

Testicular sperm extraction in azoospermic patients with gonadal germ cell tumors prior to chemotherapy - a new therapy option

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Abstract Background: In view of the high cure rates in patients with testicular germ cell tumors (TGCT), increasing clinical importance is attached to protection of fertility. Long-term infertility due to cytostatic therapy may be expected in more than 50% of the patients at a cumulative dose of cisplatin $> 0.6 \text{ g/m}^2$. The standard procedure for fertility protection in cancer patients includes cryopreservation of ejaculated spermatozoa. Considering that some patients have tumor-induced azoospermia, we examined the usefulness of testicular sperm extraction before therapy. **Method:** A survey of the literature served as a basis for investigating biological and clinical aspects of the impact of chemotherapy on male fertility. A study of our patient population also enabled us to explore the option of extracting sperm from the contralateral healthy testis prior to treatment in 14 azoospermic patients with testicular germ cell tumors. **Results:** We were able to successfully recover haploid germ cells in 6/14 testicular biopsies from azoospermic patients with testicular germ cell cancer prior to treatment. Maturation arrest was found in 3/14 cases and Sertoli-cell-only syndrome in the rest. None of the patients had secondary healing or a treatment delay because of the testicular biopsy. **Conclusion:** Since the post-therapeutic fertility status is difficult to predict in cancer patients, we think that TESE should be regarded as a general option prior to cancer treatment and offered to azoospermic cancer patients. New guidelines should be established in this connection. (*Asian J Androl 2002 Mar; 4: 9-15*)

1 Introduction

Cytotoxic chemotherapy for malignant disease has markedly improved the chances of long-term remission or cure in young patients who have not yet started a

family. Thus chemotherapy-induced impairment of fertility has gained increasing clinical importance. The cure rate is about 90% with an upward tendency, and almost all of these patients receive polychemotherapy and/or irradiation. This has led to a rapidly increasing incidence of post-therapeutic reproductive dysfunction. The aim of this article is to survey the biological basis and clinical aspects of chemotherapy and fertility and to present testicular sperm extraction as a treatment option in patients with testicular germ cell tumors.

2 Assessment of chemotherapy-induced fertility impairment

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The assessment of chemotherapy-induced fertility impairment is problematic for many reasons. Even though the time to conception or the conception rate is the best parameter for assessing the fertility of couples seeking parenthood [1], it is not very useful in monitoring the course after chemotherapy, since fertility is only relevant in patients who want to father children [2].

For practical reasons, most studies have evaluated chemotherapy-induced impairment of fertility on the basis of laboratory tests, i.e., follicle-stimulating hormone (FSH) [3], sperm-cell concentration and morphology in the ejaculate or histopathology of testicular biopsies [4-9]. Though the sperm concentration and total sperm count correlate with the time to pregnancy [10] and were used as the main parameters for postoperative fertility in most studies, they may only partially describe the impact of chemotherapy on fertility [11]. Trasler et al. have demonstrated in an animal model, for example, that current tests of male reproductive function cannot predict deleterious effects of a paternally administered agent on the offspring [12].

3 Does infertility in cancer patients originate from the treatment, the tumor, or the testis?

Impairment of spermatogenesis can already be found before treatment in the majority of young patients with TGCT [13, 14] and is thus unrelated to cytotoxic chemotherapy (see Table 1). This impairment of spermatogenesis is reversible in some cases after surgical treatment [15].

Table 1. Testicular function in patients with testicular germ cell cancer (TGCT) before onset of treatment.

Author	No. of patients with TGCT	No. of patients with azoospermia [#]	Sperm count (10 ⁶ /mL)*
Petersen et al., 1998 ^[14]	29	7/29	41 (0-433)
Schrader et al., 2001 ^[48]	31	14/39	17 (0-175)
Caroll et al., 1987 ^[49]	15	6/15	20.4 (0-465)
Nijman et al., 1987 ^[50]	17	4/17	24 (0-68)

[#] Denominator represents total no. of patients, * Median (Range).

The mechanisms underlying the preexisting impairment of gametogenesis [16] are poorly understood [17]. The following causes are under discussion: disorders of urogenital development and/or primary endocrine dys-

function [17] and the presence of contralateral testicular pathology (atrophy or unclassified intratubular germ cell neoplasia) [18, 19]. Possible tumor-related factors include endocrine activity of β -human chorionic gonadotropin [20], elevated concentrations of total serum estradiol and serum estradiol not bound to sex-hormone-binding globulin, and blocking of multiple enzymes necessary for steroidogenesis [17]. It is assumed that tumor-produced human chorionic gonadotropin stimulates estradiol production by "normal" testicular tissue but not tumor tissue and that the high estradiol levels then impair spermatogenesis [21].

A further mechanism under discussion is enhanced aromatization and in situ estrogen production in Leydig cells of the nonneoplastic testis and in interstitial or stromal cells of the tumor in patients with NS [21]. Moreover, anti-sperm autoantibodies have been detected in germ cell tumor patients [22, 23]. Emotional stress has also been discussed as another factor contributing towards reduced fertility of tumor patients [24].

Evidence of a carcinoma-induced alteration of gametogenesis is supported by studies demonstrating that the sole removal and/or successful treatment of germ cell cancer is associated with an improvement of spermatogenesis in at least some of the patients [15, 25, 26].

When certain cumulative chemotherapeutic doses are exceeded, however, a number of studies have demonstrated that gametogenesis disorders correlate significantly with therapy irrespective of its success [8, 22, 27-31]. Infertility is thus clearly due to therapy, at least after higher doses of chemotherapy.

4 Cytotoxic treatment and gametogenesis

The majority of patients develop azoospermia about 8-12 weeks after the initiation of cytostatic chemotherapy. Type A dark spermatogonia, which do not proliferate under normal conditions, and type A pale spermatogonia, which divide at 16-day intervals [32], are less responsive to cytostatics than the rapidly destroyed type B spermatogonia because they have little or no mitotic activity.

If certain cumulative doses of cytotoxic drugs are not surpassed, these stem cell spermatogonia survive polychemotherapy and form the basis for the recovery of spermatogenesis. At low cytostatic doses, a recovery of spermatogenesis may be expected around 12 weeks after polychemotherapy [8]. The destruction of type A spermatogonia at higher doses leads to a sustained or irreversible loss of sperm cell production. Detection of spermatogonia in testicular tissue specimens after chemotherapy is of limited value for predicting a recovery of spermatogenesis, since some investigators point out

that azoospermia may persist in spite of existing spermatogonia [33]. However, Petersen et al. reported that a recovery of spermatogenesis took up to 9 years in individual cases [14].

5 Cytostatic- and dose-specific impact on gonadal function

Comparative studies examining the impact of cytotoxic chemotherapy on gametogenesis demonstrated significant cytostatic- and dose-specific differences. These studies mainly compared testicular cancer patients who achieved complete remission after cytotoxic chemotherapy with those who were treated by surgery alone or enrolled in a surveillance program. This study design inevitably led to a slight distortion of the results, since tumor stages were not matched in these patients. Thus tumor patients in advanced and less advanced stages were compared without considering cytostatic therapy as an impact factor.

5.1 Acute toxicity

Studies on acute toxicity have shown that immediate gonadal dysfunction is induced by cisplatin-based chemotherapy (e.g., PEB = cisplatin, etoposide, bleomycin; PEI = cisplatin, etoposide, ifosfamide) in testicular germ cell tumor patients.

Azoospermia does not develop immediately after the initiation of chemotherapy but only after 8 - 12 weeks. The decreased sperm concentration is accompanied by

increased serum levels of follicle-stimulating hormone (FSH) in most patients [7, 30, 34-36].

5.2 Long-term toxicity

Numerous studies on the long-term effects of chemotherapy disclosed reduced fertility after polychemotherapy in the majority of cases. However, matched-pair analyses of germ cell tumor patients treated with and without cytotoxic chemotherapy reveal that significant differences in the serum levels of FSH and LH as well as in the percentage of patients with azoospermia are only detectable above a cumulative cisplatin dose of >400 mg/m², corresponding to >4 cycles of PEB (cisplatin, etoposide and bleomycin) [8]. (see Table 2)

On the other hand, some of the studies found no significant differences in hormone levels or in the percentage of patients with azoospermia > 2 years after chemotherapy after a cumulative cisplatin dose of < 400 mg/m² [4, 37]. However, permanent azoospermia may be expected in more than 50% of the patients at a cumulative cisplatin dose > 600 mg/m² [8, 30]. In a follow-up of more than 8 years after chemotherapy, some patients achieved a recovery of spermatogenesis even after a dose exceeding 600 mg/m², which indicates that the time to recovery is dose-dependent and difficult to prognosticate [30, 38].

However, the impact of chemotherapy on the specific fertility parameters of patients is extremely difficult to predict in individual cases [2].

Table 2. Testicular function after chemotherapy.

Author	No. of Patients	Treatment	Cumulative dose of cisplatin (mg/m ²)*	Observation period (months)	No. with azoospermia [#]	No. with FSH>RV [#]	No. with LH>RV [#]	No. with T <RV [#]
Hansen et al., 1990 ^[51]	28	6×PVB	487 (346-614)	15-28 (some cases >54)	18/28	22/28	18/28	7/28
Petersen et al., 1994 ^[30]	21 30	3×PEB 4×PEB	618 (486-1197) 400 (307-450)	31-103 61-103	8/17 5/27	20/21 22/30	9/21 8/30	2/21 1/30
Palmieri et al., 1996 ^[26]	28 10	4×PE(V)B 6×PE(V)B	(?) (?)	37-49 31-42	6/28 3/10	11/28 3/10	4/28 6/10	3/28 2/10
Bokemeyer et al., 1996 ^[52]	72	PVB, PEB, PEBV, PE	360-1050	15-159	(?)	40/63	21/63	6/63
Brennemann et al., 1997 ^[53]	73 28	≥2×PEB, PEBI, CEB, CVBIE	(?)	12 >96	(?) (?)	65/73 18/28	24/73 11/28	5/73 1/28
Reiter et al., 1998 ^[54]	22	Carbo	(?)	12-48	0/22	14/22	(?)	(?)
Petersen et al., 1999 ^[38]	22	6×PVB	(?)	>120	4/22	19/22	8/22	1/22

B = bleomycin; Carbo = carboplatin; E = etoposide; I = ifosfamide; P = cisplatin; V = vinblastine; T=testosterone; LH=lutenizing hormone; FSH=follicle-stimulating hormone.

* Median (Range), # Denominator represents total no. of patients

5.3 Fertility

Most of the studies mentioned have focused on the issue of semen quality and FSH serum levels, though the time to conception or the conception rate is the best parameter for assessing the fertility of couples seeking parenthood. As a parameter for male fertility, however, the conception rate has some disadvantages that can lead to a distortion of the results.

One difficulty is the lack of exact normal values, since the prevalence of infertility in the age-matched normal population is unclear. Moreover, female sterility factors are not considered, and the influence of cancer itself on the risk of infertility is difficult to estimate. Another problem is that only about one third of the patients seek paternity after treatment [2, 39].

The mean rate of couples who remain infertile after chemotherapy ranges between 15% and 30% in the literature [14, 31, 40-43]. Petersen et al. have demonstrated that the cumulative cisplatin dose per m² is of decisive importance in this connection. Paternity after chemotherapy was reported by 5 of 33 men in the group that had received less than 600 mg/m² and by none of the 21 treated with higher doses [30].

6 Testicular sperm extraction prior to treatment in azoospermic cancer patients

Cryopreservation of ejaculated spermatozoa prior to treatment is the gold standard for fertility protection in cancer patients. Many patients do not have this option, however, since they are azoospermic prior to treatment due to various factors such as those described above. The usual procedure in such patients with azoospermia confirmed by two spermiograms has thus far been to initiate therapy independently of germ cell cryopreservation. If azoospermia persists after successful cytotoxic therapy, a treatment option recently described by Chan et al. [44] is postoperative testicular sperm extraction combined with intracytoplasmic sperm injection therapy. Chan et al. demonstrated successful sperm retrieval in 9/17 azoospermic patients previously submitted to chemotherapy.

Based on our experience, we raise the question of whether TESE should be regarded as a general treatment option in cancer patients and offered in the pretreatment phase to azoospermic cancer patients who may later seek parenthood.

Two consecutive semen analyses detected azoospermia in 14/39 patients with testicular germ cell tumors, and the clinical tumor stage correlated with the semen analysis. The incidence of azoospermia increased in advanced tumor stages (see Table 3).

Table 3. Azoospermia prior to treatment in patients with different stage testicular germ cell cancer.

Clinical stage	Total No. of patients	No. of patients with azoospermia
I	22	3
II A – II B	10	6
>II C	7	5

This preliminary study did not disclose an association between the histological subtype of the tumors and the number of patients with azoospermia (see Table 4).

Table 4. Azoospermia prior to treatment in patients with different subtype testicular germ cell cancer.

Medical condition	No. of patients	No. of patients with azoospermia
Nonseminomatous germ cell tumors	20	8
Seminoma	19	6

When performing the contralateral testicular biopsy to exclude an intratubular germ cell neoplasia, we cryopreserved part of the specimen from all azoospermic patients in analogy to testicular sperm extraction (TESE). This revealed a Sertoli-cell-only syndrome in 5/14 patients and maturation arrest in 3 patients without detection of haploid germ cells. We were able to successfully recover haploid germ cells in 6 of the 14 patients.

In contrast to the TESE after cytotoxic therapy recommended by Chan et al., the pretherapeutic TESE we perform in testicular germ cell tumor patients has the advantage of sperm being extracted before spermatogenesis is additionally impaired by cytotoxic therapy. Moreover, TESE does not require an additional intervention if performed during the contralateral testicular biopsy.

The disadvantages of the procedure are: 1) It is unclear at this time whether the patient will be cured; 2) TESE may be superfluous in view of the possible spontaneous recovery of spermatogenesis after therapy. However, We think these are outweighed by the advantages already mentioned.

Summing up, our results indicate that testicular sperm extraction is a useful technique for obtaining haploid germ cells prior to cytotoxic therapy of azoospermic cancer patients. We think this procedure should be considered as an option for fertility preservation in selected azoospermic cancer patients.

7 Discussion

It is difficult to predict the influence of chemotherapy on testicular function in individual cancer patients. The risk of post-therapeutic azoospermia is related to the cumulative cisplatin dose of $>0.6 \text{ mg/m}^2$.

Moreover, the fertility status after chemotherapy is thought to correlate with pretreatment fertility and the influence of the malignant disease itself. The spermatogenesis recovery interval ranging up to 9 years or more causes couples seeking parenthood strong psychic stress and reduces their quality of life.

Valid data have not yet been obtained on the teratogenic effect of chemotherapy, though the few studies available show no increase in the number of children with congenital abnormalities [39, 45]. Hormonal protection from chemotherapy-induced testicular damage by pretreatment with gonadotropin-releasing hormone (GnRH) agonists combined with nonsteroidal antiandrogens [46] or with testosterone plus 17 beta-estradiol has thus far only succeeded in animal models [47].

Another approach found to stimulate the recovery of spermatogenesis in animals is hormonal treatment with GnRH agonists or continuous testosterone administration after cytotoxic treatment [33]. However, clinical studies have not been performed in azoospermic men after cytotoxic therapy.

Thus the only effective measure is cryopreservation of sperm prior to chemotherapy. Since subsequent intracytoplasmic sperm injection therapy requires only a minimal number of sperm cells, cryopreservation is advisable regardless of the quality of the ejaculate. This should be offered to the patient irrespective of the planned therapy, since the clinical course and response to chemotherapy cannot be predicted in individual cases.

Another option is testicular sperm extraction in patients submitted to a contralateral biopsy for germ cell tumors or nongonadal malignancies in the presence of azoospermia. We performed this successfully before chemotherapy in 6 of 14 testicular germ cell tumor patients with azoospermia.

Gametogenesis recovery after polychemotherapy in cases of azoospermia can be partially estimated, as described above, by analyzing the chemotherapeutic agents applied and their cumulative dose/ m^2 . A testicular biopsy can be helpful in individual patients with azoospermia. The described studies point to a possible recovery of spermatogenesis within 9 years in cases where spermatogonia are detected after a histological workup or molecular-diagnostic analysis. The detection of a Sertoli-cell-only syndrome as proof of persistent infertility also provides important information for active personal

family planning.

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