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blood advances

Stem cell transplantation for children with hemophagocytic lymphohistiocytosis: results from the HLH-2004 study

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Key Points

- In 187 children with a transplant, 5-year OS post-HSCT was 66% in the entire cohort and 71% in children with FHL.
- To spare children from unnecessary HSCT, pretransplant analyses, including possible confirmation of FHL, are recommended.

We report the largest prospective study thus far on hematopoietic stem cell transplantation (HSCT) in hemophagocytic lymphohistiocytosis (HLH), a life-threatening hyperinflammatory syndrome comprising familial/genetic HLH (FHL) and secondary HLH. Although all patients with HLH typically need intensive anti-inflammatory therapy, patients with FHL also need HSCT to be cured. In the international HLH-2004 study, 187 children aged <18 years fulfilling the study inclusion criteria (5 of 8 diagnostic criteria, affected sibling, or molecular diagnosis in FHL-causative genes) underwent 209 transplants (2004-2012), defined as indicated in patients with familial/genetic, relapsing, or severe/persistent disease. Five-year overall survival (OS) post-HSCT was 66% (95% confidence interval [CI], 59-72); event-free survival (EFS) was 60% (95% CI, 52-67). Five-year OS was 81% (95% CI, 65-90) for children with a complete response and 59% (95% CI, 48-69) for those with a partial response (hazard ratio [HR], 2.12; 95% CI, 1.06-4.27; $P = .035$). For children with verified FHL (family history/genetically verified, n = 134), 5-year OS was 71% (95% CI, 62-78) and EFS was 62% (95% CI, 54-70); 5-year OS for children without verified FHL (n = 53) was significantly lower (52%; 95% CI, 38-65) ($P = .040$; HR, 1.69; 95% CI, 1.03-2.77); they were also significantly older. Notably, 20 (38%) of 53 patients without verified FHL had natural killer cell activity reported as normal at diagnosis, after 2 months, or at HSCT, suggestive of secondary HLH; and in addition 14 (26%) of these 53 children had no evidence of biallelic mutations despite having 3 or 4 FHL genes analyzed (natural killer cell activity not analyzed after 2 months or at HSCT). We conclude that post-HSCT survival in FHL remains suboptimal, and that the FHL diagnosis should be carefully investigated before HSCT. Pretransplant complete remission is beneficial but not mandatory to achieve post-HSCT survival. This trial was registered at www.clinicaltrials.gov as #NCT00426101.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition with an uncontrolled accumulation of macrophages and lymphocytes.[1] HLH typically comes in a primary (genetic) form, which is almost always fatal, and a secondary (acquired) form. The most common form of primary HLH is familial

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The full-text version of this article contains a data supplement.

HLH (FHL), which is autosomal recessive and caused by biallelic mutations in 1 of 4 genes (*PRF1*, *UNC13D*, *STX11*, and *STXBP2*), all associated with defective lymphocyte cytotoxicity.[2-7] Primary HLH also includes other diseases such as X-linked lymphoproliferative disease (XLP), Griscelli syndrome type 2 (GS2), and Chédiak-Higashi syndrome (CHS). Secondary HLH (sHLH), on the contrary, is typically triggered by acquired conditions, the most common being severe infections, malignancies, and inflammatory disorders.[3,8-10]

Clinical manifestations of HLH include prolonged fever, splenomegaly, cytopenias, hypertriglyceridemia, hypofibrinogenemia, and hyperferritinemia.[11-13] The most frequent severe sequelae in pediatric HLH are associated with central nervous system (CNS) involvement, often leading to neurologic deficits.[1,14,15] Treatment of HLH aims to downregulate the hyperactive immune system; in addition, for primary HLH, hematopoietic stem cell transplantation (HSCT) is required for cure, as first shown by Fischer et al.[16-18] HSCT is also needed for certain forms of sHLH, such as chronic active Epstein-Barr virus infection, and some cases of malignancy-associated HLH.[19-21]

As a result of collaborations worldwide and new treatment protocols, survival in HLH has improved dramatically.[1,22-26] In 1994, the Histiocyte Society launched the first international therapeutic study on HLH, HLH-94, which recruited >200 eligible patients.[27] It resulted in remarkably improved outcome, with a 5-year probability of overall survival (OS) of 54%.[28] Transplant-related mortality (TRM) caused 26 of 31 deaths after HSCT in HLH-94 and is reportedly higher in HLH than in other nonmalignant conditions, with a large proportion of noninfectious pulmonary toxicity, infections, and sinusoidal obstruction syndrome/veno-occlusive disease.[29-34]

In HLH-2004, the second international HLH study launched by the Histiocyte Society, 5-year OS reached 61%, and pre-HSCT mortality was 19% compared with 26% in HLH-94 (using the same criteria).[35] The current article summarizes the data on HSCT in children recruited to the HLH-2004 study; detailed pre-HSCT information is provided by Bergsten et al.[35]

Patients and methods

Patients

Data on HSCT from 187 eligible children (aged <18 years; fulfilled ≥5 of 8 diagnostic HLH-2004 criteria or had a molecular diagnosis consistent with FHL; no previous cytotoxic or cyclosporine treatment; and no known other underlying disease) were reported to the HLH-2004 study from 22 countries (Argentina, Austria, Bahrain, Brazil, Canada, Czech Republic, Denmark, Germany, Italy, Japan, Malaysia, The Netherlands, Norway, Oman, Portugal, Serbia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States).[12,35] In relation to our previous HLH-2004 report, follow-up data from 2 additional patients were available.[35] Patient characteristics are detailed in Table 1.

A total of 134 patients were defined as having FHL if they had verified biallelic mutations in *PRF1*, *UNC13D*, *STX11*, or *STXBP2* (n = 130)[2,4-7] and/or a sibling with HLH (n = 34 [4 of whom were not analyzed for biallelic mutations]). FHL could not be verified in 53 of 187 eligible children (n = 24 aged <1 year and n = 29 aged ≥1 year at the start of HLH-2004 treatment). Of the 53 nonverified FHL patients, 15, 16, 6, and 13 patients were screened for 4, 3, 2, and 1 disease-causative genes, respectively. *STXBP2* was identified first in 2009.[2,6] As stated in the HLH-2004 protocol, children with other primary HLH diseases (GS2, n = 11; XLP1, n = 6; XLP2, n = 2; and CHS, n = 1) were evaluated separately.[12]

Patients underwent transplantation from 1 January 2004 to 31 December 2012; the last day of data entry was 31 December 2017. The Histiocyte Society, the ethics review board of Stockholm, Sweden, and the Swedish Medical Products Agency (EUDRACT 2005-003279-18) approved the HLH-2004 study, which was also registered at clinicaltrials.gov (#NCT00426101). Data were reported on clinical report forms associated with the study at transplantation, 100 days post-HSCT, and yearly thereafter. Reports on serious adverse events and mortality reports were sent to the principal investigator and were subsequently reviewed by the external Data Safety Monitoring Board.

Donor typing, conditioning, and graft-versus-host-disease prophylaxis

HLA typing results were reported for 6 loci, class I (A, B) and class II (DR). At first transplant, 44 patients (24%) had a matched-related donor (MRD), 60 (32%) had a matched unrelated donor (MUD), 11 (6%) had a haploidentical donor, 6 (3%) had a mismatched unrelated donor (MMUD), and 53 (28%) had umbilical cord blood (UCB) donor (no data, n = 13 [7%]) (Table 2).

According to the HLH-2004 treatment protocol, the modalities of the transplant procedure were decided by the transplantation team. A myeloablative conditioning regimen containing busulfan was suggested. Because only limited data on reduced-intensity conditioning were available at the time of writing the HLH-2004 protocol, it was not possible to make evidence-based suggestions on such regimens.[12,36] In this report, in line with an article by Messina et al,[32] the conditioning regimen when known was reported as busulfan based (n = 99) or treosulfan based (n = 20) if these drugs were included in the conditioning, independent of whether fludarabine was included, and fludarabine based if fludarabine but neither busulfan nor treosulfan was included (n = 39) (others, n = 3; no data, n = 26) (Table 2; supplemental Table 1). No information on the doses of each drug was reported in the clinical report forms.

As graft-versus-host disease (GVHD) prophylaxis, cyclosporine and methotrexate (MTX)/mycophenolate mofetil (MMF) were suggested in the HLH-2004 protocol. A total of 118 patients received cyclosporine; as monotherapy (n = 22), with short-course MTX (n = 52), MMF (n = 28), or prednisone/methylprednisolone (n = 24). Twenty-two received tacrolimus; as monotherapy (n = 4), with short-course MTX (n = 8), MMF (n = 5), or prednisone/methylprednisolone (n = 4). Some patients had multiple combinations; 35 had no data. In 10 patients, grafts were T-cell depleted.

HLH disease status at HSCT conditioning

HLH disease activity was assessed at the start of HSCT conditioning, analyzed both as single clinical parameters (using cutoff values or as continuous variables) or by combined parameters. Complete response was defined as no fever, no splenomegaly, absolute neutrophil count (ANC) ≥1.0 × 10^9/L, platelets ≥100 × 10^9/L, fibrinogen >1.5 g/L, and ferritin analyzed both for <500 μg/L or <2000 μg/L; one missing parameter (ie, 6 of 6 or 5 of 5 normal parameters) was allowed. Evaluations regarding the ferritin level of

Table 1. Patient characteristics at HSCT conditioning, for all evaluated children, children with and without verified FHL, and children without verified FHL aged <1 year and aged ≥1 year at the start of HLH-2004 treatment

Characteristic	All children (N = 187)	Children with FHL (n = 134)	Children without verified FHL (n = 53)	Children without verified FHL aged <1 y at start of HLH-2004 (n = 24)	Children without verified FHL aged ≥1 y at start of HLH-2004 (n = 29)
No. yes/no. evaluated/% yes of evaluated/no. missing					
Male sex	90/187/48/0	61/134/46/0	29/53/55/0	16/24/67/0	13/29/45/0
Age at start of HLH-2004, y					
<1	124/187/66/0	100/134/75/0	24/53/45/0	24/24/100/0	0/29/0/0
1-2.99	34/187/18/0	20/134/15/0	14/53/26/0	0/24/0/0	14/29/48/0
3-5.99	12/187/6/0	7/134/5/0	5/53/9/0	0/24/0/0	5/29/17/0
≥6	17/187/9/0	7/134/5/0	10/53/19/0	0/24/0/0	10/29/34/0
Age at HSCT, y					
<1	91/187/49/0	78/134/58/0	13/53/25/0	13/24/54/0	0/29/0/0
1-2.99	60/187/32/0	37/134/28/0	23/53/44/0	11/24/46/0	12/29/43/0
3-5.99	15/187/8/0	9/134/7/0	6/53/11/0	0/24/0/0	6/29/21/0
≥6	21/187/11/0	10/134/8/0	11/53/21/0	0/24/0/0	11/29/38/0
Time to HSCT from HLH-2004 start, mo					
<6	120/187/64/0	94/134/70/0	26/53/49/0	12/24/50/0	14/29/48/0
≥6	67/87/36/0	40/134/30/0	27/53/51/0	12/24/50/0	15/29/52/0
Fever	17/149/11/38	13/106/12/28	4/43/9/10	1/21/5/3	3/22/14/7
Splenomegaly	35/145/24/42	23/102/23/32	12/43/28/10	8/21/38/3	4/22/18/7
Hepatomegaly	62/145/43/42	47/103/46/31	15/42/36/11	6/20/30/4	9/22/41/7
Hemoglobin, g/L					
<90	49/145/34/42	34/105/32/29	15 /40/38 /13	9/20/45/4	6/20/30/9
Neutrophils (ANC), $\times 10^9$/L					
<1.0	36/134/27/53	27/95/28/39	9/39/23/14	6/19/32/5	3/20/15/9
Platelets, $\times 10^9$/L					
<100	25/145/17/42	14/106/13/28	11/39/28/14	4/19/21/5	7/20/35/9
Triglycerides, mmol/L					
≥3.0	45/108/42/79	34/73/47/61	11/35/31/18	4/16/25/8	7/19/37/10
Fibrinogen, g/L					
≤1.5	10/111/9/76	8/79/10/55	2/32/6/21	0/15/0/9	2/17/12/12

All clinical parameters and laboratory values were analyzed at the time of HSCT conditioning.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSF, cerebral spinal fluid.
†Information on NK cell activity, soluble CD25, and CSF was reported as not analyzed in 95, 102, and 97 children, respectively.
‡Pathologic levels by age at HSCT conditioning.
§Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L; 6 of 6 or 5 of 5 (allowing missing information on 1 variable).
‖Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L), and the rest of the parameters abnormal.
¶Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <2000 μg/L; 6 of 6 or 5 of 5 (allowing missing information on 1 variable).
#Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <2000 μg/L), and the rest of the parameters abnormal.
*P = .011 for time to HSCT; FHL children vs not verified FHL children. ***P < .001 for age at start HLH-2004 for FHL children vs not verified FHL children, and for age at HSCT conditioning for FHL children vs not verified FHL children.

Table 1. (continued)

Characteristic	All children (N = 187)	Children with FHL (n = 134)	Children without verified FHL (n = 53)	Children without verified FHL aged <1 y at start of HLH-2004 (n = 24)	Children without verified FHL aged ≥1 y at start of HLH-2004 (n = 29)
Ferritin, μg/L					
≥500	58/114/51/73	38/81/47/53	20/33/61/20	6/14/43/10	14/19/74/10
≥2000	20/114/18/73	14/81/17/53	6/33/18/20	1/14/7/10	5/19/26/10
NK cell activity absent /low†	24/36/67/151	17/20/85/114	7/16/44/37	2/6/33/18	5/10/50/19
Soluble CD25, U/L†					
≥2400	8/29/28/158	4/18/22/116	4/11/36/42	2/4/50/20	2/7/29/22
CSF cells or CSF protein abnormal†‡	24/54/44/133	17/36/47/98	7/18/39/35	4/9/44/15	3/9/33/20
Neurologic symptoms	30/150/20/37	24/108/22/26	6/42/14/11	2/20/10/4	4/22/18/7
Response (ferritin <500 μg/L)					
Complete§	42/129/33/58	30/95/32/39	12/34/35/19	6/15/40/9	6/19/32/10
Partial‖	87/129/67/58	65/95/68/39	22/34/65/19	9/15/60/9	13/19/68/10
Response (ferritin <2000 μg/L)					
Complete¶	55/131/42/56	38/96/40/38	17/35/49/18	8/15/53/9	9/20/45/9
Partial#	76/131/58/56	58/96/60/38	18/35/51/18	7/15/47/9	11/20/55/9
Median (mean; range; no. missing)					
Age at start of HLH-2004, d	138 (700; 3-6412; 0)	103 (487; 3-5729; 0)	441 (1239; 34-6412; 0)***	120 (153; 34-363; 0)	1360 (2138; 384-6412; 0)
Age at HSCT, d	371 (925; 86-7287; 0)	309 (701; 86-6403; 0)	651 (1490; 87-7287; 0)***	323 (363; 87-682; 0)	1493 (2423; 578-7287; 0)
Time to HSCT, d	148 (225; 25-2105; 0)	129 (214; 25-2105; 0)	190 (251; 25-1295; 0)*	178 (210; 25-489; 0)	194 (286; 35-1295; 0)
Hemoglobin, g/L	96 (95; 25-151; 42)	97 (97; 58-151; 29)	95 (93; 67-127; 13)	91 (93; 70-127; 4)	96 (92; 67-109; 9)
Neutrophils, ANC, $\times 10^9$/L	1.82 (2.34; 0.00-12; 53)	1.83 (2.40; 0.00-12; 39)	1.73 (2.14; 0.00-9.50; 14)	1.61 (1.80; 0.00-5.30; 5)	2.14 (2.47; 0.00-9.50; 9)
Platelets, $\times 10^9$/L	288 (300; 3-925; 42)	319 (327; 3-925; 28)	244 (227; 13-493; 14)	249 (245; 29-493; 5)	201 (210; 13-484; 9)
Triglycerides, mmol/L	2.53 (3.45; 0.45-20.90; 79)	2.59 (3.81; 0.66-20.90; 61)	1.90 (2.70; 0.45-8.28; 18)	1.77 (2.13; 0.50-4.34; 8)	2.37 (3.19; 0.45-8.28; 10)
Fibrinogen, g/L	2.80 (3.00; 0.09-8.65; 76)	2.77 (2.94; 0.09-6.63; 55)	2.99 (3.17; 1.08-8.65; 21)	2.60 (2.88; 1.60-5.90; 9)	3.60 (3.43; 1.08-8.65; 12)
Ferritin, μg/L	515 (2186; 11-74651; 73)	460 (1637; 11-38934; 53)	657 (3529; 19-74651; 20)	344 (1054; 19-8219; 10)	932 (5354; 97-74651; 10)
Soluble CD25, U/L†	1559 (2389; 69-16200; 158)	1266 (2743; 252-16200; 116)	1682 (1810; 69-3126; 42)	2588 (2362; 1390-2880; 20)	1559 (1495; 69-3126; 22)
AST, U/L	37 (62; 7-408; 52)	38 (63; 9-408; 39)	36 (60; 7-387; 13)	33 (36; 7-103; 3)	52 (87; 23-387; 10)
ALT, U/L	34 (66; 5-634; 95)	34 (72; 5-634; 82)	34 (58; 8-313; 13)	26 (31; 8-65; 3)	49 (86; 13-313; 10)

All clinical parameters and laboratory values were analyzed at the time of HSCT conditioning.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSF, cerebral spinal fluid.
†Information on NK cell activity, soluble CD25, and CSF was reported as not analyzed in 95, 102, and 97 children, respectively.
‡Pathologic levels by age at HSCT conditioning.
§Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L; 6 of 6 or 5 of 5 (allowing missing information on 1 variable).
‖Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L), and the rest of the parameters abnormal.
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*P = .011 for time to HSCT; FHL children vs not verified FHL children. ***P < .001 for age at start HLH-2004 for FHL children vs not verified FHL children, and for age at HSCT conditioning for FHL children vs not verified FHL children.

Table 2. Information on donor and conditioning for all children, and for children with and without verified FHL

Variable	All children (N = 187)	Children with FHL (n = 134)	Children without verified FHL (n = 53)
Type of donor, n (% of entire group)			
MRD	44 (24)	31 (23)	13 (25)
MUD	60 (32)	45 (34)	15 (28)
Haploidentical donor	11 (6)	7 (5)	4 (8)
MMUD	6 (3)	3 (2)	3 (6)
UCB	53 (28)	39 (29)	14 (26)
Missing information on donor type	13 (7)	9 (7)	4 (8)
Conditioning, n (% of entire group)			
Busulfan-based	99 (53)	69 (52)	30 (57)
Fludarabine-based	39 (21)	26 (19)	13 (25)
Treosulfan-based	20 (11)	18 (13)	2 (4)
Missing information on conditioning	29 (16)	21 (16)	8 (15)

Donor typing was performed on HLA class I (A, B) and class II (DR), as stated in the protocol.[12]

2000 μg/L were post hoc analyses performed due to literature suggesting that this was a more appropriate cutoff value.[37-39] Altogether, 131 patients (70%) had sufficient information for response evaluation (ie, ≥5 parameters evaluated). Partial response was defined as having 3 to 5 normal parameters of 6 measured, or 3 to 4 of 5 measured, in addition to 1 to 3 abnormal parameters. No response was defined as having >3 abnormal parameters.

Statistical methods

For comparison between groups, Fisher's exact or Pearson's χ^2 test was used for categorical variables, and the Mann-Whitney *U* test was used for continuous variables. The distribution of continuous variables was assessed by using histograms and normal qq-plots. Ferritin, ANC, triglycerides, aspartate aminotransferase, alanine aminotransferase, and soluble CD25 were ln-transformed to limit the influence of outliers.

Kaplan-Meier estimates were used for OS and event-free survival (EFS) (calculated from the day of HSCT until last follow-up, where death, and death or second HSCT, were defined as events, respectively). Confidence intervals (CIs) (95%) for survival estimates were calculated by using the log-minus-log transformation.[40] Cox proportional hazards models were used to calculate hazard ratios (HRs) with two-sided 95% CIs. We fitted adjusted Cox regression models for our main findings, adjusting for age at diagnosis and time to HSCT, respectively. Restricted cubic splines were used in Cox proportional hazards models to test the linearity assumption of the continuous variables listed in Table 1. No serious deviations from linearity were found. Cumulative incidence of TRM or death due to HLH relapse, or second HSCT, was compared between groups by using Gray's test.[41] $P < .05$ was considered significant. All analyses were performed for the entire cohort (N = 187), for children with verified FHL (n = 134), and for children without verified FHL (n = 53), separately. IBM SPSS for Windows (version 25; IBM SPSS Statistics, IBM Corporation), the function *rcspline.plot* in R package rms,[42] or the function *cuminc* in R package *cmprsk*[43] in R version 3.6.0 (R Core Team 2019) were used for the aforementioned analyses.[44]

Results

Patient characteristics

Of the187 eligible children, 134 had verified FHL; of the remaining 53 children, 24 were aged <1 year and 29 were aged ≥1 year at HLH-2004 treatment start (Table 1). The median age at the start of HLH-2004 treatment and at HSCT conditioning for the entire cohort was 138 and 371 days, respectively; 91 (49%) were aged <1 year at transplantation, and 21 (11%) were aged ≥6 years. The median time to transplantation was 148 days; 42% (n = 79), 64% (n = 120), and 86% (n = 160) underwent transplant within 4, 6, and 12 months from HLH-2004 therapy start.

Among the 134 children with verified FHL, 41 (31%) displayed biallelic mutations in *PRF1,* 66 (49%) in *UNC13D,* five (4%) in *STX11,* and 18 (13%) in *STXBP2*. Thirty-four of these 134 children (25%) had a sibling with HLH, of whom 4 were not analyzed for mutations. Seventy-eight (58%) of the verified FHL patients were aged <1 year at transplantation, and only 10 (8%) were aged ≥6 years. The median age was 103 days at the start of treatment and 309 days at HSCT. Median time to transplantation was 129 days; 48% (n = 64), 70% (n = 94), and 88% (n = 118) underwent transplant within 4, 6, and 12 months after the start of HLH-2004 therapy, respectively.

For children without verified FHL (n = 53), median time to transplantation (190 days) was significantly longer compared with children with verified FHL ($P = .011$), and about one-half of these children (n = 27 [52%]) had their transplant ≥6 months after the start of HLH-2004 therapy. These children were older than those with verified FHL; their median age was 441 days at the start of therapy ($P < .001$) and 651 days at HSCT ($P < .001$), and only 25% (n = 13) were aged <1 year at HSCT, whereas 21% (n = 11) were aged ≥6 years.

Survival

As of 31 December 2017, a total of 120 children (64%) were alive, with a follow-up of ≥3 years in 107, ≥4 years in 96, and ≥5 years

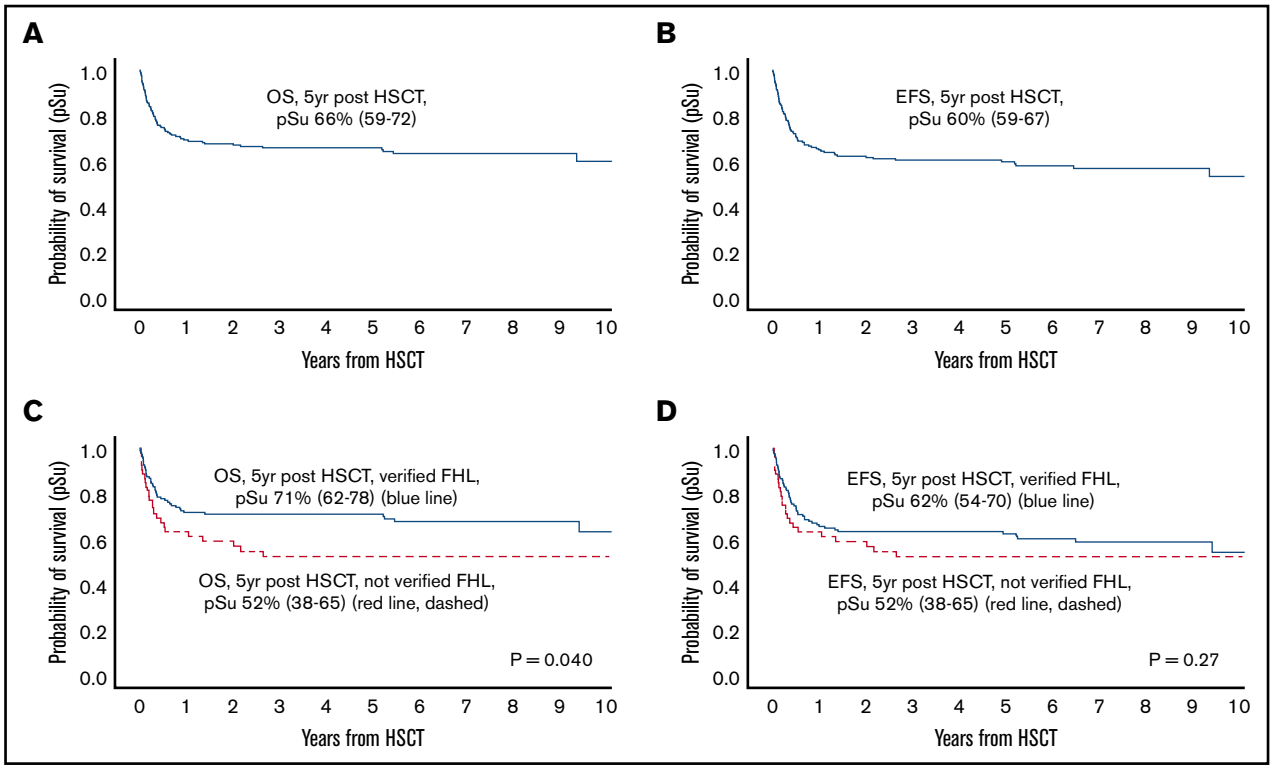

Figure 1. Kaplan-Meier estimates of survival after HSCT in the HLH-2004 study. Five-year probabilities of survival (pSu) are indicated with a 95% CI. OS or EFS is displayed, where death, and death or second HSCT, were defined as events, respectively. *P* values from Cox proportional hazards models. (A) OS for the entire HLH-2004 cohort (n = 187, events n = 67). (B) EFS for the entire HLH-2004 cohort (n = 187, events n = 78). (C) OS for children with verified FHL (n = 134, events n = 43, blue line) and children without verified FHL (n = 53, events n = 24, red line dashed); *P* = .040. (D) EFS for children with verified FHL (n = 134, events n = 54, blue line) and children without verified FHL (n = 53, events n = 24, red line, dashed); *P* = .27.

in 81 from the first HSCT (supplemental Table 2). The 5-year OS post-HSCT was 66% (95% CI, 59-72) and EFS was 60% (95% CI, 52-67) (Figure 1A-B; Table 3). For children with verified FHL, 5-year OS was 71% (95% CI, 62-78) and EFS was 62% (95% CI, 54-70), whereas the 5-year OS for children without verified FHL was significantly lower (52%; 95% CI, 38-65) (*P* = .040; HR, 1.69; 95% CI, 1.03-2.77) and their EFS was nonsignificantly lower (52%; 95% CI, 38-65) (*P* = .27; HR, 1.32; 95% CI, 0.81-2.22) (Figure 1C-D). Nonverified FHL remained a statistically significant risk factor (*P* = .032; HR, 1.74; 95% CI, 1.05-2.88) for post-HSCT death when adjusting for time to HSCT but not when adjusting for age at diagnosis. For children with mutations in *PRF1*, *UNC13D*, *STX11*, and *STXBP2*, 5-year OS was 70% (95% CI, 64-82), 70% (95% CI, 54-79), 80% (95% CI, 20-97), and 71% (95% CI, 44-87), respectively, and OS in children with an affected sibling was 79% (95% CI, 62-90). OS in girls without verified FHL was 44% (95% CI, 24-63) compared with 75% (95% CI, 63-83) in those with verified FHL (*P* = .006), and for boys it was 61% (95% CI, 40-76) and 66% (95% CI, 52-76) (*P* = .90); the interaction analysis (nonverified FHL/verified FHL; female/male) yielded *P* = .062.

OS did not improve over time when comparing transplantation years 2004 to 2006, 2007 to 2009, and 2010 to 2012 in the entire cohort (*P* = .20). From 2010 to 2012, OS exceeded 80% (82%; 95% CI, 55-94) for FHL children with MUD (n = 17) (supplemental Table 3).

Natural killer cell activity in children without verified FHL undergoing transplant

Decreased natural killer (NK) cell activity at the onset of HLH is associated with deficient function of cytotoxic lymphocytes (typical for most patients with primary HLH) or with a low number of circulating cytotoxic cells (mostly seen in sHLH).[45-47] To better understand if some of the children without verified FHL (n = 53) who underwent transplant could have possible sHLH, reported data on genetics and NK cell activity were reviewed in detail. Notably, 20 (38%) of 53 had NK cell activity reported normal at diagnosis, after 2 months, or at HSCT, which is suggestive of sHLH. Moreover, in addition 14 (26%) of these 53 additional children had no evidence of biallelic mutations despite having 3 or 4 FHL genes analyzed (as well as NK cell activity not analyzed after 2 months or at HSCT). Altogether, these 34 patients without evidence of primary HLH, who instead may have had sHLH, comprise 64% of this cohort of 53 patients (supplemental Table 4).

Notably, 11 (21%) of 53 children without verified FHL had low or absent NK cell activity after 2 months or at HSCT, possibly suggesting primary HLH. For 8 (15%) of 53 children, NK cell activity was not assessed/missing at 2 months or at HSCT, and mutation analysis was only performed for 2 (n = 1), 1 (n = 6), or no (n = 1) FHL genes; thus, for these children, the limited data cannot help in differentiating between primary vs secondary HLH.

Table 3. Kaplan-Meier estimates of OS and EFS for all evaluated children, and for children with and without verified FHL

Variable	All children (N = 187), 5-y pSu (95% CI) (no. evaluated) (no. events)		Children with FHL (n = 134), 5-y pSu (95% CI) (no. evaluated) (no. events)		Children without verified FHL (n = 53), 5-y pSu (95% CI) (no. evaluated) (no. events)	
	OS	EFS	OS	EFS	OS	EFS
General	66 (59-72) (187) (67)	60 (52-67) (187) (78)	71 (62-78) (134) (43)	62 (54-70) (134) (54)	52 (38-65) (53) (24)	52 (38-65) (53) (24)
Female	68 (57-76) (97) (32)	62 (52-71) (97) (38)	75 (63-83) (73) (19)	68 (56-77) (73) (25)	44 (24-63) (24) (13)	44 (24-63) (24) (13)
Male	64 (53-73) (90) 35)	57 (46-66) (90) (40)	66 (52-76) (61) (24)	55 (41-67) (61) (29)	61 (40-76) (29) (11)	61 (40-76) (29) (11)
Complete response (ferritin <500 μg/L)*	81 (65-90) (42) (10)	63 (47-76) (42 (17)	83 (64-93) (30) (7)	59 (39-75) (30) (14)	75 (41-91) (12) (3)	75 (41-91) (12) (3)
Partial response (ferritin <500 μg/L)†	59 (48-69) (87) (37)	56 (44-65) (87) (40)	64 (51-75) (65) (25)	59 (47-70) (65) (28)	43 (21-62) (22) (12)	43 (21-62) (22) (12)
Complete response (ferritin <2000 μg/L)‡	76 (62-85) (55) (15)	61 (47-73) (55) (23)	82 (65-91) (38) (9)	60 (43-74) (38) (17)	64 (36-82) (17) (6)	64 (36-82) (17) (6)
Partial response (ferritin <2000 μg/L)§	61 (49-71) (76) (31)	59 (47-69) (76) (33)	65 (51-76) (58) (22)	62 (48-73) (58) (24)	48 (24-69) (18) (9)	48 (24-69) (18) (9)
No neurologic symptoms	69 (59-76) (120) (38)	61 (52-70) (120) (46)	74 (63-82) (84) (23)	63 (52-73) (84) (31)	57 (39-71) (36) (15)	57 (39-71) (36) (15)
Neurologic symptoms	67 (47-80) (30) (13)	60 (41-75) (30) (15)	71 (48-85) (24) (10)	63 (40-78) (24) (12)	50 (11-80) (6) (3)	50 (11-80) (6) (3)
CSF cells or protein normal‖	63 (44-78) (30) (11)	60 (41-75) (30) (12)	68 (43-84) (19) (6)	63 (38-81) (19) (7)	55 (23-78) (11) (5)	55 (23-78) (11) (5)
CSF cells or protein pathologic‖	62 (40-78) (24) (11)	49 (28-67) (24) (14)	71 (43-87) (17) (7)	53 (28-73) (17) (10)	38 (6-72) (7) (4)	38 (6-72) (7) (4)
Age at HSCT conditioning <1 y	67 (56-76) (91) (32)	62 (51-73) (91) 836)	69 (58-78) (78) (26)	64 (52-73) (78) (30)	54 (25-76) (13) (6)	54 (25-76) (13) (6)
Age at HSCT conditioning 1-2.99 y	71 (57-81) (60) (19)	59 (45-70) (60) (26)	80 (63-90) (37) (9)	60 (43-74) (37) (16)	55 (33-73) (23) (10)	55 (33-73) (23) (10)
Age at HSCT conditioning 3-5.99 y	60 (32-80) (15) (6)	60 (32-80) (15) (6)	56 (20-81) (9) (4)	56 (20-81) (9) (4)	67 (20-90) (6) (2)	67 (20-90) (6) (2)
Age at HSCT conditioning ≥6 y	51 (28-70) (21) (10)	51 (28-70) (21) (10)	60 (25-83) (10) (4)	60 (25-83) (10) (4)	44 (15-70) (11) (6)	44 (15-70) (11) (6)
Time to HSCT <6 mo	69 (60-76) (120) (40)	64 (55-72) (120) (45)	70 (60-78) (94) (31)	64 (54-73) (94) (36)	65 (43-80) (26) (9)	65 (43-80) (26) (9)
Time to HSCT ≥6 mo	60 (47-71) (67) (27)	51 (38-62) (67) (33)	72 (55-83) (40) (12)	57 (40-70) (40) (18)	41 (22-59) (27) (15)	41 (22-59) (27) (15)
HSCT time interval 2004-2006	70 (56-80) (54) (17)	61 (46-72) (54) (22)	73 (56-84) (37) (11)	59 (42-73) (37) (16)	63 (35-82) (17) (6)	63 (35-82) (17) (6)
HSCT time interval 2007-2009	60 (48-70) 875) (32)	55 (43-66) (75) (34)	65 (51-76) (52) (20)	59 (44-71) (52) 22)	48 (27-66) (23) (12)	48 (27-66) (23) (12)
HSCT time interval 2010-2012	70 (56-80) (58) (18)	65 (51-76) (58) (22)	75 (60-86) (45) (12)	69 (53-80) (45) (16)	50 (21-74) (13) (6)	50 (21-74) (13) (6)
Donor: MRD	58 (42-71) (44) (19)	54 (38-67) (44) (21)	64 (45-78) (31 (12)	58 (38-73) (31) (14)	44 (17-69) (13) (7)	44 (17-69) (13) (7)
Donor: MUD	73 (60-83) (60) (18)	62 (48-73) (60) (24)	78 (63-88) (45) (12)	65 (48-77) (45) (18)	55 (26-77) (15) (6)	55 (26-77) (15) (6)
Donor: UCB	60 (45-72) (53) (22)	58 (44-70) (53) (23)	69 (52-81) (39) (13)	66 (49-79) (39) (14)	38 (13-59) (14) (9)	38 (13-59) (14) (9)
Conditioning busulfan-based	63 (53-72) (99) (38)	59 (48-68) (99) (42)	70 (58-79) (69) (23)	64 (51-74) (69) (27)	46 (27-63) (30) (15)	46 (27-63) (30) (15)
Conditioning fludarabine-based	69 (51-81) (39) (13)	50 (32-66) (39) (18)	76 (55-89) (26) (7)	50 (28-68) (26) (12)	53 (23-75) (13) (6)	53 (23-75) (13) (6)
Conditioning treosulfan-based	80 (55-92) (20) (4)	80 (55-92) (20) (5)	78 (51-91) (18) (4)	78 (51-91) (18) (5)	100 (—) (2) (0)	100 (—) (2) (0)

For OS and EFS, death, and death or second HSCT, were defined as events, respectively. All clinical parameters and laboratory values were analyzed at the time of HSCT conditioning.
—, cannot be calculated; CSF, cerebral spinal fluid; pSu, probability of survival.
*Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L; 6 of 6 or 5 of 5 (allowing missing information on 1 variable).
†Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L), and the rest of the parameters abnormal.
‡Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <2000 μg/L; 6 of 6 or 5 of 5 (allowing missing information on 1 variable).
§Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <2000 μg/L), and the rest of the parameters abnormal.
‖Pathologic levels by age at HSCT conditioning.

Donor groups

The 5-year EFS for MRD, MUD, and UCB was 54% (95% CI, 38-67), 62% (95% CI, 48-73), and 58% (95% CI, 44-70), respectively, and the corresponding OS was 58% (95% CI, 42-71), 73% (95% CI, 60-83), and 60% (95% CI, 45-72) (Table 3; Figure 2A; supplemental Figure 1A). For children with verified FHL, the 5-year EFS was 58% (95% CI, 38-73), 65% (95% CI, 48-77), and 66% (95% CI, 49-79) for MRD, MUD and UCB. The corresponding 5-year OS was 64% (95% CI, 45-78), 78% (95% CI, 63-88), and 69% (95% CI, 52-81) (Table 3; Figure 2B; supplemental Figure 1B).

Eleven children had a haploidentical donor (EFS, 82%; 95% CI, 45-95), all from a few transplantation centers, and six had an MMUD (EFS, 67%; 95% CI, 20-90). Neither EFS nor OS was significantly influenced by the choice of donor (Figure 2A-2B; supplemental Figure 1).

Conditioning, engraftment, GVHD, and chimerism

The choice of conditioning regimen did not statistically significantly affect the 5-year EFS for all children (busulfan based, 59% [95% CI, 48-68]; fludarabine based, 50% [95% CI, 32-66]; treosulfan based, 80% [95% CI, 55-92]). The corresponding 5-year OS values were 63% (95% CI, 53-72), 69% (95% CI, 51-81), and 80% (95% CI, 55-92) (Table 3; Figure 2C; supplemental Figure 2A). In neither of the subgroups (with or without verified FHL) did conditioning

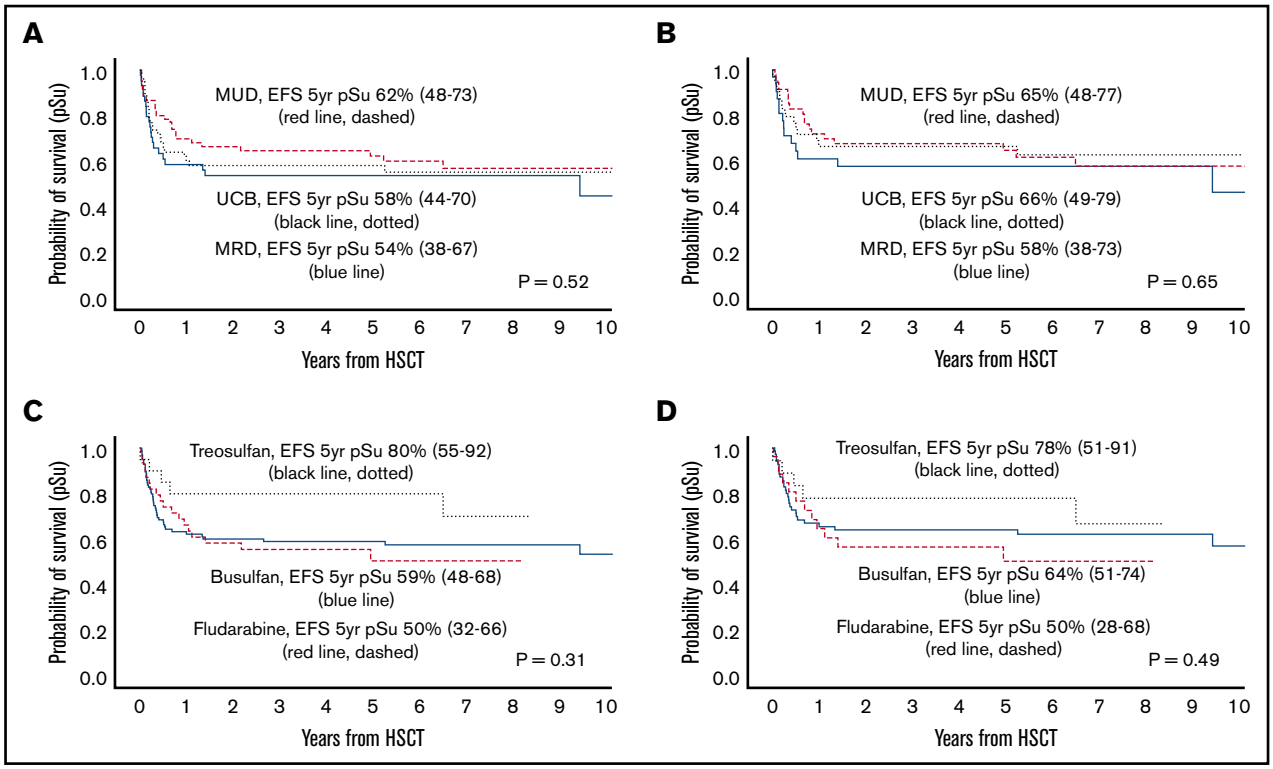

Figure 2. Kaplan-Meier estimates of EFS based on HSCT donor and conditioning in the HLH-2004 study. Five-year probabilities of survival (pSu) are indicated with a 95% CI. Death or second HSCT were defined as events. *P* values from Cox proportional hazards models. (A) EFS for the entire cohort based on donor: MRD (n = 44, event n = 21, blue line); MUD (n = 60, event n = 24, red line, dashed); UCB (n = 53, event n = 23, black line, dotted)], *P* = .52. [MUD vs MRD, *P* = .26; UCB vs MRD, *P* = .61]. (B) EFS for children with verified FHL based on donor (MRD, n = 31, event n = 14, blue line; MUD, n = 45, event n = 18, red line, dashed; UCB, n = 39, event n = 14, black line, dotted; *P* = .65 (MUD vs MRD, *P* = .40; UCB vs MRD, *P* = .44). (C) EFS for the entire cohort based on conditioning (busulfan-based, n = 99, events n = 42, blue line; fludarabine based, n = 39, events n = 18, red line dashed; treosulfan based, n = 20, events n = 5, black line, dotted; *P* = .31 (fludarabine vs busulfan, *P* = .74; treosulfan vs busulfan, *P* = .16). (D) EFS for children with verified FHL based on conditioning (busulfan based, n = 69, events n = 27, blue line; fludarabine based, n = 26, events n = 12, red line, dashed; treosulfan based, n = 18, events n = 5, black line, dotted; *P* = .49 (fludarabine vs busulfan, *P* = .49; treosulfan vs busulfan, *P* = .43).

statistically significantly influence EFS or OS (Table 3; Figure 2D; supplemental Figure 2B-D).

Information on engraftment by day 100 was available for 150 children (no data, n = 37), of whom 137 (91%) were reported to have engraftment (100 of 107 [93%] for children with verified FHL and 37 of 43 [86%] for children without FHL). Thirty-one (22%) of 138 had acute GVHD grade II to IV reported at 100 days, and 21 had chronic GVHD (years 1-5 post-HSCT). Complete chimerism at day 100 was reported in 72 children (39%), and mixed chimerism was reported in 47 (25%) (no data, n = 68).

Disease activity at conditioning

Clinical and laboratory parameters were evaluated as single parameters at time of HSCT conditioning, either with normal cutoff values or as continuous variables. Using cutoff values, no statistically significant differences regarding survival were found. Of children with verified FHL, 27 (28%) had ANC $<1.0 \times 10^9$, and 17 (85%) had low/absent NK cell activity, compared with 3 (15%) and 5 (50%), respectively, of children aged ≥1 year without verified FHL (Table 1). One-fifth (n = 30) of all patients had neurologic alterations at HSCT conditioning, and 24 (44%) displayed pathologic cerebrospinal fluid cells and/or protein.

When using continuous variables, a statistical significance in OS was found for ferritin (ln-transformed) both in the entire cohort (HR for a 1-unit increase in the natural logarithm of ferritin (μg/L) = 1.23; 95% CI, 1.03-1.48; *P* = .023) and in the children with verified FHL (HR, 1.29; 95% CI, 1.01-1.66; *P* = .040) (Table 4). In the FHL cohort, the difference remained statistically significant when adjusting for age at diagnosis and time to HSCT, both separately and in the same model.

With regard to response at HSCT, the 5-year OS for all children with complete response (using ferritin <500 μg/L) was 81% (95% CI, 65-90) and 59% (95% CI, 48-69) for those with partial response (HR, 2.12; 95% CI, 1.06-4.27; *P* = .035) (Tables 3 and 4; Figure 3A). Partial response compared with complete response remained statistically significant for post-HSCT death (HR, 2.03; 95% CI, 1.01-4.11; *P* = .048) when adjusting for age at diagnosis and time to HSCT. By using ferritin levels <2000 μg/L as the cutoff, 5-year OS for all children with complete remission was 76% (95% CI, 62-85) and 61% (95% CI, 49-71) with partial response (HR, 1.74; 95% CI, 0.94-3.22; *P* = .080) (Tables 3 and 4; Figure 3B).

Second transplantation

Twenty children (18 with verified FHL) had a second HSCT, 4 within 100 days from the first HSCT and altogether 12 during the first year

Table 4. HRs, 95% CIs, and *P* values from Cox proportional hazards models, for all evaluated children, and for children with and without verified FHL

Reference group	Group compared with the reference group	All children (N = 187), HR (95% CI; *P*)		Children with FHL (n = 134), HR (95% CI; *P*)		Children without verified FHL (n = 53), HR (95% CI; *P*)	
		OS	EFS	OS	EFS	OS	EFS
Sex							
Female	Male	1.26 (0.78-2.03; .35)	1.22 (0.78-1.90; .38)	1.68 (0.92-3.07; .092)	1.59 (0.93-2.71; .092)	0.65 (0.29-1.46; .30)	0.64 (0.29-1.44; .28)
Age group at HSCT							
Age at HSCT conditioning <1 y	Age at HSCT conditioning 1-2.99 y	0.84 (0.49-1.49; .56)	1.06 (0.64-1.75; .83)	0.66 (0.31-1.40; .28)	1.07 (0.58-1.96; .83)	0.78 (0.28-2.14; .62)	0.77 (0.28-2.13; .62)
	Age at HSCT conditioning 3-5.99 y	1.10 (0.46-2.63; .83)	0.97 (0.42-2.37; .99)	1.38 (0.48-3.97; .55)	1.20 (0.42-3.43; .73)	0.53 (0.11-2.61; .43)	0.55 (0.11-2.76; .47)
	Age at HSCT conditioning ≥6 y	1.51 (0.74-3.07; .26)	1.34 (0.66-2.70; .42)	1.17 (0.41-3.36; .77)	1.03 (0.36-2.93; .96)	1.19 (0.39-3.71: .76)	1.19 (0.38-3.70; .76)
Time to HSCT and interval							
Time to HSCT <6 mo	Time to HSCT ≥ 6 mo	1.22 (0.75-1.99; .42)	1.35 (0.86-2.11; .20)	0.89 (0.46-1.73; .73)	1.18 (0.67-2.08; .56)	1.63 (0.71-3.73; .25)	1.58 (0.69-3.20; .28)
HSCT time interval 2004-2006	HSCT time interval 2007-2009	1.60 (0.87-2.90; .12)	1.28 (0.74-2.19; .39)	1.55 (0.73-3.37; .25)	1.12 (0.58-2.14; .74)	1.76 (0.66-4.70; .26)	1.71 (0.64-4.56; .28).
	HSCT time interval 2010-2012	1.08 (0.55-2.10; .83)	0.99 (0.55-1.80; .97)	1.08 (0.47-2.48; .87)	0.94 (0.47-1.90; .86)	1.18 (0.38-3.67; .77)	1.14 (0.37-3.54; .82)
Donor and conditioning							
Donor: MRD	Donor: MUD	0.60 (0.32-1.15; .12)	0.71 (0.40-1.28; .26)	0.60 (0.27-1.33; .21)	0.74 (0.37-1.49; .40)	0.72 (0.24-2.15; .55)	0.73 (0.25-2.19; .58)
	Donor: UCB	0.92 (0.50-1.70; .79)	0.86 (0.47-1.55; .61)	0.85 (0.39-1.87; .68)	0.75 (0.37-1.57; .45)	1.16 (0.43-3.12; .77)	1.20 (0.45-3.22; .72)
Conditioning busulfan-based	Conditioning fludarabine-based	0.85 (0.45-1.60; .62)	1.10 (0.63-1.91; .74)	0.83 (0.35-1.94; .66)	1.27 (0.64-2.52; .49)	0.80 (0.31-2.08; .65)	0.79 (0.31-2.05; .63)
	Conditioning treosulfan-based	0.47 (0.17-1.32; .15)	0.51 (0.20-1.29; .16)	0.67 (0.23-1.95; .46)	0.68 (0.26-1.77; .43)	†	†
Disease activity at conditioning							
Complete response (ferritin <500 μg/L)‡	Partial response (ferritin <500 μg/L)§	2.12 (1.06-4.27; .035)*	1.30 (0.74-2.30; .36)	1.89 (0.82-4.37; .14)	0.99 (0.52-1.88; .96)	2.96 (0.83-10.51; .094)	3.01 (0.85-10.69; .089)
Complete response (ferritin <2000 μg/L)‖	Partial response (ferritin <2000 μg/L)¶	1.74 (0.94-3.22; .080)	1.16 (0.68-1.98; .58)	1.88 (0.86-4.09; .11)	1.01 (0.54-1.89; .97)	1.68 (0.60-4.73; .32)	1.71 (0.61-4.82; .31)
No neurologic symptoms	Neurologic symptoms	1.34 (0.72-2.52; .36)	1.26 (0.70-2.25; .45)	1.49 (0.71-3.13; .30)	1.30 (0.67-2.53; .45)	1.24 (0.36-4.19; .74)	1.24 (0.36-4.30; .73)
CSF cells or protein normal#	CSF cells or protein abnormal#	1.32 (0.57-3.04; .52)	1.64 (0.76-3.55; .21)	1.44 (0.48-4.29; .52)	1.93 (0.73-5.09; .19)	1.27 (0.34-4.75; .72)	1.22 (0.33-4.55; .77)
Ferritin, 1-unit increase in ln		1.23 (1.03-1.48; .023)*	1.08 (0.90-1.28; .42)	1.29 (1.01-1.66; .040)*	1.03 (0.82-1.28; .82)	1.13 (0.86-1.50; .37)	1.14 (0.87-1.51; .35)

All clinical parameters and laboratory values were analyzed at the time of HSCT conditioning. For OS and EFS, death, and death or second HSCT, were defined as events, respectively. Cox proportional HRs compared with given reference group. Two-sided 95% CIs are presented.

CSF, cerebrospinal fluid.

*$P < .05$ was considered significant.

†Too few patients (n = 2) to evaluate, both alive.

‡Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5, ferritin <500 μg/L; 6 of 6 or 5 of 5 (allowing missing information on one variable).

§Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <500 μg/L), and the rest of the parameters abnormal.

‖Complete response was defined as no fever, no splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <2000 μg/L; 6 of 6 or 5 of 5 (allowing missing information on 1 variable).

¶Partial response was defined as 3 to 5 of 6 normal parameters (fever, splenomegaly, ANC >1.0 × 10^9/L, platelets >100 × 10^9/L, fibrinogen >1.5 g/L, ferritin <2000 μg/L), and the rest of the parameters abnormal.

#Pathologic levels by age at HSCT conditioning.

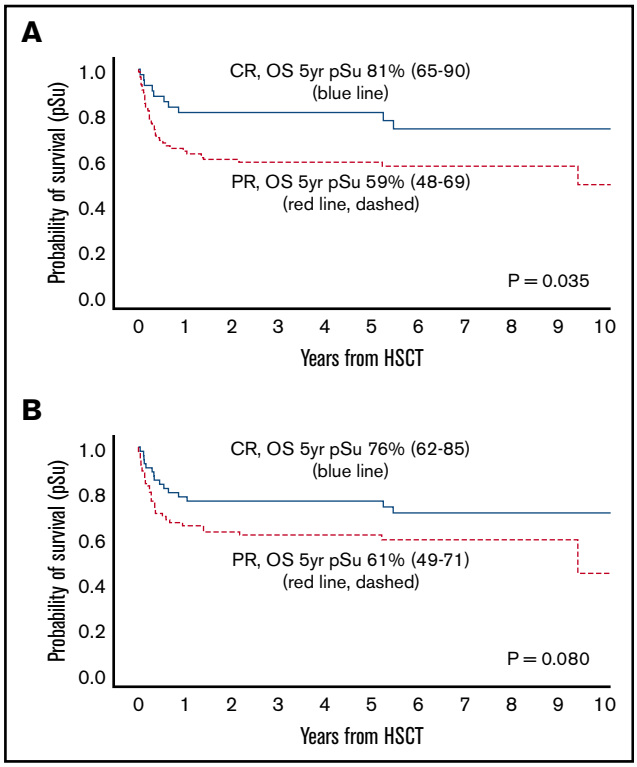

Figure 3. Kaplan-Meier estimates of OS based on complete or partial response at HSCT in the HLH-2004 study. Displayed are OS where death was defined as event. Five-year probabilities of survival (pSu) are indicated with a 95% CI. *P* values from Cox proportional hazards models. Complete response (CR) was defined as no fever, no splenomegaly, ANC ≥1.0 × 10^9/L, platelets ≥100 × 10^9/L, fibrinogen >1.5 g/L, and a ferritin cutoff of either <500 μg/L or <2000 μg/L (allowing missing information on one of the these parameters). Partial response (PR) was defined as having 3 to 5 normal parameters. (A) OS for the entire cohort based on CR (n = 42, events n = 10, blue line) or PR (n = 87, events n = 37, red line dashed); *P* = .035, using the ferritin cutoff of <500 μg/L. (B) OS for the entire cohort based on CR (n = 55, events n = 15, blue line) or PR (n = 76, events n = 31, red line dashed); *P* = .080, using the ferritin cutoff of <2000 μg/L.

(missing data, n = 3). Donors at first HSCT were MUD (n = 8), UCB (n = 6), MRD (n = 4), haploidentical (n = 1), and MMUD (n = 1). Eight children had another donor for the second HSCT, whereas 5 children had the same (missing information, n = 7). Conditioning for the first HSCT was busulfan based (n = 9), fludarabine based (n = 8), and treosulfan based (n = 1) (missing information, n = 2). Notably, the 5-year cumulative incidence of a second transplantation was 23% (95% CI, 12-43) after fludarabine-based conditioning and 8% (95% CI, 4-14) after busulfan/treosulfan-based conditioning (*P* = .029).

Ten of 20 children were alive after the second HSCT, all with >3 years of follow-up after the second HSCT, whereas 7 (MRD, n = 1; MUD, n = 1; UCB, n = 5) had died (exact duration of follow-up is missing for 3 additional survivors). Two children had a third HSCT, and both were alive at last follow-up.

Mortality

Of the deceased children (n = 67 of 187 [36%]), 36 (54%) died within 100 days and 58 (87%) died during the first year post-HSCT. Nine died ≥1 year post-HSCT (six during years 2 and 3, and three after year 5). These 67 children included 43 (32%) of 134 with verified FHL and 24 (45%) of 53 without verified FHL. Thirty-six children (54%) were reported to have died of TRM (including five after their second HSCT), 18 (27%) due to HLH relapse (nine <100 days after HSCT; six with nonverified FHL, and three with FHL [one after second HSCT]). The cause of death (TRM or HLH relapse) could not be determined in 5 children, was reported as other causes in 4, and information was missing in 4.

Children without verified FHL were more likely to die of HLH relapse than children with verified FHL. Their 5-year cumulative incidence of HLH relapse death was 18% (95% CI, 10% to 32%) compared with 6% (95% CI, 3% to 12%) for children with verified FHL (*P* = .0123). Nine children without verified FHL died of HLH relapse, 8 aged ≥1 year at the start of HLH-2004 treatment and 4 aged >6 years. In children with verified FHL, all 9 who died of HLH relapse were aged <1 year at treatment start.

The causes of death are detailed in supplemental Table 5. About one-half of the deaths within 100 days were attributed to treatment-related lung and liver complications. One 17-year-old boy died of acute myeloid leukemia 2 years after start of HLH-2004 treatment, but etoposide was only administered for 3 weeks due to pneumonia and bacteremia, after which he was in remission.

Outcome of other primary HLH patients

In addition to the 134 children with verified FHL included in the study, 20 children with primary HLH underwent HSCT following HLH-2004 treatment (XLP, n = 8; GS2, n = 11; CHS, n = 1). Donors used were MRD (n = 5), MUD (n = 9), haploidentical (n = 3), MMUD (n = 1), and UCB (n = 1) (no data, n = 1). The conditioning regimens were busulfan based (n = 8), fludarabine based (n = 8), and treosulfan based (n = 1) (no data, n = 3). At last follow-up, 14 of 20 children were alive (5 of 6 children with XLP1, 1 of 2 with XLP2, 7 of 11 with GS2 and the 1 child with CHS), with a 5-year OS of 70% (95% CI, 45-85) and a 5-year EFS of 70% (95% CI, 45-85).

Discussion

We report the largest prospective study on HSCT for HLH thus far, comprising 187 children receiving 209 transplantations within the HLH-2004 study conducted with wide international collaboration. Survival in HLH improved dramatically worldwide after the introduction of the HLH-94 treatment protocol.[28] In the subsequent study (HLH-2004), 5-year OS reached 61%, and pre-HSCT mortality was reduced from 26% in HLH-94 to 19% (using the same criteria).[35] However, the overall 5-year OS post-HSCT of 66% (71% in children with verified FHL) in HLH-2004 reported here was not better than the 64% reported in HLH-94.[31] In both protocols, myeloablative conditioning containing busulfan was suggested, which may in part explain the similar results. Nevertheless, HLH-2004 data could help to understand how to improve HSCT survival in HLH.

Notably, 53 (28%) of 187 children who underwent transplant had no family history, cytotoxicity data, or biallelic mutations suggesting verified FHL. Because not all children were analyzed for all FHL-related genes, some children reported as "without verified FHL" may still have had FHL. Remarkably, the age at start of HLH-2004 treatment was significantly higher in children without verified FHL

compared with those with FHL (median, 441 days vs 103 days, respectively; $P < .001$); their median time to HSCT was significantly longer ($P = .011$), and, moreover, their 5-year OS was significantly lower (52% vs 71%; $P = .040$). Did these children really have FHL? Indeed, 34 (64%) of these 53 children had either no evidence of defective NK cell activity (n = 20) or, in patients for whom NK-cell activity was not analyzed after 2 months or at HSCT, no biallelic mutations in 3 or 4 FHL-related genes analyzed (n = 14). This suggests that a considerable proportion of the children undergoing transplant may actually not have had FHL, although we cannot exclude other relevant indications to transplant. Possible alternative diagnoses include various forms of sHLH, such as undetected malignancies (malignancy-associated HLH) as well as infection-associated HLH and MAS-HLH (eg, reactivating autoimmune/autoinflammatory diseases), particularly in children aged ≥1 year at the start of HLH-2004 (n = 29; median starting age, 1360 days).[21] Similarly, children without verified FHL died of HLH relapse significantly more often than children with verified FHL ($P = .012$). With the aim to refine the diagnosis of FHL, because it is known that NK cell activity can be transiently reduced during active sHLH,[46,47] we suggest retesting cytotoxicity function (including CD107a mobilization, perforin expression, and SAP/XIAP expression) pretransplant at a time of no HLH disease activity in patients lacking evidence of biallelic FHL-causing mutations.[48-50]

Timing of HSCT may be critical in FHL, also because prolonged disease activity increases the risk of irreversible CNS damage, which is the most important long-term complication in FHL.[15] Disease control achieved by initial treatment is associated with a better outcome, as illustrated according to ferritin level: a one unit increase in the natural logarithm was associated with significantly increased risk of death in all patients (HR, 1.23; $P = .023$) as well as in verified FHL patients (HR, 1.29; $P = .040$). Notably, although the 5-year OS for all children with complete response (using a ferritin cutoff of <500 μg/L) at HSCT conditioning was better (81%) than for those with partial response (59%; $P = .035$), partial remission should not preclude performing HSCT in FHL.[31,32,51] Therefore, children for whom nonactive disease is difficult to achieve (particularly in those with CNS involvement) would likely benefit from prompt HSCT as previously suggested.

The choice of HSCT donor may be crucial. The 78% 5-year OS in FHL patients with MUD compared favorably with the 64% outcome with MRD ($P = .21$), thus confirming that the use of MUD is a safe and valuable option (Table 4; supplemental Figure 1B). Whether the nonsignificantly inferior outcome associated with MRD represents a true phenomenon remains to be determined.

Is the choice of the conditioning regimen related to outcome? The 5-year OS for treosulfan-, fludarabine-, and busulfan-based regimens in children with verified FHL was 78%, 76%, and 70%, respectively. Remarkably, rescue transplantations may be beneficial in selected patients, as 13 (65%) of 20 patients with a second transplant and both patients with a third transplant were alive. Not surprisingly, second transplants were significantly more frequent after fludarabine-based conditioning than after busulfan-based/treosulfan-based conditioning ($P = .029$). Conversely, fludarabine-based reduced-intensity condition reportedly has less TRM than traditional busulfan-based myeloablative conditioning, albeit a higher degree of mixed chimerism, secondary graft failure, and relapse rates.[32,36,52-57]

This study has limitations, including the rate of missing values, likely due to its multicenter design. Another limitation is that, due to the year of writing the protocol, information on only 6 HLA loci for donor typing were requested. Because only 1 child was aged <3 months at the time of transplant, the study cannot advise on HSCT in such very young patients. Notably, results of this pediatric cohort cannot be fully translated to adult patients with HLH, because underlying diseases, as well as induction and conditioning therapy, differ significantly.[21]

We conclude that: (1) patients with nonverified FHL (possibly including sHLH cases) seem to do worse than verified FHL patients; (2) a thorough patient selection with pretransplant analyses, including confirmation of FHL, is recommended; and (3) pretransplant complete remission is beneficial but not mandatory to achieve post-HSCT survival. Finally, we are optimistic that post-HSCT survival in FHL can be improved based on these lessons, on rapid availability of functional and genetic diagnostic tests leading to earlier transplants, on promising studies of treosulfan-based conditioning that reportedly have less extramedullary toxicity than other myeloablative conditioning regimens,[52,54] on targeted sub-myeloablative busulfan administration,[58] and on more experience with fludarabine-based reduced-intensity condition.[36,53,55-59]

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Authorship

Contribution: J.-I.H., A.H., M.A., G.J., S.L., and K.L.M. planned the study, with J.-I.H. as principal investigator and A.H. as study coordinator; J.-I.H., M.A., I.A., E.I., G.J., K.L., K.L.M., M.M., V.N., D.A.R., and E.S. recruited patients; I.H.M. provided statistical advice and analyses; J.W. served as HSCT advisor; E.B. performed data entry, compiled data, and performed statistical analyses; E.B., A.H., and J.-I.H. analyzed results; and E.B. and J.-I.H. drafted the manuscript, which was reviewed and approved by all authors.

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References

1. Janka GE. Familial hemophagocytic lymphohistiocytosis. *Eur J Pediatr*. 1983;140(3):221-230.
2. Côte M, Ménager MM, Burgess A, et al. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest*. 2009;119(12):3765-3773.
3. Emile JF, Abla O, Fraitag S, et al; Histiocyte Society. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. *Blood*. 2016;127(22):2672-2681.
4. Feldmann J, Callebaut I, Raposo G, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell*. 2003;115(4):461-473.
5. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science*. 1999;286(5446):1957-1959.
6. zur Stadt U, Rohr J, Seifert W, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. *Am J Hum Genet*. 2009;85(4):482-492.
7. zur Stadt U, Schmidt S, Kasper B, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet*. 2005;14(6):827-834.
8. Cetica V, Sieni E, Pende D, et al. Genetic predisposition to hemophagocytic lymphohistiocytosis: report on 500 patients from the Italian registry. *J Allergy Clin Immunol*. 2016;137(1):188-196.e4.
9. Demirkol D, Yildizdas D, Bayrakci B, et al; Turkish Secondary HLH/MAS Critical Care Study Group. Hyperferritinemia in the critically ill child with secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction syndrome/macrophage activation syndrome: what is the treatment? *Crit Care*. 2012;16(2):R52.
10. Ramos-Casals M, Brito-Zerón P, López-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. *Lancet*. 2014;383(9927):1503-1516.
11. Henter JI, Aricò M, Egeler RM, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH Study Group of the Histiocyte Society. *Med Pediatr Oncol*. 1997;28(5):342-347.
12. Henter JI, Horne A, Aricó M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-131.
13. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Eur J Pediatr*. 2007;166(2):95-109.
14. Deiva K, Mahlaoui N, Beaudonnet F, et al. CNS involvement at the onset of primary hemophagocytic lymphohistiocytosis. *Neurology*. 2012;78(15):1150-1156.
15. Horne A, Trottestam H, Aricò M, et al; Histiocyte Society. Frequency and spectrum of central nervous system involvement in 193 children with haemophagocytic lymphohistiocytosis. *Br J Haematol*. 2008;140(3):327-335.
16. Dürken M, Horstmann M, Bieling P, et al. Improved outcome in haemophagocytic lymphohistiocytosis after bone marrow transplantation from related and unrelated donors: a single-centre experience of 12 patients. *Br J Haematol*. 1999;106(4):1052-1058.
17. Fischer A, Cerf-Bensussan N, Blanche S, et al. Allogeneic bone marrow transplantation for erythrophagocytic lymphohistiocytosis. *J Pediatr*. 1986;108(2):267-270.
18. Jabado N, de Graeff-Meeder ER, Cavazzana-Calvo M, et al. Treatment of familial hemophagocytic lymphohistiocytosis with bone marrow transplantation from HLA genetically nonidentical donors. *Blood*. 1997;90(12):4743-4748.
19. Ehl S, Astigarraga I, von Bahr Greenwood T, et al. Recommendations for the use of etoposide-based therapy and bone marrow transplantation for the treatment of HLH: consensus statements by the HLH Steering Committee of the Histiocyte Society. *J Allergy Clin Immunol Pract*. 2018;6(5):1508-1517.
20. Kawa K, Sawada A, Sato M, et al. Excellent outcome of allogeneic hematopoietic SCT with reduced-intensity conditioning for the treatment of chronic active EBV infection. *Bone Marrow Transplant*. 2011;46(1):77-83.
21. La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood*. 2019;133(23):2465-2477.
22. Aricò M, Janka G, Fischer A, et al; FHL Study Group of the Histiocyte Society. Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. *Leukemia*. 1996;10(2):197-203.
23. Fischer A, Virelizier JL, Arenzana-Seisdedos F, Perez N, Nezelof C, Griscelli C. Treatment of four patients with erythrophagocytic lymphohistiocytosis by a combination of epipodophyllotoxin, steroids, intrathecal methotrexate, and cranial irradiation. *Pediatrics*. 1985;76(2):263-268.
24. Henter JI, Elinder G. Familial hemophagocytic lymphohistiocytosis. Clinical review based on the findings in seven children. *Acta Paediatr Scand*. 1991;80(3):269-277.
25. Imashuku S, Hibi S, Ohara T, et al; Histiocyte Society. Effective control of Epstein-Barr virus-related hemophagocytic lymphohistiocytosis with immunochemotherapy. *Blood*. 1999;93(6):1869-1874.
26. Stéphan JL, Donadieu J, Ledeist F, Blanche S, Griscelli C, Fischer A. Treatment of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins, steroids, and cyclosporin A. *Blood*. 1993;82(8):2319-2323.
27. Henter JI, Samuelsson-Horne A, Aricò M, et al; Histocyte Society. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood*. 2002;100(7):2367-2373.
28. Trottestam H, Horne A, Aricò M, et al; Histiocyte Society. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. *Blood*. 2011;118(17):4577-4584.

29. Baker KS, DeLaat CA, Steinbuch M, et al. Successful correction of hemophagocytic lymphohistiocytosis with related or unrelated bone marrow transplantation. *Blood*. 1997;89(10):3857-3863.

30. Baker KS, Filipovich AH, Gross TG, et al. Unrelated donor hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Bone Marrow Transplant*. 2008;42(3):175-180.

31. Horne A, Janka G, Maarten Egeler R, et al; Histiocyte Society. Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis. *Br J Haematol*. 2005;129(5):622-630.

32. Messina C, Zecca M, Fagioli F, et al. Outcomes of children with hemophagocytic lymphohistiocytosis given allogeneic hematopoietic stem cell transplantation in Italy. *Biol Blood Marrow Transplant*. 2018;24(6):1223-1231.

33. Naithani R, Asim M, Naqvi A, et al. Increased complications and morbidity in children with hemophagocytic lymphohistiocytosis undergoing hematopoietic stem cell transplantation. *Clin Transplant*. 2013;27(2):248-254.

34. Ouachée-Chardin M, Elie C, de Saint Basile G, et al. Hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis: a single-center report of 48 patients. *Pediatrics*. 2006;117(4):e743-e750.

35. Bergsten E, Horne A, Aricó M, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. *Blood*. 2017;130(25):2728-2738.

36. Cooper N, Rao K, Gilmour K, et al. Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis. *Blood*. 2006; 107(3):1233-1236.

37. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2008;50(6):1227-1235.

38. Lehmberg K, McClain KL, Janka GE, Allen CE. Determination of an appropriate cut-off value for ferritin in the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2014;61(11):2101-2103.

39. Trottestam H, Berglöf E, Horne A, et al. Risk factors for early death in children with haemophagocytic lymphohistiocytosis. *Acta Paediatr*. 2012;101(3):313-318.

40. Aalen O, Borgan Ø, Gjessing HK. Survival and event history analysis: a process point of view. New York, NY: Springer; 2008.

41. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16(3):1141-1154.

42. Harrel Jr F, E,. rms: Regression Modeling Strategies; 2019.

43. Gray B. cmprsk: Subdistribution Analysis of Competing Risks; 2014.

44. R Core Team. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2019.

45. de Saint Basile G, Ménasché G, Fischer A. Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. *Nat Rev Immunol*. 2010;10(8):568-579.

46. Halstead ES, Carcillo JA, Schilling B, Greiner RJ, Whiteside TL. Reduced frequency of CD56 dim CD16 pos natural killer cells in pediatric systemic inflammatory response syndrome/sepsis patients. *Pediatr Res*. 2013;74(4):427-432.

47. von Bahr Greenwood T, Palmkvist-Kaijser K, Chiang SC, et al. Elevated ferritin and soluble CD25 in critically ill patients are associated with parameters of (hyper) inflammation and lymphocyte cytotoxicity. *Minerva Anestesiol*. 2019;85(12):1289-1298.

48. Bryceson YT, Pende D, Maul-Pavicic A, et al. A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. *Blood*. 2012;119(12):2754-2763.

49. Marcenaro S, Gallo F, Martini S, et al. Analysis of natural killer-cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13-4 defect and discriminates between genetic subtypes of the disease. *Blood*. 2006;108(7):2316-2323.

50. Marsh RA, Bleesing JJ, Filipovich AH. Using flow cytometry to screen patients for X-linked lymphoproliferative disease due to SAP deficiency and XIAP deficiency. *J Immunol Methods*. 2010;362(1-2):1-9.

51. Sparber-Sauer M, Hönig M, Schulz AS, et al. Patients with early relapse of primary hemophagocytic syndromes or with persistent CNS involvement may benefit from immediate hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2009;44(6):333-338.

52. Burroughs LM, Nemecek ER, Torgerson TR, et al. Treosulfan-based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. *Biol Blood Marrow Transplant*. 2014;20(12):1996-2003.

53. Cooper N, Rao K, Goulden N, Webb D, Amrolia P, Veys P. The use of reduced-intensity stem cell transplantation in haemophagocytic lymphohistiocytosis and Langerhans cell histiocytosis. *Bone Marrow Transplant*. 2008;42(suppl 2):S47-S50.

54. Lehmberg K, Albert MH, Beier R, et al. Treosulfan-based conditioning regimen for children and adolescents with hemophagocytic lymphohistiocytosis. *Haematologica*. 2014;99(1):180-184.

55. Marsh RA, Kim MO, Liu C, et al. An intermediate alemtuzumab schedule reduces the incidence of mixed chimerism following reduced-intensity conditioning hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Biol Blood Marrow Transplant*. 2013;19(11):1625-1631.

56. Marsh RA, Vaughn G, Kim MO, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood*. 2010;116(26):5824-5831.

57. Sawada A, Ohga S, Ishii E, et al. Feasibility of reduced-intensity conditioning followed by unrelated cord blood transplantation for primary hemophagocytic lymphohistiocytosis: a nationwide retrospective analysis in Japan. *Int J Hematol*. 2013;98(2):223-230.

58. Malär R, Sjöö F, Rentsch K, Hassan M, Güngör T. Therapeutic drug monitoring is essential for intravenous busulfan therapy in pediatric hematopoietic stem cell recipients. *Pediatr Transplant*. 2011;15(6):580-588.

59. Güngör T, Teira P, Slatter M, et al; Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation. Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: a prospective multicentre study. *Lancet*. 2014;383(9915):436-448.