Malignancy-associated hemophagocytic lymphohistiocytosis in pediatric cases: a multicenter study from Turkey

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This study evaluates the clinical and laboratory data of children with secondary hemophagocytic lymphohistiocytosis (sHLH) related to malignancy.

Charts of patients who met the diagnostic criteria for sHLH associated with malignancy between January 2000-2006 at six different hospitals in Turkey were reviewed retrospectively. The diagnosis of HLH had been established by bone marrow aspiration in 27 patients, cerebrospinal fluid and bone marrow aspiration in one patient and lung-liver biopsy in another.

Twenty-nine children were diagnosed as having sHLH related to malignancy. Twenty cases (18 ALL and 2 AML) with acute leukemia (10 girls/10 boys, median age: 8 years [3-14 years]) were found to have sHLH. Five patients with acute leukemia had HLH at the time of diagnosis (Group 1a), and 15 patients with acute leukemia were diagnosed as having sHLH during therapy (Group 1b), namely reactive sHLH associated with the chemotherapy. Nine patients, including two cases each of rhabdomyosarcoma, neuroblastoma, Hodgkin disease, and non-Hodgkin lymphoma (NHL) and one case with Langerhans cell histiocytosis, were diagnosed as having concomitant hemophagocytosis at the initial evaluation of the tumor (Group 2).

***Fever, anemia,* and *hypertriglyceridemia* were present in all sHLH cases of all three groups.**

***Hepatomegaly* was detected in 60.0%, 73.3%, and 88.8% of the three groups, respectively. *Splenomegaly* was more frequent in patients of Groups 1a (60.0%) and 2 (88.8%) than in those of Group 1b, the reactive ones (13.3%). *Hypofibrinogenemia* was detected in all patients of Group 1a and Group 2. Low level of fibrinogen was present in 91.6%% of patients in Group 1b. All patients in Group 1b (100%) had neutropenia and thrombocytopenia. Neutropenia was found at rates of 60.0% and 55.5% in Group 1a and Group 2, respectively. Thrombocytopenia was detected in 80.0% of patients in Group 1a and 77.7% in Group 2.**

The overall mortality rate was 34.4% (10 cases) in our series of 29 children with sHLH; 50% of deaths were directly attributable to HLH.

Pediatric malignancy-associated HLH patients have been commonly described as case presentations or in a review of the literature. We believe that our cohort, compiling 29 children regarding the association between malignancy and HLH, will be useful for pediatricians who are interested in this still mysterious topic.

Key words: hemophagocytic lymphohistiocytosis, malignancy, chemotherapy-related, acute leukemia, solid tumors.

Hemophagocytic lymphohistiocytosis (HLH) has been described as a rare disease characterized by fever, hepatomegaly, splenomegaly, cytopenia, coagulopathy, hypertriglyceridemia, hyperferritinemia and hypofibrinogenemia. This disorder includes two different subgroups including primary and secondary forms, and distinction between them is difficult[1,2]. It is a life-threatening disorder resulting in infiltration of lymphocytes and macrophages with extensive hemophagocytosis in different organs, especially the liver, spleen, bone marrow and central nervous system (CNS)[3]. There is no distinction between the two types of HLH in terms of clinical symptoms, documented infection and the initial course of disease[4,5]. Relapses and family history are important clues for primary HLH. Primary HLH is an autosomal recessive disease that has been shown to be due to mutations in the perforin, the UNC13D and the syntaxin 11 genes in approximately half of the patients[6-8]. No gene mutation has been described in the remaining familial HLH patients. Secondary HLH (sHLH) is associated with a systemic viral, bacterial, fungal, or parasitic infection in individuals with an underlying immunological disorder[9]. On the other hand, one of the common triggers of sHLH is malignancy[10,11]. In contrast with adult malignancy-associated HLH, data about pediatric cases is very limited[12,13]. The mortality rate in children diagnosed with primary HLH and without bone marrow transplantation is quite high; approximately 50% of the secondary form may also result in death[5,14].

In this study, we aimed to report our experience with pediatric malignancy cases associated with sHLH at the time of diagnosis or as a reactivation during the treatment of malignancy.

Material and Methods

Between January 2000-January 2006, 29 children were diagnosed as having HLH associated with malignancy in six Pediatric Hematology-Oncology Departments of different hospitals in different regions of Turkey.

The diagnosis of HLH was made according to the guidelines of the Histiocyte Society[1], which requires either a molecular diagnosis consistent with primary HLH, family history or fulfillment of five of eight diagnostic criteria. These criteria include: fever, splenomegaly, cytopenias (affecting ≥2 of 3 lineages in the peripheral blood), hypertriglyceridemia and/or hypofibrinogenemia (fasting triglycerides >3 mmol/L, fibrinogen <1.5 g/L), hemophagocytosis in bone marrow or lymph nodes, decreased or absent natural killer (NK) cell activity, ferritin >500 μg/L and soluble CD25 (IL-receptor) >2400 U/ml. In this study, the criteria for case inclusion were based on clinical and pathologic findings of bone marrow samples revealing hemophagocytosis.

Patients with the clinical features of HLH, but whose diagnoses were not confirmed histopathologically, were excluded. The medical records of the patients were reviewed. Details of presentation, diagnosis, treatment and outcome were analyzed. No genetic analyses were done for HLH.

Chi-square, Student's t test and ANOVA were used for the statistical analysis.

Results

Of the 29 children who were diagnosed with sHLH associated with malignancy, 20 (18 acute lymphoblastic leukemia [ALL] and 1 acute myeloblastic leukemia [AML] M2, 1 AML M3) were associated with acute leukemia (Group 1). There were 10 girls/10 boys, with a median age of 8 years (range: 3-16 years). Only one case had a history of consanguinity between parents. The patients with acute leukemia were analyzed into two subgroups:

Group 1a: sHLH was diagnosed concomitantly with acute leukemia, before chemotherapy was initiated (n=5).

Group 1b: sHLH was diagnosed at any time interval of the chemotherapy that was being received for the treatment of acute leukemia (n=15). Fourteen of 15 patients were treated with BFM-based leukemia protocols; one was given St. Jude regimen.

The remaining 9 patients, including rhabdomyosarcoma (RMS; 2 cases), neuroblastoma (NB; 2 cases), Hodgkin disease (2 cases), non-Hodgkin lymphoma (NHL; 2 cases) and Langerhans cell histiocytosis (LCH; 1 case), were diagnosed as having concomitant hemophagocytosis at the time of diagnosis (Group 2).

From the total study group, 14 of the patients (5 leukemia, 9 solid tumors) were diagnosed to have sHLH at the time of diagnosis of malignancy

before the initiation of the treatment (Group 1a and 2) and 15 patients were diagnosed as sHLH during the treatment of malignancy (Group 1b).

The clinical features and laboratory findings of the sHLH cases are summarized in Table I.

Clinical Characteristics

Fever was present in all of the patients (100%) with sHLH. Hepatomegaly was more frequent in patients of Group 2 (88.8%) than in the patients of Groups 1a (60.0%) and 1b (73.3%). Splenomegaly was also more common in Group 2 patients (88.8%) than in patients of Groups 1a (60.0%) and 1b (13.3%).

Laboratory Data

Anemia and *hypertriglyceridemia* were found in all patients of all three groups (100%). *Thrombocytopenia* was present in all patients (100%) in Group 1b, 4 of 5 patients (80.0%) in Group 1a, and 7 of 9 patients (77.7%) in Group 2, respectively. *Neutropenia* was detected in all patients (100%) of Group 1b, 3 of 5 patients (60.0%) of Group 1a, and 5 of 9 patients (55.5%) in Group 2, respectively. Hypofibrinogenemia was observed in all patients who were tested in Group 1a and 2 (100%), and of the 12 tested patients in Group 1b, 11 patients (91.6%) were found to have hypofibrinogenemia (Table I).

Hemophagocytosis was documented in bone marrow examinations of all children except two cases, one after lung and liver biopsy and the other with cerebrospinal fluid (CSF) evaluation and bone marrow aspiration.

Infection

Of 29 patients, six (20.6%) developed infections including *Staphylococcus aureus* (2 cases), fungal pneumonia (1 case), aspergillosis (1 case), methicillin-resistant *S. aureus* (MRSA) (1 case), and hepatitis (1 case).

CNS Involvement

Central nervous system involvement was very rare in the patient group, with only one patient found to have hemophagocytosis in the cytological evaluation of the CSF. Neither macrophages nor protein elevation was found in the other CSF samples.

Treatment

Ten patients were given intravenous immunoglobulin (IVIG) associated with corticosteroid (CS). Two patients had only CS therapy. One patient was treated with HLH-2004 protocol and one with only IVIG. The patient who was given HLH-2004 treatment was in a dismal condition and deteriorated rapidly due to fungal pneumonia. Treatment with either IVIG or CS, or in combination, provided good relief,

Table I. Characteristics of the Groups

	Group 1a (n=5)	Group 1b (n=15)	Group 2 (n=9)
Age (mean, year)	8.8±5.0	7.8±3.0	8.6±6.6
Fever	5/5 (100.0%)	15/15 (100.0%)	9/9 (100.0%)
Hepatomegaly	3/5 (60.0%)	11/15 (73.3%)	8/9 (88.8%)
Splenomegaly	3/5 (60.0%)	2/15 (13.3%)	8/9 (88.8%)
Hypertriglyceridemia	3/3 (100.0%)	10/10 (100.0%)	8/8 (100.0%)
Hypofibrinogenemia	3/3 (100.0%)	11/12 (91.6%)	7/7 (100.0%)
Anemia	5/5 (100.0%)	15/15 (100.0%)	9/9 (100.0%)
Neutropenia	3/5 (60.0%)	15/15 (100.0%)	5/9 (55.5%)
Thrombocytopenia	4/5 (80.0%)	15/15 (100.0%)	7/9 (77.7%)
Outcome			
Alive	2/5 (40.0%)	12/15 (80.0%)	5/9 (55.6 %)
Deceased	3/5 (60.0%)	3/15 (20.0%)	4/9 (44.4%)
Progression (M)	–	1	2
Recurrence (M)	–	1	3
Infection	1	1	–
Liver insufficiency	1	–	–

M: Malignancy.

especially for fever and pancytopenia, in a 1-2-day period. The remaining 15 patients received no specific treatment for HLH, but received treatment for the primary malignancy.

When the therapy-related cases were analyzed, the HLH development time coincided with days 50 to 64 of induction therapy (Protocol I, Phase 2 of BFM regimens) in 11 cases; with the 15th day of St. Jude Total XIII induction protocol in one case; between days 40 to 50 in consolidation therapy (Protocol II, Phase 2 of BFM regimens) in two cases; and in one case after the first high-risk block of BFM protocol just after the induction regimen.

Outcome

Ten patients, including five with acute leukemia and five with solid tumors, were lost. The overall mortality rate in our series of 29 children with sHLH was 34.4%.

Of the 10 deceased patients, seven (70%) had been diagnosed as having sHLH at the time of diagnosis of the primary malignancy. Of these seven patients, three were from Group 1a and four from Group 2. Disease progression and recurrence were the primary causes of death in Group 1b and Group 2 patients; however, infection and liver insufficiency were the causes of death in Group 1a patients. The data of the deceased patients is shown in Table II.

We found no statistically significant differences in clinical and laboratory features between patients detected at diagnosis or during therapy except for leukopenia/neutropenia and also when the patients were compared with respect to having solid tumors or hematologic malignancy ($p>0.05$).

Discussion

Malignancy is one of the subgroups of disorders related to the development of sHLH. Presumably, malignant cells secrete pro-inflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6) that contribute to immune dysregulation. Malignancy-related sHLH has been reported previously in the literature, most commonly described with lymphomas and leukemia. However, most of these were reported in adult patients[10-12]. The diagnosis of HLH associated with malignancy is usually difficult. HLH manifestations may be masked or modified by the malignant process or therapeutic measures that often lead to delay in diagnosis. HLH is sometimes the first presentation of malignancies[10-12]. In a recent study, in which a literature review was done for pediatric cases of HLH in the setting of ALL, it was suggested that the underlying immunodeficient state due to the malignancy, combined with chemotherapy, predispose to infections and defects in T-cell and NK-cell function, which triggered HLH[13].

In the present study, 29 patients with malignancy who developed HLH were analyzed. In 14 of these 29 patients (48.3%), HLH was associated with acute leukemia (in 5 patients) or with solid tumors (in 9) at the time of diagnosis. This association may be due to cytokines induced by activated T cells and macrophages. In 15

Table II. Data of the Deceased Patients

Number	Age, gender	Primary disease	Primary disease state	Specific treatment state of HLH	Infection
1	14, F	ALL	Maintenance	IVIG	None
2	10, M	ALL	End of HR1	IVIG, CS	Aspergillosis
3	4, F	ALL	At diagnosis	No treatment	None
4	13, F	ALL	Induction	HLH 2004	Fungal pneumonia
5	3.5, F	ALL	At diagnosis	No treatment	None
6	14, M	RMS	At diagnosis	No treatment	None
7	10, M	RMS	At diagnosis	No treatment	*S. aureus*
8	10/12, F	NB	At diagnosis	No treatment	None
9	11/12, M	NB	At diagnosis	No treatment	None
10	2, M	LCH	At diagnosis	No treatment	*S. aureus*

HLH: Hemophagocytic lymphohistiocytosis. F: Female. M: Male. ALL: Acute lymphoblastic leukemia. AML: Acute myeloblastic leukemia. RMS: Rhabdomyosarcoma. NB: Neuroblastoma. LCH: Langerhans cell histiocytosis. IVIG: Intravenous immunoglobulin. CS: Corticosteroid.

(51.7%) of the patients, HLH developed during treatment. In our study, the HLH cases that occurred during treatment of the malignancy developed only in patients with acute leukemia. It is interesting that HLH did not develop during the period of chemotherapy in patients with solid tumors. According to diagnostic guidelines, events associated with massive tissue destruction (such as chemotherapy) may cause HLH[1,2]. We documented the leukemia patients with respect to whether or not they manifested HLH during therapy or had a larger malignant cell load and a rapid response to therapy in order to support this notion, but we found no correlation between them.

It is well known that HLH usually develops after viral, bacterial, fungal, parasitic and other infections[5,9,15,16]. On the other hand, some therapeutic agents used for treatment of acute leukemia may also cause HLH[17]. In the present study, the development of HLH during treatment of acute leukemia could be attributed to the infections concomitant with treatment or to the chemotherapeutic agents themselves. Another possibility is that acute leukemia patients are more immunocompromised than patients with solid tumors. As has been well described, sHLH may develop as a result of the strong immunologic activation commonly occurring in an immunocompromised host[18]. Although each leukemia patient manifesting HLH during chemotherapy was re-evaluated serologically for a possible viral infection, no presence of a responsible agent was detected in our cohort. The development of malignancy in previously undiagnosed genetic HLH mutations might trigger HLH, particularly when combined with infection. O'Brien et al.[13] found that significant infection was frequent in ALL patients with HLH. They suggested evaluating any patient with ALL who develops HLH for an occult infection.

When the therapy-related cases were documented, the treatment period was found to be between days 50 to 64 of induction therapy (Protocol I, Phase 2 of BFM regimens) in the majority of cases (11 cases); on the 15th day of St. Jude Total XIII induction protocol (1 case); between days 40 to 50 of consolidation therapy (Protocol II, Phase 2 of BFM regimens) (2 cases); and after the first high-risk block just after the induction regimen (1 case). During these periods, the cytokine storm may be out of control after CS withdrawal.

When the clinical and laboratory data of the malignancy patients who developed HLH were analyzed, fever, anemia and hypertriglyceridemia were detected in all patients in all three groups, suggesting that these three parameters are valuable for diagnosis of HLH in patients with malignancy (Table I). In addition to anemia, neutropenia and thrombocytopenia were also present in all patients in Group 1b. This finding may be explained as an effect of chemotherapy. Hypofibrinogenemia was also detected in almost all of the cases, at rates of 100%, 91% and 100%, respectively, in Groups 1a, 1b and 2. The low fibrinogen levels may be attributed to HLH, but can also be related to the L-asparaginase treatment in Group 1b patients[19].

It is notable and difficult to explain that although at the time of sHLH diagnosis, splenomegaly was present in only 13% of Group 1b patients, who developed sHLH during treatment of acute leukemia, hepatomegaly was present in 73%. This may be related to the hepatotoxic effects of chemotherapeutic agents, as well as to the occult infections that may have caused hepatomegaly more commonly than splenomegaly, which we were not able to detect. Although splenomegaly is an important diagnostic criterion for HLH diagnosis, it is obvious from our study that hepatomegaly is a more important finding in pediatric malignancy patients under treatment.

Lymphoma–associated hemophagocytic syndrome is a more common problem for adults than for children[20-22]. It has recently been reported that some lymphoma patients associated with familial HLH had bi-allelic perforin gene mutation. The authors have suggested that perforin may play a role in preventing tumor growth and development in the mechanism of immune surveillance[23]. On the other hand, acute nonlymphoblastic leukemia and myelodysplastic syndrome have also been described in patients with syntaxin 11 mutation[24]. The perforin A91V polymorphism leads to reduced perforin protein expression, found with increased frequency in children with ALL[25-27]. These observations suggest that there is a tendency to malignancy in patients with hereditary HLH. The relative frequency of HLH observed in children with a defined malignancy would be important to know, especially since mutations associated with HLH are distinct in the Turkish population. We found no familial cases of malignancies in the patients reported.

Of the 14 patients (Group 1a and 2) who developed HLH at the time of diagnosis, seven (50%) died. (in Group 1a, 3 of the 5 patients, 60%; in Group 2, 4 of the 9 patients, 44%). As expected, the mortality rate was lower (20%) in patients who were under treatment for the primary malignancy (Group 1b).

In conclusion, the development of sHLH in pediatric malignancy patients is not uncommon. Since the HLH cases diagnosed concomitantly at the time of diagnosis of malignancy resulted in mortality in 50% of the patients, cancer patients with high fever at presentation should be further investigated with measurement of serum ferritin, triglyceride and fibrinogen levels, and bone marrow aspiration samples should also be carefully investigated in order to detect hemophagocytosis. The majority of diagnostic features in HLH are largely "characteristic but non-specific". It should prove to be beneficial for the exact diagnosis if there was an opportunity to determine the sCD25 levels of the patients.

Earlier detection of malignancy-associated sHLH may decrease the high mortality rate in these patients with addition of etoposide to treatment protocols and additional supportive treatments such as IVIG. On the other hand, mutation analysis for HLH may be useful in malignancies associated with HLH[11,13].

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