Ovarian reserve assessment in girls and women after hematopoietic stem cell transplantation (HSCT) treatment underwent in childhood

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The authors have NOTHING TO DISCLOSE.

Background

Gonadal dysfunction is the most frequent endocrine complication in long-term female survivors of hematopoietic stem cell transplantation (HSCT). It had been thought that young age prevented from ovarian damage during HSCT. The analysis performed in children after HSCT in ALL has shown that in about 80% of these children there is an impairment of gonadal function. In girls the visual pubertal development may be adequate, but menstruation is only achieved in half of the individuals [4]. The question what is the ovarian reserve in girls and young women after HSCT leaves unanswered.

The classical tests used to the evaluation of the ovarian reserve have some inconvenience, especially in young patients after HSCT. Serum levels of follicle-stimulating hormone (FSH), inhibin B and estradiol (E2) are interdependent and should be performed in the early follicular phase. Nevertheless, the changes of serum levels of FSH, inhibin B, and estradiol occur relatively late. It is not until age seven that elevated basal gonadotropins point to gonadal damage. Moreover in young patients with low gonadotropins level, we cannot exclude the central or the composed etiology of hypogonadism. Assessment of the number of antral follicles by ultrasonography best predicts the quantitative aspect of ovarian reserve, but using an transvaginal ultrasound during in the early follicular phase is often impossible in young females.

Anti-Muellerian Hormone (AMH) is a relatively new marker of the ovarian function and the ovarian reserve. This is the serum marker that reflects the number of follicles that have made the transition from the primordial pool into the growing follicle pool, and that is not controlled by gonadotropins. AMH is the earliest marker of diminishing ovarian reserve, with relatively minimal intra- and intercycle variation, and its serum levels decrease well before any increase in baseline FSH. There is a transient rise in AMH in the neonatal period due to a transient activation of the reproductive axis at that time, followed by a more sustained rise through childhood and adolescence. Some studies reported that there is a plateau or even a decline in AMH during puberty. AMH continues to rise after adolescence, with peak concentration, at approximately, the age of 24, and then decrease gradually with age, and the levels become undetectable after menopause. AMH expression is affected relatively little by use of the oral or vaginal administration of synthetic sex steroids. Additionally being not controlled by gonadotropins, AMH would benefit both patients and clinicians.

Objective

The aim of the study was to assess ovarian reserve in young patients after HSCT using evaluation of AMH hormone with comparison to classical hormonal tests.

Methods

Patients

Twenty-eight girls and women, mean 14.8±5.0 yr-old, after HSCT, and 28 healthy girls and women, mean 15.3±.9 yr-old were included in the study (Table 1). The Local Ethical Committee approved the study. The participants were consecutive female HSCT survivors during 2001-2014 from a single pediatric transplantation centre, over 7 yr-old at the time of the recruiting to the study, who gave or whose parents gave their written, informed consents. The age of patients at transplantation time was mean 9.1±5.0 years, the time from HSCT amounted mean 5.6±3.7 years. The patients had been treated using HSCT due to different hematological (14), oncologic (11) and non-malignant diseases (3). Autologous HSCTs (auto-HSCT) were performed in nine patients and allogeneic HSCTs (allo-HSCT) in 19. The details of pretransplantation regimen and type of the graft are presented in Table 1 (Table 1). Before the study 13/28 patients had received hormone replacement therapy (HRT) to induce puberty (9), and in the treatment of amenorrhea secundaria (4). Five of them discontinued HRT from different reasons, at least six months before the study. The spontaneous puberty after HSCT had been observed in three patients, the spontaneous menarche in one patient, and the recovery of normal menstrual cycles in three patients. Sexual development as prepubertal was assessed in ten patients 7.2-11.9-yr-old.

Fasting blood samples for measurement of FSH, luteinizing hormone (LH), E2, prolactin (PRL), sex hormone binding globulin (SHBG), thyroid-stimulating hormone (TSH), free thyroxin (fT4), anti-thyroid peroxidase (anti-TPO) antibodies, AMH, and Inhibin-B were taken from the antecubital vein, at 8.00-10.00 AM, in patients with normal menstrual cycles in 3rd - 5th day of the cycle.

Biochemical methods

Hormones: FSH (Siemens Healthcare Diagnostics Inc., USA), LH (Siemens Healthcare Diagnostics Inc., USA), PRL (Siemens Healthcare Diagnostics Inc., USA), SHBG (Siemens Healthcare Diagnostics Inc., USA), TSH (Siemens Healthcare Diagnostics Inc., USA), fT4 (Siemens Healthcare Diagnostics Inc., USA), AMH (Beckman Coulter Eurocenter S.A., Switzerland), and Inhibin B (Beckman Coulter Eurocenter S.A., Switzerland) were measured by immunochemistry.

The decreased ovarian reserve was defined as the occurrence of elevated gonadotropins (mainly FSH), decreased Inhibin B and decreased AMH levels.

Statistical analysis

Statistical analysis was performed using the Staf Soft Statistica 12 package. T-Student test was used for the analysis.

Results

AMH and Inhibin-B levels were significantly lower and FSH and LH levels were significantly higher in patients after HSCT than in age-matched healthy controls (Table 2). There were no differences in TSH, fT4, SHBG, PRL levels between HSCT patients and controls. Three patients in clinical and hormonal euthyreosis have positive anti-TPO antibodies. Clinical data on patients and results of ovarian function assessment are presented in Table 1 (Table 1).

Decreased level of AMH was observed in 26/28 (90%) patients after HSCT. In 20/28 (71%) patients AMH level was very low, below 0.08 ng/ml (6/9 in auto-HSCT group, 14/19 in allo-HSCT group). All auto-HSCT patients have decreased AMH levels, lower than the lowest level in the control group. Among patients after allo-HSCT, only two patients treated due to severe aplastic anemia have normal AMH, normal other hormones and normal menstruations. Six patients with AMH levels 0.14-0.87 ng/ml, presenting with prepubertal status with low FSH (3), spontaneous puberty (1), and normal menstruations (2), have normal inhibin B level. On the base of AMH level measurement, decreased ovarian reserve and abnormal ovarian function can be diagnosed in 90% of patients after HSCT.

Table 1.
Results of ovarian function (OF) assessment in patients after autologous and allogeneic HSCT. (SE – Sarcoma Ewing, NBL – neuroblastoma, NHL – non-Hodgkin lymphoma, HD – Hodgkin disease, BuMel – busulphan, melphalan, CEM - cyclophosphamide, etoposide and melphalan, BEAM - carmustine (BiCNU), etoposide, cytarabine (Ara-C) and melphalan, HRT – hormone replacement therapy, SCID - severe combined immunodeficiency, ALL – acute lymphoblastic leukemia, CGD - chronic granulomatous disease, FA – Fanconi anemia, SAA – severe aplastic anemia, ABD - Blackfan-Diamond anemia, NHL - non-Hodgkin lymphoma, haplo – haploidentical, MSD – matched sibling donor, MUD – matched unrelated donor, Bu - busulphan, Cy – cyclophosphamide, TBI – total body irradiation, VP-16 – etposide, ATG – antithymocyte globulin, Campath - alemtuzumab, GvHD – graft versus host disease, CMV reactive. – cytomegalovirus reactivation, rhGH – recombinant human growth hormone, HRT – hormone replacement therapy).

Dgn	Follow-up time after HSCT [yrs]	Age at HSCT [yrs]	Type of HSCT	Condition regimen	Age [yrs]	AMH [ng/ml]	Inh B [pg/ml]	FSH [mIU/ml]	LH [mIU/ml]	E2 [pg/ml]	Additional factors influencing OF	Sexual development history	Clinical status at study time
SE	11.2	11.1	Auto	BuMel	22.3	<0.08	7.1	78.73	31.09	391.1		No spontaneous puberty and menarche after HSCT,	Tanner V HRT,
NBL	11	5.8	Auto	BuMel	16.8	<0.08	6.9	<0,3	<0,07	46,9	Abdomen radiotherapy	No spontaneous puberty and menarche after HSCT	Tanner V HRT
SE	9	12.4	Auto	BuMel	21.4	<0.08	5.8	77	155	17,5		No spontaneous puberty and menarche after HSCT	Tanner V Stop HRT
NBL	7.4	2.8	Auto	BuMel	10.2	<0.08	4	39.31	8.79	7.3	Abdomen radiotherapy	No spontaneous puberty after HSCT	Tanner I (before HRT)
NBL	7.4	2.8	Auto	CEM	10.2	0.14	40	8,93	0,68	40,9	Abdomen radiotherapy	Spontaneous puberty after HSCT	Tanner II
NBL	6.8	0.4	Auto	BuMel	7.2	<0.08	4.4	6,87	0,17	11,8	Abdomen radiotherapy	prepubertal	Tanner I (before HRT)
SE	5.9	17.6	Auto	BuMel	23.5	<0.08	5.5	123,37	47,97	12,6		Spontaneous puberty and menarche before HSCT, secondary amenorrhea	Tanner V Stop HRT
HD	3.8	13.6	Auto	BEAM	17.4	0.21	40.1	28,23	30,04	236		Spontaneous puberty and menarche before HSCT	Tanner V Normal menstruation cycles
NHL	1.1	10.1	Auto	BEAM	11.2	0.52	12.6	8,66	0,57	13,8		Spontaneous puberty after HSCT	Tanner II
SCID	14.09	1.03	haplo	BuCy	15.12	<0.08	4.2	37.82	78.03	77.6	GvHD III g EBV infection	No spontaneous puberty and menarche after HSCT	Tanner V HRT Hashimoto disease
ALL	10.44	10.76	MSD	TBI-VP16	21.20	<0.08	6.9	46.40	20.52	20	GvHD III, sepsis	Spontaneous puberty and menarche after HSCT, Then secondary amenorrhea and HRT for 3 yrs	Tanner V STOP HRT Normal menstrual cycles Hypothyroidism Diabetes mellitus
ALL	9.32	9.26	MSD	TBI-VP16	18.58	<0.08	11	50.74	24.75	23.1	GvHD I	No spontaneous puberty and menarche after HSCT	Tanner V HRT stop
ALL	7.27	4.69	MSD	TBI-VP16	11.96	<0.08	4.6	40.48	110.84	19.5	GvHD I	No spontaneous puberty and menarche after HSCT	Tanner I (before HRT), hypothyroidism
CGD	7.24	13.17	MUD	ByCy-ATG	20.41	<0.08	6	21.74	44.2	106.7		Spontaneous puberty and menarche before HSCT Then secondary amenorrhea	Tanner V HRT stop
FA	7.06	4.01	MUD	BuFluCy-ATG	11.07	0.87	6.2	<0.07	1.93	11.6		No spontaneous puberty and menarche after HSCT	Tanner I
ALL	6.71	8.73	MUD	TBI-VP16 ATG	15.44	<0.08	4.2	19.26	24.56	42.2	GvHD I, acute renal insuff, CMV reactiv.	No spontaneous puberty and menarche after HSCT	Tanner V, HRT rhGH-treatment
ALL	5.88	10.67	MUD	TBI-VP16 ATG	16.55	<0.08	8.8	54.03	71.79	13.1	GvHD I, CMV reactiv.	Spontaneous puberty and menarche before HSCT, Then secondary amenorrhea	Tanner V HRT
SAA	5.2	13.28	MUD	FluCy ATG	18.48	4.02	46.6	5.88	7.93	92.2		Spontaneous puberty and menarche before HSCT	Tanner V, normal menstrual cycles
ABD	4.04	3.16	MUD	ByCy-ATG	7.2	<0.08	6.8	1.61	<0.07	9.9		prepubertal	Tanner I
FA	3.25	6.95	MUD	BuCyFlu ATG	10.20	0.19	8	0.1	8.53	21.3		Spontaneous puberty	Tanner II
FA	3.18	4.36	MUD	BuCyFlu ATG	7.54	<0.08	5.1	<0.07	1.66	8.8	GvHD II	prepubertal	Tanner I
SAA	2.98	14.48	MSD	Cy-ATG	17.46	3.71	15	2.56	2.16	130		Spontaneous puberty and menarche before HSCT	Tanner V, normal menstrual cycles
FA	1.92	16.70	MUD	BuCyFlu	18.62	0.14	45.9	17.18	2.83	29.5	First graft rejection	Spontaneous puberty and menarche before HSCT, Then secondary amenorrhea	Tanner V, HRT
NHL	1.66	17.54	MSD	TBI-Thio-VP-16	19.20	<0.08	5.8	<0.07	<0.03	74.3		Spontaneous puberty and menarche before HSCT, Then secondary amenorrhea	Tanner V, HRT
ALL	0.91	11.50	MSD	TBI-VP16	12.41	<0.08	6.6	16.87	58.64	9.2	GvHD II	Spontaneous puberty and menarche before HSCT, Then secondary amenorrhea	Tanner II, HRT
CGD	0.85	11.46	MUD	BuFlu Campath	12.31	<0.08	6	54.38	4.75	16.7		Spontaneous puberty before HSCT and no menarche after HSCT	Tanner I (before HRT)
ALL	0.69	6.47	MSD	TBI-VP16	7.16	<0.08	4.4	10.21	0.57	8.6		prepubertal	Tanner I (before HRT)
ALL	0.52	9.36	MUD	TBI-VP16 ATG	9.88	<0.08	5.1	37.99	-	-	GvHD II, CMV infection. Sepsis Gram-	No spontaneous puberty and menarche after HSCT	Tanner I (before HRT)

Nineteen/28 patients (68%) had inhibin-B level lower than the lowest inhibin level in the control group. Twenty-four/28 (86%) patients after HSCT had inhibin-B level lower than 40 pg/ml, what result allows to diagnose impaired ovarian function and decreased ovarian reserve [12].

Eighteen/28 patients presented with hypergonadotropic hypogonadism, 3/28 have prepubertal values of sex hormones with elevated FSH (together 75%), 7/28 (25%) patients have normal values of sex hormones (including 3/28 prepubertal). On the base of the results of gonadotropins, abnormal ovarian function and decrease ovarian reserve can be diagnosed in 75% of patients after HSCT.

Table 2. Ranges and median values of hormones in patients after HSCT and in age-matched healthy controls.

	Age [yrs]	AMH [ng/ml]	Inh-B [pg/ml]	FSH [mIU/ml]	LH [mIU/ml]	E2 [pg/ml]	SHBG [nmol/ml]	PRL [µIU/ml]	TSH [uIU/ml]	fT4 [pmol/l]
HSCT	**15.2**	**<0.08-4.02**	**4-46.6**	**<0.07-123**	**<0.07-155**	**7.3-391.1**	**20.5-266**	**40.7-298**	**0.67-3.66**	**10.1-22.2**
median		**0.37**	**6.4**	**24.99**	**14.6**	**20**	**93.8**	**109.9**	**1.78**	**14.3**
Control	**15.8**	**0.67-9.88**	**7.1-224.7**	**<0.07-28.01**	**<0.07-155**	**8.3-152,9**	**9.8-173**	**61.5-722.7**	**0.59-3.76**	**11.4-19.9**
median		**3.3**	**46.8**	**4.79**	**3.23**	**49.8**	**105.9**	**124.8**	**1.82**	**14.5**
P	**0.4**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**0.46**	**0.43**	**0.28**	**0.49**	**0.19**

Conclusions

Patients after HSCT have impaired ovarian reserve. The ovarian reserve is mostly related to the conditioning therapy before HSCT. AMH as well as Inhibin-B and FSH are specific and good markers for the assessment of ovarian reserve.

Our study results demonstrate that in young women, HSCT recipients AMH is independent and more sensitive and specific test than other markers in the evaluation of ovarian function and reserve after HSCT. The measurement of AMH level allows to suspect hypogonadism in prepubertal girls, to diagnose central or composed hypogonadism, moreover lets to diagnose the ovarian reserve in the patients receiving HRT, without discontinuation of this therapy.

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