RESEARCH ARTICLE

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Late Effects Following Hematopoietic Stem Cell Transplantation Among Childhood Transplant Survivors with Fanconi Anemia

Çocuklukta Nakil Yapılan Fanconi Anemili Hastalarda Hematopoietik Kök Hücre Nakli Sonrası Geç Dönem Etkiler

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Abstract

Objective: This study was planned to evaluate long-term post-transplant complications in patients who underwent transplantation with the diagnosis of Fanconi anemia (FA) in childhood in our bone marrow transplantation unit and who were still being followed. It was predicted that the results would show the critical importance of determining disease-specific post-transplant long-term follow-up plans and putting them into practice in terms of early detection of complications and improving the survival rates and quality of life of FA patients.

Materials and Methods: In this single-center, cross-sectional study, according to current recommendations, we analyzed the long-term outcomes of 36 patients with FA with a median age of 18.1 years (range: 6.1-36 years, male/female ratio: 24/12) who underwent HSCT in the Pediatric Bone Marrow Transplantation Unit between 1995 and 2019 and survived at least 1 year following the transplantation.

Results: The median long-term follow-up time was 8 years (range: 1-25 years). Gonadal dysfunction was detected in approximately

Öz

Amaç: Bu çalışma, kemik iliği transplantasyonu ünitemizde çocukluk döneminde Fanconi anemisi (FA) tanısı ile nakil yapılan ve halen izlemde olan hastalarımızda nakil sonrası uzun dönem komplikasyonlarını değerlendirmek amacı ile planlanmıştır. Elde edilecek sonuçların, hastalığa özel nakil sonrası uzun dönem izlem planlarının belirlenmesinin ve pratik uygulamaya geçilmesinin, FA hastalarında komplikasyonların erken tespiti ve yaşam oranlarının, hayat kalitelerinin iyileştirilmesi açısından kritik önemini göstereceği öngörülmektedir.

Gereç ve Yöntemler: Bu çalışma kapsamında 1995 ve 2019 yılları arasında Pediatrik Kemik İliği Nakil Ünitesi'nde hematopoietik kök hücre nakli (HKHN) yapılan, nakil sonrası en az bir yıl sağ kalan 36 FA hastasının (minimum-maksimum: 6,1-36 yaş, erkek/kadın, 24/12) uzun dönem takip sonuçları kesitsel bir çalışma kapsamında değerlendirilmiştir.

Bulgular: Uzun dönem takip süresi ortanca değeri 8 yıl (aralığı: 1-25 yıl) olarak bulunmuştur. Hastaların yaklaşık %35'inde gonadal işlev

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Abstract

35% of our patients; more specifically, 31% of the patients had hypergonadotropic hypogonadism and 4% had hypogonadotropic hypogonadism. When the patients were evaluated for growth impairment, 7 of 12 patients who had reached their final adult heights and 12 of 21 patients who had not yet completed their growth had height standard deviation (SD) scores below -2 SDs. Three patients (9%) developed subclinical hypothyroidism, 2 (6%) had overt hypothyroidism, and 1 (3%) had central hypothyroidism. Although none of our patients fully met the criteria for metabolic syndrome, 23% had insulin resistance and 39% had dyslipidemia. Evaluation of organ dysfunctions revealed that nearly 50% of the patients had obstructive and 21% had restrictive changes in their pulmonary function tests. Hepatosteatosis was detected in 15% of the patients and mild valve dysfunction was detected in 50% of evaluable patients. Three patients developed secondary malignancies. Squamous cell cancer developed in 2 patients and basal cell cancer in 1 patient.

Conclusion: A risk-defined multidisciplinary approach for the long-term follow-up of children with FA undergoing HSCT is essential for early detection and management of late effects.

Keywords: Fanconi anemia, Hematopoietic stem cell transplantation, Late effects, Children, Long-term follow-up

Öz

bozukluğu gözlemlenmiş olup; bunların %31'inde hipergonadotropik hipogonadizm ve %4'ünde ise hipogonadotropik hipogonadizm saptanmıştır. Büyüme bozukluğu yönünden değerlendirilen toplam 33 hastanın 19'unda (%7'si nihai erişkin boyutuna ulaşan 12 hasta ve %12'si nihai boyutuna ulaşmayan 21 hasta) boy standart sapma (SS) skoru -2 SS altında tespit edilmiştir. Üç hastada (%9) subklinik hipotiroidi, 2 hastada (%6) aşikar hipotiroidi ve 1 hastada (%3) santral hipotiroidi saptanmıştır. Değerlendirilen hastaların %23'ünde insülin direnci ve %39'unda dislipidemi belirlenmiştir. Organ disfonksiyonları için yapılan değerlendirmeler sonrası, pulmoner fonksiyon testleriyle hastaların yaklaşık %50'sinde obstrüktif değişiklikler ve %21'inde restriktif değişiklikler geliştiği görülmüştür. Hepatosteatozun hastaların %15'inde ve hafif kardiak kapak disfonksiyonu kardiolojik değerlendirilmesi yapılanların %50'sinde geliştiği izlenmiştir. Ayrıca, iki hastada skuamöz hücreli kanser ve bir hastada bazal hücre kanser olmak üzere toplam 3 hastada HKHN'den sonra sekonder malignite gelişmiştir.

Sonuç: FA tanısı ile HKHN yapılan hastaların uzun dönem takip sürecinde, geç etkilerin erken tespiti ve yönetimi için risk sınıflamasına dayanan multidisipliner bir yaklaşım gerekmektedir.

Anahtar Sözcükler: Fanconi anemisi, Hematopoietik kök hücre transplantasyonu, Geç dönem etkiler, Çocuklar, Uzun dönem izlem

Introduction

Over the past two decades, survival rates for children with Fanconi anemia (FA) receiving transplants have significantly improved due to fludarabine-based reduced-intensity preoperative regimens. Graft failure and graft-versus-host disease (GvHD), as major contributors to early post-transplant mortality, now occur in less than 10% of cases, with survival rates reaching up to 90% after matched sibling hematopoietic stem cell transplantation (HSCT) [1,2,3,4,5]. As more children with FA survive into adulthood following transplantation, long-term adverse effects are becoming more evident. Despite the improved survival rates, FA patients remain susceptible to long-term transplant complications due to their inherent DNA repair defects and increased susceptibility to apoptosis [6,7]. Thus, these children require ongoing comprehensive screening for potential long-term adverse effects to enhance their survival and overall health. Existing studies have mainly focused on endocrine disorders and secondary cancers following transplantation [5,8,9,10]. Comprehensive reports on survival rates and late effects for children with FA who have undergone transplantation, in which organ system-based guidelines for surveillance of late effects in children are incorporated, are still needed [9,11,12]. In this study, we report late effects and long-term health issues among childhood transplant survivors with FA with a follow-up time reaching 25 years, who had undergone transplantations at younger ages. We performed this long-term follow-up study to emphasize the enduring impact of stem cell transplantation in individuals with FA who undergo that procedure during childhood. Extended organ system-specific monitoring programs may help in assessing and identifying any potential complications that may arise over time early on. As one of the most extensive reports on late effects of HSCT in children with FA, this study offers valuable insights for shaping long-term follow-up programs according to institutional resources.

Materials and Methods

In this single-center, cross-sectional study, transplantation and post-transplant clinical data were collected retrospectively. Late-effect data were obtained prospectively from 36 eligible FA patients during the study period. The prevalence of post-transplant late effects was then evaluated in accordance with the current recommendations [7,9]. The patients had undergone HSCT at the Hacettepe University İhsan Doğramacı Children's Hospital Pediatric Bone Marrow Transplantation Unit between 1995 and 2019, survived for at least 1 year after the transplant, and had a median age of 18.1 years (range: 6.1-36 years, male/female ratio: 24/12). Out of 46 FA patients diagnosed and transplanted at our center, 10 patients were excluded from the study, including 9 patients with early transplant-related deaths and 1 patient lost to follow-up. The details of the system-specific assessments of the patients for late effects are summarized in Table 1 [13,14,15,16,17,18,19]. The study was approved by

Table 1. System-based diagnostic assessment of patients with Fanconi anemia for late effects of hematopoietic stem cell transplantation.

System and/or organ		References
Hematology and immunology		
Hematological tests	Complete blood count, peripheral smear, and ferritin; T2* magnetic resonance imaging if ferritin is high	
Immunological tests	Serum immunoglobulin levels and lymphocyte subsets (age-matched reference ranges)	
Endocrine and metabolism		
Growth impairment	Growth hormone deficiency; insulin-like growth factor-1, insulin-like growth factor binding protein-3 (age- and sex-adjusted standard deviation scores)	Ertl et al. [13]
Glycemic status	Insulin resistance/glucose intolerance; fasting glucose, fasting insulin, homeostatic assessment of insulin resistance, HbA1c, and oral glucose tolerance test	Standards of Medical Care in Diabetes [14] Mayer-Davis et al. [15] Madeira et al. [16] Singh et al. [17]
Obesity and dyslipidemia	Body mass index (age- and sex-adjusted standard deviation scores)	World Health Organization [18]
Thyroid function	Thyroid-stimulating hormone Thyroxine (free T4)	
Gonadal function	Hypogonadism, infertility; follicle-stimulating hormone, luteinizing hormone, estradiol, testosterone (age- and sex-specific reference ranges); anti-Mullerian hormone; pubertal development assessment; Tanner staging	
Bone metabolism	25-OH vitamin D, serum calcium, alkaline phosphatase, magnesium, parathyroid hormone, serum osteocalcin, bone mineral density (dual-energy X-ray absorptiometry), and age-, sex-, and height-adjusted Z-scores Bone age	Zemel et al. [19]
Cardiac	Echocardiography Electrocardiography	
Pulmonary		
Respiratory function tests	Spirometry Carbon monoxide diffusion capacity, plethysmography	
Imaging	Chest X-ray and thoracic computed tomography if indicated	
Hepatic		
Liver function tests	Screening for liver function tests, chronic cholestasis, prothrombin time and international normalized ratio, ferritin; if needed, liver T2* magnetic resonance imaging	
Hepatitis serology	HBsAg, anti-HBs, and anti-HCV	
Imaging	Abdominal ultrasound Abdominal magnetic resonance imaging	
Renal		
Kidney function tests	Blood pressure monitoring Blood urea nitrogen Uric acid Creatinine Electrolytes Imaging studies as needed	
Urine tests	Complete urine analysis Spot urine protein/creatinine Spot urine albumin/creatinine 24-hour urine albumin/creatinine 24-hour urine protein/creatinine Beta 2-microglobulin Glomerular filtration rate	
Head and neck	Ophthalmology and hearing evaluation Ear-nose-throat exam for cancer screening	
Dermatology	Skin evaluation and biopsy if indicated for chronic graft-versus-host disease and cancer screening	

HBsAg: Hepatitis B surface antigen; anti-HBs: hepatitis B surface antibody; anti-HCV: hepatitis C virus antibody.

the Institutional Review Board of Hacettepe University (study approval number: GO-19/893, 2019/20-68, date: 03.09.2019) and written informed consent was obtained from patients or their legal guardians.

Statistical Analysis

Patients' demographic data were reported with the use of mean, median, and range values for continuous variables. Survival rates were calculated using the Kaplan-Meier method. All surviving FA patients (n=36) were contacted and evaluated for late effects during the study period. Post-transplant late complications were determined from the data collected and their prevalence rates were given as percentages. Demographic data for certain numerical parameters such as pre- and post-HSCT values of body mass index (BMI) standard deviation score (SDS) and height SDS were compared using Student's t-test and the chi-square test, and statistically significant results were defined as findings with values of $p<0.05$.

Results

Patient and Transplantation Characteristics and Outcomes

The median age of the patients at the time of diagnosis was 6 years (range: 0.2-15.4 years) and the median age at the time of HSCT was 9.2 years (range: 4.2-17.6 years). The median follow-up duration was 7.8 years (range: 1-25.4 years). Bone marrow failure was the most common indication for HSCT (Table 2) [20]. The conditioning regimens varied based on the year of transplant, as total abdominal irradiation or busulfan-based regimens were used from 1995 to 2003 and fludarabine-based regimens were used from 2004 to 2019 (Table 3).

Post-transplant neutrophil and platelet engraftment occurred at 14.3±0.5 days (range: 9-19 days) and 22.7±1.3 days (range: 7-46 days), respectively. Following transplantation, 5 patients (14%) developed acute GvHD and 3 patients (8%) developed chronic GvHD. Among the patients who developed acute GvHD, 3 had stage 1 skin involvement, 1 had stage 1 gastrointestinal system involvement, and 1 had stage 2 gastrointestinal system involvement. All 3 patients who developed chronic GvHD had former stage 1 skin involvement. For patients with stage 1 GvHD with isolated skin involvement, topical steroid therapy was added to cyclosporine treatment. Patients who developed higher stages of GvHD were started on systemic steroid therapy in addition to cyclosporine. Based on the severity of GvHD and the response to treatment, some patients also received treatment with mycophenolate mofetil, tumor necrosis factor inhibitors, or photopheresis. All patients responded to the treatment protocols administered.

In the initial 100 days following HSCT, cytomegalovirus infections were observed in 7 patients (19%). First-year donor chimerism was assessed for a total of 34 patients, with 22 demonstrating over 95% chimerism and 12 ranging from 5% to 95%.

None of the patients had graft failure requiring a second transplantation. The mean survival time of 45 patients (1 patient was lost to follow-up) who underwent transplantation was 20.4±1.5 years. The 1-year survival rate of our FA patients was 82% and the 10-year survival rate was 80%. Of the 9 early post-transplant deaths, 8 occurred within the first year, primarily from infections (3 due to invasive pulmonary fungal infection and 1 due to septicemia) and acute GvHD (2 patients). One patient died from multiorgan failure and another died from severe hepatic veno-occlusive disease. One patient developed donor-derived leukemia and died 19 months after transplantation. Mortality decreased significantly from 5 out of 8 in 1995-2003 to 4 out of 37 in 2004-2019 with the introduction of a reduced-toxicity fludarabine-based regimen ($p<0.001$).

Table 2. Patients' characteristics before transplantation.

Characteristics	n (%)
Sex, male/female	24/12
Age at diagnosis, years Median (range)	6 (0.2-15.4)
Malformations*	
Limited	23 (64)
Extensive	13 (36)
Phase of the disease	
Cytopenia	16 (44)
Aplastic anemia	19 (53)
Not evaluable	1 (3)
Cytogenetic abnormalities	
No clonal abnormalities	24 (67)
Clonal abnormalities	11 (31)
Not done/no metaphases	1 (3)
Genetic subtyping by screening	
FANCA	3 (8)
FANCC	1 (3)
FANCD2	1 (3)
FANCG	1 (3)
Patients with no mutation detected	1 (3)
Patients not evaluated	29 (81)
Previous treatment	
Blood transfusions	20 (56)
Androgens	9 (25)
Other treatments (steroids, intravenous immunoglobulin)	5 (14)
No treatment	10 (28)

*Malformations were classified as extensive when at least three sites were involved, including at least one deep organ such as the kidneys, gastrointestinal tract, urogenital tract, or cardiovascular system [20].

Table 3. Transplantation characteristics.

Characteristics	n (%)
Transplantation period	
1995-2003	3 (8)
2004-2019	33 (92)
Time between diagnosis and transplantation, years	
Median (range)	2.7 (0.6-8)
Age at transplantation, years	
Median (range)	9.2 (4.2-17.6)
Age at last follow-up, years	
Median (range)	17.5 (6.1-36)
Patients ≥18 years at the last follow-up	15 (42)
Transplantation preparative regimens	
Cyclophosphamide (60 mg/kg) + busulfan (6 mg/kg)	1 (3)
Cyclophosphamide (60 mg/kg) + anti-T serotherapy	1 (3)
Cyclophosphamide (60 mg/kg) + fludarabine (175 mg/m^2) ± anti-T serotherapy	32 (89)
Cyclophosphamide (20 mg/kg) + total abdominal irradiation	2 (6)
Graft-versus-host disease prophylaxis	
Cyclosporine and low-dose methotrexate	32 (89)
Cyclosporine and steroids	2 (6)
Cyclosporine and mycophenolate mofetil	1 (3)
Cyclosporine	1 (3)
Source of stem cells	
Bone marrow	28 (78)
Cord blood + bone marrow	2 (6)
Peripheral blood stem cells	6 (17)
Type of donor	
Matched sibling	26 (72)
Other matched related donor	10 (28)
Donor-recipient sexes	
No difference	18 (50)
Male donor to female recipient	6 (17)
Female donor to male recipient	12 (33)

Post-transplantation Late Effects

Hematological Outcomes and Immune Reconstitution

At the last outpatient follow-up appointment, patients showed complete hematopoietic recovery with normal hemoglobin, white blood cell, and platelet values. The absolute lymphocyte count, CD3+ lymphocyte count, CD4+ lymphocyte count, CD8+ lymphocyte count, CD19+ lymphocyte count, and natural killer cell counts were all within the normal ranges. Immunoglobulin A, immunoglobulin M, and immunoglobulin G values were also within the normal ranges compared to age-matched controls, indicating successful immune reconstitution.

Post-transplant Late Endocrine Effects

Growth Impairment

Thirty-three patients were evaluated for growth impairment. Among the 12 patients who had reached their final adult height, 7 (58%) exhibited short stature. Among the patients who had not yet reached their final heights, 12 of 21 (57%) had a height below -2 standard deviations (SDs) based on age. The mean height SDS before HSCT was -1.8±1.1 SDs while it was -2±1.4 SDs at the latest evaluation, with no significant difference ($p>0.05$). The mean insulin-like growth factor-1 SDS was -0.3±2.6 and the mean insulin-like growth factor binding protein-3 SDS was -1.6±0.5, again falling within all relevant age- and sex-specific reference ranges.

Obesity, Glycemic Status, and Dyslipidemia

Before transplantation, 1 patient (3%) was obese, with a BMI above +2 SDs, and 6 (18%) were overweight, with BMI above +1 SD. After the transplantations, obesity and overweight were observed in 2 (6%) and 7 (21%) patients, respectively. Some patients transitioned from normal weight to overweight or obesity following HSCT, but there was no significant difference in pre- and post-transplant BMI SDSs ($p>0.05$).

Fasting blood glucose levels were normal in all patients. However, 6 of 26 patients (23%) showed signs of insulin resistance based on Homeostatic Model Assessment for Insulin Resistance scores [21] and 4 of 14 patients (29%) were prediabetic based on HbA1c levels. None had HbA1c values above the diabetes cut-off [22] or showed impaired glucose tolerance or diabetes based on an oral glucose tolerance test. Dyslipidemia was found in 39% of patients, with 10% exhibiting hypertriglyceridemia. No patients met the full criteria for metabolic syndrome, but those overweight with dyslipidemia and/or glucose intolerance were monitored for potential metabolic and cardiovascular issues.

Thyroid Dysfunction

Before transplantation, 4 of 36 patients (11%) were diagnosed with hypothyroidism. At the last follow-up, 27 of 33 patients (82%) were euthyroid, 3 (9%) had subclinical hypothyroidism, 2 (6%) had overt hypothyroidism, and 1 (3%) had central hypothyroidism. Those with overt and central hypothyroidism were already receiving thyroid hormone replacement therapy.

Gonadal Dysfunction

At the time of HSCT, 25 of 36 patients (70%) were prepubertal and 11 (31%) were pubertal or older. At the last evaluation, 6 (17%) remained prepubertal, while 30 (83%) had reached puberty. Among these 30 patients, 26 were assessed for gonadal

functions based on physical examination and laboratory findings and 17 patients (65%) had normal levels of gonadotropins and sex hormones. Eight (31%) showed hypergonadotropic hypogonadism (partial or complete) and 1 (4%) had partial hypogonadotropic hypogonadism. Three patients with hypergonadotropic hypogonadism received sex-specific gonadal steroid therapy. Spermiogram results were available for 2 patients with hypergonadotropic hypogonadism, both of whom had azoospermia. Ovarian reserve was assessed for female patients based on anti-Mullerian hormone (AMH) levels (median: 0.5 µg/L; range: 0.03-2.38 µg/L). Eight of 9 evaluated female patients (89%) had low AMH values (<1 µg/L) [23].

Bone Health

Biochemical evaluations revealed that 9 of 29 patients (31%) had vitamin D insufficiency and 10 of 29 (34%) had vitamin D deficiency. Three patients exhibited mildly elevated parathyroid hormone levels without any other biochemical or radiological signs of rickets. Bone mineral density (BMD) evaluations based on dual-energy X-ray absorptiometry showed low lumbar spine BMD (Z-score below -2) for only 2 of 26 patients (8%), while low femur neck BMD was detected for 8 of 20 patients (40%). However, when adjusted for height for age, all patients' BMD scores were above -2, suggesting that their BMD was within the expected range.

Iron Overload

The median ferritin value was 87 µg/L (range: 17-1244), with 4 patients exceeding values of 500 µg/L. Two of these patients showed moderate liver iron overload on T2* magnetic resonance imaging, but they had no heart iron accumulation and had respective ferritin levels of 668 µg/L and 1034 µg/L at the last follow-up. Both were on iron chelator treatment, one with a post-transplant phlebotomy history. The other patients with elevated ferritin did not have a known history of iron overload. Both patients had a prior transfusion history (red blood cell transfusions, <10).

Post-transplant Organ Dysfunction

Cardiovascular Disease

Before transplantation, 4 patients (11%) had congenital heart disease, including 1 with a patent ductus arteriosus requiring closure and 3 with atrial septal defects, 1 of whom also had a mitral cleft and regurgitation. Twenty patients, all of whom received fludarabine-based regimens, underwent comprehensive cardiac evaluations. In long-term follow-up, none of them developed cardiomyopathy, congestive heart failure, pericarditis, coronary artery disease, or cardiac iron accumulation and no arrhythmias were observed. Echocardiography detected mild valve dysfunctions in 12 (60%) patients, most commonly mild mitral (55%) or tricuspid regurgitation (45%). There were no long-term complications observed in patients with preexisting heart conditions.

Pulmonary Disease

Of the 19 patients who underwent spirometry, 15 (79%) showed at least one abnormality, including obstruction in 10 (53%), small airway obstruction in 3 (16%), and restrictive findings in 4 (21%). Air trapping was observed in 60% of those who underwent plethysmography. Despite these abnormalities, all patients had normal results for carbon monoxide diffusion tests and lung clearance index. Notably, no patients with impaired pulmonary function had a history of chronic GvHD. One patient had undergone surgeries for metastatic pulmonary lesions due to osteosarcoma before transplantation.

Renal Disease

All patients had normal serum creatinine levels, but 5 of 27 (19%) showed elevated β2-microglobulin, indicating renal tubulopathy. Proteinuria was detected in 5 of 28 (18%) patients. One patient with left renal agenesis had hypertension with normal renal Doppler ultrasonography but reduced dimercaptosuccinic acid uptake in the right kidney's lower pole. Two patients received prophylactic antibiotics for recurrent urinary tract infections. Another patient underwent ureteroneocystostomy due to severe vesicourethral reflux 3 years after transplantation. A different patient had a smaller right kidney with parenchymal damage and increased serum creatinine, β2-microglobulin, and urine protein/creatinine ratio. One patient presented with a smaller right kidney with a dilated collecting system and parenchymal damage in both kidneys, along with abnormal renal function tests.

Liver Disease

Of 35 evaluable patients, 22 (63%) had at least one liver function test abnormality. Hepatobiliary ultrasonography was performed for 20 patients, revealing hepatosteatosis in 3 (15%), increased liver parenchymal echogenicity in 2 (10%), and hepatomegaly in 1 (5%). Three patients with hepatosteatosis showed dyslipidemia, elevated liver function tests, and abnormal lipid profiles. Two patients with moderate liver iron overload had elevated liver function tests. Hepatitis serology was normal in 34 of 35 patients. One patient with iron overload had a previous diagnosis of chronic hepatitis B, treated with lamivudine and then tenofovir. The liver biopsy showed a Knodell hepatic activity index score of 4.

Secondary Malignancies

Three of 36 surviving patients (transplantation ages of ≥10 years) developed secondary malignancies following the transplantation, including esophageal squamous cell cancer (20 years after transplantation, at the age of 25), buccal mucosa squamous cell carcinoma (14 years after transplantation, at the age of 25), and anterior chest basal cell carcinoma (12 years after transplantation, at the age of 29). None had a history

of smoking, alcohol, or excessive exposure to ultraviolet light. The patient with esophageal cancer was previously exposed to total abdominal irradiation and had a history of cutaneous chronic GvHD. The patient with buccal mucosa carcinoma, who underwent tumor mass excision and unilateral radical neck dissection due to submandibular region tumor extension, also had metastatic lung lesions. Two patients with squamous cell carcinoma had died of cancer. The patient with basal cell carcinoma had the lesion successfully excised and is under regular follow-up. For further details on long-term dermatological issues in such cases, please refer to our recently published report [24]. After the end of the study period, another patient was diagnosed with mandibular gingiva squamous cell cancer (10 years after transplantation, at the age of 24) and died of cancer.

Discussion

Proper management of FA-associated morbidities, including growth failure, endocrinopathies, and congenital anomalies, is crucial for improving the quality of life of affected individuals [25,26,27,28]. In this study, we evaluated the late effects of HSCT and its outcomes in children with FA, most of whom had received a fludarabine-based preparative regimen with anti-thymocyte globulin and no irradiation and underwent matched-related HSCT at a younger age (<10 years), all of which are defined as criteria for better transplant outcomes and fewer post-transplant complications. We aimed to characterize some important aspects of allogeneic transplantation in FA, focusing on the occurrence of late effects that impacted the functions of main organs and systems while emphasizing the importance of comprehensive screening for potential long-term adverse effects.

In the context of late effects, primary hypothyroidism has been frequently observed in patients with FA, with reported rates of up to 60% [29]. In a series comprising 120 patients with FA, 53% of those who underwent HSCT and 48% of those who did not undergo transplantation were found to have hypothyroidism. Undergoing HSCT before the age of 10 and the use of radiation- or busulfan-based preparative regimens have been defined as major risk factors for the development of hypothyroidism [30]. In our cohort, prior to transplantation, only 12% of the patients had thyroid dysfunction. During follow-up, we identified 6 patients (18%) with hypothyroidism. Gonadal dysfunction is a frequent complication observed in patients with FA, both before and after transplantation, with a prevalence of approximately 35% in our patient cohort. Female patients have been reported to experience reproductive dysfunction, including late menarche, premature menopause, and subfertility [31]. The risk of gonadal dysfunction further increases following transplantation [5,32]. Childhood transplant survivors, including those with FA, are also at increased risk of reduced BMD, which has been reported in up to 25% of childhood survivors together with avascular necrosis [33]. Thus, routine screening of BMD for all allogeneic bone marrow transplantation survivors is recommended [34]. Several well-established risk factors contribute to abnormal BMD, including drug exposure to corticosteroids and calcineurin inhibitors, untreated endocrinopathies, and regimens involving irradiation, as well as factors such as aging and immobility [12]. In our cohort, none of the patients exhibited low BMD or osteoporosis based on age- and height-adjusted lumbar spine BMD Z-scores. It is worth noting that adjusting the Z-scores according to height is particularly important for FA patients, who have a high incidence of short stature. This adjustment most likely prevented the overdiagnosis of osteoporosis in our cohort.

Growing evidence suggests that survivors of HSCT are at a heightened risk of developing metabolic syndrome, which in turn increases their susceptibility to premature cardiovascular-related mortality [35]. In our patient cohort, although none of the individuals met the complete criteria for metabolic syndrome, 23% exhibited insulin resistance and 39% had dyslipidemia. These rates of insulin resistance and dyslipidemia align with the reported incidences observed by other research groups [36,37,38]. It is important to note that individuals with FA are already at an increased risk of metabolic syndrome and early cardiovascular effects, even in the absence of a history of HSCT. Therefore, it is crucial to implement thorough surveillance for impaired glucose tolerance and diabetes, particularly for patients with additional risk factors such as overweight/obesity and dyslipidemia.

Considering other post-transplant late effects affecting main organs and systems, pulmonary complications stood out among other complications in our patients, with approximately 50% of the evaluable patients displaying abnormal spirometry parameters indicative of obstructive lung disease. Surprisingly, 21% of the patients exhibited parameters suggestive of restrictive lung disease. Notably, none of the patients reported any specific pulmonary complaints, underscoring the importance of proactive screening for late pulmonary complications in all transplant survivors regardless of their underlying disease or transplant-related risk factors. Renal congenital abnormalities are frequently observed in patients with FA, with reported incidence rates reaching 46% [39]. Structural abnormalities of the kidneys or urinary tract were identified in 36% of our patients at the time of diagnosis. It is important to note that none of our patients were diagnosed with chronic kidney disease; however, 3 patients required close monitoring and follow-up for renal disease. Chronic kidney disease is a recognized complication in transplant recipients, with an incidence of approximately 20%, depending on various risk factors such as GvHD, acute kidney injury, and drug-related nephrotoxicity [40,41]. However, the data from our cohort and other studies suggest that long-

term renal complications after HSCT may be more common in childhood survivors with FA compared to children transplanted for other indications [5,10,39,42]. The higher prevalence of preexisting renal anomalies and the increased sensitivity of the kidneys to genotoxic stress due to the underlying DNA repair defect in FA may contribute to these findings. Liver-related late effects in patients with FA can be attributed to various factors, including medications, chronic GvHD, infections, or iron overload [43]. In our study, hepatobiliary ultrasonography revealed the presence of hepatosteatosis in 15% of the patients evaluated. It is important to note that insulin resistance plays a significant role in the development of hepatosteatosis and potentially steatohepatitis. Additionally, certain medications, such as steroids, antiepileptics, and chemotherapeutics, can contribute to the development of hepatosteatosis or steatohepatitis. Therefore, close monitoring of liver function is crucial in patients with FA [44,45]. Since liver disease is usually a treatment-related complication in FA patients, previous treatments, morbidities, and transplant-related risk factors should be evaluated carefully in the patients with abnormal liver functions.

The long-term survival of patients with FA after HSCT is primarily influenced by the development of secondary malignancies and chronic GvHD. In our cohort, three patients developed secondary malignancies, including squamous cell cancer in 2 patients and basal cell cancer in 1 patient. It is important to note that squamous cell carcinoma and skin cancer in patients with FA may exhibit more aggressive behavior and occur at relatively younger ages compared to the general population. Therefore, lifelong surveillance for solid tumors, and particularly squamous cell carcinoma and skin cancer, should be implemented, with close attention to patients with chronic GvHD or *BRCA-2* mutation.

Conclusion

Our findings emphasize the continued need for rigorous and long-term monitoring of late effects following HSCT in children with FA, despite improved decision-making for HSCT and significant improvements in transplant outcomes. Special consideration should be given to screening for secondary malignancies, and especially head/neck squamous cell carcinoma, as they are still a significant cause of late morbidity/mortality and early detection might impact long-term survival.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Review Board of Hacettepe University (study approval number: GO-19/893, 2019/20-68, date: 03.09.2019).

Informed Consent: Written informed consent was obtained.

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Footnotes

Authorship Contributions

Concept: F.V.O., D.U.Ç.; Design: F.V.O., Ç.Ö.; Data Collection or Processing: F.V.O., Ç.Ö., B.A., N.Ç., H.D., B.K., B.G., H.H.A., H.D., D.D.E., U.C., H.S.D., E.N.G., H.Y.B., G.B., Ş.Ü., Ş.S.A.E., R.T., Z.A.Ö., F.G.; Analysis or Interpretation: B.A., N.Ç.; Literature Search: B.A., N.Ç., F.V.O., Ç.Ö., B.A., N.Ç.; Writing: F.V.O., D.U.Ç., Ç.Ö.

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