ELSEVIER

"ONCO-TESE": TESTICULAR SPERM EXTRACTION IN AZOOSPERMIC CANCER PATIENTS BEFORE CHEMOTHERAPY—NEW GUIDELINES?

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ABSTRACT

Objectives. To examine the usefulness of pretreatment testicular sperm extraction because some patients have tumor-induced azoospermia. In view of the high cure rates for testicular germ cell tumors and malignant lymphomas, increasing clinical importance is attached to protecting fertility. High-dose cytostatic therapy may be expected to cause long-term infertility. Thus, the standard procedure for fertility protection is cryopreservation of ejaculated spermatozoa before therapy.

Methods. Contralateral testicular biopsies were taken from 14 azoospermic patients with malignant testicular germ cell tumors. In addition, 17 patients with malignant lymphomas underwent unilateral (n = 6) or bilateral (n = 11) testicular biopsy. The tissue specimens were cryopreserved, and the histologic workup was performed at the same time.

Results. Of the 14 patients with malignant testicular germ cell tumors, 6 had spermatozoa in their testicular biopsies. Sertoli cell-only syndrome was found in 5 patients, and 3 had maturation arrest without detection of spermatozoa. Successful sperm recovery was possible in 8 of the 17 patients with malignant lymphoma, 4 had Sertoli cell-only syndrome, and 5 had maturation arrest. None of the patients had evidence of secondary wound healing or treatment delay because of the testicular biopsy.

Conclusions. Our results show that testicular sperm extraction is a useful technique for obtaining spermatozoa before cytotoxic therapy in azoospermic cancer patients. This procedure should be considered as an option for fertility preservation in azoospermic cancer patients, because high cumulative cytostatic doses can cause irreversible fertility alterations. UROLOGY **61:** 421–425, 2003. © 2003, Elsevier Science Inc.

Cytotoxic chemotherapy for malignant disease has markedly improved the chances of long-term remission or cure in patients with testicular germ cell tumors and malignant lymphoma. Depending on the clinical stage and risk factor profile, some of these patients have cure rates greater than 80% to 90%, with an upward tendency.[1,2] Most of them undergo polychemotherapy with pronounced gonadal toxic side effects leading to transient post-treatment reproductive dysfunction and, in some cases, to destruction of type A spermatogonia, the stem cell spermatogonia, with sustained or irreversible loss of spermatogenesis.

Fertility protection is gaining increasing clinical importance because most of these young patients have not yet completed or even started their family planning at the time of diagnosis. The reference standard is cryopreservation of ejaculated spermatozoa before chemotherapy. In cases of azoospermia after therapy, these cryopreserved spermatozoa can be used for assisted reproduction procedures (in vitro fertilization and intracytoplasmic sperm injection). Recent publications have emphasized, however, that many patients are not eligible for this procedure, because they already have tumor-induced azoospermia before the planned chemotherapy.[3,4] The possible causes that have been discussed include diverse parameters responsible for severe fertility disorders (eg, high serum interleukin-1, interleukin-6, and beta-human chorionic gonadotropin levels).[5] The standard procedure for these patients with azoospermia has

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thus far been to initiate therapy even without germ cell preservation. Successful cancer therapy led to long-term restoration of fertility in some patients, but others have had an additional alteration of spermatogenesis from chemotherapy-related side effects that resulted in irreversible infertility, as described above.[5] Recent studies have pointed out that testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection in azoospermic cancer patients after polychemotherapy is a promising approach for overcoming chemotherapy-induced fertility disorders.[6] However, that study showed that sperm retrieval was not possible in 12 of 20 testicular biopsies taken from azoospermic men after chemotherapy, which the investigators attributed, in part, to the gonadal toxic side effects of the chemotherapeutic drugs.

The aim of our study was to determine whether TESE before therapy is an effective treatment option for preserving fertility in azoospermic cancer patients and may thus be regarded as an alternative to the technique generally applied thus far.

MATERIAL AND METHODS

Patients

The institutional review board approved this study. All patients signed a consent form approved by the Committee on Human Rights in Research of the Freie Universität, Berlin, Germany or the Tottori School of Medicine, Yonago, Japan.

Two consecutive spermiograms preceding cryopreservation of ejaculated sperm disclosed azoospermia before treatment in 31 patients with testicular germ cell tumors (n = 14) or malignant lymphoma (n = 17). They underwent TESE, because they had not yet completed their family planning. The procedure followed inguinal orchiectomy in all patients with testicular germ cell tumors (n = 14) and was performed in conjunction with the contralateral testicular biopsy done to exclude carcinoma in situ.[7] The patients with malignant lymphoma (n = 17) underwent TESE either under local anesthesia with sedation or under general anesthesia.

We initially performed only a unilateral testicular biopsy in patients with malignant lymphoma (n = 6) and only a contralateral one in those with testicular germ cell tumors (n = 5). This did not delay or complicate therapy in any of the patients. These findings and the increased sperm recovery rate reported for multiple testicular biopsies led us to change our original concept.[8,9] We subsequently performed bilateral testicular biopsies in patients with malignant lymphomas (n = 11) and two contralateral ones in azoospermic patients (n = 9) with malignant testicular germ cell tumors.

Testicular Biopsy and Processing of Testicular Biopsy Material

In brief, a small incision was made in the tunica albuginea to reach at least three testicular lobules. The tissue samples were subdivided into five fragments, and three were immediately placed in 1.0 mL of Sperm-Freeze solution (Medicult, Hamburg, Germany) and transferred to liquid nitrogen by a computer-guided system. The human sperm Kryoprogramm (Planer 10, Messer-Griesheim, Griesheim, Germany) was used according to the manufacturer's instructions.

One sample of testicular tissue from each patient was placed in a Petri dish containing Sperm-Prep solution (Medicult, Hamburg, Germany) and examined within 10 minutes. Minced tissue was examined by phase-contrast microscopy at 400× magnification to detect cells of spermatogenesis, especially spermatozoa. In the case of negative findings, the tissue was treated with type I collagenase (Sigma, Heidelberg, Germany) following a modified form of the protocol published by Schulze *et al.*[10]

In brief, a sample of the tissue specimens was incubated for 2 hours in a gas-controlled incubator at 37°C and supplemented with 0.8 mg of type AI collagenase (Sigma, Heidelberg, Germany) and 0.2 g of trypsin inhibitor (Sigma, Heidelberg, Germany) dissolved in 1 mL of preheated Sperm-Prep medium (Medicult) and submitted to sterile filtration. The digest solution was centrifuged for 10 minutes at 800*g* and 37°C. The supernatant was removed, and the resultant pellet was analyzed using a light microscope at 400× magnification. The estimated spermatozoa per microscopic field were evaluated for vitality. Vital spermatozoa were considered to be those showing a degree of independent movement or progressive movement. In some cases, the eosin test was used to check the vitality of spermatozoa.

A post-thaw evaluation of aliquots was performed in 13 patients with malignant lymphoma and 9 with testicular germ cell tumors.

Another part of the sample was placed in Stieve's solution (formaldehyde DAB 10, 20.0 g, acetic acid 100% DAB 10, 4.0 g, aqueous saturated 7% mercuric [II] chloride solution 76.0 g/1000 mL), paraffin embedded, and prepared in 5-μm slices. The slices were stained with hematoxylin-eosin, and placenta-like alkaline phosphatase was used to detect carcinoma in situ. The biopsy material was histologically evaluated for spermatogenesis according to a modified Johnsen score (1, no seminiferous tissue; 2, no spermatogonia but seminiferous tissue [Sertoli cell-only syndrome (SCOS)]; 3, spermatogonia only; 4, no spermatids and few spermatocytes; 5, no spermatids and many spermatocytes; 6, no late and few early spermatids; 7, no late and many early spermatids; 8, few late spermatids; 9, many late spermatids and disorganized epithelium; and 10, full spermatogenesis).[11,12]

RESULTS

One (n = 5) or two (n = 9) contralateral testicular biopsies were taken in 14 azoospermic patients with a malignant testicular germ-cell tumor. The patient characteristics are shown in Tables I and II.

Spermatozoa were successfully recovered in 6 of 14 patients who had a histologic picture of mixed atrophy with focal islands of full spermatogenesis. SCOS was found in 5 of 14 patients, and maturation arrest (Johnsen score 3 to 5) without detection in 3 patients. One of the 9 patients with two biopsies of the contralateral testis had discrepant findings (biopsy 1, maturation arrest and biopsy 2, focal islands of full spermatogenesis). The percentage of patients with severe spermatogenesis disorders increased in proportion to the clinical tumor stage. On the other hand, the distribution of histologic results from testicular biopsies did not differ when patients were classified as having seminomas (haploid germ cells in 4; maturation arrest in 1, and SCOS in 3) or nonseminomas (haploid germ cells in 2; maturation arrest in 2, and SCOS in 2).

TABLE I. *Patients with testicular germ cell tumors and azoospermia before chemotherapy*

Clinical Stage	Patients with Azoospermia (n)	Patients with Successful Sperm Retrieval (n)	Patients with Maturation Arrest (JS 3–5) (n)	Patients with SCOS (JS 1–2) (n)
I	2	2/2	0/2	0/2
IIA–IIB	8	3/8	3/8	2/8
>IIC	4	1/4	0/4	3/4

KEY: JS = Johnsen score; SCOS = Sertoli cell-only syndrome.
Histologic examination results according to clinical tumor stage; classification into clinical tumor stages followed World Health Organization guidelines.

TABLE II. *Histologic findings of azoospermic cancer patients with detection of haploid germ cells* in testicular biopsies*

	Early and Elongated Spermatids Only (n)	<1 Sp/MF (n)	1–10 Sp/MF (n)	>10 Sp/MF (n)	Vital Sp† >20–80% (n)	Vital Sp† 1–20% (n)
Germ cell tumors (n = 6)	1	1	1	3	4	2
Hodgkin's/non-Hodgkin's disease (n = 8)	1		3	4	5	3

KEY: Sp = testicular spermatozoa; MF = microscopic field (×400).
**Round spermatids, elongated spermatids, and testicular spermatozoa.*
†Percentage of vital sperm in post-thaw evaluation.

Patients in the malignant lymphoma subgroup (n = 17) underwent unilateral (n = 6) or bilateral (n = 11) testicular biopsy. The patient characteristics are shown in Tables II and III.

Spermatozoa were successfully recovered in 8 of the 17 patients with malignant lymphoma. SCOS was found in 4 patients, and 5 had maturation arrest (Johnsen score 3 to 5). Two patients in the subgroup with a bilateral testicular biopsy had discrepant findings (patient 1, maturation arrest in biopsy 1 and focal islands of full spermatogenesis in biopsy 2; patient 2, focal islands of spermatogonia in biopsy 1 and SCOS in biopsy 2; Table III). None of the patients with testicular germ cell tumors or malignant lymphoma had secondary wound healing or a treatment delay because of the testicular biopsy.

COMMENT

The increasing cure rate in patients with testicular germ cell tumors and malignant lymphoma has awakened a growing interest in preserving their fertility. Because aggressive gonadal toxic chemotherapy is often indispensable with a view to a successful curative outcome (eg, cisplatin versus carboplatin), the reference standard thus far has been the cryopreservation of ejaculated sperm before chemotherapy for use in assisted reproduction measures if azoospermia persists after treatment.[13,14] This approach was a treatment option even in patients with a strongly restricted spermiogram, because subsequent intracytoplasmic sperm injection therapy requires only a minimal number of spermatozoa.[14]

However, the procedure is useless in patients with azoospermia before therapy, because cryopreservation of ejaculated sperm is not possible in such cases. However, azoospermia is detected relatively often in patients with advanced testicular germ cell tumors or lymphoma.[3,15]

Various parameters have been discussed as possible causes: disorders of urogenital development and/or primary endocrine dysfunction and the presence of contralateral testicular pathologic findings (atrophy or carcinoma in situ).[16,17] Possible tumor-related factors include endocrine activity of beta-human chorionic gonadotropin, elevated concentrations of total serum estradiol and serum estradiol not bound to sex hormone-binding globulin, and blocking of multiple enzymes necessary for steroidogenesis.[16,18] Moreover, it is assumed that tumor-generated human chorionic gonadotropin stimulates estradiol production by "normal" testicular tissue but not tumor tissue and that the high estradiol levels then impair spermatogenesis.[19]

The standard procedure in patients with tumor-induced azoospermia before therapy has thus far been to initiate chemotherapy even if cryopreservation of ejaculated sperm cannot be achieved. In some of the patients, successful therapy has led to recovery of spermatogenesis by eliminating the

TABLE III. ***Patients with Hodgkin's/non-Hodgkin's disease and azoospermia before chemotherapy***

Disease	Patients with Azoospermia (n)	Patients with Successful Sperm Retrieval (n)	Patients with Maturation Arrest (JS 3–5)	Patients with SCOS (JS 1–2)
Hodgkin's disease	7	3/7	2/7	2/7
Non-Hodgkin's disease	10	5/10	3/10	2/10

KEY: JS = Johnsen score; SCOS = Sertoli cell-only syndrome.
Histologic examination results according to the disease type.

azoospermia-inducing tumor-related factors.[15] If high cumulative cytostatic doses were exceeded, however, the gonadal toxic side effects of chemotherapy will cause an additional impairment of spermatogenesis that results in irreversible infertility.[20]

Hormonal protection from chemotherapy-induced testicular damage has been achieved in an animal model by pretreatment with gonadotropin-releasing hormone agonists combined with nonsteroidal antiandrogens or with testosterone plus 17β-estradiol but has not yet been established in humans.[21,22] Thus, no clinical protection can be offered against chemotherapy-related gonadal toxic side effects at present. The same is true for stimulation of spermatogenesis after chemotherapy. Although successful in animals, hormonal treatment with gonadotropin-releasing hormone agonists or continuous testosterone administration after cytotoxic therapy is not yet a clinically established method.[23]

A very interesting study recently published by Chan *et al.*[6] points to a successful new treatment approach. The study group showed that testicular sperm extraction combined with intracytoplasmic sperm injection in azoospermic cancer patients after polychemotherapy was effective in overcoming chemotherapy-induced fertility disorders (9 successful sperm retrievals from 20 TESE procedures and 2 live births in 9 TESE-intracytoplasmic sperm injection cycles). However, the study showed that sperm retrieval was not possible in 12 of 20 testicular biopsies taken from azoospermic men after chemotherapy. The high rate of patients without germ cell detection in the testicular biopsies is probably attributable at least in part to the gonadal toxic side effects of chemotherapy.

Our study is the first to show that TESE before chemotherapy provides another promising treatment option. Spermatozoa were successfully obtained in 14 of 31 azoospermic cancer patients before chemotherapy. With successful sperm recovery in 2 of 31 patients, bilateral TESE for patients with malignant lymphoma or two contralateral biopsies for those with a testicular germ cell tumor was superior to a simple biopsy in this initial study and should thus be given preference. This recommendation is substantiated by the findings of Ostad *et al.*,[9] who achieved a higher sperm recovery rate with multiple biopsies than with a single biopsy approach in patients with nonobstructive azoospermia.

The approach we selected has the following advantages over the aforementioned procedures:

1. The potential additional alteration of spermatogenesis by gonadotoxic side effects of chemotherapy is of no consequence as it would be with the above-mentioned TESE after chemotherapy.
2. Irrespective of whether these germ cells are used for measures of assisted reproduction, the knowledge that germ cells were cryopreserved before the initiation of therapy enhances the patient's quality of life and sense of security.
3. Possible teratogenic effects of chemotherapy are avoided with this procedure. The many studies on teratogenic effects of chemotherapy provide no evidence for an increased risk of malformations in offspring of patients with a history of chemotherapy.[24] However, their scope and follow-up may still be inadequate, and they have not been able to eliminate the fear of an increased risk of malformations among children of chemotherapy patients.[24] The potential risks of cytotoxic therapy for the offspring of patients were pointed out by Trasler *et al.*,[25] who demonstrated in a rat model that paternal cyclophosphamide treatment causes fetal loss and malformations without affecting male fertility. Sega[26] also called attention to the risk of DNA alterations after chemotherapy.
4. We were also able to show that TESE before chemotherapy did not lead to a treatment delay in any of the patients.

We believe these advantages outweigh the disadvantages of the procedure (eg, the uncertainty of whether the patient will be cured and the superfluity of TESE in patients with spontaneous recovery of spermatogenesis after therapy). Cryopreserved spermatozoa should only be injected after patients have received genetic counseling to exclude such

diseases as Klinefelter syndrome, mucoviscidosis, and so forth, and have discussed the risks of intracytoplasmic sperm injection. Cryopreserved spermatozoa from 2 patients with malignant lymphoma were used for intracytoplasmic sperm injection. Fertilization succeeded both times with a miscarriage in the 11th week of gestation in 1 case and the birth of a healthy daughter in the other.

CONCLUSIONS

Our results indicate that testicular sperm extraction is a useful technique for obtaining haploid germ cells before cytotoxic therapy of azoospermic cancer patients. We believe this procedure should be considered as an option for preserving fertility of azoospermic cancer patients in selected cases. It would seem expedient to establish new guidelines in this connection.

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