RESEARCH ARTICLE
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Late Effects After Hematopoietic Stem Cell Transplantation Among Childhood Transplant Survivors with Fanconi Anemia

Ozkocer C. et al.: Post-transplant Late Effects and Fanconi Anemia

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Abstract
Background: Fanconi anemia is the most common inherited bone marrow failure syndrome. HSCT remains the only curative treatment for hematological manifestations of FA. Despite restoration of long-term hematopoiesis, patients continue to remain at risk of late effects.
Objectives: In our study, we aimed to reveal the problems that occur in the long-term follow-up of FA patients, and point out an ongoing need for the improvement of long-term follow-up guidelines for childhood transplant survivors with FA.
Study Design: In this single centered, cross-sectional study, we analyzed the long-term outcome of 36 patients with FA according to current recommendations with a median age of 18.1 years (range: 6.1-36 years, male/female, 24/12)

who underwent a HSCT at Pediatric Bone Marrow Transplantation (BMT) Unit between 1995 and 2019 and survived at least one year post-transplant.
Results: The median long-term follow-up time was 8 years (range, 1-25 years). Gonadal dysfunction was detected in about 35% of our patients. 31% of the patients had hypergonadotropic hypogonadism, 4 % had hypogonadotropic hypogonadism. When the patients were evaluated for growth impairment, 7 of 12 patients who reached their final adult height and 12 of 21 patients who didn't complete their growth, had height standard deviation score below –2 SD. Three patients (9%) developed subclinical hypothyroidism, two (6%) had overt hypothyroidism and one (3%) had central hypothyroidism. Although, none of our patients fully met the criteria for metabolic syndrome, 23% of the patients had insulin resistance and 39% had dyslipidemia. Evaluation of organ dysfunctions revealed that almost 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 one patient.
Conclusion: A risk-defined multidisciplinary approach for long-term follow up of children with FA undergoing HSCT is essential for early detection and management of late effects.
Key words: Fanconi anemia, hematopoietic stem cell transplantation, late effects, children, long-term follow-up

Öz
Amaç: Bu çalışma, KİT Ünitemizde çocukluk döneminde 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 Yöntem: Bu çalışma kapsamında 1995 ve 2019 yılları arasında Pediatrik Kemik İliği Nakil Ünitesi'nde HKHN yapılan, nakil sonrası en az bir yıl sağ kalan 36 FA hastasının (min-maks: 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 (min-maks: 1-25 yıl) olarak bulunmuştur. Hastaların yaklaşık %35'inde gonadal işlev 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 skoru -2 SD'nin 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'nden 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 kelimeler: Fanconi anemisi, hematopoietic kök hücre transplantasyonu, geç komplikasyonlar, uzun dönem izlem

1. 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 preperative regimens. Graft failure and Graft versus Host Disease (GvHD), 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) (1-5). As more children with FA survive into adulthood posttransplant, 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 defect 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 mainly focus on endocrine disorders and secondary cancers post-transplant (5, 8-10). Comprehensive reports on survival rates and late effects for children with FA who have undergone transplantation are still needed, in which organ-system-based guidelines for surveillance of late effects in children are incorporated (9, 11, 12). Herein, we report the late effects and long-term health issues among childhood transplant survivors with FA with a follow-up time reaching 25 years, who underwent transplantation at a younger age. We have performed

this long-term follow-up study in order to emphasize the enduring impact of stem cell transplantation in individuals with Fanconi anemia who underwent the 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.

2. Patients and Methods

In this single-centered, cross-sectional study, transplantation and posttransplant clinical data were collected retrospectively. Late effects data was obtained prospectively from 36 eligible FA patients during the study period. Then prevalence of posttransplant late effect was evaluated in accordance with current recommendations (7, 9). The patients underwent HSCT at the Hacettepe University Ihsan Dogramacı Children's Hospital Pediatric Bone Marrow Transplant (BMT) Unit between 1995-2019, survived at least one-year post-transplant, and had a median age of 18.1 years (range: 6.1-36 years, male/female, 24/12). Out of 46 FA patients diagnosed and tranplanted at our center, 10 patients were excluded from the study: 9 due to early transplant-related deaths, and 1 lost to follow-up. The details of system-specific assessments of the patients for late effects are summarized in Table 1 (13-19).

3. Statistical Analysis

Patients' demographic data is reported with the use of range, mean and median values for continuous variables. Survival rates were calculated for using Kaplan-Meier method. All of the alive FA patients (n=36) had been contacted and evaluated for the late effects during the study period. Post-transplant late complications were determined from the data collected and their prevelance was given as percentage. Demographic data for certain numeric parameters such as pre- and post-HSCT values of BMI SDS and height SDS were compared using student T test and a statistically significant result is defined as a p-value < 0.05.

4. Results

4.1. Patient, Transplant Characteristics and Outcome

The median age of the patients at diagnosis was 6 years (range 0.2-15.4 years) and 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 (TAI) or busulfan-based regimens used from 1995 to 2003, and fludarabine-based regimens used from 2004 to 2019 (Table 3).

Post-transplant neutrophil and platelet engraftment occurred at 14.3 ± 0.5 days (9-19 days) and 22.7 ± 1.3 days (7-46 days) respectively. Following the transplant, 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, one had stage 1 gastrointestinal system involvement, and one 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 treatments with mycophenolate mofetil, TNF inhibitors, or photopheresis. All patients responded to the treatment protocols administered.

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

None of the patients had a graft failure and required a second transplantation.The mean survival time of 45 patients (one patient was lost to follow-up) who underwent transplantation was 20.4 ± 1.5 years. The one-year survival rate of our FA patients was 82% and 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, another 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 (1995-2003) to 4 out of 37 (2004-2019) with the introduction of a reduced toxicity Flu-based regimen ($p<0.001$).

4.2. Post-Transplantation Late Effects

4.2.1. Hematological Outcome and Immune Reconstitution

At the last outpatient control, 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 (NK) cell count were all within the normal

range. Immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) values were also within the normal range, compared to age-matched controls, indicating successful immune reconstitution.

4.2.2. 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 of them (58%) exhibited short stature. Among the patients who had not yet reached their final height, 12 out of 21 (57%) had a height below -2 standard deviation scores (SDS) based on age. The mean height SDS pre-HSCT was -1.8 ± 1.1 SD, and -2 ± 1.4 SD at the latest evaluation, with no significant difference ($p > 0.05$). The mean insulin-like growth factor 1 (IGF-1) SDS was -0.3 ± 2.6, and the insulin-like growth factor binding protein 3 (IGFBP-3) SDS was -1.6 ± 0.5, all within age and gender-specific reference ranges.

Obesity, gylcemic status and dyslipidemia

Before transplantation, one patient (3%) was obese (Body mass index, BMI SDS>+2), and 6 (18%) were overweight (BMI SDS>+1). Post-transplant, obesity and overweight status were observed in 2 (6%) and 7 (21%) patients, respectively. Some patients transitioned from normal weight to overweight or obesity post-HSCT, but there was no significant difference in pre- and post-transplant BMI SDS scores ($p>0.05$).

Fasting blood glucose levels were normal in all patients. However, 6 of the 26 patients (23%) showed signs of insulin resistance based on HOMA-IR scores (21) and 4 out of 14 patients (29%) indicated prediabetes based on HbA1c levels. None had HbA1c values above the diabetes cutoff (22) or showed impaired glucose tolerance or diabetes 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 are monitored for potential metabolic and cardiovascular issues.

Thyroid dysfunction

Before transplantation, 4 out of 36 patients (11%) were diagnosed with hypothyroidism. At the last follow-up, 27 out 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 on thyroid hormone replacement therapy**.**

Gonadal dysfunction

At HSCT, 25 out 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 one (4%) had partial hypogonadotropic hypogonadism. Three patients with hypergonadotropic hypogonadism received gender-specific gonadal steroid therapy. Spermiogram results were available for 2 patients with hypergonadotropic hypogonadism, both of whom had azoospermia. Ovarian reserve was assessed in female patients with Anti-Mullerian hormone levels (median: 0.5 mcg/L range: 0.03-2.38 mcg/L). Eight out of nine evaluated females (89%) had low AMH values (<1 mcg/L) (23).

Bone health

Biochemical evaluation demonstrated 9 out of 29 patients (31%) had vitamin D insufficiency, and 10 out 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) evaluation using DXA analysis showed low lumbar spine BMD (Z-score < -2) in only 2 out of 26 patients (8%), while low femur neck BMD was detected in 8 out of 20 patients (40%). However, when adjusted for height-for-age (height-for-age Z-score: HAZ), all patients' BMD scores were above -2, suggesting their BMD was within the expected range.

4.2.3. Iron overload

The median ferritin value was 87µg/L (range 17-1244), with four patients exceeding 500 µg/L. Two of these showed moderate liver iron overload on T2* MRI, but no heart iron accumulation, and had 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 two patients with elevated ferritin did not have a known history of iron overload. Both patients had a prior transfusion history (red blood cell transfusion <10).

4.2.4. Post-transplant Organ Dysfunctions

Cardiovascular Disease

Before transplantation, 4 patients (11%) had congenital heart disease, including one with a patent ductus arteriosus requiring closure, and three with atrial septal defects (ASD), one of whom also had mitral cleft and regurgitation.

Twenty patients, all received a Flu-based regimen, underwent comprehensive cardiac evaluation. In long-term, none 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%) and tricuspid regurgitation (45%). There were no long-term complications observed in patients with pre-existing heart conditions.

Pulmonary Disease

Out of 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 in 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 out of 27 (19%) showed elevated beta-2 microglobulin, indicating renal tubulopathy. Proteinuria was detected in 5 out of 28 (18%). One patient with left renal agenesis had hypertension, with normal renal Doppler ultrasonography but reduced dimercapto succinic acid (DMSA) 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 post-transplant. A separate case showed a smaller right kidney with parenchymal damage and increased serum creatinine, beta-2 microglobulin, and urine protein/creatinine ratio. One case presented 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 on 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 out of 35 patients.One patient with iron overload had a previous diagnosis of chronic hepatitis B, treated with lamivudine then tenofovir. The liver biopsy showed a Knodell-hepatic activity index (HAI) score of 4.

4.2.5. Secondary Malignancy

Three out of 36 patients (transplantation age ≥ 10 years) developed secondary malignancies post-transplant: esophageal squamous cell cancer (20 years post-transplant, at the age of 25), buccal mucosa squamous cell carcinoma (SCC) (14 years post-transplant, at the age of 25), and anterior chest basal cell carcinoma (12 years post-transplant, at the age of 29). None had a history of smoking, alcohol, or excessive Ultraviolet (UV) exposure.The patient with esophageal cancer, previously exposed to total abdominal irradiation and with 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 has metastatic lung lesions. Two patients with squamous cell carcinoma 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 dermatologic issues, 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 post-transplant, at the age of 24) and died of cancer.

5. 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 (25-28). In this study, we evaluated the late effects of HSCT and the outcomes in children with FA, who mostly received a fludarabine-based preparative regimen with ATG and no irradiation, underwent matched-related HSCT at a younger age (<10 years), all of which are defined criteria for better transplant outcome and fewer post-transplant complications. We sought to characterize some important aspects of allogeneic transplantation in FA focusing on the occurrence of late effects that affected the main organ functions and systems by 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 involving 120 patients with FA, 53% of those who underwent HSCT and 48% of those who did not undergo transplant were found to have hypothyroidism. Undergoing HSCT before the age of 10 and the use of radiation- or busulfan-based preparative regimens are 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 the follow-up, we identified 6 patients (18%) with hypothyroidism. Gonadal dysfunction is a frequent complication observed in patients with Fanconi anemia (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 bone mineral density which has been reported in up to one quarter of childhood survivors and 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 bone mineral density, including drug exposure to corticosteroids and calcineurin inhibitors, untreated endocrinopathies, regimens involving irradiation, as well as factors such as aging and immobility (12). In our cohort, none of the patients exhibited low bone mineral density 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 in FA patients, who have a high incidence of short stature. Probably, this adjustment 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 (36-38). 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 HSCT. Therefore, it is crucial to implement thorough surveillance for impaired glucose tolerance and diabetes, particularly in patients with additional risk factors such as overweight/obesity and dyslipidemia.

Considering other posttransplant 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 of 46% (39). Structural abnormalities of the kidney 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, three 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, 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 pre-existing renal anomalies and the increased sensitivity of 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.

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 two patients and basal cell cancer in one 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 a relatively younger age compared to the general population. Therefore, lifelong surveillance for solid tumors, particularly squamous cell carcinoma and skin cancer, should be implemented, with close attention to patients with chronic GvHD and BRCA-2 mutation.

In 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 (especially head/neck SCC) since they are still a significant cause of late morbidity/mortality and early detection might impact long-term survival.

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AUTHOR CONTRIBUTIONS
The study was conceived by F.V.O. and D.U.C., who also served as co-corresponding authors and contributed equally. F.V.O. and C.O. were responsible for the study design, patient evaluation at the BMT Clinic, and data collection from patients' files. They also contributed to the writing of the manuscript. BA and NC provided assistance in data collection from patients' files and their analyses. HD, BBK, BG, HHA, HD, DDE, NA, OT, MDB, MUT, UC, HSD, ENG, HYB, GB, SSAE, RT, MUA, ZAO, SUC, and FG were involved in the long-term follow-up of FA patients. All authors reviewed and approved the final draft of the manuscript and made contributions to the text

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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.
AVAILABILITY OF DATA AND MATERIALS
All data is available through the corresponding author upon request.
ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Written informed consent for research purposes were obtained and the study is approved by the Institutional Review Board of Hacettepe University (Study approval number: GO-19/893, 2019/20-68)
CONSENT FOR PUBLICATION
Not applicable.

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Table 1. System-based diagnostic assessment of FA patients for late effects of HSCT

System and/or Organ		References
Hematology and Immunology		
Hematological tests	Complete blood count, peripheral smear and ferritin, T2* magnetic resonance imaging (MRI) 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 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3) (age and sex adjusted standard deviation (SD) scores)*	*Ertl DA et al. (13)
Glycemic status	Insulin resistance* / glucose intolerance; fasting glucose**, fasting insulin**, homeostatic assessment of insulin resistance (HOMA-IR), glycated hemoglobin (HbA1c)** and oral glucose tolerance test	**Standards of Medical Care in Diabetes (14) **Mayer-Davis EJ et al. (15) *Madeira IR et al.(16) *Singh Y et al. (17)
Obesity and dyslipidemia	Body mass index (age and sex adjusted SD scores)* Fasting lipid profile	* WHO. Obesity and overweight (18)
Thyroid Function	Thyroid-stimulating hormone (TSH) Thyroxine (free T4)	
Gonadal Function	Hypogonadism, infertility; Follicle stimulating hormone (FSH), Luteinizing hormone (LH), estradiol, testosterone (age and sex specific reference ranges) Anti-Mullerian hormone (AMH) Pubertal development assessment; Tanner staging	
Bone metabolism	25-OH vitamin D, serum calcium (Ca), alkaline phosphatase (ALP), magnesium (Mg), parathyroid hormone (PTH), serum osteocalcin, bone mineral density (dual-energy x-ray absorptiometry DXA), age, sex and height adjusted Z-scores* Bone age	*Zemel BS et al. (19)
Cardiac	Echocardiography (ECHO) Electrocardiography (ECG)	
Pulmonary		
Respiratory function test	Spirometry Carbon monoxide diffusion capacity Plethysmography	
Imaging	Chest X-ray and thoracic computed tomography (CT) if indicated	
Hepatic		
Liver Function tests	Screening for liver function tests (LFT), chronic cholestasis, prothrombin time and international normalised ratio (INR), ferritin, if needed, liver T2* MRI	

Hepatitis serology	HBsAg, Anti-HBs and Anti-HCV	
Imaging	Abdominal ultrasound Abdominal MRI	
Renal		
Kidney function tests	Blood pressure monitoring Blood urea nitrogen (BUN) 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 β2 microglobulin Glomerular filtration rate	
Head and Neck	Ophthalmology and hearing evaluation Ear Nose & Throat (ENT) exam for cancer screening	
Dermatology	Skin evaluation and biopsy if indicated for chronic GvHD and cancer screening	

Table 2. Patients' characteristics before transplantation

Characteristics	N(%)
Gender, M/F	24/12
Age at diagnosis (yr),	
Median	6
(range)	(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 are classified as extensive when at least three sites are 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	
Median (range), yr	2.7 (0.6-8)
Age at transplantation	
Median	9.2
(range), yr	(4.2-17.6)
Age at the last follow-up, median (range), yr	
Median	17.5
(range), yr	(6.1-36)
Patients ≥18 yr 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 (60mg/kg) + Fludarabine (175 mg/m2) ± Anti-T serotherapy	32(89)
Cyclophosphamide (20 mg/kg) + total abdominal irradiation	2 (6)
GvHD 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 gender	
No difference	18 (50)
Male donor to female recipient	6 (17)
Female donor to male recipient	12 (33)