Endocrine Sequelae in 157 Pediatric Survivors of Hematopoietic Stem Cell Transplantation (HSCT)

Maria Güemes,1,2 Álvaro Martín-Rivada,1,2 Marta Bascuas Arribas,1 Eva María Andrés-Esteban,3 Blanca Molina Angulo,4 Jesús Pozo Román,1,2,5,6 and Jesús Argente1,2,5,6,7

1Departments of Pediatrics & Pediatric Endocrinology, Hospital Infantil Universitario Niño Jesús, 28009 Madrid, Spain
2Research Institute “La Princesa,” 28009 Madrid, Spain
3Department of Statistics, Hospital Infantil Universitario Niño Jesús, 28009 Madrid, Spain
4Department of Hematology and Bone Marrow Transplant, Hospital Infantil Universitario Niño Jesús, 28009 Madrid, Spain
5Department of Pediatrics, Universidad Autónoma de Madrid, 28029 Madrid, Spain
6Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain
7IMDEA, Food Institute, CEIUAM + CSI, 28049 Madrid, Spain

Correspondence: Jesús Argente, MD, PhD, Department of Pediatrics & Pediatric Endocrinology, Hospital Infantil Universitario Niño Jesús, Avenida Menéndez Pelayo, 65, 28009 Madrid, Spain. Email: jesus.argente@uam.es or jesus.argente@fundacionendo.org.

Abstract

Context: Successful rates of hematopoietic stem cell transplantation (HSCT) face paralleled escalation of late endocrine and metabolic effects.

Objective: This work aimed to characterize these sequelae distinguishing between the underlying pathologies and treatments received.

Methods: A retrospective descriptive study was conducted in 157 children post-HSCT (hematopoietic pathology [N=106], solid tumors [N=40], and rare entities [N=11]) followed at a single endocrine department between 2009 and 2019. Regression analysis was used to ascertain association.

Results: Of all patients, 58.7% presented with at least one endocrine abnormality. Endocrinopathies post HSCT were most frequently developed in lymphoblastic leukemia (60.5% of them), whereas myeloid leukemias had the fewest. A total of 64% of patients presented with primary hypogonadism; 52% short stature, and 20% obesity. Endocrinopathy was associated with older age at HSCT (9.78 years [6.25-12.25] vs 6.78 years [4.06-9.75]) (P<.005), pubertal Tanner stage V (P<.001), chronic graft-vs-host disease (GVHD) (P=.022), and direct gonadal therapy (P=.026). The incidence of endocrinopathies was higher in girls (15% more common; P<.02) and in patients who received radiotherapy (18% higher), steroids (17.4% increase), androgenic HSCT (7% higher), thymoglobulin, or cyclophosphamide. Those on busulfan presented with a 27.5% higher rate of primary hypogonadism (P=.003).

Conclusion: More than half of children surviving HSCT will develop endocrinopathies. Strikingly, obesity has risen to the third most frequent endocrine disturbance, mainly due to steroids, and partly adhering to the general population tendency. Lymphoblastic leukemia was the condition with a higher rate of endocrine abnormalities. Female sex, older age at HSCT, pubertal stage, allogenic transplant, radiotherapy, alkylating drugs, and GVHD pose risk factors for endocrine disturbances.

Key Words: endocrinopathies, pediatric, hematopoietic stem cell transplantation

Successful rates of hematopoietic stem cell transplantation (HSCT) for conditions with poor prognosis, encompassing hematologic malignancies, bone marrow failure, immune system deficiencies, hemoglobinopathies, and inborn errors of metabolism, remain on the rise. Late effects of these survivors have thus occurred in parallel, with endocrine and metabolic disruptions being among the most frequent manifestations [1, 2].

Before HSCT intravenous infusion, the bone marrow must be depleted or “conditioned” with myeloablative agents, namely high-dose chemotherapy, total body irradiation (TBI), or total lymphoid irradiation [3]. Following HSCT, immunosuppressive agents, mainly high-dose glucocorticoids, are added to avoid rejection of the donor cells and/or treat graft-vs-host disease (GVHD) [4].

The overall detection of endocrinopathies post HSCT has been reported to be approximately 65.2% [5], representing nearly 60% in those receiving HSCT before age 10 years [1]. Risk factors reported for endocrinopathies include the underlying diseases and age at HSCT, high-dose chemotherapy agents, cranial/neck/mediastinal/TBI (its cumulative dose and administration schedule), and longer duration of
follow-up [5-7]. While hypogonadism seems to be the most common endocrine alteration detected across the series, a spectrum of abnormalities in the other pituitary hormone axes as well as in the metabolic profile have been reported [5, 7].

The aim of this study was to characterize the endocrine and metabolic abnormalities that emerged in patients who underwent HSCT, followed at a single tertiary referral center in Spain.

Materials and Methods
Participants
A total of 157 child and adolescent (age < 18 years) recipients of HSCT and with full endocrine follow-up were identified (62 girls and 95 boys). The children came from diverse provinces within the Spanish territory. The underlying diagnoses for which HSCT was received were hematopoietic pathology (42 cases of lymphoblastic leukemia, 34 myeloblastic leukemia, 5 lymphomas, 13 congenital anemias, 12 cytopenias), solid tumors (40 cases: 13 Ewing sarcoma, 9 neuroblastomas, 13 central nervous system [CNS] tumors, 4 Wilms tumors, 1 nasopharyngeal metastatic carcinoma), and 11 rare entities (2 congenital dyskeratosis, 3 malignant osteopetrosis, 2 X-linked adrenoleukodystrophies, 2 mucopolysaccharidosis, 1 hyperesinophilic syndrome, 1 hyper-IgE syndrome).

Study Design
A retrospective longitudinal descriptive study was performed among pediatric post-HSCT patients followed at a single tertiary endocrine service (Hospital Infantil Universitario Niño Jesús). All patients who received an HSCT between the years 2009 and 2019 in our center were included.

Data regarding the therapies received for the underlying condition (chemotherapy, radiotherapy, surgery, HSCT conditioning agents), as well as endocrine and metabolic abnormalities, were collected and analyzed.

Ethical Approval
For all patients, informed consent was obtained from patients or their parents/legal guardians before transplantation. The institutional review board of our center approved the data collection. Data were fully anonymized. This study conforms to the Declaration of Helsinki ethical principles.

Hematopoietic Stem Cell Transplantation Aspects
The types of HSCT performed in our center include allogeneic and autologous transplantation. TBI was used as a conditioning regimen for HSCT only in patients with congenital anemias, like Fanconi anemia, and other rare diseases, such as congenital dyskeratosis, at a low dose (300 cGy) or in certain patients with very high-risk acute lymphoblastic leukemia (ALL) transplanted before the year 2010 (12 Gy). Most of the patients without TBI received a myeloablative conditioning regimen based on alkylating agents such as busulfan, thiotepa, or melphalan. Reduced-intensity conditioning regimens were applied in patients with congenital anemias, cytopenias, congenital dyskeratosis or second transplants.

Prophylaxis for GVHD in allogeneic transplant recipients consisted mainly of cyclosporine alone or in combination with glucocorticoids and/or thymoglobulin. In case of acute GVHD, the standardized glucocorticoid protocol was to initially commence with intravenous methylprednisolone at a dose of 2 mg/kg/day, divided into 3 doses. If response was not achieved within the next 48 to 72 hours, another line of treatment was added (etanercept, extracorporeal photopheresis, ruxolitinib, or cyclosporine, among others).

Assessment of Endocrine and Metabolic Disturbances
The endocrine follow-up protocol of children who underwent HSCT involved the following visits to the endocrine clinic:

| Total N subject to HSCT 413 | Survivors with full endocrine follow-up 157 | ≥ 1 endocrine abnormality 88 (58.7%) |
|-----------------------------|---------------------------------------------|---------------------------------------|
| Hypogonadism:               |                                             |                                       |
| -Primary                    |                                             |                                       |
| -Central                    |                                             |                                       |
| Short stature               |                                             |                                       |
| - being GH deficient        |                                             |                                       |
| Obesity                     |                                             |                                       |
| BMD z-score < -2 SDS       |                                             |                                       |
| Exogenous Cushing syndrome  |                                             |                                       |
| Thyroid nodules             |                                             |                                       |
| Cortisol deficiency         |                                             |                                       |
| Hypothyroidism:             |                                             |                                       |
| -Primary                    |                                             |                                       |
| -being subclinical          |                                             |                                       |
| -Central                    |                                             |                                       |
| Dyslipidemia                |                                             |                                       |
| Central precocious puberty  |                                             |                                       |
| Glucose disturbance         |                                             |                                       |

Figure 1. Endocrine abnormalities detected before and after HSCT. BMD, bone mineral density; GH, growth hormone; HSCT, hematopoietic stem cell transplantation; Post-HSCT, endocrine abnormalities detected after transplantation; Pre-HSCT, endocrine abnormalities detected before transplantation; T2DM, type 2 diabetes mellitus.
including auxology and pubertal Tanner stage: before HSCT, 6 and 12 months after it, and every 6 to 12 months afterward (depending on whether endocrine abnormalities were present or not, respectively), up to age 18 years. A fixed protocol of blood tests was analyzed at each of these appointments: insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (in boys) and estradiol (in girls), levothyroxine (FT4), thyrotropin (TSH), prolactin, vitamin D and calcium-phosphate metabolism, lipids, glucose, insulin, and glycated hemoglobin A1c. Bone age was performed before HSCT and yearly thereafter (data not presented in this manuscript).

Bone mineral density (BMD) was evaluated by dual-energy x-ray absorptiometry (DXA) scan, where spinal L1 to L4 Z score SD was interpreted in relation to the patient’s bone age and adjusted for height. BMD was analyzed before HSCT—if the patient was clinically stable—and 12 months after HSCT. If the BMD in the latter assessment had a Z score less than −1 SD, it was reevaluated yearly by DXA scan; conversely, if the Z score was greater than −1 SD, then the DXA scan was performed every 5 years thereafter.

Height SD were calculated according to the Spanish growth charts of the 1988 longitudinal study by Hernández et al [8], in which short stature was defined as height below −2 SD. Target height ±5 cm was estimated with the formula: (maternal height + paternal height)/2; 6.5 was added in the case of boys, whereas 6.5 was subtracted for girls. Growth hormone (GH) deficiency (GHD) was evaluated on inappropriate growth velocity, with growth factors (IGF-1 and IGFBP-3) below the normal range for age and puberty. GH peak concentrations following stimulation tests (clonidine test—predominantly—and/or insulin tolerance test) below 7 mg/L (in Spain only one GH stimulation test is required if former CNS lesion). Sex steroid priming for GH testing, which is controversial, was not performed.

To correctly identify patients with hypogonadism, this definition was reserved only for girls older than 13 years and boys beyond age 14 years, in the face of absent pubertal onset or pubertal arrest. Hypergonadotropic hypogonadism was defined in basal blood samples by LH greater than 10 UI/L or FSH greater than 15 UI/L, with concentrations of testosterone (in boys) and estradiol (in girls) below the cutoff for pubertal stage [5, 7]. Hypogonadotropic hypogonadism was established in the presence of Tanner stage I, prepubertal serum concentrations of testosterone (in boys), and estradiol (in girls), accompanied by LH-releasing hormone stimulation test with LH peak below 5 mU/mL.

Hypothyroidism was defined by FT4 below normal range for age, with either TSH over the cutoff level (primary hypothyroidism) or normal/inappropriately normal TSH (central hypothyroidism). Subclinical hypothyroidism was considered as raised TSH with normal FT4 concentrations.

Cortisol status of patients who had discontinued prolonged steroid use was assessed by high-dose adrenocorticotropin stimulation test (250 mcg). Should cortisol concentrations reach 18 mg/dL and/or double the baseline value, sufficiency was considered (polyclonal assay [Access 2 Beckman Coulter chem immunoassay]). Obesity was defined as body mass index (BMI) higher than 2 SD according to the Spanish reference charts of the 1988 longitudinal study by Hernández et al [8]. Baseline fasting glucose and insulin concentrations and results of an oral glucose tolerance test as well as lipid values (triglycerides, total cholesterol, and fractions) were collected.

### Statistical Analysis

Demographic and clinical variables were described using the median and interquartile range for quantitative variables, since the variables did not have a normal distribution, and by frequency distribution for qualitative variables. Shapiro-Wilk test was used to check for normality. To analyze the relationship between the different diagnoses (GVHD, GHD, short stature, among others) with the demographic and clinical variables, the non-parametric Mann-Whitney U test was used for the quantitative variables and χ² for qualitative.

The analyses were performed using STATA/SE v16.0 software, and P values below 5% were considered statistically significant.

### Results

Of the 157 patients with complete endocrine follow-up, 88 (58.7%) had at least one endocrine abnormality. The follow-up time (median [range]) of the whole cohort was 3.5 years (0.5-10.6 years). No association was identified between the presence of endocrinopathies and duration of follow-up. Fig. 1 shows

| Table 1. Treatments received for underlying conditions and age when they were administered |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Leukemias       | Anemias         | Lymphomas       | Cytopenias       | Solid tumors    | Rare entities   |
| Chemotherapy                   | Lymphoblastic   | Myeloblastic    |                 |                 |                 |                 |
| Age, y (median (IQR))          |                 |                 |                 |                 |                 |                 |
| Chemotherapy                   | N (%)           | 42 (100%)       | 35 (100%)       | 0 (0%)          | 5 (100%)        | 41.6% (54.1%)   | 5 (97.4%)       | 1 (9%)          |
| Radiotherapy                   |                 |                 |                 |                 |                 |                 |
| Age, y (median (IQR))          |                 | 8.0 (7.1)       | 12.6 (7.1)      | 7.7 (12.1)      | 7.5 (6.3)       | 9.8 (0%)        |
| Direct gonadal therapy         | N (%)           | 7.7 (1.6)       | 6.2 (4.8)       | 6.2 (4.8)       | 6 (5.8)         | 1.9 (0%)        |
| HSCT                            |                 | 8.0 (5.2)       | 9.3 (7.3)       | 13.7 (6.9)      | 9.0 (5.2)       | 8.5 (6.4)       | 10.2 (10.6)     |

Two patients with posterior fossa tumor received proton beam therapy before HSCT, one developed no endocrine abnormalities during follow-up, and the other patient was diagnosed with central precocious puberty before HSCT.

Direct gonadal therapy refers to gonadal radiotherapy, gonadal surgical removal, or combination of both.

Abbreviations: HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; N/A, not applicable.
the endocrine abnormalities detected both before transplant, the “pre-HSCT” group, and following the transplant, the “post-HSCT” group, taking all of the underlying diagnostic entities as a whole. Table 1 includes the treatments received for the underlying conditions and age when they took place.

Types of Hematopoietic Stem Cell Transplantation
The types of HSCT received were allogenic in 126 patients (81.5%) and autologous in 31 children (19.5%). Table 2 shows the conditioning agents used. Most of the patients received a myeloablative conditioning regimen. There were 17 different combinations of chemotherapy agents for conditioning, the most common ones, in order of frequency, being fludarabine + busulfan + thiotepa (used in the majority of acute leukemias), busulfan + melphalan (used in solid tumor patients who received an autologous transplantation), cyclophosphamide + fludarabine + thymoglobulin (a reduced toxicity regimen used in congenital anemias and some cytopenias), and fludarabine + busulfan (used in some rare nonmalignant diseases). TBI but with a very low nonmyeloablative dose (3 Gy) was used only in 4 cases of Fanconi anemia and 2 cases of congenital dyskeratosis. In 2 ALLs type-B, TBI was administered at a dose of 12 Gy.

Graft-vs-Host Disease
GVHD was present in 34 (21.6%) patients in the whole cohort. GVHD was found in 18 (52.9%) lymphoblastic leukemias, 5 (14.7%) myeloblastic leukemias, 2 (5.8%) lymphomas, 1 (2.9%) congenital anemia, 3 (8.8%) solid tumors, 2 (5.8%) rare entities, and in 3 (8.8%) cytopenias. Chronic GVHD was associated with developing at least one endocrinopathy (P = .022), as endocrinopathies were 16% more common in those that developed GVHD (P = .022). In particular, those with GVHD had a 50% higher rate of iatrogenic Cushing (P < .0001) and a 39% higher number of cases of dyslipidemia (P = .048). The difference between height and target height SD in the last clinic visit was −1.27 ± 1.30 in those who developed GVHD vs −0.46 ± 1.12 in those who did not develop it (P = .0063).

Table 2. Conditioning agents employed for hematopoietic stem cell transplantation

| Conditioning regimen | Total No. receiving conditioning |
|----------------------|----------------------------------|
| Yes                  | %                                | No |
| Busulfan             | 132                              | 83.54% | 26 |
| Fludarabine          | 103                              | 65.19% | 55 |
| Thiotepa             | 88                               | 53.70% | 70 |
| Thymoglobulin        | 19                               | 12.03% | 139 |
| Cyclophosphamide     | 20                               | 12.66% | 138 |
| Melphalan            | 34                               | 21.52% | 124 |
| Cisplatin            | 1                                | 0.63%  | 157 |
| Carboplatin          | 3                                | 1.90%  | 155 |
| Etoposide            | 2                                | 1.27%  | 156 |
| Total body irradiation | 8                          | 5.06%  | 150 |

Table 3. Endocrinopathies identified in different underlying diagnoses

| Endocrine abnormalities | Leukemias | Lymphomas | Anemias | Cytopenias | Solid tumors | Rare entities |
|-------------------------|-----------|-----------|---------|------------|--------------|---------------|
|                         | Lymphoblastic | Myeloblastic | N = 38 | N = 5 | N = 11 | N = 12 | N = 37 | N = 11 |

Abbreviations: BMD, bone mineral density; Hypogonad, hypogonadism; Hypothyroid, hypothyroidism; IQR, interquartile range.
of endocrinopathies, there was no statistical association between conditioning agents, or combination of them, and endocrinopathies.

**Endocrine Abnormalities**

In both the group with endocrinopathies detected pre HSCT and in those in whom endocrinopathies were identified post HSCT, the most common finding was the detection of 1 single endocrine abnormality (75% of cases), followed by 2 (18.7%), and then 3 endocrinopathies (6.2%). The entities that most frequently developed any form of endocrinopathy were anemias (82% of them) and rare entities (72% of them); however, no statistical association was identified between endocrinopathies and an underlying diagnostic entity (note the small sample size for some underlying conditions). Girls presented with 15% more endocrinopathies than boys. A later age at HSCT (median [25th-75th percentile]: 9.78 years [6.25-12.25] vs 6.78 [4.06-9.75]) was associated with endocrinopathy (P = .005), so that 75% of individuals transplanted at age 10 years or older developed endocrinopathies as opposed to 48.4% of those younger than 10 years (P = .001). Similarly, pubertal Tanner stage V at the time of HSCT was associated with development of endocrinopathy (P < .001).

The endocrinopathies identified in the different underlying diagnoses are presented in Table 3. Several aspects are here under taken into consideration.

**Gonadal axis**

At the time HSCT took place, 121 patients (77%) remained in pubertal Tanner stage I. Following the HSCT, 51 (78.4%) patients were at an appropriate age to be considered accountable for the diagnosis of hypogonadism (girls aged > 13 years, boys aged > 14 years). Of the 42 patients with primary hypogonadism post HSCT, 32 patients had already commenced hormone replacement therapy accordingly. Post-HSCT patients significantly developed hypogonadism (P < .001). Girls had an 18.8% higher rate of primary hypogonadism than boys (45.7% vs 26.9%; P = .019). Hypogonadism was associated with an advanced Tanner stage at the time of HSCT (P = .009), so much so that primary hypogonadism occurred in 26.4% of those at Tanner stage I vs 64.1% in those at Tanner stages II, III, IV, and V.

Busulfan recipients presented with a 27.5% higher rate of primary hypogonadism (P = .003), and those who received direct gonadal therapy had a 45.2% higher rate of hypogonadism (P = .001). Hypogonadism was due to bilateral orchiectomy in 4 cases; 5 of 8 (62%) patients with gonadal radiotherapy developed hypogonadism; and 5 of 6 (83%) patients with unilateral orchiectomy + contralateral radiotherapy developed hypogonadism (P = .018). Table 4 provides further statistical associations identified with hypogonadism post HSCT.

**Thyroid state**

Patients who had received previous radiotherapy had a 14% higher incidence of hypothyroidism (P = .010). Particularly, in case of previous CNS radiotherapy, the incidence of central hypothyroidism was 29% higher (P < .001). Central hypothyroidism was identified pre HSCT in 2 patients with a CNS tumor who received radiotherapy, whereas post HSCT only primary hypothyroidism was identified 4 patients (3 of them received local radiotherapy). Table 4 provides other statistical associations identified with hypothyroidism post HSCT. Neither of the

Table 4. Statistical associations identified with endocrine deficiencies

|                          | Patients who developed hormone deficiency (mean ± SD) | Patients who did not develop hormone deficiency (mean ± SD) | P       |
|--------------------------|------------------------------------------------------|----------------------------------------------------------|---------|
| Hypogonadism associated with | Age at diagnosis of underlying pathology 9.70 ± 4.25 (n = 41) | 5.97 ± 4.22 (n = 97) | .0025  |
|                          | Age at chemotherapy 11.11 ± 3.56 (n = 37) | 6.00 ± 4.35 (n = 76) | .0009  |
|                          | Age at HSCT 12.09 ± 3.58 (n = 41) | 7.38 ± 4.26 (n = 96) | .0001  |
|                          | Age at last clinic appointment 15.84 ± 2.46 (n = 40) | 10.52 ± 4.13 (n = 93) | .0001  |
|                          | BMI at last clinic appointment 21.03 ± 3.67 (n = 40) | 18.74 ± 3.81 (n = 94) | .0008  |
|                          | Age at first endocrinopathy 11.78 ± 3.34 (n = 39) | 7.00 ± 5.46 (n = 32) | .0003  |
| Hypothyroidism associated with | Radiotherapy dose 40.3 ± 23.0 (n = 4) | 6.23 ± 18.01 (n = 118) | .01    |
|                          | Age of radiotherapy 5.14 ± 3.76 (n = 4) | 2.19 ± 4.02 (n = 138) | .048   |
| GH deficiency associated with | Radiotherapy dose 57.00 ± 42.58 (n = 4) | 6.30 ± 16.75 (n = 119) | .021   |
|                          | Age radiotherapy 6.46 ± 4.97 (n = 4) | 2.25 ± 3.98 (n = 141) | .036   |
|                          | Height SD at last clinic appointment −2.72 ± 1.89 (n = 4) | −1.01 ± 1.25 (n = 139) | .039   |
|                          | BMI SD at last clinic appointment 1.13 ± 1.38 (n = 4) | −0.06 ± 1.14 (n = 140) | .047   |

Pearson χ² test was employed to search for significant relationships between the variables in the first 2 columns. The numerical values represented in columns 3 and 4 are the mean ± SD in patients who did or did not manifest the particular endocrine deficiency being analyzed, respectively.

Abbreviations: BMI, body mass index; GH, growth hormone; HSCT, hematopoietic stem cell transplantation.
2 patients who underwent to proton beam therapy developed hypothyroidism. The relationship between hypothyroidism/ TSH status and thyroid nodules could not be accurately assessed given the small number in each category.

**Growth**

Short stature was 26.4% more common in children with 1 or more endocrine abnormalities before HSCT (P = .007), and 27.3% more common in children with endocrine abnormalities post HSCT (P < .0001). The age of the first endocrinopathy was 7.56 ± 5.68 years in those with short stature, vs 11.38 ± 3.81 years in those without short stature (P = .0095).

Patients who had received previous CNS radiotherapy had a 21% higher incidence of GHD (P < .001). Also, patients with a pre-HSCT endocrine abnormality had a 7.0% higher rate of GHD (P = .057 trend toward significance). Additional statistical associations detected with GHD, post HSCT, are included in Table 4.

**Obesity**

Post-HSCT patients had significantly higher BMI (P = .01), although only 3 cases were obese, all the latter having developed GVHD. The age of HSCT was 13.00 ± 4.08 years in those who developed obesity, as opposed to 8.38 ± 4.64 years in those who were of normal weight (P = .0508). Patients with endocrine follow-up before HSCT had an 8.9% less incidence of obesity (trend P = .07).

**Cortisol status**

Post-HSCT patients significantly developed iatrogenic cushingoid features (P = .05). Also, patients with exogenous hypercortisolism had a 49% higher presence of hypogonadism (P = .003). Conversely, patients with hypocortisolism had a 30% higher incidence of GHD (P < .001) and an 18% higher rate of hypothyroidism (P = .07 trend toward significance).

**Discussion**

Pediatric studies of endocrinopathies post HSCT are limited, with smaller cohorts and usually multicentric. This recent study represents systematic cooperation between pediatric endocrinologists with pediatric endocrinologists describing HSCT endocrine late effects in children. Endocrine morbidity was examined distinguishing among the different underlying pathologies for practical classification purposes, although causality relies heavily on the treatments received. The group of children described here differs in many aspects from data previously published as it contains relatively few children conditioned by TBI.

During the entire follow-up, myeloid leukemia patients were the group with fewer overall endocrinopathies. Explanations for this may include the low rate of radiotherapy administration, lack of direct gonadal exposure, and age younger than 10 years at HSCT. By contrast, the conditions with the highest rates of endocrinopathies were congenital anemias. Nonetheless, a bias must be taken into account as certain conditions, for instance, Fanconi anemia, may present with short stature as an inherent feature. Comparing the adjusted height-SD at the first year following HSCT and at the last visit for Fanconi anemia patients might have accounted for this issue, but data were not available.

Lymphoblastic leukemia presented with the highest rate of post-HSCT endocrinopathies, explained by the patients who received TBI irradiation for conditioning as well as those who received chemotherapy conditioning with busulphan + thiotepa + fludarabine. Lymphomas have one of the highest rates of post-HSCT endocrinopathies as many of them have received potent chemotherapy, radiotherapy, and direct gonadal therapy. Interestingly, there were no cases of short stature in this group, potentially due to having the most advanced age at transplantation (13 years), hence the biggest percentage of growth had already taken place.

Busulfan (an alkylating drug) and fludarabine (a purine analogue) were the most commonly used agents in our center. Conditioning with high-dose chemotherapy and irradiation have been considered the highest risk factors for endocrine late effects [9], and TBI particularly if delivered as a single fraction [10]. Thus, to decrease the rate of complications, TBI today tends to be administered in 3 to 9 fractions, achieving a dose between 10 and 16 Gy [11]. It also seems that conditioning regimens with lower-intensity-only chemotherapy could decrease toxicity [12]. In our hospital most of the patients have not received myeloablative TBI in allogeneic transplantation since the year 2005. Only in some patients with Fanconi or congenital dyskeratosis is a low dose of TBI administered. Prolonged steroid therapy, to manage GVHD, has been described to lead to endocrine late effects [13]. We identified an association between developing GVHD and developing endocrinopathies, in concurrence with other studies [7]. Also, graft-related issues including transmission of autoimmune disease can contribute to endocrinopathies [14], but this aspect was not assessed in our study. Most of the conditioning regimens used herein were based on alkylating anti-neoplastic agents, since this is the most commonly used regimen both in reduced-intensity and myeloablative transplant procedures for treating immune deficiency.

As opposed to other studies [6], we did not find an association between endocrinopathy detection and length of follow-up, perhaps due to the shorter duration of follow-up in our study, which is limited to the pediatric age.

**Gonadal Axis**

Although this study includes slightly more boys (as certain conditions are X-linked, such as adrenoleukodystrophy and severe combined immunodeficiency), girls were found to present with a higher rate of endocrinopathies, mainly as hypogonadism was very common in this sex. Alkylating agents impair follicular function, even in reduced-intensity regimens [15], with busulfan generally being considered the most gonadotoxic one [16, 17]. A recent study in adults found that women who developed primary hypogonadism were more frequently treated with busulfan for pre-HSCT conditioning therapy (40.6% vs 0%) [18]. Additionally, high rates of ovarian function preservation with melphalan-based reduced-intensity conditioning have been reported [19].

The caveat is that in the pediatric milieu, when analyzing the gonadal function, it is only sex steroid generation that is being assessed, which in males is usually preserved (testosterone from Leydig cells). Unfortunately, neither antimüllerian hormone, spermiogram, nor other parameters of assessing fertility capacity (spermatogenesis from Sertoli cells) were measured in this study. Sertoli cells more often fail, as they are more sensitive to chemotherapy and radiotherapy, which explains smaller testicular volume.

Primary hypogonadism post HSCT has been described in 36% [5, 7] of pediatric patients, up to 75% of girls [20], and even higher rates in adults [6]. Studies have associated gonadal
failure with: higher age (> 10 years) at transplant (in younger individuals there can be gonadal recovery), underlying malignant disease (acute myeloid leukemia/ALL/lymphomas), second leukemia remission, cranial/pelvic/total body irradiation, alkylating agents, cisplatin, and nitrosoureas [5, 7, 20].

HSCT performed in postpubertal patients (vs prepubertals), in girls (vs boys) and in allo-HSCT (vs auto-HSCT) determined higher rates of hypogonadism. Recovery from ovarian insufficiency has been described [16]. Interestingly, also in adults, older patients have more hypogonadism [18] but the probability of gonadal function recovery declines by a factor of 0.8 per year of age [21]. It is worth mentioning that those patients without evidence of hypogonadism and those with recovery of the hypothalamus-pituitary-gonadal axis may still experience a premature menopause [15, 20].

On the other hand, central precocious puberty was less commonly identified: in one male detected pre HSCT (mixed germinal tumor in a patient with tuberous sclerosis who received craniospinal radiotherapy) and in another case post HSCT who was a female affected by a posterior fossa embryonal tumor with multilayered rosettes who received proton beam therapy. Radiotherapy has been described to activate the hypothalamus-pituitary-gonadal axis [22], mainly in females and at low radiation doses.

Thyroid

Hypothyroidism has been described in 13% [7] to 21% [5] to 52% [23] of cases post HSCT. Thyroid dysfunction has been associated in the literature with neck/mediastinum/TBI, in certain studies with busulfan and cyclophosphamide conditioning, and in those with relapse of Hodgkin lymphoma [5, 7, 23] (likely due to mantle radiation and not disease). There is controversy regarding the association with autologous HSCT and influence of age at HSCT.

Growth

Linear growth impairment has been reported in 20% [20, 24] to 84% of cases [7, 11, 25, 26], with differing figures due to outcome measures (final height not considered in all reports) and cohort heterogeneity. Growth failure is multifactorial, where contributors include hormone deficiencies (GH, gonadotropins, thyroid hormones), craniospinal or TBI limiting spinal growth [11], exogenous steroids, immunosuppressives, malnutrition, poor underlying condition, allogenic HSCT, and younger age at HSCT (particularly <10 years). Many HSCT patients seem to lose potential height, even if their final stature does not lie below ~2 SDS; thus, for future studies we aim to estimate it as final height below ~1 SD for target height or difference between final height and target height. This study found a low incidence of GHD post HSCT (2/65 = 3%), although, surprisingly, GHD has been documented in up to 20% to 40% of patients following HSCT [5, 25-27]. We speculate the difference relies on different GH peak cutoffs, obesity blunting GH peak response, and overdiagnosing GHD in other cohorts.

Obesity and Metabolic Syndrome

Obesity has been described in 3.9% [5] of cases post HSCT. Interestingly, in our study this was the third most common endocrine abnormality detected. The most plausible explanation for this involves the use of high doses of steroids causing cushingoid features in these children, many of whom, even once the iatrogenic Cushing is resolved, persist with weight excess. Truncal obesity and sarcopenia favor this explanation. Additional etiopathogenic factors include the pandemic of obesity in Western countries in recent decades as our obesity data herein fit perfectly with Spanish schoolchildren data on overweight (23.3%) and obesity (17.3%) [28].

Despite the minority of obese patients reported by other groups, insulin resistance resulting from chronic inflammation caused by GVHD and redistribution of fat tissue toward central accumulation has been reported [29]. Metabolic syndrome has been found to be associated with age over 10 years at transplantation, TBI conditioning, evolving time from HSCT, as well as untreated GHD and hypogonadism. Conversely, Shalitin et al [5] did not find an association between obesity and any other independent variable. Sarcopenic obesity has been described in these patients in the context of marrow adiposity, increased visceral adipose tissue, and decreased lean mass in the presence of a relatively normal BMI [30].

B-cell function may be impaired from tacrolimus and glucocorticoids [31], with new-onset diabetes after transplant and impaired fasting glucose having been described in up to 5% of patients receiving HSCT in certain series [32]. However, in our study only one case of impaired glucose tolerance was found.

Dyslipidemia

Childhood HSCT survivors show a high prevalence of dyslipidemia [33], having been described in 18% [5] to 26% [7] to 40% of patients post HSCT. Etiopathogenic factors could include chemotherapy agents and obesity. Dyslipidemia has been associated in studies with hematological malignancies, younger age at transplantation, hypothyroidism, hypogonadism, chronic GVHD, and treatments such as steroids and TBI conditioning, longer follow-up, and positive family history [5].

Bone

Decreased BMD has been described in 21% [34] to 37.8% [7] or 48% [35] of patients following HSCT. These studies have found association with malnutrition, low intake of calcium and vitamin D, sedentariness, untreated GHD and hypogonadism, and treatment modalities that damage osteoprogenitor cells such as cyclosporine, tacrolimus, methotrexate, and steroids. Unfortunately, DXA scan was unavailable for many patients before the HSCT as they were clinically unstable, or following the transplant as patients come from other parts far from the Spanish territory we studied.

Limitations of the study include its retrospective nature, the small number of patients in certain diagnostic groups, and the heterogeneity in conditioning regimens due to the evolution of protocols over time.

Conclusions

More than half of the children surviving an HSCT will develop endocrine abnormalities, with hypogonadism and growth impairment being the most frequent. Obesity has risen to be the third most common endocrine abnormality, mainly due to steroid use, and also probably in the context of the increase of this condition in the general population. Lymphoblastic
leukemia and lymphomas were the conditions with a higher rate of endocrine abnormalities. Regardless, ascribing causality to the underlying pathology and to former therapies is challenging, although alkylating agents and body irradiation are associated with higher rates of endocrinopathies. Factors such as female sex, older age, pubertal stage, allogenic HSCT, therapies (radiotherapy, steroids, busulfan, thymoglobulin, cyclophosphamide), and GVHD should alert care providers to potential endocrine disturbances. Adhering to established pre- and post-HSCT protocols will enable a prompt diagnosis and treatment of hormone and metabolic alterations. In recent years novel conditioning regimens have continued to be developed with the goal of maintaining efficacy while reducing toxicity, with the aim of improving patients’ quality of life.

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Data Availability
Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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