High prevalence of testicular cancer in azoospermic men without spermatogenesis

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BACKGROUND: An increased risk of testicular cancer in men with infertility and poor semen quality has been reported. Our aim was to investigate the prevalence of testicular nodules and cancer in azoospermic subjects with different spermatogenetic patterns. METHODS: A total of 1443 consecutive infertile men were investigated, out of which 145 (10.0%) were found to be azoospermic. By using clinical examination and testicular ultrasound, 11 out of the 145 patients showed testicular nodules (2.8–26 mm). To obtain spermatozoa for assisted reproduction, 97 subjects required testicular sperm extraction (TESE) and biopsy, including the 11 patients with nodules. They were divided into two groups according to biopsy results: Group A (n = 38) with complete Sertoli cell-only syndrome (SCOS) and Group B (n = 59) with varying spermatogenetic patterns. Ten nodules were found in Group A and one in Group B. RESULTS: In azoospermic men, the overall prevalence of nodules was 7.5%. In complete SCOS, the prevalence of nodules and cancer was 10/38 (26.3%) and 4/38 (10.5%), respectively. Amongst the cancers, one embryonal carcinoma, one seminoma and two in-situ carcinomas were found. CONCLUSIONS: The prevalence of testicular nodules and cancer in azoospermic men with complete SCOS is very high. In these subjects, the role of clinical evaluation, ultrasound and biopsy should be emphasized.

Key words: azoospermia/cancer/male infertility/SCOS/testicular nodule

Introduction

A relationship between spermatogenesis impairment, testicular cancer, hypospadias and cryptorchidism was suggested, and a new pathogenic picture called testicular dysgenesis syndrome (TDS) was coined (Lutke Holzik et al., 2003; Skakkebaek, 2004; Boisen et al., 2004). A possible correlation between these developmental disorders supports the hypothesis of a common factor leading to abnormal semen and testicular cancer (Botchan et al., 1997; Moller and Skakkebaek, 1999).

In this syndrome, testicular germ cells seem to fail to correctly differentiate, predisposing to in-situ carcinomas (ISCs) and developing into cancer in adulthood (Skakkebaek et al., 1987). Impaired intrauterine growth could be responsible for an increased risk of TDS (Main et al., 2006). Fetal exposure to toxicants also induced focal dysgenetic areas in rats, associated with large Leydig cell clusters, similar to damage observed in TDS (Mahood et al., 2006). The impact of environmental factors on testicular tissue with genomic instability is suggested as a model in the pathogenesis of testicular cancer in TDS (Rajpert-De Meyts, 2006). The crucial influence of small genetic disorders in developing a severe histological damage within testicular tubules is well known (Krausz and Degl’Innocenti, 2006).

The question of whether a specific spermatogenic testicular defect is more related to testis cancer may be gained by conducting a prevalence study of testicular cancer in infertile men with different spermatogenetic patterns.

Azoospermic patients are the best clinical population to be investigated because many of them require a surgical procedure for testicular sperm extraction (TESE), which includes testicular biopsy. Furthermore, the highest incidence of testis cancer is also reported in these subjects (Jacobsen et al., 2000).

The aim of this study was to determine the prevalence of testicular nodules and cancer in azoospermic men with different spermatogenetic patterns in the biopsy.

Materials and methods

A total of 1443 consecutive, otherwise well, but infertile, men were admitted to our Male Infertility Centre from January 2000 to December 2003: all of them underwent a clinical examination followed by two semen analyses performed according to World Health Organization (WHO, 1999) criteria (Rowe et al., 2000). Unilateral or bilateral vas deferens agenesia were excluded, because
of a possible misleading influence in histological diagnosis caused by abnormal pressure within the tubules. All patients with abnormal genetic evaluation (karyotype, Y chromosome and cystic fibrosis) or any other clinical known causes of azoospermia (previous cancer therapy, toxicant contact and seminal tract infections) were excluded from the analysis.

Azoospermia was found in 145 patients (10.0%) and confirmed in repeated tests with vigorous semen centrifugation and pellet examination.

All 145 patients underwent scrotal ultrasonography (US). Sonographic evaluation was performed by using Esaote AU5 device with a 7.5 MHz B mode linear array transducer and a pulsed Doppler investigation with colour flow mapping capability.

Testicular ultrasonographic nodules were found in 11 out of 145 patients (7.5%) (Figure 1). All the nodules were detected by scrotal sonography (Figure 2) and two of them, although asymptomatic, could be recognized by palpation.

Ninety-seven out of 145 azoospermic subjects, including the 11 with testicular nodules, where asked to submit to TESE (plus biopsy) to search for spermatozoa.

All 97 patients (mean age, 34.9 ± 5.7 years) completed a diagnostic work-up including more detailed historical data, hormonal (FSH, LH, testosterone) and genetic evaluation and transrectal ultrasound.

On the basis of what the spermatogenetic status showed upon testicular biopsy, the 97 azoospermic subjects were retrospectively divided into two groups: Group A (n = 38) with complete Sertoli cell-only syndrome (SCOS) and Group B (n = 59) with histological findings other than complete SCOS.

A single testicular biopsy was analysed by examining all the tubules in the entire histological sample. In Group A, we placed patients in whom no spermatogenesis in any tubule was found, i.e. complete SCOS (Cooperberg et al., 2005) and in Group B all others, including patients in whom a small percentage of tubules showed germ cells.

Nine out of the 11 subjects with testicular nodules underwent a more extensive microsurgical exploration to remove the suspicious area (Figure 3) (Colpi et al., 2005).

The Institutional Review Board approved this study, and all patients granted their written informed consent. Two out of 11 patients with testicular nodules gave their informed consent for TESE only, without a more extensive surgical exploration to enucleate the suspected area. These two patients were admitted to a clinical and ultrasound follow-up.

All statistical analyses were performed with Graph Pad Instat using a two-tailed Student’s t-test with Welch correction (Graphpad Software). \( P < 0.05 \) was considered statistically significant. The data are presented as mean ± SD.

Results

In azoospermic men examined for infertility by ultrasound and palpation, the prevalence of testicular nodules was 11 out of 145 (7.5%) (Figure 1).

In the 97 patients admitted to TESE and biopsy, 10 nodules were found in Group A (n = 38; 26.3%) and 1 in Group B (n = 59; 1.7%). One patient in each group refused nodule removal.

The nine nodules that were removed from Group A were submitted to histological examination, showing as a result one seminoma, one embryonal carcinoma, three Leydig cell tumours and four Leydig hyperplasias. Furthermore, the biopsies which were routinely performed during TESE made it possible to detect two additional ISCS in Group A (Table I). The maximum diameter of echographic nodules ranged between 2.8 and 26 mm. One out of three Leydig cell

![Figure 1](https://academic.oup.com/humrep/article-abstract/22/4/1042/699071)
tumours and the embryonal carcinoma were recognized later by palpation in clinical setting. All the above mentioned patients affected by cancer underwent orchidofuniculectomy.

Groups A and B were similar in terms of age (34.9 ± 6.4 versus 34.9 ± 5.3 years). Group A (SCOS) presented with a statistically reduced right (9.9 ± 3.9 versus 13.0 ± 4.3 ml) and left testicular volume (9.3 ± 3.5 versus 12.1 ± 4.2 ml) (P < 0.001) and with a statistically increased FSH (20.8 ± 11.9 versus 10.2 ± 7.1 U/L) (P < 0.0001) and LH (8.0 ± 5.2 versus 4.4 ± 2.2 U/L) and testosterone (5.5 ± 3.2 versus 4.5 ± 1.5 ng ml⁻¹) (P < 0.05). No typical hormonal pattern was found in Leydig cell tumours. Seventeen out of 97 (17.5%) patients presented with previous undescented testis, but there was no significant difference between the two groups (Group A = 10/38; Group B = 7/59). Five tumours were found in the 17 previous undescented testes (the embryonal carcinoma, two Leydig cell tumours, one Leydig hyperplasia and one ISCs). Six tumours were found in the remaining 80 regularly undescented testes.

A further statistical analysis showed that a history of cryptorchid in an azoospermic population represents a statistical increased risk for tumours with P = 0.022 calculated by a two-sided Fisher’s exact test. No hypospadias was found.

Discussion

In azoospermic patients with complete SCOS (Group A), the prevalence of testicular nodules was higher (10/38 = 26.3%) than in Group B (1/59 = 1.7%) (P < 0.001), where biopsy showed normal to severely impaired spermatogenesis (Group B: average spermatids/tubule: 7.5 ± 6.3; median value: 6.3). Two out of nine nodules enucleated from Group A were cancers (2/38 = 5.2%): one seminoma and one embryonal carcinoma. Adding the two ISCs, which were detected in Group A, the prevalence of testicular cancer (4/38 = 10.5%) in severely impaired testes turned out to be high. Therefore, in azoospermic, but otherwise well, infertile men, the high prevalence of testicular nodules (11/145 = 7.5%) should suggest a routine clinical screening by a clinician.

Only two cases out of nine surgically explored were palpable by the clinician (one Leydig cell tumour and the embryonal carcinoma).

Studies incorporating non-selected infertile patients showed a prevalence of non-palpable tumours ranging between 0.1 and 1.1% (Horstman et al., 1994; Pierik et al., 1999; Carmignani et al., 2004).
When one considers that in our azoospermic population, the majority of detected nodules were non-palpable, suggestion of routine ultrasound screening could be envisaged. In our case, ultrasonography detected two Leydig cell tumours and one seminoma, which would have otherwise gone undetected without ultrasonography.

Concerning the Leydig cell tumour, a large size seems to be important for malignancy (Kim et al., 1985). In healthy subjects, Leydig cells are interspersed between the seminiferous tubules. An increased number of Leydig cells have been noted in specific genetic and clinical conditions correlated to male infertility: androgen insensitivity syndrome (Singh et al., 2006), human chorionic gonadotropin overexpression (Ahtiainen et al., 2005), oligospermia (Buckspan et al., 1989), varicocele (Sirvent et al., 1990) and sporadic data concerning cryptorchidism, Klinefelter syndrome and testicular atrophy. A very high cell number has usually been defined as the Leydig cell hyperplasia and, in some cases, these cells acquire morphological features of aggregates. The histological analysis of testis biopsies in infertile patients showed a high presence of Leydig cell micronodules (Holm et al., 2003). Some authors consider it a consequence induced by the Leydig cell dysfunction, able to induce Leydig cells aggregation, as time goes (Holm et al., 2003).

The possible Leydig cell development into micronodules, should suggest a precocious evaluation able to recognize early ultrasound signs of these cellular aggregations. The hypothesis of a linkage between these aggregates and their increased size with malignant development is still speculative. At the moment, we need to enucleate the nodule and suggest ultrasound follow-up only in the testes of infertile men with the Leydig cell micronodule which has been confirmed by surgery.

Finally, because of the high prevalence of ISCs detected by biopsy in azoospermic men, a routine testicular biopsy should be suggested during the TESE procedure (Kliesch et al., 1997).

In summary, a high number of non-palpable testicular nodules may be found by the combined use of testicular ultrasound and biopsy in azoospermic, but otherwise well, men examined for infertility.

This increased prevalence of testicular nodules and cancer in Group A supports the hypothesis that SCOS may be strictly linked to predisposing factors leading to testis neoplasms and suggests the need for complete testicular diagnosis in azoospermic males screened for severe infertility before assisted-reproduction technology is applied.

### Table 1. Biopsy results from 97 azoospermic patients

<table>
<thead>
<tr>
<th>Sertoli cell-only syndrome (Group A)</th>
<th>No Sertoli cell-only syndrome (Group B)</th>
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<tr>
<td>Patients</td>
<td>Ultrasound nodule</td>
</tr>
<tr>
<td>38</td>
<td>10</td>
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<td>59</td>
<td>1</td>
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This predisposing factor could be related to dysgenetic damage and mainly to severe germ cell line impairment like complete SCOS. In fact, in Group B a very small percentage of nodules were found and all degrees of spermatogenesis were shown, including 35.5% (21/59) showing normal spermatogenesis with the number of spermatis/tubule >10. Further investigations will be necessary in the azoospermic population to increase our knowledge of testicular nodules and histological features.

To sum up, according to our results, scrotal sonography should be mandatory before TESE in azoospermic patients, based on the high prevalence of non-palpable testicular nodules detected as small cancer upon surgery. Finally, owing to the increased risk of cancer in testes with severely impaired spermatogenesis (complete SCOS), a biopsy for histological examination should always be performed during TESE in order to detect any ISCs.

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### References
Holm M, Rajpert-De Meyts E, Andersson AM and Skakkebaek N (2003) Leydig cell micronodules are a common finding in testicular biopsies from men with impaired spermatogenesis and are associated with decreased testosterone/LH ratio. J Pathol 199,378–386.


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