Outcome of primary hemophagocytic lymphohistiocytosis: a report on 143 patients from the Italian Registry

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Abstract

Primary hemophagocytic lymphohistiocytosis (pHLH) is a severe, life-threatening hyperinflammatory syndrome caused by defects in genes of the granule-dependent cytotoxic pathway. Here we investigated the clinical presentation and outcome in a large cohort of 143 patients with pHLH diagnosed in the last 15 years and enrolled in the Italian registry. The median age at diagnosis was 12 months (interquartile range, 2-81), and 92 patients (64%) fulfilled the HLH-2004 criteria. Of 111 patients who received first-line combined therapy (HLH-94, HLH-2004, Euro-HIT protocols), 65 (59%) achieved complete response and 21 (19%) partial response. Thereafter, 33 patients (30%) reactivated, and 92 (64%) received hematopoietic stem cell transplantation, 78 of whom (85%) survived and were alive at a median follow-up from diagnosis of 67 months. Thirty-six patients (25%) died before hematopoietic stem cell transplantation and 14 (10%) after. Overall, 93 patients (65%) were alive after a median follow-up of 30 months. Unadjusted predictors of non-response were age <6 months and high ferritin and bilirubin levels, while predictors of pre-transplant and overall mortality were high ferritin and bilirubin levels. At multivariable analysis, high levels of ferritin predicted non-response, while high levels of bilirubin predicted pre-transplant and overall mortality. Despite recent advances in therapeutic management, pHLH remains a life-threatening condition with significant early mortality. Liver dysfunction is the main predictor of poor prognosis.

Introduction

Primary hemophagocytic lymphohistiocytosis (pHLH) is a rare and severe genetic syndrome first described in 1952.[1] It encompasses different monogenic disorders caused in most cases by defects in genes of the granule-dependent cytotoxic pathway of natural killer (NK) or CD8 T cells, which were first described in the late '90s.[2-6] Before that, the diagnosis of pHLH relied on the presence of clinical and laboratory criteria, the demonstration of deficient NK-cell

function, and the anamnestic finding of familial recurrence of the syndrome. Since the "genetic era", pHLH is classified as follows, based on the detected mutation: familial hemophagocytic lymphohistiocytosis (FHL), defined by biallelic mutations of *PRF1* (FHL2), *UNC13D* (FHL3), *STX11* (FHL4), or *STXBP2* (FHL5); pigmentary disorders associated with HLH, defined by mutations of *RAB27A* (Griscelli syndrome type 2, GS2), *LYST* (Chediak-Higashi syndrome, CHS), or *AP3B1* (Hermansky-Pudlak syndrome type 2, HPS2); X-linked lymphoproliferative disease (XLP), caused by mutations of *SH2D1A* (XLP1) or *XIAP* (XLP2). Other HLH-associated syndromes include gain-of-function mutations of *NLRC4*, *CDC42* heterozygous mutations, Epstein-Barr virus (EBV) susceptibility diseases other than XLP, primary immune deficiencies, and inborn errors of metabolism.[7,8]

The clinical presentation of pHLH is indistinguishable from that of secondary HLH, and includes fever, cytopenias, and hepatosplenomegaly in most cases. Involvement of the central nervous system (CNS) and multiorgan failure (typically affecting the liver) are also common. Typical laboratory abnormalities include low fibrinogen, high triglycerides, and abnormal elevation of ferritin and soluble IL2 receptor (sIL2R). Moreover, before genetic testing is performed, pHLH in patients with FHL2-5 or pigmentary disorders can be postulated based on the demonstration by flow cytometry of impaired either intracytoplasmic perforin or surface CD107a expression by NK and CTL cell.[9]

Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for pHLH. A chemotherapy regimen based on the combination of etoposide and dexamethasone has been developed in two consecutive trials promoted by the Histiocyte Society as a bridge to HSCT,[10,11] and is now considered the standard of care for the disease. Alternative therapeutic strategies include an immunotherapeutic approach based on antithymocyte globulin (ATG) and methylprednisolone[12] and, more recently, biologic drugs such as alemtuzumab,[13] emapalumab,[14] and ruxolitinib.[15] Nevertheless, pHLH remains a difficult-to-treat disease with high early mortality and long-term disabilities.

In the last decades, there has been a rapid evolution of diagnostic strategies, allowing more rapid genetic diagnosis in patients with HLH syndrome. However, many aspects of the syndrome, in terms of presentation and outcome of the genetic forms, remain to be understood. Herein, we analyzed a large cohort of patients with pHLH diagnosed in the last 15 years and enrolled in the Italian HLH registry, to analyze their clinical presentation and long-term outcome. In addition, we sought to identify predictors of poor response and of early and late mortality.

Methods

Study population

Clinical data and biologic samples from patients who received a diagnosis of HLH[16] are collected in the Italian HLH registry held at the Meyer Children's Hospital in Florence, Italy. For the purposes of the present study, we retrieved the registry data on patients who received a genetic diagnosis of pHLH in the last 15 years. Data on genetic testing are reported in the *Online Supplementary Appendix*. Patients diagnosed after 2007 were eligible for study inclusion if they had available data on clinical and laboratory presentation, and outcome, with a minimum follow-up duration of 12 months. Patients referred to our laboratory from other countries or with unavailable follow-up data were excluded. Although most patients included in the registry were infants or children, also adults were considered for study inclusion if they met the inclusion criteria.

Data collection

Baseline data included demographics, family history, and clinical features of patients at diagnosis. The complete laboratory assessment, as recommended by the HLH-2004 diagnostic criteria (full blood count, liver enzymes, triglycerides, fibrinogen, ferritin, sIL2) was also included. Follow-up data included information on treatment received (including HSCT), response to treatment, disease reactivation, *sequelae*, and the status at the last follow-up. All these data were pseudo-anonymized, collected in dedicated forms, and stored in an electronic, password-protected database. Response to first-line treatment was categorized as complete response (CR), partial response (PR), and non-response (NR). Details on outcome definition are provided in the *Online Supplementary Appendix*.

Statistical analysis

Continuous variables were presented as median and interquartile range (IQR) while categorical variables as absolute number and percentage. For continuous variables, multiple-group comparisons were performed by the Kruskal-Wallis test, while for two-group comparisons the Mann–Whitney U test was used. For the analysis of frequency, statistical analyses were performed using χ^2 test, if applicable, or Fisher's exact test with the associated odds ratio (OR). For each OR, a 95% confidence interval (95% CI) was applied. For tables larger than 2X2, Fisher's exact test with hybrid approximation and Monte Carlo simulation were used to compute the *P* value. For time-to-event (survival analysis) we designed Kaplan-Meier curves with the log-rank test. In addition, the Cox proportional hazards regression model was used for multivariate analysis. For multivariate analyses, we also applied generalized linear models (binomial and Gaussian family). Some continuous variables were binarized by drawing a ROC curve using the maximum sum of sensitivity and specificity to obtain an optimal threshold or applying a threshold based on relevant clinical values. Two-sided $P<0.05$ was considered significant. Statistical analysis was performed using R software version 4.2.2.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and its later amendments.[17] Written informed consent was obtained for all patients included in the registry.

Results

Three hundred and eighty-one patients with pHLH were included in the Italian HLH registry. Of them, 116 were excluded because they were diagnosed before 2007, 42 because they were not followed in a national center, 63 because of insufficient follow-up data, 12 because they had received a *post mortem* diagnosis, and five because of a revision of the genetic diagnosis. Finally, 143 patients were included in the study (Table 1).

Patients' characteristics at diagnosis

The baseline characteristics of the 143 included patients are detailed in Table 1. The median age at diagnosis was 12 months (interquartile range [IQR], 2-81). While most patients were diagnosed before the second year of life, 14 patients (10%) were older than 14 years at diagnosis and 11 (8%) were adults. Around two thirds of patients were males (N=88, 62%). They were in most cases of Caucasian origin (N=113, 79%), while the remaining patients originated from North Africa (N=12, 8%), the Indian subcontient (N=9, 6%), Latin America, Middle East and sub-Saharan Africa (N=3, 2%, for each of them). Thirty patients (21%) had related parents, and 38 (27%) had familial disease.

Ninety-two patients (64%) fulfilled the HLH-2004 criteria at diagnosis.[16] A hundred and twenty-eight patients (90%) had fever, 120 (84%) splenomegaly, and 84 (59%) hepatomegaly. CNS involvement (defined as neurological impairment and/or abnormalities at magnetic resonance imaging or cerebrospinal fluid analysis) was reported in 49 patients (34%). At least bilinear cytopenia was found in 97 patients (68%): the median hemoglobin level was 8 g/dL (IQR, 7.7-10), the median neutrophil count 7.6x10^9/L (IQR, 0.33-1.72), and the median platelet count 48x10^9/L (IQR, 24-100). Seventy-two patients (50%) had liver enzyme abnormalities: the median aspartate and alanine aminotransferase values were 164 and 132 U/L (IQR, 66-386 and 56-279, respectively), and the median value of total bilirubin 1.1 mg/dL (IQR, 0.5-2.9). The median values of ferritin, triglycerides, and fibrinogen were 2,538 ng/mL (IQR, 1,061-11,550), 278 mg/dL (IQR, 185-428), and 121 mg/dL (IQR, 80-228), respectively. Infectious triggers were detected in 42 patients (29%) and mostly consisted of EBV (N=21, 50%) and cytomegalovirus (N=4, 10%). Four patients were asymptomatic and were diagnosed based on familial screening.

Genetic diagnosis

The genetic diagnoses of the 143 patients are reported in Figure 1. Forty-seven patients (33%) had FHL2, 41 (29%) FHL3, two (1%) FHL4, 15 (11%) FHL5, 12 (8%) XLP1, nine (6%) XLP2, eight (6%) CHS, and nine (6%) GS2. We found

Table 1. Demographics and clinical characteristics at diagnosis.

Charactersitics	Included patients N=143
Age in months, median (IQR)	12 (2-81)
Female sex, N (%)	55 (38)
Ethnic origin, N (%) Caucasian Sub-Saharan African North African Middle East, Arabic Indian subcontinent Latin American	 113 (79) 3 (2) 12 (8) 3 (2) 9 (6) 3 (2)
Familial disease, N (%)	38 (27)
Parental consanguinity, N (%)	30 (21)
Complete HLH-2004 criteria, N (%)	92 (64)
Fever , N (%)	128 (90)
Splenomegaly, N (%)	120 (84)
Hepatomegaly, N (%)	84 (59)
CNS involvement*, N (%)	49 (34)
Cytopenia (at least bilinear), N (%)	97 (68)
Hemoglobin g/dL, median (IQR)	8 (7.7-10)
ANC x10^9/L, median (IQR)	0.76 (0.33-1.73)
PLT x10^9/L, median (IQR)	48 (24-100)
Fibrinogen mg/dL, median (IQR)	121 (80-228)
Triglycerides mg/dL, median (IQR)	278 (185-428)
Ferritin ng/mL, median (IQR)	2,538 (1,061-11,550)
ALT UI/L, median (IQR)	132 (56-279)
AST UI/L, median (IQR)	164 (66-386)
Bilirubin mg/dL, median (IQR)	1.1 (0.5-2.9)
Infectious trigger, N (%) EBV CMV HHV6-7 Parvovirus Others# NA	42 (29) 21 (50) 4 (10) 2 (5) 3 (7) 3 (7) 9 (21)

*Central nervous system (CNS) involvement is defined by the presence of at least 1 of the following: i) neurological symptoms; ii) magnetic resonance imaging abnormalities; iii) CSF abnormalities; #others include *C. difficile, E. coli, Dengue, Leishmania, M. pneumonia,* SARS-CoV-2, RSV and VZV. IQR: interquartile range; ANC: absolute neutrophil count; PLT: platelets; ALT: alanine transaminases; AST: aspartate transaminases; EBV: Epstein-Barr virus; CMV: cytomegalovirus; HHV6: human herpesvirus-6; NA: not available.

differences in baseline characteristics depending on the genetic diagnosis (*Online Supplementary Table S1; Online Supplementary Figure S2*) in terms of sex, age at diagnosis, consanguinity, and severity of clinical presentation (including the prevalence of complete HLH criteria, hematologic abnormalities, ferritin and ALT values, and infectious triggers). In particular, patients with XLP had higher age and lower rates of hematologic involvement at diagnosis (higher platelet and neutrophil count, lower rates of cytopenia). When we analyzed the distribution of the genetic diagnosis in three age groups (0-6 months, 7-24 months, and >24 months; *Online Supplementary Figure S1*), the proportion of patients with FHL2 and FHL3 remained stable in the three age groups, while most patients with XLP were older than 2 years, and patients with FHL5, CHS, and GS2 were typically younger.

Response to treatment

Thirteen patients (9%) did not receive any treatment, either for the rapidly fatal outcome (N=5), a mild clinical presentation (N=3), or the absence of symptoms (N=5). First-line therapies in the 130 treated patients included chemotherapy according to the HLH-94[10] and HLH-2004[11] trials in 29 (22%) and 75 (58%) patients, respectively, while seven patients (5%) were included in the Euro-HIT trial (EUDRACT2011-002052-14). The 19 remaining patients (15%) received steroid-based therapy (Figure 2; *Online Supplementary Table S2*).

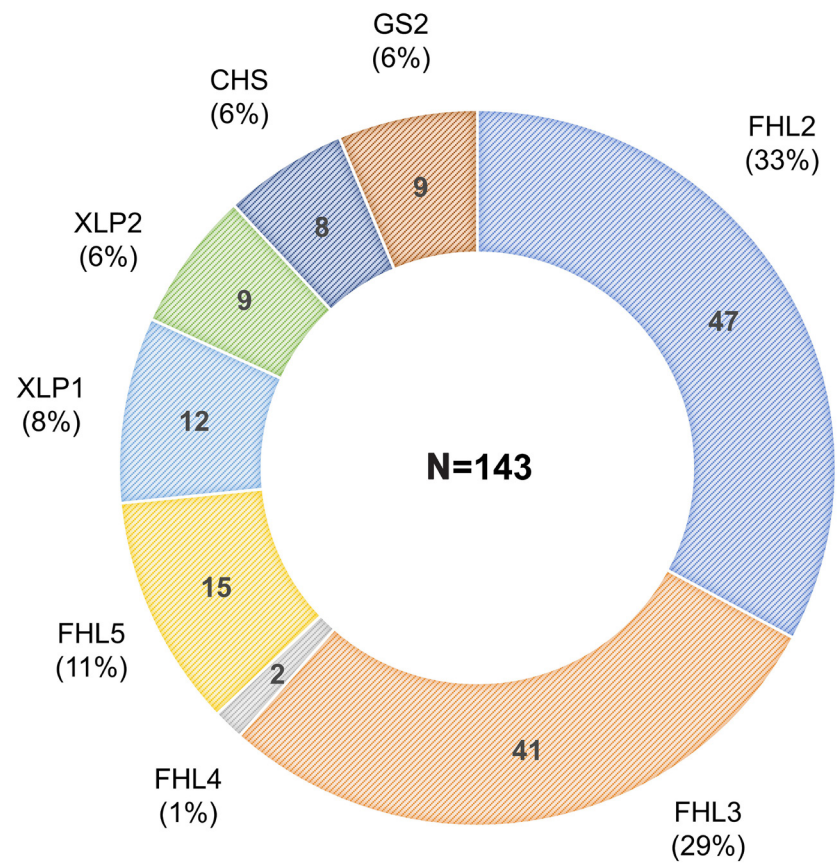

Figure 1. Distribution of the genetic diagnoses in the 143 patients. FHL2: familial hemophagocytic lymphohistiocytosis type 2; FHL3: familial hemophagocytic lymphohistiocytosis type 3; FHL4: familial hemophagocytic lymphohistiocytosis type 4; FHL5: familial hemophagocytic lymphohistiocytosis type 5; GS2: Griscelli syndrome type 2; CHS: Chediak-Higashi syndrome; XLP1: X-linked lymphoproliferative disease type 1; XLP2: X-linked lymphoproliferative disease type 2; GS2: Griscelli syndrome type 2.

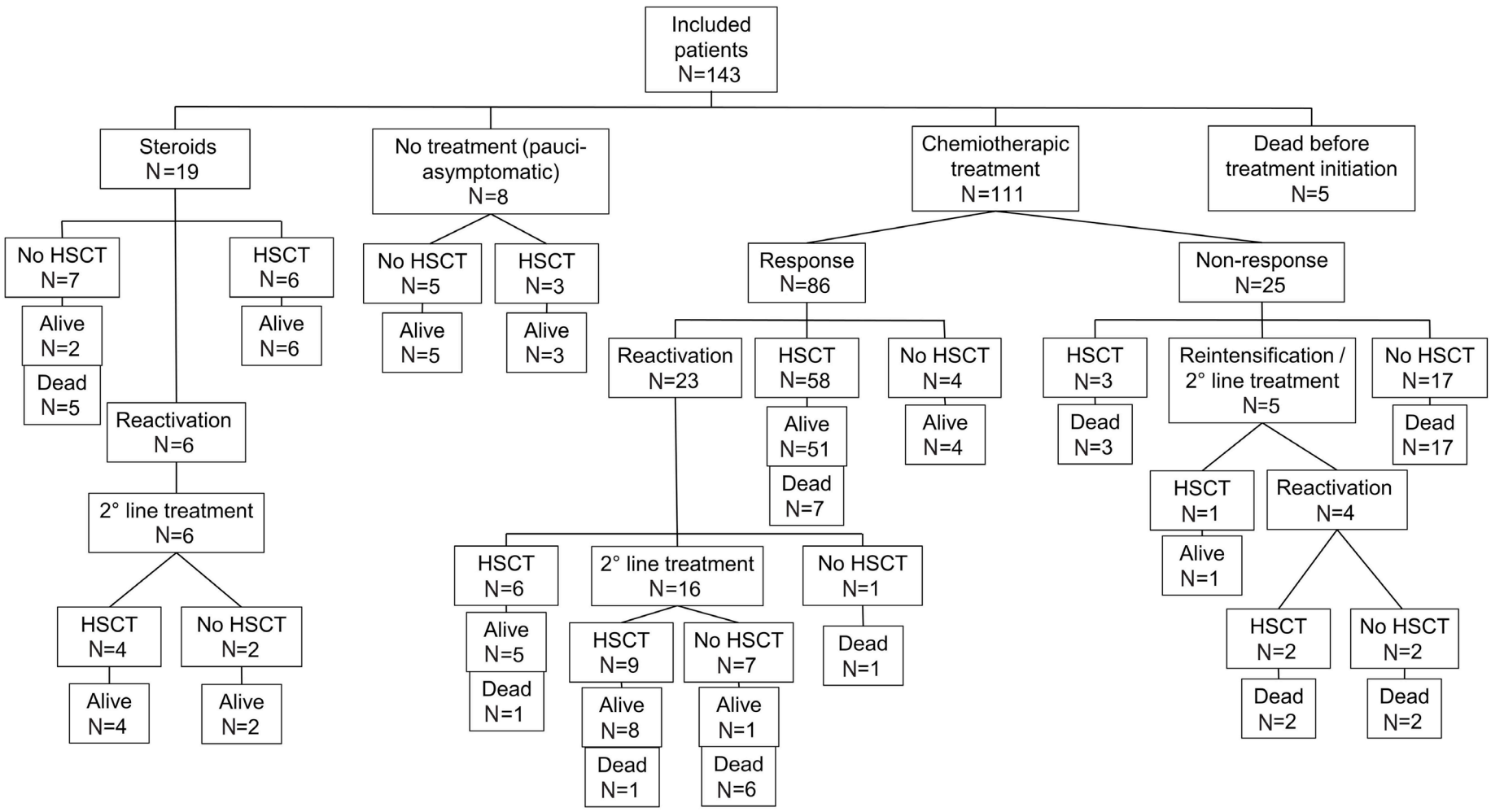

Figure 2. Flow-chart of treatment and outcome. HSCT: hematopoietic stem cell transplantation.

The overall response rate to chemotherapy was 78% (86/111; Figure 2). CR was documented in 65 of 111 patients and PR in 21 additional patients (*Online Supplementary Table S2*). In 11 of these patients, additional treatment was required: rituximab in four patients with EBV infection, ATG, ruxolitinib, and infusion of maternal NK cells in two each, and tacrolimus in one. Twenty-five of 111 patients (22%) did not respond to chemotherapy: one received emapalumab as salvage therapy and was successfully transplanted, the remaining 24 patients (including 4 patients who responded to re-intensification therapy but reactivated), died at a median follow-up of 1 month (IQR, 0-3) (Figure 2). Among patients who received a steroid-based treatment, 16 of 19 (84%) responded (11 CR, 5 PR), and five died. Of note, all these five patients had severe central nervous system (CNS) involvement at diagnosis.

Eight patients were not treated either because they refused treatment (N=1), were asymptomatic (the 4 patients diagnosed due to familial screening), or paucisymptomatic (N=3); these latter patients were transplanted and were alive at a median follow-up of 28 months (IQR, 23-67) (Figure 2).

No significant differences in terms of response to treatment or survival were detected depending on the received therapeutic protocol (*Online Supplementary Table S2*), but patients who responded to first-line treatment had older age, higher platelets, and lower ferritin values at diagnosis (Figure 3).

Reactivation

Thirty-three of 100 (33%) patients who responded to treatment reactivated, at a median time from diagnosis of 6 months (IQR, 3-11). Of them, 23 patients responded to first-line chemotherapy and then reactivated at a median time from diagnosis of 5.5 months (IQR, 2.5-9): one child died for abrupt disease progression without receiving treatment, while six received frontline HSCT and were all alive at last follow-up except for one patient that died for disease progression despite HSCT (Figure 2). The remaining 16 patients received additional treatment, including chemotherapy according to HLH-2004 (alone in 8, associated with alemtuzumab, ATG, and ruxolitinib in 1 each) and emapalumab (5 patients; *Online Supplementary Table S3*).

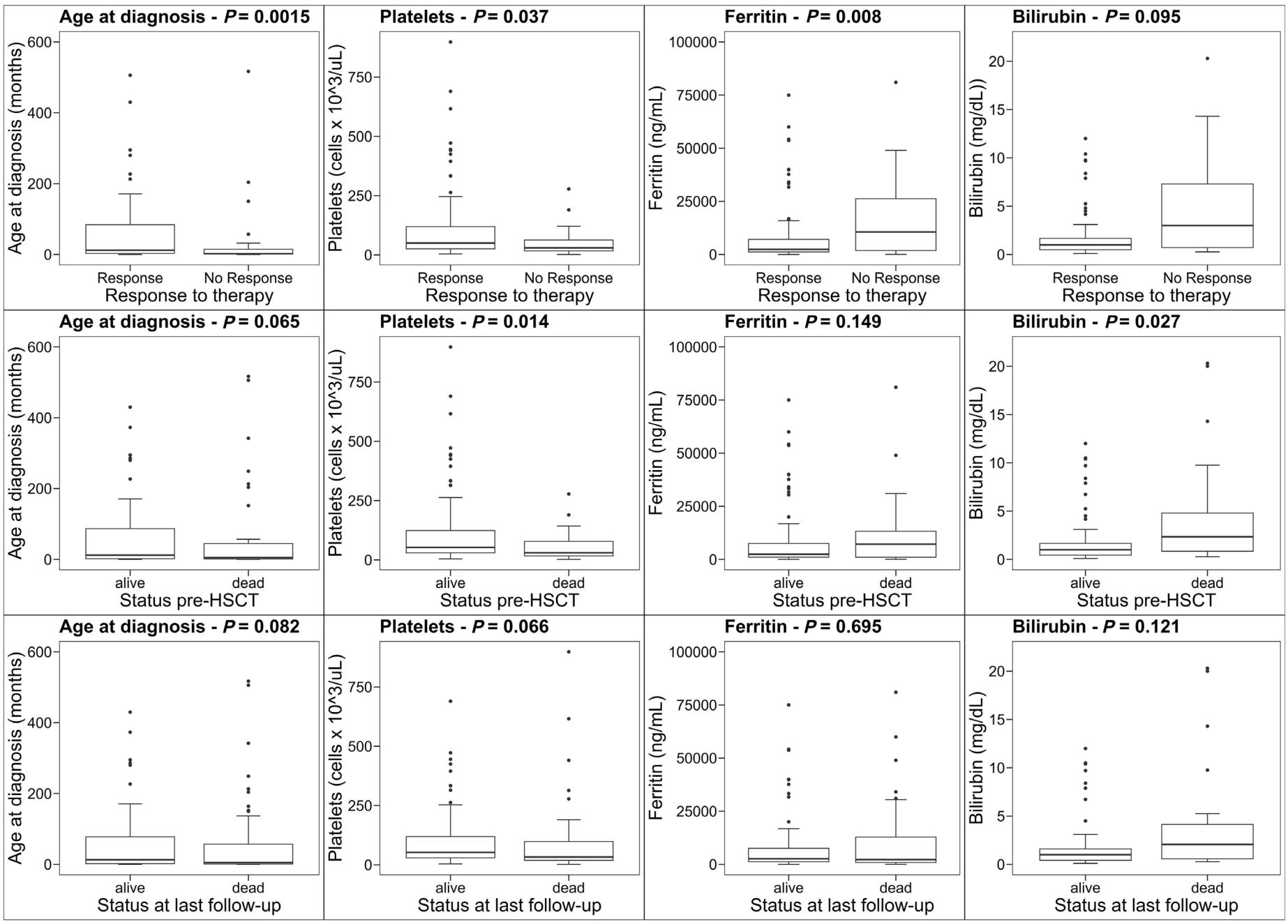

Figure 3. Clinical and laboratory features at baseline and outcome. HSCT: hematopoietic stem cell transplantation.

Nine of them were transplanted and eight survived, while six of the seven patients who did not receive HSCT died (Figure 2). In four patients, after initial non-response to chemotherapy, partial disease control was obtained after re-intensification treatment, but they rapidly reactivated: two received HSCT but all four eventually died at a median time from diagnosis of 4.5 months (IQR, 2-6; *Online Supplementary Table S3;* Figure 2). Six patients reactivated after steroid treatment and received second-line treatment, and four of them were transplanted; all six were alive after a median follow-up duration of 61 months (IQR, 32-64). The reactivation-free survival of the study cohort is reported in Figure 4. No differences in reactivation-free survival were found according to the genetic diagnosis and the study period (*Online Supplementary Figures S3-5*).

Transplantation

Ninety-two of 143 patients (64%) received HSCT, at a median time from diagnosis of 5 months (IQR, 4-8.5). Four of them required a second HSCT for graft loss, and one an additional third transplant. Among the 92 transplanted patients, 78 patients (85%) survived and were alive at a median time of 67 months (IQR, 34-106) from diagnosis and 56 months (IQR, 25-97) from HSCT (Figure 4). Of the 14 patients who died after HSCT, three were transplanted due to treatment failure and died from disease progression, and 11 for transplant-related mortality. Patients who were able to receive HSCT were older and had higher platelet counts and lower bilirubin values at diagnosis (Figure 3). Interestingly, 15 patients (10%) did not receive HSCT and were alive at a median follow-up duration of 34 months (IQR, 13-55; *Online Supplementary Table S4*). Ten patients responded to first-line treatment, including four patients who reactivated and then responded to second-line treatment. Five patients did not receive any treatment: four were diagnosed due to familial screening and remained disease-free through follow-up, while one patient with isolated CNS disease refused treatment and was lost after follow-up at 14 months.

Survival

Ninety-three patients (65%) were alive at the last follow-up (Figure 4). The median follow-up duration was 30 months (IQR, 6-74) in the whole cohort and 57 months (IQR, 29-97) for patients alive at the last follow-up. Among the 50 patients who died, five (10%) did not receive treatment because they died within day 30 from diagnosis, and five (10%) received only steroids (median time to death 1 month; IQR, 1-6). The remaining 40 patients received chemotherapy (9 HLH-94, 28 HLH-2004, and 3 Euro-HIT). Among these 40 patients, 16 died after initial response to first-line treatment (9 for transplant-related mortality, 6 after disease reactivation, and 1 for severe CNS disease), while 24 did not respond and died at a median time from diagnosis of 1 month (IQR, 0-3) for disease progression (Figure 2). Thirty-six patients of 143 (25%) died before receiving HSCT (Figure 4). Causes of death included disease progression leading to multi-organ failure in 32 of 50 patients (64%, following HSCT in 3 patients), transplant-related events in 11 (22%), infections in five (10%) and severe CNS sequelae in two (4%) (*Online Supplementary Table S5*). Interestingly, of the ten patients with *PRF1* A91V mutations (for which exist conflicting data on pathogenicity), three died before HSCT and three received HSCT for active HLH.

When evaluating survival, either at HSCT or at the last follow-up, according to the genetic diagnosis, we found no

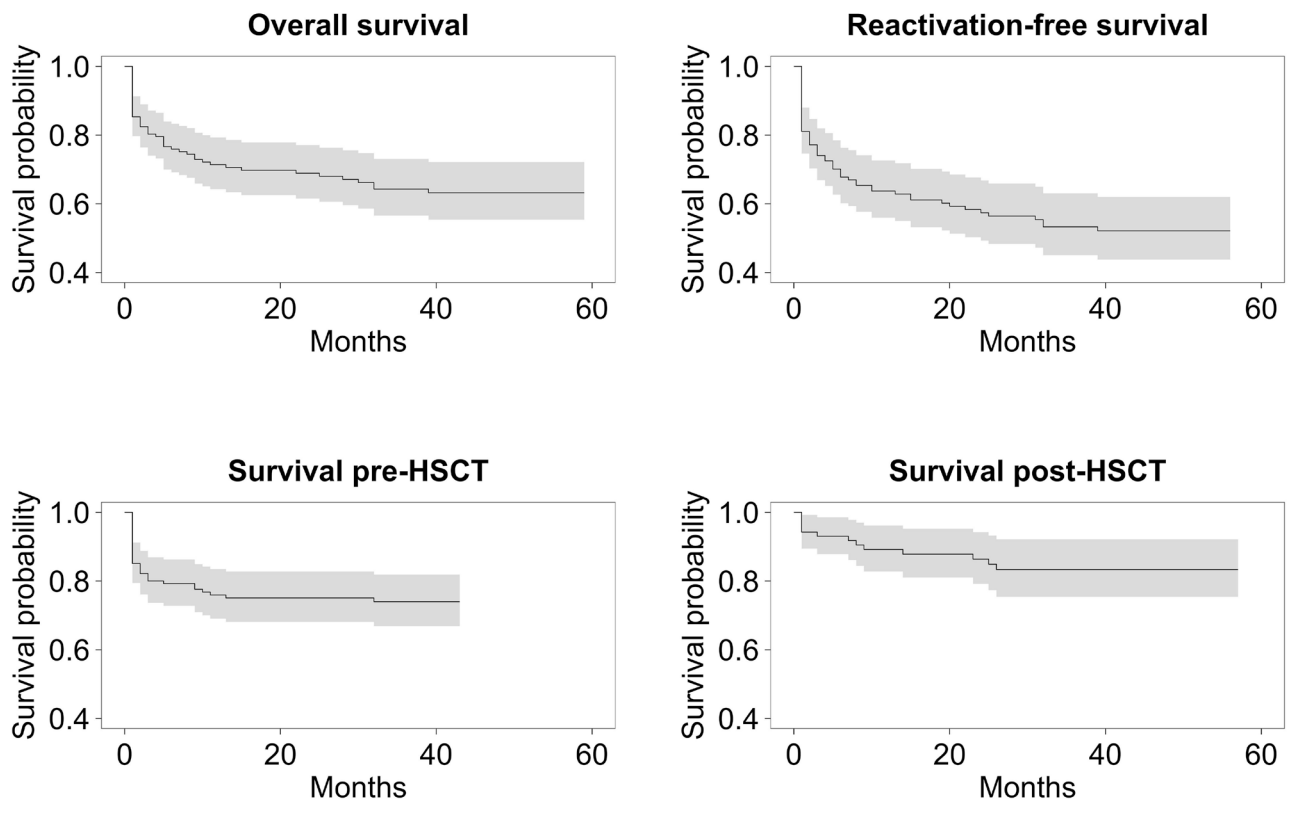

Figure 4. Survival analysis. HSCT: hematopoietic stem cell transplantation.

significant difference (*Online Supplementary Figure S3*). Similarly, no difference in survival was found when analyzing patients with XLP compared to patients with FHL and pigmentary disorders (*Online Supplementary Figure S4*), nor between the first and the second study period (2007-2014 *vs*. 2015-2022); however, a trend toward better post-transplant survival was noticed in the last period (*Online Supplementary Figure S5*).

No significant difference was found in baseline characteristics of patients alive and dead at the last follow-up, while patients who died before HSCT had lower platelet counts and higher bilirubin values at diagnosis, compared to those who received HSCT (Figure 3).

Predictors of non-response and mortality

At unadjusted analysis, predictors of non-response were age <6 months, high ferritin values (>5,000 ng/mL), and high bilirubin levels (>2 mg/dL) at diagnosis. Predictors of pre-transplant and overall mortality were high ferritin and bilirubin levels at diagnosis (Table 2). At multivariable analysis, high ferritin values were confirmed as predictors of non-response (overall response [OR] 5.66, 95% CI: 1.12-42.4; *P*=0.049), while bilirubin levels >2 mg/dL were confirmed as predictors of pre-transplant (OR 4.89, 95% CI: 1.39-18.6; *P*=0.014) and overall mortality (OR 2.98, 95% CI: 1.02-8.86; *P*=0.045; Table 3).

Sequelae

Sequelae were reported in 31 of 143 patients (22%). In 11 of 92 transplanted patients (12%) *sequelae* were transplant-related (chronic graft-*versus*-host disease in 7; endocrine, vascular, and immunologic in 1 each); three had malignancies (lymphoma, acute lymphoblastic leukemia, and gastric cancer); two had a nutritional impairment, following col-

Table 2. Unadjusted analysis of predictors of non-response, pre-transplant mortality, and overall mortality.

	Non-response		Death pre-HSCT		Death at last follow-up	
	Crude OR (95% CI)	*P*	Crude OR (95% CI)	*P*	Crude OR (95% CI)	*P*
Female sex	0.93 (0.37-2.32)	0.881	0.83 (0.37-1.84)	0.642	0.68 (0.33-1.41)	0.303
Age at onset <6 months	2.95 (1.19-7.33)	0.017	1.75 (0.81-3.78)	0.153	1.99 (0.99-4.02)	0.052
Diagnosis						
FHL2 (*vs*. others)	-	-	-	-	-	-
FHL3	2.17 (0.75-6.89)	0.167	1.82 (0.69-5.08)	0.237	1.79 (0.74-4.43)	0.198
FHL4	inf (0-NA)	0.997	inf (0-NA)	0.995	inf (0-NA)	0.995
FHL5	2.47 (0.56-17.42)	0.282	1.88 (0.50-9.12)	0.381	1.11 (0.34-3.79)	0.862
XLP1	1.20 (0.29-6.12)	0.813	0.94 (0.25-3.96)	0.925	1.04 (0.29-3.95)	0.956
XLP2	inf (0-NA)	0.994	inf (0-NA)	0.990	inf (0-NA)	0.990
GS2	3.14 (0.49-61.80)	0.307	0.59 (0.14-2.66)	0.470	0.59 (0.13-2.51)	0.475
CHS	inf (0-NA)	0.995	3.28 (0.52-64.2)	0.286	5.19 (0.83-101)	0.138
Clinical presentation						
Complete HLH criteria	0.75 (0.30-1.90)	0.541	1.34 (0.57-3.12)	0.497	1.43 (0.67-3.05)	0.355
Fever	1.72 (0.20-81.3)	1.000	4.44 (0.62-196.2)	0.187	1.96 (0.48-11.54)	0.383
Splenomegaly	1.04 (0.25-6.23)	1.000	1.48 (0.46-4.75)	0.506	1.42 (0.52-3.92)	0.494
Hepatomegaly	0.59 (0.23-1.49)	0.259	1.16 (0.51-2.66)	0.719	1.08 (0.52-2.25)	0.829
CNS involvement	1.58 (0.57-4.36)	0.372	1.61 (0.67-3.86)	0.283	1.68 (0.78-3.63)	0.187
Hemoglobin <8 g/dL	0.90 (0.35-2.34)	0.835	1.12 (0.49-2.57)	0.792	1.06 (0.50-2.23)	0.883
ANC <0.5x10^9/L	1.07 (0.39-2.91)	0.900	1.39 (0.58-3.31)	0.455	1.96 (0.90-4.26)	0.088
PLT <20x10^9/L	2.77 (0.87-8.46)	0.075	2.14 (0.84-5.51)	0.107	1.92 (0.80-4.60)	0.139
Cytopenia at least bilinear	1.27 (0.46-3.53)	0.643	1.15 (0.48-2.78)	0.756	0.98 (0.45-2.13)	0.959
Ferritin ≥5,000 ng/mL	6.73 (2.36-19.20)	<0.001	3.47 (1.48-8.13)	0.003	2.17 (1.01-4.67)	0.044
Triglycerides ≥265 mg/dL	0.99 (0.38-2.55)	0.980	1.51 (0.65-3.51)	0.336	0.73 (0.34-1.54)	0.409
Fibrinogen ≤150 mg/dL	1.22 (0.44-3.34)	0.705	1.86 (0.74-4.68)	0.182	1.01 (0.46-2.22)	0.976
AST >2xULN	0.85 (0.21-4.21)	0.750	1.41 (0.46-4.34)	0.550	0.76 (0.30-1.94)	0.567
ALT >2xULN	1.50 (0.34-9.25)	0.747	1.14 (0.39-3.34)	0.807	1.08 (0.42-2.79)	0.865
Bilirubin >2 mg/dL	5.53 (1.23-29.29)	0.012	5.96 (1.90-18.7)	0.001	3.41 (1.41-9.88)	0.007
Infectious trigger	0.35 (0.11-1.10)	0.065	0.79 (0.33-1.90)	0.606	0.70 (0.32-1.53)	0.368

HLH: hemophagocytic lymphohistiocytosis; FHL2: familial hemophagocytic lymphohistiocytosis type 2; FHL3: familial hemophagocytic lymphohistiocytosis type 3; FHL4: familial hemophagocytic lymphohistiocytosis type 4; FHL5: familial hemophagocytic lymphohistiocytosis type 5; XLP1: X-linked lymphoproliferative disease type 1; XLP2: X-linked lymphoproliferative disease type 2; GS2: Griscelli syndrome type 2; CHS: Chediak-Higashi syndrome; CNS: central nervous system; ANC: absolute neutrophil count; PLT: platelets; ALT: alanine transaminases; AST: aspartate transaminases; ULN: upper level of normal; OR: odds ratio; CI: confidence interval; HSCT: hematopoietic stem cell transplantation; NA: not applicable.

ectomy in one and requiring long-term parenteral nutrition in both (these patients had XLP2 and FHL4, respectively); two had liver function impairment, requiring liver transplant in one of them, two had chronic kidney disease, and one pulmonary hypertension; two patients had peripheral nervous system impairment, while six patients had CNS complications (paresis in two, and intellectual delay and epilepsy in one each; the remaining two patients had fatal spastic tetraplegia). Finally, one patient had prolonged hypogammaglobulinemia and one osteoporosis.

Discussion

Primary HLH is a severe condition that carries a high burden of early mortality and long-term complications. The clinical course of pHLH can be misleading, and its management is critical.[8] In this study, we describe the presentation and investigate the long-term outcome of a large cohort of patients with pHLH and identify predictors of non-response and mortality.

In historic cohorts, most pHLH patients were diagnosed within the first year of age, but in recent years the rate of diagnosis in older children - or even in adults - has increased.[18,19] Similarly, in our cohort, only half the patients were younger than 1 year at diagnosis, and 10% of them were adolescents or adults. This data is probably due to the improvement of familial screening but also reflects an increased awareness of this condition outside the pediatric domain. Moreover, compared to previous studies,[7] we report a higher rate of XLP, accounting for around 12% of patients in our cohort. These patients were older at diagnosis and had less hematologic involvement at presentation. Thus, unsurprisingly, the proportion of patients presenting with the traditional combination of HLH-related symptoms was lower compared to data from an older international cohort[20] and from large international HLH studies.[11,21]

With regard to treatment, two large international trials coordinated by the Histiocyte Society[11,21] have defined the standard of care, providing the first, marked improvement of survival of patients with HLH. Yet, HLH-2004 did not provide any further improvement compared with HLH-94, with mortality rate remaining up to about 35%. Thus, HLH-94 remained the standard of care. In our study, most patients received one of the two standard chemotherapy regimens, HLH-94 or HLH-2004. Not surprisingly, overall survival and early mortality rates did not differ from the study results, to which those patients largely contributed. Two more recent Turkish studies on pHLH reported higher mortality rates, of 63%[22] and 81%.[19] Compared to the HLH-94 and HLH-2004 studies, we found a lower rate of post-transplant mortality (15% *vs*. 34% and 30%, respectively), which probably partially reflects the improvement of transplant procedures and supportive care in more recent years.

A minority of patients received novel treatments (i.e., emapalumab, ruxolitinib, and alemtuzumab) at reactivation, and these molecules were effective in most cases. However, we were unable to properly evaluate their impact due to the limited number of treated patients. In contrast, patients not responding to first-line treatment experienced adverse outcomes in almost all cases, even those few patients who were able to receive second-line treatment (only 1 patient was successfully rescued using emapalumab). This subgroup of patients is more likely to benefit from alternative treatments.

Thus, early identification of a subset of HLH patients at highest risk who could benefit from alternative therapeutic approaches remains of interest. Data from the HLH-94 study indicated that jaundice, edema, and kidney function abnormalities were associated with early mortality.[21] Additional analysis of the same study reported bilirubin >50 μmol/L, hyperferritinemia, and CSF pleocytosis as adjusted predictors of early mortality.[23] More recently, a monocentric Turkish study identified hepatic involvement as associated, at unadjusted analysis, with inferior 5-year survival in patients with pHLH younger than 2 years.[19] In the present study, we analyzed predictors of non-response to first-line treatment, and of pre-transplant and overall mortality in patients with pHLH. Younger age at diagnosis was significantly associated with non-response, while higher ferritin values were associated with non-response, pre-transplant, and overall mortality. Of note, the ferritin cut-off of 2,000 ng/mL previously used by Trottestam *et al*.[23] did not reach statistical significance in our cohort, probably depending

Table 3. Multivariable analysis of predictors of non-response, pre-transplant mortality, and overall mortality.

	Non-response		Death pre-HSCT		Death at last follow-up	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age at onset <6 months	1.51 (0.29-7.62)	0.611	0.60 (0.14-2.14)	0.450	1.16 (0.40-3.25)	0.773
PLT <20x10^9/L	3.24 (0.57-17.9)	0.173	1.51 (0.33-6.21)	0.579	1.58 (0.42-5.70)	0.486
Ferritin ≥5,000 ng/mL	5.66 (1.12-42.4)	0.049	2.72 (0.79-9.68)	0.112	1.26 (0.43-3.53)	0.667
Bilirubin ≥2 mg/dL	1.69 (0.29-9.19)	0.538	4.89 (1.39-18.6)	0.014	2.98 (1.02-8.86)	0.045

PLT: platelets; OR: odds ratio; CI: confidence interval; HSCT: hematopoietic stem cell transplantation.

on the different study population (HLH *vs*. pHLH). Moreover, bilirubin values >2 mg/dL were significantly associated with all three outcomes. At multivariable analysis, liver dysfunction, demonstrated by hyperbilirubinemia, was confirmed as a reliable predictor of early and overall mortality, and high burden of inflammation, suggested by hyperferritinemia, predicted non-response to first-line treatment. In contrast, we did not confirm the association between CNS involvement and outcome, while data on kidney function were not analyzed.

HSCT is considered the only curative treatment for pHLH. In previous studies, the finding of pHLH patients surviving without HSCT was anecdotic (2/168 in the HLH-2004 study, both harboring a p.Ala91Val *PRF1* mutation; and none in the 60 pHLH patients enrolled in the HLH-94 study). Unexpectedly, 15 patients (10%) in the present series were alive at a median follow-up of 34 months without receiving HSCT. Out of them, four were asymptomatic and diagnosed due to familial screening: they neither developed HLH nor received HLH-directed treatments, at a time to last follow-up ranging from 12 to 105 months. One patient with isolated CNS disease refused treatment, while the remaining ten patients responded to first line HLH-directed treatment and remained disease free after 13 to 86 months.

Overall, our study included a large pHLH cohort, diagnosed over the last 15 years, allowing us to depict an updated picture of the genetic subtype distribution and the outcome of patients with pHLH in Italy. However, we must acknowledge that this study has limitations. First, to include such a large number, an extended time interval is embraced during which medical and transplant practices have changed. However, since the second therapeutic study HLH-2004 has not shown a significant improvement in treatment results, accumulation of the two cohorts should not result in a significant bias in outcome and prognostic investigation. When looking at diagnostic studies, the diagnostic criteria have remained unchanged, but we selected patients based on genetic diagnoses. It has to be acknowledged that a minority of the included patients had mutations with a debatable pathogenicity, but in these cases the genetic diagnosis was supported by abnormal functional studies and/or stringent clinical criteria (HLH-2004). These patients were clinically managed as pHLH and were therefore included in the analyses of the present study. Of note, we also included ten patients harboring homozygous or compound heterozygous A91V *PRF1* mutations, whose pathogenicity is still debated. However, while four of them were alive without HSCT, three received HSCT for active HLH and three died before being able to be transplanted. Although it increases the heterogeneity of the cohort, we believe that reporting these patients could support clinical management in these ambiguous cases.

In conclusion, pHLH is a rare and very severe inheritable condition that still carries an invariably dismal prognosis, if not rapidly and appropriately identified and treated. Patients failing to achieve disease control with first-line treatment remain at highest risk of fatal outcome. These patients usually present with high levels of ferritin and bilirubin, and an early shift to alternative approaches including new agents, appears warranted. For patients achieving disease control allowing HSCT, improved transplant skills may offer a higher chance of cure. Identification of the small subset of patients with pHLH with potential to maintain disease control after initial therapy even without HSCT, may profit from a careful, pin-point analysis, maybe supported also by an accurate genotype-phenotype study.

Disclosures

No conflicts of interest to disclose.

Contributions

FP and ES conceived the work and wrote the manuscript. FP, AC, LB, and MLC collected and analyzed the data. IT, CDF, CM, VB, SC, SG, FDA, AT, MA, FT, CF, AT, and ES followed the patients. AC, LB, and MLC performed diagnostic testing. MT performed the statistical analyses.

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Data-sharing statement

Data will be shared upon appropriate request to the corresponding author.

Appendix: AIEOP Histiocytosis Working Group

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