

Primary Hemophagocytic Lymphohistiocytosis: A Severe Immune Dysregulatory Disease with Various Genotypic Features and Outcomes: A Cross-Sectional Study from a Tertiary Pediatric Center

Primer Hemofagositik Lenfohistiyositoz: Çeşitli Genotip Özellikleri ve İzlem Sonuçları Olan Ciddi Bir İmmün Disregülasyon Bozukluğu: Üçüncü Basamak Bir Pediatri Merkezinden Kesitsel Bir Çalışma

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Abstract

Objective: Inborn errors of immunity (IEIs) are caused by deficiencies or functional abnormalities in the immune system, leading to increased susceptibility to infections, autoimmunity, autoinflammatory diseases, allergies, and/or malignancies. Primary hemophagocytic lymphohistiocytosis (HLH) arises from genetic mutations affecting the function of cytotoxic T lymphocytes and natural killer cells, while secondary HLH is triggered by infections, malignancies, rheumatologic disorders, or immune deficiencies. Treatment consists of remission induction, control of triggers, maintenance of remission, rescue treatment, and hematopoietic stem cell transplantation (HSCT) as curative steps. The aim of this study is to evaluate the clinical and laboratory features, as well as the outcomes, of primary HLH patients who were diagnosed and treated in a multidisciplinary manner over the past 25 years.

Materials and Methods: The study included 30 patients with primary HLH/IEI who were diagnosed and treated in the departments of pediatric hematology, immunology, and oncology of the Ankara University Faculty of Medicine Children's Hospital and Bone Marrow Transplantation Unit from 2000 to 2025.

Results: Of the 30 patients, 18 were boys and 12 were girls. The median age at the onset of the first symptom was 10 months (range: 0.5-204 months), while the median age at the time of admission to our center was 12.5 months (range: 1-204 months). Pedigree analysis showed that 21 patients were born to consanguineous parents. All

Öz

Amaç: İmmün sistemin doğuştan hataları (İSDH), bağışıklık sistemindeki eksiklikler veya fonksiyonel anormalliklerden kaynaklanır ve enfeksiyonlara, otoimmüniteye, otoenflamatuvar hastalıklara, alerjilere ve/veya malignitelere karşı artan duyarlılığa yol açar. Primer hemofagositik lenfohistiositoz (HLH) sitotoksik T lenfositlerin ve doğal öldürücü hücrelerin işlevini etkileyen genetik mutasyonlardan kaynaklanırken, sekonder HLH enfeksiyonlar, maligniteler, romatolojik bozukluklar veya immün yetersizlikler tarafından tetiklenir. Tedavi; remisyon indüksiyonu, tetikleyicilerin kontrolü, remisyonun sürdürülmesi, kurtarma tedavisi ve küratif olarak HKHT'den oluşur. Bu çalışmanın amacı, son 25 yılda multidisipliner olarak tanı konulan ve tedavi edilen primer HLH hastalarının klinik ve laboratuvar özellikleri ile izlem sonuçlarını paylaşmaktır.

Gereç ve Yöntemler: Ankara Üniversitesi Tıp Fakültesi Çocuk Hastanesi Çocuk Hematoloji, İmmünoloji ve Onkoloji Bölümleri ile Kemik İliği Transplantasyon Ünitesi'nde 2000-2025 yılları arasında tanı konulan ve tedavi edilen primer HLH/İSDH tanılı 30 HLH olgusu çalışmaya dahil edildi.

Bulgular: Otuz hastanın 18'i erkek, 12'si kızdı. İlk semptomun başlangıcındaki ortalama yaş 10 ay (dağılım: 0,5-204 ay), merkezimize başvuru sırasındaki ortalama yaş ise 12,5 ay (dağılım: 1-204 ay) idi. Yirmi bir hastanın ebeveynleri akrabaydı. Tüm hastalarda beş günden uzun süren ateş vardı ve ortalama ateş süresi 13,30±14,05 gündü (dağılım: 5-60 gün). Splenomegali 29 hastada (%96,6) ve hepatomegali 25 hastada



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Abstract

patients had a fever lasting longer than 5 days, with a mean duration of 13.30 ± 14.05 days (range: 5-60 days). Splenomegaly was detected in 29 patients (96.6%) and hepatomegaly in 25 (83%). Anemia was observed in 27 patients (90%), neutropenia in 23 (76.6%), and thrombocytopenia in 30 (100%). Genetic evaluation was performed for all patients and a causative gene was identified in 19 of 30 cases (63%). The most common genetic diagnosis was perforin deficiency (FHLH2), detected in 8 patients (26.6%), followed by UNC13D defect (FHLH3) in 4 patients (13.3%). HSCT was performed for 17 patients (56.6%), with 6 receiving transplants from matched related donors, 4 from matched sibling donors, 5 from matched unrelated donors, and 2 from mismatched related donors. Thirteen patients remain alive, with mean survival of 119.89 months. Seventeen patients (56.6%) died, primarily due to multiorgan dysfunction syndrome, acute respiratory distress syndrome, HLH reactivation, septic shock, or heart failure. HSCT patients had a significantly longer survival (mean: 165.6 months) compared to patients who did not undergo HSCT (45.36 months; $p < 0.01$). Admission to the pediatric intensive care unit, organ failure, and neurological involvement were identified as adverse prognostic factors, all significantly associated with higher mortality ($p < 0.05$).

Conclusion: Given the increasing recognition of HLH as a possible manifestation of IEIs, comprehensive immunological and genetic evaluations should be pursued without delay in suspected cases. Our findings, in line with the results of national and international cohorts, confirm that HSCT remains the only curative option for familial HLH and should be performed as early as possible after achieving disease remission. Improving access to early diagnostics and HSCT could significantly enhance outcomes, particularly in genetically predisposed populations.

Keywords: Primary immunodeficiency, Hemophagocytic lymphohistiocytosis, Hematopoietic stem cell transplantation

Öz

(%83) saptandı. Anemi 27 hastada (%90), nötropeni 23 hastada (%76,6) ve trombositopeni 30 hastada (%100) gözlemlendi. Tüm hastalarda genetik değerlendirme yapıldı ve 30 olgunun 19'unda (%63) genetik mutasyon tanımlandı. En yaygın genetik tanı 8 hastada (%26,6) saptanan perforin eksikliği (FHLH2) olup, bunu 4 hastada (%13,3) UNC13D gen defekti (FHLH3) izlemiştir. HKHN 17 hastaya (%56,6) uygulanmış, 6 hastaya tam uygun akraba donördeb, 4 hastaya tam uygun kardeş donörden, 5 hastaya tam uygun akraba dışı donörden ve 2 hastaya da haploidentik akraba donörden nakil yapılmıştır. On üç hasta halen hayatta olup ortalama sağkalım süresi 119,89 aydır. On yedi hasta (%56,6) ölmüştür; ölüm nedenleri arasında çoklu organ disfonksiyon sendromu, akut solunum yetmezliği sendromu, HLH reaktivasyonu, septik şok ve kalp yetmezliği yer almaktadır. HKHT hastaları, HKHT olmayan hastalara kıyasla (45,36 ay, $p < 0,01$) önemli ölçüde daha uzun bir sağkalım süresine (ortalama 165,6 ay) sahipti. Çocuk yoğun bakım ünitesine yatış, organ yetmezliği ve nörolojik tutulum kötü prognostik faktörler olarak tanımlanmış ve bunların tümü daha yüksek mortalite ile anlamlı şekilde ilişkilendirilmiştir ($p < 0,05$).

Sonuç: HLH'nin İSDH'lerin olası bir belirtisi olarak giderek daha fazla tanındığı göz önüne alındığında, şüpheli olgularda gecikmeden kapsamlı immünolojik ve genetik değerlendirmeler yapılmalıdır. Bulgularımız, ulusal ve uluslararası kohortlarla uyumlu olarak, HKHT'nin ailesel HLH için tek küratif seçenek olmaya devam ettiğini ve hastalık remisyonuna ulaşıldıktan sonra mümkün olduğunca erken yapılması gerektiğini doğrulamaktadır. Erken tanı ve HKHT'ye erişimin iyileştirilmesi, özellikle genetik olarak yatkın popülasyonlarda sonuçları önemli ölçüde artırabilir.

Anahtar Sözcükler: Primer immün yetmezlik, Hemofagositik lenfohistiositoz, Hematopoetik kök hücre nakli

Introduction

Inborn errors of immunity (IEIs) are caused by deficiencies or functional abnormalities in the immune system, leading to increased susceptibility to infections, autoimmunity, autoinflammatory diseases, allergies, and/or malignancies. The 2024 IEI classification categorizes these disorders into 10 subgroups, with immunodysregulation disorders defined by abnormalities in the immune system's regulatory mechanisms. These disorders encompass a spectrum of conditions associated with autoimmunity, hyperinflammation, and immune dysfunction. Unlike other immune-related diseases, these disorders are not primarily defined by an increased susceptibility to infections [1].

Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome in which various underlying conditions trigger a similar inflammatory response profile that is ultimately fatal if not treated. Due to granule-mediated cytotoxicity defects in CD8⁺ T-cells and natural killer (NK) cells, infected cells are not effectively eliminated and antigen presentation is not suppressed. Consequently, uncontrolled proliferation of T-cells

and macrophages occurs, leading to excessive cytokine secretion. This process results in macrophage and histiocyte infiltration into tissues, ultimately causing tissue damage and organ failure. Primary HLH arises from genetic mutations affecting the function of cytotoxic T lymphocytes and NK cells, while secondary HLH is triggered by infections, malignancies, rheumatologic disorders, or immune deficiencies [2].

Fever, maculopapular and/or petechial rash, hepatosplenomegaly, weight loss, and irritability are the most commonly encountered symptoms. Primary HLH typically presents in infancy due to genetic defects in NK cell cytotoxicity, often also affecting a family member, with recurrent episodes observed after treatment. Secondary HLH generally occurs in adolescence or adulthood, usually triggered by underlying conditions such as infections, malignancies, medications, or hematopoietic stem cell transplantation (HSCT). Secondary HLH can be seen with metabolic disorders, storage diseases, rheumatologic diseases such as juvenile idiopathic arthritis, systemic lupus erythematosus, and immune-activating therapies such as CAR-T cells or immune checkpoint inhibitors [3]. A diagnosis is established if 5 out of 8 clinical and laboratory

criteria are met, as outlined in Table 1, as recognized by the Histiocyte Society [4,5].

Primary HLH can be divided into two subgroups: familial HLH (FHLH) and HLH associated with IEI. FHLH occurs in 1 in 50,000-100,000 individuals and is inherited in an autosomal recessive manner, with 70% of patients diagnosed under the age of 1. It includes five subtypes, with mutations in four of them affecting cytotoxic granule function and exocytosis. Genetic defects causing FHLH include perforin deficiency (FHLH2), UNC13D deficiency (FHLH3), munc13-4 deficiency, syntaxin 11 deficiency (FHLH4), STXBP2 (FHLH5) deficiency, FAAP 24 deficiency, and SLC7A7 deficiency [6].

Common features of IEI that lead to primary HLH include hypopigmentation, seen in conditions such as Chediak-Higashi syndrome, Griscelli syndrome type 2, and Hermansky-Pudlak syndrome types 2 and 10. Other IEIs associated with HLH include severe combined immunodeficiency, chronic granulomatous disease, STAT1 and STAT3 gain-of-function mutations, DOCK8 deficiency, autoimmune lymphoproliferative syndrome, and conditions like Wiskott-Aldrich syndrome and ataxia-telangiectasia syndrome [7]. In addition to these defects, there are other primary immunodeficiencies that can cause HLH, including STAT2 deficiency, TIM3 deficiency, PIK3CG deficiency, and NCKAP1L deficiency [1].

Treatment consists of remission induction, control of triggers, maintenance of remission, rescue treatment, and HSCT as curative steps. The HLH-1994 and HLH-2004 protocols, including etoposide, corticosteroids, cyclosporine, and intrathecal methotrexate combinations, are used for the first step of treatment. In some cases, intravenous immunoglobulin (IVIG) and steroid treatment may be used. Some sources recommend antithymocyte globulin (ATG) and cyclosporine A (CSA) for

maintenance treatment. HSCT should be performed as soon as possible after achieving the first remission to prevent recurrences. For successful HSCT, active disease might be a risk factor, but it is not necessary to have the remission of all clinical symptoms. Ideally, HSCT should be performed prior to HLH activation. In particular, active neurological disease requires aggressive treatment including immediate HSCT [7,8,9].

The aim of this study is to evaluate the clinical and laboratory features, as well as the outcomes, of primary HLH patients who were diagnosed and treated in a multidisciplinary manner over the past 25 years.

Materials and Methods

Patients

This study included 30 patients with primary HLH/IEI who were diagnosed and treated in the departments of pediatric hematology, immunology, and oncology of the Ankara University Faculty of Medicine Children's Hospital and Bone Marrow Transplantation Unit from 2000 to 2025.

Hemophagocytic Lymphohistiocytosis Diagnosis

The diagnosis of HLH was established based on the HLH-1994 and HLH-2004 diagnostic criteria [5]. The diagnosis of primary HLH required the presence of family members with similar clinical features, a history of consanguineous marriage, and/or the identification of a genetic mutation associated with familial or primary HLH through genetic diagnostic methods. Patient data were retrospectively collected from medical records and the hospital information system, including demographic characteristics, clinical symptoms and findings, laboratory results, genetic diagnosis, treatment details, response to treatment, HSCT characteristics, pediatric intensive care unit (PICU) admission data, prognosis, and follow-up duration.

Table 1. Diagnostic criteria of hemophagocytic lymphohistiocytosis [5].

The diagnosis of HLH can be established if one of either (1) or (2) is positive:
(1) A molecular diagnosis consistent with HLH
(2) Diagnostic criteria for HLH fulfilled (5 out of the following 8 criteria):
(A) Initial diagnostic criteria (to be evaluated in all patients with HLH)
Fever
Splenomegaly
Cytopenia (affecting 2 of 3 lineages in the peripheral blood): hemoglobin of <90 g/L (in infants of <4 weeks of age: hemoglobin <100 g/L)
Platelets <100x10 ⁹ /L
Neutrophils <1.0x10 ⁹ /L
Hypertriglyceridemia and/or hypofibrinogenemia: Fasting triglycerides 3.0 mmol/L (i.e., 265 mg/dL), fibrinogen 1.5 g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
No evidence of malignancy
(B) New diagnostic criteria
Low or absent NK-cell activity (according to local laboratory reference)
Ferritin ≥500 µg/L
Soluble CD25 (i.e., soluble IL-2 receptor) 2400 U/mL
HLH: Hemophagocytic lymphohistiocytosis; NK: natural killer; IL: interleukin.

Central nervous system involvement in patients was assessed based on the presence of at least one of the following clinical or laboratory findings: abnormal neurological examination, central nervous system abnormalities on computed tomography or magnetic resonance imaging associated with HLH, cerebrospinal fluid (CSF) findings suggestive of HLH (protein level of >30 mg/dL or an elevated white blood cell count of $>50 \times 10^6/L$, with >5 white blood cells/ μL), or evidence of hemophagocytosis in the CSF [10].

Immunological evaluations were conducted in the pediatric immunology laboratory, including assessments of immunoglobulin (Ig) levels (IgG, IgA, IgM, and total IgE), IgG antibody responses to the hepatitis B and rubella vaccines, isohemagglutinin titers, peripheral blood lymphocyte subsets, recent thymic emigrants (RTEs), lymphocyte activation responses, and burst tests. Ig levels were evaluated based on reference values for healthy Turkish children [11]. Peripheral blood lymphocyte subsets were assessed using the age-specific reference values of our laboratory [12]. For RTE values, age-related reference intervals established for healthy Turkish children were also applied [13].

Treatment responses were classified as complete response, partial response (PR), or unresponsiveness to treatment. Complete remission was defined as the normalization of all HLH-2004 clinical and laboratory diagnostic criteria 8 weeks after any treatment. Partial remission was defined as an improvement in at least two diagnostic criteria without the emergence of additional findings. Treatment unresponsiveness was characterized by an improvement in fewer than two diagnostic criteria and the development of new findings. Reactivation was defined as disease progression in patients who had previously achieved a complete or PR to treatment.

Hematopoietic Stem Cell Transplantation

Neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count greater than $500/mm^3$.

Platelet engraftment was defined as a platelet count of $\geq 20 \times 10^9/L$, sustained for 7 days without the need for platelet transfusion. Full chimerism was defined as the presence of $>95\%$ donor-derived cells in the recipient's hematopoietic tissues at any time after HSCT, while mixed chimerism was defined as the presence of donor-derived cells between 5% and 95% [14].

According to the human leukocyte antigen identification definitions of the International BFM Working Group, donors were classified into four groups: matched sibling donors (MSDs), matched related donors (MRDs), matched unrelated donor (MURDs), and mismatched related donors (MMRDs) [15].

The doses and application days of the conditioning regimens were organized according to the guidelines of the European Society for Blood and Marrow Transplantation. The regimens used were as follows: busulfan/cyclophosphamide/etoposide, treosulfan/fludarabine, and busulfan/fludarabine. CSA, methotrexate, and mycophenolate mofetil were used for graft-versus-host disease (GvHD) prophylaxis. ATG was used as a serotherapy agent for 8 HSCTs.

Ethical Approval

The study was approved by the ethics board of the Ankara University Faculty of Medicine on July 21, 2023, with decision number 2023/388.

Statistical Analysis

Descriptive statistics for continuous data were presented using mean, standard deviation, median, minimum, and maximum values. For discrete data, numbers and percentages were used. The Shapiro-Wilk test was applied to assess the normality of continuous data. Chi-square or Fisher exact tests were used for group comparisons of nominal variables (cross-tabulations). IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA) was used for data analysis with the significance threshold set at $p < 0.05$. For survival analysis (event-free survival and overall survival), Kaplan-Meier survival analysis was employed, and the log-rank test was used to compare survival differences between independent groups.

Results

Demographic Features

Of the 30 patients, 18 were boys and 12 were girls. The median age at the onset of the first symptom was 10 months (range: 0.5-204 months, 27.69 ± 46.47 months), while the median age at the time of admission to our center was 12.5 months (range: 1-204 months, 33.28 ± 48.88 months). Twelve patients (40%) were admitted before the age of 1 and 18 patients (60%) before the age of 2. Pedigree analysis showed that 22 patients were born to consanguineous parents. Family history revealed that 12 patients had relatives who had experienced recurrent fever and abdominal distension or family members who had died of sepsis with clinical features highly resembling HLH. The median follow-up duration was 37 months (range: 0-247 months, 61.93 ± 72.00 months). Of the 11 patients without a genetic diagnosis, 5 had both a family history and consanguinity, while 3 had only consanguinity. The other 3 patients had neither consanguinity nor a family history; however, as no etiology explaining secondary HLH was identified in these patients, they were also considered to have primary HLH.

Clinical Features

All patients had a fever lasting longer than 5 days, with a mean duration of 13.30 ± 14.05 days (median [min-max]: 10

[5-60] days, interquartile range: 5-13 days). Splenomegaly was detected in 29 patients (96.6%) and hepatomegaly in 25 (83%). On physical examination, the mean and median palpable spleen sizes were 5.97 ± 2.29 cm and 6.5 cm (range: 2.0-10.0 cm), respectively, while the mean and median liver sizes were 5.57 ± 2.14 cm and 5 cm (range: 3.0-9.5 cm). Lymphadenopathy was observed in 6 patients (20%), rash was observed in 5 (16.6%), and neurological involvement was observed in 5 (16.6%). In 1 patient, lumbar puncture revealed a significantly elevated CSF protein level, and cytological analysis of the CSF sample showed atypical lymphohistiocytic infiltration. The other 3 patients with neurological involvement had normal CSF biochemistry and cytology; however, magnetic resonance imaging revealed lesions associated with HLH involvement.

Laboratory Findings

Anemia was observed in 27 patients (90%), neutropenia in 23 (76.6%), and thrombocytopenia in 30 (100%). Bicytopenia was present in 10 patients (33.3%), while pancytopenia was detected in 21 (70%). Ferritin was elevated in all patients and triglyceride levels were elevated in 28 (93%). Hypofibrinogenemia was identified in 20 patients (66.6%). Alanine transaminase elevation was observed in 15 (50%) and aspartate transaminase was elevated in 27 (90%) patients. Seven patients (23%) had increased total bilirubin and 6 (20%) had increased indirect bilirubin. Elevated lactate dehydrogenase levels were observed concurrently in 26 (86.6%) patients. Other laboratory findings included elevated increased C-reactive protein levels in 19 patients (63%). Coagulation profile analysis revealed prolonged prothrombin time in 9 patients, elevated international normalized ratio in 11, prolonged activated partial thromboplastin time in 10, and elevated D-dimer levels in 15. Additionally, biochemical abnormalities included hyponatremia in 14 patients and hypoalbuminemia in 16. Hemophagocytosis was detected in the bone marrow of 25 patients. One patient with XIAP deficiency had an Epstein-Barr virus (EBV) viral load of 6556 copies/mL at admission, while another patient with CD3 zeta chain (CD247) deficiency presented with a cytomegalovirus (CMV) viral load of 3000 copies/mL. In a 17-year-old female patient for whom whole-exome sequencing did not reveal any disease-causing mutation, the EBV viral load was measured at 9,263,000 copies/mL.

Immunological evaluation, including serum Ig levels and peripheral blood lymphocyte subset analysis, was performed for 20 patients (66%). Among the assessed patients, low IgG levels were detected in 5 patients, low IgA in 4, and low IgM in 2. Lymphocyte subgroup analysis revealed low CD3⁺ T cells in 2 patients, low CD4⁺ T cells in 6, low CD8⁺ T cells in 2, low CD16⁺56⁺ NK cells in 3, low CD19/20⁺ B cells in 9, and low RTEs in 2. Lymphocyte activation responses to phytohemagglutinin (PHA) were evaluated for 7 patients, all of whom had results within the normal range.

NK cell activity and soluble CD25 (soluble IL2 receptor) levels could not be measured for any patients.

Genetics

Genetic evaluation was performed for all patients and a causative gene was identified in 19 of 30 cases (63%). The most common genetic diagnosis was perforin deficiency (FHLH2), detected in 8 patients (26.6%), followed by UNC13D gene defect (FHLH3) in 4 patients (13.3%). Other identified mutations included STXBP2 (FHLH5) in 1 patient (3.3%), LYST gene defect associated with Chediak-Higashi syndrome in 1 patient (3.3%), CD3 zeta chain defect in 1 patient (3.3%), and XIAP deficiency leading to X-linked lymphoproliferative syndrome type 2 (XLP-2) in 1 patient (3.3%). Additionally, one patient (3.3%) developed CMV viremia at 1 month of age and was admitted to the PICU with symptoms of HLH. This patient was subsequently diagnosed with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. Another patient met the HLH criteria and was diagnosed with a STAT1 gain-of-function mutation while being followed for mucocutaneous candidiasis. In 1 patient, a homozygous LPIN2 mutation was detected, leading to a diagnosis of Majeed syndrome.

Eleven patients had no identifiable genetic mutations linked to primary HLH. However, due to their early-onset presentation and/or family history, and/or consanguinity, and/or in the absence of secondary HLH triggers, they were classified as primary HLH cases.

Treatment

Twenty-one patients received the HLH-2004 protocol, 3 received the HLH-1994 protocol, and 6 received high-dose pulse steroids combined with IVIG. The general condition of the patients receiving IVIG/steroid therapy instead of standard protocols was not sufficient to handle the toxicity of the agents included in the protocol and multiple organ dysfunctions were present. Outside of the HLH-1994 and HLH-2004 protocols, therapeutic plasma exchange was performed as bridging therapy prior to HSCT for 2 of the 6 patients receiving IVIG/steroid therapy. One patient received tocilizumab therapy.

Among the 3 patients treated with the HLH-1994 protocol, one with a STAT1 gain-of-function mutation received plasma exchange, while another with XIAP deficiency and EBV positivity received rituximab. Of all patients, 15 achieved complete remission and 3 had partial remission. HLH reactivation occurred in 8 patients, 4 of whom were non-responders. Examining the treatment responses of 11 patients without a genetic diagnosis at the end of the second month, 4 were in complete remission, 1 was in partial remission, and 6 were experiencing HLH reactivation.

HSCT was performed for 17 patients (56.6%), with 6 (35%) receiving transplants from MRDs, 4 (23.5%) from MSDs, 5 (29.4%) from MURDs, and 2 (11.7%) from MMRDs. Stem cell sources included bone marrow for 11 (64.7%) patients and peripheral blood for 6 (35.3%) patients. HSCT was performed for 13 of the 19 patients with an identifiable genetic diagnosis.

Conditioning regimens varied. Nine patients (52.9%) received busulfan/cyclophosphamide/etoposide, 5 (29.4%) received treosulfan/fludarabine, and 3 (17.6%) received busulfan/fludarabine. CSA was used for GvHD prophylaxis in 3 (17.6%) patients, and 12 (70.5%) received CSA combined with methotrexate and mycophenolate mofetil. ATG was the most common serotherapy (8 patients, 47%). Neutrophil engraftment was achieved in 12 patients at a median time of 15.5 days (range: 11-23 days), while platelet engraftment occurred at a median of 26 days (range: 15-57 days) in the same group. One patient with perforin deficiency experienced graft failure on the 28th day after transplantation. Two patients died before engraftment could be achieved. Of the transplanted patients, 11 (64.7%) were followed with full donor chimerism and 3 (17.6%) with mixed chimerism.

Among the HSCT-related complications, mucositis occurred in 7 (41.1%), hypertension in 3 (17.6%), and acute kidney injury in 4 (23.5%) patients. Additionally, 3 (17.6%) patients developed capillary leak syndrome, 6 (35.2%) patients experienced infections with various pathogens (including CMV and *Pneumocystis jirovecii*), 3 (17.6%) patients developed sinusoidal obstruction syndrome, and 5 (29.4%) patients experienced acute GvHD.

Among the patients who developed acute GvHD, the most frequently affected sites were the skin and gastrointestinal tract. All patients initially received methylprednisolone as first-line therapy. Of the two patients who were unresponsive to methylprednisolone, 1 patient received budesonide, mesenchymal stem cell infusion, and anti-interleukin-6 (tocilizumab) for gastrointestinal GvHD and subsequently developed liver involvement. The other patient also required mesenchymal stem cell therapy due to steroid-refractory GvHD.

Thirteen patients remain alive, with mean survival of 119.89 months. Seventeen patients (56.6%) died, primarily due to multiorgan dysfunction syndrome, acute respiratory distress syndrome, HLH reactivation, septic shock, or heart failure. HSCT patients had significantly longer survival (mean: 165.6 months) compared to patients who did not undergo HSCT (45.36 months; $p < 0.01$). PICU admission, organ failure, and neurological involvement were identified as adverse prognostic factors, all significantly associated with higher mortality ($p < 0.05$). No significant differences in survival were noted based on GvHD development, genetic diagnosis, donor type, or conditioning regimen ($p > 0.05$). Poor prognostic factors included PICU admission, the need for mechanical ventilation, multiorgan

failure, and neurological involvement, with significantly higher mortality rates among these patients. The demographic, clinical, laboratory, and therapeutic characteristics of the patients are summarized in Table 2. The characteristics of the patients who underwent HSCT are provided in Table 3 and Supplementary Table 1. Overall and event-free survival characteristics are shown in Figure 1.

Discussion

Primary HLH is a life-threatening immune dysregulation syndrome that predominantly affects infants and young children. HLH is increasingly being recognized as a manifestation of underlying IEs. Research indicates that HLH may not only be a familial hemophagocytic syndrome; it can also serve as a sentinel event for broader immune dysregulation [16,17]. This perspective is especially relevant in cases involving atypical presentations or those lacking a confirmed genetic mutation. Advances in molecular diagnostics and treatment approaches over the past two decades have significantly improved our understanding and management of this rare disease. However, challenges remain, particularly in early recognition, timely genetic diagnosis, and access to HSCT, which remains the only curative treatment [16,17]. In primary HLH, there is an underlying genetic defect that can lead to FHLH. Mutations of PRF1, UNC13D, STX11, and STXBP2 are some of these defects [18,19]. Primary and secondary HLH are clinically similar and cannot always be differentiated from each other. Clinically, patients can have fever (90%-100%), hepatosplenomegaly (70%-100%), maculopapular rash (10%-60%), lymphadenopathy (20%-50%), respiratory distress (40%-90%), jaundice, weight loss, and irritability. These cases resemble hyperferritinemic septic shock. When there is neurological involvement, patients may present with seizure and coma. Consistent with the literature [20], the most prevalent clinical manifestation in our series was fever, followed by splenomegaly, hepatomegaly, rash, and lymphadenopathy. A multicenter study from Türkiye by Akyol et al. [21] reported high rates of consanguinity (72.2%) and positive family history for HLH-related symptoms or deaths (39.8%). Similarly, in our cohort, 67.9% of the patients were born to consanguineous parents and 32.1% had a family history suggestive of HLH. Although primary HLH most often appears during the first two years of life, it can also be seen later [20,22]. In our series, the median age at diagnosis was 12.5 months, with the youngest patient admitted at 3 months of age while the oldest patient at admission was 12.5 years. The percentage of patients diagnosed before 1 year of age was 40% and the percentage of patients diagnosed before 2 years of age was 60%. Our cohort reveals that diagnostic delay is still an issue, with a median time from symptom onset to diagnosis of 2.5 months. This is consistent with previously published data from Türkiye, emphasizing the need for improved awareness and early referral strategies [21]. Fever was the most important finding, being observed in all patients. Given that several HLH patients

Table 2. Demographic, clinical, laboratory, and follow-up results of patients diagnosed with hemophagocytic lymphohistiocytosis.		
Demographic features (n=30)		
Sex, n (%)	Boy Girl	18 (60%) 12 (40%)
Age at first symptom, months		
Median (min-max)		10 (0.5-204)
Mean \pm SD		27.69 \pm 46.47
Age at admission to our center, months		
Median (min-max)		12.5 (1-204)
Mean \pm SD		33.28 \pm 48.88
Follow-up duration, months		
Median (min-max)		37 (0-247)
Mean \pm SD		61.93 \pm 72.00
Consanguineous marriage, n (%)		22 (73.3%)
Family history, n (%)		12 (40%)
Clinical features (n=30), n (%)		
Fever		
Fever duration, days		30 (100%)
Mean \pm SD		13.30 \pm 14.05
Median (min-max)		10 (5-60)
Interquartile range		5-13
Splenomegaly		29 (96.7%)
Hepatomegaly		25 (83.3%)
Lymphadenopathy		6 (20%)
Rash		5 (16.7%)
Neurological involvement		5 (16.6%)
Laboratory features		
Anemia (n=30)		27 (90%)
Neutropenia (n=30)		23 (76.7%)
Thrombocytopenia (n=30)		30 (100%)
Bicytopenia (n=30)		10 (33.3%)
Pancytopenia (n=30)		21 (70%)
Ferritin elevation (n=30)		30 (100%)
Ferritin level, ng/mL		
Median (min-max)		2290 (489-37.879)
Mean \pm SD		5669.40 \pm 7730.48
Interquartile range		1603-7212
Hypertriglyceridemia (n=30)		28 (93.3%)
Hypofibrinogenemia (n=30)		20 (66.7%)
AST elevation (n=30)		27 (90%)
ALT elevation (n=30)		15 (50%)
Hyperbilirubinemia (n=30)		
Total hyperbilirubinemia		7 (23.3%)
Unconjugated hyperbilirubinemia		6 (20%)
LDH elevation (n=30)		26 (86.7%)
CRP elevation (n=26)		19 (73.1%)
PT elevation (n=23)		9 (39.1%)
INR elevation (n=24)		11 (45.8%)
aPTT elevation, s (n=23)		10 (43.5%)
D-dimer elevation, ng/mL (n=22)		15 (68.2%)
Hyponatremia (n=29)		14 (48.3%)
Hypokalemia (n=29)		0 (0%)

Table 2. Continued.

Demographic features (n=30)		
Hypoalbuminemia (n=29)	16 (55.2%)	
Immunological evaluation		
Low IgG (n=22)	5 (22.7%)	
Low IgM decrease (n=23)	3 (13%)	
Low IgA decrease (n=23)	5 (21.7%)	
CD3 decrease (n=21)	2 (9.5%)	
CD4 decrease (n=22)	7 (31.8%)	
CD8 decrease (n=22)	2 (9.1%)	
CD16 ⁺ 56 ⁺ (NK) cell decrease (n=20)	5 (25%)	
RTE decrease (n=13)	2 (15.4%)	
Low lymphocyte activation response (CD3 ⁺ CD25 ⁺ , CD3 ⁺ CD69 ⁺ with PHA) (n=9)	0	
Hemophagocytosis in bone marrow aspiration/biopsy (n=30)		
Treatment (n=30) HLH-1994 HLH-2004 Other	HLH-1994	3 (10%)
	HLH-2004	21 (70%)
	Other	6 (20%)
Treatment response (n=30) Partial remission Reactivation No response	Complete remission	15 (50%)
	Partial remission	3 (10%)
	Reactivation	8 (26.6%)
	No response	4 (13.3%)
Prognosis (n=30)	Alive	13 (43.3%)
	Deceased	17 (56.7%)
AST: Aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; PT: prothrombin time; INR: international normalized ratio; aPTT: activated partial thromboplastin time; Ig: immunoglobulin; CD: cluster of differentiation; RTE: recent thymic emigrant, NK: natural killer; SD: standard deviation.		

in our cohort lacked a genetic diagnosis but fulfilled clinical criteria and had strongly suggestive family histories, a broader immunological workup may be warranted in future studies. As proposed by Ricci et al. [17], expanded genetic tests may uncover novel IELs or immune dysregulation pathways involved in the pathogenesis of HLH, especially in consanguineous populations. The most frequently identified genetic defect in our cohort was PRF1 deficiency, consistent with findings from previous national and international series. Additionally, rare mutations in genes such as STX11 and UNC13D were detected in this study, further underscoring the genetic heterogeneity of the disease.

In a primary HLH series reported by Pegoraro et al. [23], from among 143 patients, 68% had bicytopenia, 50% had elevation of liver function tests, and all patients had high ferritin levels. The series reported by Cleves et al. [24] included 21 patients and 61.9% of those patients had bicytopenia, 81% had elevated ferritin, and 76.2% had hypertriglyceridemia or hypofibrinogenemia. In our series, cytopenia was most often observed in thrombocytes and it was present in all patients. Pancytopenia was observed more often than bicytopenia (67.9%). Ferritin elevation was observed in all patients. This was followed by hypertriglyceridemia (96.4%), aspartate transaminase elevation (89.3%), lactate dehydrogenase

elevation (85.7%), and hypofibrinogenemia (64.3%). Although hemophagocytosis was not pathognomonic for bone marrow aspiration, it was seen in 85.7% of the patients.

During immunological evaluation, the lymphocyte activation response was normal in HLH but NK cell activity and T-cell cytotoxic activity were significantly lower. In active HLH, CD8 T-cells and, to a lesser extent, CD4 T-cells may be increased. Soluble CD8 and CD25 may also be increased in the process of cytokine storms [25]. In our series, we did not have access to the results of immunological evaluations for all patients. However, lymphocyte activation response with PHA was normal for all tested patients (n=9). The most frequently observed immunological abnormalities (n=20) included reduced CD4⁺ T-cell counts (30%), decreased RTEs (18.2%), low NK cell counts (16.7%), and decreased IgG levels (25%).

Cetinkaya et al. [26] evaluated 28 patients with primary HLH and found that hypoalbuminemia was significantly associated with mortality. Similarly, in a cohort of 41 patients, Bayram et al. [27] reported that hypernatremia and elevated blood urea nitrogen levels were significantly more frequent among deceased patients. In our study, hypernatremia was not observed; however, hyponatremia and hypoalbuminemia were

Table 3. Characteristics of 17 patients who underwent HSCT.

HSCT	17 (56.6%)	n	%
Donor type (n=17)	MRD	6	35
	MSD	4	23.5
	MURD	5	29.4
	MMRD	2	11.7
Conditioning regimen (n=17)	Busulfan/etoposide/cyclophosphamide	9	52.9
	Treosulfan/fludarabine	5	29.4
	Busulfan/fludarabine	3	17.6
GvHD prophylaxis (n=17)	Missing data	2	11.7
	Cyclosporine	3	17.6
	Cyclosporine/MTX or MMF	12	70.5
Chimerism follow-up (n=17)	Tacrolimus/MMF	1	5.8
	Cyclophosphamide/MTX/tacrolimus	1	5.8
	Full chimerism	11	64.7
Acute GvHD	Mixed chimerism	3	17.6
	Died before engraftment	2	11.7
	Graft failure	1	5.8
Acute GvHD treatment (n=5)	5/17 (29.4%)		
	Steroids	5	
	Mesenchymal stem cells	2	
	Tocilizumab	1	

HSCT: Hematopoietic stem cell transplantation; GvHD: graft-versus-host disease; MTX: methotrexate; MMF: mycophenolate mofetil; MRD: matched related donor; MSD: matched sibling donor; MURD: matched unrelated donor; MMRD: mismatched related donor.

detected in 48.1% and 51.9% of patients, respectively. In another series reported by Pegoraro et al. [23], hyperferritinemia and total bilirubin levels exceeding 2 mg/dL were associated with poor prognosis. Despite these findings in the literature, our statistical analysis did not reveal a significant correlation between biochemical parameters and clinical outcome ($p>0.05$). This lack of association may be attributed to the relatively small sample size in our study.

The most important type of organ involvement in HLH is neurological involvement. In different studies, the rate of neurological involvement ranges between 10% and 70% [28]. In T2 flair magnetic resonance imaging, hyperintensities can be observed in the gray and white matter in the supratentorial and infratentorial fields. At the same time, pleocytosis (50%-90%) might be identified in CSF analysis. Clinically, a loss of balance, seizures, headaches, and loss of strength in the extremities are the most commonly encountered findings [20]. In our series, 4 patients had neurological involvement. Two patients were being followed with PRF1 and UNC13D gene mutations. Only one patient had pleocytosis and atypical cells in the CSF. In the cranial magnetic resonance imaging of the other 3 patients, hyperintense areas observed in the white and gray matter were interpreted as HLH involvement. The common findings in the clinical presentations of these 4 patients were seizures and

impaired general condition. All 4 patients died during follow-up. In statistical evaluations, the presence of neurological involvement was found to be a significantly unfavorable prognostic factor ($p=0.001$).

The diagnostic process for HLH consists of two stages. The first stage involves the application of the HLH-2004 diagnostic criteria. In the second stage, genetic testing is promptly initiated for patients who meet the aforementioned criteria. Importantly, treatment should not be delayed while awaiting genetic results. In parallel to the genetic diagnosis, certain markers can be analyzed with flow cytometry to support the diagnosis. Perforin (for FHLH2), CD107a (for UNC13D, STXBP2, STX11, RAB27A, LYST, and AP3B1), SAP (for SH2D1A), and XIAP (for XLP2) can be measured [18,29]. Due to the unavailability of functional assays such as NK cell activity and CD107a expression at our center, genetic testing served as the primary diagnostic approach; however, as noted by Canna and Marsh [19], the integration of both genetic and functional data enhances diagnostic accuracy in FHLH. However, Henter et al. [30] evaluated 366 FHLH patients in their study and concluded that the sensitivity and specificity of the diagnostic criteria did not differ in instances where NK cell activity could not be measured. In our series, we tried to establish a genetic diagnosis for all patients, but we could identify genetic mutations in only 18 cases (64.2%). Most frequently, we observed

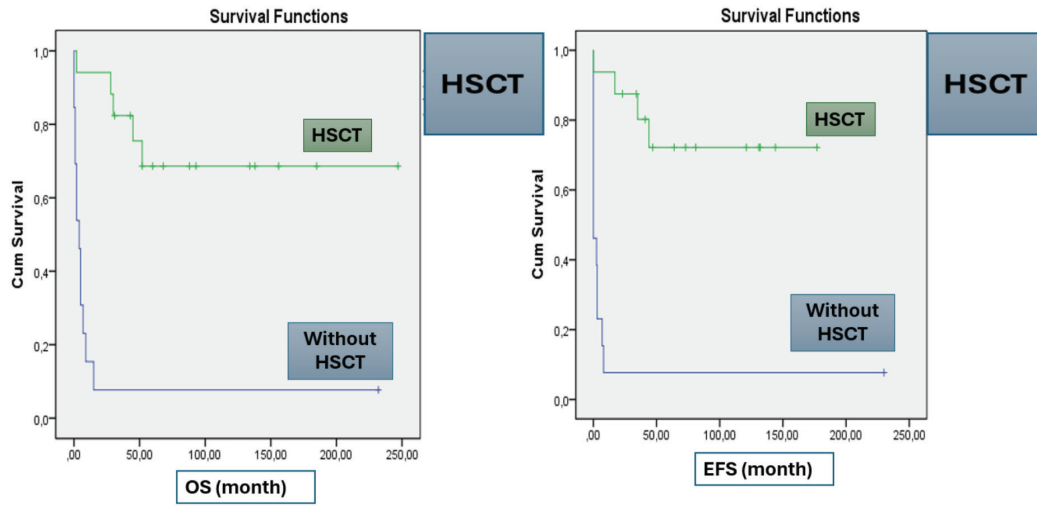


Figure 1a. Overall survival (OS) and event-free survival (EFS) for patients with (green line) and without (blue line) hematopoietic stem cell transplantation (HSCT).

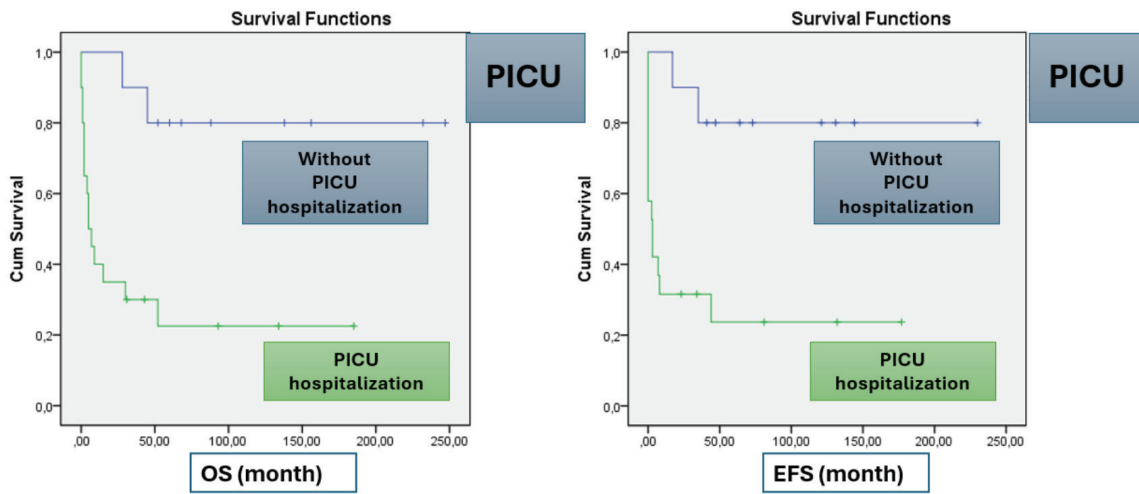


Figure 1b. Overall survival (OS) and event-free survival (EFS) for patients with (green line) and without (blue line) hospitalization in the pediatric intensive care unit (PICU).

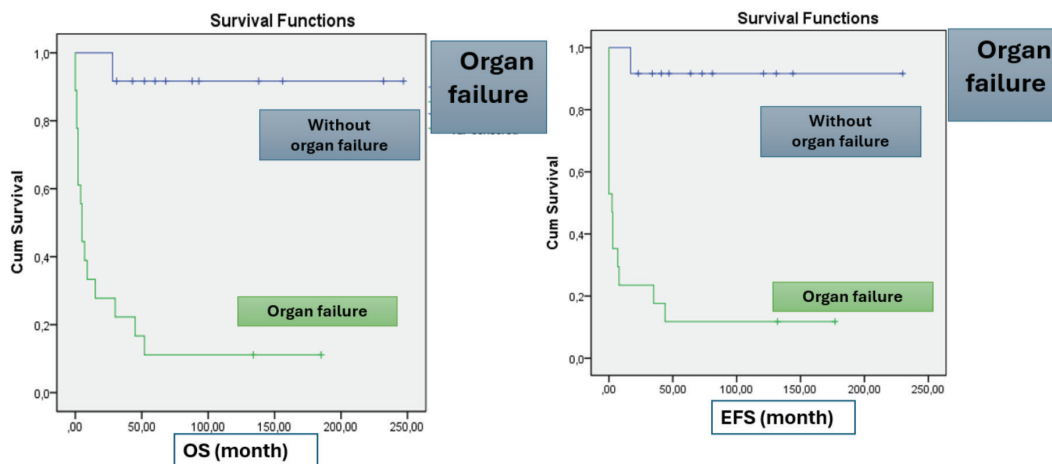


Figure 1c. Overall survival (OS) and event-free survival (EFS) for patients with (green line) and without (blue line) organ failure.

FHLH, and in this group we most commonly identified perforin and UNC13D mutations.

The objectives of HLH treatment are to induce remission, control the triggers, maintain remission/rescue treatment, and provide curative treatment. Timely treatment of HLH is very important for prognosis. If active HLH is left untreated, survival decreases significantly within the first 2 months. Cellular targeted therapies (e.g., etoposide, cyclosporine, steroids, ATG, alemtuzumab, intrathecal methotrexate) and cytokine targeting therapies (e.g., emapalumab, ruxolitinib, tadekinig-alfa) can be used as treatment approaches [7]. The HLH-94 and HLH-2004 protocols rely on immunotherapy including dexamethasone, etoposide, and CSA to induce remission and to maintain it; the main difference between these protocols is the time to start CSA [5]. In our study, the most commonly used treatment regimen was HLH-2004 (75%). A statistically significant difference could not be found among HLH-2004, HLH-94, and other treatment methods ($p>0.05$). In the cohort of Pegoraro et al. [23], comprising 143 patients, HLH-2004 was found to provide better outcomes compared to HLH-94 for post-transplant mortality. However, this difference was also attributed to the improvements in HSCT procedures and care over the years.

HSCT currently remains the only curative treatment for HLH, and it is recommended to perform allogeneic HSCT as early as possible following the achievement of remission to reduce the risk of relapse. In our cohort, 17 patients (60.7%) underwent HSCT, most commonly from MRDs. The preferred conditioning regimen was busulfan, etoposide, and cyclophosphamide. Acute GvHD developed in 5 patients and 5 patients died following transplantation. Despite these complications, the overall survival rate was significantly higher among transplanted patients compared to those who did not receive HSCT ($p=0.001$), consistent with the findings of Bayram et al. [27], who reported a 9.4-fold survival benefit in HSCT recipients.

Recent data from Öztürk et al. [22] further emphasize that genetic mutations, particularly in PRF1, UNC13D, and STX11, significantly influence post-transplant outcomes in pediatric HLH. In our study, PRF1 and UNC13D were also the most frequently identified mutations. However, these genetic findings did not significantly affect transplant-related survival in our cohort, likely due to the smaller sample size. Nonetheless, the findings reported by Öztürk et al. [22] underscore the importance of early genetic diagnosis to optimize transplant timing and donor selection.

Our findings are also in line with those of Messina et al. [31], who reported that MSDs were the most common source for transplantations and that busulfan-based regimens were frequently used. In their study, partial MRDs were associated

with poorer event-free survival and higher rates of graft failure. Notably, the presence of active disease at the time of the transplant did not negatively impact HSCT outcomes, highlighting the importance of timely transplantation, even during times of disease activity [31]. In contrast, Greental Ness et al. [32] found significantly lower 5-year event-free survival in patients receiving reduced-intensity conditioning regimens and in transplants from non-MSD donors. In our series, donor type, conditioning regimen, acute GvHD, genetic diagnosis, and stem cell source were not statistically associated with differences in overall survival or event-free survival, which may again be attributed to the limited size of the cohort.

In terms of prognosis, 17 of the 30 patients (56.6%) died. Poor prognostic factors included PICU admission, the need for mechanical ventilation, multiorgan failure, and neurological involvement, with significantly higher mortality rates in these cases. Among patients who underwent HSCT, survival rates were significantly higher. In the cohort reported by Chinn et al. [33] with 122 patients, the overall survival rate was 45%, while for the cohort of Abbasi et al. [34] with 51 patients, it was 43.9%. Consistent with the literature, the survival rate in our series was 46.5%.

Conclusion

HLH is not a single disease entity but rather a clinical syndrome characterized by a shared hyperinflammatory phenotype triggered by diverse genetic and acquired conditions. In countries such as Türkiye with a high prevalence of consanguineous marriages, HLH should be carefully considered in the differential diagnosis of patients presenting with persistent fever, hepatomegaly, cytopenia, and rash. Early recognition and prompt referral, particularly to immunology and hematology specialists, are critical for timely intervention.

Given the increasing recognition of HLH as a possible manifestation of IEs, comprehensive immunological and genetic evaluations should be pursued without delay in suspected cases. Our findings, in line with other national and international cohorts, confirm that HSCT remains the only curative option for FHLH, and it should be performed as early as possible after achieving disease remission. Improving access to early diagnostics and HSCT could significantly enhance outcomes, particularly in genetically predisposed populations.

Ethics

Ethics Committee Approval: The study was approved by the ethics board of the Ankara University Faculty of Medicine on July 21, 2023, with decision number 2023/388.

Informed Consent: Verbal and written consent forms were obtained from the legal guardians of the patients for this study.

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Footnotes

Authorship Contributions

Concept: A.G.Ö., Z.Ş.H., H.F.Ç., C.İ., T.İ., E.İ., S.İ.Ö., H.U.D., N.T., E.C.Ü., F.D., M.E., A.İ.; Design: A.G.Ö., Z.Ş.H., H.F.Ç., C.İ., T.İ., E.İ., S.İ.Ö., H.U.D., N.T., E.C.Ü., F.D., M.E., A.İ.; Data Collection or Processing: A.G.Ö., Z.Ş.H., H.F.Ç., C.İ., F.D., M.E., A.İ.; Analysis or Interpretation: A.G.Ö., Z.Ş.H., H.F.Ç., C.İ., T.İ., E.İ., S.İ.Ö., H.U.D., N.T., E.C.Ü., F.D., M.E., A.İ.; Literature Search: A.G.Ö., Z.Ş.H., H.F.Ç., C.İ., F.D., M.E., A.İ.; Writing: A.G.Ö., Z.Ş.H., H.F.Ç., C.İ.

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Supplementary Table 1. Characteristics of 17 patients who underwent hematopoietic stem cell transplantation.								
Patient number	Genetic diagnosis if applicable	Donor type	Conditioning regimen/graft-versus-host prophylaxis	Myeloid/thrombocyte engraftment (days)	Stem cell source	CD34 count (10 ⁶ /kg)	Complications	Prognosis
P1	N/A	Matched family donor (mother)	Busulfan, cyclophosphamide, etoposide/ cyclosporine, methotrexate	11/57	Peripheral	9.45	Chronic GvHD (skin, oral, eye)	Dead (due to sudden cardiac arrest)
P2	N/A	Matched sibling donor	Busulfan, cyclophosphamide, etoposide/ cyclosporine, methotrexate	12/19	Bone marrow	11.12	Mucositis, sinusoidal obstruction syndrome, splenic candidiasis, acute kidney injury	Alive
P3	Perforin	Matched family donor (mother)	Busulfan, cyclophosphamide, etoposide/ cyclosporine	Graft failure	Peripheral	2.62	Mucositis, capillary leak syndrome, acute GvHD (gastrointestinal system), acute pulmonary edema, acute kidney injury, hemorrhagic cystitis	Dead (multiple organ dysfunction syndrome)
P4	N/A	Matched family donor (father)	Busulfan, cyclophosphamide, etoposide/ cyclosporine, methotrexate	23/18	Bone marrow	8.58	Mucositis	Alive
P5	Perforin	Matched family donor (father)	Busulfan, cyclophosphamide, etoposide/ cyclosporine	19/29	Bone marrow	15.5	Mucositis	Alive
P6	Perforin	Matched family donor (father)	Busulfan, cyclophosphamide, etoposide/ cyclosporine, methotrexate	23/15	Bone marrow	3	Acute GvHD (skin)	Alive
P7	N/A	Matched sibling donor	Fludarabine, treosulfan, ATG/ tacrolimus, methotrexate	15/33	Bone marrow	5	None	Alive
P8	UNC13D	Matched sibling donor	Fludarabine, treosulfan, ATG/ cyclosporine	19/26	Bone marrow	10.3	Acute GvHD (skin)	Alive
P9	STXBP2	Matched unrelated donor	Busulfan, cyclophosphamide, etoposide/ cyclosporine, methotrexate	15/42	Bone marrow	5.6	Capillary leak syndrome, <i>Pneumocystis jirovecii</i> pneumonia, autoimmune hemolytic anemia	Dead (acute respiratory distress syndrome)
P10	Perforin	Matched unrelated donor	Busulfan, cyclophosphamide, etoposide, ATG/ cyclosporine, methotrexate	16/19	Peripheral	5.41	Hypertension, cytomegalovirus viremia	Alive

Continued.								
Patient number	Genetic diagnosis if applicable	Donor type	Conditioning regimen/graft-versus-host prophylaxis	Myeloid/thrombocyte engraftment (days)	Stem cell source	CD34 count (10 ⁶ /kg)	Complications	Prognosis
P11	Perforin	Matched family donor (father)	Busulfan, fludarabine, ATG/cyclosporine, methotrexate	N/A	Bone marrow	4.38	None	Alive
P12	UNC13D	Matched unrelated donor	Busulfan, cyclophosphamide, etoposide, ATG/cyclosporine, methotrexate	23/19	Peripheral	4	Catheter infection	Alive
P13	LYST (Chediak-Higashi syndrome)	Matched unrelated donor	Treosulfan, fludarabine, ATG, rituximab/cyclosporine, mycophenolate mofetil	15/26	Peripheral	5	Acute GvHD (skin, gastrointestinal), mucositis	Alive
P14 (P8's sister)	UNC13D	Matched sibling donor	Busulfan, fludarabine, ATG/cyclosporine, methotrexate	15/47	Bone marrow	10.5	Mucositis, sinusoidal obstruction syndrome, hypertension	Alive
P15	STAT1 gain-of-function	Mismatched family donor (father)	Fludarabine, treosulfan, ATG/cyclophosphamide, methotrexate	None	Bone marrow	2.95	Gastrointestinal and pulmonary hemorrhage	Dead
P16	CD247	Mismatched family donor (mother)	Fludarabine, treosulfan, thiotepa/N/A	N/A	Bone marrow	N/A	Acute kidney injury, BCGitis, acute skin GvHD	Dead (respiratory failure due to chronic lung GVHD)
P17	Perforin	Matched unrelated donor	Busulfan, fludarabine, ATG/cyclophosphamide, methotrexate, mycophenolate mofetil	None	Peripheral	6.75	Mucositis, diarrhea, sinusoidal obstruction syndrome	Dead (septic shock)

N/A: Not applicable; ATG: antithymocyte globulin; GvHD: graft-versus-host disease; BCG: Bacillus Calmette-Guerin.