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Original Article

Hemophagocytic Lymphohistiocytosis in Adults: Low Incidence of Primary Neoplasm as a Trigger in a Case Series from Turkey

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Abstract. Hemophagocytic Lymphohistiocytosis (HLH) is an indicator of an exaggerated immune response and eventually adverse outcomes. This study aimed to investigate the clinical and laboratory features and outcomes of patients with HLH. The medical records of 26 HLH adult patients (≥ 16 years of age) were retrospectively analyzed. Gender, age, the duration of fever, time to diagnosis, etiology and laboratory data were extracted from the records. The mean age was 38 ± 18 years, and 15 (58%) patients were female. A total of nine cases had infectious diseases; four cases had rheumatologic diseases, three cases had hematological malignancies while nine cases could not have a definitive diagnosis. The median time to detection of HLH was 20 days (IQR: 8-30 d). Of the 25 patients, 11 (44%) died. The erythrocyte sedimentation rates of the surviving and non-surviving patients were 39 ± 22 mm/h and 15 ± 13 mm/h, respectively. When a long-lasting fever is complicated by bicytopenia or pancytopenia (especially), clinicians should promptly consider the possibility of HLH syndrome to improve patients' prognosis.

Keywords: Hemophagocytic lymphohistiocytosis, Ferritin, Cytopenia, Fever of unknown origin.

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Introduction. Hemophagocytic Lymphohistiocytosis (HLH) is a life-threatening clinical condition caused by an exaggerated immune response that results in tissue destruction.[1] Its primary form generally occurs due to an underlying genetic immune dysfunction and primarily affects infants and young children, whereas the secondary form occurs due to various underlying conditions ranging from viral infections to autoimmune diseases and cancers in adults.[2,3]

The clinical features of HLH include fever, hepatosplenomegaly, lymphadenopathy, neurological symptoms, and skin manifestations. Cytopenia, elevated liver enzymes, high serum ferritin levels, hypertriglyceridemia, and hypofibrinogenemia are laboratory abnormalities that have frequently been reported in HLH patients.[4] Although extremely elevated ferritin levels represent a highly sensitive marker of HLH, this disease may be difficult to recognize and diagnose.[5] Early diagnosis and prompt treatment of the underlying cause are important to decrease morbidity and mortality in patients with HLH.[6] In this study, we aimed to investigate the clinical features and outcomes of patients with HLH in tertiary care centers in Turkey through a retrospective chart review.

Materials and Methods. The medical records of adult patients (≥ 16 years of age) diagnosed with HLH between January 2010 and April 2016 at six university hospitals in Turkey were reviewed retrospectively.

Infectious disease, rheumatology, and hematology specialists were contacted for the records. The Istanbul Medipol University Ethical Committee approved this study.

All the patients who met the diagnostic guidelines of the Histiocyte Society (HLH-2004) were included in this study (**Table 1**).[7] Molecular parameters, such as NK cell activity and soluble CD25, were not available in this study due to a lack of laboratory facilities. The following features were evaluated: fever (type, duration), time to diagnosis, splenomegaly, hepatomegaly, lymphadenopathy, rash, serosal involvement, respiratory symptoms, neurological symptoms, and opportunistic infections. Hepatomegaly and splenomegaly were defined as the long axis of the organs exceeding 155 mm and 130 mm on radiological investigations, respectively.[8]

Table 1. Hemophagocytic Lymphohistiocytosis 2004 Trial Diagnostic and Modified Criteria.

HLH 2004 criteria
Fever ≥ 38.5°C
Splenomegaly
Cytopenia (at least two of the following): Hemoglobin < 9 g/dL Platelets < 100,000/μL ANC < 1,000/μL
Triglycerides > 265 mg/dL and/or fibrinogen < 150 mg/dL Ferritin > 500 ng/mL
Hemophagocytosis (bone marrow, spleen, lymph node, or liver)
Low or absent NK cell activity
Elevated soluble CD25 (soluble IL-2 receptor alpha)

ANC, absolute neutrophil count; NK, natural killer, IL-2, interleukin 2. Measurements of the underlined, italicized markers were not available in this study.

Hemogram, ferritin, lactate dehydrogenase (LDH), transaminase, bilirubin, triglyceride, high-density lipoprotein (HDL), and fibrinogen levels and erythrocyte sedimentation rates (ESRs) were analyzed. Hemophagocytosis was defined as histological evidence of activated macrophages engulfing blood cells in the bone marrow and/or other tissues.

Opportunistic infections were defined as a new clinical condition with ongoing immunosuppression due to HLH. When positron emission tomography with computed tomography (PET/CT) was performed, the presence of fluorodeoxyglucose (FDG) uptake was evaluated. Underlying triggering diseases and treatment modalities, including types of initial therapy, secondary therapy, and adjunctive therapy (supportive or underlying disease-specific treatment), were evaluated. Patients without both ferritin and triglyceride levels in their medical records were excluded.

The basic statistical analysis was performed using R version 3.0.4. Results are expressed as numbers (percentages) for categoric variable and as mean (standard deviation) or median (interquartile range) for continuous variables. The chi-square test and contingency table were used to compare subgroups of patients. *p* values of less than 0.05 were considered as statistically significant.

Results. 26 patients met the inclusion criteria. The mean age of the patients was 38 years (range, 16-74), and 15 (58%) females were included. The triggering etiologies were established in 17 cases (65%) and were as follows: infection in 9 cases

(Crimean-Congo hemorrhagic fever [CCHF] in 1 case, Epstein-Barr virus [EBV] in 4 case, cytomegalovirus [CMV] in 1 case, influenza virus in 1 case, toxoplasmosis in 1 case, and histoplasmosis in 1 case), rheumatologic disease in 3 cases (adult-onset Still's disease [AOSD] in 1 cases, rheumatoid arthritis in 1 case, and systemic lupus erythematosus [SLE] in 1 case), hematologic malignancy in 3 cases (diffuse large B-cell lymphoma in 2 cases, intravascular lymphoma in 1 case), and ulcerative colitis in 1 case. Of the 26 patients, 11 (42%) died. Of the fatal cases, five had an infectious etiology, one lymphoma, and the other five were associated with an unknown etiology.

Fever was the most common sign among the HLH patients. It was the primary presenting symptom in all cases. The median duration of fever was 19 days (IQR, 10-30), and the median time to diagnosis was 21 days (IQR 8-30). Hepatomegaly and splenomegaly were detected in 21 (84%), and 23 (92%) patients, respectively, and 18 (72%) patients had lymphadenopathies (peripheral in 2 patients and systemic in 16 patients).

Neurological manifestations, including encephalopathy, seizures, and an altered level of consciousness, were observed in nine cases (38%). Bilaterally thalamic involvement and demyelinization findings were revealed in two different patients who had not apparent neurological symptoms. In two patients, the neurological findings were associated with herpes simplex virus (HSV) reactivation. HSV DNA was detected in the cerebrospinal fluid (CSF) and blood of two patients with encephalitis. Eight patients who received immunosuppressive drugs for HLH developed concomitant opportunistic infections (CMV in three patients, HSV in three patients, invasive aspergillosis in one patient, and *Pneumocystis jiroveci* pneumonia in one patient).

The erythrocyte sedimentation rates of the surviving and non-surviving patients were 39 ± 22 mm/h and 16 ± 14 mm/h, respectively. The median ferritin level was 8826 ng/ml (IQR 1656-27386 ng/ml, range 566-100000 ng/ml), the median LDH level was 1562 IU/ml (range; 342-5251 IU/ml) and total bilirubin level median was 1.85 mg/dl (IQR 0.8-6.1 mg/dl). The mean triglyceride level was 528±321 mg/dl, and the median HDL levels were 7 mg/dl (IQR 5-12 mg/dl).

Bone marrow biopsy showed hemophagocytosis in 22 of 26 (84%) patients. Additionally, there was bone marrow involvement in two of the lymphoma cases. Activated macrophages with maturation arrest (in 2 patients) and chronic lymphoproliferation were the other findings in bone marrow biopsies. Hemophagocytosis and hemosiderosis were observed in liver biopsies of two of these patients.

Of the 26 patients, eight were evaluated by PET/CT scans; their findings are summarized in **Table 2**.

All the patients with HLH received specific treatment for the hemophagocytic syndrome. Three patients (12%) were treated only with glucocorticoids, whereas the others received both glucocorticoids and another drug(s) as initial treatment. The underlying diseases, HLH-associated laboratory values and initial, secondary, and adjunctive treatment modalities of the patients after HLH diagnosis are summarized in **Table 2**.

Discussion. Various underlying conditions that predispose patients to HLH have been defined in previous studies.[9,10,11] In children, underlying genetic defects play a predominant part in the development of HLH.[11,12] Although malignancies, frequently hematologic, seem to be the leading cause of adult-onset HLH,[11] the predominant cause of HLH may differ by country because of different genetic/ethnic backgrounds or differences in triggering agents, particularly infections.[11,12] However, only 3 of 26 patients in our study were diagnosed with lymphoma. Although, this finding may reflect the underrepresentation of hematology units at participating centers or underdiagnosed lymphoproliferative diseases, a similar distribution of causes of this disease in the Mediterranean Region has been found in Spain but not in Italy and in France.[11]

Many clinical and laboratory features were consistent with those reported previously, e.g., high fever, cytopenia, splenomegaly, hepatomegaly, and hyperferritinemia.[4] Fever was the primary presenting symptom in all of our patients, which is similar to a report from Riviere et al.[4] Hyperferritinemia is a sensitive marker of HLH. No cutoff value has been defined as critical; however, high levels of ferritin (> 50,000 µg/L) are associated with a poor prognosis.[13] Hypofibrinogenemia is the main factor for blood

Table 2. Underlying Diseases, PET/CT Involvement, Treatments and Outcomes of Hemophagocytic Lymphohistiocytosis Patients.

Patient	Secondary disease	Ferritin levels (µg/L)	Triglycerides (mg/dL)	PET/CT involvement area	First treatment	Secondary treatment	Adjunctive treatment	Outcome
1	DLBCL	1381	480	BM, Spleen, LN	VP-16 + DEX + CyA		R-CHOP	Alive
2	Idiopathic	7300	468	BM, Spleen, Liver, LN	VP-16 + DEX + CyA			Alive
3	DLBCL	78286	528	BM, Spleen, LN	VP-16 + DEX			**Died**
4	EBV	90000	1170		DEX + IVIG		Acyclovir	Alive
5	CMV	100000	589	BM, Liver	mPSL		GCV	**Died**
6	SLE	1230	245	NA	mPSL + IVIG	Rituximab	GCSF + Plasmapheresis	Alive
7	Idiopathic	909	259	NA	mPSL + IVIG			Alive
8	RA	1656		NA	IVIG	mPSL + IVIG		Alive
9	Idiopathic		1010	NA	mPSL + CyA			Alive
10	Ulcerative colitis	15100	789	NA	mPSL + IVIG			Alive
11	Influenza	21191	281	NA	mPSL + IVIG		Oseltamivir	Alive
12	Idiopathic	10027	501	NA	mPSL + IVIG			**Died**
13	Toxoplasmosis	34000	1206	BM, Spleen, Liver, LN	mPSL + IVIG		TMP/SMZ	**Died**
14	CCHF	1405		BM, Spleen, Liver	mPSL			Alive
15	Histoplasmosis (foreign country)	2500	921	BM, Spleen	mPSL		Itraconazole	**Died**
16	EBV	7029	525	NA	VP-16 + DEX + CyA			**Died**
17	EBV	88967	654	NA	VP-16 + DEX + CyA		Acyclovir	**Died**
18	Idiopathic	27386	103	NA	VP-16 + DEX + CyA			**Died**
19	EBV	10500	417	NA	DEX + IVIG		Acyclovir	**Died**
20	Idiopathic	85024	266	NA	VP-16 + DEX			**Died**
21	Idiopathic	8826	374	NA	VP-16 + DEX + CyA			**Died**
22	Still's disease	2551	232	NA	mPSL + IVIG			Alive
23	Idiopathic	15000	43	NA	IVIG			**Died**
24	Intravascular DLBCL	562	416	BM, Spleen, Lung	R-CHOP		ASCT	Alive
25	Idiopathic	639	561	BM, Spleen, Liver	NA			**Died**
26	İdiopatic	9650	165	Spleen, liver	mPSL + IVIG			Alive

NA, not available; BM, bone marrow; LN, lymph node; mPSL, methylprednisolone; DEX, dexamethasone; CyA, cyclosporine; DLBCL, diffuse large B-cell lymphoma; IVIG, intravenous immunoglobulins; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; AOSD, adult-onset Still's disease; VP-16, etoposide; GCV, ganciclovir; R-CHOP, rituximab, hydroxydaunorubicin [doxorubicin], Oncovin [vincristine], and prednisone; GCSF, granulocyte colony-stimulating factor; TMP/SMX, trimethoprim-sulfamethoxazole; CCHF, Crimean-Congo hemorrhagic fever. ASCT: Autologous Hematopoietic Stem Cell Transplantation.

cells velocity that also stated as bad prognostic factor for HLH. Riviere et al. found higher LDH levels in patients with hemophagocytic syndrome (positive cases) than in negative and undetermined patients.[4]

In our case series, the baseline ESR values, the median ferritin, LDH and total bilirubin levels of the non-survivors were higher than those of the survivors. But, due to limited numbers of patients to conclude statistical significance with parametric and non-parametric tests, we do not give any hypothetical test results here. We may say that more tissue destruction like liver as cholestatic hepatitis and blood cells hemolysis can explain the higher LDH and total bilirubin levels in non-survivors as bad prognostic factors.

Neurological symptoms may manifest as different clinical presentations, ranging from depression and convulsions to progressive encephalopathy. In one study, HSV reactivation was not found in patients with hemophagocytosis and multi-organ failure.[14] In contrast, two of the five cases with neurological findings in our study involved HSV reactivation.

Hemophagocytosis has been demonstrated in HLH patients, especially in the bone marrow, spleen, liver, and lymph nodes. It is a diagnostic criterion. Hemophagocytosis was reported in bone marrow aspirates in 84% of HLH cases of the literature, which is similar to our findings. Hemophagocytosis by itself is not a pathognomonic finding for the diagnosis of HLH.[15] Because hemophagocytosis may be a late finding in progressive HLH, repeat biopsies may be needed to confirm the diagnosis in some cases.[1]

PET/CT is a recently developed technique that is especially useful in cases of malignancy, and this method may be valuable for diagnosing underlying malignancies versus isolated HLH. In several reported cases, the diffuse involvement of the bone marrow associated with spleen, liver, and lymph node involvement has been found on PET/CT scans, which is consistent with hemophagocytosis.[16] Little information is available regarding whether PET/CT findings were related to underlying or opportunistic diseases versus HLH. The underlying conditions of our patients may have been responsible for the PET/CT findings; however, one idiopathic case showed a diffuse involvement of the reticuloendothelial system, which was considered to be associated with HLH (**Figure 1**).

In 2004, a new HLH treatment protocol was published (HLH-2004).[7] Corticosteroids, cyclosporine, and etoposide constitute the backbone of treatment. Among our cases, only two patients were treated with this backbone therapy, and they recovered completely with good prognoses. Etoposide-based steroid combination regimens have been confirmed to improve HLH-associated outcomes in most trials.[17]

All the patients had been treated with at least one antibiotic regimen preceding the diagnosis of suspected sepsis (data not presented). Of the 25 patients, 3 received only high-dose corticosteroids as initial management, and only 1 case (a CCHF patient) resolved clinically. All the patients with rheumatologic disease-related HLH responded to treatment with good prognoses. While etoposide and cyclosporine combination with high dose corticosteroid is the recommended regime for HLH, in our study, eight patients were treated with etoposide containing regimes, and six of them died. Mortality in rheumatological diseases is significantly lower than that in infection- or malignancy-related HLH.[18]

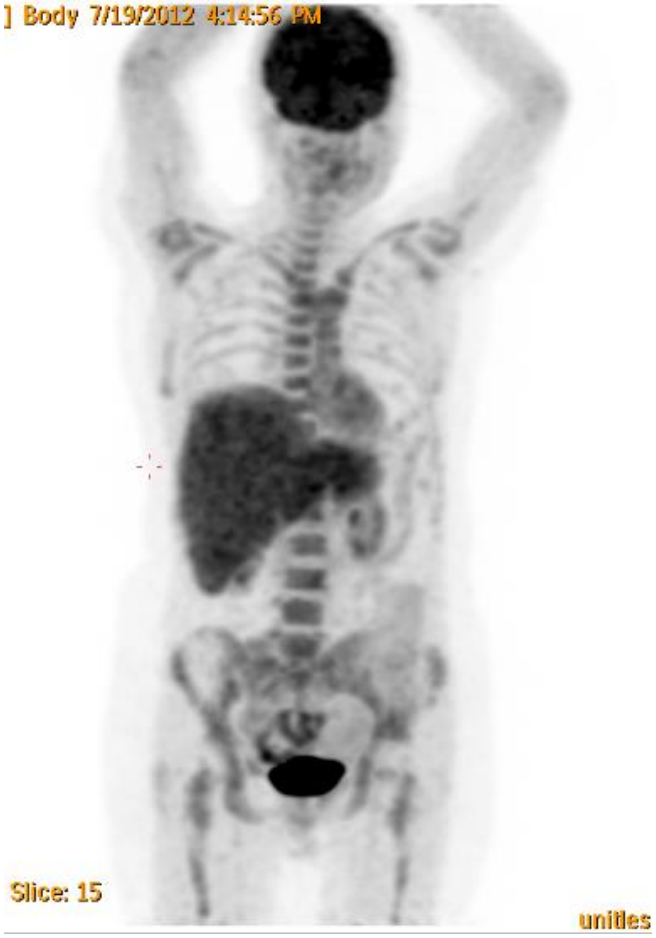

Figure 1. (Maximum intensity projection) The anterior view of a patient showing diffuse F18-FDG uptake of the liver with a SUVmaxvalue of 4.5 (normal: 3.2±0.8) and an increase in liver dimensions.

The reported mortality of secondary HLH in adult case series varies from 20-74.8%, which is similar to the rate of 44% in our case series.[4,11] Our cases with underlying rheumatological diseases also showed a good prognosis.

Advanced age, the presence of lymphoma or infectious disease, transplantation, and persistent fever within three days after the first treatment have been clinically defined as poor prognostic factors.[4] Although a statistical comparison was not possible, ten of the fatal cases in this study had an infectious (~50%) and idiopathic (~50%) etiology.

This retrospective case series study was intended to present an additional adult HLH case series to the literature. Its retrospective nature and small sample size are important limitations. In conclusion, HLH occurs secondary to many diseases observed in internal medicine practice. When a long-lasting fever is complicated by bicytopenia or pancytopenia (especially), clinicians must promptly consider the possibility of HLH syndrome since early diagnosis improves

patients' prognosis. "In our case series, the baseline ESR values, the median ferritin, LDH and total bilirubin levels of the non-survivors were higher than those of the survivors.

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