Testicular Function in Patients With Testicular Cancer Treated With Orchiectomy Alone or Orchiectomy Plus Cisplatin-Based Chemotherapy

Peter Vejby Hansen,* Henrik Trykker, Poul Erik Helkjaer, Jørn Andersen

Gonadal function was evaluated in 25 patients with metastatic testicular cancer who were treated with orchiectomy plus chemotherapy with cisplatin, vinblastine, and bleomycin (PVB) and in 21 patients with clinical stage I disease who were treated only with orchiectomy and then kept under surveillance. Four years after PVB treatment, none of the patients were azoospermic; after 5 years, the total sperm counts in 46% of the patients had reached their pretreatment levels. In the group under surveillance, sperm counts below the reference level persisted or developed in 55% of the patients. Sperm production was similar in the two groups of patients 1.5 years after treatment and beyond. We conclude that spermatogenesis is not restored in all patients treated with PVB because of both preexisting germ cell defects and treatment toxicity. [J Natl Cancer Inst 81:1246-1250, 1989]

Cisplatin-based chemotherapy has improved the survival of patients with disseminated germ cell tumors of the testis (1). This effective salvage therapy has led to a treatment policy of close surveillance and no other treatment after orchiectomy in patients with clinical stage I disease (2,3). Little information is available about the gonadal long-term toxic effects of combination chemotherapy with cisplatin, vinblastine, and bleomycin (PVB) (4,5). Patients with testicular cancer often have low sperm counts after unilateral orchiectomy (6,7), and in some patients the sperm counts apparently remain low (6). Therefore, investigation of the testicular toxic effects of PVB requires a prospective study design and a control group of patients treated with orchiectomy alone.

The aims of the present study were (a) to describe the testicular function in patients under surveillance after treatment with orchiectomy alone and in patients treated with orchiectomy plus PVB and (b) to assess the long-term gonadal toxic effects of PVB by a comparison of the results obtained from these two groups of patients. Sperm counts and the serum levels of gonadotropin and testosterone were investigated at orchiectomy and at follow-up after the end of treatment in a group of young patients with testicular cancer.

Patients and Methods

From January 1977 to December 1986, we examined the testicular function following unilateral orchiectomy in 97 patients aged ≤40 years with testicular germ cell cancer (8). Informed consent was obtained from the patients according to the guidelines given in the Declaration of Helsinki. Forty-six of these 97 patients were included in the present study; 25 patients were treated with chemotherapy, and the other 21 patients were kept under surveillance. The remaining 51 patients, who received subdiaphragmatic irradiation, will be reported on elsewhere (Hansen PV, Trykker H, Svennekjær IL, et al.: unpublished data). Histologic classification and clinical staging of the disease were performed according to criteria of the Danish Testicular Carcinoma Study (9). The patient characteristics are summarized in table 1.

Semen samples, serum follicle-stimulating hormone, serum luteinizing hormone, and serum testosterone were analyzed after orchiectomy and at follow-up on a yearly basis, starting 1 year after treatment. Spermatogenesis was quantitated, with the total sperm count per ejaculate used as an end point for germ cell activity. Blood and semen samples were obtained on the same day. The sampling continued until sperm counts had reached the pretreatment level for at least 2 years or on request from the patient. Two patients treated with PVB did not supply samples at follow-up because of progressive disease, and one patient under surveillance declined to supply a semen sample at follow-up. Gonadal function was evaluated for a median of 4.3 years (range, 0.9-6.8 yr) after PVB and 2.9 years (range, 1.3-7.0 yr) after orchiectomy in patients under surveillance. All follow-up samples were obtained from patients free of disease.

The cytostatic treatment consisted of six courses of PVB (10), and three of the patients received maintenance chemotherapy.

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therapy with vinblastine and dactinomycin. Data from one patient treated with PVB alone who on presentation had azoospermia were included in the comparison with those on patients under surveillance, but they were excluded from the life-table calculation so as not to confound the estimates. The median total doses of PVB received by the remaining 22 patients were 487 mg of cisplatin/m² (range, 346-614 mg/m²), 40 mg of vinblastine/m² (range, 14-55 mg/m²), and 164 mg of bleomycin/m² (range, 124-203 mg/m²); the total doses of the maintenance therapy received by these three of these patients were 10-33 mg of vinblastine/m² and 2-8 mg of dactinomycin/m². Reduction of the doses of the cytostatics below the recommended doses (table 1) was due to general toxicity. The 22 patients were divided into two groups ("low-dose" and "high-dose") according to the amount of cytostatics given.

The low-dose group consisted of 10 patients who received total doses of at least one of the cytostatics below 75% (cisplatin and bleomycin) or 50% (vinblastine) of the recommended doses; none of these patients received maintenance therapy. The high-dose group consisted of 12 patients who received total doses of all three drugs exceeding 75% (cisplatin and bleomycin) and 50% (vinblastine) of the recommended doses; three of these patients received maintenance therapy.

We used the Mann-Whitney U test to compare groups of observations and the Wilcoxon signed rank test to compare paired observations. Life-table estimates were calculated on data grouped into years to illustrate the relationship of time to gonadal recovery after PVB treatment. The life-table estimates were compared by the log rank test.

### Results

The 20 patients under surveillance had their first follow-up <2 years after orchiectomy. Sperm counts remained low at the first follow-up in nine of 13 patients with postorchiectomy sperm counts below the reference value (11), but they returned to normal levels in four of 13. Sperm counts decreased permanently in two of seven patients with postorchiectomy values exceeding the reference value and remained normal in five of seven. Sperm counts tended to increase from orchiectomy to the first follow-up, but the trend was not significant (P > .05). Serum follicle-stimulating hormone values were unchanged from orchiectomy to first follow-up (P > .05). Table 2 presents the total sperm counts per ejaculate and the serum follicle-stimulating hormone levels at orchiectomy and at various follow-up intervals.

Most, if not all, patients became azoospermic during PVB treatment. At the first follow-up <2 years after the treatment, the semen samples from nine of 23 patients showed azoospermia, and those from another seven of 23 patients showed sperm counts below 1 million. Compared with the patients under surveillance, the PVB-treated patients had significantly lower sperm counts and significantly higher serum follicle-

### Table 1. Clinical characteristics of 25 patients treated with PVB and 21 patients under surveillance

<table>
<thead>
<tr>
<th></th>
<th>PVB†</th>
<th>Surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in yr (range)</td>
<td>23 (16-35)</td>
<td>31 (19-39)</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminoma</td>
<td>2 (8)</td>
<td>10 (48)</td>
</tr>
<tr>
<td>Nonseminoma</td>
<td>23 (92)</td>
<td>11 (52)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1 (4)†</td>
<td>21 (100)</td>
</tr>
<tr>
<td>II</td>
<td>15 (60)</td>
<td>—</td>
</tr>
<tr>
<td>III</td>
<td>9 (36)</td>
<td>—</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>3 (12)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Proven fertility§</td>
<td>6 (16)</td>
<td>8 (22)</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, values are number of patients (%).
†PVB: 20 mg of cisplatin/m² on days 1-5; 6 mg of vinblastine/m² on days 1-2; and 15 mg of bleomycin/m² on days 2, 9, and 16, given intravenously every 3 wk for a total of six courses.
‡In the PVB group, six patients had fathered at least one child, and 19 had not desired children. In the surveillance group, eight patients had fathered at least one child, two patients had infertility problems, and 11 had not desired children.

### Table 2. Total sperm counts and serum follicle-stimulating hormone levels at orchiectomy and at follow-up after orchiectomy plus chemotherapy or after orchiectomy alone

<table>
<thead>
<tr>
<th></th>
<th>PVB</th>
<th>Surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of patients</td>
<td>Sperm count (×10⁶)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>After orchiectomy</td>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>Follow-up (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-1.4</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>1.5-2.4</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>2.5-3.4</td>
<td>15</td>
<td>82</td>
</tr>
<tr>
<td>3.5-4.4‡</td>
<td>11</td>
<td>48</td>
</tr>
<tr>
<td>≥4.5‡</td>
<td>6</td>
<td>52</td>
</tr>
</tbody>
</table>

*Serum follicle-stimulating hormone 95% reference, 0.5-10 IU/L (Second International Reference Preparation, World Health Organization standard 78/549); reference value, total sperm count exceeding 80 million (11).
†A significant difference (P < .05) was observed when the PVB group and the surveillance group were compared.
‡Number of observations on patients under surveillance too low to allow analysis.
stimulating hormone levels at follow-up 0.5–1.5 years after PVB treatment (P < .05). No significant differences were noted for the ensuing observation intervals (P > .05).

Life-table estimates illustrating the relationship of time to gonadal recovery after PVB treatment are shown in figure 1(a–d). A median time of 1.5 years elapsed before the first recovery of spermatozoa in the semen, and spermatogenesis was estimated to have started in all patients 4 years after PVB treatment. Five years after PVB treatment, 46% of the patients were estimated to have reached their pretreatment total sperm counts, 60% to have total sperm counts exceeding 80 million, and 60% to have normal serum follicle-stimulating hormone levels.

The total sperm counts and the serum follicle-stimulating hormone levels in the patients who received high-dose cytostatic treatment and those who received low-dose cytostatic treatment were not significantly different before treatment (P > .05). The life-table estimates calculated for these patients are also presented in figure 1 (a–d). Patients in the high-dose group tended to recover more slowly than those in the low-dose group. The differences reached significance in patients with normal serum follicle-stimulating hormone levels (X² = 6.32; P < .05). However, they were not significant in patients without azoospermia (X² = 2.86; P > .05), in patients who had total sperm counts at or above the pretreatment value (X² = 3.13; P > .05), or in patients who had total sperm counts of >80 million (X² = 3.48; P > .05).

The serum luteinizing hormone levels in patients after PVB treatment temporarily were significantly higher than those in the patients under surveillance (P < .05) (data not shown). However, no differences were noted between the serum testosterone values in the two groups (P > .05) (data not shown). The serum levels of luteinizing hormone and testosterone in the high-dose group and the low-dose group were similar in patients before and after PVB treatment (P > .05). A serum testosterone value below the reference level or a normal serum testosterone value in combination with an elevated serum luteinizing hormone level was observed at follow-up in 43% of the patients under surveillance and in 73% of the patients treated with PVB. These abnormalities persisted for at least two follow-up periods in 24% of the patients under surveillance and in 18% of the PVB-treated patients. Biochemical evidence indicating a lasting impairment in Leydig cell functioning was observed in one patient under surveillance but in none of the patients treated with PVB.

Discussion

Sperm counts are low in most patients with testicular germ cell cancer after unilateral orchiectomy (6–8,12), and combination chemotherapy can induce additional impairment of spermatogenesis (4,5). In the present study, 70% of the PVB-treated patients had a severe but reversible reduction in their total sperm counts, whereas 55% of the patients under surveillance had follow-up total sperm count levels below the reference value. Sperm production was similar in the two patient groups 1.5 years after the treatment and beyond. Regeneration of spermatogenesis following PVB-induced impairment of spermatogenesis was also demonstrated by others (4,5,13–17). However, the gonadal long-term toxic effects of PVB were not reported previously, and the significance of a control group of patients without surveillance has not been thoroughly investigated (5,18).

The reduction in the sperm production after orchiectomy can be explained partly by cryptorchidism and/or carcinoma in situ of the remaining testis (19,20). Berthelsen and Skakkebæk (6) studied biopsy specimens from the contralateral testis of patients with testicular cancer. They reported irreversible arrest of spermatogenesis in patients without cryptorchidism or carcinoma in situ. In the present study, sperm counts below the reference value were accordingly observed at follow-up in 11 of 20 of the patients under surveillance. The patients under surveillance tended to be older than the PVB-treated patients, partly because of the higher number of patients in the surveillance group with seminoma. However, age cannot explain the high incidence of impairment of spermatogenesis in the patients under surveillance because their initial sperm counts were similar to those of
the PVB-treated patients. The impairment of spermatogenesis is potentially reversible in some patients (6). It may be induced by β-human chorionic gonadotropin or α-fetoprotein produced by the tumor (6,8,12). In the present study, normalization of sperm counts was observed in four of 13 patients under surveillance with postorchiectomy levels below the reference value. The serum levels of β-human chorionic gonadotropin, α-fetoprotein, or both tumor markers were elevated in all four of these patients at orchietomy.

Azoospermia was reported to occur in nearly all patients during PVB treatment (4,5), and a similar severe impairment of spermatogenesis was observed in patients after combination therapy with vindesine, etoposide, and bleomycin (21). In the present study, azoospermia was not observed in all PVB-treated patients, possibly because of the rather long interval between the end of treatment and the first follow-up. However, spermatogenesis was severely depressed in 70% of the PVB-treated patients. Gonadal recovery seems to occur in most PVB-treated patients, and spermatooza have been reported to be present in the semen samples from 70%–75% of the patients 2 years after PVB treatment (4,5). Regeneration occurs even later, as demonstrated in the present study showing that the percentage of PVB-treated patients with spermatooza in semen increased from 59% to 100% between the 2nd and the 4th years of follow-up. Spermatogenesis was not completely restored, and 54% of the patients had sperm counts below the pretreatment level 5 years after PVB treatment. This incomplete recovery was apparently related to the size of the dose and may, therefore, be due to cytotoxicity. A lasting decrease in sperm count developed in two of the patients under surveillance, probably because of preexisting germ cell defects. The progression of such a defect present at orchietomy may also explain the incomplete recovery of spermatogenesis in some of the patients treated with PVB.

Etoposide has recently replaced vindesine in the treatment of testicular cancer, and only three to four cycles of bleomycin, etoposide, and cisplatin (BEP) seem to be needed to cure most patients (22). The germ cell toxic effects of etoposide and vinblastine are comparable in mice (23,24), and the damage induced by BEP therapy to spermatogenesis in man may therefore be similar to that induced by PVB. We observed less gonadal damage in the patients who received the lowest doses of cytostatics. Germ cell destruction may, therefore, be reduced because of the administration of fewer treatment cycles and the ensuing drop in the total amount of cytostatics given.

Serum luteinizing hormone levels exceeding the reference value and/or subnormal serum testosterone levels were observed at follow-up in a significant number of the patients under surveillance. Therefore, a compensatory, slight impairment in Leydig cell functioning seems to be present in the unaffected testis of some patients with testicular cancer. Some investigators (4,5,17) have reported evidence suggesting that impairment of the Leydig cell functioning may be caused by PVB, whereas others (13,15) have reported no effect on serum luteinizing hormone and testosterone levels after PVB treatment. Except for a temporary increase in serum luteinizing hormone levels, the serum levels of luteinizing hormone and testosterone in PVB-treated patients were similar to those observed in patients under surveillance. Therefore, PVB treatment does not seem to induce any changes in the long-term functioning of Leydig cells.

In conclusion, spermatogenesis was regenerated in all patients treated with PVB, but recovery to the pretreatment sperm count levels was not achieved in 54% of the patients 5 years after treatment. This failure to recover may be due partly to cytostatic toxic effects and partly to preexisting germ cell defects. In 55% of the patients under surveillance, defective spermatogenesis persisted or developed Elevated serum levels of luteinizing hormone and/or subnormal serum levels of testosterone were common findings in both PVB-treated patients and patients under surveillance. A control group consisting of patients under surveillance is, therefore, important for the study of the gonadotoxic effects of cytostatics on patients with testicular cancer.

References

Resistance to Drugs Associated With the Multidrug Resistance Phenotype Following Selection With High-Concentration Methotrexate

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To study patterns of resistance at extreme but nevertheless clinically relevant drug concentrations, we developed a series of methotrexate-selected CCRF-CEM sublines, all of which were highly resistant to this antifolate (relative resistance, 10²- to >10⁵-fold). The least methotrexate-resistant subline was completely sensitive to drugs associated with the multidrug resistance phenotype. However, more highly methotrexate-resistant sublines were significantly cross-resistant to vincristine, vinblastine, and daunorubicin (maximum relative resistance, 40-fold). These sublines were not cross-resistant to doxorubicin, daunorubicin, and teniposide. Regression analysis indicated that relative resistance to methotrexate was correlated with relative resistance to vincristine \( (r = 0.96) \) and vinblastine \( (r = 0.99) \). Such cross-resistance in highly methotrexate-resistant cells may have important clinical implications. [J Natl Cancer Inst 81:1250-1254, 1989]

The antifolate methotrexate (MTX) is one of the most widely employed agents in cancer chemotherapy. This drug is of major therapeutic benefit in the treatment of many malignancies, including childhood acute lymphoblastic leukemia (1). Among the features that have contributed to the widespread use of MTX is the fact that, to date, exposure of cells to MTX has not been associated with the development of cross-resistance to any other structurally unrelated antineoplastic agents (2). As such, MTX has been clearly distinguished from drugs associated with the multidrug resistance (MDR) phenotype, in which selection for resistance of cells to certain “natural product” drugs, including the vinca alkaloids, anthracyclines, and epipodophyllotoxins, results in the development of cross-resistance to other members of the MDR family. This cross-resistance occurs even though the cells have never been exposed to MDR phenotype-associated drugs (3). The association of this phenomenon with a protein (termed P-glycoprotein) that enhances drug efflux has been well described in a variety of rodent and human cells (4,5).

The P-glycoprotein-mediated drug efflux system operating in MDR cells appears to function only with hydrophobic, lipophilic compounds. Therefore, we are not surprised that an anionic drug such as MTX does not appear to be a substrate for this system. It is becoming apparent, however, that antifolates per se are not necessarily distinct from the MDR family, since at least two recent reports indicate that cells possessing the MDR phenotype are cross-resistant to the lipophilic antifolate trimetrexate (6,7).

MTX-resistant cell lines derived in vitro are commonly 10 to 1,000 times more resistant than the sensitive parent line (8-10). While such laboratory-derived models of drug resistance might therefore seem extreme, these models often do not achieve the levels of resistance that may be anticipated in the clinical context. For example, in vitro-derived MTX-resistant cell lines are commonly selected with the use of micromolar concentrations of drug (8-10), even though steady-state serum concentrations some five to 1,000 times these levels are routinely achieved with established clinical protocols (11). While cell lines with extreme levels of MTX resistance have been described (12,13), they have not been investigated with respect to cross-resistance to other drugs.

To address this issue, we have exposed CCRF-CEM cells to progressively higher levels of MTX. This has generated a series of extremely resistant cell populations that are capable of routine growth in the presence of the highest clinically achievable concentrations of MTX. We have examined these cells to determine their cross-resistance to other cytotoxic drugs and report here, for the first time, the emergence of progressively increasing resistance to vinca alkaloids and dactinomycin in MTX-resistant cell populations. The observed cross-resistance to drugs normally associated with the MDR phenotype only becomes evident at extreme levels of MTX resistance, and the results are discussed in terms of their clinical significance.

Materials and Methods

Cytotoxic drugs were purchased from Australian subsidiaries of the manufacturers as follows: MTX from David Bull Laboratories; vinblastine and vincristine from Eli Lilly & Co.; doxorubicin from Farmitalia; daunorubicin from May and Baker; dactinomycin from Merck, Sharpe and Dohme Int.; and teniposide from Bristol Laboratories.

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