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The effects of radiotherapy and chemotherapy on female reproduction

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High dose chemotherapy and radiotherapy have radically increased long-term survival of young cancer patients, but major side effects of these treatments are ovarian failure and infertility. Knowledge of the risks and probabilities of ovarian failure caused by treatment is crucial for patients and physicians in order to make informed choices that will best serve patients' interests. This review presents data on ovarian damage and failure following exposure to radiotherapy, chemotherapy and ablative therapy. The risk is evaluated from the published literature according to patient's age, treatment protocol and also according to the diagnosis of some common malignancies. Many of these patients will not be sterilized immediately following treatment, but will suffer from premature menopause. In order to prevent sterilization, ovarian transposition before pelvic irradiation is mandatory. Besides cryopreservation of ovarian tissue and embryos before administration of chemotherapy, the possible protective effect of pituitary–ovarian down-regulation is discussed. The mechanism of primordial follicles damage induced by radio/chemotherapy is presented as well as the role of apoptosis signalling pathways underlying destruction. Increased knowledge of these mechanisms could help to identify potential effective inhibitors that can block the path of primordial follicles destruction and reduce ovarian failure rate.

Key words: bone marrow transplantation/chemotherapy/fertility/ovarian transposition/radiotherapy

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Introduction

The continued refinements in high dose chemotherapy and radiotherapy have significantly improved the cure rates of many young patients with certain haematological malignancies and solid tumours (Boring, 1994). Looking at survival data is very efficient for auditing the effect of cancer treatment over the last 25 years. The treatment of leukaemia in children is a story of success. Five-year survival in the period 1971–1975 was 33% for all leukaemias diagnosed in children between 1986–1990 is approaching 80%. Data from England and Wales has indicated that during 1986–1990 the 5-year survival rate for Hodgkin's disease was >90%, and for acute lymphocytic leukaemia and non-Hodgkin's lymphoma it was ~75%. The treatment of solid tumours, such as a Wilm's tumour and hepatoblastoma, have shown increased survival rates from 61 to 84% and 15 to 43% respectively. In sarcomas there have been equally spectacular improvements from 17 to 51% for osteosarcoma, 33 to 61% for Ewing's sarcoma and 41 to 66% for soft tissue sarcoma. As for adult cancers, the overall increase in relative survival, comparing those adults diagnosed in 1971–1975 with those diagnosed in 1986–1990, indicates that the increase in 5-year survival is clearly due to improved cancer treatment for conditions such as cancer of the testes, bone, bladder and colon and for Hodgkin's disease and leukaemia (McVie, 1999).

However, treatment is associated with significant morbidity, and the long-term physical and psychological effects of treatment have been subjected to wider attention. Of these, ovarian toxicity is an important and common long-term side effect of curative chemo/radiotherapy. Since many of these patients are young, with expectations of a normal reproductive life-span, premature menopause and sterilization can impact their quality of life and self-esteem dramatically.

Unlike other late effects of chemotherapy which are mostly informative for the patient and physician, there are certain options

to increase the potential reproductive abilities of survivors of radio/chemotherapy. These involve invasive and expensive procedures while the patient is in active disease and there is only a limited window of opportunity for appropriate action while the ovary is functional, a chance that may not exist later. These acts include collection and cryopreservation of embryos, oocytes or ovarian tissue (Nugent *et al*., 1997) and ovarian transposition prior to high dose radiotherapy to the pelvis (Williams *et al.*, 1999). Knowledge of the risks and factors affecting sterilization odds is important before administration of cancer treatment in order to give appropriate consultation as to whether and to what extent acts to preserve fertility are indicated. In the chapter on epidemiology, the risk of ovarian failure after chemotherapy and radiotherapy treatment for cancer is presented. The risk is assessed in relation to parameters such as patient's age, treatment protocol (drug regimens and radiation doses used) and type of malignancy.

The long-term results following chemotherapy and radiotherapy treatments show reduced follicle stores or ovarian atrophy. However, the acute effects and the direct mechanisms are only partially understood. Recent studies have stressed the role of apoptotic pathways underlying germ cell destruction. A good knowledge of the mechanisms involved in ovarian damage caused by radio/chemotherapy is important in order to introduce agents that block or reduce damage effectively.

Radiotherapy

Ionizing radiation has adverse effects on gonadal function at all ages, the degree and persistence of the damage depending on the dose, irradiation field and patient's age, with older women being at greater risk of damage. The ovaries are exposed to significant doses of irradiation when radiotherapy is used to treat pelvic and abdominal disease, such as cervical and rectal cancer, and with craniospinal radiotherapy for central nervous system malignancies. This is also the case when pelvic lymph nodes are irradiated for haematological malignancies such as Hodgkin's disease and with total body irradiation as part of the conditioning regimen prior to bone marrow transplantation. In many of these cases patients are young, i.e. before or at child-bearing age. Where possible, shielding of the gonads is used, or the radiation field is restricted to avoid direct irradiation to the ovaries, but in some cases there is no alternative. The estimated dose at which half of the follicles are lost in humans (LD_{50}) is 4 Gy (Wallace *et al.*, 1989). Lashbaugh and Casarett have indicated that women <40 years of age are less sensitive to radiation-induced ovarian damage, with an estimated dose of 20 Gy required to produce permanent ovarian failure compared with 6 Gy in older women (Lashbaugh and Casarett, 1976). Bath *et al.* studied the effect of total body irradiation (TBI; 14.4 Gy) on ovarian function in childhood and adolescence. Six out of eight women treated with TBI had ovarian failure. Biochemical evidence of incipient ovarian failure was seen in two girls treated pre-puberty (Bath *et al.*, 1999).

Thibaud *et al.* showed that TBI of $\leqslant$10 Gy given in a single dose before puberty causes a high ovarian failure rate (55–80%). However, fractionated TBI is less toxic to the ovaries even in higher doses. With fractionated TBI of $\geqslant$15 Gy, ovarian failure is present in all cases. After the age of 25 years TBI is more toxic to the ovaries (Thibaud *et al.*, 1998).

In order to study the long-term effects of treatment in young women who were not sterilized with cancer treatment, several studies examined the risk of infertility and early menopause after treatment of childhood cancer. The study of the American Cancer Institute, Bethesda, MA (Byrne *et al*., 1987) found a relative fertility of 0.78 in women who received radiation below the diaphragm. In another study, Chiarelli *et al.* evaluated female childhood cancer survivors who received abdominal pelvic irradiation and/or chemotherapy with alkylating agents and compared the risk of premature menopause and infertility with survivors who were treated by non-sterilizing surgery only. The results indicated that the risk of premature ovarian failure increased significantly with increasing dose of abdominal pelvic irradiation: with doses <20 Gy the relative risk was 1.02; with irradiation of 20–35 Gy the relative risk increased to 1.37; and with doses >35 Gy the relative risk of premature ovarian failure was 3.27. The proportion of females who suffered from treatment-related infertility correlated with patients' age at the time of treatment and was restricted only to women who were irradiated after puberty. Also, the percentage of women who suffered from infertility was correlated with increasing dose of abdominal pelvic irradiation—treatment doses of 20–35 Gy caused 22% infertility and doses >35 Gy caused 32% infertility (Chiarelli *et al*., 1999). A common example for early menopause in young women as a result of ovarian exposure to radiation is the use of inverted Y irradiation in the treatment of Hodgkin's disease with pelvic lymph node involvement. The direct effects of radiation dose on primordial follicle reserve in mouse ovaries was studied by Gosden and associates (Gosden *et al.*, 1997). Radiation doses of 0.1, 0.2 and 0.3 Gy reduced in a dose-related effect the total number of surviving primordial follicles. This explains sterilization with total depletion of primordial follicles reserve following exposure to high doses of radiotherapy and premature ovarian failure with lower doses that cause only partial depletion of primordial follicle reserve.

Ovarian Transposition (Oophoropexy)

Repositioning the ovaries out of the irradiation field can preserve ovarian function and should be considered in women of reproductive age with pelvic malignancies or before pelvic lymph node irradiation. Ovarian transposition can be done by laparotomy during the surgical treatment for the malignancy, as for cervical cancer, or by laparoscopic procedure just prior to pelvic irradiation. The procedure may reduce the damage caused by radiotherapy but does not protect against damage caused by systemic chemotherapy.

The indications, effectiveness and complications of ovarian transposition were evaluated in 107 patients treated for cervical cancer (Morice *et al.*, 2000). The authors evaluated the endocrine function of the ovaries post-transposition. Ovarian function was preserved in 11/11 patients treated exclusively by surgery, in 53/59 patients (90%) in the group treated by post-operative vaginal brachytherapy, and in 15/25 patients (60%) treated by post-operative external radiation therapy and vaginal brachytherapy.

In patients who need pelvic irradiation but are not planned for abdomino-pelvic surgery as with Hodgkin's disease, cauda equina

tumours or patients with other haematological malignancies, laparoscopic ovarian transposition should be considered. Although uncommon, a laparoscopic procedure just prior to pelvic radiation should also be considered in patients who have had staging laparotomy with oophoropexy several months before and whose ovaries have migrated back to their original positions. Had the repeat ovarian transposition not been performed the therapy would have resulted in ovarian failure (Williams *et al.*, 1999). These authors concluded that laparoscopy is an attractive alternative to laparotomy for ovarian transposition in young women who require pelvic radiotherapy and are not planned for abdomino-pelvic surgery. Others have stated that the laparoscopic procedure is highly efficient, requires only a short period of hospitalization, and associates with few post-operative complications (Classe *et al*., 1998).

The proper location to fix the transposed ovaries depends on the planned irradiation field. For cervical cancer the ovaries are transposed high and lateral above the pelvic brim, whilst for pelvic lymph node irradiation (as in Hodgkin's disease) the ovaries can be medially or laterally transposed. However, lateral transposition is preferred to medial transposition procedure, because ovaries laterally transposed are located outside the radiation field, while in those medially transposed only few are completely outside the radiation field (Hadar *et al*., 1994).

The surgical procedure of laparoscopic lateral ovarian transposition consists of releasing the right ovary from its pelvic attachments and placing the ovary as high and as laterally as possible in the right paracolic gutter, after creating a pedicle on the infundibulopelvic ligament (Figure 1) (Clough *et al.*, 1996; Tulandi and Al-Took, 1998). As the procedure for right ovarian transposition is easier due to interference by the sigmoid colon in the left side of the pelvis, the feasibility, morbidity and efficacy of unilateral laparoscopic ovarian transposition were evaluated (Clough *et al.*, 1996). The study has indicated that the mean dose of irradiation received by the transposed ovary was 1.75 Gy (range 0.4–3.7) and all patients <40 years old did not suffer from premature ovarian failure.

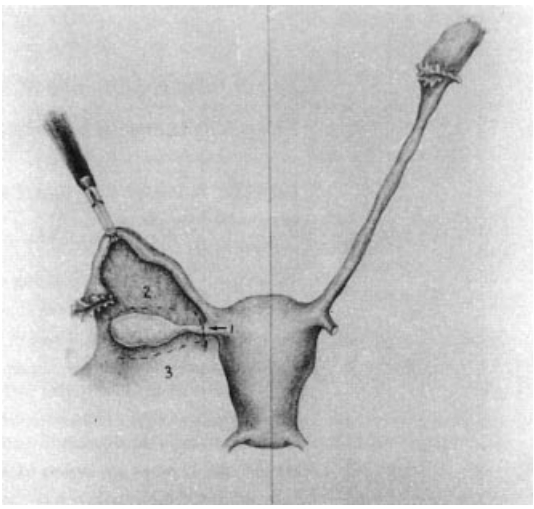

Figure 1. Laparoscopic lateral ovarian transposition. The ovarian ligaments (1—see arrow) and the mesovarium (2) are divided. If mobility is inadequate, a relaxing incision on the peritoneum inferior to the ovary (3) may be needed. (from Tulandi and Al-Took, reprinted by permission from the American Society from Reproductive Medicine; *Fertility and Sterility* 1998, **70**, 381–383).

Calculations to estimate the amount of radiation each ovary received following lateral transposition (mean distance 14.4 cm) were performed for the intracavitary radiation, as well as the dosage that would have been received had external pelvic (45 Gy) irradiation with or without para-aortic nodal irradiation (45 Gy) been required. The mean dose of radiation each transposed ovary received was estimated to be 1.26 Gy for intracavitary radiation, whereas for external pelvic +/– para-aortic nodal irradiation the dose was 1.35–1.90 and 2.30–3.10 Gy respectively (Covens *et al.*, 1996).

In conclusion, laparoscopic ovarian transposition is an important procedure in women of reproductive age before pelvic irradiation. The operation is highly effective, but the surgical procedure should not be under-estimated; its main complications are injury to the vasculature, Fallopian tube infarction in some patients and a high rate (~20%) of ovarian cyst formation (Gabriel *et al.*, 1986; Morice *et al.*, 2000). In some patients the irradiation may be sufficiently attenuated to avoid precipitating ovarian failure, but still enough to increase the risk of an early menopause. Following ovarian transposition, IVF technology will often be required to restore fertility.

Chemotherapy

Chemotherapeutic agents can be grouped into five classes of drugs based on their mode of action: alkylating agents, aneuploidy inducers, topoisomerase II inhibitors, antimetabolites and radiomimetics. Frequently chemotherapeutic agents are used in combination because their anti-tumour effects are commonly additive, but in many occasions their adverse effects are increased as well. Late complications associated with chemotherapy treatments, such as secondary malignancies and adverse effects on the female gonads, are assuming greater significance. Ovarian damage and failure is unfortunately a common long-term side effect of curative chemotherapy. The patient's age at treatment and chemotherapy regimen both influence the risk level facing patients. Knowledge of these parameters can help to predict, before treatment, the chances of a patient suffering from ovarian failure and whether procedures to preserve ovarian function, such as cryopreservation of ovarian tissue, are indicated.

The effect of age on ovarian function

Clinical studies have stressed the importance of age as a significant factor in determining the effects of chemotherapy on subsequent ovarian function. Older women had a much higher incidence of complete ovarian failure and permanent infertility as compared with younger women (Fisher and Cheung, 1984; Sanders *et al*., 1996; Moore, 2000). The effect of age has been studied in 44 adult females who have previously received chemotherapy MVPP (nitrogen mustard, vinblastine, procarbazine, prednisone) for Hodgkin's disease. The median age at treatment was 23 years. Seventeen women maintained regular menses (median age: 22 years), 10 developed oligomenorrhoea (median age: 23 years) and 17 older women stopped menstruating during chemotherapy (median age: 30 years) (Whitehead *et al.*, 1983). While young girls and adolescents can also be affected by

chemotherapy they are more resistant, most probably because of larger follicle stores prior to treatment (Sanders *et al.*, 1988).

The effect of treatment protocol on ovarian function

For each cancer few treatment protocols are accepted and when a patient is first diagnosed one of those protocols is administered. It is important for patients and clinicians to realise the risk of becoming sterile before administration of chemotherapy. In breast cancer patients, Lower *et al.* determined the prevalence and timing of menstrual abnormalities in 109 premenopausal early-stage breast cancer patients undergoing adjuvant methotrexate or anthracycline-based combination chemotherapy protocol. In both groups cyclophosphamide was administrated as part of the treatment protocol. Amenorrhoea occurred in about one-third of patients during chemotherapy (methotrexate group 31%, anthracycline group 33%), and a higher proportion were amenorrhoeic 1 year after chemotherapy was completed (methotrexate group 45%, anthracycline group 46%). Although abnormalities were more likely to occur in older premenopausal patients, 28% of patients <35 years old developed persistent abnormal menses (Lower *et al.*, 1999). Bines *et al*. reviewed reports on ovarian failure post adjuvant chemotherapy in premenopausal breast cancer survivors and reported that in regimens based on cyclophosphamide, methotrexate and fluorouracil (CMF) the average chemotherapy-related amenorrhoea rate was as high as 68% (Bines *et al.*, 1996). Several protocols are accepted for the treatment of Hodgkin's disease: Howell and Shalet reviewed the risk of ovarian damage in Hodgkin's disease females following aggressive treatment with combination cytotoxic chemotherapy and radiotherapy (Howell and Shalet, 1998). Treatment with MVPP, COPP (cyclophosphamide, vincristine, procarbazine, prednisone) and ChlVpp (chlorambucil, vinblastine, procarbazine and prednisolone) results in ovarian failure in 38–57% of patients. In two groups of high-grade non-Hodgkin's lymphoma and Hodgkin's disease patients <45 years of age (Bokemeyer *et al.*, 1994) gonadal dysfunction was documented in 1/10 women (10%) with non-Hodgkin's lymphoma, and 13/26 (50%) women with Hodgkin's disease suffered from premature ovarian failure. The frequency of gonadal dysfunction was markedly higher in patients treated for Hodgkin's lymphoma, probably due to infradiaphragmatic radiotherapy and regimens including procarbazine.

The use of combined modality therapy in early-stage Hodgkin's disease can significantly reduce long-term ovarian toxicity. Brusamolino *et al.* observed that after four courses of ABVD chemotherapy (doxorubicin, bleomycin, vinblastine and dacarbazine) followed by radiotherapy, fertility was preserved in young women. Transient amenorrhoea was reported by 12/33 patients <45 years old, and no cases of permanent amenorrhoea were observed in 17 patients <25 years (Brusamolino *et al.*, 2000). In 36 young patients (median age at diagnosis: 14 years) treated by five cycles of COP (cyclophosphamide, oncovin and procarbazine), alternated with four cycles of ABVD and low-dose (20 Gy) regional radiotherapy, the majority of female patients have had regular menstrual cycles post-treatment. Six patients developed ovarian failure (17%), and 10 have had a total of 17 pregnancies (Hudson *et al.*, 1993).

Gershenson *et al.* evaluated 40 patients treated for malignant ovarian germ cell tumours. The median age at diagnosis was 15 years and median age at the time of the study was 25.5 years. All patients had surgery and unilateral oophorectomy. Various regimens of chemotherapy were used, mostly VAC (vincristine, dactinomycine, cyclophosphamide). In this group 13% of patients had irregular menses, 15% oligomenorrhoea or amenorrhoea and 8% suffered from persistent amenorrhoea (Gershenson *et al.*, 1988).

Chemotherapy is a common treatment modality in some groups of patients with autoimmune diseases. Pulse cyclophosphamide therapy is frequently used for active lupus nephritis or neuropsychiatric lupus. The risk for secondary amenorrhoea after pulse cyclophosphamide therapy was evaluated in 39 premenopausal women with systemic lupus erythematosus. Out of 16 women who received short cyclophosphamide pulses (0.5–1.0 g/m^2 body surface area) monthly for a total of seven doses, two (12%) developed sustained amenorrhoea compared with nine out of 23 (39%) in the long cyclophosphamide pulses protocol (⩾15 doses). The increased risk for sustained amenorrhoea in the long cyclophosphamide pulses group was most evident in patients >25 years old (Boumpas *et al.*, 1993) In another study the incidence of premature ovarian failure after cyclophosphamide treatment for systemic lupus erythematosus was 26% (18/70 patients). The major determinants for the development of ovarian failure in patients with systemic lupus erythematosus are the age at the start of therapy and the cumulative cyclophosphamide dose (Mok *et al.*, 1998).

We have evaluated ovarian failure rate in young cancer patients following chemotherapy treatment, according to patient's age, treatment protocol and the type of disease, in order to assess the risk facing each group (Meirow, 1999). Patients were evaluated prior to chemotherapy administration: age, diagnosis of disease and treatment protocol (drugs and doses) were recorded. Only patients with normal menstrual cycles were enrolled into the study. Post-treatment gynaecological evaluation including gonadotrophin profile was performed. The study included 168 patients with cancer who were treated with conventional chemotherapy and completed pretreatment and post-treatment evaluation: 47 had acute myelocytic leukaemia (AML), 36 had non-Hodgkin's lymphoma, 47 had Hodgkin's disease and 38 women had breast cancer. Ovarian failure rate for the entire group was 34% and it was affected by age; the older the patients the higher were the chances of ovarian failure ($P = 0.0001$) (Table I). All patients in this group were treated by combination chemotherapy, the fraction contributed by each of the chemotherapeutic classes (alkylating agents, platinum derivatives, plant alkaloids, antimetabolites and antibiotics) was analysed by the odds ratio (OR) of exposed against unexposed patients. Alkylating agents were found to impose the highest risk in causing ovarian failure (OR = 3.98) and cisplatin caused ovarian failure with an OR of 1.77 (Figure 2). Recognizing the risks of becoming sterile is crucial for both patients and medical staff before different treatments are started. On many occasions treatment may be shifted from one protocol to another, but for each disease only a few characteristic protocols are commonly used. Therefore it is very practical to analyse the risk of ovarian failure according to disease type. Our study showed that the ovarian failure rate was

Table 1. Ovarian function, by age at chemotherapy, in 168 young cancer patients

Characteristic	Ovarian function		Total	*P* value
	Lost	Normal		
No. of patients	57	109	168	–
All patients				
Age in years (mean ± SD)	34.7 ± 8.0	27.4 ± 8.3	29.9 ± 8.9	< 0.0001
Range	14–44	11–43	11–44	–
Disease type				
Age in years (mean ± SD)				
AML	38.4 ± 3.6	26.1 ± 8.3	27.9 ± 8.9	< 0.001
NHL	34.8 ± 7.9	29.5 ± 8.3	31.8 ± 8.4	0.06
HD	26.2 ± 7.5	23.6 ± 7.5	24.4 ± 7.5	0.3
Breast CA	39.8 ± 2.6	34.3 ± 4.4	37.0 ± 4.5	< 0.001

AML = acute myelocytic leukaemia; NHL = non-Hodgkin's lymphoma; HD = Hodgkin's disease; CA = cancer.

15% for AML, 44% for non-Hodgkin's lymphoma, 32% for Hodgkin's disease and 50% for breast cancer (Figure 3).

Partial ovarian injury

At birth the human ovary contains approximately 2 million follicles, but due to a continual process of atresia this reserve is progressively eroded over time. With ageing the primordial follicles population falls below a key threshold number, menstrual cycle ceases and natural menopause occurs. Chemotherapy and radiotherapy reduce primordial follicle reserves, which can result in immediate ovarian failure. However, with many young patients cyclic menses may proceed with discontinuation of therapy, which does not necessarily imply that the ovaries escaped damage. Clinical studies use indirect measurements such as menstrual history, endocrine profiles and pregnancy rates to examine the effects of chemotherapy on the ovary, necessarily defining the outcome in dichotomous terms—whether or not ovarian failure occurred. Nor do these studies demonstrate the effects of increasing doses of chemotherapy within non-sterilizing range on primordial follicles population and ovarian reserve. Several studies have observed that a significant proportion of younger patients who continued regular menstruation after chemotherapy were at risk of undergoing premature menopause a number of years after treatment (Byrne *et al.*, 1992; Wallace *et al.*, 1993). The risk of premature menopause and infertility was evaluated in 719 female childhood cancer survivors; only females who were non-menopausal at the end of treatment were included in the analyses. Survivors who received both alkylating agents and abdominal–pelvic radiation were more likely to suffer from premature menopause than those who did not (RR = 2.58). The risk of premature menopause and infertility increased with increasing dose of abdominal–pelvic radiation and alkylating agent's dose (Chiarelli *et al.*, 1999).

In an animal study, primordial follicle loss following exposure to different doses of cyclophosphamide was directly measured and compared with reproductive outcome post-treatment (Meirow *et al.*, 1999). Young mice were treated with different doses of cyclophosphamide, and the total number of follicles remaining in

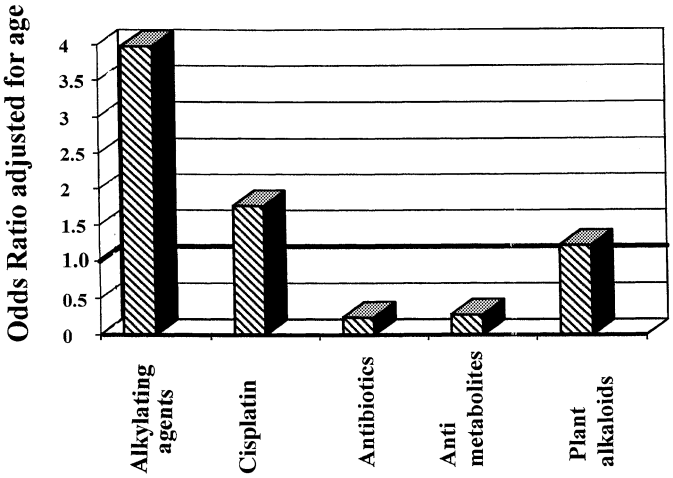

Figure 2. In 168 cancer patients treated by combination chemotherapy, the overall ovarian failure rate was 34% representing an odds ratio of 1. All medications were set in five drug categories (alkylating agents, platinum derivatives, plant alkaloids, antimetabolites and antibiotics) and analysis was performed on these groups. The fraction contributed by each of the chemotherapeutic classes was analysed by the odd ratio of exposed against not exposed patients (from Meirow *et al.*, 1997 with permission).

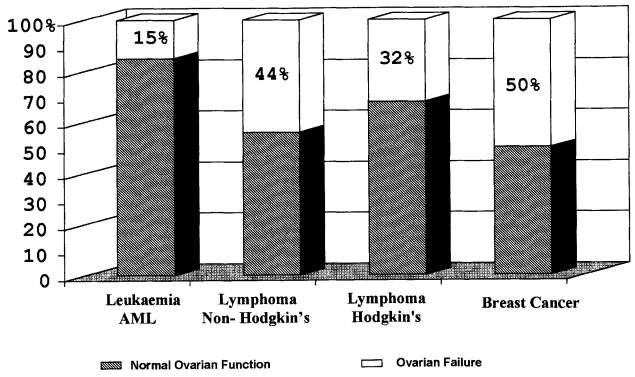

Figure 3. The rate of ovarian failure following chemotherapy in 168 young cancer patients (mean age: 29 years) with proved normal ovarian function prior to treatment in patients with acute myeloid leukaemia (AML), Hodgkin's disease, non Hodgkin's disease (NHD), and breast cancer. A disease oriented study (from Meirow, 1999 with permission from *Leukemia and Lymphoma* **33**, 65–76).

both ovaries was counted. Results have indicated that the effect of chemotherapy on the ovary is not an 'all or none' phenomenon and that cyclophosphamide causes follicular destruction in exponential proportion to increasing doses (P = 0.0001). The study showed that exposure to doses of chemotherapy strong enough to destroy half of the ovarian primordial reserve, did not affect reproductive performance (ovulation, mating and pregnancy rates) post-treatment (Figure 4). These results strongly indicate that clinical information such as regular menses and normal reproductive outcome after chemotherapy are not reassuring parameters from which it is possible to conclude that the ovaries survived treatment unaffected. Reduced follicular reserve post-treatment also explains the tendency of women subjected to chemotherapy to undergo premature menopause.

With these clinical data and animal studies we strongly need to support the recommendation that patients who regain ovarian function post-high dose chemotherapy or radiotherapy treatments

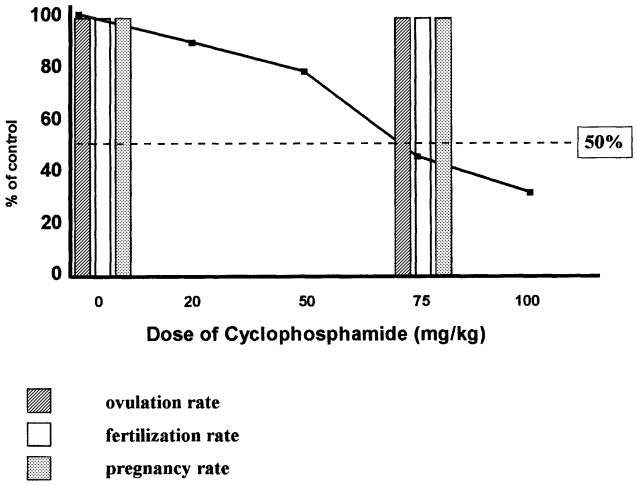

Figure 4. Reproductive performance in mice as indicated by ovulation, fertilization and pregnancy rates following exposure to 75 mg/kg cyclophosphomide as a % of control values. Line graph indicates the total number of primordial follicles counted in both ovaries of mice exposed to cyclophosphamide at doses of 0/20/50/75/100 mg/kg. Reproductive performance is not altered and does not disclose the ovarian injury even when >50% of follicles are destroyed—see text (from Meirow *et al.*, 1999 with permission).

should not delay child bearing for too many years. These patients should try to conceive, if possible, after few years of disease free interval, but not <6–12 months after treatment due to possible toxicity of treatment on growing oocytes (Meirow *et al.*, 2001).

Bone marrow transplantation

Bone marrow transplantation (BMT) has been widely used in the last three decades. In 1997 >20 000 patients were treated on a regular basis in dozens of centres worldwide. One of the commonest indications for such treatments is onco-haematological malignancy, for which a clear dose–response relationship with chemotherapy exists. In this population BMT is usually performed on children or young women mainly during the reproductive years. The conditioning regiments used for BMT include high dose chemotherapy, with or without body irradiation. There is a very high risk of ovarian failure among children and adult patients cured by BMT. In a survey of 38 000 male and female patients, or their partners, who had received high dose chemotherapy or total body irradiation with allogeneic/autologous stem cell transplantation, the fecundity rate was extremely low with only 129 pregnancies reported (Apperley and Reddy, 1995).

We have studied a group of 63 female cancer patients (mean age: 29.6 years) who were treated with ablative radio/chemotherapy followed by BMT. All had previous chemotherapy treatments several months to years before BMT, but all had cyclic menstrual activity with gonadotrophin levels (FSH and LH) within normal limits. Continuous monitoring of these patients (menses cycles and gonadotrophin concentrations) for >5 years following BMT have clearly demonstrated persistent ovarian failure in all but five patients. There was no correlation with age, cytotoxic regimen given or suppression of endogenous hormonal secretion during BMT including the administration of GnRH agonists, oral contraceptives or progestins (Meirow, 1999).

Teinturier *et al.* evaluated 21 girls who had received BMT without total body irradiation for malignant tumours at ages 2–17 years (Teinturier *et al.*, 1998). Ten girls in this group who were treated with the alkylating agent busulfan (140mg/m^2) with or without cyclophosphamide all had persistent ovarian failure, even in those treated prepubertally. Of the 11 girls who were conditioned without alkylating agents, two had ovarian failure and three had gonadotrophin concentrations above or at the upper normal limit. In a group of 31 girls aged 3.2–17.5 who had BMT with or without irradiation, those who had menstruated before treatment suffered from permanent amenorrhoea. Six years after BMT, 80% of this group had permanent ovarian failure (Thibaud *et al.*, 1998).

A common non-total body irradiation conditioning protocol for BMT is the busulfan- cyclophosphamide (Bu-Cy) regimen. In adult females (mean age: 38 years) who were treated with 'big Bu-Cy' (200 mg/kg Cy), only one out of 73 patients recovered ovarian function (Sanders *et al.*, 1996). Also with decreasing Cy dose ['little Bu-Cy', 120 mg/kg)] ovarian failure was the result in all 19 adult female patients aged 16–40 years (mean: 30) who were tested ⩾2 years following BMT (Grigg *et al.*, 2000). With the use of a non-ablative approach for conditioning before BMT (high dose melfalan) a study has shown that this treatment was as effective as more aggressive regimens in Hodgkin's disease and non-Hodgkin's lymphoma, but had the substantial advantage of preserving fertility in female patients. Of 30 women transplanted, 10 females had successful pregnancies after BMT (Jackson *et al.*, 1997)

These studies indicate that there is extremely high risk of persistent ovarian failure in female patients who undergo BMT. In children, growth and sexual development are impaired and sterility is common in adults. In this group of patients (children and adults) we should make every effort prior to BMT to actively preserve future fertility by means such as ovarian tissue cryopreservation.

Suppression of the ovaries to minimize gonadotoxicity

Since dividing cells are more sensitive to the cytotoxic effects of chemotherapy than quiescent cells, it has been hypothesized that inhibition of the pituitary–gonadal axis by sex steroid hormones or GnRH agonists would render the germinal epithelium less susceptible. Clinical studies have clearly indicated that chemotherapy has much more profound effects on the ovaries of adult women compared with prepubertal girls. It is possible, therefore, that an artificial prepubertal state through pituitary–ovarian desensitization and down-regulation may provide ovarian protection. However, a possible explanation for the decreased ovarian damage in prepubertal girls is that young patients have much larger follicle numbers and may therefore sustain less damage for any given dose.

Montz and co-workers found that progesterone or GnRH agonists maintained fertility and fecundity rates similar to those of untreated control animals (Montz *et al.*, 1991). In another study, GnRH agonist administration given before and throughout cyclophosphamide treatment significantly increased the pregnancy/mating rate, the number of implantations/mated animal and reduced the need for remating (Ataya and Ramahi-Ataya, 1993). Subsequently, Ataya and colleagues demonstrated in Rhesus monkeys that 65% of the primordial follicle population were lost following cyclophosphamide treatment and that this was sig-

nificantly lowered to 29% in GnRH agonist-treated monkeys (Ataya *et al.*, 1995a). However, unlike the protective effect showed for chemotherapy, following radiation the follicle counts were not preserved in GnRH agonist-treated monkeys (Ataya *et al.*, 1995b).

A clinical prospective evaluation of GnRH agonist administration during combination chemotherapy for malignant and non-malignant conditions (62 patients and 50 controls aged 15–40 years) have shown a protective effect of GnRH agonist. While only one patient of the GnRH agonist and chemotherapy group experienced ovarian failure, <50% of the chemotherapy only group resumed regular cyclic activity (Blumenfeld, 2001). The effects of GnRH agonist in patients undergoing cytotoxic treatment for systemic lupus erythematosus was recently assessed. In eight women receiving GnRH agonist in parallel to alkylating agent chemotherapy (cyclophosphamide or chlorambucil) none suffered premature ovarian failure, while five of nine patients treated with alkylating agents alone experienced premature ovarian failure (Blumenfeld *et al.*, 2000). However, as previously presented with high dose ablative chemotherapy in conjunction with BMT, ovarian failure is almost invariably induced, irrespective of the suppression of endogenous hormone secretion given during the therapy (Meirow, 1999).

Thus, some studies suggest a protective effect with the addition of a GnRH agonist to chemotherapy regimens while other studies do not support such an effect. Also, the mechanism of such a protective effect remains unknown. The primordial follicle population constitutes the vast majority of the follicles in the human ovary, but these are not under the influence of gonadotrophins. More basic studies are needed to understand the mechanism of primordial follicles destruction and how inhibiting the pituitary–gonadal axis may protect the ovaries from damage. Also, more clinical studies are needed in order to elucidate the indications and effectiveness of such treatment.

Radio/chemotherapy-mediated female germ cell destruction

Ovarian follicles are remarkably vulnerable to agents that cause DNA damage such as ionizing radiation and chemotherapeutic agents. A number of studies have examined the ovarian histology following chemotherapy and radiotherapy treatments. The commonest observation is ovarian atrophy and a reduced follicle store (Himelstein-Braw *et al.*, 1978; Chapman *et al.*, 1979; Marcello *et al.*, 1990; Familiari *et al.*, 1993). These studies of human ovarian biopsies did not provide information about the acute mechanism of injury. In recent years, the mechanism of radiotherapy and chemotherapy-induced damage is extensively investigated.

In order to visualize and study the direct mechanism of chemotherapy-induced ovarian damage and primordial follicles injury, healthy human cortical ovarian slices were exposed *in vitro* to therapeutic doses of cisplatin (Meirow *et al.*, 1998). Histology and immunohistochemical staining showed that chemotherapy induced pregranulosa cell swelling, marked pregranulosa cell nuclear swelling and primordial follicles architecture disruption with disappearance of the lumen and its oocyte. Positive apoptotic staining was obtained in the pregranulosa cells exposed to chemotherapy but not in controls (Figure 5). Therefore, in the human ovary cisplatin acts primarily on the pregranulosa cells inducing apoptotic changes, and the cells exhibit marked swelling. Familiari *et al.* examined ovarian follicles by electron microscopy following exposure to chemotherapy (Familiari *et al.*, 1993). Results have shown that follicular cells were enlarged containing cytoplasmic elements and the nuclei within the pregranulosa cells were also enlarged. Also the primordial follicles population was often surrounded by an abnormally thick basal lamina.

Recent studies have initiated mapping the apoptosis signalling pathway underlying germ cell destruction by chemotherapy, and identifying key genes and proteins as potential inhibitors to block the path of primordial follicles destruction. A study by Perez *et al.* has shown that in mice the chemotherapeutic agent doxorubicin induces apoptosis in the primordial follicles and the first steps of apoptosis occur in the pregranulosa cells. The protein p53 was not required for drug-induced oocyte destruction; however, members of the caspase gene family were required for oocyte death (Perez *et al.*, 1997). The pathways of $K^{(+)}$ regulation showed that potassium efflux during ovarian cell death appears early in oocytes and granulosa cells and may regulate a number of

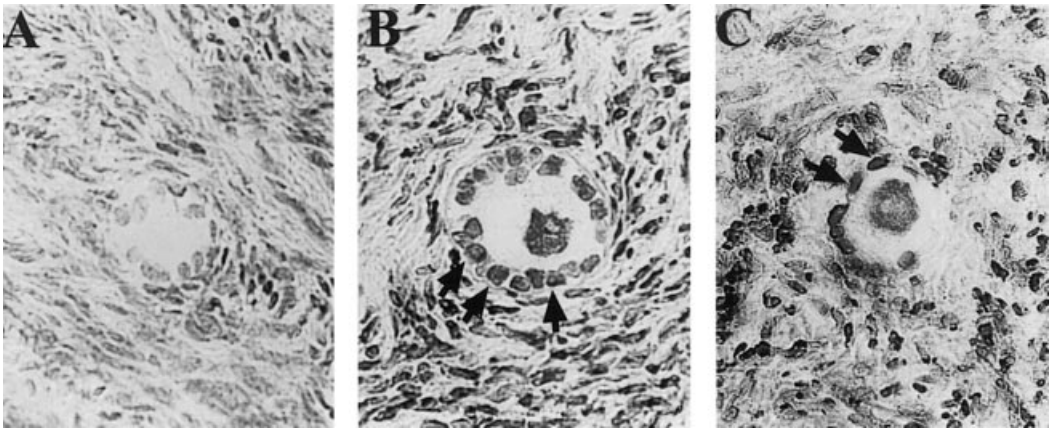

Figure 5. In-situ immunohistochemical staining for apoptosis of human ovarian cortex examined under light microscopy showing (**A**) Normal primordial follicles lacking apoptosis staining after culture without cisplatin (control tissue) for 36 hours and (**B** and **C**) primordial follicle with positive staining for apoptosis in pregranulosa cells and the oocyte. Magnification ×100 (from Meirow et al., 1998 with permission).

apoptotic events including caspase activity and internucleosomal DNA cleavage (Perez *et al.*, 2000).

In the hope of finding treatments to preserve fertility of women undergoing cancer therapy, chemical or genetic manipulations are currently being investigated to reduce the drug's capacity to impose damage. Morita *et al*. have stressed the sphingomyelin pathway in regulation of oocyte death. Disruption of the gene for acid sphingomyelinase in female mice suppressed the normal apoptotic deletion of fetal oocytes, and oocytes treated *ex vivo* with sphingosine-1-phosphate resisted the apoptosis induced by anti-cancer therapy. Moreover, radiation-induced oocyte loss was completely prevented by in-vivo therapy with sphingosine-1-phosphate. Thus, the sphingomyelin pathway regulates developmental death of oocytes, and sphingosine-1-phosphate provides a new approach to preserve ovarian function *in vivo (*Morita *et al.*, 2000). More research is needed in this area; it seems crucial to have good knowledge of the pathway involved in oocyte apoptosis in order to introduce agents that arrest apoptosis effectively. An in-vitro model may serve to investigate and understand the sterilizing effects of toxins on the human ovary and to investigate the protective effects of specific agents.

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