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CASE REPORT

Dumbbell-shaped Ewing's sarcoma family of tumor of thoracic spine in a child

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Abstract An 8-year-old boy was referred due to difficulty in walking. T1-weighted MRI detected a well-marginated lesion expanding from the epidural region in the spinal canal to the paravertebral area through the Th9 and Th10 intervertebral foramen. The patient underwent a biopsy under video-assisted thoracoscopy and the tumor was diagnosed as Ewing's sarcoma family of tumor (ESFT). Imaging confirmed that the tumor completely disappeared and his neurologic functions were recovered perfectly at the end of treatment. Very few cases of skeletal ESFT of epidural extension in childhood have been documented. Video-assisted thoracoscopic surgery remains the best option for the diagnosis of endothoracic tumors in children.

Keywords Ewing's sarcoma family of tumor (ESFT) · Dumbbell-shaped · Video-assisted thoracoscopic surgery (VATS) · Biopsy · EWS-FLI1

Introduction

Ewing's sarcoma family of tumor (ESFT) is a malignant tumor consisting of uniform small round cells. ESFT mostly arises from skeletal bones or soft tissue in the leg, pelvis and rib. Although neurogenic tumors such as a neuroblastoma or schwannoma are representative "dumbbell-shaped" tumors in childhood, ESFT presenting "dumbbell-shaped" is extremely rare. This report describes a case of a "dumbbell-shaped" ESFT presenting in a child.

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Case report

An 8-year-old boy was referred for the difficulty in ambulation without any particular cause. When he visited the hospital 3 days after the symptoms appeared, he could not stand up by himself. The family history was negative for congenital malformation and cancer. A neurologic examination on admission revealed hypesthesia affecting the superficial sensory modalities from T8 to T10 on his right side and apparent muscle weakness in the lower extremities. He did not have any difficulties in either defecation or urination. All laboratory tests including tumor markers such as NSE, HVA, and VMA were within the normal range. A pyriform-like mass shadow was found on a chest roentgenogram behind of the right side of the heart. A chest CT revealed a mass shadow of 3.5 × 1.9 cm in the posterior mediastinum adjacent to the thoracic vertebra (Th9–11). T1-weighted MRI detected a well-marginated homogeneous lesion expanding from the epidural region in the spinal canal to the paravertebral area through Th9 and Th10 intervertebral foramen and revealed abnormal low intensity at the vertebral body of Th9, thus suggesting a vertebral bone originating tumor (Fig. 1a, b). A T2-weighted image demonstrated a high intensity lesion markedly compressing the spinal cord. A T1-weighted image with gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) enhancement showed homogeneous high intensity lesion at the same site. A Tc-99 m HDMP bone scintigram revealed a hot spot on the vertebral body of

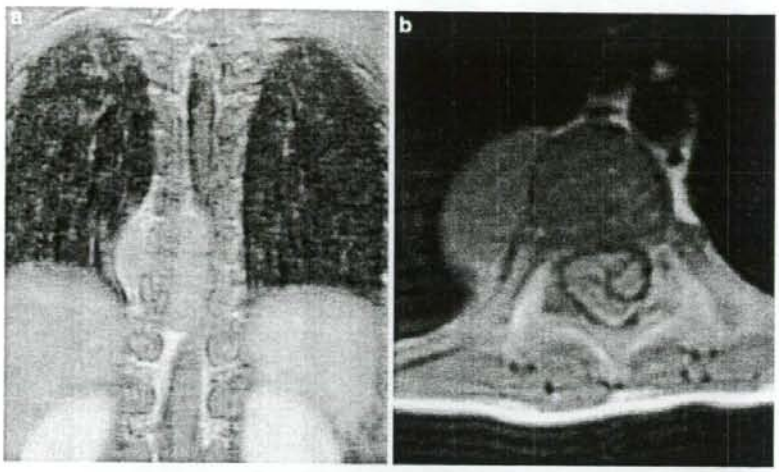

Fig. 1 T1-weighted magnetic resonance imaging at the time of diagnosis showing a well-marginated homogeneous lesion (3.5 × 1.9 cm) expanding from the epidural region in the spinal canal to the paravertebral area through the Th9 and Th10 intervertebral foramen and revealed an abnormal low intensity at the vertebral body of Th9. **a** Coronal section, **b** horizontal section

Th9. He underwent a biopsy under video-assisted thoracoscopic surgery (VATS) with a presumptive diagnosis of a neuroblastoma that is a common "dumbbell-shaped" tumor occurring in childhood. The tumor was located at the right side of the thoracic vertebra and covered with pleura. Its surface was smooth and yellowish in color. It was fragile and partially removed in a piece-by-piece fashion for the pathological diagnosis (Fig. 2). The operation time was 90 min and blood loss was 20 ml. No intraoperative and postoperative complication was encountered. A histological examination revealed a diffuse proliferation of monomorphic small round tumor cells and immunohistochemical staining showed the tumor was clearly positive for MIC2 (CD99), but negative for NSE and c-kit. In addition, RT-PCR detected transcripts of the fusion gene, *EWS-FLI1*, but not *EWS-ERG*. Finally a chromosomal translocation (t(11;22) (q24; q12)) was detected in tumor cells, therefore the tumor was diagnosed as ESFT. Although the definite diagnosis was not made immediately after the biopsy, the postoperative chemotherapy under the possible diagnosis as neuroblastoma or ESFT was initiated on the day of surgery since the neurological symptoms were becoming worse from hour to hour. After one course of chemotherapy consisting of cylophosphamide (CPA) tetrahydropyranyladriamycin (THP-ADR), etoposide (VP-16) and cisplatin (CDDP), the tumor was markedly reduced and the leg paralysis was decreased. After the complete histopathological diagnosis was made as ESFT, the induction chemotherapy designed for ESFT with vincristine (VCR), THP-ADR, CPA, ifosfamide and VP-16 followed by local treatment with radiotherapy was conducted. His motor and sensory functions were recovered perfectly to the normal range and he could walk without any help by the end of the induction chemotherapy.

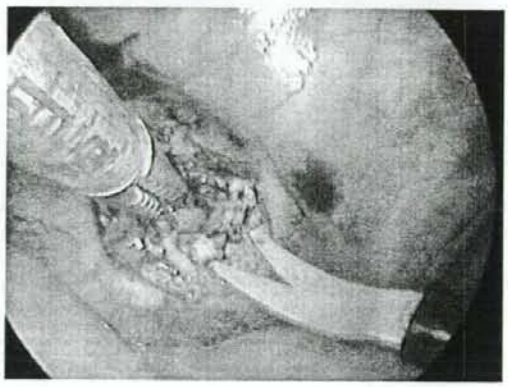

Fig. 2 Video-assisted thoracic biopsy of the tumor. The surface of tumor was smooth and yellowish in color. The tumor was fragile and partially removed in a piece-by-piece fashion by scissors for the pathological diagnosis

Imaging confirmed that the tumor had completely disappeared at 6 months after the operation.

Discussion

The ESFT is an infrequent neoplasm, only 20 new cases are registered per year in Japan [1]. In North America, it is more frequent, and 225 patients younger than 20 years are diagnosed per year [2]. Ewing's sarcoma usually arises from the long bones and pelvis, with the spinal column being the site in only 3.5–9% of all cases [3]. The typical radiographic features of spinal Ewing's sarcoma include varying degrees of osteolytic and osteosclerotic changes in the vertebral bone [4]. On the other hand, the lesion in the

spinal column without osseous changes are diagnosed as extraskeletal Ewing's sarcoma [5]. In the present case, although the tumor was located in the extradural space in the right side of the spinal canal, the lesion was clearly associated with osseous change at the vertebral body of Th9 based on MRI examination and a Tc-99 m HDMP bone scintigram. Therefore, the lesion was diagnosed to be skeletal Ewing's sarcoma arising from the vertebral bone. Very few cases of skeletal Ewing's sarcoma of epidural extension have been documented as described by Song et al. [6], although one report indicated that epidural extension was thought to be a common feature [3]. In addition, skeletal Ewing's sarcoma derived from the vertebral bone of epidural extension associated with intrathoracic extension through vertebral foramen in the present case is definitely rare.

Koizumi et al. [7] reported successful treatment of "dumbbell-shaped" ESFT in a child with thoracoscopic examination followed by the complete removal of tumor by VATS after chemotherapy. In the present case, the tumor was so chemo-sensitive that the tumor completely disappeared with induction chemotherapy and the surgical removal of the tumor after chemotherapy was not needed.

It is well known that neurogenic tumors such as neuroblastoma or schwannoma sometimes present as "dumbbell-shaped" lesions in childhood [8]. Since it is indistinguishable symptomatically and radiologically from other types of neurogenic tumors prior to a surgical biopsy, a histopathological examination is critical for the diagnosis of ESFT. The correct and definitive diagnosis can be made only by determining the immunoreactivity for MIC2 [9] and the detection of the chimeric EWS-FLI1 transcript [10] using a biopsy specimen. Since the consecutive treatment beginning with induction chemotherapy should be started as soon as obtaining the diagnosis of ESFT, a minimally invasive surgical biopsy that never interferes with the start of induction chemotherapy should be applied. In the treatment of malignant disease, VATS was reported to be useful in establishing a quick and reliable diagnosis, and assisting the appropriate treatment including chemotherapy, radiotherapy and/or surgery [11–13]. Close observation with thoracoscopy provides useful information macroscopically for the diagnosis of pediatric tumors. The rapid postoperative recovery in VATS also allowed the immediate use of postoperative chemo-radiotherapy in comparison to a thoracotomy [12]. Spinal cord compression is seen in 3–5% of patients with pediatric malignancies [14]. It is reported that in patients with pediatric malignancies with difficulty in walking at the beginning of treatment, approximately 50% of them could walk following emergent therapy [15]. Therefore, in this case, it was necessary to immediately begin the treatment. The brief surgical biopsy with VATS was successfully performed with small amount of blood loss, and the treatment for the eradication of tumor was immediately started on the day of the biopsy.

In summary, ESFT can present as a "dumbbell-tumor", and VATS remains the best option for the diagnosis of endothoracic tumors in children.

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Umbilical cord-blood transplantations from unrelated donors in patients with inherited metabolic diseases: Single-institute experience

Tokimasa S, Ohta H, Takizawa S, Kusuki S, Hashii Y, Sakai N, Taniike M, Ozono K, Hara J. Umbilical cord-blood transplantations from unrelated donors in patients with inherited metabolic diseases: Single-institute experience.
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Abstract: We evaluated the feasibility of UCBT from unrelated donors and a myeloablative preparative regimen that did not involve anti-thymocyte globulin in five children with lysosomal and peroxisomal diseases. Patients with MPS II (n = 1), adrenoleukodystrophy (n = 1), metachromatic leukodystrophy (n = 2), and Krabbe disease (n = 1) received UCBT between December 2001 and September 2005. All patients received oral Bu (600 mg/m^2), CY (200 mg/kg IV), and fludarabine (180 mg/m^2 IV). Prophylaxis for GVHD consisted of a combination of tacrolimus and a short methotrexate course. Neutrophil engraftment occurred a median of 24 days (range, 21–25) after transplantation. None had graft rejection. One patient developed grade III acute GVHD and the other four patients had grade I acute GVHD; none had extensive chronic GVHD. One patient developed hemorrhagic cystitis. There were no treatment-related deaths. Although one child with MPS II died of PTLD 10 months after the UCBT, four of the five children are alive 14, 20, 31, and 55 months after transplantation with complete donor chimerism. These results suggest the feasibility of the UCBT with Bu, fludarabine, and CY-preparative regimen for patients with inherited metabolic diseases.

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Key words: inherited metabolic disease – cord blood transplantation – busulfan – cyclophosphamide – fludarabine

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Inherited metabolic diseases are rare congenital disorders, in which toxic substances accumulate in various tissues because of mutations of various metabolic enzymes. Some lysosomal and peroxisomal storage diseases are characterized by severe neurologic deterioration and eventual death in the first or second decade of life (1).

Abbreviations: ATG, anti-thymocyte globulin; Bu, busulfan; CBU, cord blood unit; CY, cyclophosphamide; EBV, Epstein–Barr virus; G-CSF, granulocyte colony-stimulating factor; GVHD, graft vs. host disease; HLA, human leukocyte antigen; IV, intravenously; MPS, mucopolysaccharidosis; NC, nucleated cells; PTLD, post-transplant lymphoproliferative disorder; UCBT, umbilical cord blood transplantation.

Recent clinical trials of enzyme replacement therapy have shown promise in the treatment of selected diseases (2). However, they also clearly demonstrated that this approach could not be used to treat patients characterized by severe neurologic deterioration, because IV administered enzymes do not cross the blood–brain barrier (3). Allogeneic bone marrow transplantation is still the only effective treatment for selected metabolic diseases (4, 5). It can prevent the progression of symptoms by providing a continuous source of normal enzymes from engrafted donor leukocytes and other macrophage-derived cells. However, the identification of suitable matched-related donors can be difficult for some patients, and recruitment of an

unrelated adult donor takes too long for the treatment of a rapidly progressive disorder. Preliminary experience suggests that UCBT may be as effective as marrow transplants (6, 7). Staba et al. reported that all evaluable patients with MPS I had either stable or improved neurocognitive function after UCBT (7). UCB grafts have certain benefits such as rapid availability and even reduced risk of GVHD, although they may have high frequency of graft failure. Hence, UCBT represents an alternative to marrow transplantation (8).

Here, we analyzed retrospectively five children with lysosomal or peroxisomal storage disease who underwent UCBT.

Patients and methods

Five Japanese children with lysosomal or peroxisomal storage disease who lacked HLA-matched related donors underwent unrelated donor UCBT at Osaka University Hospital between December 2001 and September 2005. The diagnosis was confirmed by the lack of specific enzyme activity in peripheral blood leukocytes or fibroblasts. The parents of each patient provided informed consent before UCBT. Multiorgan assessment was performed before UCBT to demonstrate the following criteria: Karnofsky performance score > 50%; creatinine clearance > 50% of the lower limits of normal for age; aspartate aminotransferase less than five times the upper limits of normal and total serum bilirubin < 2.5 mg/dL; cardiac status asymptomatic or left ventricular ejection fraction > 50%; and asymptomatic pulmonary function or oxygen saturation > 90% on room air. The selected CBU was required to provide a minimum of 2×10^7 total NCs/kg of recipient weight before cryopreservation.

Selection of donors

HLA matching between patient and UCB donor was based on serologic class I HLA typing and high-resolution molecular class II typing (DRB1). The cord blood unit was selected to provide the highest number of NCs with matching at a minimum of four to of six HLA loci. A unit genetically matched at three of six alleles could be selected without serologic mismatches. All cord blood units were provided through the Japan Cord Blood Bank Network.

Conditioning regimen

All patients received oral Bu (37.5 mg/m^2 per dose) for 16 doses on days −9 through −6, CY 50 mg/kg per dose IV on days −5 through −2, and fludarabine (30 mg/m^2 per dose IV on days −6 through −1. Anticonvulsants were given during Bu therapy to prevent seizures, and mesna was given immediately after CY infusion to prevent hemorrhagic cystitis. The cord blood was infused on day 0.

GVHD prophylaxis and treatment

All patients received short-term methotrexate (15 mg/m^2 on day 1, 10 mg/m^2 on days 3, 6, and 11) and tacrolimus. Whole blood concentration of tacrolimus was maintained at a trough level of 5–15 ng/mL. Patients with grades II and above GVHD received methylprednisolone (2 mg/kg per day IV every eight h).

Supportive therapy

All patients were treated in laminar air flow room, and received total parenteral nutrition and antibiotics. Administration of 5 μg/kg of G-CSF (lenograstim or filgrastim) was started on day 1. Empiric treatment with broad-spectrum IV antibiotics was started at the first episode of neutropenic fever and continued until neutropenia resolved. Continuous infusion of low-dose heparin was administered from the initiation of the preparative regimen through post-transplantation day 28 for prophylaxis against veno-occlusive disease. All patients were supported as needed with transfusions of leukocyte-depleted, irradiated packed red blood cells and platelets.

Evaluation of toxicity

Neutrophil recovery was defined as a blood neutrophil count $> 0.5 \times 10^3/\mu L$. Platelet recovery was defined as a blood platelet count $> 20 \times 10^3/\mu L$, independent of platelet transfusion. Visceral toxicity was evaluated according to the criteria defined by Common Terminology Criteria for Adverse Events, version 3.

Results

Patient characteristics

Primary diseases are one Krabbe disease (juvenile type), one adrenoleukodystrophy (childhood-onset type), two metachromatic leukodystrophy (late infantile type) and one Hunter's syndrome (MPS type II). Median age at transplantation was five yr (range, two to nine years). There were four males and one female. Patient characteristics are shown in Table 1.

Engraftment

Neutrophil and platelet engraftment occurred a median of 24 days (range, 21–25) and 33 days (range, 28–46), respectively, after transplantation (Table 2). Red cell and platelet transfusions were no longer required after a median interval of 26 days (range, 10–41) and 41 days (range, 26–43), respectively. As of the last follow-up evaluation, all five patients had complete donor chimerism (Table 2).

GVHD

Patient 2 had skin rash, high fever, and diarrhea, indicating the occurrence of grade III acute GVHD (skin: stage 1, liver: stage 0, gut: stage 3) on day 16. It gradually resolved after treatment with 2 mg/kg methylprednisolone. The other four patients had grade I acute GVHD (Table 2). None had chronic GVHD.

Table 1. Characteristics of patients undergoing UCBT

Patient no.	Primary disease	Transplantation date	Initial manifestation	Age at onset	Age at diagnosis	Age at transplantation	Sex	HLA mismatch	Infused NCs ($\times 10^7$/kg)	CD34+ cell dose ($\times 10^5$/kg)
1	Krabbe disease, late onset	2003/12/12	Blindness	5yr 0m	9yr 6m	9yr 11m	M	DR0405/0410 DR0407/0410	2.6	3.7
2	Adrenoleukodystrophy, childhood cerebral form	2001/12/3	Disturbances of vision	5yr 0m	5yr 1m	5yr 3m	M	B61/52	5.9	9.2
3	Metachromatic leukodystrophy, late infantile	2004/11/12	Gait disturbance	1yr 7m	2yr 7m	2yr 10m	F		4.5	4.4
4	Metachromatic leukodystrophy, late infantile	2005/4/28	Gait disturbance	2yr 4m	3yr 4m	3yr 9m	M	B61/55	4.0	4.0
5	Hunter's syndrome (MPS II)	2005/9/1	Mental retardation	3yr 6m	3yr 8m	5yr 10m	M	A0601/0206 DR0406/0410 DR1406/1402	2.3	12.0

NCs, neutrophil cells; MPS, mucopolysaccharidosis; yr, years; m, months; M, male; F, female; HLA, human leukocyte antigen.

Toxicity

No treatment-related death was observed. No neurologic toxicity occurred. Hemorrhagic cystitis occurred in one patient (grade 2) and septicemia was observed in two patients. No patient has had clinically significant cardiac dysfunction.

Outcome

Four of five patients were alive for 24, 29, 40, 64 months as of May 1, 2007. The patient with metachromatic leukodystrophy developed acute encephalopathy 21 months after the UCBT *and recovered.* One child with MPS II died of PTLD 10 months after the UCBT. The PTLD was histopathologically proven in the adenoids. Six courses of rituximab administration were not effective. It was not associated with EBV.

Discussion

We describe the outcome in five children with inherited metabolic storage diseases who received UCBT from unrelated donors after the administration of a chemotherapy-based conditioning regimen. Allogeneic bone marrow transplantation has been the only effective treatment for selected metabolic diseases (1). Unfortunately, most patients lack an HLA-identical sibling. Registries of potential unrelated donors have been established to provide hematopoietic transplants for patients lacking a histocompatible relative. There are now over 10 million potential unrelated bone marrow donors accessible in registries worldwide, and approximately half of patients can access an HLA-A, -B, and -DR-identical donor. Banked UCB is prospectively HLA typed, screened for infections and other risk factors, and readily available for transplantation. The time from diagnosis to definitive treatment may represent a crucial period in which a readily available source of stem cells is extremely desirable to prevent further disease progression (7). Escolar et al. demonstrated that UCBT in newborns with infantile Krabbe disease before the development of symptoms can favorably alter the natural history of the disease (9). UCBT also offers an advantage of potential reduced risk of GVHD (10), although one of our patients (No. 2) had grade III acute GVHD. The cause of this severe GVHD developing despite only one serotype mismatch within the HLA-B locus is unknown. Mismatch of class I antigen more strongly influences the severity of acute GVHD than class II antigen in Japanese population (11). In addition, there might be a disparity at the split antigen HLA-A26, which both the donor and recipient had.

Table 2. Outcome of patients undergoing UCBT

Patient no.	Chimerism	Days to WBC >1000/μL	Days to neutrophils >500/μL	Days to PLT>20 000/μL	Acute GVHD	Toxicity/event	Outcome	Follow-up (m)
1	Complete chimera FISH 98.8%	21	24	28	I	Hemorrhagic cystitis	Alive	40
2	Complete chimera VNTR	24	24	45	III	Septicemia (*Streptococcus mitis*)	Alive	64
3	Complete chimera VNTR	21	24	33	I	–	Alive	29
4	Complete chimera VNTR	26	25	36	I	–	Alive	24
5	Complete chimera VNTR	23	21	46	I	Septicemia (MRSA)	Dead (PTLD)	10

WBC, white blood cell; Neut, neutrophils; PLT, platelets; GVHD, graft vs. host disease; m, months; FISH, fluorescence *in situ* hybridization; VNTR, variable number of tandem repeat; MRSA, methicillin-resistant Staphylococcus aureus; PTLD, post-transplant lymphoproliferative disorder.

However, disadvantages of UCBT include poor engraftment (12). Children with congenital metabolic disorders might be considered to be at a higher risk for engraftment failure than children with malignancies, because they have not been exposed to large amounts of anticancer drugs before transplantation. In fact, the Eurocord group has reported that the cumulative incidence of neutrophil recovery at day 100 in two different cohorts of patients with malignant and non-malignant disease was 77.4% and 69.3%, respectively (13).

The combination of CY, Bu, and ATG has been frequently used for patients with congenital disorders (6, 7). Toxicity of ATG has resulted from the large amount of foreign protein in ATG. Immediate anaphylaxis can occur and fever, chills, and urticaria are common with the several infusions of ATG (14). In this series of hematopoietic stem-cell transplantations using ATG for 98 patients with hematological or inherited storage disease, the adverse events of ATG were mainly fever (76%), chills (56%), flush (12%), and anaphylactic shock (2%) (14).

In our study, ATG was substituted by fludarabine, which has strong immunosuppressive properties and demonstrates few severe toxicities. Fludarabine-based regimens are often used in reduced intensity stem-cell transplantation. Resnick et al. has developed a basic non-myeloablative stem-cell transplantation protocol, using a conditioning regimen with fludarabine, Bu, and ATG for patients including three children with adrenoleukodystrophy (15). Jacobsohn et al. reported that they performed reduced intensity hematopoietic stem-cell transplantation with a uniform regimen consisting of fludarabine, Bu, and ATG for 13 children with non-malignant diseases including two lysosomal storage diseases, and 72% of evaluable patients achieved full donor engraftment (16). However, the doses of these drugs in this study were sufficient for myeloablation and complete chimerism.

No deaths were associated with GVHD and none of the children had chronic GVHD in our study. By contrast, among children with MPS I who receive bone marrow transplants from unrelated donors, the rates of GVHD have been reported to be between 30% and 55% and to account for up to half of transplantation-associated mortality (17, 18).

Although a few cases of PTLD have been reported after UCBT (19), the risk of this complication has not been well analyzed. A recent analysis by Brunstein et al. found no significant difference for the risk of this disorder between unrelated donor UCB or unmanipulated marrow (20). However, these authors also reported that patients undergoing a non-myeloablative UCBT with ATG are at a uniquely higher risk for the development of EBV-related complications, in particular PTLD (20).

We have not mentioned the neurologic outcome of these patients, as this small series contains various diseases and the observational periods are relatively short. The preliminary neurologic evaluation of these patients revealed that the condition of the patient with Krabbe disease has gradually improved after the brief deterioration during the transplantation, while the patients with adrenoleukodystrophy progressed during UCBT. Metachromatic leukodystrophy, which had been progressive, remained stable for one yr post-transplantation.

In summary, our results of UCBTs for patients with inherited metabolic diseases are promising, although the number of patients is too low to draw any definitive or statistically significant conclusions. ATG could be replaced with fludarabine in the Bu and CY-based regimen. Future long-term follow-up and detailed neurologic examinations will evaluate the complete effects of UCBT.

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Endocrinological Analysis of 122 Japanese Childhood Cancer Survivors in a Single Hospital

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Abstract. With recent improvements in the diagnosis and treatment of cancer, the number of childhood cancer survivors (CCSs) has been increasing in Japan. The importance of quality of life during the lifetime of CCSs has now been recognized, and the late effects of cancer treatments are essential and important issues. In this study we analyzed the endocrinological abnormalities of CCSs by retrospectively evaluating 122 outpatients (62 males and 60 females) who had been referred from pediatric oncologists to our follow-up clinic among 151 CCSs attending our hospital more than two years after their cancer treatment. Follow-up duration varied from 2 to 30 (median 8.0) years. Their average age was 17.3 (range 4–36, median 17.0) years, and 38 patients (31.1%) reached adulthood. Endocrinological abnormalities were found in 82 (67%) of 122 survivors. Gonadal dysfunction was observed in 60 patients (49%). Thirty-nine patients (32%) were short or grew at a slower rate. Twenty-six patients (21%) showed thyroid dysfunction. Other abnormalities were as follows: obesity in 20 patients (16%), leanness in 10 (8%), central diabetes insipidus in 11 (9%) and adrenocortical dysfunction in 9 (7%). Low bone mineral density was observed in 41 (42%) of 98 patients evaluated. These endocrinological abnormalities were caused by the combined effects of cancer itself and various treatments (chemotherapy, radiation therapy, surgery, and hematopoietic stem cell transplantation). Lifetime medical surveillance and continuous follow-up are necessary for CCSs, because treatment-related complications may occur during childhood and many years after the therapy as well. Endocrinologists should participate in long-term follow-up of these survivors in collaboration with pediatric oncologists.

Key words: Childhood cancer survivors, Late effects, Endocrinological abnormality, Growth retardation, Quality of life
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WITH recent improvements in the diagnosis and treatment of children with cancer, the number of childhood cancer survivors (CCSs) is surmised to be rapidly growing in Japan as well as other countries, including the U.S.A. and Europe [1–3]. Approximately 80% of children with cancer now survive longer than five years [2, 3], however, this improved survival has been accompanied by the occurrence of late treatment-related complications, late effects, such as second neoplasm, organ dysfunctions and psychosocial problems, which have become essential and important issues [4–7]. Among late effects, endocrinological abnormalities are the most common problems, often requiring early interventions [8, 9]. Several reports from the U.S.A. and Europe have described the late effects or adverse health outcomes in a large number of CCSs, and guidelines to follow-up CCSs from abroad are now available [3, 10–14], however, only a few studies on CCSs, especially focused on endocrinological abnormalities, have been published in Japan [15, 16, 17]. The aim of the present retrospective study was to assess the endocrine abnormalities of CCSs referred from pediatric oncologists to our follow-up clinic in our hospital situated in the western Japan.

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Table 1. Patient profile and endocrinological late effects of 122 CCSs

		Total (n = 122)	Hematologial (n = 67)	Brain tumor (n = 26)	Solid tumor (n = 29)
Therapy	Chemotherapy	116 (95%)	67	20	29
	Surgery	57 (47%)	5	23	29
	Radiation therapy	72 (59%)	42	20	10
	HSCT	65 (53%)	36	10	19
	Conditioning: TBI	25	25	0	0
	Conditioning: non-TBI	40	11	10	19
Endocrine disorder	Total	82 (67%)	39 (58%)	25 (96%)*	18 (62%)
	Growth disturbance	39 (32%)	14	17*	8
	Obesity	20 (16%)	11	6	3
	Leanness	10 (8%)	5	1	4
	Hypogonadism	60 (49%)	23	20*	17
	Primary	51 (M 26, F 25)	23 (M 11, F 12)	11 (M 5, F 6)	17 (M 10, F 7)
	Central	9 (M 4, F 5)	0	9 (M 4, F 5)	0
	Thyroid dysfunction	26 (21%)	8	16*	2
	Adrenocortical dysfunction	9 (7%)	0	9*	0
	Central diabetes insipidus	11 (9%)	0	10*	1

* Significant high incidence: $p<0.01$ by chi square test

Patients and methods

Patients

The records of 151 CCSs who had survived for more than two years after cancer treatment and been followed regularly in Osaka University Hospital were reviewed, retrospectively. This study analyzed 122 patients (62 males and 60 females), who had been referred from oncologists to our follow-up group among 151 CCSs in our pediatric department from January 1997 to December 2007. The unreferred 29 patients were excluded in the study: hematological disease (n = 6), brain tumor (n = 5) and solid tumor (n = 18). Follow-up duration varied from 2 to 30 (mean 8.8, median 8.0) years. Average age was 17.3 (range 4–36, median 17.0) years, and 38 patients (31%) reached adulthood. Mean age at diagnosis of the underlying disease was 6.4 (range 0–15) years. The underlying disease and treatment regimens [chemotherapy, radiation therapy, surgery and hematopoietic stem cell transplantation (HSCT)] were assessed from the medical records. Table 1 lists the patient profiles. Diagnosis was hematological disease (67), brain tumor (26) and solid tumor (29). Hematological malignant diseases were classified as follows: acute lymphoblastic leukemia (34), acute myelocytic leukemia (12), non-Hodgkin lymphoma (8), chronic myelocytic leukemia (3), juvenile myelomonocytic leukemia (2), Hodgkin lymphoma (2) and myelodysplastic syndrome (1). Non-malignant hematological diseases included aplastic anemia (3), Wiskott-Aldrich syndrome (1) and leukocyte adhesion deficiency (1). Brain tumors were classified as germinoma (11), medulloblastoma (9) and craniopharyngioma (6). Solid tumors were classified as neuroblastoma (7), rhabdomyosarcoma (8), hepatoblastoma (4), primitive neuroectodermal tumor (2), Wilms' tumor (2), germinoma (1), upper pharyngeal tumor (1), teratoma (1), eosinophilic granuloma (1), ovarian undifferentiated germinoma (1) and granulosa cell tumor of the ovary (1). Number in each parenthesis indicates the number of patients.

Treatment

Treatment protocols for cancer have changed over the years. The treatment regimens of the 122 survivors included 116 chemotherapy (95%), 72 radiotherapy (59%) and 57 surgery (47%). Sixty-five patients (53%) underwent HSCT, including 33 allogeneic and 32 autologous sources. Five patients underwent two transplants, and one patient, three transplants. Hematopoietic stem cells were derived from bone marrow (n = 45), peripheral blood (n = 23) and umbilical cord blood (n = 3). Twenty-five patients with hematological diseases received radiation as a conditioning

for HSCT: total body irradiation (TBI) at a dose of 8–12 Gy in 4 to 8 fractions in 22 patients, TBI without cranial irradiation at a dose of 6–10 Gy in 2 to 4 fractions in 2 patients and total lymphoid irradiation at a dose 6 Gy in 3 fractions in 1 patient. The conditioning regimen for HSCT in the other 40 patients was chemotherapy only, including 8–16 mg/kg of busulfan and/or 100–200 mg/kg of cyclophosphamide for hematological disease, or 800 mg/m^2 of thio-TEPA and 280 mg/m^2 of melphalan for brain tumor and solid tumor. The dose of radiation varied with the case: 3–24 Gy to the whole brain for hematological diseases, 10–54 Gy to the local cerebral region plus 18–30 Gy to the whole brain with 18–32 Gy to the whole spine for brain tumors and 20–45 Gy to the local lesion for solid tumors.

Evaluation methods

The current physical and psychological status, use of medication and school health check were inquired from patients and their parents. Height and weight were measured at every visit. Current height was expressed as the standard deviation score (SDS) for age and gender. Short stature was defined as a stature <–2 SD for chronological age or slow growth as growth velocity 1.5 SD below the mean for more than two years. Patients under GH therapy were all included in this category. GHD (growth hormone deficiency) was defined as a low IGF-1 level and low peak GH level on the GH provocation tests. Subjects with peak GH levels <10 ng/ml on insulin, arginine, clonidine and dopamine were considered to have abnormal GH secretion. Obesity was defined as >20% obese for ideal weight, and leanness as <20% lean for ideal weight. Pubertal status was assessed by the method of Tanner. Serum levels of basal FSH, LH and testosterone (boys)/estradiol (girls) were measured. For boys, testicular volume was determined using an orchidometer. Failure of spontaneous puberty was defined as the absence of breast development in girls at 14 years of age or more and of testicular enlargement in boys at 15 years of age or more. Tubular damage of male patients was defined by little (<10 ml) [16] or no increase in testicular volume combined with a high basal FSH level (>20 mIU/ml) at pubertal age. For girls, the age at initiation and regularity of menstruation were inquired. FSH and LH values were considered high or low in primary and central hypogonadism. The cut-off point for early pubertal signs was 9 years of age for boys and 7.5 years for girls. Thyroid status was evaluated by palpation of the thyroid gland, and also measurement of serum TSH and FT4 levels. Primary hypothyroidism was defined as TSH≥10 μU/ml and central hypothyroidism as FT4 <0.8 ng/dl and inappropriately elevated TSH. In hypothyroidism, antithyroid peroxidase (TPO) antibody and/or anti-thyroglobulin (Tg) antibody were measured. Adrenocortical function was assessed by serum ACTH and cortisol levels and serum DHEAS. These endocrine tests were measured at outpatient clinic in the morning to avoid diurnal variation of hormones. Central diabetes insipidus was defined as polyuria and necessity for DDAVP therapy.

Bone mineral density

BMD (bone mineral density) (g/cm^2) of the lumbar spine (L2–L4) was measured by dual energy X-ray absorptiometry (DXA) in 98 out of 122 patients: DPX-L (LUNAR) until March 2006 and Discover QDR 1000 (HOLOGIC) after April 2006. The conversion formula was as follows: [QDR 1000] = 0.827 × [DPX-L] + 0.042. In adult cases, osteopenia was defined as between 70 and 80% of the Japanese young adult mean (YAM) and osteoporosis as less than 70% of YAM [18]. The BMD in childhood was expressed as age-normalized Z-scores (SD from the mean for age- and sex-matched controls) according to the report by Tanaka [19]. In children, osteopenia was defined as a Z-score between 1.7 and 2.6 SD below the mean, and osteoporosis as a Z-score of more than 2.6 SD below the mean.

Statistical Analysis

The chi-square test was used to assess the difference between each group in Table 1 and Table 3. The two-tail Wilcoxon t-test was used to compare the differences of mean height-SDS in Table 2. These statistical analyses were carried out using Excel Ystat 2006.

Results

Endocrinological abnormalities were found in 82 (67%) of 122 CCSs in our follow-up clinic: 39 (58%) of 67 patients with hematological diseases, 25 (96%)

of 26 patients with brain tumors and 18 (62%) of 29 patients with solid tumors. Frequency of endocrinological disorders was significantly higher in patients with brain tumors compared to other types of tumors (Table 1, $p<0.01$). Hormone replacement therapies were prescribed as follows: sex hormone (n = 29: male 7, female 22), levothyroxine (n = 22), GH (n = 13), DDAVP (n = 10), and corticosteroid (n = 9). These endocrinological problems have emerged at different sites and times on a case-by-case basis even when common protocols were used for the same diseases.

Physical growth

Thirty-nine patients (32%) had a height <–2 SD for age or slow growth at the last assessment. Sixteen patients were diagnosed with GHD, and 13 patients had been treated with recombinant human GH (Table 2). The average age at GH initiation was 11.6 years old (range, 5.4 to 14.6), 5.3 years after the end of cancer treatment (range, 1.5 to 11.3 years). Mean height-SDS at initiation of GH therapy was –3.4 (range, –6.2 to –2.2), and current height-SDS was –1.8 SD (range, –5.0 to the mean). Height gain was significant according to these improved SD scores as a group ($p<0.01$). However, a few cases with delayed or short duration of GH therapy showed poor improvement in height. We started sex hormone replacement therapies at relatively late ages to avoid closure of growth plate and to ensure longer duration of GH therapy. GH therapy was stopped in two patients: one patient (case 3) with germinoma due to poor compliance and an abnormal oral glucose tolerance test, and an extremely obese patient (case 8) with craniopharyngioma due to the occurrence of type 2 diabetes mellitus. In the last evaluation, 20 (16%) of 122 patients were obese, and 10 patients (8%) were lean for their ideal weight.

Thyroid status

Twenty-six patients (21%) were diagnosed with thyroid dysfunctions, and 22 (18%) were treated with levothyroxine replacement therapy among 122 CCSs. Sixteen patients had primary hypothyroidism and 9 patients had central hypothyroidism. One patient (meduloblastoma), who had received radiation therapy to the brain and spine, alternately showed both types of hypothyroidism. Anti-Tg antibody and/or anti-TPO antibody were positive in 6 patients; 4 had allogeneic transplantations. Nine of 10 patients with primary hypothyroidism who had no anti-thyroid autoantibody had been irradiated to the neck for their underlying disease or as a conditioning regimen for HSCT. All 9 patients with central hypothyroidism had been treated for suprasellar brain tumors, 3 germinoma and 6 craniopharyngioma. No case of hyperthyroidism or thyroid cancer was seen in our study.

Gonadal function and puberty

Males

Thirty (48%) pubertal boys out of 62 male CCSs had abnormal gonadal functions: 4 with central hypogonadism and 26 with primary hypogonadism. All 4 patients with central hypogonadism manifested panhypopituitarism after the therapy of suprasellar brain tumors. All 26 primary hypogonadic males, 11 patients with hematological diseases, 5 with brain tumors and 10 with solid tumors, had been treated with chemotherapy including alkylating agent, cyclophosphamide. Eight of 11 patients with hematological diseases had also been treated with TBI for HSCT. Most male patients with primary hypogonadism had begun spontaneous puberty. Sex hormone replacement therapy was administered to only 7 patients, 4 with central hypogonadism and 3 with primary hypogonadism. It was difficult to identify gonadal dysfunctions in prepubertal patients. Sperm evaluations were not routinely performed. Three males with β-HCG-producing germinoma presented with precocious puberty and advanced bone age at diagnosis. Their accelerated puberty was stopped when cancer treatment was initiated, and they showed primary hypogonadism after chemotherapy including cyclophosphamide.

Females

Thirty (50%) out of 60 female CCSs were found to have abnormal gonadal functions: 5 with central hypogonadism and 25 with primary hypogonadism. All 5 patients with central hypogonadism had suprasellar brain tumors. Among 25 hypogonadic females, 12 patients with hematological diseases, 6 with brain tumors and 7 with solid tumors, had been treated with chemotherapy including alkylating agent, cyclophosphamide. Nine of 12 female patients with hematological diseases had also been treated with TBI for HSCT. Sex hormone replacement therapy was administered to 22 (19 primary, 3 central) hypogonadic patients. These re-

Table 2. Patient profiles of the 13 CCSs treated with GH therapy

Case	Disease	Age	Sex	Age (yr) at diagnosis	CT	Surgery	RT	HSCT	Age (yr) at GH start	Height-SDS at GH start	Height-SDS at present	Age (yr) at HRT start
1	ALL	13	F	0	+	–	+	+	8.5	–2.7	–2.9	12
2	AML	18	F	1	+	–	–	+	14.6	–2.9	–2.4*	–
3	Germinoma	15	F	11	+	–	+	–	14.1	–3.8	–2.5*	not yet
4	Germinoma	16	F	9	+	+	+	+	13.9	–3.2	–1.5*	15
5	Craniopharyngioma	11	F	5	–	+	–	–	9.7	–2.2	–1.1	not yet
6	Craniopharyngioma	13	M	3	–	+	–	–	5.4	–2.4	–0.6	13
7	Craniopharyngioma	17	M	11	–	+	–	–	14.1	–2.5	–0.3	15
8	Craniopharyngioma	21	F	11	–	+	–	–	13.1	–3.7	–1.3*	16
9	Craniopharyngioma	32	F	1	–	+	–	–	6.1	–5.8	Mean*	15
10	Medulloblastoma	12	F	7	+	–	+	–	11.3	–2.5	–2.1	–
11	Medulloblastoma	15	F	4	+	+	+	+	13.8	–6.2	–5.0	not yet
12	Hepatoblastoma	13	F	0	+	+	–	+	10.8	–2.6	–2.6	not yet
13	Neuroblastoma	17	F	2	+	+	–	+	13.7	–3.2	–1.0	16

CT, chemotherapy; RT, radiotherapy; HSCT, hematopoietic stem cell transplantation; SDS, standard deviation score; HRT, hormone replacement therapy for hypogonadism

* GH therapy stopped

placement therapies were initiated relatively late at pubertal age for the purpose of height gain as mentioned above. Although one girl showed transient normalization of FSH/LH, none of the girls with ovarian failure recovered to normal function. One girl showed central precocious puberty, and two girls showed borderline age of pubertal initiation. These three girls had been treated with 15–18 Gy cranial irradiation therapy at the age of three.

Bone mineral density

BMD (g/cm^2) of the lumbar spine (L2–L4) in 98 (44 males and 54 females) of 122 patients was measured. Low BMD was observed in 41 patients (42%): thirty patients (16 males and 14 females) showed osteopenia (31%), and 11 patients (2 males and 9 females) showed osteoporosis (11%). Nine (36%) out of 25 adult patients showed decreased BMD. Twenty-one (58%) of 36 short stature patients and 7 (58%) of 12 GHD showed low bone density. Eight (35%) of 23 hypogonadic males and 17 (57%) of 30 hypogonadic females also showed low BMD. Among 11 extremely low BMD group, namely osteoporosis patients, 9 (82%) patients had short stature, 5 (45%) had GHD, and 10 (91%) had hypogonadism. Although the number of osteoporosis patients is small, growth disturbance, GHD and hypogonadism were considered to be risk factors for low BMD (Table 3). One male with chronic graft-versus-host disease (GVHD) experienced compressed fracture of the lumbar spine.

Other endocrine abnormalities

Nine (7%) patients with suprasellar brain tumor showed panhypopituitarism including adrenocortical dysfunction. All were treated with corticosteroid replacement therapy. Central diabetes insipidus was demonstrated in 11 (9%) patients: craniopharyngioma (n = 6), germinoma (n = 4) and Langerhans cell histiocytosis (n = 1). DDAVP replacement therapy was stopped in one patient with craniopharyngioma due to improved symptoms two years after the operation.

Discussion

As the survival rates for childhood cancers have improved, the importance of quality of life over a long lifetime and the late effects of CCSs have become recognized [20]. Cure and care of long-term survivors of childhood cancer has been recently reported from abroad [21] and a high rate of illness affecting chronic health has been documented [5, 6]. In the present study, we investigated late effects, especially endocrinological complications of CCSs in a single Japanese hospital. The endocrine organs are sensitive to both chemotherapy and radiation. Endocrine disturbance have been documented in 20% to 50% of CCSs [22].

Table 3. Lumbar bone mineral density of 98 CCSs

	No. of patients evaluated	Low BMD	Osteoporosis
Total	98 (M 44, F 54)	41 (M 18, F 23)	11 (M 2, F 9)
Hematological	54	23	7
Brain tumor	24	11	3
Solid tumor	20	7	1
Adulthood	25	9	1
Radiation	66	29	9
Post HSCT	60	32*	9
Growth disturbance	36	21**	9*
GHD	12	7	5*
Hypogonadism	53 (M 23, F 30)	25 (M 8, F 17)	10 (M 1, F 9)**

Significant high incidence: *$p<0.01$, ** $p = 0.01$ by chi square test

CCSs are generally defined as children who have survived more than 5 years after the cancer treatment. However, we included 2-year survivors in this study, because late effects such as growth retardation and hypogonadism should be managed as early as possible. This is a retrospective study, which may have a bias by selection at the time of reference. Although simple comparison is not appropriate in terms of the rate of complications, it is noteworthy to recognize the high incidence of endocrine complications, especially in CCSs associated with brain tumor, in the present study.

Growth retardation is one of the most common endocrinological complications emerged during cancer treatment and also after therapy in CCSs [8, 9, 23, 24]. It was also a frequent complication in the present study (32%). The cause of the growth disturbance was multifactorial: inadequate nutrition, GHD, hypothyroidism, insufficient pubertal height gains due to hypogonadism, and corticosteroid therapy for chronic GVHD. Injury to the epiphyseal plate of the spine was also suspected, especially in patients treated with spinal irradiation and/or TBI. GH therapy has been reported to be beneficial for height gain. The height SD scores were improved by GH therapy in our study, as reported previously [25]. On the other hand, there are also concerns about the risk of recurrence of tumors and second neoplasm associated with GH therapy [26, 27]. We observed the relapse of germinoma during GH therapy in one patient, not included in the present study because of less than two years of follow-up. From a study of 361 GH-treated CCSs, and 12,963 non-GH-treated CCSs, Sklar *et al.* concluded that GH therapy does not appear to increase the risk of disease recurrence or death in CCSs, although this therapy may increase the number of second tumors [28]. It is well known that higher circulating levels of IGF-1 are associated with an increased risk of malignancies especially colon cancer [29, 30]. Thus, a regular monitoring of serum IGF-1 level is important for GH-treated CCSs.

Abnormal gonadal function is also a common problem, but it could not be evaluated before puberty. In male patients, it is sometimes difficult to recognize gonadal abnormalities from their appearance, because testosterone secretion is relatively reserved. In such cases, a high level of FSH (>20 mIU/ml) and decreased testis volume (<10 ml) would be a key factor to make a diagnosis [16]. Pubertal girls showed ovarian damage with an extremely high level of FSH/LH, and they required hormonal supplementation. Only two adult patients, one male and one female, among 38 adult patients had healthy childbirth at present. We have not routinely performed sperm cryopreservation nor sperm evaluation. Fujita *et al.* reported that transplantation of spermatogonial stem cells isolated from leukemic mice restored fertility without seeding leukemia [31]. Although a few hopeful childbirths have been reported [32], preservation of fertility in women is a prolonged and important problem in CCSs [33]. Longer follow-up is needed in consideration with fertility.

As another long-term complication in CCSs, the risk of low BMD has been reported [34]. The leading method of assessing BMD is dual energy DXA, and age-, race-, and sex-specific reference curves can be

used to help identify children with bone deficits and to monitor changes in the bone response to chronic diseases or therapies [35]. The standard of BMD in normal Japanese children may remain to be improved in view of small number of subjects; however, according to the report of Japanese childhood BMD [19], a decrease in BMD was observed in 42% cases in our study. The prevalence of osteopenia and osteoporosis in 98 CCSs was 31% and 11%, respectively. In addition, vertebral fracture was observed in one patient with GVHD. Peak bone mass, achieved at the end of sexual development, is also reduced in our study. The reported etiology of bone loss in CCSs is multifactorial [36], and includes negative effects of the malignant disease itself, chemotherapeutic agents such as glucocorticoids and methotrexate, irradiation, GVHD, poor nutrition, prolonged physical inactivity, hypogonadism and growth hormone deficiency. Adequate bone mineral acquisition is especially important in children because it determines peak bone mass. Although our study has the limitation of small number of subjects, patients with a high risk of low BMD, such as GHD, gonadal dysfunction (especially female) and chronic GVHD, should be closely followed for a long time. Further studies are needed to evaluate the long-term effect on BMD in CCSs and to prevent pathological fractures later in life.

Multimodal therapy, consisting of chemotherapy, surgery and radiotherapy with or without HSCT, has recently been developed. Susceptibility to chemotherapy and radiotherapy differs depending on the age of patients and the dosage. Complicated side effects are serious problems, and it is not easy to predict each prognosis. The appearance of clinical or laboratory abnormalities take years. Especially gonadal dysfunction and growth disorders are more evident in adolescence. Annual testing before the initiation of puberty is thus strongly recommended, even though many of these patients are unaware of signs and symptoms of organ failure during childhood. Measurement of height and growth velocity and evaluation of pubertal stage should be performed at 3 to 6 months interval during growth period. Evaluation should be modified on a case-by-case basis even when both the diagnosis and therapy protocol are the same.

The present study did not include patients with less than 2 years of follow-up, patients not referred to endocrinologists or patients who had been lost to follow-up. The timing of patient reference from oncologist might have given a bias to selection of study population. Recently HSCT has been applied to an extended spectrum of diseases, including immunologic, genetic and metabolic nonhematological diseases, which should be evaluated in future study. Our study also has limitations because it is retrospective analysis of a cohort of a rather small number of patients in a single hospital; however, the therapy protocol, the condition for which oncologists refer to endocrinologists and clinical assessment are rather consistent among patients.

In summary, our study suggests that CCSs tend to develop late endocrine dysfunctions after cancer therapy. Lifetime medical surveillance and continuous follow-up are necessary for CCSs because treatment-related complications may occur during childhood and many years after the therapy as well. Endocrinologists should participate in the follow-up of these children in collaboration with pediatric oncologists.

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