Gonadal function after multimodality treatment in men with testicular germ cell cancer


Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica. Facoltà di Medicina e Chirurgia. Università “Federico II”. Napoli. Italy


We evaluated gonadal function in 63 patients with testicular cancer both within 1 month of unilateral orchectomy before further treatment (pretreatment) and 3 years after treatment discontinuation (post-treatment). Sixteen patients underwent orchectomy alone (group 1), nine patients underwent infradiaphragmatic radiotherapy (group 2) and 28 patients received four cycles (group 3) and 10 patients received six cycles (group 4) of cisplatin-based chemotherapy (cisplatin, vinblastine and bleomycin—PVB, or cisplatin, etoposide and bleomycin—PEB). Pretreatment semen analyses showed reduced sperm cell density, motility and impaired morphology of spermatogenesis in all four groups (p > 0.05). At the same time elevated estradiol and decreased serum follicle-stimulating hormone (FSH) levels in 28.5% of subjects were correlated with high serum beta human chorionic gonadotropin concentrations. Semen analyses revealed the lowest values for all parameters after infradiaphragmatic radiotherapy. Sperm cell count, motility and morphology were significantly better in patients treated with orchectomy alone or with a conventional dose of chemotherapy than in the groups that received radiotherapy or high doses of chemotherapy (p < 0.05). We also observed a correlation between serum FSH values and sperm cell density for both pretreatment and post-treatment in every group of patients (p < 0.05). Persistent subclinical Leydig cell dysfunction in groups treated with radiotherapy or high doses of chemotherapy was expressed by increased basal luteinizing hormone levels (78% of patients in group 2 vs 60% of patients in group 4) (p < 0.05) and by normal testosterone serum values (89% of patients in group 2 vs 80% of patients in group 4). Spermatogenesis and Leydig cell function are, therefore, persistently impaired in the majority of testicular cancer patients treated with radiotherapy or with more intensive chemotherapy.


Testicular germ cell tumors are the most common malignancy in young adult males. These are one of a few neoplasms that can be cured in 80–90% of cases (1). Much of the success in the treatment of this disease is related to the introduction of cisplatin-based chemotherapy (cisplatin, vinblastine and bleomycin (PVB) in the 1970s, and cisplatin, etoposide and bleomycin (PEB) in the 1980s) (2), in addition to the classic treatments (surgery and radiotherapy). This multimodality treatment has substantially improved the survival of patients with testicular cancer. Most of these patients are at a reproductive age and consequently the influence of therapy on testicular function is a matter of concern.

The semen quality after orchectomy and before further treatment is decreased in the majority of patients (3–5). Furthermore, it has been shown that both radiotherapy (6–11) and chemotherapy (4, 5, 12–15) lead to additional impairment of spermatogenesis and Leydig cell function.

The aim of the present report is to describe the effect of the combined treatments on the spermatogenesis and the pituitary–gonadal axis, through the evaluation of semen and hormonal analysis, and to give a better definition of treatment damage to gonadal function and an assessment of the possibilities of recovery.

Patients and methods

Between 1982 and 1990, we evaluated 63 patients affected by testicular cancer (21 with seminoma and 42 with non-seminoma). Treatment was based on the histology and the extent of disease, assessed according to the classification of the National Tumor Institute of Milan (16).

Seminoma patients in low stages (I or II A–B) underwent infradiaphragmatic radiotherapy (para-aortic and ipsilateral iliac lymph nodes). Non-seminoma patients in clinical stage I either followed a surveillance protocol or underwent retroperitoneal lymph node dissection (RPLND). All patients in clinical stage II underwent RPLND. No further treatment was given. More advanced stage seminoma and non-seminoma
patients were treated with three to four cycles of chemotherapy. A further group of patients, non-responders to standard drug treatment, received six cycles of chemotherapy.

For a better evaluation, we divided all patients into four groups on the basis of the treatment they had undergone:

Group 1 consisted of 16 patients with non-seminoma stage I or II A–B (median age 27 years) managed with no chemotherapy or radiotherapy.

Group 2 included 9 patients with low-stage seminoma who underwent infradiaphragmatic radiotherapy (median age 41 years).

Group 3 was composed of 28 patients (eight seminoma and 20 non-seminoma patients, median age 25 years) with advanced disease (stage II C–D, III, IV) treated with three to four cycles of cisplatin-based chemotherapy (12 with PVB and 16 with PEB).

Group 4 included 10 patients (four with seminoma and six with non-seminoma, median age 34 years) who underwent six cycles of chemotherapy (4 with PVB and six with PEB) (see Table 1 for all patients’ characteristics).

All patients were without evidence of metastases and they had a Karnofsky performance status ≥ 90 when post-treatment sperm cell analyses were performed after 3 years. Serum \( \beta \)-hCG levels were elevated in 24 patients (57.7%) with non-seminoma tumors and in six patients (28.5%) with seminoma (total of 30 patients: 47.6%) at the time of diagnosis.

One month after orchiectomy (pretreatment) only 28.5% of all patients had high serum values of \( \beta \)-hCG. These values gradually decreased and at the time of semen and hormonal analyses all patients had normal serum \( \beta \)-hCG levels. Semen and hormonal analyses were performed simultaneously both within 1 month of unilateral orchiectomy but before further treatment (pretreatment) and 3 years after discontinuation of all treatment (post-treatment). Two semen samples were obtained from each patient at 2-week intervals by masturbation after 72 h of sexual abstinence. Volume of ejaculate together with sperm cell density, motility and morphology were assessed 1 h after ejaculation. The mean values of the two samples from each patient were considered in this study. We defined azoospermia as the absence of spermatozoa in the ejaculate, normozoospermia as a sperm density of 20 million/ml or more, normal motility as 50% or more spermatozoa with progressive motility at 1 h and normal morphology as 30% or more cells with normal morphology (17).
Patients with dry ejaculation were excluded from the present study. The levels of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estradiol, prolactin, androstenedione, dehydroepiandrosterone sulfate (DHEAS), dihydrotestosterone (DHT), 17-hydroxyprogesterone, free testosterone, sex hormone-binding globulin (SHBG) and beta human chorionic gonadotropin (β-hCG) were measured by radioimmunoassay both pretreatment and post-treatment. The mean value of two serum samples from each patient was used for calculations. Normal laboratory ranges (95% confidence limits) were as follows: FSH, 5–15 mIU/ml; LH, 5–15 mIU/ml; total testosterone, 3.5–10 ng/ml; estradiol, 15–45 pg/ml; prolactin, 2–15 ng/ml; androstenedione, 0.4–1 ng/ml; DHEAS, 2000–3500 ng/ml; DHT, 40–200 ng/ml; 17-hydroxyprogesterone, 0.2–2 ng/ml; free testosterone, 13.9–37.3 pg/ml; SHBG, 13–55 nmol/l; β-hCG, 0–5 mIU/ml. Post-treatment gonadotropin response to 100 μg gonadotropin-releasing hormone (GnRH) iv was observed at 15, 30, 45, 60 and 120 min. Statistical analyses were performed using Wilcoxon’s rank sum test and Spearman’s rank correlation test, with a significance limit of 0.05 (two-tailed).

**Results**

After orchietomy but before any other treatment, median values of sperm cell density were reduced and did not differ substantially among the four groups (p > 0.05) (Table 2). Furthermore, before treatment the majority of all the subjects had decreased motility and impaired morphology in their seminal fluids (Table 2). After 3 years, sperm cell density, motility and morphology were significantly worse in the groups who had received infradiaphragmatic radiotherapy (group 2) or high-dose chemotherapy (group 4) than in groups 1 and 3 (p < 0.05) (Table 3), while there was no significant difference when group 3 was compared with group 1 (p > 0.05) (Table 3). Nevertheless, reduction of motile spermatozoa and increase of sperm cells with abnormal morphology were present in each

### Table 2. Pre-treatment gonadal function.

<table>
<thead>
<tr>
<th></th>
<th>No chemotherapy or radiotherapy (group 1)</th>
<th>Radiotherapy (group 2)</th>
<th>Chemotherapy 3–4 cycles (group 3)</th>
<th>Chemotherapy 6 cycles (group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>16</td>
<td>9</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>Basal FSH (mIU/ml)</td>
<td>9.4 (1.3–32)</td>
<td>8.2 (1.9–23.1)</td>
<td>7.7 (2.4–38.2)</td>
<td>8.7 (1.9–27)</td>
</tr>
<tr>
<td>Basal LH (mIU/ml)</td>
<td>7.5 (5.9–41)</td>
<td>10.4 (6.2–22)</td>
<td>9.5 (5.3–27.9)</td>
<td>9.2 (5.4–38.5)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>6.4 (3.6–18.7)</td>
<td>6.1 (4.2–9.3)</td>
<td>7.0 (2.9–19.4)</td>
<td>7.3 (4.5–23.4)</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>27.5 (20.1–83.3)</td>
<td>32.1 (19.8–95.7)</td>
<td>32.8 (17.1–74.8)</td>
<td>40.8 (22.9–104.2)</td>
</tr>
<tr>
<td>Sperm cell density (x 10 million/ml)</td>
<td>12.5 (0–45)</td>
<td>8 (0–42)</td>
<td>10.5 (0–34)</td>
<td>6 (0–15)</td>
</tr>
<tr>
<td>% spermatozoa with progressive motility</td>
<td>28 (3–73)</td>
<td>32.5 (0–45)</td>
<td>37 (0–78)</td>
<td>26 (5–39)</td>
</tr>
<tr>
<td>% spermatozoa with normal morphology</td>
<td>23.5 (15–41)</td>
<td>24 (17–28)</td>
<td>26.5 (15–56)</td>
<td>22.5 (14–26)</td>
</tr>
<tr>
<td>Volume of ejaculate (ml)</td>
<td>2.8 (0.2–6.1)</td>
<td>3.5 (1.2–5.4)</td>
<td>2.5 (0.3–5.6)</td>
<td>2.4 (0.5–4.5)</td>
</tr>
<tr>
<td>No. of patients with azoospermia</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

a Values are medians (ranges in parentheses) and number of patients with pathological values/total number of evaluable patients: p > 0.05 between groups 1, 2, 3, 4.

b Not analyzed samples from patients with azoospermia.
Table 3. Post-treatment gonadal function.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>No chemotherapy or radiotherapy (group 1)</th>
<th>Radiotherapy (group 2)</th>
<th>Chemotherapy 3–4 cycles (group 3)</th>
<th>Chemotherapy 6 cycles (group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>16</td>
<td>9</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>Basal FSH\textsuperscript{b} (mIU/ml)</td>
<td>8.3 (5.4–29.9)</td>
<td>19.6 (10–31.1)</td>
<td>11.1 (5–41.7)</td>
<td>19.8 (8.3–52.4)</td>
</tr>
<tr>
<td>Basal LH\textsuperscript{b} (mIU/ml)</td>
<td>9.7 (5–18.2)</td>
<td>16.4 (6–20.9)</td>
<td>10.3 (5–17.6)</td>
<td>18.7 (5.5–27)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>7.1 (2.1–9.2)</td>
<td>6.6 (3.3–8)</td>
<td>5.1 (2.5–8.2)</td>
<td>4.4 (1.7–6.2)</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>31.4 (16.3–52.6)</td>
<td>27.3 (19.2–41.5)</td>
<td>28.7 (12.2–70.5)</td>
<td>38.2 (21.7–74.6)</td>
</tr>
<tr>
<td>Sperm cell density\textsuperscript{b} (x 10 million/ml)</td>
<td>30 (0–70)</td>
<td>7 (0–16)</td>
<td>35 (0–90)</td>
<td>7.5 (0–18)</td>
</tr>
<tr>
<td>% spermatozoa with progressive motility\textsuperscript{b,c}</td>
<td>46 (0–81)</td>
<td>23 (0–38)</td>
<td>42.5 (0–69)</td>
<td>28 (2–44)</td>
</tr>
<tr>
<td>% spermatozoa with normal morphology\textsuperscript{b,c}</td>
<td>28 (16–80)</td>
<td>20 (14–28)</td>
<td>27.5 (15–66)</td>
<td>21 (15–28)</td>
</tr>
<tr>
<td>Volume of ejaculate (ml)</td>
<td>3/2 (1–5.8)</td>
<td>4 (0.5–6.2)</td>
<td>2.5 (0.5–4.4)</td>
<td>2.2 (0.6–5)</td>
</tr>
<tr>
<td>No. of patients with azoospermia</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are medians (ranges in parentheses) and number of patients with pathological values/total number of evaluable patients.

\textsuperscript{b} Group 1 or 3 vs groups 2 or 4. p < 0.05; group 1 vs group 3. p > 0.05; group 2 vs group 4. p > 0.05.

\textsuperscript{c} Not analyzed in samples from patients with azoospermia.

Discussion

Using present therapeutic strategies, a growing number of patients with testicular cancer can be cured. The LH values were significantly (p < 0.05) higher in groups 2 and 4 than in the other two groups: basal LH concentrations were elevated in seven of nine irradiated patients (78%) and in six of ten patients (60%) treated with more intensive chemotherapy (Table 3). After GnRH injection, post-treatment maximal LH and FSH concentrations were elevated significantly (p < 0.05) in groups 2 and 4 when compared with peak FSH and LH values of the remaining groups of subjects. In groups 1 and 3 median baseline and GnRH-stimulated FSH and LH levels were, therefore, within the normal range without a significant difference between the groups (p > 0.05) (Table 3). We also observed no statistically significant difference in the post-treatment normal median serum values of testosterone and of all the other hormones (data not shown) among the four groups (p < 0.05) (Table 3).
The possibility of evaluating gonadal function after treatment for testicular germ cell tumors presupposes the availability of data concerning pretreatment spermatogenesis and steroidogenesis. Several reports have shown that most untreated patients develop an impairment of spermatogenesis (18–20).

Before radiotherapy or chemotherapy our data revealed reduced sperm cell density, decreased motility and impaired morphology of spermatozoa, as shown in previous studies (19, 21). We also found a significant negative correlation between pretreatment sperm cell density and serum FSH values. In most patients, pretreatment oligozoospermia or azoospermia combined with high serum FSH levels represents an index of pre-existent disturbances of spermatogenesis in both testes (15).

In accordance with previous results (10, 14, 15), we found that a complete recovery of the number of spermatozoa in ejaculate and normalization of testicular steroid overproduction in patients with hCG-producing tumors, occurs within 3 years after orchiectomy alone when following a surveillance protocol. Nevertheless, semen quality parameters continue to be impaired with regard to sperm motility and morphology, as found in a former report (13).

Using infradiaphragmatic radiotherapy for seminoma, the importance of gonadal shielding in order to preserve testicular function has been emphasized by several reports (7, 10, 22). In fact, radiation-induced injury is dependent on scattered dose to contralateral testis (7, 8, 10). Our 3-year data, referring to a limited number of patients, seem to support Hansen’s observation about persistent exocrine gonadal damage with impairment of functional recovery in irradiated patients (10). Persistent long-term serum FSH elevation and substantial reduction of sperm cells following irradiation may therefore not always be due to treatment, because a decrease in the number of stem cells with increasing age, pretreatment FSH serum values and sperm counts are all factors that seem to add to the radiation effects (10, 14, 15). In contrast with other investigators (14) but in agreement with previous indications about permanent radiation-induced Leydig cell dysfunction (23), we found that increased basal and GnRH-stimulated LH levels and normal testosterone serum values could denote a less-efficient compensation mechanism in older seminoma patients. However, such negative results need further investigation.

Spermatogenesis is highly disturbed 6–12 months after cytotoxic treatment. Thereafter, a gradual improvement of semen parameters occurs with time but the degree of this recovery is a matter for discussion (4, 6, 12, 21). Different dosages of chemotherapeutic agents and pretreatment serum FSH values have been related to 3-year post-treatment sperm cell count and to serum FSH levels (14, 15, 24). In agreement with other authors (12, 14, 15), we observed normal serum FSH values and normal sperm cell density 3 years after discontinuation of standard cytotoxic treatment (group 3), whereas other semen quality parameters remain impaired. Also in

Fig. 1. Post-treatment corresponding values of serum FSH and sperm density: (— —) 95% reference interval for serum FSH and lower reference value for sperm density.
agreement with other authors (10, 14, 21), high doses of cisplatin-based chemotherapy produce persistent damage to exocrine testicular function in the majority of patients, particularly of high age. Elevated post-treatment serum basal and GnRH-stimulated FSH levels are biological indicators of an impaired spermatogenetic process with functional Sertoli cell insufficiency, as found in rats (25). Furthermore, elevated pretreatment serum FSH values represent a negative predictive factor as regards recovery of post-treatment spermatogenesis (15, 23, 24).

Results concerning Leydig cell function after chemotherapy are conflicting. Using standard doses of cisplatin-based treatment, our study indicates that no effect on Leydig cell function was caused by cytotoxic treatment, as shown by Aass (14). Subclinical Leydig cell dysfunction has been found transiently (21) or persistently (13) after six cycles of chemotherapy for advanced non-seminoma testicular cancer, whereas in another study this finding was not confirmed (14). We report evidence suggesting that in the majority of these patients cisplatin-based chemotherapy causes persistent but compensated damage to Leydig cells. Therefore, increased post-treatment basal and GnRH-stimulated LH levels and normal testosterone concentrations are predicting values that cisplatin-induced Leydig cell dysfunction may, with time, become permanent at a subclinical level. The importance of the patient’s age in evaluation of both spermatogenesis and Leydig cell function was emphasized previously by some authors who demonstrated that testicular cancer patients over 25 years of age at treatment are less resistant to the negative effects of standard cisplatin-based chemotherapy (26) or irradiadiaphragnmatic radiotherapy (9, 10) than younger patients.

The GnRH-stimulated tests that we carried out determine better the activity of the hypothalamus–pituitary–gonad axis and give important confirmation of the base levels of the gonadotropins.

In conclusion, after standard and intensive treatment for testicular germ cell tumors, gonadal function seems to be influenced negatively by the patient’s age. The question of whether, in the long period, radiation and cisplatin damage to spermatogenesis will become important for a patient needs further investigation with more protracted observation periods and more sophisticated methods of research.

References


Received May 11th, 1995
Accepted January 23rd, 1996