Spermatogenesis After Cancer Treatment: Damage and Recovery

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Treatment with cytotoxic chemotherapy and radiotherapy is associated with significant gonadal damage in men, and alkylating agents are the most common agents implicated. The vast majority of men receiving procarbazine-containing regimens for the treatment of lymphomas are rendered permanently infertile, whereas treatment with doxorubicin hydrochloride (Adriamycin), bleomycin, vinblastine, and dacarbazine appears to have a significant advantage, with a return to normal fertility in the vast majority of patients. Cisplatin-based chemotherapy for testicular cancer results in temporary azoospermia in most men, with a recovery of spermatogenesis in about 50% of the patients after 2 years and 80% after 5 years. The germinal epithelium is very sensitive to radiation-induced damage, with changes to spermatogonia following as little as 0.2 Gy. Testicular doses of less than 0.2 Gy had no significant effect on FSH levels or sperm counts, whereas doses between 0.2 and 0.7 Gy caused a transient dose–dependent increase in FSH and reduction in sperm concentration, with a return to normal values within 12–24 months. No radiation dose threshold has been defined above which permanent azoospermia is inevitable; however, doses of 1.2 Gy and above are likely to be associated with a reduced risk of recovery of spermatogenesis; the time to recovery, if it is to occur, is also likely to be dose dependent. [J Natl Cancer Monogr Inst 2005;34:12–7]

Impairment of spermatogenesis has been demonstrated before treatment in patients with various malignancies. In addition, germinal epithelial damage resulting in oligo- or azoospermia is a recognized consequence of certain chemotherapeutic agents and

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radiotherapy. Testicular damage is drug specific and dose related *(1–4)*. The chance of recovery of spermatogenesis following cytotoxic insult, and also the extent and speed of recovery, are related to the agent used and the dose received. It has also been suggested that the germinal epithelium of the adult testis is more susceptible to damage than that of the prepubertal testis *(5)*, implying that patient age or maturation of the testis at the time of cytotoxic insult may influence the degree of damage. Radiotherapy-induced testicular damage is similarly dose dependent, with speed of onset, chance of reversal, and time to recovery of spermatogenesis all related to the testicular dose of irradiation *(6)*.

Chemotherapy

Many drugs, particularly alkylating agents, have been shown to be gonadotoxic; the agents most commonly implicated are listed in Table 1. Most research has focused either on cyclophosphamide given alone for immunologically mediated disease or on combination chemotherapy used in the treatment of hematological malignancies and testicular cancer (results summarized in Table 2). Although the ultimate assessment of germinal cell function is the achievement of fatherhood, there are a number of confounding factors that make the use of this end point more difficult to interpret. Survivors of cancer may be less likely to find a partner or may be less inclined to want children because of the psychological effects of the disease or treatment and because of the perception of an increased risk of congenital malformations in the offspring of parents who have received cytotoxic treatment. Female factors that may determine fertility for the couple are also difficult to determine. The majority of studies have therefore focussed on semen analysis and biochemical markers of fertility rather than on number of births.

Cyclophosphamide

Rivkees and Crawford *(6)* published an analysis of 30 studies that examined gonadal function after various chemotherapy regimens, which included a total of 116 males who had been treated with cyclophosphamide alone. Gonadal function or histology were assessed by a number of different methods, with semen analysis, basal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, and testicular biopsy being the most commonly used. Of the 116 patients, 52 (45%) had evidence of testicular dysfunction following treatment. The incidence of gonadal dysfunction correlated with the total dose of cyclophosphamide, occurring in over 80% of postpubertal patients who received more than 300 mg/kg.

Table 1. Gonadotoxic drugs

Group	Definite gonadotoxicity
Alkylating agents	Cyclophosphamide *(5)*
	Chlorambucil
	Mustine
	Melphalan
	Busulfan *(20)*
	Carmustine *(41)*
	Lomustine *(41)*
Antimetabolites	Cytarabine
Vinca alkaloids	Vinblastine *(42)*
Others	Procarbazine
	Cisplatin *(43)*

Table 2. Summary of fertility in adult men following treatment of different malignancies

Diagnosis and treatment	Fertility posttreatment
Hodgkin disease	
MVPP	Azoospermia in >90%
MOPP	Azoospermia in >90%
ChlVPP/EVA hybrid	Azoospermia in >90%
COPP	Azoospermia in >90%
ABVD	Temporary azoospermia with normal sperm count in all at 18 months
Non-Hodgkin lymphoma	
CHOP	Permanent azoospermia in ~30%
VAPEC-B	Normospermia in >95%
VACOP-B	Normospermia in >95%
MACOP-B	Normospermia in >95%
VEEP	Normospermia in >95%
Bone marrow transplant for a variety of malignancies	
Cyclophosphamide alone	FSH raised in 40%
Busulphan and Cyclophosphamide	FSH raised in 80%
CBV	FSH raised in >95%
High-dose melphalan	FSH raised in >95%
BEAM	FSH raised in >95%
Testicular cancer	
Cisplatin/carboplatin based therapy	Normospermia in 50% at 2 years and 80% at 5 years

MVPP = mustine, vinblastine, procarbazine and prednisolone; MOPP = mustine, vincristine, procarbazine and prednisolone; ChlVPP/EVA = chlorambucil, vinblastine, prednisolone, procarbazine, doxorubicin, vincristine, and etoposide; COPP = cyclophosphamide, vincristine, procarbazine, and prednisolone; ABVD = doxorubicin hydrochloride, bleomycin, vinblastine, and dacarbazine; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisolone; VAPEC-B = vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin; VACOP-B = vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide, and bleomycin; MACOP-B = mustine in place of vinblastine; VEEP = vincristine, etoposide, epirubicin, and prednisolone; CBV = cyclophosphamide, carmustine, and etoposide; BEAM = carmustine, etoposide, Ara-C, and melphalan; FSH = follicle-stimulating hormone.

Treatment of Hematological Malignancy

The effect on testicular function of chemotherapy used in the treatment of lymphomas, especially Hodgkin disease, has been widely reported. Several studies have reported azoospermia with raised FSH levels in over 90% of men following cyclical chemotherapy with MVPP (mustine, vinblastine, procarbazine, and prednisolone) *(7,8)*.

In an attempt to reduce the gonadotoxic effect of MVPP by halving the alkylating drug and reducing the procarbazine dose, a hybrid combination of chlorambucil, vinblastine, prednisolone, procarbazine, doxorubicin, vincristine, and etoposide (ChlVPP/EVA) has been used. However, in a direct comparison with MVPP, hybrid chemotherapy was found to have the same effect on gonadal function *(9)*. This may in part relate to the replacement of mustine with chlorambucil in the hybrid regime. An alternative hybrid regime, however, consisting of ABVD (doxorubicin hydrochloride [Adriamycin], bleomycin, vinblastine, and dacarbazine) alternating with MOPP (similar to MVPP but with vinblastine replaced by vincristine) has been shown to be less gonadotoxic, with recovery of spermatogenesis in 17 of 42

patients (40%) a median of 27 months following treatment *(10)*. Furthermore, direct comparison of ABVD alone with MOPP showed further reductions in gonadal toxicity.

Viviani et al. *(11)* studied a total of 53 men treated with combination chemotherapy for Hodgkin disease. Of 29 men treated with MOPP (similar to MVPP but with vinblastine replaced by vincristine), 28 were azoospermic a median time of 6 months after the completion of therapy. Of these men, 21 were retested 18–58 months after the initial analysis, and in only three of these patients was any recovery of spermatogenesis seen. The effect of ABVD was, however, considerably less, with a normal sperm count in 11 of 24 patients and oligospermia in a further five. Furthermore, full recovery of spermatogenesis occurred within 18 months of the first evaluation in all 13 men in whom the sperm count was repeated.

Other chemotherapy regimens used for the treatment of lymphomas have also been investigated. The effect on adult testicular function has been assessed in patients treated during childhood with ChlVPP for Hodgkin disease. Testicular dysfunction, as indicated by raised gonadotrophin levels, was found in a significant proportion of a cohort of 46 male patients treated with ChlVPP reported by Mackie et al. *(12)*, with 89% and 24% having raised FSH and LH levels, respectively. The use of COPP (cyclophosphamide, vincristine, procarbazine, and prednisolone), which includes the gonadotoxic agent cyclophosphamide in addition to procarbazine, is associated with even more marked gonadal dysfunction. Charak et al. *(13)* found azoospermia in each of 92 patients following treatment with six or more cycles of COPP, along with significant rises in gonadotrophin levels compared with pretreatment values. Median follow-up in this study was 6 years, with 17% of patients treated more than 10 years previously, indicating that germinal epithelial failure is likely to be permanent.

There are few data concerning parenthood rates following treatment for Hodgkin disease. Swerdlow et al. *(14)* published data on a large cohort of patients who had received chemotherapy, radiotherapy, or both for Hodgkin disease over a 21-year period. Of 101 men who responded to a questionnaire, only 18 had fathered a child, and 12 of these men had been treated with radiotherapy only. Improved fatherhood rates were reported by Aisner et al. *(15)*, with 25 of 51 (49%) of men desirous of conception able to produce offspring following chemotherapy or radiotherapy for Hodgkin disease. Surprisingly, there was no influence of the modality of treatment, with similar fatherhood rates in those who had and those who had not received chemotherapy.

Chemotherapy regimens used for the treatment of non-Hodgkin lymphoma (NHL) are generally less gonadotoxic than those used for Hodgkin disease. Pryzant et al. *(1)* reported on 71 patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-based chemotherapy. All men were rendered azoospermic during treatment, but by 5 years posttreatment 67% had recovered to normospermic levels, with a further 5% being oligospermic. The reduced incidence of permanent infertility in men treated for NHL compared with that of Hodgkin disease patients is probably related to the absence of procarbazine in the standard regimens used for NHL *(16)*, although the reduction in the dose of alkylating agents may also be important. The absence of procarbazine and alkylating drugs is also the likely explanation for the reduced toxicity of ABVD reported by Viviani et al. *(11)*.

Other regimens not containing procarbazine, which have been used for NHL, have also been shown to be less gonadotoxic. VAPEC-B (vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin) *(17)*, VACOP-B (vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide, and bleomycin) *(18)*, MACOP-B (mustine in place of vinblastine) *(18)*, and VEEP (vincristine, etoposide, epirubicin, and prednisolone) *(19)* have all been associated with normal posttreatment fertility in the vast majority of men.

Testicular function following high-dose chemotherapy used as preparation for bone marrow transplantation has also been studied. Sanders et al. *(20)* reported on a total of 155 men treated with cyclophosphamide (200 mg/kg) or busulphan and cyclophosphamide (busulphan 16 mg/kg, cyclophosphamide 200 mg/kg). After an average of 2–3 years posttransplant, 67 of 109 (62%) patients who received cyclophosphamide, but only 8 of 46 (17%) patients treated with busulphan and cyclophosphamide, had recovery of testicular function defined by normal LH, FSH, and testosterone levels with evidence of sperm production. Fatherhood was reported in 28 of the 109 men receiving cyclophosphamide (26%), but in only two men (plus an additional one who used cryopreserved sperm) following treatment with busulphan.

The only prospective study to examine testicular function following high-dose treatment reported data in 13 men who received either BEAM (carmustine, etoposide, Ara-C, and melphalan; n = 11) or melphalan and single-fraction total-body irradiation (TBI; n = 2) *(21)*. All of these patients had previously received multiagent chemotherapy, and four had abnormal semen parameters before transplantation. All patients were azoospermic 2–3 months posttransplantation, which was associated with raised FSH levels.

These findings were also confirmed by Howell et al. *(22)*, who studied 68 patients treated with high-dose chemotherapy (cyclophosphamide, carmustine, and etoposide; busulphan and cyclophosphamide; or carmustine, etoposide, doxorubicin, and melphalan) as conditioning for bone marrow transplant. The authors demonstrated a raised FSH in 60 patients (88%).

Testicular Cancer

The other group of patients in whom the effects of chemotherapy on testicular function have been widely investigated is that of men with testicular cancer *(23–26)*. To attempt to delineate which abnormalities are a result of cytotoxic chemotherapy, several of these studies also examined pretreatment testicular function or have compared chemotherapy-treated patients with those who underwent orchidectomy alone. Lampe et al. *(25)* analyzed data concerning 170 patients with testicular germ cell cancers who underwent treatment with either cisplatin- or carboplatin-based chemotherapy. Forty of these men (24%) were azoospermic pretreatment, with a further 41 (24%) being oligospermic. A median of 30 months after the completion of chemotherapy, only 64% of those patients who were normospermic before therapy remained normospermic, while 54 (32%) of the total cohort were azoospermic and 43 (25%) were oligospermic. The probability of recovery to a normal sperm count was found to be higher for those men with a normal pretreatment sperm count, in those who received carboplatin- rather than cisplatin-based therapy, and in those treated with fewer than five cycles of chemotherapy. Recovery continued for more than 2 years, with the calculated chance of spermatogenesis at 2 years being 48% and at 5 years 80%. Several authors have compared testicular function in patients following chemotherapy with that of patients treated with orchidectomy

alone *(23,24,26)*. All of these studies have demonstrated greater testicular dysfunction in the cytotoxic-treated groups, with evidence of germinal epithelial damage indicated by raised FSH levels or reduced sperm counts. Fatherhood rates have been reported following treatment for testicular cancer. Huyghe et al. *(27)* studied a total of 451 men following treatment for testicular cancer with orchidectomy ± chemotherapy or radiotherapy. Of 164 men who attempted to achieve pregnancy, 110 succeeded, with greater success rates in those who received chemotherapy alone compared with those treated with local radiotherapy.

Other Malignancies

Similar results have been demonstrated in patients treated with cisplatin-based chemotherapy for osteosarcoma *(2,28)* and lung cancer *(29)*. A threshold level of 600 mg/m^2 was observed for cisplatin treatment, above which significant impairment of spermatogenesis was seen *(2)*. The majority of patients treated with cytotoxic chemotherapy for leukemia, however, do not have persistent gonadal dysfunction. Wallace et al. *(30)* found long-term germinal epithelial dysfunction in only six out of 36 (17%) patients treated during childhood for acute lymphoblastic leukemia, although the period of follow-up in this study was considerable (median time of 10.7 years post-chemotherapy) and the majority of patients had evidence of germinal epithelial damage on testicular biopsy immediately after chemotherapy (which included cyclophosphamide and cytosine arabinoside).

Radiotherapy

The testis is one of the most radiosensitive tissues, with very low doses of radiation causing significant impairment of function. Damage may be caused during direct irradiation of the testis or, more commonly, from scattered radiation during treatment directed at adjacent tissues.

Spermatogenesis Following Single-Dose Irradiation

The effects of relatively low-dose single-fraction irradiation on spermatogenesis in healthy fertile men have been well documented *(6)* and are illustrated in Fig. 1. The more immature cells are more radiosensitive with doses as low as 0.1 Gy, causing morphological and quantitative changes to spermatogonia. Doses of 2–3 Gy result in overt damage to spermatocytes, leading to a reduction in spermatid numbers. At doses of 4–6 Gy, numbers of spermatozoa are significantly decreased, implying damage to spermatids. The decline in sperm count following damage to more immature cells, with doses of up to 3 Gy, takes 60–70 days, with doses above 0.8 Gy resulting in azoospermia and doses below 0.8 Gy giving rise to oligospermia. A much faster fall in sperm concentration occurs following doses of 4 Gy and above because of damage to spermatids.

Recovery of spermatogenesis takes place from surviving stem cells (type A spermatogonia) and is dependent on the dose of radiation. Complete recovery, as indicated by a return to preirradiation sperm concentrations and germinal cell numbers, takes place within 9–18 months following radiation with 1 Gy or less, 30 months for 2–3 Gy, and 5 years or more for doses of 4 Gy and above. Irradiation doses exceeding 6 Gy may result in permanent azoospermia.

Spermatogenesis Following Scattered Irradiation

Animal data indicate that fractionation of radiotherapy increases its gonadal toxicity, and the evidence suggests that this is also the case in humans. Speiser et al. *(31)* studied 10 patients who received a testicular dose of radiation of 1.2–3 Gy, in 14–26 fractions, during inverted Y-inguinal field irradiation for Hodgkin disease. All patients were azoospermic following treatment, and recovery was not seen in a single patient despite follow-up of over 15 months in four patients and of up to 40 months in one. An update of these data, published in 1994 *(32)*, revealed no recovery of spermatogenesis in patients receiving doses of 1.4–2.6 Gy after 17–43 months' follow-up but a return of fertility in the two patients with testicular radiation doses of 1.2 Gy, indicating that this may represent a threshold for permanent testicular damage. Hahn et al. *(33)* carried out serial semen analysis on 11 cancer patients who had received large pelvic field irradiation, or interstitial ^{125}I seeds implanted in the prostate gland. The dose of radiation to the testis was 1.18–2.28 Gy, delivered in 24–34 fractions. All patients became azoospermic, and recovery to oligospermia (three men) or normospermia (two men) was only seen in five patients. The other six remained azoospermic during a follow-up period of 35–107 weeks.

Lower doses of radiation to the testes are, however, associated with better recovery rates for spermatogenesis. Centola et al. *(32)* reported a return of spermatogenesis in all eight patients who received radiation doses of 0.28–0.9 Gy for testicular seminoma,

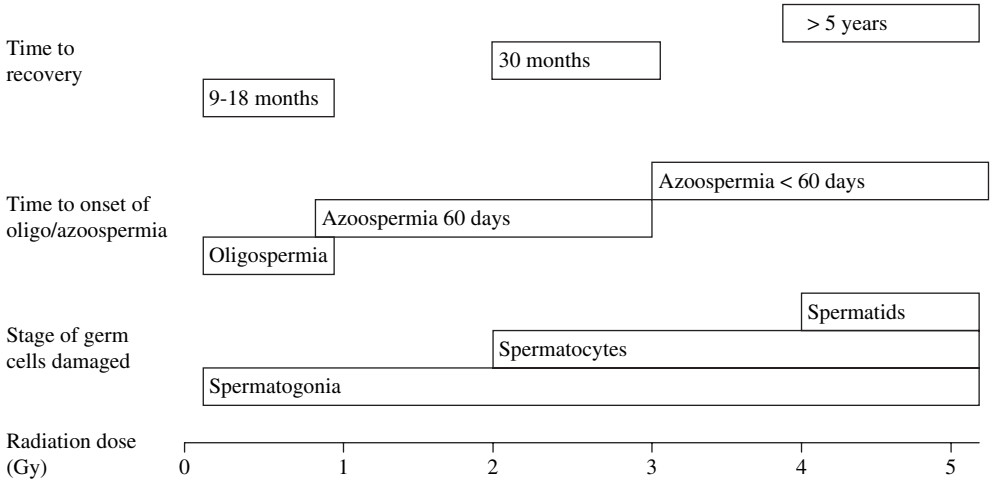

Fig. 1. Impairment of spermatogenesis following single-dose irradiation; the effect of radiation dose on stage of germ cell damage and time to onset and recovery from germ cell damage. Adapted from the data of Rowley et al. *(6)*.

with four out of five reviewed at 12 months having normal sperm counts. Kinsella et al. *(34)* published data concerning 17 patients who had received low-dose scattered irradiation during treatment of Hodgkin disease. Testicular doses of less than 0.2 Gy had no significant effect on FSH levels or sperm counts, but doses between 0.2 and 0.7 Gy caused a transient dose-dependent increase in FSH and reduction in sperm concentration, with a return to normal values within 12–24 months.

TBI

TBI used as conditioning for bone marrow transplant is associated with appreciable gonadal toxicity. Sanders et al. *(20)* reported on a total of 463 men treated with 10.0, 12.0, or 14.0–15.5 Gy TBI. After median follow-up of over 20 years, 81 (18%) of these men had testicular recovery as defined by normal LH, FSH, and testosterone levels with evidence of sperm production. Fatherhood, however, was reported in only five men (1%) and in only two of 392 (0.5%) who received 12.0 Gy or more. Similar rates of germinal epithelial failure were documented by Anserini et al. *(35)*. Following treatment with TBI (9.9 or 13.2 Gy) and cyclophosphamide, azoospermia was found in 41 of 48 men (85%), with oligospermia in the remaining seven patients.

Genetic Damage Following Cytotoxic Therapy

In addition to impairment of steroidogenesis and sperm production, there has been concern that cytotoxic chemotherapy may also result in transmissible genetic damage. Animal studies have demonstrated untoward effects in offspring of animals treated with cytotoxic agents, but no clear evidence for this has been reported in humans. Increased aneuploid frequency has been observed in human sperm following chemotherapy for Hodgkin disease *(36,37)*, and an increase in chromosomal abnormalities has been demonstrated several years after treatment for testicular cancer *(38)*. In addition, techniques such as single-cell gel electrophoresis (Comet assay) and sperm chromatin structured assay have demonstrated DNA damage in sperm following cytotoxic insult *(39)*. Data concerning the outcome of pregnancies have not shown any increase in genetically mediated birth defects, altered sex ratios, or birthweight effects in offspring of cancer survivors *(40)*, possibly as a result of selection bias against genetically abnormal sperm. Although this is reassuring, there is a theoretical potential for modern reproductive techniques such as intracytoplasmic sperm injection to bypass sperm selection mechanisms, and continued surveillance will be necessary. However, data concerning the outcome of pregnancies have not shown any increase in genetically mediated birth defects, altered sex ratios, or birthweight effects in offspring of cancer survivors *(40)*, possibly as a result of selection bias against genetically abnormal sperm. On the evidence thus far, it is therefore reasonable to conclude that patients treated with cytotoxic chemotherapy who remain fertile are not at increased risk of fathering children with genetic abnormalities.

Summary

Treatment with cytotoxic chemotherapy and radiotherapy is associated with significant gonadal damage in men. Alkylating agents, such as cyclophosphamide and procarbazine, are the most common agents implicated. The vast majority of men receiving procarbazine-containing regimens for the treatment of lymphomas are rendered permanently infertile. Treatment with ABVD appears to have a significant advantage in terms of testicular function, with a return to normal fertility in the vast majority of patients. Cisplatin-based chemotherapy for testicular cancer results in temporary azoospermia in most men, with a recovery of spermatogenesis in about 50% after 2 years and 80% after 5 years. The germinal epithelium is very sensitive to radiation-induced damage, with changes to spermatogonia following doses as little as 0.1 Gy and permanent infertility after fractionated doses of 2 Gy and above.

All men should be counseled regarding the possible effects of treatment on testicular function, and sperm banking should be offered to all patients undergoing potentially sterilizing therapy. Hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular tissue are possible future methods of preserving fertility, but they are as yet unproven. Regular semen analyses should be offered to men following cytotoxic treatment to allow appropriate family planning.

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