

Impaired Testicular Function in Patients With Carcinoma-In-Situ of the Testis

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Purpose: To elucidate the biologic association between germ cell neoplasia and testicular dysfunction, through investigation of Leydig cell function and semen quality in men with carcinoma-in-situ (CIS) of the testis.

Patients and Methods: We examined two groups of men, unilaterally orchidectomized for testicular cancer. Biopsy of the contralateral testis had showed CIS in a group of 24 patients and no evidence of CIS in the other group of 30 patients. Semen quality and serum levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were compared in these two groups of men after orchidectomy but before further treatment for testicular cancer.

Results: Significantly higher LH levels (median, 8.1 IU/L v 4.8 IU/L; $P < .001$) and generally lower testosterone levels (median, 12.5 nmol/L v 15.5 nmol/L; $P = .13$) were found in the CIS group. The proportion of patients with Leydig cell dysfunction was higher in the group of

patients with CIS (11 of 24) than in the group of patients without (two of 30) ($P = .01$). Sperm concentration and total sperm count were significantly lower ($P < .001$) in patients with CIS (median, $0.03 \times 10^6/\text{mL}$ and 0.10×10^6 , respectively) than in patients without (median, $9.1 \times 10^6/\text{mL}$ and 32×10^6 , respectively), whereas the levels of FSH were significantly higher ($P < .001$) in the former group of men (median, 19.6 IU/L v 9.0 IU/L).

Conclusion: Not only spermatogenesis but also Leydig cell function is impaired in testes with CIS. This impairment could be due to common factors in the pathogenesis of germ cell neoplasm and testicular dysfunction. Alternatively, CIS cells may have a negative impact on Leydig cell function.

J Clin Oncol 17:173-179. © 1999 by American Society of Clinical Oncology.

IT IS WELL ESTABLISHED that testicular germ cell cancer (TGCC) is associated with poor spermatogenesis¹⁻⁸ and occurs more frequently in men with other types of testicular dysfunction, such as testicular atrophy and cryptorchidism.⁹⁻¹⁴ However, it remains to be established whether these testicular abnormalities cause germ cell cancer or whether they are associated with germ cell cancer because of common etiologic factors. Increased incidence of genital abnormalities and germ cell cancer in patients with testicular dysgenesis and the androgen insensitivity syndrome indicates that changes in the fetal endocrine milieu could be involved in the pathogenesis of testicular cancer.¹⁵⁻¹⁷ Moreover, even though the data are ambiguous, some epidemiologic studies have shown that factors, which are associated with variations in intrauterine hormone levels, are correlated with the risk of testicular cancer.^{14,18-20} It is thus likely that testicular neoplasia is associated with fetal exposure to harmful endocrine factors. If this is true, one would expect that not only spermatogenesis but also Leydig cell function could be impaired in a testis with germ cell neoplasia, even before the development of invasive disease. The finding of decreased Leydig cell function in patients orchidectomized for testicular cancer compared with patients orchidectomized for other causes is in line with these suggestions.²¹ However, little is known about Leydig cell function in these patients.

To elucidate the hypothesis of an association between germ cell neoplasia and testicular dysfunction, we compared

testicular function in men orchidectomized for testicular cancer with and without carcinoma-in-situ (CIS) in the contralateral testis.

PATIENTS AND METHODS

Subjects

Group 1 consisted of 25 consecutive patients who had CIS in the contralateral testis at the time of orchidectomy for TGCC. They were examined from 1986 to 1993 after orchidectomy but before further treatment.

Group 2 included 31 consecutive patients who had unilateral testicular cancer and no evidence of CIS in the contralateral testis. They were examined from 1995 to 1996 after orchidectomy but before further treatment.

Biopsy of the contralateral testis was performed in all patients at the time of orchidectomy. The diagnosis of CIS was in all cases established

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Submitted June 17, 1998; accepted September 23, 1998.

Supported by the Danish Cancer Society.

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0732-183X/99/1701/0173\$3.00/0

by histologic examination of the biopsy specimens. The amount of CIS was assessed semiquantitatively by determining the percentage of tubules containing CIS. In each group, one patient was excluded because of elevated levels of human chorionic gonadotropin beta in serum. Characteristics of patients in the study are shown in Table 1. All patients were examined less than 6 months after orchidectomy, and there was no statistically significant difference in age between the two groups. Semen analyses and analyses of serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estradiol were performed as described in the next sections.

Semen Analysis

All analyses of ejaculates were performed in our laboratory, in accordance with the guidelines of the World Health Organization.^{22,23} The following parameters were analyzed: volume, total sperm count, sperm concentration, morphology, motility, and duration of abstinence. Ejaculates for analysis were obtained from 23 patients in group 1 and 26 patients in group 2.

Hormone Analyses

Serum values of FSH and LH were measured by time-resolved immunofluorometric assay (DELFLIA, Wallac, Turku, Finland). Luteinizing hormone and FSH levels were expressed in international units per liter. Detection limits and interassay variations for LH and FSH were 0.05 and 7% and 0.05 and 10%, respectively. Testosterone and estradiol were analyzed by radioimmunoassay (Coat-a-Count, DPC, Los Angeles, CA). Detection limits and interassay variations for testosterone and estradiol were 0.23 and 13% and 18 and 13%, respectively. Reference values for LH, FSH, testosterone, and estradiol are given in Table 2. Blood samples for hormone analyses were obtained from all men. All analyses were performed during the years 1995 and 1996. Blood samples from patients examined before 1995 had been stored at -20°C. The consequence of freezing has been tested and no detectable effect on the results of hormonal analyses was seen.

Table 1. Patient Characteristics

| | Group 1 (n = 24) | Group 2 (n = 30) | P* |
|---|---------------------|---------------------|--------|
| Age at orchidectomy, years | | | |
| Median | 26 | 29 | .49 |
| Range | 19-52 | 21-39 | |
| Time from orchidectomy to examination, months | | | |
| Median | 1 | 5 | < .001 |
| Range | 0-4 | 0-6 | |
| Diagnosis | | | |
| Seminoma/nonseminoma | 17/7 | 17/12† | .57 |
| Cancer stage | | | |
| I | 21 | 25 | .43 |
| II | 3 | 3 | |
| III | 0 | 1 | |

*Group 1 versus group 2.

†One patient had unilateral CIS.

Table 2. Results of Hormone Analyses

| | Group 1 (n = 24) | Group 2 (n = 30) | Reference Values | P* |
|--------------------------|---------------------|---------------------|------------------|--------|
| FSH, IU/L | | | | |
| Median | 19.6 | 9.0 | 1.2-22 | < .001 |
| Range | 6.3-87 | 0.31-47 | | |
| LH, IU/L | | | | |
| Median | 8.1 | 4.8 | 1.1-11 | < .001 |
| Range | 3.3-56 | 0.82-27.4 | | |
| Testosterone, nmol/L | | | | |
| Median | 12.5 | 15.5 | 6.2-32.5 | .13 |
| Range | 2.8-23.8 | 7.3-35.9 | | |
| Estradiol, pmol/L | | | | |
| Median | 73† | 76† | 44-212 | .32 |
| Range | 23-118 | 30-153 | | |
| Leydig cell dysfunction§ | 11 | 2 | | .01 |

NOTE. Values are expressed as median, range, or n.

*Group 1 versus group 2.

†n = 17.

‡n = 14.

§LH level above reference value and/or testosterone level below reference value.

Statistical Methods

The Mann-Whitney test was used for comparison of the data in the two groups because neither response parameters nor log-of-response parameters were normally distributed. The correlations between time from orchidectomy to examination and semen characteristics and hormone levels were tested by calculation of Spearman rank correlation coefficient. The χ^2 test was used to test the statistical significance of the differences between the groups in the numbers of patients with seminomas and nonseminomas, the number of patients with azoospermia, and the number of patients with Leydig cell dysfunction. The relationship between amount of CIS and response parameters was examined by dividing the patients in group 1 into two groups of 12 patients with the highest and lowest amounts of tubules containing CIS. The data were analyzed using Mann-Whitney test.

RESULTS

Semen Analysis

The results of semen analyses are shown in Table 3 and Fig 1. The duration of abstinence was not statistically different between the two groups. The differences in sperm concentration and sperm count between group 1 and group 2 were highly significant. Sufficient numbers of spermatozoa to allow analysis of morphology were present in only seven patients in group 1 and 20 patients in group 2, and motility could be analyzed in only 10 patients in group 1 and 21 patients in group 2. In this small subset of patients, we did

Table 3. Results of Semen Analyses

| | Group 1 (n = 23) | Group 2 (n = 26) | P* |
|---|---------------------|---------------------|--------|
| Abstinence time, days | | | |
| Median | 3.0 | 2.5† | .48 |
| Range | 0.5-10 | 0.5-8 | |
| Total sperm count, ×10 ⁶ | | | |
| Median | 0.10 | 32 | < .001 |
| Range | 0-25 | 0-200 | |
| Sperm concentration, ×10 ⁶ /mL | | | |
| Median | 0.03 | 9.1 | < .001 |
| Range | 0-13.3 | 0-69 | |
| Volume, mL | | | |
| Median | 2.3 | 2.9 | .09 |
| Range | 0.5-6.1 | 1.4-12.1 | |
| Motile sperm, %‡ | | | |
| Median | 57§ | 65 | .60 |
| Range | 35-75 | 0-87 | |
| Sperm with normal morphology, %‡ | | | |
| Median | 33¶ | 36# | .62 |
| Range | 27-45 | 21-61 | |
| Azoospermia | 11 | 1 | .002 |

NOTE. Values are expressed as median, range, or n.

*Group 1 versus group 2.

†n = 19.

‡Patients with azoospermia and severe oligozoospermia were not included.

§n = 10.

||n = 21.

¶n = 7.

#n = 20.

not find any statistically significant differences between the two groups in proportions of sperm with normal morphology nor in proportions of motile sperm. No correlation was seen between time from orchidectomy to examination and sperm concentration or total sperm count. In group 1, the groups with the highest and lowest amounts of CIS had similar sperm concentrations and total sperm counts ($P = .38$ and $P = .41$), respectively.

Hormone Analyses

The results of the hormone analyses are presented in Table 2. The serum values of FSH were significantly higher in group 1 than in group 2 (Fig 1), and serum LH concentrations were significantly higher in group 1 than in group 2 (Fig 2). Moreover, serum testosterone showed a tendency toward lower values in group 1 than in group 2 (Fig 2). All patients in group 2 had serum levels above the lower limit of the reference values, whereas three patients in group 1 had testosterone levels below the lower limit of normality. Furthermore, the number of patients with Leydig cell dysfunction, as indicated by testosterone levels below reference values and/or LH levels above the reference interval, was significantly higher in group 1 than in group 2. Estradiol levels in the two groups were not significantly different. No correlation was seen

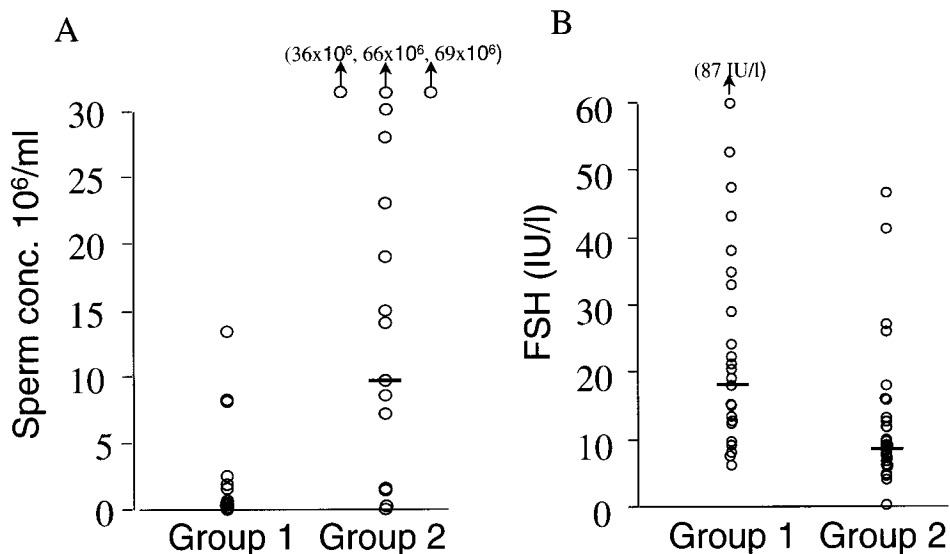


Fig 1. Sperm concentrations (A) and serum FSH values (B) in patients with (group 1) and without (group 2) CIS in the contralateral testis after orchidectomy for testicular cancer. The differences in sperm concentration and FSH levels were highly significant ($P < .001$). Bar = median value. The median sperm concentration in group 1 was 0.03×10^6 /mL.

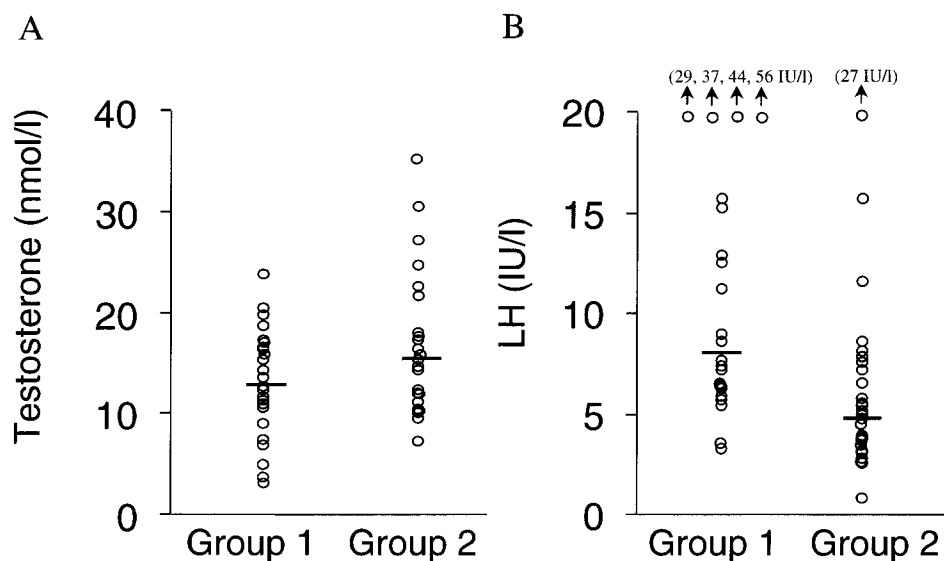


Fig 2. Serum testosterone (A) and LH (B) values in patients with (group 1) and without (group 2) CIS in the contralateral testis after orchidectomy for testicular cancer. The difference in testosterone levels did not reach statistical significance ($P = .13$), whereas the difference in LH levels was highly significant ($P < .001$). Bar = median values.

between time from orchidectomy to investigation and any of the hormone levels. In group 1, the groups with the highest and lowest amounts of CIS had similar levels of FSH, LH, and testosterone ($P = .99$, $P = .55$, and $P = .98$), respectively.

DISCUSSION

We found a pronounced impairment of both Leydig cell function and spermatogenesis in testes with CIS. This is to our knowledge the first study that demonstrates this relationship between CIS in the testis and Leydig cell dysfunction.

The Leydig cell dysfunction in the CIS group was expressed as testosterone levels below reference values and/or LH levels above reference values in a higher proportion of men, significantly higher LH levels, and a tendency toward lower testosterone levels, compared with levels in a similar group of patients without CIS.

The low sperm output and high FSH levels in patients with CIS in the contralateral testis are in line with the observations in histologic studies that have shown generally poor spermatogenesis in testes harboring germ cell neoplasia (Fig 3).^{24,25} The purpose of the biopsies was to assess whether the testis harbored CIS. The histologic investigation did not reveal any apparent relationship be-

tween the amount of CIS and semen-related parameters or hormone levels.

In the present study, no correlation was seen between time from orchidectomy to examination and semen characteristics or levels of any of the reproductive hormones in the two groups of patients. Thus, the differences between semen characteristics and hormone levels in men with and without CIS cannot be explained by differences in time from orchidectomy to investigations.

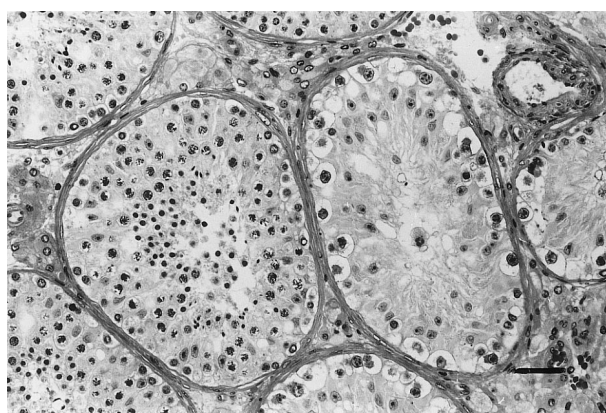


Fig 3. Histologic section of testicular tissue from a patient with CIS of the testis. Right: tubule with CIS cells and Sertoli cells only. Left: impaired spermatogenesis in a tubule without CIS cells. Hematoxylin and eosin stain. Bar: 50 μ m.

Our findings could be explained by a common etiology of germ cell neoplasia, Leydig cell dysfunction, and impairment of spermatogenesis or impairment of Leydig cell function due to presence of CIS cells in the seminiferous tubules. In laboratory animals, exposure to excessive amounts of estrogens in fetal life causes disturbances of Leydig cell and of Sertoli cell development, resulting in permanent Leydig cell dysfunction and impairment of spermatogenesis.^{26,27} Fetal exposure to diethylstilbestrol has been shown to increase the risk of development of gonadal malformations and poor semen quality in humans,²⁸⁻³⁰ although the studies were inconclusive regarding the risk of developing testicular cancer.³¹ Epidemiologic investigations have shown that conditions associated with abnormal intrauterine hormone levels are correlated with risk of testicular cancer.^{14,18-20} Thus, even though the data are ambiguous,³² hormonal involvement in the pathogenesis of TGCC seems likely.

Possibly CIS cells may inhibit Leydig cell function directly, via Sertoli cells or because of germ cell depletion. We have no information on factors produced by CIS cells that may inhibit Leydig cell function. However, because tubules with CIS are depleted of germ cells and tubules in the same testis without CIS often contain germ cells (Fig 3), CIS may have some influence on surrounding cells. Impaired Sertoli cell function in tubules with CIS and in tubules with impaired spermatogenesis due to other causes has been indicated by abnormal expression of cytokeratins in Sertoli cells of these men.³³ Inhibition of Sertoli cell function and germ cell depletion could possibly cause secondary alterations in Leydig cell function because these cells interact in a complex manner.³⁴ Several paracrine factors, including activin, LH-releasing hormone-like factor, and growth factors such as insulin-like growth factor I, transforming growth factor-beta, and fibroblast growth factor, produced by Sertoli cells, may be involved in these interactions with Leydig cells,³⁴⁻³⁷ but we have no data indicating alterations in production of these paracrine factors in tubules with CIS. At present, it is not known whether germ cell depletion has any effect on Leydig cell function.

Our findings have important clinical implications because patients with CIS require treatment that has serious impact on their gonadal function. The options for treatment of CIS are either radiotherapy (RT), with a dose level that eradicates CIS cells without severe impairment of Leydig cell function,³⁸ or surveillance followed by orchidectomy when a second invasive testicular cancer evolves. The reason for the

latter strategy might be a desire to preserve fertility until the development of a second testicular cancer. It has been shown that almost all, if not all, cases of CIS will progress to invasive cancer if left untreated.³⁹ Permanent androgen insufficiency after bilateral orchidectomy would, therefore, be the final outcome in all patients with CIS in the contralateral testis if the strategy of surveillance was followed. In the present study, 11 of 23 patients with bilateral disease had azoospermia and only three patients had sperm concentrations above 5 times 10^6 /mL, which in infertile men has been shown to be the critical limit for inducing pregnancy without assisted fertilization.⁴⁰ Therefore, few if any patients with bilateral germ cell neoplasm are expected to be able to induce pregnancy without assisted reproductive techniques. Thus, *in vitro* fertilization with intracytoplasmic sperm injection is needed to achieve paternity in the vast majority of patients, irrespective of whether they are treated with RT at the moment of diagnosis or whether the treatment is postponed until development of invasive cancer. The present results are in line with the observation that none of 38 men with bilateral testicular cancer achieved fatherhood between diagnoses of first and second tumors.⁴¹

We therefore recommend that patients orchidectomized for testicular cancer in whom CIS is present in the contralateral testis receive RT at a dose that eradicates CIS cells but preserves Leydig cell function.³⁸ The treatment should be preceded by cryopreservation of semen in patients with viable sperm in the ejaculate. A report has indicated that patients with CIS may actually be fertile,⁴² and treatment can be individualized and delayed for a few months in patients with good semen quality and a keen desire for fatherhood. The high number of patients with Leydig cell dysfunction (11 of 24) indicates that andrologic follow-up is necessary in all patients with CIS in the contralateral testis in order to fulfill any need for androgen substitution.

In conclusion, we demonstrated that Leydig cell function and spermatogenesis are severely affected in testes with CIS. It remains to be established whether there is a pathogenic link between the development of testicular germ cell neoplasia and Leydig cell dysfunction. However, the present results are in line with the assumption that hormonal factors are involved in the etiology of testicular germ cell neoplasia. In light of the poor semen quality associated with CIS in the testis, local RT at a dose that eradicates the CIS cells but allows preservation of Leydig cell function is the optimal treatment for patients with CIS in the contralateral testis.

Careful surveillance of androgen status in these patients is needed before and after RT to detect and treat androgen insufficiency.

ACKNOWLEDGMENT

We thank Kenneth M. Grigor, Department of Pathology, Edinburgh University, Edinburgh, Scotland, for revising the manuscript.

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