Patient-centered value-based healthcare in long-term follow-up after pediatric stem cell transplantation for nonmalignant diseases

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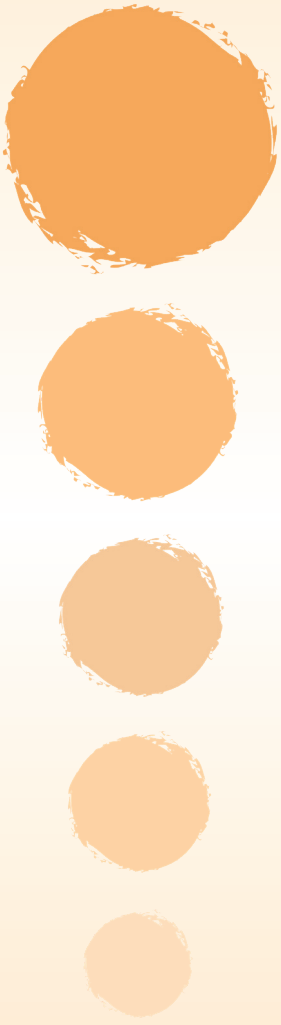

Chapter 3

Late endocrine effects after hematopoietic stem cell transplantation in children with nonmalignant diseases

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ABSTRACT

The number of children undergoing hematopoietic stem cell transplantation (HSCT) for nonmalignant diseases has increased in recent years. Endocrine complications are common after HSCT for malignant diseases, while little is known about long-term prevalence and risk factors in children transplanted for nonmalignant diseases. We retrospectively evaluated gonadal function, near adult height and thyroid function in 197 survivors of pediatric HSCT for hemoglobinopathies (n = 66), inborn errors of immunity/metabolism (n = 74) and bone marrow failure disorders (n = 57); median follow-up was 6.2 years (range 3.0-10.5). Gonadal dysfunction occurred in 55% of (post)pubertal females, was still present at last assessment in 43% and was more common after busulfan- than treosulfan-based conditioning (HR 10.6, CI 2.2-52.7; adjusted for HSCT indication). Gonadal dysfunction occurred in 39% of (post)pubertal males, was still present at last assessment in 32% and was less common in those who were prepubertal compared to (post)pubertal at HSCT (HR 0.11; CI 0.05-0.21). Near adult height was more than 2 SDS below mean parental height in 21% of males and 8% of females. Hypothyroidism occurred in 16% of patients; 4% received thyroxin treatment. In conclusion, endocrine complications, especially gonadal dysfunction, are common after pediatric HSCT for nonmalignant conditions. In females, treosulfan seems less gonadotoxic than busulfan. Careful long-term endocrine follow-up is indicated.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) provides an established curative treatment for an increasing variety of nonmalignant diseases (1). Nonmalignant indications for HSCT include hemoglobinopathies (HBP), bone marrow failure syndromes (BMF), inborn errors of immunity (IEI) and inborn errors of metabolism (IEM). The growing number of indications, continuous developments in HSCT approaches and the ongoing improvements in survival have led to a growing population of survivors who are at risk of developing late effects. Endocrine complications, such as growth impairment and gonadal dysfunction, are among the most frequent late effects after HSCT for malignant diseases. However, little is known about the prevalence and risk factors of long-term endocrine complications in children transplanted for nonmalignant diseases. Factors that may affect the incidence and severity of endocrine complications such as underlying disease, age at HSCT, pretransplant therapies, conditioning agents including irradiation, use of high dose steroids, chronic graft versus host disease (GvHD) and iron overload differ between children with malignant and nonmalignant diseases (2-4). Therefore, knowledge gained from studies of late effects after HSCT for malignant diseases may not apply to patients with nonmalignant diseases.

The aim of this study is to evaluate the cumulative incidence of gonadal dysfunction, thyroid dysfunction and growth failure in individuals who had received an HSCT in childhood for nonmalignant diseases. We hypothesize that the incidence of late endocrine effects in children transplanted for nonmalignant diseases is lower than what has been described after HSCT for malignant indications, as we expect they received less toxic treatment pre-HSCT, less toxic conditioning regimens and less irradiation therapy. Identifying the incidence of, and risk factors for, late endocrine effects is crucial for 1) optimizing treatment regimens to improve outcome after HSCT in children, 2) improving shared decision making before HSCT by providing patients and their parents accurate and complete information on potential risks and benefits of HSCT and 3) developing optimal clinical guidelines for screening of endocrine late effects (5).

METHODS

Study design

This retrospective non-interventional single-center study included all patients, aged 0-18 years, with a nonmalignant disease who had received an HSCT at the Department of Pediatrics at the Leiden University Medical Center in the Netherlands between 1997 and 2018 and were alive 2 years post-transplant (Fig 1). Exclusion

criteria were re-transplantation, no data available on the outcome measures of the study and death within 2 years post-transplant. Patients were evaluated by a transplantation specialist and pediatric endocrinologist for a clinical and laboratory assessment pre-transplantation and yearly after transplantation at the outpatient clinic. Clinical assessment included assessment of symptoms suggestive of endocrine complications (such as amenorrhea), auxological measurements, evaluation of the growth chart, assessment of bone age with an X-ray of the left hand, evaluation of pubertal stage and palpation of the thyroid gland. At each visit the following laboratory measurements were performed: gonadotrophins, testosterone in boys, estradiol in girls, thyroid stimulating hormone (TSH), Free Thyroxin (FT4), Insulin-like Growth Factor 1 (IGF-1) and Insulin-like Growth Factor Binding Protein-3 (IGF-BP3). The study protocol was assessed by the local medical ethical committee who determined that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study. The need for informed consent was waived.

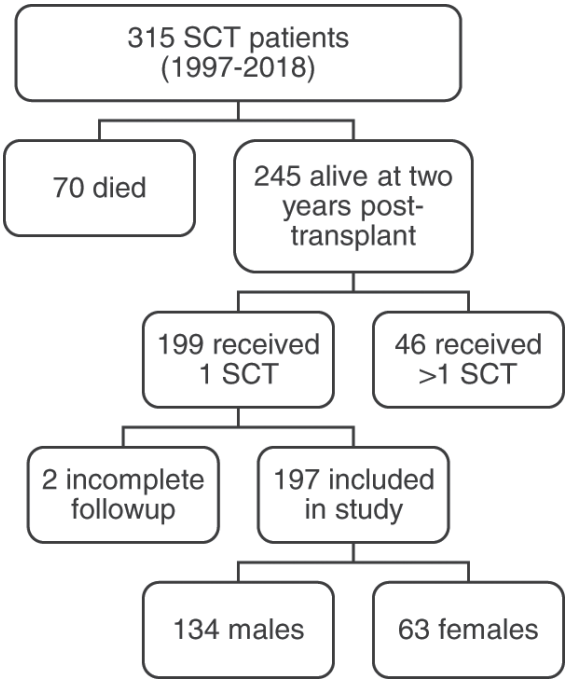

Fig 1. Flow chart showing inclusion and exclusion of patients.

Data collection

Patient characteristics were collected from the medical files including sex, age, underlying disease and conditioning regimen. Indications for HSCT were classified as IEI/IEM, HBP or BMF; blood cell disorders such as paroxysmal nocturnal hemoglobinuria were included in the BMF group (for all diagnoses included in these groups see Supplementary Table 1). Conditioning regimens were divided into five main categories: busulfan-based, treosulfan-based, chemotherapy with total abdominal irradiation (TAI)/total body irradiation (TBI), others and no conditioning (for specific regimens see Supplementary Table 2).

Data were collected on three main endocrine late effects after transplantation: gonadal dysfunction, thyroid complications and growth failure. Data were abstracted from medical charts by two of the authors (LCdK and JEB).

Definitions and outcome measures

Gonadal dysfunction

Patients at Tanner stage ≥G2 or ≥B2 were classified as (post)pubertal and were included in the analysis of gonadal dysfunction (6, 7). Patients diagnosed with gonadal dysfunction before HSCT were excluded from this analysis. Gonadal dysfunction (elevated gonadotrophins) and hypogonadotropic hypogonadism (low estradiol/testosterone with gonadotrophins below/within reference range) were recorded (for exact definitions see Supplementary Table 3) (8). Use of hormone replacement therapy (HRT) or oral contraceptives after HSCT, ovarian tissue cryopreservation prior to HSCT, and the use of a GnRH agonist (GnRHa) at the time of HSCT were recorded.

Thyroid complications

Patients diagnosed with hypothyroidism before HSCT were excluded from this analysis. Overt primary hypothyroidism (elevated TSH and FT4 below reference range), subclinical hypothyroidism (elevated TSH with normal FT4), central hypothyroidism (TSH within/below reference range with FT4 below reference range) and hyperthyroidism (suppressed TSH and elevated FT4) were recorded (for exact definitions see Supplementary Table 3) (9, 10).

Growth failure

Patients (temporarily) treated with growth hormone before or after HSCT were excluded from this analysis. Height standard deviation scores (SDS) for age and sex were calculated using reference data reported by de Onis et al. and Garza et al. (11, 12). Near adult height (NAH) was analyzed in all patients with a chronological age ≥14 years for boys and ≥12 years for girls, who fulfilled at least one of the following

criteria: height velocity <2 cm/year or bone age >16 years for boys and >14 years for girls according to Greulich and Pyle (13). NAH was compared to mid-parental height (MPH) (14). Short stature (SS) was defined as NAH < -2 SDS.

Statistical analysis

Continuous outcomes were compared between groups with a Mann-Whitney U test. Differences in categorical factors between groups were analyzed by the Pearson's chi-square test or Fisher's exact test. Two-tailed P-values of <0.05 were considered statistically significant. Univariate logistic regression analyses were performed to evaluate risk factors for outcomes calculating odds ratios. Risk factors evaluated were age at HSCT, gender, diagnosis, conditioning regimen, donor type, puberty stage at HSCT and acute GVHD and, in the analysis of gonadal dysfunction, the use of a GnRHa. When large and significant differences were seen in follow-up duration multistate Cox models (Supplementary Fig 1) were used to compare groups and to calculate hazard ratios (HR) using R 4.1.0 (15).

RESULTS

Patient characteristics

The study included 197 patients, 134 males and 63 females. Median age at HSCT was 5.7 years (IQR 2.9-11.3 years) and median follow-up was 6.2 years (IQR 3.0-10.5 years). Underlying diseases were IEI/IEM (n=74), HBP (n=66), and BMF (n=57) (Table 1). Patients with IEI/IEM were significantly younger at HSCT and follow-up duration was significantly longer. The majority of patients had received busulfan-based (46%) or treosulfan-based (34%) myeloablative conditioning. The remainder was treated with chemotherapy with low dose irradiation (4%), no conditioning (2%), or others (fludarabine with cyclophosphamide (11%), cyclophosphamide (2%), other (2%)). The conditioning regimen was significantly different between IEI/IEM, HBP and BMF (Table 1).

Table 1. Patient characteristics.

	Total ***N*=197**	**IEI/IEM** ***N*=74**	**HBP** ***N*=66**	**BMF** ***N*=57**	***p*** **value**
Male / female	134/63	55/19	46/20	33/24	0.13
Age at transplantation, years, median (IQR)	5.7 (2.8-11.3)	3.0 (0.9-6.7)	8.5 (4.9-14.1)	7.9 (4.4-13.1)	<0.001
Age at last assessment, years, median (IQR)	14.7 (9.7-18.7)	13.7 (8.9-17.8)	15.7 (12.4-18.4)	14.9 (9.9-19.2)	0.3
Follow-up duration, years, median (IQR)	6.2 (3.0-10.5)	8.4 (4.4-12.4)	5.4 (2.9-8.5)	5.1 (2.6-10.1)	0.01
Conditioning regimens					<0.001
Busulfan based	90 (46%)	50 (68%)	22 (33%)	18 (32%)	
Treosulfan based	66 (34%)	20 (27%)	41 (62%)	5 (9%)	
TAI-based	8 (4%)	0 (0%)	1 (2%)	7 (12%)	
Others	30 (15%)	1 (1%)	2 (3%)	27 (47%)	
None	3 (2%)	3 (4%)	0 (0%)	0 (0%)	
Donor relation					<0.001
Matched related donor	76 (39%)	20 (27%)	30 (45%)	26 (46%)	
Mismatched related donor	23 (12%)	4 (5.4%)	15 (23%)	4 (7.0%)	
Unrelated donor	98 (50%)	50 (68%)	21 (32%)	27 (47%)	
Stem cell source					0.1
BM	159 (81%)	54 (73%)	53 (80%)	52 (91%)	
CB	15 (7.6%)	10 (14%)	4 (6.1%)	1 (1.8%)	
Other/Undefined	1 (0.5%)	0 (0%)	1 (1.5%)	0 (0%)	
PBSC	22 (11%)	10 (14%)	8 (12%)	4 (7.0%)	
aGvHD					0.14
Grade 0-I	175 (89%)	62 (84%)	59 (89%)	54 (95%)	
Grade II-III[a]	22 (11%)	12 (16%)	7 (11%)	3 (5%)	

IEI/IEM inborn errors of immunity/metabolism, HBP hemoglobinopathies, BMF bone marrow failure disorders, BM bone marrow, CB cord blood, PBSC peripheral blood stem cells, aGvHD acute graft versus host disease.
[a]There were no patients with grade IV aGvHD.

Gonadal function in females

At last follow-up 44 of 63 females were (post)pubertal and included for evaluation of gonadal function. At time of HSCT 19 of them (43%) were (post)pubertal; median age at HSCT was 8.9 years (IQR 6.1-14.2 years) and median age at last visit 17.5 years (IQR 15.6-21.2).

Gonadal dysfunction occurred in 24 of these 44 (55%) patients and was still present at last assessment in 19/44 females (43%) (Table 2 and Fig 2). Median time from HSCT to diagnosis of gonadal dysfunction was 1.0 year (IQR 0.6-7.8 years), median age at diagnosis of gonadal dysfunction was 14.0 years (IQR 11.5-15.3 years). In five females, gonadotrophin levels decreased over time with eventual recovery of gonadal function. No females were diagnosed with hypogonadotropic hypogonadism.

Twenty-one patients received HRT, which could be discontinued in six. Four out of eight patients who had undergone cryopreservation of one ovary developed gonadal dysfunction, of whom three received HRT. Ten patients received GnRHa treatment during HSCT; five of them developed gonadal dysfunction and from two no follow-up data on gonadal function were available. Gonadal dysfunction was significantly more common in females who received busulfan-based compared to treosulfan-based conditioning, 16/17 (94%) versus 5/15 (33%). Bivariate multistate analysis, including HSCT indications, showed a HR for busulfan versus treosulfan of 10.6 (95%CI 2.2-52.7, p=0.004) and a HR for BMF versus IEI/IEM of 0.2 (95%CI 0.05-0.8, p=0.03, Supplementary Table 4).

Table 2. Female gonadal dysfunction and risk factors

	Gonadal dysfunction	**No gonadal dysfunction**	**p-value[a]**
	N=24	**N=20**	
Pubertal status at HSCT			0.4
Prepubertal	12 (48%)	13 (52%)	
(Post)pubertal	12 (63%)	7 (37%)	
Conditioning			<0.001
Busulfan based	16 (94%)	1 (6%)	
Treosulfan based	5 (33%)	10 (67%)	
TBI/TAI based	2 (100%)	0 (0%)	
Others	1 (10%)	9 (90%)	
Underlying disease			0.01
Inborn errors of immunity/metabolism	11 (85%)	2 (15%)	
Bone marrow failure disorders	5 (31%)	11 (69%)	
Hemoglobinopathies	8 (53%)	7 (47%)	

HSCT hematopoetic stem cell transplantation, TAI total abdominal irradiation, TBI total body irradiation.
[a]Univariate analysis by Fisher's exact test.

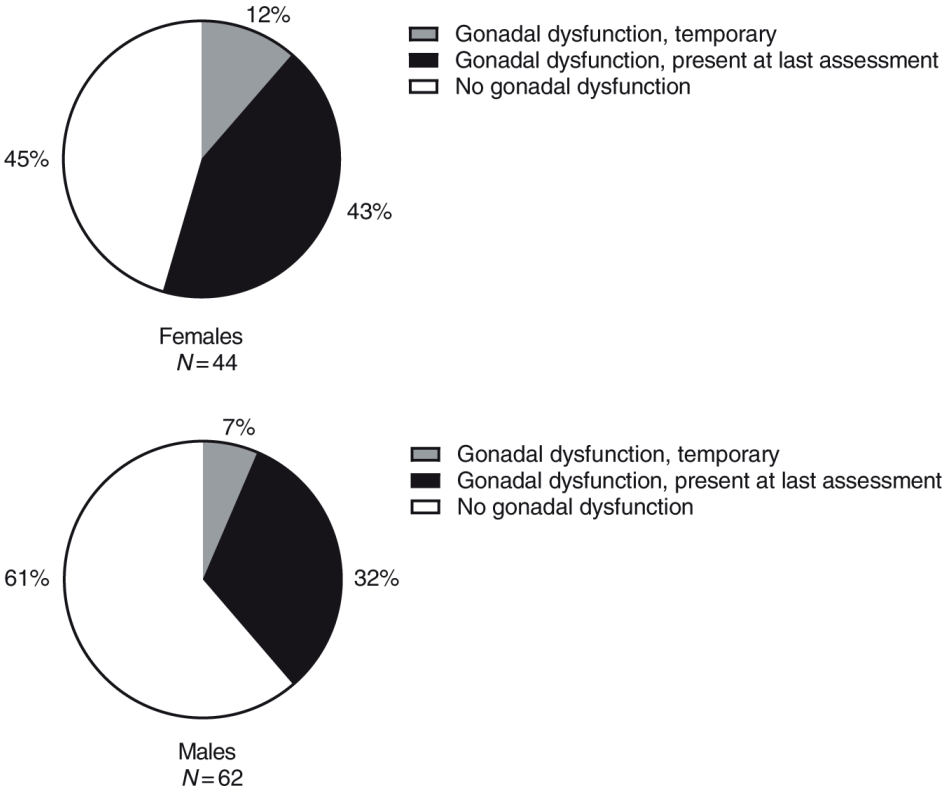

Fig 2. Gonadal dysfunction by gender

Gonadal function in male patients

At last follow-up 67 of 134 males were (post)pubertal of whom 62 patients were included for evaluation of gonadal function. One patient was excluded due to missing data and four patients were excluded because of gonadal dysfunction prior to HSCT. At time of HSCT 19 (31%) were (post)pubertal, median age at HSCT was 7.6 years (IQR 4.1-13.2 years) and median age at last visit was 18.6 years (IQR 15.1-21.1 years).

In 24/62 (39%) gonadal dysfunction was observed, which was still present at last assessment in 20/62 (32%) (Table 3 and Fig 2). Median time from HSCT to diagnosis of gonadal dysfunction was 4.4 years (IQR 1.0-11.1 years), median age at diagnosis of gonadal dysfunction was 15.9 years (IQR 14.9-17.7 years). Hypogonadotropic hypogonadism was seen in two males. In one pituitary iron overload was suspected based on high serum ferritin levels after chronic transfusion therapy because of beta-thalassemia. Two males received HRT because of hypergonadotropic (n=1) or mixed (n=1) hypogonadism.

Table 3. Male gonadal dysfunction and risk factors

	Gonadal dysfunction	No gonadal dysfunction	p value[a]
	N=24	N=38	
Pubertal status at HSCT			
Prepubertal	12 (28%)	31 (72%)	0.04
(Post)pubertal	12 (63%)	7 (37%)	
Conditioning			0.009
Busulfan based	17 (46%)	20 (54%)	
Treosulfan based	2 (14%)	12 (86%)	
TBI/TAI based	2 (40%)	3 (60%)	
Others	3 (50%)	3 (50%)	
Underlying disease			0.13
Inborn errors of immunity/metabolism	7 (29%)	17 (71%)	
Bone marrow failure disorders	9 (53%)	8 (47%)	
Hemoglobinopathies	8 (38%)	13 (62%)	

HSCT hematopoetic stem cell transplantation, TAI total abdominal irradiation, TBI total body irradiation.
[a]Univariate analysis by Fisher's exact test.

Gonadal dysfunction was significantly less common in males who were prepubertal versus (post)pubertal at HSCT (28% versus 63%, HR 0.11; 95%CI 0.05-0.21, p<0.001, Table 3). In males who received busulfan-based conditioning 46% developed gonadal dysfunction, compared to 14% in patients with treosulfan-based conditioning; this difference was not statistically significant (HR 3.1; 95%CI 0.7-13.3, p=0.14)

Thyroid complications

Data on thyroid function were available from 189 of 197 patients. Two were excluded from this analysis because of a diagnosis of hypothyroidism prior to HSCT. Thirty-four patients (18%) developed thyroid complications after a median duration of 21.0 months (IQR 11.0 - 27.0 months) post-HSCT. One female was diagnosed with papillary thyroid carcinoma 16 years after HSCT; she had received TAI (4 Gy). In 29 patients (16%) hypothyroidism was diagnosed (overt, n=7; subclinical, n=19; or central, n=3). Primary or central hypothyroidism was still present at last assessment in 8 of 187 patients (4%). All six patients (3%) with primary hypothyroidism had positive thyroid peroxidase (TPO) antibodies. In one patient the sibling donor had (in retrospect) also been diagnosed with auto-immune hypothyroidism. No association was found between the underlying diagnosis of patients with auto-immune hypothyroidism compared to patients with normal thyroid function after HSCT. All six patients showed complete chimerism and had no endocrine dysfunction before HSCT. Fourteen patients (7%) required thyroxin treatment. In patients who

received busulfan-based conditioning 24% developed hypothyroidism, compared to 8% in patients with treosulfan-based conditioning (HR 1.6; CI 0.6-4.5, p=0.31). Hypothyroidism was observed in 1/8 patients who received TAI (4 Gy).

Transient hyperthyroidism occurred in four patients (2%). Anti-TSH receptor antibodies were positive in one and anti-TPO antibodies were positive in two of these patients.

Growth

Growth hormone treatment was used in 18 of 197 patients and these were excluded from the analysis. At last visit, 79 of 179 patients had reached NAH (44%) and in 60 of them MPH was known (Supplementary Table 5). Overall, median height SDS did not significantly change between HSCT and last follow-up, with a median change of 0.0 SDS (IQR -0.6-0.4). Nineteen percent of patients had SS (NAH < -2 SDS). In 7 of 34 males (21%) and 2 of 26 females (8%) NAH was more than 2 SDS below MPH; five of them already had a height more than 2 SDS below MPH at HSCT (Fig 3 and Supplementary Table 5). Univariate regression analysis did not identify factors associated with distance of NAH to MPH more than 2 SDS.

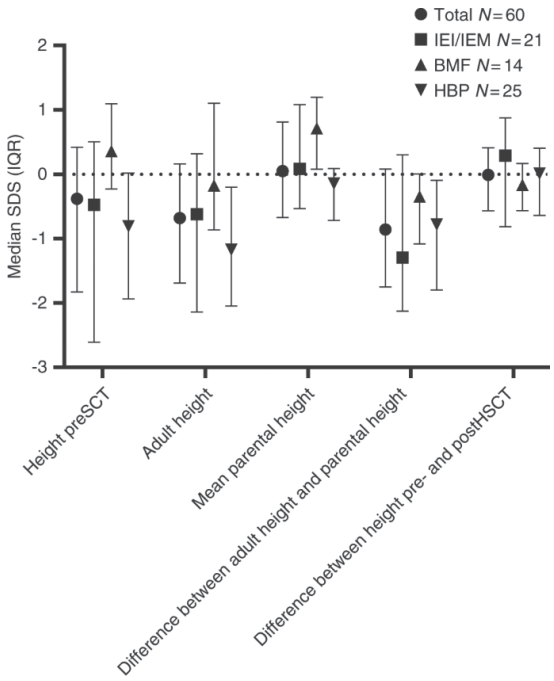

Fig 3. Growth outcomes by underlying condition

Multiple endocrine complications

To investigate to what extent gonadal dysfunction, thyroid complications and growth failure cluster, we calculated the number of endocrine complications per patient in 54 patients who had reached NAH and were (post)pubertal at last visit. In total, 33 patients (61%) had at least one endocrine complication and 25 patients (46%) had a complication that was still present at last assessment (Fig 4). Eleven (20%) had more than one endocrine complication and seven (13%) more than one complication present at last assessment.

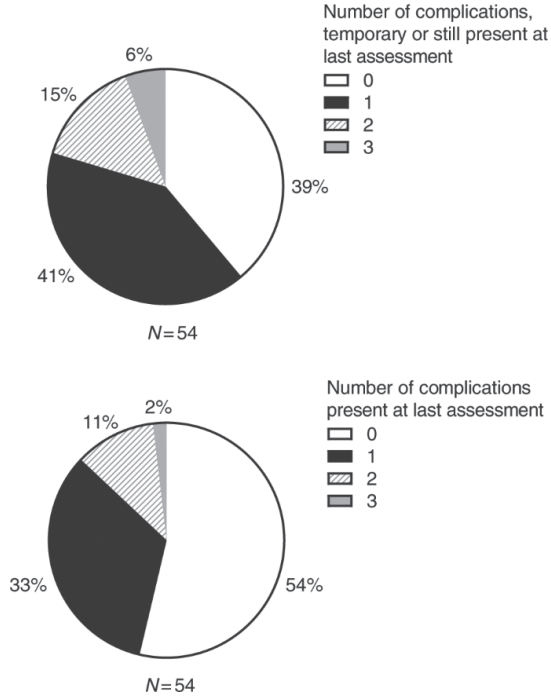

Fig 4. Frequency of multiple complications

DISCUSSION

The aim of our study was to identify the prevalence of, and risk factors for, late endocrine effects after HSCT in children transplanted for nonmalignant diseases to optimize treatment regimens, improve shared decision making before HSCT with patient and parents and aid in developing optimal clinical guidelines for screening of late effects. Our study reports a high cumulative incidence of 61% of endocrine complications in survivors of pediatric HSCT for nonmalignant diseases. Previous studies, mainly in malignant diseases, showed a similar incidence of endocrine complications after HSCT, ranging from 59 to 65% (16-18). This may seem surprising, as children with malignant disease have usually received more extensive chemotherapy and/or irradiation prior to HSCT. However, in children transplanted for nonmalignant diseases factors other than the conditioning may contribute to endocrine complications, such as iron overload due to chronic transfusions, the use of immunosuppressive agents and the underlying diseases themselves.

Gonadal dysfunction

Gonadal dysfunction was the most frequent endocrine complication in both females and males, seen in respectively 55% and 39%. We demonstrate that females were more likely to develop gonadal dysfunction after busulfan- than after treosulfan-based conditioning and in males a similar trend was seen. Previous studies, mainly of HSCT for malignant diseases, reported a similarly high risk of gonadal dysfunction after busulfan-based conditioning in both males and females (19-25). We also observed an increased risk of gonadal dysfunction in males who were (post)pubertal at HSCT. This is in line with other reports (26-28), although the opposite has also been reported (29). All parents and patients should be counseled about the risk of gonadal dysfunction and should be offered fertility preservation prior to HSCT, in particular patients with busulfan conditioning and males that are (post)pubertal at HSCT. The significantly higher risk of gonadal dysfunction after busulfan needs be taken into account when selecting a conditioning regimen. In addition, animal studies are providing insight into the mechanisms by which gonadal damage occurs, which will hopefully lead to future strategies to prevent gonadal toxicity due to conditioning (30, 31). However, factors other than conditioning-related toxicity may also play a role in certain patient groups, as a recent study found that endocrinopathies including primary ovarian insufficiency frequently occurred among individuals with immunodeficiencies who had not been transplanted (32). We also observed a particularly high rate of gonadal dysfunction among females with IEI/IEM (11/13). Further research is necessary to unravel the role of underlying genetic defects and immune dysregulation in the pathophysiology of gonadal dysfunction in order to identify ways to improve outcome.

Several guidelines on follow-up after HSCT for HBP, BMF or SCID, recommend annual assessment of pubertal progress and laboratory evaluation of gonadal function

starting 12 months after HSCT or without specifying when screening should start (5, 33, 34). Based on the high incidence of gonadal insufficiency among females, the finding that half of them were diagnosed with this complication within 1 year post-HSCT and the fact that most required treatment we recommend earlier screening, from 6 months post-HSCT to ensure early detection and appropriate treatment of hypogonadism (Table 4). Importantly, several individuals had temporary gonadal dysfunction with elevated gonadotrophin levels in the initial post HSCT period that normalized over time indicating recovery of gonadal function, as has been reported in previous studies in patients transplanted for severe aplastic anemia (SAA) or malignancies (35-38). Patients should be counselled about the possibility of ovarian recovery and advised about contraceptive measures.

Table 4. Recommendations for endocrine follow-up in nonmalignant HSCT survivors

Endocrine system	Follow-up recommendations
Growth	Measure height and weight annually. In case of short stature and/or growth deflection perform laboratory evaluation for short stature and determine bone age. Refer patients with poor growth to an endocrinologist to consider GH treatment.
Thyroid	Perform thyroid function tests (TSH, free T4) annually. Refer patients with abnormal results to an endocrinologist for advice on treatment.
Gonads - females	Evaluate Tanner stage and menstrual cycle annually until adulthood. In females aged ≥11 years: • Measure sex steroid and gonadotropin levels (FSH, LH, estradiol) annually until menarche has occurred; thereafter repeat in case of menstrual irregularities amenorrhea or in case of wish to evaluate with regard to fertility. • Consider measuring Anti-Mullerian hormone (AMH) at least once. Refer patient with pubertal delay, amenorrhea and/or abnormal laboratory results to an endocrinologist or gynaecologist for advice on treatment.
Gonads - males	Evaluate Tanner stage annually until adulthood. In males aged ≥12 years: • Measure sex steroid and gonadotropin levels (FSH, LH, testosterone). Consider repeating these measures annually. • Repeat measures in case of symptoms/signs suggestive of hypogonadism. • Consider measuring Inhibin B at least once. Refer patient with pubertal delay, abnormal progression of puberty and/or abnormal laboratory results to an endocrinologist or andrologist for advice on treatment.

We recommend screening at 6 months post HSCT, 12 months and then annually.

Thyroid function

In our cohort 18% of patients developed thyroid complications and 7% were treated with thyroxin. The overall reported incidence of thyroid disease in survivors of HSCT in childhood for malignant and nonmalignant diseases is similar, with incidences ranging from 10-24% (39-41). A number of mechanisms may explain abnormalities in thyroid function after HSCT. In our cohort, all patients with persistent primary hypothyroidism (3%) had anti-TPO antibodies pointing to immune mediated endocrine dysfunction. Auto-immune hypothyroidism in transplanted children has been observed in previous studies with a similar incidence (42), while in the general population a lower incidence has been reported, around 1-2% (43). An unexplained GvHD-like phenomenon is suggested to play a role (40, 42, 44, 45), which is supported by a study that found thyroid dysfunction to be 8.4 times more likely after HSCT with an unrelated donor compared to matched sibling donors (46) and the absence of development of auto-immune thyroid dysfunction with use of T cell-depleted grafts (42). Furthermore, the underlying conditions are suggested to explain some of the thyroid complications as the risk of thyroid disease is elevated in for example IPEX, Fanconi anemia and beta-thalassemia (44, 47-49). Our study did not show an association with diagnosis, GvHD, donor type, serotherapy or chimerism and although thyroid dysfunction was more common in the group with busulfan- compared to treosulfan-based conditioning this difference was not statistically significant. Previous studies have shown that even radiation free conditioning seems to increase the risk of thyroid dysfunction and found a trend toward more thyroid dysfunction after myeloablative compared to reduced intensity conditioning (40, 41). In total, 35% of patients developed thyroid dysfunction more than 2 years post HSCT and were asymptomatic at diagnosis stressing the importance of prolonged annually screening.

Growth

In our study height SDS did not change between HSCT and last follow-up, in line with several previous studies in both nonmalignant (SCD and TM) and malignant diseases when using radiation-free conditioning (50-53). Nonetheless, almost 20% of patients ended with a short NAH and 21% of males and 8% of females had a NAH more than 2 SDS below MPH. One study reported a similar incidence of SS of 15% at last visit (not final height) in TM patients treated with HSCT. However, another study reported no significant difference between height at last follow-up and mid-parental height in patients with SCD (16, 52).

The high incidence of growth failure, frequently already present preSCT, suggests underlying conditions themselves rather than HSCT may play a large role in growth impairment. Many diagnoses are associated with SS including TM, SCD, Fanconi

anemia and several IEI/IEM syndromes (54-57). Our study did not show that SS or difference between adult height and MPH was associated with factors as age at HSCT, gender, pubertal stage, diagnosis, GvHD and donor type.

Previous studies report conflicting results to what extent busulfan based conditioning causes growth impairment (58, 59). The current study did not find an association of poor growth outcome with any of the conditioning regimens. Given the high prevalence of SS, which was likely underestimated due to exclusion of patients treated with growth hormone, growth monitoring is warranted and in those with growth failure growth hormone treatment may be considered (60-62).

To our knowledge this is one of the largest studies investigating multiple endocrine complications after HSCT in children with nonmalignant diseases. The strengths of the study are the large cohort and the systematic long-term follow-up in which dedicated pediatric endocrinologists performed a yearly clinical and biochemical evaluation. The variety of primary diagnoses also is a strength on the one hand, but this, together with the range of different conditioning regimens, also makes it difficult to determine the individual factors associated with endocrine complications. Large registries, such as the EBMT registry, should allow future studies among larger cohorts with a specific diagnosis rather than the broad diagnostic groups used in the current study, and assessment of the interaction of that specific diagnosis with the transplant process and other relevant factors that may modify outcome (such as iron overload in HBP). This will be necessary to define diagnosis-specific strategies to prevent endocrine complications and to develop diagnosis-specific recommendations for follow-up, as are currently available for some diagnoses (5, 33, 34).

Although the median duration of follow-up in the current study was over 6 years, this might still have been insufficient to evaluate long-term complications and potential recovery over time in all patients. Other limitations are the exclusion of patients with re-transplantation or death within 2 years after HSCT which constitutes a risk of bias and exclusion of 18 patients treated with growth hormone which might have caused an underestimation of the negative impact of HSCT on growth. Lastly, we were unable to assess infertility due to the relatively young age of the cohort in the current study, but this is an essential part of gonadal function and certainly deserves further investigation. In addition, impact on quality of life of the various endocrine complications needs to be evaluated.

In conclusion, this study shows that the majority of patients treated with HSCT for nonmalignant conditions during childhood developed one or more endocrine complications. Gonadal dysfunction was the most common late effect seen in

55% of females and 39% of males, whereas SS and thyroid dysfunction occurred in nearly 20%. Therefore, we recommend counseling about endocrine late effects and the option of fertility preservation before HSCT and at least yearly evaluation of growth, pubertal development and thyroid function starting 6 months post-HSCT. Busulfan based conditioning was associated with a significantly higher risk of developing gonadal dysfunction in females. This should be taken into account when deciding upon conditioning regimens. Further research is necessary to gather diagnosis-specific knowledge on endocrine complications after HSCT and the pathophysiology in order to develop strategies to prevent these complications and refine recommendations for follow-up.

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