Hemophagocytic syndrome in patients with acute myeloid leukemia undergoing intensive chemotherapy

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ABSTRACT

Hemophagocytic lymphohistiocytosis is a condition of immune dysregulation characterized by severe organ damage induced by a hyperinflammatory response and uncontrolled T-cell and macrophage activation. Secondary hemophagocytic lymphohistiocytosis typically occurs in association with severe infections or malignancies. Patients with acute myeloid leukemia may be prone to develop hemophagocytic lymphohistiocytosis because of an impaired immune response and a high susceptibility to severe infections. In a series of 343 patients treated by intensive chemotherapy over a 5-year period in our center, we identified 32 patients (9.3%) with fever, very high ferritin levels, and marrow hemophagocytosis (i.e. patients with hemophagocytic lymphohistiocytosis). Compared to patients without hemophagocytic lymphohistiocytosis, these 32 patients had hepatomegaly, pulmonary or neurological symptoms, liver abnormalities, lower platelet count and higher levels of C-reactive protein as well as prolonged pancytopenia. A microbial etiology for the hemophagocytosis was documented in 24 patients: 14 bacterial infections, 9 *Herpesviridae* infections and 11 fungal infections. The treatment of hemophagocytic lymphohistiocytosis consisted of corticosteroids and/or intravenous immunoglobulins along with adapted antimicrobial therapy. Patients with hemophagocytic lymphohistiocytosis had a median overall survival of 14.9 months, which was significantly shorter than that of patients without hemophagocytic lymphohistiocytosis (22.1 months) (P=0.0016). Hemophagocytic lymphohistiocytosis was significantly associated with a higher rate of induction failure, mainly due to deaths in aplasia. Hemophagocytic lymphohistiocytosis can be diagnosed in up to 10% of patients with acute myeloid leukemia undergoing intensive chemotherapy and is associated with early mortality. Fever, very high ferritin levels and marrow hemophagocytosis represent the cornerstone of the diagnosis. Further biological studies are needed to better characterize and recognize this syndrome in patients with acute myeloid leukemia.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a condition of immune system dysregulation characterized by both severe inflammation and uncontrolled activation of T cells and macrophages inducing severe, sometimes fatal, organ damage observed in the bone marrow, liver, and central nervous system.[1] Patients with HLH have rapid deterioration of their general condition, fever, hepatosplenomegaly, profound pancytopenia, disseminated intravascular coagulation, very high ferritin levels and hemophagocytosis in the bone marrow, spleen or lymph nodes. Polyadenopathy, jaundice, rash, and neurological symptoms are less frequent. This clinical syndrome can be encountered in association with various underlying diseases, including inherited genetic defects (primary or familial HLH), malignancies, severe infections and autoimmune disorders (secondary HLH). Primary HLH often occurs in young children with inherited defects in genes of the perforin cytotoxic pathway such as *PRF1*, *UNC13D*, *Munc 18-2*, *Rab27a*, *STX11*, *SH2D1A* and *BIRC4*. Secondary HLH typically occurs in association with severe infections (i.e, infection-associated hemophagocytic syndrome), malignancies (i.e, malignancy-associated hemophagocytic syndrome) or autoimmune disorders (i.e, macrophage activation syndrome). Diagnostic criteria have evolved over time and now combine both clinical and biological features including impaired natural killer (NK)-cell activity.[2-4] However, while primary HLH has been well characterized in light of the recent discovery of mutations in several genes involved in T-cell cytotoxic activity, it is often challenging to distinguish between true secondary HLH in adults and severe inflammatory conditions, in which features of hemophagocytosis are encountered (e.g., critically ill patients).[5-7] Moreover, hypomorphic mutations in *PRF1*, *UNC13D* and *STBXBP2* have recently been discovered in sporadic cases of HLH in adults.[8] Thus, there may be an overlap between HLH and severe inflammation in some clinical contexts. An emerging concept suggests that HLH could be a unique syndrome associated with a continuum of underlying genetic risk factors and triggered by an immune challenge of varying intensity determined by the genetic background of patients.[6]

In hematologic malignancies, HLH is classically associated with specific entities, such as T-cell or NK/T-cell lymphoma and intravascular large B-cell lymphoma, or induced by treatment-related bacterial, viral or fungal infections and is, thus,

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frequently described as malignancy- or infection-associated hemophagocytic syndrome.[9-11] In patients with acute myeloid leukemia (AML), HLH has been occasionally described in case-reports. AML patients may be prone to develop HLH due to their disease- and/or treatment-related impaired immune response and their high susceptibility to severe infections, which act as triggering factors.[12] Alerted by several cases of HLH in our department, we sought to determine the frequency and patterns of HLH presentation as well as its impact on prognosis in a series of consecutive AML patients treated with intensive chemotherapy.

Methods

Patients

Between January 1, 2006, and December 31, 2010, all consecutive patients with a new diagnosis of AML (except acute promyelocytic leukemia) admitted to our center and eligible for intensive chemotherapy were registered for this study. The diagnostic workup and treatment modalities have been described elsewhere.[13,14] All patients had a central venous catheter placed and were given bacterial digestive tract decontamination, antibiotic therapy for febrile neutropenia (piperacillin-tazobactam/amikacin and imipenem/vancomycin/ciprofloxacin as first- and second-line treatments, respectively) and antifungal prophylaxis with posaconazole. Clinical and biological data were recorded by four of the authors (KD, AS, SB and CR). Biological data, including fibrinogen, C-reactive protein, ferritin and triglyceride levels were assessed at diagnosis and every week thereafter. Bone marrow aspiration was performed routinely at diagnosis, on day 15 of induction chemotherapy (for patients <60 years old), assessment of response (~day 35), in cases of unexpected prolonged cytopenias (> 35 days) or in suspected cases of HLH (i.e, patients receiving antimicrobial treatments for febrile neutropenia, and/or sudden increases in ferritinemia and/or unexpected cytopenias). The presence of features of hemophagocytosis was recorded regardless of clinical presentation. Cytological analysis of May–Grünwald–Giemsa-stained bone marrow smears was performed routinely. Hemophagocytosis was defined by evidence of macrophage-dependent phagocytosis of erythrocytes, leukocytes, platelets and/or their precursors, regardless of the percentage of macrophages (*Online Supplementary Figure S1*). The findings of the bone marrow aspirates for each patient were discussed between cytologists and physicians during weekly meetings. This study was approved by the Institutional Ethics Committee.

Study population

Three hundred and forty-three patients were included. Their characteristics at diagnosis of AML are shown in Table 1. The criteria used to assign patients to the HLH group were hemophagocytosis in the bone marrow with unexplained fever under broad antimicrobial treatment, and/or sudden increases in ferritin and/or unexpected non-blastic pancytopenia. Twenty-nine patients fulfilled these criteria. Three other patients without bone marrow hemophagocytosis had bio-clinical evidence strongly suggestive of a diagnosis of HLH: (i) one patient with fever (40°C) for 12 days, hepatomegaly, a cutaneous rash, a rapid rise in ferritinemia (from 1200 to 21000 μg/L) and pancytopenia with a "dry tap" on bone marrow aspiration; (ii) one patient with fever, pancytopenia, neurological symptoms, hyperferritinemia (106000 μg/L), and macrophages in the cerebrospinal fluid without features of hemophagocytosis; and (iii) one patient with fever, multi-organ failure, pancytopenia and hyperferritinemia (30000 μg/L). These three patients were included in the HLH group, making the total number of patients in this group 32 (HLH^+ group). A group of 22 patients with features of hemophagocytosis in the bone marrow but without any clinical or biological symptoms of HLH was also identified (HLH^-/$HemoPh^+$ group). The remaining 289 patients neither met the criteria for HLH nor had bone marrow hemophagocytosis (HLH^-/$HemoPh^-$ group). Bio-clinical data were collected at diagnosis of AML for all patients and at the time of HLH or hemophagocytosis onset for HLH^+ and HLH^-/$HemoPh^+$ groups. Bone marrow aspiration was performed in the HLH^+ and HLH^-/$HemoPh^+$ groups

Table 1. Characteristics of patients at diagnosis of AML.

	HLH^-/$HemoPh^-$ n=289 (84.3%)	HLH^-/$HemoPh^+$ n=22 (6.4%)	HLH^+ n=32 (9.3%)
Age (years)			
Median (IQR)	59 (49-66)	55.5 (41-61)	62 (46-69)
Gender			
Male, n. (%)	166 (57.4)	12 (54.5)	19 (59.4)
Female, n. (%)	123 (42.6)	10 (45.5)	13 (40.6)
ECOG performance status			
0-1, n. (%)	196(67.8)	20 (90.9)	21 (65.6)
≥2, n. (%)	38 (13.1)	0 (0)	4 (12.5)
Unknown, n. (%)	55 (19)	2 (9.1)	7 (21.9)
AML status			
De novo, n. (%)	231 (79.9)	18 (81.8)	23 (71.9)
Secondary, n. (%)	54(18.7)	4 (18.2)	8 (25)
Unknown, n. (%)	4 (1.4)	0 (0)	1 (3.1)
Previous MDS, n. (%)	27 (9.3)	1 (4.5)	5 (15.6)
Previous autoimmune disorder, n. (%)	1 (0.4)	0 (0)	1 (3.1)
Extramedullary involvement			
Yes, n. (%)	73 (25.3)	5 (22.7)	12 (37.5)
No, n. (%)	171 (59.2)	16(72.7)	17 (53.1)
Unknown, n. (%)	45 (15.6)	1 (4.5)	3 (9.4)
WBC (10^9/L)			
Median (IQR)	10.6 (3.1-42.3)	7.4 (2.2-41.3)	7.7 (2.9-20.6)
Hemoglobin (g/dL)			
N.	289	22	31
Median (IQR)	9.6 (8.4-11.3)	11.1 (7.8-12.8)	9.9 (9-11.1)
Platelets (10^9/L)			
N.	286	21	31
Median (IQR)	70 (38-118)	96 (67-134)	53 (16-162)
Lymphocytes (10^9/L)			
N.	277	22	31
Median (IQR)	2.1 (1.1-3.6)	1.7 (1.2-6.3)	1.8 (0.9- 2.8)
Bone marrow blasts (%)			
N.	278	21	29
Median (IQR)	64.5 (38-85)	51 (37-82)	44 (24-78)
Multilineage dysplasia			
Yes, n. (%)	40 (13.8)	4 (18.2)	8 (25)
No, n. (%)	233 (80.6)	16 (72.7)	21 (65.6)
Unknown, n. (%)	16 (5.5)	2 (9.1)	3 (9.4)
Cytogenetics			
Favorable, n. (%)	26 (9)	3 (13.6)	2 (6.3)
Intermediate, n. (%)	198 (68.5)	14 (63.6)	24 (75)
Adverse, n. (%)	62 (21 .5)	5 (22.7)	5 (15.6)
Unknown, n. (%)	3 (1)	0 (0)	1 (3.1)
Ferritin (μg/L)			
N.	153	15	25
Median (IQR)	725 (362-1377)	384 (188-1074)	685 (392-1594)

Total percentages differ from 100% because of rounding. HLH: hemophagocytic lymphohistiocytosis; HemoPh+: bone marrow hemophagocytosis; MDS: myelodysplastic syndrome; IQR: interquartile range.

at: (i) clinical suspicion of HLH (respectively, n=14, 44% *versus* n=0); (ii) prolonged pancytopenia (n=13, 41% *versus* n=3, 14%); and (iii) systematically at diagnosis (n=1, 3% *versus* n=2, 9%) or for response evaluation (n=4, 13% *versus* n=17, 77%) (*P*<0.0001).

Endpoints and statistical analysis

The primary endpoint of the study was overall survival. Other outcomes evaluated were duration of neutropenia, response to treatment, resistant disease, relapses, and death. The endpoints and statistical analysis are described in the *Online Supplementary Material*.

Results

Characteristics of patients at diagnosis of acute myeloid leukemia

As shown in Table 1, there were no differences in the main clinical and biological parameters between the three groups at the time of AML diagnosis. Of note, the level of ferritin was above the upper normal limit in most patients, the median ferritin levels being 725 (IQR, 362-1377), 384 (IQR, 188-1074) and 685 (IQR, 392-1594) μg/L in the HLH$^-$/HemoPh$^-$, HLH$^-$/HemoPh$^+$ and HLH$^+$ groups, respectively. All patients received intensive chemotherapy for remission induction, including daunorubicin + cytarabine (n=146), idarubicin + cytarabine (n=50), idarubicin + cytarabine + lomustine (n=101), time sequential induction with daunorubicin and cytarabine (n=13) or idarubicin + high-dose cytarabine (n=11). Other drugs, such as gemtuzumab ozogamycin (n=19), fludarabine (n=1) and imatinib (n=2) were added occasionally.

Characteristics of patients at diagnosis of hemophagocytic lymphohistiocytosis or bone marrow hemophagocytosis

Twenty-two patients developed HLH during first induction chemotherapy, seven during consolidation and three during salvage therapy after relapse. The median time from the diagnosis of AML diagnosis to the onset of HLH was 40 days (IQR, 27-71) in the 22 patients who developed HLH during first induction chemotherapy. Table 2 compares the characteristics of HLH$^+$ and HLH$^-$/HemoPh$^+$ patients at diagnosis of HLH$^+$ or HLH$^-$/HemoPh$^+$, respectively. Clinically, patients in the HLH$^+$ group had fever (81%), hepatomegaly (21.9%) and respiratory symptoms (59.4%) significantly more often than HLH$^-$/HemoPh$^+$ patients (Table 2). Splenomegaly (18.8%), jaundice (25%), rash (18.8%) and neurological symptoms (12.5%) also occurred frequently in the HLH$^+$ group, albeit not significantly more frequently than in the HLH$^-$/HemoPh$^+$ group. Biologically, HLH$^+$ patients had significantly lower platelet counts, prothrombin time and serum albumin level as well as higher levels of C-reactive protein, whereas there were no differences in serum sodium, creatinine, triglyceride and fibrinogen levels between the two groups. Liver abnormalities were significantly associated with HLH, as indicated by higher levels of bilirubin and markers of cholestasis, including γ glutamyltransferase and alkaline phosphatase.

Variations of ferritin levels

At the time bone marrow aspiration was performed for the diagnosis of HLH or assessment of response, the median serum ferritin level was 5093 μg/L (IQR, 2826-11307) in HLH$^+$ patients compared to 1866.5 μg/L (IQR, 660-2789) in HLH$^-$/HemoPh$^+$ patients (*P*=0.0004) (Table 3). During the period following the bone marrow aspiration (until response to HLH treatment or death), the median maximal value of ferritin was 6953.5 μg/L (IQR, 4425.5-15305.5) in the HLH$^+$ group compared to 3373 μg/L (IQR, 1808-5149) in the HLH$^-$/HemoPh$^+$ patients (*P*=0.0071). Moreover, 15 patients (47%) in the HLH$^+$ group and only two (11%) in the HLH$^-$/HemoPh$^+$ group had a ferritin level higher than 8000 μg/L (*P*=0.0104). We also studied the variation in serum ferritin levels in the week prior to the diagnosis of

Table 2. Comparisons of HLH$^+$ and HLH$^-$/HemoPh$^+$ patients at diagnosis of HLH$^+$ or HemoPh$^+$.

	HLH$^+$ n=32	HLH-/HemoPh$^+$ n=22	*P* value
Fever, n. (%)	26 (81.3)	6 (27.3)	0.0001
Splenomegaly, n. (%)	6 (18.8)	0 (0)	0.0706
Hepatomegaly, n. (%)	7 (21.9)	0 (0)	0.0335
Icterus, n. (%)	8 (25)	1 (4.5)	0.0666
Rash, n. (%)	6 (18.8)	1 (4.5)	0.2197
Neurological symptoms, n. (%)	4 (12.5)	0 (0)	0.1368
Respiratory symptoms, n. (%)	19 (59.4)	2 (9.1)	0.0002
WBC (10^9/L)-Median (IQR)	0.4 (0.2- 1.6)	0.9 (0.5-4.1)	0.0723
Hemoglobin (g/dL)-Median (IQR)	9 (8.3-9.7)	9.7 (9- 10.8)	0.0628
Platelets (10^9/L)-Median (IQR)	18.5 (11- 28)	47.5 (12-155)	0.0494
Prothrombin time (%)			
N.	30	20	0.0013
Median (IQR)	66.5 (57- 78)	84 (69.5-98)	
Fibrinogen			
N.	26	18	0.1042
Median (IQR)	5.1 (4.2-5.9)	4.4 (3.1-5.3)	
Natremia (mmol/L) -Median (IQR)	135.5(133-138)	138 (134-139)	0.2351
Triglycerides (mmol/L)			
N.	30	22	0.3788
Median (IQR)	1.3 (0.9- 2.5)	1.2 (0.8-1.8)	
Creatinine (>1.5 x ULN)-n (%)	5 (15.6)	1 (4.5)	0.3826
Albumin (g/L)			
N.	29	22	0.0005
Median (IQR)	27 (25- 30)	33 (29-37)	
AST (IU/L)-Median (IQR)	31.5 (18- 103.5)	26 (19-38)	0.1835
ALT (IU/L)-Median (IQR)	31 (15.5-107.5)	29 (16-37)	0.2241
AST or ALT (>5 x ULN)-n (%)	7 (21.9)	0 (0)	0.0335
Alkaline phosphatase (IU/L)			
Median (IQR)	427 (251.5-635.5)	214 (175-276)	0.0005
>2 x ULN, n. (%)	10 (31.3)	1 (4.5)	0.0189
γGT (IU/L)			
Median (IQR)	213 (136-333.5)	59.5(34-119)	0.0001
>5 x ULN, n. (%)	19 (59.4)	3 (13.6)	0.0008
Bilirubin (μmol/L)			
N.	30	21	0.0066
Median (IQR)	13.5 (10-49)	10 (8-11)	0.0328
>ULN, n. (%)	13(40.6)	3 (13.6)	
C-reactive protein (mg/L)			
N.	30	19	0.0005
Median (IQR)	115.5 (57- 178)	16 (5- 92)	

HLH: hemophagocytic lymphohistiocytosis; HemoPh+: bone marrow hemophagocytosis. WBC: white blood cells; ULN: upper limit of normal; AST: aspartate transaminase; ALT: alanine transaminase; γGT: gamma-glutamyltranspeptidase; N is only mentioned when there are unknown data. Bio-clinical data were collected at the onset of HLH or hemophagocytosis in the HLH$^+$ and HLH$^-$/HemoPh$^+$ groups.

HLH in 22 patients or prior to hemophagocytosis on bone marrow aspiration in 12 HLH$^-$/HemoPh$^+$ patients. The median increase in ferritin was 1421 µg/L (IQR, -287-8746) and 73.5 µg/L (IQR, -836-802.5) in HLH$^+$ and HLH$^-$/HemoPh$^+$ patients, respectively (P=0.1395). The median number of red cell packs received by HLH$^+$ patients in the 3-month period before the diagnosis of HLH was 14 (IQR, 8-20) compared to 7 (IQR, 2-10) in the HLH$^-$/HemoPh$^+$ patients (P=0.0048). There was no significant correlation between the maximal level of serum ferritin and the number of red cell packs in HLH$^+$ patients (Spearman correlation coefficient, Rho=-0.14; P=0.46).

Etiology

A potential infectious etiology functioning as a trigger for HLH was found in 24 patients (75%): 14 had bacterial infections, which were mainly septicemia and pneumonia, 9 had *Herpesviridae* infections and 11 had fungal infections, which were mainly invasive aspergillosis (Table 4). No mycobacterial infections were documented. During the induction phase, bacterial or fungal infections were documented in 15 HLH$^+$ patients (46.9%) and in 91 HLH$^-$/HemoPh$^-$ patients (31.5%) (P=0.079). Parenteral nutrition and growth factors have occasionally been associated with hemophagocytosis.[15,16] Parenteral nutrition was delivered to ten HLH$^+$ (31%) and six HLH$^-$/HemoPh$^+$ (27%) patients (P=0.75). Before the diagnosis of HLH and bone marrow hemophagocytosis, 20 HLH$^+$ (62.5%) and 6 HLH$^-$/HemoPh$^+$ (27%) patients had received granulocyte colony-stimulating factor (P=0.01).

Treatment of hemophagocytic lymphohistiocytosis

Twenty-nine patients (90.6%) received a specific treatment for HLH in a median time of 24 h after diagnosis. First-line treatment was corticosteroids (CS) in five patients, intravenous immunoglobulins (IVIg) in 16 patients, CS + IVIg in five patients, CS + etoposide in two patients and CS + IVIg + cyclosporine in one patient. Overall, CS and IgIV were used as the first-line treatment in 13 (44%) and 22 (76%) patients, respectively. The distribution of treatment modalities according to the type of infection associated with HLH is shown in Figure 1. No patient with invasive fungal infection received CS as part of the first-line treatment. Patients could receive second-line treatment with CS, IVIg or cyclosporine according to initial response. Although no response criteria are specifically defined for HLH, we judged that rapid disappearance of fever (within 48 h) and resolution of cytopenias were the most relevant markers of response. According to our data, the decrease in ferritin in the first few days (day 0 to day 7 or day 15) following treatment of HLH$^+$ patients (n=29), did not enable differentiation between responders and non-responders. Of the 13 patients treated with CS, nine (69%) had complete resolution of fever whereas only six out of 16 patients (37.5%) treated by IVIg alone became apyretic. Improvement of cytopenias was observed in one of five (20%) patients treated with CS alone and in seven of 16 (44%) treated with IVIg alone. In the five patients treated with CS + IVIg as first-line therapy, fever resolved in four and three had improvement of cytopenias. Of note, the response rate was not associated with the type of underlying infection, being 43% (n=6; P=0.688) in patients with bacterial infections, 33% (n=3; P=0.397) in those with viral infections and 36% (n=4; P=0.388) in patients with fungal infections. Overall, 20 patients (62.5%) had resolution of symptoms after adapted antibiotic or antifungal therapy and specific treatment of the HLH.

Outcomes of patients

The median overall survival was 14.9 months (IQR, 2.9-41.7) in the HLH$^+$ group and 22.1 months (IQR, 9.2-85.6) in

Table 3. Ferritin levels in HLH$^+$ and HLH$^-$/HemoPh$^+$ patients.

	HLH+ N=32	HLH-/HemoPh+ N=22	*P* value
Ferritin (µg/L)			
At HLH/HemoPh$^+$ diagnosis			
N.	31	18	0.0004
Median (IQR)	5093 (2826-11307)	1866.5 (660-2789)	
Maximal value during evolution*			
N.	32	18	0.0071
Median (IQR)	6953.5 (4425.5-15305.5)	3373 (1808-5149)	
> 500, n. (%)	32 (100)	17 (94.4)	0.3600
> 3000, n. (%)	26 (81.3)	11 (61.1)	0.1797
> 8000, n. (%)	15 (46.9)	2 (11.1)	0.0104
> 10000, n. (%)	13 (40.6)	2 (11.1)	0.0287

*HLH: hemophagocytic lymphohistiocytosis; HemoPh+: bone marrow hemophagocytosis. *Evolution represents the time between HLH diagnosis and response to anti-HLH treatment or death.*

Table 4. Infections at diagnosis of HLH$^+$ or HemoPh$^+$.

	HLH+ N=32	HLH-/HemoPh+ N=22
Bacterial infection	14	7
Site		
Septicemia	6	2
Pneumonia	4	0
Soft tissues	2	4
Other	2	1
Bacteria		
Enterobacteria	5	0
Gram-positive cocci	3	6
Stenotrophomonas maltophlia	3	0
Other	3	1
Viral infection	9	
EBV	2	1
HHV6	4	0
HHV8	1	0
HSV1	1	1
CMV	1	0
Influenzae	0	1
Rhinovirus	0	1
Fungal infection	11	0
Invasive aspergillosis		
Lung (possible)	2	0
Lung (probable)	6	0
Sinus	2	0
Mucormycosis		
Lung	1	0

HLH: Hemophagocytic lymphohistiocytosis; HemoPh+: bone marrow hemophagocytosis. Of note, four HLH$^+$ patients had concomitant viral and bacterial infections, three had viral and fungal infections and three others had bacterial and fungal infections. Two HLH$^-$/HemoPh$^+$ patients had concomitant viral and bacterial infection.

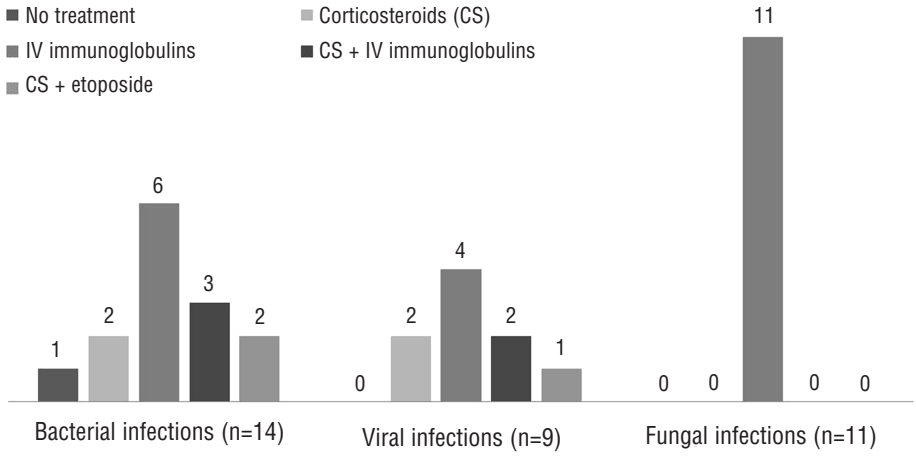

Figure 1. Distribution of anti-HLH treatments according to underlying infections. CS: corticosteroids (either prednisolone 1 mg/kg/day or dexamethasone 10 mg twice a day for 3 days then taper); IV immunoglobulins (IVIg): intravenous immunoglobulins (0.4 g/kg/day for 5 days or 1 g/kg/day for 2 days). Of note, four patients had concomitant viral and bacterial infections (1 was treated with IVIg, 2 with CS + IVIg and 1 with CS + etoposide), three had viral and fungal infections treated with IVIg and three others had bacterial and fungal infections treated with IVIg.

the HLH$^-$ group (HLH$^-$/HemoPh$^-$ and HLH$^-$/HemoPh$^+$) (log-rank test, P=0.0016) (Figure 2). In Cox proportional hazards models adjusted for prognostic factors of AML, including age, secondary AML, white blood cell count, cytogenetics, performance status and consolidation treatment by autologous or allogeneic stem cell transplantation, HLH$^+$ patients had a significantly increased risk of death compared to HLH$^-$ patients (hazard ratio, 2.05, 95% CI, 1.31 to 3.22; P=0.002) (Table 5). The adjusted risk of HLH$^-$/HemoPh$^+$ patients did not differ significantly from that of HLH$^-$/HemoPh$^-$ patients. To describe the impact of HLH in the context of AML patients in more detail, we focused on the 22 patients (and 16 patients) in whom HLH (or HLH$^-$/HemoPh$^+$) was diagnosed in the course of intensive chemotherapy for first remission induction. The duration of neutropenia tended to be longer in the HLH$^+$ patients (37 days, IQR 29-59) than in the HLH$^-$/HemoPh$^+$ patients (28.5 days, IQR 24-38; P=0.158). In the HLH$^+$ group, 9/22 patients (40.9%) reached a complete response or complete response with incomplete blood count recovery compared to 214/289 (74.1%) and 15/16 (93.8%) in the HLH$^-$/HemoPh$^-$ and HLH$^-$/HemoPh$^+$ groups, respectively (P=0.001) (Table 6). The lower response rate in the HLH$^+$ group was associated with higher rates of hypoplastic death and resistant disease, resulting in a significantly higher mortality rate at 3 months (36.4% in the HLH$^+$ group compared to 12.5% in the HLH$^-$/HemoPh$^-$ and HLH$^-$/HemoPh$^+$ groups, P=0.011).

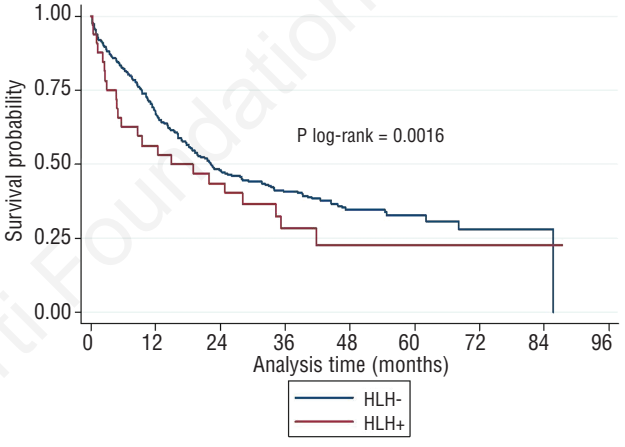

Figure 2. Overall survival of HLH$^+$ and HLH$^-$ patients. HLH: hemophagocytic lymphohistiocytosis; HemoPh$^+$: bone marrow hemophagocytosis.

Discussion

It has been recently suggested that hyperinflammatory disorders, such as HLH, macrophage activating syndrome, malignancy-associated hemophagocytic syndrome or systemic inflammatory response syndrome, could be linked by a common pathophysiology involving aberrant cytokine release, the so-called cytokine storm.[6,17] We show here that up to 10% of AML patients undergoing intensive chemotherapy had a clinical presentation consistent with HLH although the strict criteria defined for pediatric patients were not met in our series.[4] It should be emphasized that there is no current consensus on the criteria for HLH in adult patients.[18] The comparison of patients without symptoms of HLH but hemophagocytosis in the bone marrow and HLH$^+$ patients showed that hemophagocytosis itself is not a specific feature of HLH. We do acknowledge that the distinction between HLH and other febrile conditions (i.e. pure infectious complications) can be challenging in the setting of intensive chemotherapy courses. Thus, a comparison with other febrile, neutropenic AML patients would be necessary to assess the specific markers of HLH in those patients better. Unfortunately, we did not collect data from all AML patients at the time of a first febrile episode during the period of this study so we could not perform such a comparison. However, virtually all AML patients undergoing intensive chemotherapy are neutropenic for a long period of time (usually 21 days) and it is quite exceptional that these patients remain afebrile.

In our database including 643 patients undergoing intensive chemotherapy between 2000 and 2010, approximately 30% had documented infections (136 patients had bacterial and 60 had fungal infections).[13] In this series, bacterial or fungal infections were documented in 31.5% of HLH$^-$/HemoPh$^-$ patients during the induction phase, indicating that a substantial proportion of neutropenic patients with documented infections did not develop the particular clinical picture of HLH$^+$ patients. Thus, we feel confident that the clinical picture we describe here in roughly 10% of

patients meets *bona fide* criteria to distinguish HLH patients from other neutropenic AML patients. Furthermore, most of the classical criteria of HLH seem unsuitable when applied to leukemia due to the nature of the disease. This is especially the case for pancytopenia related to bone marrow involvement and intensive chemotherapy and the ferritin levels that are nearly always greater than 500 μg/L at the time of AML diagnosis and further increase as a result of repeated red blood cell transfusions.

Overall, we have identified criteria that could help to identify HLH in AML patients (Table 7). These criteria could be further improved by specific prospective studies assessing T-cell and NK-cell activity and sCD25, genetic screening and cytokine profiling in the setting of AML patients treated by intensive chemotherapy. Indeed, it will be interesting to determine whether these patients have a genetic background (mild hypomorphic mutations, polymorphisms or complex polygenic traits) predisposing them to HLH during intensive chemotherapy.[17] Additionally, a large microbiological screen including viral testing should be part of the initial workup in HLH$^+$ patients. In this study, the onset of HLH in the course of AML therapy had a strong impact on prognosis as shown by the multivariate analysis. We show that HLH$^+$ patients had higher rates of death in aplasia and resistant disease as well as a shorter overall survival. As various infectious causes have been identified, with an overrepresentation of *Herpesviridae* and invasive aspergillosis, these early deaths are probably due to severe infections. However, HLH induced liver and/or pulmonary involvement and prolonged pancytopenia that undoubtedly worsened these infections. Although not significant in our study, it is also noteworthy that the rate of failure of induction therapy was higher in HLH$^+$ patients, suggesting that uncontrolled disease could also have affected survival. The fact that fewer patients with HLH failed to achieve a complete response could underline the role of the malignant disease as a trigger for HLH. Indeed, one could argue that HLH is a surrogate marker for failure of chemotherapy. Another somewhat provocative hypothesis is that the inflammatory response could improve leukemia cell survival and subsequent resistance to chemotherapy.

It is, therefore, critical to detect the onset of HLH early in AML patients. A typical presentation is a patient with a high fever even under broad-spectrum antibiotic treatment, a rapid rise in ferritin levels (sometimes to extraordinarily high levels) and liver abnormalities. This clinical picture should suggest bone marrow aspiration for determination of hemophagocytosis, although this criterion is not an absolute prerequisite for diagnosis. An extensive workup for infections is also required to maximize antimicrobial treatment since we documented an infectious agent as a potential trigger of HLH in 75% of cases. HLH treatments, which should be started promptly, remain challenging, particularly in aplastic patients who have received intensive chemotherapy. CS, etoposide-based regimens, cyclosporine and antithymocyte globulin are currently administered to pediatric patients.[18]

Table 5. Multivariate Cox proportional-hazards model for overall survival in HLH$^+$ patients *versus* HLH$^-$ patients (including HLH$^-$/HemoPh$^+$ and HLH$^-$/HemoPh$^-$).

	Hazard Ratio	95% Confidence Interval	*P* value
HLH-	1.00		
HLH+	2.05	1.31-3.22	0.002
Age	1.01	1.00-1.02	0.028
De novo AML	1.00		
Secondary AML	1.63	1.18-2.26	0.003
WBC ≤ 50x10^9/L	1.00		
WBC > 50x10^9/L	1.64	1.16-2.30	0.005
Favorable cytogenetic risk	1.00		
Intermediate cytogenetic risk	1.96	1.01-3.83	0.047
Adverse cytogenetic risk	3.52	1.72-7.19	0.001
WHO performance status=0	1.00		
WHO performance status=1	1.32	0.93-1.87	0.116
WHO performance status=2	1.84	1.10-3.11	0.022
WHO performance status=3	1.92	0.89-4.15	0.096
No SCT	1.00		
Autologous SCT	0.32	0.14-0.73	0.007
Allogeneic SCT	0.63	0.42-0.95	0.027

HLH: hemophagocytic lymphohistiocytosis; HemoPh$^+$: bone marrow hemophagocytosis. WBC: white blood cell count; SCT: stem cell transplantation.

Table 6. Outcome of patients after induction remission chemotherapy.

	HLH$^-$/HemoPh$^-$ n=289	HLH$^-$/HemoPh$^+$ n=16	HLH$^+$ n=22	*P* value
Response, n. (%)				
CRi	33 (11.4)	0 (0)	5 (22.7)	0.093
CR	181 (62.6)	15 (93.8)	4 (18.2)	<0.001*
CR or CRi	214 (74.1)	15 (93.8)	9 (40.9)	0.001**
Induction failure, n. (%)				
Early deaths	11 (3.8)	0 (0)	0 (0)	1
Deaths in aplasia	15 (5.2)	0 (0)	7 (31.8)	<0.001***
Resistant disease	40 (13.9)	1 (6.3)	6 (27.3)	0.167
Relapse[&], n. (%)	107 (48.2)	5 (33.3)	5 (62.5)	0.387
3-month mortality, n. (%)	36 (12.5)	2 (12.5)	8 (36.4)	0.011****

*HLH: hemophagocytic lymphohistiocytosis; HemoPh+ : bone marrow hemophagocytosis; CRi: complete response with incomplete blood count recovery; CR: complete response. [&]In patients with CR or CRi or complete response with two cycles of induction. *According to Bonferroni correction* P *value=0.033 for HLH-/HemoPh-* vs. *HLH-/HemoPh+*, P *value<0.001 for HLH-/HemoPh-* vs. *HLH+ and* P *value<0.001 for HLH-/HemoPh+* vs. *HLH+. **According to Bonferroni correction* P *value=0.396 for HLH-/HemoPh- vs HLH-/HemoPh+*, P *value=0.003 for HLH-/HemoPh-* vs. *HLH+ and* P *value=0.003 for HLH-/HemoPh+* vs. *HLH+. ***According to Bonferroni correction* P *value=1.000 for HLH-/HemoPh-* vs. *HLH-/HemoPh+*, P *value<0.001 for HLH-/HemoPh-* vs. *HLH+ and* P *value=0.042 for HLH-/HemoPh+* vs. *HLH+. ****According to Bonferroni correction* P *value=1.000 for HLH-/HemoPh-* vs. *HLH/HemoPh+*, P *value=0.018 for HLH-/HemoPh-* vs. *HLH+ and* P *value=0.429 for HLH-/HemoPh+* vs. *HLH+.*

Table 7. Proposed criteria for the diagnosis of HLH in AML patients.

AML patient undergoing intensive chemotherapy
and
Fever
Hepatosplenomegaly
Ferritin > 5000 μg/L
Prolonged neutropenia and/or thrombocytopenia (outside the expected range for chemotherapy-induced myelosuppression, on day 35)
Liver abnormalities

*These criteria should lead to the performance of bone marrow aspiration to reveal hemophagocytosis. The proposed criteria should be improved by classical criteria for HLH such as genetic defects in genes of the cytotoxic pathway (*PRF1, UNC13D, STXBP2, RAB27A, STX11, SH2D1A, *or* XIAP*), natural killer cell activity and soluble CD25.*

Immunomodulation with IVIg has also been used in virus-associated HLH.[19] Because of the profound pancytopenia induced by chemotherapy, etoposide was used only exceptionally in our patients. In fact, most were treated with CS (either prednisolone or dexamethasone) or IVIg depending on the underlying infection. Although our study does not establish a standard of care in this situation, we believe that the combination of IVIg and CS (short pulse then tapering) could be the treatment of choice. Indeed, CS induced a rapid improvement in symptoms and overall condition, while IVIg were associated with a better survival in HLH$^+$ patients (*data not shown*). Other rational therapies, such as anti-cytokines (e.g., tocilizumab), should be assessed in this setting.[20]

Our study also revealed quite unexpectedly that patients with bone marrow hemophagocytosis but without HLH did particularly well in terms of complete response and overall survival, which was a median of 85.6 months (IQR, 27.9-85.6). Bone marrow aspiration was performed in most of the HLH$^-$/HemoPh$^+$ patients to assess the response to chemotherapy. Although it is speculative, hemophagocytosis in this setting could be a marker of innate immunity against leukemic cells. Indeed, macrophages express signal regulatory protein alpha (SIRPα) which, when activated by its ligand, CD47, induces intracellular signaling resulting in inhibition of phagocytosis.[21] It has been demonstrated that increased CD47 expression by leukemic stem cells inhibits macrophage activity and is associated with a poor prognosis in AML.[22,23] Conversely, patients with low CD47 expression are more chemosensitive, suggesting that there may be a correlation between marrow hemophagocytosis and CD47 expression in AML patients.

To conclude, HLH can be encountered in up to 10% of AML patients undergoing intensive chemotherapy and is associated with induction failure and early mortality. Fever, a very high ferritin level and marrow hemophagocytosis represent the cornerstones of the diagnosis, which must be rapidly established to adapt antimicrobial treatments and to introduce immunomodulation promptly. Further research, using genetic screening, cytokine monitoring or T-cell and NK-cell functional studies, is needed to characterize this syndrome better in AML patients.

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