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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Gonadal status in long-term male survivors of childhood cancer

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Abstract
Purpose To evaluate the prevalence of gonadal dysfunction and the associated risk factors in a cohort of male childhood cancer survivors (CCS).
Methods Gonadal function was evaluated measuring FSH, LH, inhibin B and total testosterone levels. Patients with total testosterone <3 ng/dl were considered to have hypogonadism. Patients with FSH >10 UI/l and inhibin B <100 pg/ml were considered to have spermatogenesis damage (SD). To assess the impact of risk factors, we estimated crude and adjusted OR performing logistic regression models.
Results One hundred and ninety-nine male CCS were enrolled; the median follow-up time was 14.01 years. SD was diagnosed in 68 patients, 16 CCS had primary hypogonadism, and 13 had central hypogonadism. The prevalence of gonadal dysfunction (SD or primary hypogonadism) was 45 %, similar in the three considered periods of pediatric cancer diagnosis (1985–1989, 1990–1999, >2000). The adjusted risk of gonadal dysfunction was higher in patients treated with radiotherapy (OR = 8.72; 95 % CI 3.94–19.30) and in those exposed to both alkylating and platinum-derived agents (OR = 9.22; 95 % CI 2.17–39.23). Sarcomas were the cancer diagnosis associated with the higher risk of gonadal dysfunction (OR = 3.69; 95 % CI 1.11–12.22). An extremely high rate of gonadal dysfunction was detected in patients who underwent hematopoietic stem cell transplantation and/or total body irradiation.
Conclusions Gonadal dysfunction still remains a significant late effect of anticancer therapies; thus, it is mandatory to inform patients (and parents) about this risk, and semen cryopreservation should be offered to all boys who are able to produce semen.

Keywords Childhood cancer survivors · Late effects · Gonadal function · Male infertility

Introduction

Considering all cancer types, the probability of recovery for a child or adolescent diagnosed with cancer is nowadays more than 80 % (SEER Cancer Statistics 2015), and it has been estimated that in 2020, in the general population 1/350 people will be a survivor of childhood cancer (CCS) (Parry et al. 2005). However, in the last years, it has been widely described that CCS are at risk of a considerable array of late effects due to previous anticancer therapies. Thirty years after cancer diagnosis, about two-thirds of CCS resulted to have at least one chronic illness related to previous treatments (Oeffinger et al. 2006). Any organ or system could be affected by these late effects that usually arise many years after treatments and frequently involve the endocrine system (Armstrong et al. 2010; Brignardello et al. 2013).

Testes are highly sensitive to the toxic effects of anticancer treatments (van Casteren et al. 2009), and gonadal

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dysfunction is one of the major endocrinological late complications among CCS. All aspects of male reproductive health (i.e., fertility and testosterone production) can be damaged by anticancer therapies.

Spermatogenesis can be impaired by direct or scatter testicular irradiation as well as by treatment with cytotoxic drugs, especially alkylating and platinum-derived agents (Meistrich 2013). Post-treatment azoospermia may be temporary or permanent, being the magnitude of damage dependent on cumulative doses, and recovery may occur some years after the end of therapies (Green et al. 2014).

Leydig cells are relatively resistant to toxicity of treatments, if compared with testicular germ cells. However, high doses of chemotherapy and/or testicular irradiation can result in hypoandrogenism, leading to clinical complications such as fatigue, impaired libido, premature osteoporosis and metabolic disorders (Kenney et al. 2012; Romerius et al. 2009).

Prepubertal status at diagnosis seems to be not protective against alkylating agent-associated gonadotoxicity, even if such protection at very young age has been suggested in the past, due to quiescent stage of the testes in the prepubertal age (van Casteren et al. 2009). As far as radiotherapy, testicular radiation dose capable to induce Leydig cell dysfunction is known to be higher after puberty (Lee and Shin 2013; Kenney et al. 2012).

Reduced fertility and inadequate testosterone production can also be due to gonadotropin deficiency, which develops when the hypothalamus and/or the pituitary gland is damaged by surgery or cranial irradiation (Lee and Shin 2013).

Besides affecting physical wellness, gonadal dysfunction could also be a source of emotional distress for male CCS. Therefore, monitoring gonadal status of these patients and recognizing treatment-associated infertility risk are important aspects of their follow-up care (Kenney et al. 2012).

Studies on hypogonadism in CCS are sparse and sometimes include relatively small patient cohorts. Furthermore, most of them are based on one type of malignancy and/or a single treatment protocol. The aim of this study is to evaluate the prevalence of gonadal dysfunction and the most important associated risk factors in a cohort of male CCS followed in a specialized adult-focused outpatients' clinic.

Materials and methods

Patients

We considered all patients referred to the "Transition Unit for Childhood Cancer Survivors" (part of the "Città della Salute e della Scienza" Hospital in Turin, Italy) from November 2001 to September 2014. Demographic and treatment characteristics of this cohort have been previously published (Brignardello et al. 2013). All clinical data (cancer diagnosis, therapies, relapses, second tumors, late toxicities, etc.) of our CCS are recorded in a dedicated database, which is regularly updated during follow-up visits. For this study, we included patients satisfying the following criteria: (a) male sex; (b) first cancer diagnosis after 1/01/1985; (c) age at cancer diagnosis <18 years; (d) ≥5 years of survival after the first cancer diagnosis; and (e) ≥1 visit after the 18th birthday. In all patients, gonadal function was evaluated measuring FSH, LH, inhibin B and total testosterone levels. Patients with clinically significant low levels of total testosterone (<3 ng/dl) were considered to have hypogonadism, further subclassified as primary or central, depending on gonadotropin levels. Due to the reluctance of CCS to provide semen samples, in the presence of FSH levels >10 IU/l and inhibin B <100 pg/ml patients were considered to have spermatogenesis damage, even in the absence of semen analysis.

Statistical analysis

The distribution of patients characteristics according to gonadal function (normal, spermatogenesis damage, primary, central hypogonadism) was summarized using frequencies and percentages.

Patients with a diagnosis of central hypogonadism were excluded from the analysis of risk factors, due to the differences in pathogenesis between primary and central hypogonadism. To assess the impact of risk factors (demographic and clinical characteristics at cancer diagnosis, treatment exposures) on gonadal dysfunction (SD or primary hypogonadism), we estimated crude and adjusted OR performing logistic regression models. Since the cancer type and treatment exposures were strictly associated, we performed two different models. We included age, period of cancer diagnosis and cancer type in the first model, whereas age, period of cancer diagnosis and treatments were considered in the second one.

Results

A total of 199 consecutive male CCS were enrolled in the study (Fig. 1). Median follow-up time was 14.01 years (IQR = 10.08–17.76 years). Demographic and clinical characteristics according to the gonadal function are detailed in Table 1.

At the last available visit, 102/194 (51.26 %) male CCS had normal gonadal function. Among the remaining patients, a spermatogenesis damage was diagnosed in 68 patients (34.17 %); this diagnosis was confirmed in all 41 patients in whom semen analysis was performed. Sixteen (8.04 %) CCS fulfill the criteria for primary hypogonadism,

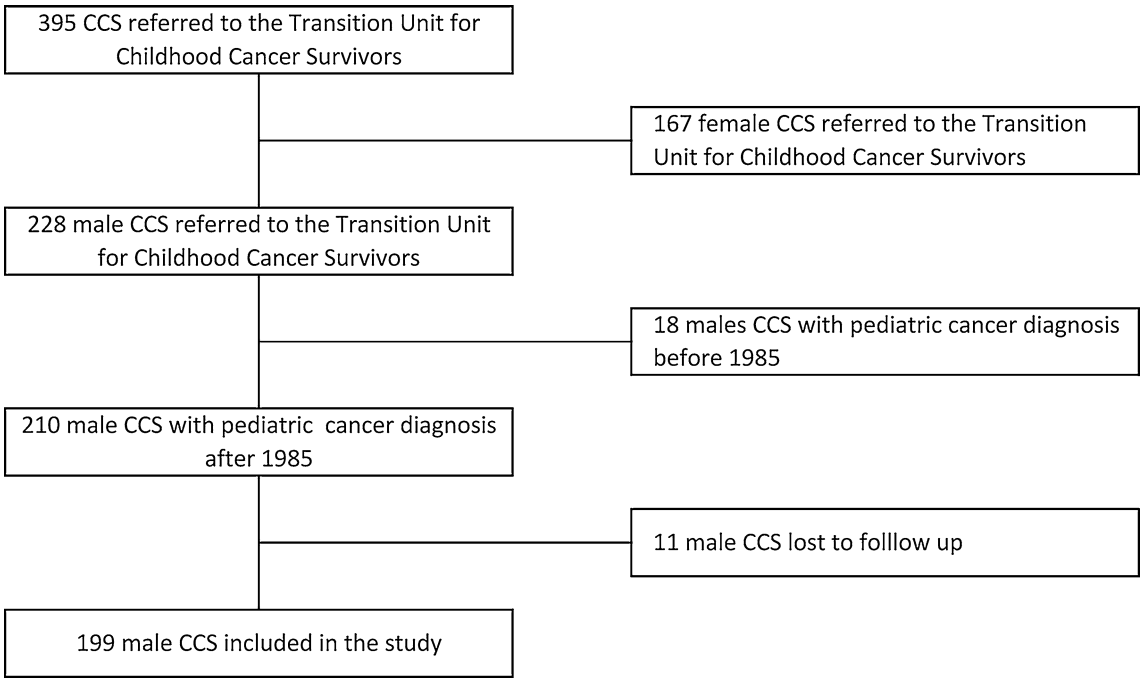

Fig. 1 Selection process of patients

whereas 13 (6.53 %) were diagnosed with central hypogonadism (possibly accompanied by testicular damage).

Among 33 patients previously treated with total body irradiation (TBI), none had normal gonadal function, 17 had spermatogenesis damage, 13 had primary hypogonadism, and 3 had central hypogonadism. An extremely high rate of gonadal dysfunction (46/48) was also detected in patients who underwent hematopoietic stem cell transplantation (HSCT).

The analysis of risk factors suggested a strong association between cancer type and gonadal dysfunction (Table 2), sarcomas being the cancer type associated with the higher risk of gonadal dysfunction (crude OR = 3.71; 95 % CI 1.13–12.23 and adjusted OR = 3.69; 95 % CI 1.11–12.22).

Considering treatment exposures, all patients treated with TBI had gonadal dysfunction. As a consequence, this exposure was not included in the models. The risk of gonadal dysfunction was higher in patients treated with radiotherapy (crude OR = 5.83; 95 % CI 2.95–11.52 and adjusted OR = 8.72; 95 % CI 3.94–19.30; Table 2) and in patients exposed both to alkylating agents and to platinum-derived agents (adjusted OR = 9.22; 95 % CI 2.17–39.23; Table 2).

After multivariate adjustment, age at pediatric cancer diagnosis seems not to affect the development of gonadal dysfunction (Table 2). The prevalence of gonadal dysfunction was similar in the three considered periods of cancer diagnosis (1985–1989, 1990–1999, >2000; Table 2).

Discussion

It is well known that the testes are highly susceptible to the toxic effects of cancer therapy, and consequently, it is not surprising that, after a very long period of observation, our data indicate a high prevalence of gonadal dysfunction in male CCS, mainly consisting in spermatogenesis damage. On the contrary, central hypogonadism resulted quite rare and mostly related to surgery or to radiotherapy involving the sellar region, in patients treated for brain tumors or hematologic malignancies.

Almost all patients treated with HSCT showed gonadal dysfunction. This result is most probably due to the use of TBI or high doses of alkylating agents as conditioning regimen for HSCT, since these treatments (also in our cohort) are strictly associated with the onset of male hypogonadism (Tromp et al. 2011; Felicetti et al. 2011). Indeed, the germinal epithelium is highly sensitive to damage induced by irradiation. Transient azoospermia can be observed after direct irradiation of the testes at doses as low as 0.1 Gy (15), whereas doses >6 Gy are able to completely destroy the pool of spermatogonial stem cells, resulting in permanent azoospermia (Rowley et al. 1974; Centola et al. 1994). Unfortunately, in our patients treated with radiotherapy, the available information did not allow to define the gonadal exposure, except for subjects submitted to TBI.

As it regards chemotherapy, it is well known that almost all chemotherapeutic agents are able to induce transient reduction in sperm count, due to damage of differentiating

Table 1 Patients' characteristics by gonadal dysfunction

	Patients without gonadal dysfunction ($N = 102$)		Patients with spermatogenesis damage ($N = 68$)		Patients with primary hypogonadism ($N = 16$)		Patients with central hypogonadism ($N = 13$)		Total ($N = 199$)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Age at pediatric cancer diagnosis (years)										
0–4	23	22.55	9	13.24	10	62.50	3	23.08	45	22.61
5–10	30	29.41	21	30.88	2	12.50	4	30.77	57	28.64
≥10	49	48.04	38	55.88	4	25.00	6	46.15	97	48.74
Period of pediatric cancer diagnosis										
1985–1989	9	8.82	5	7.35	3	18.75	6	46.15	23	11.56
1990–1999	62	60.78	42	61.76	8	50.00	6	46.15	118	59.3
2000–2007	31	30.39	21	30.88	5	31.25	1	7.69	58	29.15
Cancer type										
Hematologic malignancies	81	79.41	48	70.59	12	75.00	4	30.77	145	72.86
ALL	41	40.20	20	29.41	9	56.25	2	15.38	72	36.18
Hodgkin's lymphoma	19	18.63	20	29.41	0	0.00	1	7.69	40	20.10
NHL	18	17.65	3	4.41	0	0.00	0	0.00	21	10.55
AML	3	2.94	5	7.35	3	18.75	1	7.69	12	6.03
Brain tumors	11	10.78	6	8.82	2	12.50	9	69.23	28	14.07
Sarcomas	4	3.92	10	14.71	1	6.25	0	0.00	15	7.54
Others	6	5.88	4	5.88	1	6.25	0	0.00	11	5.53
Treatments										
Any radiation	45	44.12	54	79.41	15	93.75	11	84.62	125	62.81
Total body	0	0.00	17	25	13	81.25	3	23.08	33	16.58
Cranial	20	19.61	7	10.29	3	18.75	8	61.54	38	19.10
Any chemotherapy	95	93.14	68	100.0	15	93.75	9	69.23	187	93.97
Alkylating agents	78	76.47	52	76.47	13	81.25	4	30.77	147	73.87
Alkylating agents + platinum-based	3	2.94	14	20.59	2	12.50	4	30.77	23	11.56
Other combinations	14	13.73	2	2.94	0	0.00	1	7.69	17	8.54
HSCT	2	1.96	27	39.71	14	87.50	5	38.46	48	24.12

ALL Acute lymphoblastic leukemia, *NHL* non-Hodgkin's lymphoma, *AML* acute myeloblastic leukemia, *HSCT* hematopoietic stem cells transplantation

germ cells (Meistrich 2013). However, in agreement with other previous reports (Tromp et al. 2011; Rivkees and Crawford 1988; Wyns et al. 2010; Anserini et al. 2002), our data confirm that a permanent azoospermia is strongly associated with the exposure to alkylating and/or platinum-based agents. These are cell cycle nonspecific drugs, and therefore, they can also damage cells with low proliferation rate, such as spermatogonial stem cells (Holoch and Wald 2011).

Leydig cells are more resistant than germ cells to the detrimental effects of chemo-/radiotherapy. In our cohort, only 13/194 CCS showed low testosterone levels. Indeed, radiation doses required to damage Leydig cells are higher than those needed to produce germ cell failure (Wallace 2011; Shalet et al. 1989).

Also regarding chemotherapy, Leydig cell dysfunction may become apparent only after higher cumulative doses of cytotoxic drugs, mainly alkylating and platinum-based agents.

As far as cancer type, we observed a strong association between gonadal dysfunction and sarcomas, likely due to the high doses of alkylating agents usually needed to treat these tumors. Moreover, about half of our 40 Hodgkin's lymphoma survivors had gonadal dysfunction, probably due to the use of procarbazine in a high percentage of these patients (65 %; data not shown).

In our series, age at pediatric cancer diagnosis did not impact on the development of gonadal dysfunction. Interestingly, the risk of developing gonadal dysfunction was similar in patients treated recently or in earlier periods. This observation highlights that testicular dysfunction and

Table 2 Crude and adjusted effect of risk factors on gonadal dysfunction (SD or primary hypogonadism)

	Total ($N = 186$)	Gonadal dysfunction ($N = 84$)		Crude effect				Adjusted effect							
								Model 1[a]				Model 2[b]			
	N	*N*	%	OR	95 CI		*p*	OR	95 CI		*p*	OR	95 CI		*p*
Age at pediatric cancer diagnosis (years)															
0–4	42	19	45.24	1				1				1			
5–9	53	23	43.40	0.93	0.41	2.10	0.858	0.95	0.41	2.21	0.910	1.08	0.40	2.93	0.877
≥10	91	42	46.15	1.04	0.50	2.16	0.922	1.03	0.45	2.34	0.949	0.64	0.25	1.68	0.369
Period of the first cancer diagnosis															
1985–1989	17	8	47.06	1				1				1			
1990–1999	112	50	44.64	0.91	0.33	2.52	0.852	0.96	0.32	2.86	0.941	1.48	0.43	5.10	0.538
2000–2007	57	26	45.61	0.94	0.32	2.79	0.916	0.93	0.28	3.15	0.914	1.24	0.32	4.87	0.756
Cancer type															
Hematologic malignancies	141	60	42.55	1				1							
Brain tumors	19	8	42.11	0.98	0.37	2.59	0.970	0.98	0.36	2.63	0.965				
Sarcomas	15	11	73.33	3.71	1.13	12.23	0.031	3.69	1.11	12.22	0.033				
Others	11	5	45.45	1.12	0.33	3.86	0.851	1.13	0.33	3.89	0.848				
Treatments															
Any radiation	114	69	60.53	5.83	2.95	11.52	<0.001					8.72	3.94	19.30	<0.001
Total body	30	30	100.00												
Cranial	30	10	33.33	0.55	0.24	1.26	0.159								
Chemotherapy															
Alkylating agents	143	65	45.45	1								1			
Alkylating agents + platinum-based	19	16	84.21	6.40	1.79	22.93	0.004					9.22	2.17	39.23	0.003
Other combination or none chemotherapy	24	3	12.50	0.17	0.05	0.60	0.006					0.19	0.05	0.76	0.001
HSCT	43	41	95.35	47.67	11.03	206.03	<0.001								
Total	186	84	45.16												

ALL Acute lymphoblastic leukemia, *NHL* non-Hodgkin's lymphoma, *AML* acute myeloblastic leukemia, *HSCT* hematopoietic stem cells transplantation

[a] Including age, period of diagnosis and cancer type

[b] Including age, period of diagnosis and treatments

male infertility remain still today significant late effects of anticancer therapies.

Therefore, it is mandatory to inform male cancer patients (and/or their parents) about the risk of gonadal damage and infertility related to anticancer therapies, and semen cryopreservation should be offered to all boys who are able to produce semen (Wallace 2011; Loren et al. 2013; Joshi et al. 2014).

For patients who have not yet entered puberty, the options for fertility preservation (namely cryopreservation of testicular tissue to perform organ culture, tissue grafting, stem cell transplantation or "in vitro" gametogenesis) remain completely experimental, and families must be informed that any of these procedures should be considered today highly investigational (Holoch and Wald 2011; Jahnukainen et al. 2011).

However, this research field is rapidly developing, and until less toxic treatments will be available, it is to be hoped that in the next years it will provide viable options for fertility preservation also in prepubertal boys.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal consent is not required.

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