.

Authors	Patients	N.	Disease	Follow up	End point	Treatment	Results	Risk Factors
Behringer *et al.* 2005[12]	Women	405	HL	3.2 years	Amenorrhea	Radiotherapy ABVD 2COPP/ABVD 4COPP/ABVD 8 BEACOPP 8BEACOPPesc	6.3% 3.9% 6.9% 37.5% 22.6% 51.4%	Alkylating agent Age Disease stage No oral contraception
Decanter *et al.* 2010[13]	Women	30	HL and NHL	1 year	AMH dosage	ABVD Non ABVD[(1)]	Normal Decrease	
Hodgson et al. 2007[16]	Women attempting pregnancy	36	HL	1 year	Parenthood	ABVD	Same fertility rate as controls	
Brusamolino *et al.* 2006[17]	Women	67	HL	10 years	Amenorrhea Parenthood	ABVD	Fertility preserved 10 became pregnant	
Franchi-Rezgui *et al.* 2003[15]	Women attempting pregnancy	84	HL and NHL	8 years	Parenthood	[(2)]	37%: preserved fertility 40% : POF 23% : relative fertility	Dose of alkylating agent Age
Kiserud *et al.* 2007[14]	Women attempting pregnancy	91	HL	10 years	Parenthood	LowChem[(3)] MedChem HDChem	55% 21% 27%	Dose of alkylating agent Age
	Men attempting parenthood	120				LowChem MedChem HDChem	85% 35% 18%	Dose of alkylating agent
Kulkarni *et al.* 1997[18]	Men	38	HL	34 months	FSH level Sperm count	ABVD COPP/ABVD	Normal FSH level 88.5% Normal FSH level 33% Azoospermia only in COPP/ABVD arm.	Alkylating agent
van der Kaaij *et al.* 2007[19]	Men	349	HL	26 months	FSH level	Without alkylating agent[(4)] With alkylating agent	Recovery of fertility 82% Recovery of fertility 30%	Dose of alkylating agent
Sieniawski *et al.* 2008[20]	Men	112	HL	17.4 months	Azoospermia	Radiotherapy ABVD 2COPP/ABVD 4COPP/ABVD 8 BEACOPP 8BEACOPPesc	1% 0% 56% 91% 93% 87%	Alkylating agent
Hobbie 2005[21]	Men	11	HL	6.5 years	Azoospermia	COPP/ABV	63%	-

HL: Hodgkin's lymphoma; Non-Hodgkin's lymphoma: COPP (cyclophosphamide, vincristine, procarbazine, prednisone), BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone); [(1)]Non ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) group: R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone), ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone), BEACOPP, BEAM (carmustine, etoposide, aracytine, melphalan), MINE (methyl-glioxal, ifosfamide, vinorelbine, etoposide). [(2)]MOPP/ABV (mechlorethamine, vincristine, procarbazine, prednisone/doxorubicin, bleomycin, vinblastine), ABVPP (doxorubicin, bleomycin, vinblastine, procarbazine, prednisone), ACVBP, ECVBP (epirubicin, cyclophosphamide, vindesine, bleomycin, prednisone), CHOP, MOPP (mechlorethamine, vincristine, procarbazine, prednisone), BEAM, IVAM (ifosfamide, etoposide, aracytine, methotrexate). [(3)]LowChem: Low = ABO(V)D (doxorubicin, bleomycin, vincristine, dacarbazine), EBVD (epirubicin, bleomycin, vinblastine, dacarbazine) MedChem: Medium Chemotherapy = ChlVPP (chlorambucil, vinblastine, procarbazine, prednisone) ≤4 courses, MVPP (mechlorethamine, vinblastine, procarbazine, prednisone) ≤4 courses, CHOP, MIME (methyl-glioxal, ifosfamide, methotrexate, etoposide); HDChemo: High-dose Chemotherapy = ChlVPP > 4 courses, MVPP > 4 courses, ASCT support BEAC/BEAM. [(4)]Chemotherapy without alkylating agents: ABVD, EBVP (epirubicin, bleomycin, vinblastine, prednisone); chemotherapy with alkylating agents: MOPP,MOPP/ABV, BEACOPP.

(45%) who reported having attempted post-treatment parenthood, 76 (63%) had been successful without using ARTs. In addition, 10 of 13 males achieved post-treatment parenthood with the use of ARTs after 15 years of follow up.

The percentage of fatherhood may increase with progress in *in vitro* fertilization techniques. Indeed, with progress in ARTs, even very oligozoospermic patients may benefit from fertilization techniques such as ICSI (IntraCytoplasmic Sperm Injection).

Treatments of refractory lymphoma or relapses and second-line chemotherapy

The main protocols used are DHAP (dexamethasone, high-dose cytarabine, cisplatin),[25] ICE (ifosfamide, carboplatin, etoposide),[26] MINE (methyl-glioxal, ifosfamide, vinorelbine, etoposide),[27] GVD (gemcitabine, vinorelbine, liposomal doxorubicin).[28] Most teams use these, but the risk in terms of fertility has not been evaluated.

The risk of infertility has not been extensively studied after hematopoietic cell transplantation. Several studies reported that the regimens used for allogeneic stem cell conditioning cause the most severe damage, especially in the case of total body irradiation (TBI), with POF and infertility almost constantly occurring in adults and in up to 81% of cases in children.[29,30] Pregnancies have been reported after conditioning with BEAM (carmustine, etoposide, cytarabine, melphalan) and autologous transplantation. A recent report from the Late Effects Working Committee of the Center for International Blood and Marrow Transplant Research (CIBMTR) concluded that some patients who received TBI and/or myeloablative conditioning can preserve their fertility. The study described 83 pregnancies in female recipients of hematopoietic cell transplantation (HCT) and 95 pregnancies in female partners of male recipients of HCT.[31]

The risk from radiation depends on the irradiated volume and dose. After radiotherapy above the diaphragm (mantle-field), the dose to the gonads is approximately 0.1%. A change in ovarian function is reported after a dose of 1.5 Gy in patients over 40 years of age. After 2.5 to 5.0 Gy, 30-40% of women between 15 and 40 years of age become infertile and over 90% of patients over 40 years of age.[32] A decrease in the sperm count was observed after a dose of 0.15 Gy, and a risk of sterilization may occur after a dose of 1-2 Gy.

Sub-diaphragmatic radiation has been abandoned for initial treatment, and indications are rare and specific. Total body irradiation is used for selected patients undergoing autologous transplantation, and in some cases of dose-reduced conditioning followed by allogeneic stem cell transplants in relapse.

Assessment of fertility

The assessment of the ovarian reserve is used to estimate the risk of infertility in patients treated for cancer. This assessment is based on a series of arguments. After chemotherapy, recovery of normal menstrual cycles does not guarantee normal fertility, but amenorrhea is a strong negative predictor of fertility. Indeed, in several studies, patients with normal cycles had abnormal hormonal markers and abnormal transvaginal ovarian ultrasound examinations. The presence of transient amenorrhea after treatment was demonstrated to be a risk factor for subsequent infertility.

There are several hormone assays to establish endocrine profile. FSH is normally elevated in cases of impaired fertility. However, most studies agree that a normal FSH determination does not exclude ovarian dysfunction. The Anti-Müllerian hormone (AMH) produced by growing follicles declines with age and is undetectable after menopause. Its level parallels that of the number of primordial follicles and seems to be the most informative determination.[33] Some authors recommend the determination of inhibin B hormone which is secreted by the follicles recruited during the ovarian cycle. This hormone is involved in the negative regulation of FSH. As the level of inhibin B decreases with age, an increase in FSH levels through the loss of negative feedback is also observed.

Numerous studies have demonstrated the usefulness of transvaginal ultrasound, particularly at day 3 of the menstrual cycle. It provides two essential parameters: the measurement of the ovarian volume and the follicular count which is the number of follicles between 2 and 10 mm in diameter, which correspond to small antral follicles. These two elements correlate significantly with ovarian reserve and fertility. In patients with amenorrhea, the usefulness of this technique is not proven.[34] Consequently, there are no standard recommendations. The diagnosis of POF is, therefore, based on a body of clinical and laboratory evidence. Most authors combine the estimation of AMH and transvaginal ultrasound.

The essential test to assess male fertility is the semen analysis. It permits a study of the sperm count, shape, vitality and mobility. This is an excellent reflection of exocrine testicular function and of fertility. As a cycle of spermatogenesis takes three months, it is pointless to conduct the test too soon after treatment. It can be repeated and often results improve over time. Some hormone assays can also provide information on gonadal function. FSH, which is secreted by the pituitary gland, rises if testicular tissue sustains injury, due to loss of negative feedback. Inhibin B is secreted by Sertoli cells and levels are low in cases of spermatogenesis dysfunction. It has been suggested that it is a better marker than FSH.[35]

Different fertility preservation methods

With an improvement in the prognosis for female patients with Hodgkin's lymphoma, the management of secondary infertility treatment has become an important goal. Several techniques are under development and require close collaboration between oncology and ART teams. For the time being, it has been established that the chemotherapy in use is a known risk for future infertility. Several options are available depending on the patient's age, the presence or absence of a partner and the patient's wishes[36,37] (Table 2).

In ART centres, *in vitro* fertilization (IVF) for embryo cryopreservation is performed in many infertile patients and good results are obtained. After ovarian stimulation, mature oocytes are collected and fertilized *in vitro*. The resulting embryos are frozen. Human embryo freezing and thawing protocols are well established and used to obtain satisfactory embryo survival. At the appropriate time, the embryo(s) is/are implanted and pregnancy can be completed. The pregnancy rate per embryo transfer is approximately 18%. However, this technique is possible only in puberal women with a stable partner. Moreover, ovarian stimulation for 9-14 days, starting on the 2nd or 3rd day of menses, can take 4-6 weeks, depending on the time

of menses. The possibility of delaying the start of treatment should be discussed but this is dependent on the stage of the lymphoma and on the patient's wishes, especially in relapse cases or if intensification is proposed.

The technique of mature oocyte cryopreservation, the equivalent to the freezing of sperm, appears to be the most logical option for women of childbearing age. However, the results are still disappointing. After ovarian stimulation, which takes 4-6 weeks, the oocytes are frozen at the metaphase of a second meiotic division (MII). After thawing, they are mixed *in vitro* with the partner's sperm and the resulting embryos are then placed in the uterine cavity of the patient. The first pregnancy was reported in 1986. However, the oocytes are fragile and are often altered by the freezing-thawing cycle. Until recently, the pregnancy rate obtained was less than 3% *per* frozen oocyte. Two new techniques have increased this percentage:

- fertilization of oocytes by IntraCytoplasmic Sperm Injection (ICSI);
- the vitrification process (ultra-rapid freezing) which significantly reduces cell loss. A 6.8% pregnancy rate achieved with vitrified oocytes.[38]

Cryopreservation of ovarian tissue (CPOT) is a promising technique but has only started to be used recently.[39] The principle is to remove a whole ovary by laparoscopy. It is then prepared in the laboratory. The cortex is isolated in order to freeze ovarian cortex segments according to a carefully established protocol in the presence of cryoprotectants. Many follicles, mostly primordial, are frozen.

Oocyte maturation is, therefore, required for fertilization. There are three theoretical methods of maturation: *in vitro* maturation and xenografting, which are currently only research avenues, and autografting of frozen-thawed ovarian cortex segments which is the technique currently used. After thawing, the ovary is implanted either in an orthotopic position, i.e. in the pelvic cavity, or in a heterotopic position (in the forearm or in the abdominal wall). About 30 attempts have been reported, including 11 patients treated for Hodgkin's lymphoma.[40] According to these reports, all the women recovered ovarian endocrine function with a return of menstruation, an improvement of symptoms such as hot flashes, and an improvement in hormone assays. However, to date, only 12 full-term pregnancies resulting in 13 live births have been reported worldwide (including 4 in patients with Hodgkin's lymphoma), with the occasional need for ovarian stimulation and, in cases of heterotopic reimplantation, routine *in vitro* fertilization (IVF).[41-43] The results of this technique are, therefore, still to be confirmed and this should be clearly explained in advance to the patients involved. However, CPOT is the only feasible technique in children and prepuberal adolescents.[44]

The main risk of this procedure is the possibility of reintroducing cancer cells. In the case of Hodgkin's lymphoma, the risk is very low; ovarian invasion was found in less than 5% of an autopsy series. Only one team has reported a case of disease invading a specific ovary removed from a young woman. This was detected at the histological analysis. Such verification is essential before pathological freezing. A pelvic relapse has never been reported after ovarian implantation.[45]

The other major drawback of the CPOT is the amputation of 50% of the ovarian reserve. There is, therefore, a risk of accelerating the process of POF, although how big a risk is not clear.[46] It should, therefore, only be proposed in women undergoing treatment with a risk of infertility of nearly 100%, such as with transplant conditioning. After conventional chemotherapy regimens, even those containing alkylating agents, this risk changes according to patient age and dose. As mentioned above, young women are likely to recover normal ovarian function and to be able to complete spontaneous pregnancies.

Regarding analogs of LH-RH, the hypothalamus secretes GnRH or LHRH in a pulsatile manner. This hormone acts on specific receptors in pituitary gonadotroph cells that in turn secrete LH and FSH. Continuous administration of LHRH (or GnRH) causes desensitization of the hypothalamic-pituitary axis which, when prolonged, induces biochemical castration and a collapse of FSH and LH.

Analogs of LHRH are substrates of decapeptide GnRH, the natural molecule. They are 50- to 200-fold more potent than the natural peptide. Their administration, therefore, neutralizes the hypothalamic-pituitary axis. This chemical menopause inhibits the initiation of growth of primordial ovarian follicles, which would protect them from the cytotoxic action. This technique is highly contested. For the time being, two randomized trials have demonstrated an improvement in tests reflecting the ovarian reserve in the treated arm, but there is an equivalent rate of spontaneous pregnancies in both arms.[47,48] Two other studies (the most recent was conducted by the German group) that randomized oral contraceptives and GnRH in patients treated with escalated BEACOPP were unable to find evidence of any efficacy of GnRH which did not improve the hormone assays.[49,50] Analogs of LHRH are, therefore, not recommended in the absence of studies demonstrating their effectiveness. For women who experience premature menopause, hormone replacement ther-

Table 2. Advantages and drawbacks of different fertility preservation methods.

	Advantages	Drawbacks
In vitro fertilization for embryo cryopreservation	Accessible technique Good pregnancy rate	Ovarian stimulation Puberal women Partner
Mature oocyte cryopreservation	No need for maturation	Ovarian stimulation Puberal women Low pregnancy rate
Cryopreservation of ovarian tissue	No previous ovarian stimulation Numerous immature oocytes	Amputation of 50% of the ovarian reserve Recent technique Oocyte maturation Risk of reintroducing cancer cells after autografting

apy may reduce the risks of estrogen deficiency, such as osteoporosis.

In male adults and adolescents after puberty, two techniques are used for sperm collection. The self-preservation of ejaculated semen is the most current. The semen is collected by masturbation and analyzed according to WHO criteria. The ejaculate is then diluted with cryoprotectant, packed in straws, frozen in liquid nitrogen vapor and placed in tanks for long-term storage in liquid nitrogen. Usually 2-4 collections are necessary and an average of 24 straws are stored for each patient. In case of azoospermia, a testicular biopsy can be performed which allows sperm to be obtained in approximately half of the cases. In most patients, even if the quality of the sperm is low, the ejaculate can be used for ART. If the patient has secondary infertility due to cytotoxic agents, the technique will be chosen based on the number of flakes, the number of motile spermatozoids *per* straw, an eventual improvement in the sperm test after treatment and the results of the female partner's tests. The different techniques available are:

- intrauterine insemination;
- simple *in vitro* fertilization;
- *in vitro* fertilization with an intra-cytoplasmic sperm injection (ICSI): ICSI allowed ART to be proposed to very oligospermic patients.

One in every 2 patients in care for ART will have a child. The current recommendation is to provide self-preservation of ejaculated semen for patients treated with the BEACOPP regimen or before salvage treatment for relapse, and it remains optional for patients treated with ABVD, considering the low risk of infertility after the ABVD regimen. Most patients recover normal spermatogenesis after ABVD, often as long as 6-18 months later, and the *Centre d'Etudes et de Conservation des Oeufs et du Sperme* (CECOS – Egg and Sperm Conservation Centers) asks patients to decide whether or not to continue conservation first.

Several techniques are being tested in pre-pubescent boys. Cryopreservation of immature testicular tissue can lead to a transplant and in case of recovery of spermatogenesis, the collected gametes could be used in ART.[51] A technique that has already been tested in humans is the cryopreservation of germ stem cells which are then reimplanted in the testis. This is still experimental, but would avoid recourse to ART.[52] *In vitro* production by induction of gametes from embryonic stem cells is still being tested in animals.

Conclusion

The risk of infertility related to chemotherapy must be discussed with patients at the time of the diagnosis. In both sexes, one of the main risk factors for infertility is the type of chemotherapy used and in particular the dose of alkylating agents. In women, being under 30 years of age is a protective factor and thus the ovarian reserve also plays a major role.

In Figure 1, we propose a decision algorithm for managing fertility preservation that can be presented to patients. Among adults and adolescents after puberty, semen cryopreservation is proposed before first-line treatment with BEACOPP or salvage treatment for relapse, and it is optional in cases of localized Hodgkin's lymphoma treated with ABVD and radiotherapy. In women, fertility preservation is not systematically proposed before first-line treatment with ABVD; however, the risk of POF

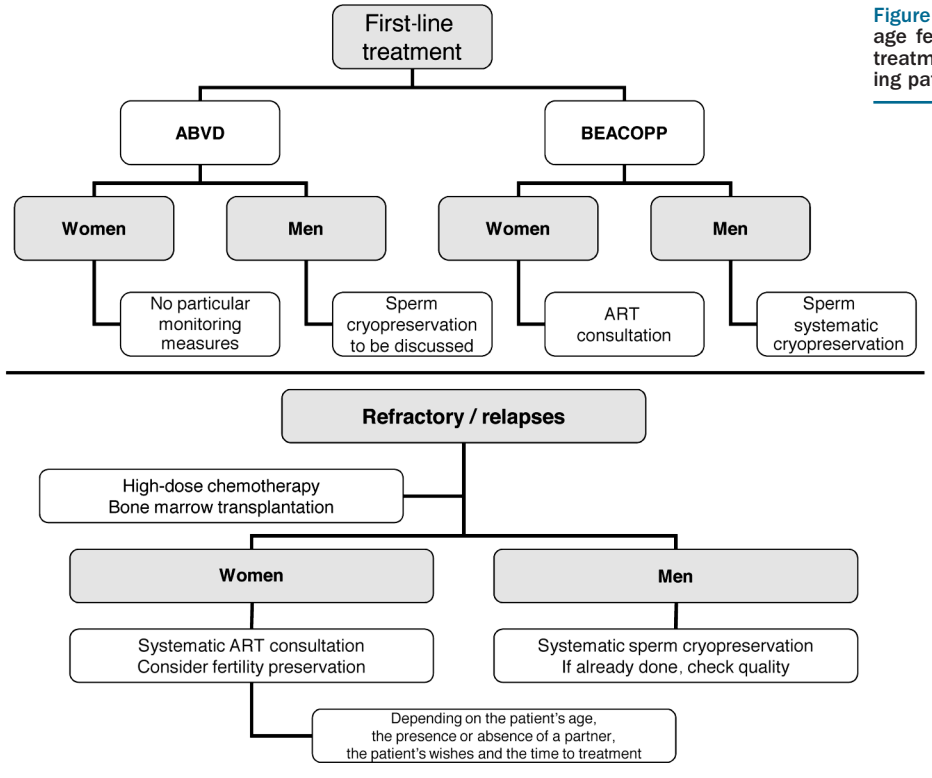

Figure 1. Decision-algorithm to manage fertility preservation in first-line treatment and for refractory/relapsing patients.

increases with age. Preservation methods can be offered to patients over 35 years of age, and decisions can be made according to the patient's wishes. ARTs are not routinely recommended for patients receiving ABVD in their early twenties to mid-thirties. For women receiving BEACOPP or the BEAM conditioning regimen and an autologous transplant, an ART consultation should be offered so that a personalized decision can be made, given that these treatments do not systematically cause sterility.

For women with a stable partner, *in vitro* fertilization for embryo cryopreservation is a standard procedure but it can only be offered to a small number of patients and requires delayed treatment initiation. Oocyte cryopreservation remains experimental, although it is increasingly being developed with new cryopreservation techniques that improve the results and which may soon make it a more attractive option. Ovarian tissue cryopreservation is still experimental and should only be proposed to women undergoing treatment with a high risk of infertility. The benefit-to-risk ratio must be discussed according to the patient's age and wishes, and also the possibility of delaying treatment initiation. Optimal management requires multidisciplinary collaboration between hematologists and Assisted Reproductive Techniques teams.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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References

1. Mancini J, Rey D, Préau M, Malavolti L, Moatti J. Infertility induced by cancer treatment: inappropriate or no information provided to majority of French survivors of cancer. Fertil Steril. 2008;90(5):1616-25.
2. van der Kaaij MA, van Echten-Arends J, Simons AH, Kluin-Nelemans HC. Fertility preservation after chemotherapy for Hodgkin lymphoma. Hematol Oncol. 2010; 28(4):168-79.
3. Johnston RJ, Wallace WHB. Normal ovarian function and assessment of ovarian reserve in the survivor of childhood cancer. Pediatr Blood Cancer. 2009;53(2):296-302.
4. Schover LR. Premature ovarian failure and its consequences: vasomotor symptoms, sexuality, and fertility. J Clin Oncol. 2008; 26(5):753-58.
5. Perez GI, Knudson CM, Leykin L, Korsmeyer SJ, Tilly JL. Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. Nat Med. 1997;3(11):1228-32.
6. Gonfloni S, Di Tella L, Caldarola S, Cannata SM, Klinger FG, Di Bartolomeo C, et al. Inhibition of the c-Abl-TAp63 pathway protects mouse oocytes from chemotherapy-induced death. Nat Med. 2009;15 (10):1179-85.
7. Meirow D, Biederman H, Anderson RA, Wallace WHB. Toxicity of chemotherapy and radiation on female reproduction. Clin Obstet Gynecol. 2010;53(4):727-39.
8. Faddy MJ, Gosden RG. A mathematical model of follicle dynamics in the human ovary. Hum Reprod. 1995;10(4):770-5.
9. Haukvik UKH, Dieset I, Bjøro T, Holte H, Fosså SD. Treatment-related premature ovarian failure as a long-term complication after Hodgkin's lymphoma. Ann Oncol. 2006;17(9):1428-33.
10. Rueffer U, Breuer K, Josting A, Lathan B, Sieber M, Manzke O, et al. Male gonadal dysfunction in patients with Hodgkin's disease prior to treatment. Ann Oncol. 2001; 12(9):1307-11.
11. van der Kaaij MAE, Heutte N, van Echten-Arends J, Raemaekers JMM, Carde P, Noordijk EM, et al. Sperm quality before treatment in patients with early stage Hodgkin's lymphoma enrolled in EORTC-GELA Lymphoma Group trials. Haematologica. 2009;94(12):1691-7.
12. Behringer K, Breuer K, Reineke T, May M, Nogova L, Klimm B, et al. Secondary amenorrhea after Hodgkin's lymphoma is influenced by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives during therapy: a report from the German Hodgkin's Lymphoma Study Group. J Clin Oncol. 2005;23(30): 7555-64.
13. Decanter C, Morschhauser F, Pigny P, Lefebvre C, Gallo C, Dewailly D. Anti-Müllerian hormone follow-up in young women treated by chemotherapy for lymphoma: preliminary results. Reprod Biomed Online. 2010;20(2):280-5.
14. Kiserud CE, Fosså A, Holte H, Fosså SD. Post-treatment parenthood in Hodgkin's lymphoma survivors. Br J Cancer. 2007;96 (9):1442-9.
15. Franchi-Rezgui P, Rousselot P, Espié M, Brière J, Marolleau JP, Gisselbrecht C, et al. Fertility in young women after chemotherapy with alkylating agents for Hodgkin and non-Hodgkin lymphomas. Hematol J. 2003;4(2):116-20.
16. Hodgson DC, Pintilie M, Gitterman L, Dewitt B, Buckley C, Ahmed S, et al. Fertility among female hodgkin lymphoma survivors attempting pregnancy following ABVD chemotherapy. Hematol Oncol. 2007;25(1):11-5.
17. Brusamolino E, Baio A, Orlandi E, Arcaini L, Passamonti F, Griva V, et al. Long-term events in adult patients with clinical stage IA-IIA nonbulky Hodgkin's lymphoma treated with four cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine and adjuvant radiotherapy: a single-institution 15-year follow-up. Clin Cancer Res. 2006;12(21):6487-93.
18. Kulkarni SS, Sastry PS, Saikia TK, Parikh PM, Gopal R, Advani SH. Gonadal function following ABVD therapy for Hodgkin's disease. Am J Clin Oncol. 1997;20:354-7.
19. van der Kaaij MA, Heutte N, Le Stang N, Raemaekers JM, Simons AH, Carde P, et al. Gonadal Function in Males After Chemotherapy for Early-Stage Hodgkin's Lymphoma Treated in Four Subsequent Trials by the European Organisation for Research and Treatment of Cancer: EORTC Lymphoma Group and the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 2007;25:2825-32.
20. Sieniawski M, Reineke T, Josting A, Nogova L, Behringer K, Halbsguth T, et al. Assessment of male fertility in patients with Hodgkin's lymphoma treated in the German Hodgkin Study Group (GHSG) clinical trials. Ann Oncol. 2008;19(10): 1795-801.
21. Hobbie WL, Ginsberg JP, Ogle SK, Carlson CA, Meadows AT. Fertility in males treated for Hodgkins disease with COPP/ABV hybrid. Pediatr Blood Cancer. 2005;44(2): 193-6.
22. Engert A, Diehl V, Franklin J, Lohri A, Dörken B, Ludwig W, et al. Escalated-dose BEACOPP in the treatment of patients with advanced-stage Hodgkin's lymphoma: 10 years of follow-up of the GHSG HD9 study. J Clin. Oncol. 2009;27(27):4548-54.
23. Aisner J, Wiernik PH, Pearl P. Pregnancy outcome in patients treated for Hodgkin's disease. J Clin Oncol. 1993;11(3):507-12.
24. Sieniawski M, Reineke T, Nogova L, Josting A, Pfistner B, Diehl V, et al. Fertility in male patients with advanced Hodgkin lymphoma treated with BEACOPP: a report of the German Hodgkin Study Group (GHSG). Blood. 2008;111(1):71-6.
25. Josting A, Rudolph C, Reiser M, Mapara M, Sieber M, Kirchner HH, et al. Time-intensified dexamethasone/cisplatin/cytarabine: an effective salvage therapy with low toxicity in patients with relapsed and refractory Hodgkin's disease. Ann Oncol. 2002; 13(10):1628-35.
26. Moskowitz CH, Bertino JR, Glassman JR, Hedrick EE, Hunte S, Coady-Lyons N, et al. Ifosfamide, carboplatin, and etoposide: a highly effective cytoreduction and peripheral-blood progenitor-cell mobilization regimen for transplant-eligible patients with non-Hodgkin's lymphoma. J Clin Oncol. 1999;17(12):3776-85.
27. Fermé C, Bastion Y, Lepage E, Berger F, Brice P, Morel P, et al. The MINE regimen as intensive salvage chemotherapy for relapsed and refractory Hodgkin's disease. Ann Oncol. 1995;6(6):543-9.
28. Bartlett NL, Niedzwiecki D, Johnson JL, Friedberg JW, Johnson KB, van Besien K, et al. Gemcitabine, vinorelbine, and pegylated liposomal doxorubicin (GVD), a salvage regimen in relapsed Hodgkin's lymphoma: CALGB 59804. Ann Oncol. 2007;18(6): 1071-9.
29. Hammond C, Abrams JR, Syrjala KL.

Fertility and risk factors for elevated infertility concern in 10-year hematopoietic cell transplant survivors and case-matched controls. J Clin Oncol. 2007;25(23):3511-7.
30. Nakayama K, Liu P, Detry M, Schover LR, Milbourne A, Neumann J, et al. Receiving information on fertility- and menopause-related treatment effects among women who undergo hematopoietic stem cell transplantation: changes in perceived importance over time. Biol Blood Marrow Transplant. 2009;15(11):1465-74.
31. Loren AW, Chow E, Jacobsohn DA, Gilleece M, Halter J, Joshi S, et al. Pregnancy after hematopoietic cell transplantation: a report from the late effects working committee of the Center for International Blood and Marrow Transplant Research (CIBMTR). Biol Blood Marrow Transplant. 2011;17(2): 157-66.
32. Ash P. The influence of radiation on fertility in man. Br J Radiol. 1980;53(628):271-8.
33. van Beek RD, van den Heuvel-Eibrink MM, Laven JSE, de Jong FH, Themmen APN, Hakvoort-Cammel FG, et al. Anti-Mullerian hormone is a sensitive serum marker for gonadal function in women treated for Hodgkin's lymphoma during childhood. J Clin Endocrinol Metab. 2007; 92(10):3869-74.
34. Broekmans FJ, Faddy MJ, Scheffer G, te Velde ER. Antral follicle counts are related to age at natural fertility loss and age at menopause. Menopause. 2004;11(6 Pt 1):607-14.
35. van Beek RD, Smit M, van den Heuvel-Eibrink MM, de Jong FH, Hakvoort-Cammel FG, van den Bos C, et al. Inhibin B is superior to FSH as a serum marker for spermatogenesis in men treated for Hodgkin's lymphoma with chemotherapy during childhood. Hum Reprod. 2007;22 (12):3215-22.
36. Lobo RA. Potential options for preservation of fertility in women. N Engl J Med. 2005; 353(1):64-73.
37. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917-31.
38. Nagy ZP, Chang C, Shapiro DB, Bernal DP, Kort HI, Vajta G. The efficacy and safety of human oocyte vitrification. Semin Reprod Med. 2009;27(6):450-5.
39. Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonckt A, Demylle D, Dolmans M. Ovarian tissue cryopreservation and transplantation: a review. Hum Reprod. Update. 2006;12(5):519-35.
40. von Wolff M, Donnez J, Hovatta O, Keros V, Maltaris T, Montag M, et al. Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy--a technique in its infancy but already successful in fertility preservation. Eur J Cancer. 2009;45(9):1547-53.
41. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364 (9443):1405-10.
42. Demeestere I, Simon P, Emiliani S, Delbaere A, Englert Y. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. Oncologist. 2007;12(12):1437-42.
43. Andersen CY, Rosendahl M, Byskov AG, Loft A, Ottosen C, Dueholm M, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Hum Reprod. 2008;23(10):2266-72.
44. Poirot CJ, Martelli H, Genestie C, Golmard J, Valteau-Couanet D, Helardot P, et al. Feasibility of ovarian tissue cryopreservation for prepubertal females with cancer. Pediatr Blood Cancer. 2007;49(1):74-8.
45. Bittinger SE, Nazaretian SP, Gook DA, Parmar C, Harrup RA, Stern CJ. Detection of Hodgkin lymphoma within ovarian tissue. Fertil Steril 2011;95(2):803.e3-6.
46. Rosendahl M, Andersen CY, Ernst E, Westergaard LG, Rasmussen PE, Loft A, et al. Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow-up study. Hum Reprod. 2008;23(11): 2475-83.
47. Falorio S, Angrilli F, Fioritoni G. Gonadotropin-releasing hormone analog treatment for the prevention of treatment-related ovarian failure and infertility in women of reproductive age with Hodgkin lymphoma. Leuk Lymphoma. 2008;49 (6):1087-93.
48. Blumenfeld Z, Avivi I, Eckman A, Epelbaum R, Rowe JM, Dann EJ. Gonadotropin-releasing hormone agonist decreases chemotherapy-induced gonadotoxicity and premature ovarian failure in young female patients with Hodgkin lymphoma. Fertil Steril. 2008;89(1):166-73.
49. Nitzschke M, Raddatz J, Bohlmann MK, Stute P, Strowitzki T, von Wolff M. GnRH analogs do not protect ovaries from chemotherapy-induced ultrastructural injury in Hodgkin's lymphoma patients. Arch Gynecol Obstet. 2010;282(1):83-8.
50. Behringer K, Wildt L, Mueller H, Mattle V, Ganitis P, van den Hoonaard B, et al. 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 Ann Oncol. 2010;21(10):2052-60.
51. Avarbock MR, Brinster CJ, Brinster RL. Reconstitution of spermatogenesis from frozen spermatogonial stem cells. Nat Med. 1996;2(6):693-6.
52. Brook PF, Radford JA, Shalet SM, Joyce AD, Gosden RG. Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. Fertil. Steril. 2001;75(2):269-74.