Effects of taxane-based chemotherapy on inhibin B and gonadotropins as biomarkers of spermatogenesis

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Objective: To assess the effects of taxane-based chemotherapy on the male reproductive axis, and, therefore, its impact on spermatogenesis and fertility.

Design: Controlled clinical study.

Setting: Patients with cancer in an academic research environment.

Patient(s): Forty male patients of reproductive age with cancer with solid tumor at diagnosis, who had been scheduled to receive taxane-based chemotherapy.

Intervention(s): The patients were given treatment with docetaxel or paclitaxel combined with gemcitabine or carboplatin. Blood sampling and testicular ultrasonography were performed before and after completion of chemotherapy.

Main Outcome Measure(s): In all patients, serum levels of inhibin B, FSH, and LH were measured, and, in half of the patients, bilateral testicular volume was also measured.

Result(s): There was a statistically significant decrease in serum inhibin B and increase in serum FSH in all patients after the completion of the taxane-based chemotherapy. The median LH levels did not exhibit a statistically significant increase after the last cycle. Bilateral testicular volume exhibited a statistically significant decrease in 19 out of 20 patients (95%) after completion of chemotherapy.

Conclusion(s): Taxane-based chemotherapy induces the reduction of inhibin B and the reciprocal elevation of FSH, which are associated with significant gonadal damage in the early stages after completion of chemotherapy.

Key Words: Chemotherapy, docetaxel, gonadotoxicity, infertility, paclitaxel, taxanes

During the last two decades, new cytostatic drugs with significant effectiveness against cancer have become available to oncologists. Among the new cytostatic drugs, the taxanes, paclitaxel and docetaxel, are effective agents against several malignancies, and their application in oncology had a favorable influence on tumor response and patients’ survival. The unique chemical structure and mechanism of action of the taxanes, combined with their broad antitumor activities, has rendered them one of the most important categories of anticancer agents (1).

The most important clinical outcomes in the care of the patient with cancer are overall and disease-free survival, as well as quality of life and toxicity (2). Chemotherapy toxicity is an important end point in clinical trials, and major effort is made toward achieving the best efficacy and minimal toxicity of each regimen. There are several toxicity grading scales to assess treatment-related toxicities. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 is a descriptive terminology that can be used for adverse event reporting. A grading (severity) scale is provided for each adverse event. According to the NCI-CTCAE the reproductive function and the endocrine system are separate categories associated with fertility and infertility (3).

Fertility is of principal concern in patients of reproductive age who have cancer and a major aspect of their quality of life. Oncologists should be aware of the potential gonadotoxic effects of the regimens they use for the treatment of reproductive-aged patients. They are also responsible to inform the patient about the risk of treatment-induced infertility. Recent surveys of male and female cancer survivors of reproductive age show that at least half of the patients have no recollection of a discussion concerning fertility before starting chemotherapy (4–8). Patients should be aware of the possibility of sperm banking before the initiation of chemotherapy. It seems that cryopreservation is the only preemptive measure available to preserve fertility in young patients with cancer. There are, however, a number of drawbacks concerning this method. Serious concerns have been raised regarding possible negative effect of cryopreservation on a variety of sperm functions, as well as on overall sperm quality. Decrease in sperm concentration and motility owing to the freezing process is considered the main cause. Furthermore, ethical and cultural considerations may be
involved in a patient’s final decision regarding sperm banking (9, 10).

The risk of infertility and the impact of cancer treatment on spermatogenesis are described in literature. The chemosensitivity variables that correlate with the spermatoxicity of chemotherapy are the category and number of antineoplastic agents used, the total dose, the duration of treatment, and the age and the individual sensitivity of each patient. Nevertheless, the gonadotoxic effects of newer chemotherapeutic agents, such as taxanes, remain unknown in human beings (4, 9, 11, 12). The only reproductive and developmental studies concerning taxanes have been performed on experimental animals (13). As far as other chemotherapeutic drugs are concerned, platinum compounds are likely to induce azoo- sperma, but this has been observed when they are coadministered with other high-stereilizing agents (9). Carboplatin seems to cause less sterility than cisplatin (14). The potential gonadotoxic effect of the antimetabolite gemcitabine has been observed only in experimental studies in male mice and ranges from moderate to severe (15).

The testis consists of the germinal epithelium arranged in tubules and of the endocrine components (Leydig cells). The seminiferous tubules contain the germ cells and the Sertoli cells, which support and regulate germ cell differentiation. Drugs enter the testis by the vasculature in the interstitial region and then can reach the Leydig and Sertoli cells, as well as the spermatogonia, which are at the outer rim of the tubules. Many chemotherapeutic agents can even penetrate the Sertoli cell barrier and damage late-stage germ cells. The final recovery of sperm production depends on the survival of the spermatogonial stem cells and their ability to differentiate (16). Sertoli cells primarily secrete a 32-kDa glycoprotein hormone, the inhibin, which suppresses FSH secretion by gonadotropes. The form of inhibin that is secreted by human Sertoli cells, inhibin B, selectively suppresses FSH secretion in gonadotropes by inhibiting transcription of the gene encoding the β subunit of FSH (17).

The loss of germ cells has secondary effects on the hypothalamic-pituitary-gonadal axis. Inhibin B secretion by the Sertoli cells decreases, and as inhibin B limits FSH secretion by the pituitary, serum FSH rises. Germinial aplasia results in decreased levels of inhibin B and increased levels of FSH. Although FSH measurements have been used as a surrogate for sperm count, they only show an imperfect correlation, partly because of interpatient variability in baseline levels; inhibin B would be more reliable but is not routinely measured. Germinial aplasia also reduces testis size (16). This study was conducted to assess the effects of taxane-based chemotherapy on the male reproductive axis and, therefore, its impact on fertility.

**MATERIALS AND METHODS**

**Patients**

From January 2005 to March 2007, 40 patients of reproductive age with cancer and solid tumor at diagnosis who had been scheduled to receive taxane-based chemotherapy were recruited to the study. Exclusion criteria were prior cancer treatment (either radiotherapy or chemotherapy), testicular cancer, medical history of infertility, genital abnormality or infection, and finally age younger than 20 years or older than 60 years. The patients were given docetaxel (70–80 mg/m²) or paclitaxel (130–200 mg/m²) combined with gemcitabine (1,000 mg/m²) or carboplatin (400 mg/m²). Treatment was repeated every 3 or 4 weeks, according to the chemotherapeutic protocol. In all patients, serum levels of inhibin B, FSH, and LH were measured before and after the completion of chemotherapy, and in half of the patients bilateral testicular volume also was measured at these stages. Bilateral testicular volume was determined as the sum of the right and left testicle volume.

All patients gave written informed consent before enrollment in the study. The study was approved by the University of Athens Ethics Committee.

**Hormone Analysis**

Blood samples were obtained between 7 and 9 AM, before the infusion of the chemotherapy, and were centrifuged after clotting. Single serum samples were stored at −80°C until analysis. Inhibin B concentrations were measured with use of a specific enzyme-linked immunosorbent assay (DSL-10-84100 active inhibin B ELISA; DSL) with an intra-assay variation of 4.1% and an interassay variation of 6.4%. The detection and captured antibodies used (two-site “sandwich” ELISA) are highly specific for the dimeric inhibin B molecule, with negligible cross-reactivity to the pro-alpha subunit, inhibin A, or activin. The detection limit was 7 pg/mL. Radioimmunoassays were used to measure serum FSH (intra-assay variation of 5.3%, interassay variation of 7.1%) and LH levels (intra-assay variation of 3.9%, interassay variation of 4.6%). The reference values of our laboratory were FSH 1.5 to 13 IU/L and LH 1.4 to 9 IU/L.

**Testicular Volume**

Testicular dimensions were measured with use of ultrasonography with a 7.5-MHz probe (Acuson Sequoia 512–Linear 8L5). This method is considered objective, accurate, and reproducible. The testicular volume was calculated with use of the formula: 

\[ V = \left(\pi/6\right) \times (\text{Longitudinal axis}) \times (\text{Transverse axis}) \times (\text{Depth axis}), \]

where \( V = \text{testis volume} \) and \( \pi/6 = 0.52 \), which is the constant generally used in the assumption that the testicle resembles an ellipsoid in shape (18–20). The measurement was performed before and after the treatment by a single experienced radiologist who had no knowledge of the patient’s hormone levels and cumulative dose of taxanes.

**Statistical Analysis**

All results are expressed as mean ± SEM or as median values. Data were computed by the SPSS statistical software package (version 15.0; SPSS, Inc., Chicago, IL). More specifically, statistical analysis of the values before and after chemotherapy
was performed with use of paired *t*-test and the Mann-Whitney test, on the basis of the data distribution (parametric or nonparametric). The correlation between the variables was determined by the Spearman correlation coefficient. A *P* value of < .05 was considered to be statistically significant.

**RESULTS**

The patients’ characteristics are presented in Tables 1 and 2. The mean age of patients was 53.05 ± 1.24 years (range 28–60 years). Thirty-seven out of 40 patients had a diagnosis of non-small-cell lung cancer, one had head and neck cancer.
TABLE 2

Statistical evaluation of patients’ characteristics.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (median-range)</td>
<td>53.05 ± 1.24 (28–60)</td>
</tr>
<tr>
<td>Type of malignancy (n) (%)</td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>37/40 (92.5)</td>
</tr>
<tr>
<td>AdenoCa ups</td>
<td>2/40 (5)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>1/40 (2.5)</td>
</tr>
<tr>
<td>Chemotherapeutic regimens (n) (%)</td>
<td></td>
</tr>
<tr>
<td>Paclitaxel-gemcitabine</td>
<td>14/40 (35)</td>
</tr>
<tr>
<td>Paclitaxel-carboplatin</td>
<td>2/40 (5)</td>
</tr>
<tr>
<td>Docetaxel-gemcitabine</td>
<td>11/40 (27.5)</td>
</tr>
<tr>
<td>Docetaxel-carboplatin</td>
<td>13/40 (32.5)</td>
</tr>
<tr>
<td>No. of cycles (median-range)</td>
<td>3 (2–6)</td>
</tr>
<tr>
<td>Taxanes total dose (mg) (median-range)</td>
<td></td>
</tr>
<tr>
<td>Docetaxel</td>
<td>695 (252–1,440)</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>940 (680–1,950)</td>
</tr>
</tbody>
</table>

Note: NSCLC = non-small-cell lung cancer; AdenoCa ups = adenocarcinoma of unknown primary site.
The data were evaluated with use of the paired t-test.


carcinoma, and two patients were given treatment for adeno- carcinomas of unknown primary site. In 24 patients, we used docetaxel (70–80 mg/m²) with gemcitabine (1,000 mg/m²) or carboplatin (400 mg/m²) and, in 16 patients, we administered paclitaxel (130–200 mg/m²) with gemcitabine (1,000 mg/m²) or carboplatin (400 mg/m²). The median number of taxane-based chemotherapy cycles received was three (range two to six cycles). The median total doses for paclitaxel and docetaxel were 940 mg (range 680–1950 mg) and 695 mg (range 252–1440 mg), respectively.

Pretreatment and postchemotherapy median levels of inhibin B, gonadotropins (FSH, LH), bilateral testicular volume, and inhibin B/FSH ratio are shown in Table 3. These levels were also evaluated separately in each of the two taxane groups (the docetaxel and the paclitaxel group of patients), as presented in Table 4. After completion of the taxane-based chemotherapy, serum inhibin B showed a statistically significant decrease in all patients from a median of 97.7 pg/mL (range 1.04–1,753 pg/mL) to a median of 40.1 pg/mL (range 0–668 pg/mL), P < .01. The decrease in the serum inhibin levels again was statistically significant when we examined the docetaxel and the paclitaxel groups separately. On the other hand, serum FSH levels showed a statistically significant increase in all men after treatment (a median of 6.65 IU/L [range 0.5–26.2 IU/L] dropped to a median of 10.3 IU/L [range: 2.6–32.3 IU/L], P ≤ .001), as well as in each taxane group separately. The inhibin B/FSH ratio exhibited a statistically significant decrease from a median of 15.6 (range 0.04–541) to a median of 4.72 (range 0–101), P ≤ .001. This difference was statistically significant in each of the taxane groups as well. Serum LH levels increased in 27 of 40 patients (67.5%) and decreased in the remaining 13 of 40 patients (33.5%). The median LH levels before chemotherapy and after the last cycle were 4.80 IU/L (range 0.9–13.6 IU/L) and 5.04 IU/L (range 2–13.7 IU/L), respectively, which did not represent a statistically significant increase (P > .05). The LH levels again did not exhibit a statistically significant increase in either of the two taxane groups (P > .05). In 19 out of 20 patients (95%), there was a statistically significant decrease in the bilateral testicular volume after completion of chemotherapy from a median of 27.6 mL (range 13.8–37.3 mL) to a median of 23.5 mL (range 9.2–32.8 mL), P ≤ .001. This decrease also was statistically significant in each of the docetaxel and paclitaxel groups of patients, when examined separately.

As was expected, a significant inverse correlation between serum inhibin B and FSH concentrations (r = −0.4827, P ≤ .001) was found, whereas LH levels did not correlate with inhibin B levels (r = −0.0883, P > .05). Finally, FSH was statistically significantly inversely correlated with bilateral testicular volume (r = −0.3166, P < .05). Inhibin B and

TABLE 3

Pretreatment and postchemotherapy levels of FSH, LH, bilateral testicular volume, and inhibin B/FSH ratio.

<table>
<thead>
<tr>
<th>Median value (range)</th>
<th>Before chemotherapy</th>
<th>After chemotherapy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibin B (pg/mL)</td>
<td>97.7 (1.04–1,753)</td>
<td>40.1 (0–668)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>6.65 (0.5–26.2)</td>
<td>10.3 (2.6–32.3)</td>
<td>≤ .001</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>4.80 (0.9–13.6)</td>
<td>5.04 (2–13.7)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>BTV (mL)</td>
<td>27.6 (13.8–37.3)</td>
<td>23.5 (9.2–32.8)</td>
<td>≤ .001</td>
</tr>
<tr>
<td>Inhibin B/FSH ratio</td>
<td>15.6 (0.04–541)</td>
<td>4.72 (0–101)</td>
<td>≤ .001</td>
</tr>
</tbody>
</table>

Note: BTV = bilateral testicular volume. The data were evaluated with use of the paired t-test.


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LH concentrations were not correlated with bilateral testicular volume.

**DISCUSSION**

This is the first report of the dynamic changes in inhibin B and gonadotropin levels of patients with cancer given treatment with taxane-based chemotherapy with respect to their gonadal function. Although the gold standard of fertility studies in men is semen analysis, measurements of inhibin B and gonadotropins contribute to an indirect evaluation of fertility and are extremely useful in cases where sperm samples are not available, for example, for cultural or ethical reasons (21). Inhibin B also can be used as a direct marker of Sertoli cell function and spermatogenesis in adult males (22–25).

Serum inhibin B and FSH levels correlate well with sperm concentration and thus can play a role as serum markers of spermatogenesis (26). Combined measurements of inhibin B and FSH have shown to be a better indicator of spermatogenesis adequacy than either measurement by itself (27).

In our study, inhibin B was decreased after completion of chemotherapy in all patients in a statistically significant manner. According to a summary of clinical studies, normal serum levels of inhibin B between the ages of 45 and 59 years are between 60 and 120 pg/mL. On the other hand, cutoff levels of inhibin B in infertile men with elevated FSH are <43 pg/mL. Very low levels of inhibin B are found in men with negligible or zero sperm production (28). It seems that taxanes are related to early gonadotoxicity by altering the levels of inhibin B from normal to levels observed in infertile men. The toxicity was induced by a median value of three cycles of taxanes. Three cycles of taxane-based chemotherapy are typical in the adjuvant or neoadjuvant setting of solid-tumor treatment. Patients of reproductive age and especially those who are scheduled to receive adjuvant chemotherapy should be informed about its toxic effects to proceed to sperm cryopreservation to preserve their fertility (9).

In addition to inhibin B, bilateral testicular volume was assessed as a direct marker of the taxane-induced gonadal toxicity. Bilateral testicular volume after completion of chemotherapy showed a statistically significant decrease in all patients, as well as in each taxane group of patients. Although the volume of the testicles was reduced, their value was within normal range (>20 mL). An indirect evaluation of gonadal dysfunction can be made by assessing the pituitary production of FSH and LH, both of which rise in complete gonadal failure (21). According to the findings of our study, serum FSH levels showed a statistically significant increase in all patients (as well as in the two separate taxane groups) but remained within the normal reference values of our laboratory. Elevation of FSH is an additional indication of the gonadotoxic effects of the taxanes. Serum LH levels, on the other hand, were increased after the last cycle, but this difference was not statistically significant and remained within normal ranges, as with FSH. Finally, the inhibin B/FSH ratio showed a statistically significant decrease, and combined measurement of these two spermatogenesis markers confirms the harmful effects of taxanes on gonads. Our study did not confirm the association between inhibin B and testicular volume that was shown by other series (21, 25). A possible explanation for this is that, although the dimensions of testes were reduced, their size continued to be within normal ranges. In contrast with previous studies, none of our patients had small testicular volumes, even after chemotherapy. Additionally, biochemical alterations usually take place before clinical changes.

We observed that results in each of the two separate taxane groups are in accordance with those we reached when examining all patients together, showing that both of the taxanes have the gonadotoxic effect being studied. It should be noted, however, that the study of Reh et al. (29), which examined amenorrhea rates in 45 patients with breast cancer, found no significant long- or short-term gonadotoxic effect of taxanes on ovarian function.

There are several limitations in our study. First, the follow-up of the patients’ fertility status only extended for the period...
they underwent chemotherapy, and therefore it is very short (2-6 months). This was due to the age of patients and type of malignancy they had. Most of them had non-small-cell lung cancer, disease progression soon was diagnosed, and they had to be excluded from follow-up because of the initiation of a different chemotherapeutic regimen. There are no data indicating whether the inhibin B reduction or the FSH increase continues to persist after a long-term period or whether their values return to normal, to determine whether taxane-induced gonadotoxicity is permanent or temporary. Furthermore, other drugs administered in combination with taxanes, such as carboplatin or gemcitabine, also may have a gonadotoxic effect. Finally, the lack of semen analysis, for ethical reasons, does not allow the evaluation of the direct effect of the taxanes on sperm production. In conclusion, taxane-based chemotherapy induces reduction of inhibin B, reciprocal elevation of FSH, as well as bilateral testicular volume decrease, which are alterations associated with significant gonadal damage in the early stages after completion of chemotherapy.

REFERENCES
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Taxane-based chemotherapy induces reduction of inhibin B, elevation of FSH, and bilateral testicular volume decrease, indicating reproductive toxicity of these drugs in the early stages after completion of chemotherapy.