

Parental desire and acceptability of spermatogonial stem cell cryopreservation in boys with cancer

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BACKGROUND: In the near future, a substantial proportion of adults will be childhood cancer survivors. The cryopreservation and transplantation of spermatogonial stem cells (SSCs) is currently successful in animals; application in humans seems likely in the near future. Cryopreserving SSCs might become an important issue in childhood cancer. Because this might require testicular biopsies or hemicastration, parental desire/acceptability for SSC collection was enquired for. **METHODS:** Three hundred eighteen parents of boys surviving at least 2 years after the diagnosis of cancer were asked about collecting SSCs by biopsy or hemicastration and collecting sperm by masturbation or electrostimulation. Opinions were assessed as if at the time of diagnosis and at the present time. **RESULTS:** Sixty-three per cent of parents responded. At diagnosis, SSC collection by means of biopsy was approved by 61%, hemicastration by 33% and collecting sperm by 70% ($P < 0.013$). The acceptability of performing hemicastration was significantly lower than all other forms of SSC/sperm collection. No differences were observed between parents' present opinion and opinion at diagnosis. No differences related to treatment intensity, presumed negative fertility effects and pubertal state were found. **CONCLUSIONS:** Infertility is a major topic for parents. For prepubertal boys, the collection of SSCs might be a great relief in respect of the fertility issue. Collecting SSCs by biopsy is desired and accepted by the majority of parents; hemicastration is accepted by one-third of parents. The translation of SSC cryopreservation and transplantation from animal models to humans is eagerly awaited.

Key words: spermatogonial stem cells/childhood cancer/hemicastration/testicular biopsy/infertility

Introduction

The incidence of childhood cancer ranges from 1.0 to 2.5 per 1000 children (Stiller and Parkin, 1996). Owing to recently developed highly effective anti-cancer treatment strategies for children, the majority of children currently survive their malignancy. It has been estimated that by the year 2010, 1 in 250 adults will be a long-term survivor of childhood cancer (Blatt, 1999). Given this apparent success in paediatric oncology, long-term adverse effects of cancer treatment are becoming more and more important. One of the major concerns with current treatment regimens is the adverse effect on fertility. Large-scale follow-up studies of prepubertal children treated for childhood cancer are scarce (Wallace *et al.*, 1989; Wallace *et al.*, 1991; Radford *et al.*, 2001; Thomson *et al.*, 2002; van den Berg *et al.*, 2004). Nevertheless, it is generally expected that fertility is severely decreased in childhood cancer survivors and almost certain in prepubertal boys treated with high-dose chemotherapy and/or irradiation involving the genital region (van den Berg *et al.*, 2004; Wallace *et al.*, 2005).

In prepubertal boys diagnosed with cancer, there are currently no means to preserve their reproductive potential, in contrast to adolescent and adult men, for whom cryopreservation of semen before the start of chemotherapy is an option. Current data from research in animal models (mouse, rat, bovine and primate) indicate that spermatogonial stem cells (SSCs) can be isolated, cultured *in vitro* and used for autologous transplantation resulting in the restoration of spermatogenesis (Brinster and Zimmermann, 1994; Clouthier *et al.*, 1996; Schlatt *et al.*, 1999; Nagano *et al.*, 2001; Izadyar *et al.*, 2003). These experiments have thus far never been performed in humans, but the technology is likely to be transferred to humans in the near future. As a result, the isolation, storage and transplantation of SSCs may become available for prepubertal boys diagnosed with cancer.

To collect SSCs from prepubertal boys, we have to perform invasive techniques. In such a future scenario, the moment of harvesting SSCs lies probably before the start of anti-cancer treatment, in a phase of utter confusion, in which parents already have to deal with a serious diagnosis and its therapeutic

consequences. The aim of this study was therefore to delineate the desire to collect SSCs as well as the acceptability of the necessary invasive procedures among parents of boys diagnosed with cancer and to compare the acceptability of SSC collection with the acceptability of sperm collection via masturbation or electrostimulation.

Materials and methods

Parents of all boys surviving childhood cancer, diagnosed in the period from September 1995 to September 2003 at the Emma Children Hospital AMC, were sent questionnaires. The first part of these questionnaires contained items on the relation of the parent/caretaker to the child, age of the child at initial diagnosis, type of malignancy and subsequent relapse. In the second part, parents were asked for their opinion on SSC collection by means of a biopsy of the testicle or by hemicastration. It was stated that the techniques are not available yet and parents were asked to fill in the questionnaire as if the techniques were available as standard (non-experimental) techniques. For pubertal boys, questions on sperm collection through masturbation or through electrostimulation under general anaesthesia were added. The acceptability of donor sperm was not asked for. Parents were asked for their opinion both at the actual moment of filling in the questionnaire and retrospectively as if they were at the moment of initial diagnosis. Both parents of each child were asked to fill in the separate questionnaires independently. The age of 12 was used to distinguish pubertal from prepubertal boys. For determining the impact of treatment intensity on parental acceptability, responses were sorted into two groups; i.e. Group A: parents of children in whom chemotherapeutic treatment would probably not endanger fertility [non-relapsing acute lymphocytic leukaemia (ALL), thyroid carcinoma, brain tumour and neuroblastoma] and Group B: parents of children in whom it was likely that fertility would be compromised (relapsing ALL, osteosarcoma, acute myeloid leukaemia, neuroblastoma, Hodgkin's disease, lymphomas, Ewing tumours and rhabdomyosarcoma).

To determine statistical significance, we used Mann–Whitney, Wilcoxon and chi-square tests, and for correlations, we calculated Pearson's ρ 's using SPSS (Statistical Package for the Social Sciences software; version 11.5.2). P -levels < 0.05 were taken as being significant.

Results

Questionnaires were sent out to both parents of 159 eligible boys (318 questionnaires), and 117 families responded (74%). Twenty-two families did not respond, whereas 20 families were excluded because of an incorrect address, refusal to participate in the survey or missing data on original disease and age at diagnosis. In 85 cases, both parents had filled in the questionnaire, and in 32 cases, only a single parent filled in the questionnaire (Figure 1).

Ages at initial diagnosis ranged from 0 to 17 years (mean 6.0 years), whereas current ages ranged from 3 to 24 years (mean 13 years). One hundred sixty-two questionnaires originated from families with prepubertal boys; in 68 cases, both parents had filled in a questionnaire. Forty questionnaires originated from families with pubertal boys; in 17 cases, both parents had filled in a questionnaire. The distribution of malignancies is summarized in Table I. In 18 boys, a relapse of the malignancy was reported.

A substantial number of parents (62%) from *prepubertal* boys would have given consent to collect SSCs by means of a testicular biopsy at the time of initial diagnosis. If hemicastration had been necessary to collect SSCs, 34% of parents would have permitted this. When asked for their present opinion, slightly higher, but not statistically significant, rates were observed: 70 and 39% for biopsy and hemicastration, respectively (Table II).

Parents from *pubertal* boys would have given consent to collect SSCs by means of a testicular biopsy at the time of initial diagnosis in 60% of cases. If hemicastration had been necessary

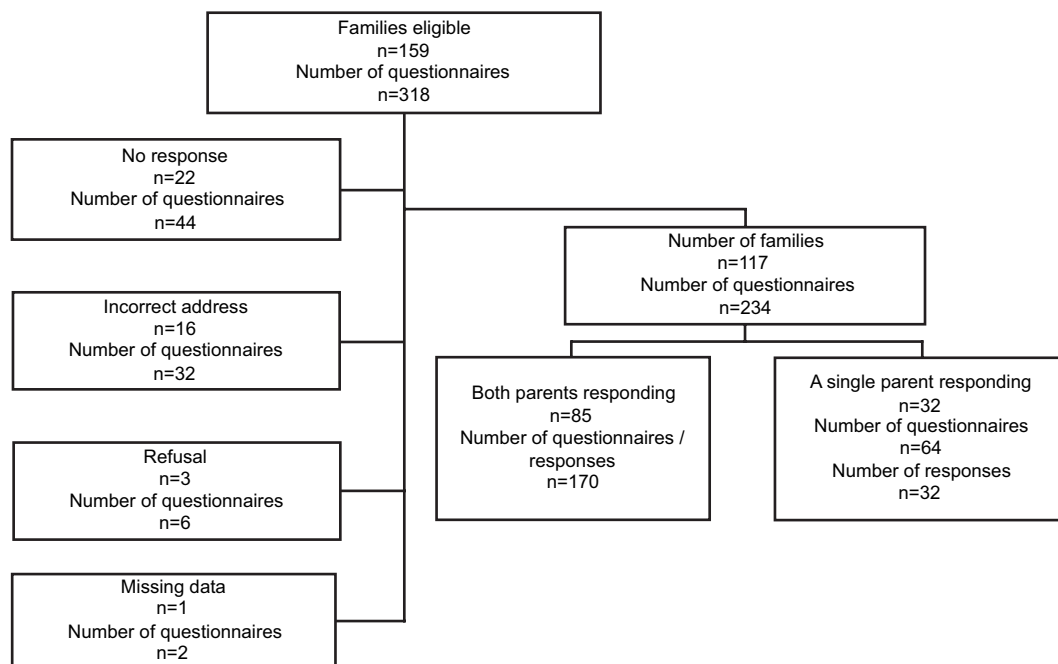


Figure 1. Consort diagram.

Table I. Diagnoses for the pubertal and prepubertal boys included in the study

Original diagnosis	Percentages of patients
Acute lymphoblastic leukaemia	28
Non-Hodgkin's lymphoma	15
Wilms' tumour	13
Neuroblastoma	10
Brain tumour	8
Ewing sarcoma	6
Rhabdomyosarcoma	5
Germ cell tumour	4
Osteosarcoma	4
Acute myeloid leukaemia	2
Miscellaneous diagnosis	5

to collect SSCs, 27% would have permitted this. By comparison, the collection of sperm by masturbation at initial diagnosis was approved by 70% of the parents. The same percentage was noted in case the sperm collection was performed by electrostimulation. When asked for their present opinion, higher, but not statistically significant, rates were observed for all forms of SSC/sperm collection: i.e. 65, 35, 78 and 82%, for biopsy, hemicastriation, electrostimulation and masturbation, respectively.

There was no statistically significant difference in the acceptability to collect SSCs via biopsy as compared with the collection of sperm via masturbation in pubertal boys. The acceptability to perform hemicastriation was significantly lower than all other forms of SSC/sperm collection (at least $P < 0.013$; Table II).

The analysis of all questionnaires showed that the acceptance rate of both biopsy and hemicastriation was not significantly

different for parents with prepubertal versus pubertal boys (Table II). Comparing the opinions of parents of children in whom chemotherapeutic treatment would probably not endanger fertility (Group A, $n = 94$) versus parents of children in whom it was likely that fertility would be compromised (Group B, $n = 108$) showed that there were no differences in acceptability to collect SSCs (Table III).

Finally, there were no statistically significant differences when comparing responses from mothers with responses from fathers (data not shown).

Discussion

Parents confronted with a malignancy in their child experience turmoil of unexpected events in the first weeks of illness. They are often overloaded with new information on subjects they never thought of before such as the effect of cancer treatment on fertility. In paediatric oncology, the preservation of fertility has always been limited to boys being able to produce an ejaculate. The preservation of fertility by means of a biopsy had been debated previously, and it is not known whether the procedure is safe (Wallace and Walker, 2001). On the basis of non-human experiments, it is however likely that in the near future, new ways of preserving reproductive potential by collection of SSCs can be introduced. This collecting of SSCs involves invasive procedures that have to be performed before the start of cancer therapy (Steele *et al.*, 2000; Tsai *et al.*, 2000). Patients with malignancies potentially residing in the testicles might be excluded from these new techniques, unless harbouring of (metastatic) disease can be ruled out or adequate

Table II. Number of parents giving consent for the various procedures of semen/spermatogonial stem cell collection

	All parents, n (%)		Parents from prepubertal boys, n (%)		Parents from pubertal boys, n (%)	
	At diagnosis ^a	Present opinion	At diagnosis	Present opinion	At diagnosis	Present opinion
Biopsy	124/202 (61) ^c	140/202 (69) ^c	100/162 (62) ^d	114/162 (70) ^c	24/40 (60) ^e	26/40 (65) ^g
Hemicastriation	66/202 (33) ^c	72/188 (38) ^c	55/162 (34) ^d	58/148 ^b (39) ^c	11/40 (27) ^{e,f}	14/40 (35) ^{c,g}
Masturbation	–	–	–	–	28/40 (70) ^f	33/40 (82) ^c
Electrostimulation	–	–	–	–	28/40 (70) ^f	31/40 (78) ^c

–, not enquired.

^aParents were asked to give their opinion retrospectively, as if at the moment of initial diagnosis.

^bMissing data (not answered; $n = 24$).

^c $P < 0.0001$.

^d $P < 0.003$.

^e $P < 0.007$.

^f $P < 0.0003$.

^g $P < 0.013$.

Table III. Number of parents giving consent for biopsy/hemicastriation

	Opinion at diagnosis		Present opinion	
	Group A	Group B	Group A	Group B
n	94	108	94	108
Consent for biopsy, n (%)	58 (62%)	66 (61%)	70 (74%)	70 (65%)
Consent for hemicastriation, n (%)	33 (35%)	33 (31%)	35 (37%)	42 (39%)

A, patient treated with protocols not considered to induce infertility; B, patient treated with protocols considered to induce infertility.

purging techniques are available. In pubertal boys, semen collection by means of masturbation might still be the first choice. However, SSCs can be of value, because semen quality can be quite poor at initial diagnosis (Redman *et al.*, 1987; Fitoussi *et al.*, 2000).

This study tried to delineate what parents accept and desire at the moment of diagnosis in case fertility is endangered in their son. Based on our data, it should be stressed that the information on the various procedures was given by means of leaflets attached to the questionnaire. As such, it is likely that after more extensive counselling, the parents may have reached a different decision. As such, mentioning that testicular biopsy with proper haemostasis of small prepubertal testicles might result in virtually no testicular mass left will certainly influence ultimate decisions.

Our data show that SSC collection by testicular biopsy is as acceptable as semen collection by masturbation or electrostimulation. One-third of all parents would ultimately even accept hemicastration as a means to collect SSCs. Although no significant change in opinions at initial diagnosis versus later on was noted, some parents might regret an initial negative decision at a later date. However, data on the validity of retrospective ratings as compared with ratings collected at the moment of the event indicate that this is not likely to be the case (Kreulen *et al.*, 2002). We also observed that the desire to cryopreserve SSCs was not related to potential harmfulness of the oncological treatment, indicating that even a minor chance of infertility is considered as a major burden in respect of ultimate quality of life. Furthermore, our survey clearly indicated that in these boys, procreation by means of their own sperm is at least as important for their fathers as for their mothers.

We conclude that the majority of parents consider future fertility of their sons as a very important health issue even if the chance of infertility is relatively low. Both SSC collection and sperm collection are highly appreciated by parents from boys diagnosed with childhood cancer. Our data indicate that the translation of current animal experiments on SSC collection and transplantation into clinical care is highly desired by parents from young boys with cancer.

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