ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

Correspondence:
Sigrid Isaksson, Reproductive Medicine Centre, Skåne University Hospital, Jan Waldenströms gata 47, 205 02 Malmö, Sweden.
E-mail: sigrid.isaksson@med.lu.se

Keywords:
azoospermia, cancer treatment, Inhibin B, testicular cancer

Received: 7-Oct-2013
Revised: 20-Dec-2013
Accepted: 28-Dec-2013

doi: 10.1111/j.2047-2927.2014.00182.x

Inhibin B concentration is predictive for long-term azoospermia in men treated for testicular cancer

[1,2]S. Isaksson, [2]J. Eberhard, [2]O. Ståhl, [2]E. Cavallin-Ståhl, [3]G. Cohn-Cedermark, [4,5]S. Arver, [6]Y. Lundberg Giwercman and [1]A. Giwercman

[1]Reproductive Medicine Centre, Skåne University Hospital, Malmö, [2]Department of Oncology, Skåne University Hospital, Lund, [3]Department of Oncology, Karolinska Institutet and University Hospital, Stockholm, [4]Centre for Andrology and Sexual Medicine, Karolinska University Hospital, Stockholm, [5]Department of Medicine, Karolinska Institutet, Stockholm, and [6]Department of Clinical Sciences, Lund University, Malmö, Sweden

SUMMARY

Azoospermia is a serious potential side effect following treatment for testicular cancer (TC). Our purpose was to examine possible predictors of long-term azoospermia in TC survivors. Ejaculates and blood samples were obtained from 217 patients at post-orchidectomy but before further treatment (T_0) and/or at one or more of the time points 6, 12, 24, 36–60 months after treatment (T_6, T_{12}, T_{24}, $T_{36–60}$). All patients delivered ejaculates at $T_{36–60}$, of which 117 also had confirmed presence of spermatozoa in the ejaculate at T_0, enabling longitudinal analyses. Types of therapy, cryptorchidism and Inhibin B before and after treatment were evaluated in relation to risk of azoospermia at T_{36}. Inhibin B levels at T_6, T_{12} and T_{24} were predictors of azoospermia at T_{36} with cut-off levels at 49.7, 55.9 and 97.8 ng/L respectively (sensitivity 100%, specificity 57–78%). The frequency of azoospermia in all patients at $T_{36–60}$ was 7.8% (95% CI 4.9–12%). As compared to surveillance patients, only those receiving >4 cycles of chemotherapy or ≥4 cycles of chemotherapy + radiotherapy (RT) had increased risk of long-term azoospermia (63% vs. 4.4% in the surveillance group; $p = 0.0018$). In conclusion, all patients with sperm production at post-orchidectomy but before further treatment and Inhibin B >56 ng/L 12 months after treatment had sperm production 3 years post-treatment. Eight per cent of TC survivors had azoospermia 3–5 years post-treatment, with highest risk in those receiving >4 cycles of chemotherapy or ≥4 cycles of chemotherapy in combination with RT.

INTRODUCTION

Testicular cancer (TC) is the most common cancer in men in their second to fourth decade of life, thus affecting men in their reproductive age. The prognosis is excellent, with a relative 5-year survival of 98% in Sweden (NORDCAN The Association of the Nordic Cancer Registries, 2013). The question of long-term toxicity of the treatment, including reproductive function, has therefore become increasingly important (Fossa & Magelssen, 2004; Kim *et al.*, 2010).

Testicular cancer survivors may have reduced fertility for different reasons. Sub-fertile men have an increased risk of developing TC (Jacobsen *et al.*, 2000; Walsh *et al.*, 2009). All patients with TC undergo orchidectomy of the affected testicle, which may contribute to deterioration of semen quality (Petersen *et al.*, 1999b). In addition, both chemotherapy and radiotherapy (RT) used in the treatment of TC can cause a dose- and time-dependent impairment of testicular function (Rowley *et al.*, 1974; Hansen *et al.*, 1990; Aass *et al.*, 1991; Centola *et al.*, 1994; Petersen *et al.*, 1994; Palmieri *et al.*, 1996; Pont *et al.*, 1996; Lampe *et al.*, 1997; Howell & Shalet, 2005; Brydoy *et al.*, 2010, 2012). All TC patients should therefore be offered cryopreservation of semen prior to treatment, to enable *in vitro* fertilization in case of post-treatment azoospermia.

Inhibin B, a negative feedback regulator of follicle stimulating hormone (Illingworth *et al.*, 1996; Anderson *et al.*, 1997) is a marker of spermatogenesis (Jensen *et al.*, 1997; Klingmuller & Haidl, 1997; Pierik *et al.*, 1998; von Eckardstein *et al.*, 1999; Myers *et al.*, 2009), and may predict pre-treatment sperm concentration in male cancer patients (van Casteren *et al.*, 2009). The synthesis of this peptide depends on interaction between germ cells and Sertoli cells (Kumanov *et al.*, 2005). Undetectable Inhibin B levels are associated with absence or arrest of spermatogenesis (Petersen *et al.*, 1999a). Therefore, Inhibin B appears to be a potential predictive factor for azoospermia after TC treatment.

Although spermatozoa from men with oligozoospermia often can be utilised for intra-cytoplasmatic spermatozoa injection (Nagy *et al.*, 1995), azoospermic subjects need to undergo a

testicular biopsy for possible recovery of spermatozoa. Today, there is no well-established method for predicting azoospermia in TC patients. As the reproductive window of a couple is time-limited, it is essential to identify predictive factors for long-term azoospermia for optimal counselling regarding future fertility potential and the possible need of using cryopreserved semen for *in vitro* fertilization.

The aim of this prospective longitudinal study was to examine the frequency of post-treatment long-term azoospermia in TC patients, and relate this risk to type of oncological treatment. We also evaluated Inhibin B and previous cryptorchidism as potential predictive markers of long-term azoospermia in those men.

MATERIALS AND METHODS

Patients

The study was carried out from 2001 at the Department of Oncology, Lund University Hospital, Lund and from 2003 at the Department of Oncology, Karolinska University Hospital, Stockholm. The inclusion was discontinued in October 2006. All TC patients 18–50 years of age referred to Lund University Hospital since March 1996, and to Karolinska University Hospital since November 1998, were eligible for a study on reproductive function following TC treatment. Patients were followed up to 5 years post-treatment. All patients gave written informed consent. The protocol was approved by the ethical review boards of Lund University and of Karolinska Institute.

A total of 459 patients were eligible for the study. Seventy-five patients (16%) declined and 52 (11%) were excluded owing to mental co-morbidity, linguistic difficulties, bilateral TC or physical disability. In total, 332 patients (72%) were included. Of these, 58 patients were excluded owing to azoospermia prior to diagnosis of TC, previous vasectomy, bilateral orchidectomy, RT to the remaining testicle because of carcinoma in situ (CIS), retrograde ejaculation, relapsing disease or development of CIS in the remaining testicle during the study period, testosterone replacement therapy 36–60 months after treatment, dying before delivering any samples, moving to another region, dropping out of the study or unwillingness to deliver ejaculates. From 57 men, no 3- or 5-years follow-up semen samples were available, that is why 217 patients were included in the present study (Fig. 1 and Table 1). Of these, 12 were included after treatment for relapsing disease. For six patients, data collected during testosterone replacement therapy were excluded. The patients included did not differ in mean age compared to those excluded or declining participation (32.6 and 33.4 years respectively). The two groups did not differ regarding proportions of seminomas vs. non-seminomas, but among participants the proportion of patients with stage I disease (78% vs. 61%) and consequently those receiving adjuvant chemotherapy (ACT) (34% vs. 22%) was somewhat higher, whereas the opposite was true for those receiving the most extensive treatment (3.7% vs. 9.9%) (Table S1).

Of the 217 patients, 119 men delivered semen samples after orchidectomy, but before further treatment. Two of these had

Figure 1 Flow chart of the inclusion of patients in present study.

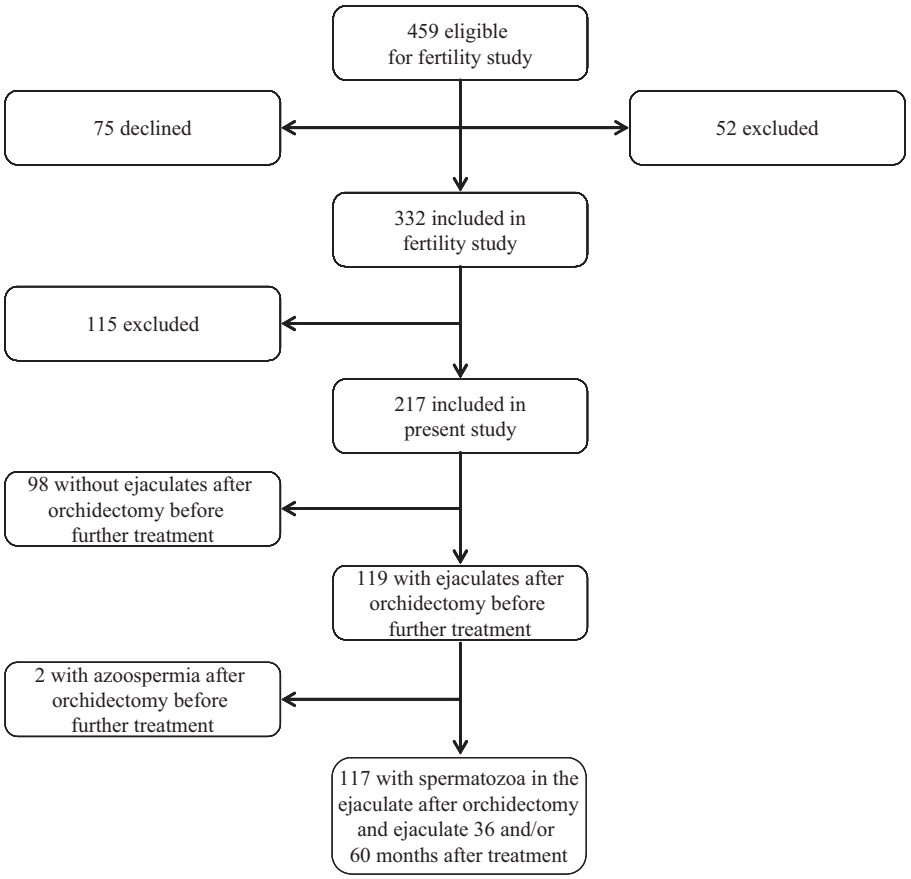

Table 1 Characteristics of 217 patients included in present study

	SO	ACT	SCT	RT	HCT	Total
Seminoma	11	12	13	62	2[a]	100
Stage I	11	12	–	62	–	85
Stage II	–	–	10	–	2[a]	12
Stage III	–	–	2	–	–	2
Stage IV	–	–	1	–	–	1
Non-seminoma	12	62	37	–	6	117
Stage I	12	62	10[a]	–	–	84
Stage II	–	–	16	–	2	18
Stage III	–	–	2	–	–	2
Stage IV	–	–	9	–	4	13

SO, no further treatment after orchidectomy; ACT, adjuvant chemotherapy: one to two cycles of cisplatin-based chemotherapy or carboplatin; SCT, standard doses of chemotherapy: three to four cycles of cisplatin-based chemotherapy; RT, adjuvant radiotherapy administrated to the para-aortic and ipsilateral iliac lymph nodes; HCT, higher doses of chemotherapy: >4 cycles of cisplatin-based chemotherapy or ≥4 cycles of cisplatin-based chemotherapy + radiotherapy at targets other than the remaining testicle. [a]Patients with relapsing disease.

post-orchidectomy azoospermia, leaving 117 patients, with spermatozoa in the ejaculate after orchidectomy and at least one follow-up ejaculate at T_{36} and T_{60} (patients with longitudinal data, Fig. 1 and Table 2). These 117 patients were on average 3 years younger than the 100 patients without longitudinal data (31.1 and 34.4 years respectively). There were no differences between the two groups regarding distribution of histological diagnoses or stages of disease. However, among the patients with longitudinal data some higher proportion (41% vs. 26%) of those who received one to two cycles of chemotherapy was observed; whereas surveillance was less frequent (2.6% vs. 20%) (Table S2).

Cancer treatment

Treatment was given according to the SWENOTECA protocols (SWENOTECA Swedish and Norwegian Testicular Cancer Group, 2000 and 2004). For staging, the Royal Marsden Hospital staging system was used (Dearnaley *et al.*, 2001).

All patients underwent orchidectomy of the affected testicle. Treatment for stage I seminoma was adjuvant RT, ACT or surveillance after orchidectomy. Adjuvant RT was administered to infra-diaphragmal para-aortic and ipsilateral iliacal lymph nodes to a total target dose of 25.2 Gy in 14 fractions. In Lund, the scattered dose to the remaining testis was estimated to 0.04–0.43 Gy retrospectively in seven randomly selected men. ACT was given with one cycle of carboplatin AUC 7. Patients with stage IIB-IV seminomas were treated with the BEP regimen (bleomycin 30 000 IU days 1, 5, 15 to a maximum dose of 3×10^5 IU, etoposide 100 mg/m^2 day 1–5, cisplatin 20 mg/m^2 per cycle day 1–5, given every third week) or EP (BEP minus bleomycin).

Patients with stage I non-seminomas without vascular invasion of tumour cells were offered ACT or surveillance. Patients with stage I non-seminomas with vascular invasion were recommended ACT. Standard chemotherapy of non-seminomas was the BEP regimen; one to two cycles in the adjuvant setting and three to four cycles for metastatic disease.

The patients were allocated into groups according to treatment schedule:

- Surveillance only: no further treatment after orchidectomy, $n = 23$ ($n = 3$ with longitudinal data).
- ACT: one to two cycles of cisplatin-based chemotherapy or carboplatin, $n = 74$ ($n = 48$ with longitudinal data).
- Standard dose chemotherapy (SCT): three to four cycles of cisplatin-based chemotherapy, $n = 50$ ($n = 31$ with longitudinal data).
- RT: adjuvant RT administrated to the para-aortic and ipsilateral iliac lymph nodes, $n = 62$ ($n = 29$ with longitudinal data).
- Higher doses of chemotherapy (HCT): >4 cycles of cisplatin-based chemotherapy or ≥4 cycles of cisplatin-based chemotherapy + radiotherapy at targets other than the remaining testicle, $n = 8$ ($n = 6$ with longitudinal data).

Adjuvant chemotherapy was one cycle of BEP ($n = 51$), CVB (BEP with vinblastine instead of etoposide, $n = 2$) or carboplatin ($n = 11$), or two cycles of BEP ($n = 4$), CVB ($n = 4$), CEB (BEP with carboplatin instead of cisplatin, $n = 1$) or carboplatin ($n = 1$). SCT was three cycles of BEP ($n = 15$) or four cycles of BEP or EP ($n = 35$). HCT was cisplatin-based chemotherapy + radiotherapy ($n = 3$) or cisplatin-based chemotherapy ($n = 5$). Number of chemotherapy cycles in HCT patients was four ($n = 2$), five ($n = 1$), six ($n = 1$) or eight ($n = 2$). Two patients received five or nine cycles plus two cycles of high dose chemotherapy with stem cell rescue.

Table 2 Characteristics of 117 patients with longitudinal data (i.e. spermatozoa in the ejaculate after orchidectomy but before further treatment and follow-up ejaculate 36–60 months after treatment)

	SO	ACT	SCT	RT	HCT	Total
Seminoma	3	9	8	29	2[a]	51
Stage I	3	9	–	29	–	41
Stage II	–	–	6	–	2[a]	8
Stage III	–	–	1	–	–	1
Stage IV	–	–	1	–	–	1
Non-seminoma	–	39	23	–	4	66
Stage I	–	39	7[a]	–	–	46
Stage II	–	–	10	–	1	11
Stage III	–	–	1	–	–	1
Stage IV	–	–	5	–	3	8

SO, no further treatment after orchidectomy; ACT, adjuvant chemotherapy: one to two cycles of cisplatin-based chemotherapy or carboplatin; SCT, standard doses of chemotherapy: three to four cycles of cisplatin-based chemotherapy; RT, adjuvant radiotherapy administrated to the para-aortic and ipsilateral iliac lymph nodes; HCT, higher doses of chemotherapy: >4 cycles of cisplatin-based chemotherapy or ≥4 cycles of cisplatin-based chemotherapy + radiotherapy at targets other than the remaining testicle. [a]Patients with relapsing disease.

Cryptorchidism, semen samples and blood samples

Patients delivered ejaculates and/or blood samples after orchidectomy, but prior to further treatment (T_0), and/or 6 (T_6), 12 (T_{12}) 24 (T_{24}), 36 (T_{36}) and 60 (T_{60}) months after treatment. Patients could enter the study at any time between T_0 and T_{60} and delivered samples at the remaining time points. At the time of inclusion, the patient was asked about a history of cryptorchidism, which was registered in 6.9% of patients in present study and 5.1% of patients with longitudinal data.

Semen samples

Long-term azoospermia was defined as the absence of spermatozoa in the ejaculate 3 or 5 years after cancer treatment. Semen samples were analysed according to the 1999 World Health Organization (WHO) guidelines (WHO, 1999).

For patients recruited in Lund, all semen analyses were performed at the Reproductive Medicine Centre, Skåne University Hospital, Malmö, except 24 T_0 ejaculates, which were analysed at the fertility laboratory, Lund University Hospital prior to cryopreservation. In Stockholm, all samples were analysed at the Centre for Andrology and Sexual Medicine, Karolinska University

Hospital. The laboratories in Malmö and Stockholm serve as reference laboratories for European Society of Human Reproduction and Embryology/Nordic Association for Andrology external quality control programme.

Inhibin B analyses

Blood sampling was performed between 08.00 and 15.00 h. Blood samples from Lund were analysed at the Department of Clinical Chemistry Malmö, Laboratory Medicine and from Stockholm at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Göteborg. At both laboratories, Inhibin B was measured with the OBI Inhibin B ELISA from DSL, Oxford, UK (Groome *et al.*, 1996). The detection limit was 15 ng/L and total CV's ranged from 15.3% at the concentration of 28 ng/L to 7.0% at the concentration of 339 ng/L. The laboratory normal range for Inhibin B was 50–330 ng/L (post-pubertal men).

Statistical analyses

The mean risk of long-term azoospermia (including 95% confidence intervals) in relation to treatment given was calculated as fraction of men without spermatozoa in the ejaculate at $T_{36–60}$ for all patients as well as for patients with longitudinal data. To obtain sufficient numbers of individuals, the results of semen samples collected at T_{36} and T_{60} were combined into one category, $T_{36–60}$. As more patients delivered semen samples only at T_{36} than only at T_{60}, the results from T_{36} were used when results from both T_{36} and T_{60} were available. For the total patient cohort, 166 T_{36}-values and 51 T_{60}-values were used. For patients with longitudinal data, 92 T_{36}-values and 25 T_{60}-values were used. For analyses of potential predictors of long-term azoospermia, only semen samples from T_{36} were used. Patients on surveillance after orchidectomy were used as controls for respective therapy group. For comparison between different treatment groups, two-tailed Fisher's exact test was used.

The following factors were tested as potential predictors for developing azoospermia at T_{36}: previous cryptorchidism and Inhibin B at T_0, T_6, T_{12} and T_{24}. For analyses of predictive factors for azoospermia, receiver operating characteristic (ROC) curve analyses (SPSS 20.0 software, SPSS Inc., Chicago, IL, USA) were performed, and as cut-off levels, we selected Inhibin B levels to give 100% sensitivity and maximal specificity in predicting azoospermia.

Positive predictive value (PPV) and negative predictive value (NPV) for Inhibin B levels at T_0, T_6, T_{12} and T_{24} in relation to the risk of azoospermia at T_{36} were calculated. PPV was calculated as proportion of patients with Inhibin B below cut-off with azoospermia at T_{36} and NPV as proportion of patients with Inhibin B above cut-off having spermatozoa in the ejaculate at T_{36}. For all statistical tests, $p < 0.05$ was considered statistically significant.

RESULTS

Inhibin B and cryptorchidism as predictors of azoospermia

Inhibin B concentrations in serum at T_0, T_6, T_{12} and T_{24} were all predictors of azoospermia at T_{36} with 100% sensitivity, but with different cut-off levels and different specificities. The cut-off levels of Inhibin B in ROC analysis, and PPV and NPV at T_0, T_6, T_{12} and T_{24} are presented in Table 3. Judged from specificity and PPV, Inhibin B at T_{12} was the best predictor of azoospermia at T_{36} and Inhibin B at T_0 had the poorest predictive value.

Table 3 Inhibin B level as predictor of azoospermia 36 months after treatment

	N	Azoo (*n*)	Inhibin B cut-off, ng/L	Sensitivity, %	Specificity, %	AUC	PPV	NPV
T_0	43	3	112.00	100	40.0	0.750	0.11	1.00
T_6	51	7	49.65	100	70.5	0.883	0.35	1.00
T_{12}	62	8	55.90	100	77.8	0.911	0.40	1.00
T_{24}	77	8	97.75	100	56.5	0.899	0.21	1.00

N, number of patients in the analysis; Azoo (*n*), number of patients with azoospermia 36 months after treatment; AUC, area under the curve; PPV, positive predictive value: proportion of patients with Inhibin B below cut-off presenting with azoospermia at T_{36}; NPV, negative predictive value: proportion of patients with Inhibin B above cut-off having spermatozoa in the ejaculate at T_{36}.

Among patients with longitudinal data, six had azoospermia at T_{12} and all these men remained azoospermic at T_{36}. However, in the total patient cohort, two men who were azoospermic at T_{12} regained sperm production at T_{36}. Both presented with Inhibin B values <14 ng/L at T_{12}.

In the total patient cohort, 14 patients had azoospermia at T_{36}, and two of them shifted to sperm production at T_{60}. One of these men was included in the longitudinal cohort and in the ROC analyses.

A history of cryptorchidism proved non-significant for the risk of azoospermia at T_{36} (data not shown).

Treatment modality and risk of azoospermia

The frequency of long-term azoospermia was 7.8% (95% CI 4.9–12%) for all patients. In this cohort, two patients shifted from azoospermia at T_{36} to sperm production at T_{60}. For patients with longitudinal data, the frequency of long-term azoospermia was 7.7 (95% CI 3.9–14%), and one patient shifted from azoospermia at T_{36} to sperm production at T_{60}. The frequencies of azoospermia in patients receiving the same treatment modalities were similar in the cohort of patients with longitudinal data compared to the total patient cohort. Significant risk of long-term azoospermia was seen only for patients receiving HCT in the total patient cohort, with 63% (95% CI 30–87%) azoospermia at $T_{36–60}$. Similar frequency but without statistically significant difference to surveillance was seen for patients with longitudinal data, with 67% (95% CI 30–91%) azoospermia in the HCT group. The proportion of men with azoospermia in relation to treatment is presented in Tables 4 and 5. In the total patient cohort, three patients receiving HCT had sperm production after treatment. Treatment details and Inhibin B levels for those men are given in Table 6.

DISCUSSION

The main findings of present study were that Inhibin B values obtained 6, 12 and 24 months after treatment were reliable clinical predictors of azoospermia risk 3 years post-treatment. The best prediction was obtained 12 months after treatment. All patients with spermatozoa in the ejaculate after orchidectomy but before further treatment, and Inhibin B > 56 ng/L 12 months after treatment, had spermatozoa in the ejaculate 3 years post-treatment. Eight per cent of TC survivors were azoospermic 3–5 years post-treatment.

A similar cut-off level of Inhibin B was previously reported predictive of azoospermia in childhood cancer survivors

Table 4 Azoospermia 36–60 months post-treatment in relation to treatment, all patients

Treatment	*N*	Azoo	Azoo (%)	95% CI	*p*[a]
SO	23	1	4.4	0.0–23	–
ACT	74	1	1.4	0.0–8.0	0.42
SCT	50	5	10	3.9–22	0.66
RT	62	5	8.1	3.1–18	1.0
HCT	8	5	63	30–87	0.0018
Total	217	17	7.8	4.9–12	–

N, total number of patients; azoo, number of patients with azoospermia; CI, confidence interval; SO, no further treatment after orchidectomy; ACT, adjuvant chemotherapy: one to two cycles of cisplatin-based chemotherapy or carboplatin; SCT, standard doses of chemotherapy: three to four cycles of cisplatin-based chemotherapy; RT, adjuvant radiotherapy administrated to the para-aortic and ipsilateral iliac lymph nodes; HCT, higher doses of chemotherapy: >4 cycles of cisplatin-based chemotherapy or ≥4 cycles of cisplatin-based chemotherapy + radiotherapy at targets other than the remaining testicle. [a]Compared to patients with no further treatment after orchidectomy.

Table 5 Azoospermia 36–60 months post-treatment in relation to treatment, patients with spermatozoa in the ejaculate after orchidectomy but before further treatment

Treatment	*N*	Azoo	Azoo (%)	95% CI	*p*[a]
SO	3	0	0	0.0–62	–
ACT	48	0	0	0.0–8.9	1.0
SCT	31	4	13	4.5–29	1.0
RT	29	1	3.5	0.0–19	1.0
HCT	6	4	67	30–91	0.17
Total	117	9	7.7	3.9–14	–

N, total number of patients; azoo, number of patients with azoospermia; CI, confidence interval; SO, no further treatment after orchidectomy; ACT, adjuvant chemotherapy: one to two cycles of cisplatin-based chemotherapy or carboplatin; SCT, standard doses of chemotherapy: three to four cycles of cisplatin-based chemotherapy; RT, adjuvant radiotherapy administrated to the para-aortic and ipsilateral iliac lymph nodes; HCT, higher doses of chemotherapy: >4 cycles of cisplatin-based chemotherapy or ≥4 cycles of cisplatin-based chemotherapy + radiotherapy at targets other than the remaining testicle. [a]Compared to patients with no further treatment after orchidectomy.

(Romerius *et al.*, 2011). In a recent study of TC survivors, after a median follow-up of 11 years, low or undetectable Inhibin B was associated with azoospermia (Brydoy *et al.*, 2012). However, in a few men, azoospermia was seen despite high Inhibin B values. This inconsistency was probably because of longer follow-up and inclusion of older men, in whom the cause of azoospermia may differ from that seen in younger men. Furthermore, nothing was known about pre-treatment sperm production in the previous study, whereas in present study, longitudinal analyses were carried out in patients with spermatozoa in the ejaculate before therapy.

In the present study, Inhibin B at T_{12} was the best biochemical predictor of azoospermia at T_{36}. In the total patient cohort, two men azoospermic at T_{12} regained sperm production at T_{36}. Thus, similar to Inhibin B measurement, semen analysis performed at T_{12} has a somewhat limited value in pointing out men in whom long-term recovery of sperm production will take place. However, the advantage of using Inhibin B is that a blood sample can be taken at the local oncological department, and the patient does not need to go to a centralized laboratory for semen analyses. Inhibin B can also be used if the patient has retrograde ejaculation or is unwilling to deliver an ejaculate.

Previous studies of possible predictive factors for azoospermia after cancer treatment have provided inconclusive results. One study found a higher probability of recovery of sperm production for patients with normal sperm counts before chemotherapy (Lampe *et al.*, 1997). On the contrary, another study found that recovery of spermatogenesis was not related to pre-treatment sperm quality (Gandini *et al.*, 2006). However, none of the previous studies identified factors with high enough sensitivity and/or specificity to predict long-term infertility in TC survivors.

We found that 8% of the TC survivors were azoospermic 3–5 years post-treatment. In a recent study of TC survivors, 15% were reported to have azoospermia after a median follow-up of 11 years (Brydoy *et al.*, 2012), However, the proportions of patients receiving different treatment modalities differed largely from our study with a higher proportion of men receiving more than four cycles of chemotherapy in the Norwegian material. The proportion of irradiated patients was also higher in the latter study, as was total target doses for some patients.

Even in men receiving three to four cycles of cisplatin-based chemotherapy, we found no statistically significant increased risk of long-term azoospermia. This is confirming previous findings that ≤4 cycles of standard cisplatin-based chemotherapy have no statistically significant negative long-term effects on sperm production (Aass *et al.*, 1991; Pont *et al.*, 1996). However, a more recent study indicated increased frequency of azoospermia in TC survivors treated with ≤4 cycles of standard cisplatin-based chemotherapy or carboplatin compared to patients on surveillance (Brydoy *et al.*, 2012). This discrepancy might be owing to larger number of patients and thereby higher statistical power in the latter study. In addition, in the Norwegian material, 10% of men treated with ≤4 cycles of chemotherapy were also irradiated.

Our patients receiving more extensive treatment had increased risk of azoospermia 3–5 years post-treatment, which also confirms previous studies showing increased risk of long-term azoospermia after treatment with cisplatin equivalent to >4 cycles of

Table 6 Patients with sperm production after extensive treatment

Patient	Treatment	T_{12}		T_{24}		T_{36}		T_{60}	
		Azoo (yes/no)	Inhibin B, ng/L	Azoo (yes/no)	Inhibin B, ng/L	Azoo (yes/no)	Inhibin B, ng/L	Azoo (yes/no)	Inhibin B, ng/L
1	Four cycles of chemotherapy + para-aortic radiotherapy with boost to vertebrae Th12-L3	Missing	Missing	No	213	No	233	No	175
2	Five cycles of chemotherapy	Yes	13.5	Yes	26.6	No	25.9	No	Missing
3	Eight cycles of chemotherapy	Missing	Missing	No	24.7	No	54	No	102

T_{12}, 12 months after treatment; T_{24}, 24 months after treatment; T_{36}, 36 months after treatment; T_{60}, 60 months after treatment; azoo, azoospermia.

standard chemotherapy (Hansen *et al.*, 1990; Petersen *et al.*, 1994; Brydoy *et al.*, 2012). Similar frequency of azoospermia, although not significantly different compared to surveillance, was seen among patients with longitudinal data. The lack of significance was probably owing to the smaller number of patients in the cohort with longitudinal data. Notably, even among those extensively treated patients some had sperm production after treatment.

Radiotherapy was not associated with a significant increased risk of azoospermia. In a previous study, with patients receiving RT with target, fraction dose and total target dose similar to ours, frequency of azoospermia 24 months after treatment was similar to that found by us 36–60 months post-irradiation (Gandini *et al.*, 2006).

The strength of this study was the large number of patients included and the longitudinal approach with known sperm production before therapy.

As Inhibin B has a diurnal variation with peak values in the morning and nadirs in the late afternoon, the blood sampling, which occurred between 08.00 and 15.00 h, must be regarded as a drawback (Carlsen *et al.*, 1999). Furthermore, as men with retrograde ejaculation were not evaluated, the proportion of azoospermic men might be slightly higher. The frequency of cryptorchidism was slightly lower in our cohort as compared to other studies of TC patients (Meirow & Schenker, 1995; Petersen *et al.*, 1998; Fraietta *et al.*, 2010). This lower frequency might be the cause why cryptorchidism was not a statistically significant risk factor of azoospermia, but should not influence the results regarding Inhibin B.

We chose to assess Inhibin B as a single predictor of long-term azoospermia, without taking into consideration treatment modality, age, period of abstinence or other factors which might influence semen quality. Although such information might be interesting from a biological point of view, for a clinical set up the use of simple diagnostic tools seems more appropriate.

Another limitation of our study is the relatively low number of azoospermic men at the different follow-up time points. Thus, additional studies are needed in order to confirm our results.

We used semen sample from T_{36} and not from T_{60} when both were available, because more samples were available at T_{36}. In a cohort of male cancer patients with a median age of 27 years at semen cryopreservation, those who requested to use their cryopreserved semen did so after a mean time of 57 months, range 15–130 months (van Casteren *et al.*, 2008). In other words the time interval for usage of cryopreserved semen was wide, but some patients used their semen within less than 2 years after cryopreservation. Three years after treatment therefore seems to be a clinically relevant endpoint, as some patients can be expected to start a family at this age. We also considered 3 years after treatment to be a clinically relevant endpoint because of the limited reproductive window of a couple.

In the present study, Inhibin B after orchidectomy but before further treatment was a less reliable predictor of long-term azoospermia. All TC patients should be offered semen cryopreservation before treatment of TC, regardless of Inhibin B value after orchidectomy.

In conclusion, all patients with spermatozoa in the ejaculate after orchidectomy but before further treatment and Inhibin B > 56 ng/L 1 year after treatment had spermatozoa in the ejaculate 3 years after treatment. Thus, our results suggest that Inhibin B 12 months after treatment can be used in the follow-up of TC survivors to identify those at risk of having azoospermia up to 3 years after treatment. Although recovery of sperm production after 5 years has not been evaluated and cannot be completely excluded, many patients may not be willing or have the possibility to postpone parenthood for more than a few years after cancer treatment. These results may therefore be important in counselling young cancer survivors if confirmed in other studies.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Cancer Society (CAN 2012/661), Swedish Governmental Funding for Medical Training and Research, Swedish Childhood Cancer Society (PROJ10/030), King Gustaf V's Jubilee Fund for Cancer Research, Stockholm (grants 44052 and 74061), and Skåne University Hospital Funds: Malmö University Hospital Cancer Fund and Gunnar Nilssons Cancer Fund.

DISCLOSURE

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

J.E., O.S., E. C.-S., Y. L.-G. and A.G. designed the study. J.E., O.S., G. C-C, S.A. and S.I. collected the data. S.I. and A.G. analysed and interpreted the data. All authors rewieved, revised and approved the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of 217 patients included in present study and 242 patients declining participation or excluded.

Table S2. Characteristics of 117 patients with spermatozoa in the ejaculate after orchidectomy but before further therapy and follow-up ejaculate 36–60 months post-treatment, and 100 patients without ejaculates after orchidectomy but before further therapy or with azoospermia after orchidectomy but before further therapy.

