A Short Review on Growth and Endocrine Long-term Complications in Children and Adolescents with β-Thalassemia Major: Conventional Treatment versus Hematopoietic Stem Cell Transplantation

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Abstract. The conventional treatment of β-thalassemia (β-TM) patients is based on the correction of anemia through regular blood transfusions and iron chelation therapy. However, allogeneic hematopoietic stem cell transplantation (HSCT) remains the only currently available technique which has the curative potential. Variable frequency and severity of long-term growth and endocrine changes after conventional treatment as well as after HSCT have been reported by different centers. The goal of this mini review is to summarize and update knowledge about long-term growth and endocrine changes after HSCT in patients with β-TM in comparison to those occurring in β-TM patients on conventional treatment. A regular surveillance, early diagnosis, treatment, and follow-up in a multi-disciplinary specialized setting are suggested to optimize the patient’s quality of life (www.actabiomedica.it).

Key words: Allogeneic hematopoietic stem cell transplantation (HCT), β-thalassemia major, growth, endocrinopathies, fertility

Introduction

Thalassemias are inherited hemoglobinopathies characterized by impaired or absent production of one of the globin chains of adult hemoglobin (Hb). The most common form is β-thalassemia related to a defective production of the β-globin chains causing an unbalanced ratio of α-globin to β-globin.

As a consequence, the unbound free α-globin chains precipitate in erythroid precursors, leading to ineffective erythropoiesis, chronic hemolytic anemia, and compensatory hemopoietic expansion (1,2). More than 50,000 children with this disease are born worldwide each year, adding to the disease burden of this condition (3).

Historically, β-thalassemia has been divided into three main subgroups based on severity of the clinical phenotype: major (β-TM), intermedia, and minor. Distinction of the various phenotypes of thalassemia is mostly based on clinical parameters, although a genotype-phenotype association is established in both α- and β-thalassemia syndromes. Nowadays, β-thalassemia patients are classified as either transfusion-dependent (TDT) or non-transfusion-dependent (NTDT) according to the severity of phenotype. This classification embraces all other forms of thalassemia syndromes such as α-thalassemia, hemoglobin E/β-thalassemia and combined α- and β-thalassemias (4). Diagnosis and management of these disorders both in the neonatal period or later using appropri-
ate approaches and uniform technology are extremely important.

Definitive diagnosis of thalassemia requires a comprehensive workup from complete blood count, Hb analysis, and molecular studies to identify mutations of globin genes (1,2). The most common mutations that cause β-thalassemia are single nucleotide substitutions, small deletions, or insertions within the β-globin gene. To date, more than 400 identified mutations of the β-globin gene (HBB) or promoter region that reduce or prevent the expression of the β-globin subunit of hemoglobin (Hb) in erythroid precursors (1,2,4).

After birth, HbF synthesis which is composed of two α-globin and two γ-globin chains (α2γ2) rapidly declines and HbF is gradually substituted by the major and minor adult hemoglobin forms, HbA and HbA2, respectively. As postnatal red blood cells (RBCs) HbF levels drop (less than 1%), children with β-TM experience severe microcytic, hypochromic anemia and become dependent on lifelong periodic blood transfusions (1,2,5,6). Unfortunately, chronic transfusions result in an increasing iron load (IOL) that lead to iron toxicity in vulnerable organs such as the heart, liver and endocrine glands, and necessitates daily iron chelation therapy (ICT), an expensive and sometimes uncomfortable treatment (7,8).

Deferoxamine (DFO), deferasiprone (DFP), and deferasirox (DFX) are used to treat IOL in an attempt to reduce morbidity and mortality related to organs siderosis (5–8). Moreover, the advances in the understanding the mechanism of iron toxicity and overloading, and the availability of noninvasive methods to monitor iron loading in the liver, heart, and pancreas, the life expectancy of β-TM patients has improved significantly in high-income countries. Nevertheless, endocrine complications are still observed and have been mainly attributed to the poor compliance to ICT, chronic liver disease (9) and cost of medication. The latter figure is even higher in patients of developing countries. The proportion of non-compliance to ICT was 24.7% amongst patients with TDT aged 9 years old and above, attending three tertiary hospitals in Malaysia (12).

In the recent decades, with the improvements of transfusion and ICT, the advances in the understanding the mechanism of iron toxicity and overloading, and the availability of noninvasive methods to monitor iron loading in the liver, heart, and pancreas, the life expectancy of β-TM patients has improved significantly in high-income countries (14). Nevertheless, the occurrence of endocrine disorders still persist and have been attributed to poor compliance to iron-chelating agents and cost of medication. The latter figure is even higher for patients in developing countries.

Unlike supportive blood transfusions, hematopoietic stem cell transplantation (HSCT) offers the hope of a definitive cure for patients with β-TM (15). Advances in transplantation techniques and supportive care strategies have resulted in a significant improvement in survival of those who have undergone treatment. However, HSCT survivors are at risk of developing long-term complications, such as endocrinopathies. The risk of these complications is influenced by pre-HSCT therapeutic exposures, transplantation-related conditioning, and post-transplantation management of graft-versus-host disease (GVHD) (16).

Gene therapy with globin lentiviral vectors and genome editing to inhibit the BCL11A gene are currently under investigation (17). Gene therapy and HSCT, however, have limitations, are feasible in a
small subset of patients and require pre-transplantation conditioning.

Moreover, the recent approval of luspatercept, an erythroid maturation agent that binds select TGF-β superfamily ligands to diminish Smad 2/3 signaling and enhance late-stage erythropoiesis, offers a new long-term therapeutic option for adult patients with β-TM to reduce red blood cell transfusion burden, anemia, and iron overload (17).

The goal of this short review is to summarize and update knowledges about long-term growth and endocrine changes after HSCT in patients with β-TM in comparison to those conventionally treated with packed RBC transfusions and iron chelating agents.

Methods

Search Strategy

The search identified articles, published in the last 30 years, retrieved from PubMed, Google Scholar and Research Gate. As keywords, we used Medical Subject Headings such as “allogeneic bone marrow transplantation”, “stem cells transplantation”, “conditioning regimens”, “β-thalassemia major”, “transfusion-dependent thalassemia”, “children”, “adolescents”, “young adults”, “growth”, “endocrine abnormalities”, “hypogonadism”, “growth hormone deficiency”, “hypothyroidism”, “diabetes mellitus”, “fertility”, “cyclophosphamide”, and “busulfan”. Exclusion criteria: all articles included publication dates prior to 1990 and related topics not listed in the inclusion criteria.

Inclusion and exclusion article selection

Studies were included if they were published in peer-reviewed journals, in English, described the protocols of conditioning regimens utilized in HSCT and were not abstracts or reviews. Titles were first screened to eliminate irrelevant articles. Abstracts were then reviewed to confirm eligibility, and selected articles were processed for full-text analysis. The search also included studies cited in relevant articles.

Results

Hemopoietic stem cell transplantation in patients with β-TM

In 1980, allogeneic HSCT was introduced as a treatment option (18). In the 1980s and early 1990s, more than 1,000 β-TM patients were transplanted in Pesaro (Italy) and in 900 consecutive unselected patients transplanted from an HLA-identical sibling donor a 20-year probability of thalassemia-free survival was reported in 73% of patients, who were divided into three classes based on presence of hepatomegaly, portal fibrosis, and a history of inadequate iron chelation (19).

Initial experiences with thalassemia patients undergoing allogeneic HSCT were limited to matched sibling donors (20). Later, various centers using modern improved transplantation approaches, and careful patient selection, reported a significant reduction of transplantation-related mortality (TRM) in young low-risk β-TM transplanted children (21). Nowadays, the availability of an international network of voluntary stem cell donor registries and cord blood banks has significantly increased the odds of finding a suitable HLA matched donor. Although acute and chronic GvHD remains the most important complication in unrelated HSCT in thalassemia, leading to significant rates of morbidity and mortality for a chronic non-malignant disease (22). For several decades little progress has been made in treating GvHD, with corticosteroids being the mainstay of first-line therapy. Over the past years, intensive pre-clinical research has led to an improved understanding of the pathophysiology of acute and to a lesser extend chronic GvHD. This has translated into the approval of several new agents for the treatment of both forms of GvHD (21-23).

Conditioning regimens for hematopoietic stem cell transplantation in patients with β-TM

The purpose of the preparative regimen is two-fold: to provide adequate immunosuppression to prevent rejection of the transplanted graft and to eradicate the disease for which the transplant is being performed. The biological aspects of allogeneic
HSCT in β-TM are different from those for hematologic malignancies. For many years, the most common conditioning regimen reported in literature included oral busulfan (Bu) at 14 mg/kg followed by cyclophosphamide (Cy) given intravenously at 120-200 mg/kg. The addition of azathioprine, hydroxyurea and fludarabine to the Bu-Cy regimen have made an important contribution to improving the results in high-risk patients (24).

In the last decade, new conditioning regimens for β-TM patients have been introduced with improved results, such as intravenous Bu, or treosulfan associated with thiotepa and fludarabine (25-27), and the preferred graft-versus-host disease (GVHD) prophylaxis in the majority of published studies of HSCT from matched sibling donors consisted of cyclosporine and methotrexate (28).

In conclusion, HSCT has been well established for several decades and now offers very high rates of cure for β-TM patients who have access to this therapy. Outcomes have improved over the last decade, even in high-risk patients. This has been possible due to better risk stratification, more effective targeted dose adjustment of intravenous Bu during conditioning, a modified conditioning regimen and continually improving supportive care. Systematic follow-up is needed to measure long term outcomes and an adequate management needs to be provided post-HSCT for all pre-existing complications particularly iron chelation to prevent further organ dysfunction (29-33).

**Pharmacology of alkylating agents**

Alkylating agents are an important component of many conditioning protocols for HSCT in both children and adults. With increasing survival rates after HSCT, long-term effects represent a major concern especially in pediatric HSCT recipients. Toxicity profiles of the two major alkylating agents (Cy and Bu), are briefly reviewed.

**Cyclophosphamide (Cy)**

Cy is a synthetic nitrogen mustard alkylating agent, with antineoplastic and immunosuppressive activities. In the liver, Cy is converted to active metabolites including phosphoramide mustard, which binds to and crosslinks DNA and RNA, thereby inhibiting DNA replication and protein synthesis. This agent, at low doses, is also a potent immunosuppressant primarily by depleting T-regulatory cells (34).

**Busulfan (Bu)**

Busulfan is a bifunctional alkylating agent of the alkylsulfonate type; it hydrolyzes in aqueous environment and releases methanesulfonate groups, leading to a reactive carbonium ion that alkylates DNA. Its metabolism is complex and not yet completely understood. It is primarily metabolized by the liver through conjugation with glutathione, mainly by glutathione-S-transferase A1 (GSTA1) (35).

**Treosulfan (Treo)**

Treo is a water-soluble bifunctional alkylating agent and a structural analogue of busulfan. Although Treo has structural similarities with Bu, its mechanism of alkylation is different. As a pro-drug, it undergoes non-enzymatic and pH-dependent conversion into active mono and diepoxide derivatives under physiological conditions. Interpatient variability of clearance in children is high; between 30 and 68% have been reported in population pharmacokinetic studies (36). Treo has a strong myeloablative potential and is considered less toxic than Bu; therefore, it has been considered in children with malignant and non-malignant disorders an appealing alternative to Bu as part of conditioning of HSCT (37,38).

**Thiotepa** is often combined with Treo and Fludarabine (Flu) or Bu and Flu in a myeloablative regimen. Thiota is primarily metabolized by the hepatic system, where it is rapidly bio transformed by CYP3A4 and CYP2B6 to its key metabolite, TEPA, which is also an alkylating agent (39,40). Thiota and TEPA are eliminated in urine, but also dermally via sweat (39,40). Thiota is approved by the European Medicines Agency in adults and children, in combination with other chemotherapeutic agents, as both autologous and allogeneic HSCT therapy in hematological diseases and solid tumors (40).
Effects of alkylating agents on growth, bone formation and gonads in animal studies

Cyclophosphamide

In rats, Cy chemotherapy altered survival or proliferation of growth plate chondrocytes and metaphyseal osteoblastic cells and reduced heights of metaphyseal spongiosa trabecular bone, which may contribute to chemotherapy side effects of this drug on bone lengthening and bone mass accumulation (41). In addition, mice treated with Cy for 6 weeks had impaired bone formation as evidenced by significant reduction of bone mineral density (BMD) and decrease in alkaline phosphatase levels compared to mice without chemotherapy. Besides its direct effect of inhibiting bone formation it also inhibits bone removal, making the resulting bone loss particularly difficult to treat with antiresorptive therapy (42).

In mice, Cy treatment (100 mg/kg/day) for 7 days led to osteoporosis through inhibition of osteoclastogenesis as shown by decreasing the number and differentiation of bone mesenchymal stem cells (MSCs) and reducing the formation and activity of osteoblasts. In addition, Cy suppressed the osteoclastogenesis by reducing the maturation and activity of osteoclasts. Authors suggested that the effect of Cy on bone formation might play a dominant role in its detrimental effects on bone remodeling (43).

The negative effects on the gonads of mice consist of irreversible follicle loss and permanent impairment of oocyte quality (44) and high gonadotoxic effects when administered before the initiation of spermatogenesis (45, 46). Multidose regimens of Cy did not eliminate the stem spermatogonia but resulted in cell loss and residual damage (47).

Decapeptyl ameliorates cyclophosphamide-induced reproductive toxicity in male Balb/C mice (48). In addition, when GH-deficient rats were treated with GH, it was demonstrated to have a protective effect on the count and motility of spermatids following treatment with Cy (49).

Busulfan

In animals, Bu is known to have a detrimental effect on differentiating spermatogonia (50). In mice, Bu disrupts spermatogenesis by interrupting meiosis that decreases the percentage of cells in late phases. In addition, it disrupts the expression of genes/proteins important for spermatogenesis and increases intracellular oxidative stress (51).

In male mice, following single injections of Bu at 15, 30 or 45 mg/kg, regardless of Bu doses, fertility was lost within 4 weeks after treatment, while more than 95% spermatogonial stem cells (SSCs) were lost within 3 days. Fertility and SSC numbers gradually recovered with time, but the recoveries were delayed at higher busulfan doses. The loss and restoration of fertility after busulfan treatment are direct consequences of SSC loss and expansion (52).

Effects of alkylating agents on growth, bone formation and gonads in human

Cyclophosphamide

In human, Cy has a significant reproductive toxicity in both males and females. It interferes with spermatogenesis and oogenesis. Sterility, sometimes reversible, can occur in both men and women treated with Cy. This toxic effect depends on the dose and duration of therapy. Cy causes premature ovarian insufficiency by inducing death and/or accelerated activation of primordial follicles and increased atresia of growing follicles. It also causes an increase in damage to blood vessels and the stromal compartment and increment inflammation (52).

In males, spermatogenesis is much more likely to be disrupted than is testosterone production because the germinal epithelium of the testis is more sensitive to damage from cytotoxic drugs than the Leydig cells. The degree of damage to the germinal epithelium is influenced by the stage of sexual maturation of the testis. In general, the post pubertal testis appears to be more susceptible to damage than the prepubertal testis (53).

Busulfan

It is generally assumed that Bu/Cy-based conditioning regimens for HSCT do not affect growth, although Bakker et al. (54) found in 4/10 non β-TM children, without a history of irradiation, an insufficient secretion of growth hormone (GH). They suggested that Bu crosses blood brain barrier and accordingly might have negative outcome on growth.
In human, the preparative regimen including Bu is associated with damage to the testicular function in as much as 83% of patients. In prepubertal children, Sanders et al. (55) and Thibaud et al. (56) reported that Bu induced a complete ovarian failure with extremely rare spontaneous recovery. Moreover, Sanders et al. (55) demonstrated that Bu administered at myeloablative doses was associated with increased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) as well as ovarian failure (57).

Comparing ovarian function in two groups of girls who received Bu versus those who did not receive Bu in the conditioning regimen showed that those treated with Bu had a significantly higher incidence of ovarian failure (100% vs 27%; P = 0.002). Among the 11 girls who did not receive Bu eight were prepubertal compared to five out of 10 in the Bu-treated group. In addition, ovarian toxicity of Bu was also severe and permanent and displayed irreversible reduction of reserve primordial follicles in the ovaries (58,59).

In males, Bu administration resulted in a permanent, more severe atrophy of the testicles that included detrimental effect on testicular histology, manifested by obliteration of the typical morphology of the seminiferous tubules and spermatogenic milieu. Zini et al. (60), reported that Bu interrupt DNA synthesis in sperm cells and lead to reduced sperm motility.

Growth and endocrine changes in patients with β-TM: conventional therapy versus after HSCT

Growth and endocrinopathies in patients with β-TM on conventional therapy:

The prevalence and severity of hypogonadism and short stature in β-TM varies among studies, depending on patients’ age, genotype, transfusion frequency and starting age, compliance, and efficiency of iron chelation therapy. Intensive combined chelation has been shown to improve patients’ iron load and consequently prevent, decrease or reverse multiple endocrine complications associated with transfusion iron overload including abnormal glucose metabolism, hypothyroidism, and hypogonadism. However, there is progressive increase of endocrinopathies in these patients related to age (longevity) and with increased hepatic iron overload. Therefore, in assessment of the prevalence of endocrine complications these important factors shall be conjoinedly interpreted (7,8).

Soliman et al. (61), described the prevalence of growth and endocrine abnormalities in adolescents and growing adults with β-TM reported, after 2020, from 10 countries (Figure 1). Growth impairment and growth hormone deficiency (GHD) occurred from 31% to 49 %, hypogonadism and delayed or absent puberty from 35% to 57 %, primary hypothyroidism from 4% to 29 %, dysglycemia (impaired glucose tolerance and diabetes mellitus) from 5% to 17% and hypoparathyroidism from 1.2% to 10.5% of patients.

Recently, a meta-analysis of 74 studies, published from 1978 to 2019, on the prevalence of growth impairment was reported in patients with β-TM treated with conventional protocols (blood transfusion and iron chelation therapy). Studies from the following countries were included: Asia (71.6%), Europe (16.2%), Africa (6.7%), America (2.7%), Oceania (1.3%), and Multicenter (2.7%) (62). The overall mean age of the participants was about 14 years. The pooled prevalence of short stature was 48.9% and was higher in males (61.9%) compared to females (50.9%). The pooled prevalence of growth retardation was 41.1% and was higher in males (51.6%) versus females (33.1%). The pooled prevalence of GHD was 26.6% (62).

Short stature was defined when the patient height is more than two standard deviations below the mean for age, gender, and ethnicity, growth retardation was when the height of the subject was lower than the mid-parental-height (MPH) and GHD was defined when the peak of GH after provocative test was below 5 ng/mL (62).

In another meta-analysis of 44 studies, conducted by He et al. (63) including 16,605 patients, aged from 2 to 28 years, was reported that the prevalence of impaired fasting glucose, impaired glucose tolerance and diabetes mellitus were 17.2%, 12.4%, and 6.5%, respectively.

In general, it was highly recognized that the high prevalence of growth retardation and endocrinopathies in patients with β-TM was primarily due to iron overload which induce oxidative damage to different organs including endocrine glands (pituitary, thyroid, pancreas and parathyroid) and the liver (leading to insulin resistance and low IGF1 secretion) (61-69).

Various combination schemes have been developed in order to maximize treatment efficacy, tolera-
Growth and endocrinopathies after HSCT in patients with β-TM:

After HSCT a variable occurrence of growth and endocrine long-term consequences very reported from different centers (Table 1). In one study (73), a strict correlation was observed between age at time of transplant and final adult height. The patients whose age at transplant was <7 years had a less impaired growth rate than did patients who were >7 years. Moreover, greatest loss in height was observed in subjects who had higher serum levels of transaminases and ferritin and these biochemical parameters were strictly correlated to the final adult height. The mean final adult height in subjects who received HSCT after 7 years of age, failed to achieve their full genetic potential.

Gonadal damage has been commonly reported in both sexes. Rahal et al. (85) evaluated long-term complications in 99 β-TM patients who successfully received HSCT. The median duration of clinical follow up was 12 years. After transplantation, 11% of patients developed thyroid dysfunction, 5% diabetes, and 2% heart failure. Hypogonadism was present in 56% of females and 14% of males. Female patients who reached a spontaneous puberty after HSCT were significantly younger at transplantation than those who experienced delayed puberty (median age 2.5 vs 8.7 years). Fertility was preserved in 9/27 females, aged 20 years or older, and 2 other patients became pregnant following oocyte donation. Moreover, male β-TM patients who underwent allogeneic HSCT had lower fertility potential, mainly in sperm parameters compared with patients treated with blood transfusion and chelation (82).

Health-related quality of life (HRQoL) measurements in β-TM patients have shown that HSCT was associated with improved health, activity, and emotional well-being compared to patients on conventional treatment chronic (80,89,90). The improvements were not as robust when HSCTs were performed in older β-TM patients with advanced disease or in the presence of chronic GVHD (83).

However, it must be kept in mind that when we interpreted the data of all these studies, there were
| Author name, Ref. and Patients's age | Study design and Sample size | Growth retardation | Gonadal dysfunction | Thyroid disorder | Comments |
|-------------------------------------|-----------------------------|--------------------|---------------------|-----------------|----------|
| Ponte et al., 1991 (70). 1.9 -18 yrs at HSCT. | Longitudinal - 8            | 7/8 children had decreased growth velocity after HSCT. | -                  | -               | 1/8 patient (with normal ferritin) had normal growth after HSCT. |
| De Sanctis et al., 1991 (71). 9.3 - 17.2 yrs. | Prospective - 30 (15 boys and 15 girls) | -                  | 12 girls and 15 boys presented hypogonadotropic hypogonadism. | -               | -        |
| De Simone et al., 1995 (72). N.A. | Longitudinal - N.A.          | Worsening of growth in patients > 7 yrs. | -                  | -               | 16 had impaired GH secretion. |
| De Simone et al., 2001 (73). N.A. | Longitudinal - 47           | Subjects who received HSCT< 7 yrs did not reach the genetic target height. | -                  | -               | -        |
| De Sanctis et al., 2002 (74). N.A. | Retrospective-68 (30 males)  | In all but 3 ex-thalassaemic females an improvement of standing height was observed. | 66% females and 38% of males had abnormal puberty. 68% had gonadal dysfunction. | -               | -        |
| Li et al., 2004 (75). N.A. | Longitudinal - 32           | 40% had Ht-SDS < -2 before HSCT 15% had Ht-SDS < -2 after HSCT. | Gonadal failure was universal in girls, but boys were less affected. | -               | 1/10 developed GH deficiency. One patient developed diabetes mellitus. |
| Valchopoulos et al., 2005 (76). N.A. | Prospective - 25 (12 males)  | -                  | 14 (1 male and 13 females). | -               | 100% of the post-menarcheal females exhibited amenorrhea. |
| Di Bartolomeo et al., 2008 (77). Median age 9 yrs (range: 11 months to 28 yrs). | Prospective - 115           | -                  | -                  | -               | Ten spontaneous pregnancies were recorded (4 women - age at HSCT: 14, 14, 13.17 yrs and 2 partners of male patients- age at HSCT: 17 and 24 yrs). The births resulted in 10 normal babies. |
| Khalil et al., 2012 (78). 1.1-32 yrs at HSCT. | Retrospective-47            | 52% had final Ht-SDS < -2 after HSCT. | Gonadal failure in 80% of females and 36% of males. | 11% HT.         | 9% DM.   |
| Poomthavorn et al., 2013 (79). Median age13.2 yrs (range: 5.9-25.8yrs). | Prospective-47              | -                  | 29 patients (62%) presented gonadal dysfunction. | -               | -        |
| La Nasa et al., 2013 (80). Mean age 34 yrs (range 21-48 yrs). | Cross sectional-109         | -                  | -                  | -               | Pregnancy: 14% women (n=6), 17% partners of male patients (n=11). |
| Aldemir-Kocaba et al., 2014 (81). Mean age 12.4 ± 5.4 yrs. | Longitudinal-41             | The height SDS were better in patients whose age was < 7 yrs at HSCT. | 14.6% low FSH, LH, testosterone or estradiol. | 10% HT         | The risk of gonadal insufficiency was significantly lower in patients who underwent HSCT <7 years of age. |
some limitations, such as: (a) endocrine complications were not systematically investigated indicating the need for systematic tracking; (b) some studies only reported small patient numbers, which makes it difficult to assess the possible influence of transplantation; (c) different combinations of conditioning agents may have influenced the patient’s outcome; and (d) due to the constantly evolving field and improvement of the transplant procedures over time, some of the regimens and procedures in the reported studies are already amended or revised/renewed.

**Conclusions**

 HSCT technologies have improved substantially during recent years, and their outcome is likely to be much better today than it was at first. Various approaches have been developed for HSCT preparation and conditioning: intravenous busulfan, targeted intravenous busulfan, treosulfan, thiotepa, and fludarabine, as well as intensive pre-transplant transfusion-chelation regimens (91). Nevertheless, with increasing numbers of long-term survivors, delayed complications, often presenting years after HSCT, are becoming a concern. Late sequela may arise as a result of the disease for which transplantation was performed or from toxicity associated with the wide variety of conditioning regimens (70-88).

Considerable growth and endocrine abnormalities have been detected in β-TM patients after HSCT. Growth retardation and short final stature occurred to a greater extent in patients transplanted after the age of 7 years (72,73). Similar results were reported, recently, by Rahal et al. (85).

### Table 1. Growth and endocrine disorders in patients with β-TM who underwent HSCT (Ref.70-88)

| Author name, Ref. and Patients’s age | Study design and Sample size | Growth retardation | Gonadal dysfunction | Thyroid disorder | Comments |
|--------------------------------------|-----------------------------|--------------------|---------------------|-----------------|----------|
| Chaudhury et al., 2017 (82). Median age at HSCT 5.5 yrs. | Retrospective - 176 | Mean SDS for height and weight were low at baseline and remained low after HSCT. (79%). | Hypogonadism occurred most frequently in older transplanted patients. | - | Hypogonadism was significantly higher in recipients ≥ 7 years at the time of HSCT and in those with pre-existing morbidity. |
| Caocci et al., 2017 (83). Median age at HSCT 12 yrs (range: 1-45 yrs). | Multicentric - 258 | - | - | - | Pregnancy: 6 women and 6 partners of male patients. |
| Hamidieh et al., 2018 (84). 10.8 ± 3.9 yrs. | Prospective - 20 (6 females) | 8 low IGF-1. | - | - | HSCT did not appear to have an overall positive or negative effect after 3 months of observation. |
| Rahal et al., 2018 (85). Age range:3.1-11.2 yrs at HSCT. | Retrospective multicenter - 99 (54 females) | The median Ht-SDS for final height was of −1.4 in males and −1.1 in females. | Hypogonadism was present in 56% of females and 14% of males. | 11% HT | 5% had diabetes and 36% of adults was overweight |
| See et al., 2018 (86) Age range:1-30 yrs | Retrospective - 40 | Mean height decreased of -0.84 SDS | 55% gonadal dysfunction. | 7.5% HT | Diabetes: 2 cases Pregnancy: 1 patient |
| Ntali et al., 2018 (87). Median age at HSCT 13 yrs (range:3–17 yrs). | Retrospective - 11(5 males) | - | 18.18% HH. | 45.5% HT | 72% had osteopenia/osteoporosis. Two women conceived spontaneously while on HRT and their pregnancies were both uneventful. |
| Rostami et al., 2020 (88). Age range :16 -41 yrs. | Cross sectional - 43 males | - | Hypogonadism was documented in 36.6% patients. | - | Lower fertility potential. |

Legend: HT: primary hypothyroidism; Ht-SDS: height-standard deviation score; GH: growth hormone; HH: hypogonadotropic hypogonadism; DM: diabetes mellitus; HRT: hormone replacement therapy.
These data support the set-up of simple universal measures that allow early identification and proper and timely management of these consequences. Therefore, patients with β-TM should be recruited and evaluated for transplantation in early stage of the disease, especially before the age of seven years.

Primary gonadal failure was observed in both sexes (in males: germ cell failure with compensated Leydig cell failure; in females: ovarian failure with elevated gonadotropins in 80% of recipients) (71).

Most but not all of these endocrine problems were a consequence of conditioning regimens because etiology is multifactorial and is also correlated to previous iron overload, class risk factors before HSCT, patient’s age, chronic GVHD, and corticosteroid treatment. The normal-low LH and FSH levels and reduced testosterone responses, after gonadotrophin releasing hormone and human chorionic gonadotrophin, noted in 15 prepubertal boys after HSCT were likely due to the association of iron deposition in the pituitary and Bu/Cy gonadal toxicity (71).

In the light of fertility impairment and uncommon recovery of gonadal damage after HSCT, (De Sanctis V, personal observation in 750 β-TM patients - unpublished data) these patients should receive comprehensive counselling regarding measures for fertility preservation. However, there is no doubt that, this must be taken into account when discussing the pros and cons of HSCT with the patient and parents. it must be also remembered that when a β-TM patient is treated with a conventional treatment manner, delayed puberty or absence of puberty is the most common endocrine disorder and many girls even though they start to menstruate may develop secondary amenorrhea or secondary hypogonadism (8, 92–98).

Today there are good possibilities for fertility preservation. Cryopreservation of spermatozoa is the first line treatment for fertility preservation in adolescent and adult male patients. Where sperm retrieval is impossible, such as in prepubertal boys, or it is unfeasible in adolescents prior to the onset of HSCT, alternative experimental treatments such as testicular tissue cryopreservation and the harvesting and banking of isolated spermatogonial stem cells can now be proposed as viable means of preserving fertility (99).

In females, egg or embryo cryopreservation are established approaches but are not achievable for many children and adolescents. Recently, the harvesting and cryopreservation of ovarian tissue represents a novel surgical option that allows for the possibility of fertility preservation to be extended to children of all ages. These procedures appear to be safe and do not add to transplant-related morbidity (100-102).

In conclusion, the growing data on these disorders in the post-transplant setting highlight the need for more research and evidence-based guidelines. After HSCT we recommend an annual assessment of growth, pubertal development, endocrine, sexual, and reproductive functions is recommended. Additionally, a regular surveillance, early diagnosis, treatment, and follow-up in a multi-disciplinary specialized setting are suggested to optimize the patient’s quality of life.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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