Hematopoietic stem cell transplantation for hemophagocytic lymphohistiocytosis: a retrospective analysis of data from the Italian Association of Pediatric Hematology Oncology (AIEOP)

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ABSTRACT

Background
Hemophagocytic lymphohistiocytosis is a life-threatening disease. Hematopoietic stem cell transplantation still represents the treatment of choice for most patients with this disease.

Design and Methods
We retrospectively analyzed 61 patients with hemophagocytic lymphohistiocytosis who underwent HSCT over a 17-year period at nine centers affiliated to the Italian Pediatric Hematology Oncology Association (AIEOP). The median time from diagnosis to hematopoietic stem cell transplantation was 0.6 years (range, 0.13–5). The donor for the first hematopoietic stem cell transplantation was either a relative (43%) or an unrelated volunteer (57%). Fifty-four patients (89%) had a complete genetic study, which led to the diagnoses of FHL2, due to perforin defect (21 patients), FHL3, due to Munc 13-4 defect (14 patients), Griscelli disease (2 patients), X-linked lymphoproliferative disease (1 patient), and CATCH22 syndrome (1 patient). No mutations were found in the remaining 15 patients. Twenty-one patients had neurological involvement at diagnosis.

Results
Three patients failed to engraft. Grade II-IV acute and chronic graft-versus-host disease occurred in 31% and 17% of patients, respectively. Overall, 39 patients are alive (64%), 15 died of toxicity, 6 of progressive disease and 1 of sudden death. The 8-year overall survival probability was 58.6% (95% confidence interval, 42-72), while the cumulative incidence of transplantation-related mortality was 25.7% (95% confidence interval, 16-40). The outcome of patients with a known genetic defect was comparable to that of patients without mutation. Neurological sequelae were reported in seven patients, six of whom had central nervous system disease at diagnosis.

Conclusions
These data confirm that hematopoietic stem cell transplantation represents a curative treatment for a large proportion of patients with hemophagocytic lymphohistiocytosis, irrespective of the underlying genetic defect.

Key words: hemophagocytic lymphohistiocytosis, perforin defect, hematopoietic stem cell transplantation, transplantation-related mortality, children.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening, sepsis-like syndrome, characterized by an uncontrolled state of hyperinflammation, caused by unremitting activation of $CD8^+$ T lymphocytes and macrophages and excessive levels of cytokines.[1-3] Genetic studies of patients with familial disease documented that HLH may result from mutations in different proteins involved in the cytotoxic machinery of natural killer and T cells,[4] including perforin (PRF1),[5] Munc 13-4,[6] syntaxin11,[7] Rab27a,[8,9] and SAP.[10] Familial HLH (FHL) is invariably fatal if left untreated. Chemo-immunotherapy with dexamethasone, etoposide and cyclosporine A or immunosuppression with rabbit anti-thymocyte globulin, cyclosporine A and prednisone, with or without intrathecal methotrexate, enables the achievement of a transient, complete or partial clinical response in most patients.[11-14] Allogeneic hematopoietic stem cell transplantation (HSCT) remains by far the most important curative approach, with a probability of long-term survival reported to range from 50% to 70%, depending on the type of donor and the intensity of the conditioning regimen.[15-17]

In this study, we report the results of HSCT in 61 consecutive patients with HLH transplanted at centers affiliated to the Italian Pediatric Hematology Oncology Association (AIEOP) network.

Design and Methods

Patients

Between January 1989 and April 2006, 102 patients were diagnosed as having HLH and their data were recorded in the AIEOP model 1.01 Registry form. Of these 102 patients, 61 (60%) underwent HSCT from either a related or an unrelated donor in nine AIEOP centers and represent the study population recorded in the AIEOP HSCT Registry.[18] All these patients met the diagnostic criteria for HLH.[13]

Table 1 shows the main characteristics of the patients included in the analysis. Consanguinity was reported in four patients (7%), and familial disease in 12 patients (20%). In 54 patients (89%), a complete genetic study for HLH-related genes was performed: 21 (39%) had bi-allelic *PRF1* mutations (FHL type 2), 14 (26%) had FHL3 due to *Munc13-4* mutations, two had Griscelli disease (4%), one had X-linked lymphoproliferative disease (2%), and one had CATCH22 syndrome (2%) while 15 patients resulted completely negative. Genetic studies were incomplete or not performed in seven patients.

Neurological involvement at diagnosis, as defined by the presence of either neurological signs, cerebro-spinal fluid pleocytosis or characteristic alterations at brain imaging, was found in 21 patients.

Forty-nine patients (80%) were treated initially with standard chemo-immunotherapy according to HLH-94 or current HLH-2004 protocols based on the use of etoposide, dexamethasone and cyclosporine A,[12-13] one patient was treated according to the LCHI protocol for hystiocytosis,[19] eight patients were treated according to personalized protocols, including etoposide and/or prednisone and/or cyclosporine A, and three patients underwent HSCT as primary treatment. Multiple intrathecal injections of methotrexate were part of treatment for patients with neurological involvement or in the case of progression of neurological symptoms.

Disease status at time of HSCT was known for 60 patients and recorded as complete regression of disease-related signs and symptoms in 18 patients (30%), significant clinical improvement but with persistence of some clinical or biological signs of HLH in six patients (partial remission, 10%), or as active HLH in 36 patients (60%). All parents or patients (when applicable) gave their informed consent to HSCT. The HLH-2004 study, including the indication for HSCT, was approved by the local institutional review boards.

HSCT supportive measures and definitions

Patients were cared for in high-efficiency particulate-filtered air (HEPA) rooms during the period that they were neutropenic; standard measures were adopted to prevent infectious complications, such as prophylaxis with non-absorbable antibiotics, fluconazole, acyclovir and cotrimoxazole (this last starting from day +30). Hyperhydration, forced diuresis and urine alkalinization were used to prevent drug-related chemical cystitis; mesna (mercaptoethanesodium sulphonate) was given to patients receiving cyclophosphamide. Erythrocyte and platelet concentrates were filtered to remove leukocytes and irradiated (25 Gy). A standard empirical approach based on broad-spectrum antibiotics and amphotericin-B deoxycholate, or its lipid/liposomal derivatives, was used for the treatment of fever or suspected infection, while specific anti-infective therapy was adopted for proven infections.[20,21] Neutrophil and platelet engraftment were defined as the first of three consecutive days on which neutrophil and platelet counts exceeded 0.5×10^9/L and 50×10^9/L, respectively.

Chimerism was assessed on whole blood by using variable nucleotide tandem repeats. The presence of >95% of donor-derived cells was defined as full donor chimerism, while the presence of > 5% of host-derived cells was considered as mixed chimerism.

Standard criteria were used to diagnose and to grade acute and chronic graft-versus-host disease (GvHD) and transplant-related toxicity.[22-24] Acute GvHD was assessed in all patients with myeloid engraftment, while chronic GvHD was evaluated only in patients surviving beyond day +100 after HSCT. The Lansky play score or Karnofsky score was used as appropriate to evaluate post-HSCT performance status.

Statistical analysis

Patients' characteristics were compared using the χ^2 or Fisher's exact test (as appropriate) in the case of discrete variables, or the Mann-Whitney test in the case of continuous variables. The end-points of the study were engraftment of neutrophils and platelets, transplantation-related mortality (TRM), cumulative incidence and grade of acute and chronic GvHD, and probability of overall survival. The follow-up time was calculated

Table 1. Clinical and demographic characteristics of the 61 patients.

Patients' characteristics	
Gender ratio (female/male)	20/41
Median age at diagnosis, (range), years	0.5 (0.03 - 12.4)
Consanguinity[a], n (%)	4 (6.8)
Family history[a], n (%)	12 (20)
Genetic studies, n (%)	
Perforin	21 (34)
Munc 13-4	14 (23)
Griscelli	2 (3)
Catch 22	1 (2)
X-linked lympho-proliferative disease	1 (2)
None of the above mutations	15 (25)
Incomplete genetic studies, n %	7 (11)
Neurological involvement at diagnosis[a], n (%)	21 (36)
Median age at HSCT, (range), years	1.4 (0.27-15.9)
Pre-HSCT therapy n (%)	
HLH 94	39 (64)
HLH 2004	10 (16)
LCH I	1 (2)
Cs-A+VP16	2 (3)
Cs-A+VP16+PDN	2 (3)
VP16	4 (7)
No therapy	3 (5)
Status at HSCT, n (%)[b]	
Complete remission	18 (30)
Partial remission	6 (10)
Active disease	36 (60)

Cs-A: cyclosporine-A; VP16: etoposide; PDN: prednisone; HSCT: hematopoietic stem cell transplantation; [a]data missing for two patients; [b]data missing for one patient.

Table 2. Summary of hematopoietic stem cell transplantation data of hemophagocytic lymphohistiocytosis patients.

Year of HSCT n (%)	
1989–1997	15 (25)
1998–2006	46 (75)
Median time from diagnosis to HSCT, (years, range)	0.6 (0.13-5)
Conditioning regimen, n (%)	
Bu + Cy ± (VP 16 or L-PAM)	34 (56)
Bu + Fludara ± (L-PAM or TT)	11 (18)
Fludara + (TT or Cy ± L-PAM)	11 (18)
VP 16 + L-PAM	1 (2)
Total body irradiation	4 (7)
Anti-thymocyte globulin	38 (62)
Alemtuzumab	2 (3)
Donor type, n (%)	
Sibling	12 (20)
Relative other than sibling	14 (23)
Unrelated	35 (57)
Source of stem cells	
Bone marrow	49 (80)
Peripheral blood	6 (10)
Cord blood	6 (10)
TNC$\times 10^8$/kg infused, bone marrow (median, range)	6.2 (1-27.3)
TNC$\times 10^7$/kg infused, cord blood (median, range)	5.9 (1.3-15)
CD34$^+\times 10^6$/kg infused, peripheral blood (median, range)	12 (9.6-22.6)
Median time to PMN recovery[a] (d, median, range)	15 (8-31)
Median time to PLT recovery[b] (d, median, range)	25 (11-122)
GvHD prophylaxis[c]	
Cs-A	15 (26)
Cs-A+Steroids	11 (19)
MTX	1 (2)
Cs-A+MTX	27 (47)
T-cell depletion[d]	4 (7)
Acute GvHD[e]	
0	19 (37)
I	17 (33)
II	14 (27)
III	2 (4)
IV	0 (0)
Chronic GvHD[f]	
0	34 (83)
Limited	2 (5)
Extensive	5 (12)

yrs: years; d: days; Bu: busulfan; Cy: cyclophosphamide; VP16, etoposide; L-PAM, melphalan; TT: thiotepa; Fludara: fludarabine; HSCT: hematopoietic stem cell transplantation; [a]data referred to 54 engrafted patients; [b]data referred to 47 engrafted patients; [c]data missing for three patients; Cs-A, cyclosporin-A; MTX, methotrexate; TNC, total nucleated cells; PMN: polymorphonuclear cells; PLT: platelet; GvHD, graft-versus-host disease; [d]T-cell depletion by CD 34$^+$ cell selection or soybean lectin agglutination; [e]data calculated on 52 patients assessable for acute GvHD (data missing for two patients); [f]data calculated on 41 patients assessable for chronic GvHD (data missing for two patients).

from the date of HSCT to the date of death or, for the patients alive, to the date of last follow-up. Engraftment and TRM for the first HSCT were estimated by the cumulative incidence method (*NCSS, Kaysville, Utah Hintze, J. 2001*) taking into account competing events; the differences between subgroups were compared with the Gray's k-sample test (*R, version 1.9; http://www.r-project.org*). A competing event for engraftment was early death (within 30 days for neutrophils and 60 days for platelets) after HSCT for disease progression or toxicity. A competing event for TRM was death due to progression of disease.[25]

Overall survival was estimated by the Kaplan-Meier method with differences between subgroups compared using the log-rank test Version 8.2 (SAS Institute, Cary, NC, USA).[26] All results are expressed as 8-year probabilities or 8-year cumulative incidences (%) with 95% confidence intervals (95% CI). Host-, donor-, and transplant-related characteristics were included in the analysis of prognostic factors for TRM and overall survival. The following variables were analyzed: patients' gender, age at HSCT, time interval between diagnosis and HSCT, status of HLH at HSCT (complete remission + partial remission *vs.* active disease), type of donor (related HLA identical *vs.* other), stem cell source (bone marrow *vs.* other sources), type of conditioning regimen (total body irradiation, yes *vs.* no; busulfan-based regimen *vs.* other; fludarabine-based regimen *vs.* other; use of anti-lymphocyte serum, yes *vs.* no), type of GvHD prophylaxis (graft manipulation, yes *vs.* no; cyclosporine-A alone *vs.* other regimens with or without cyclosporine-A), median number of total nucleated cells or CD34$^+$ cells infused, median time to neutrophil and platelet engraftment, acute GvHD occurrence (grade 0-I *vs.* II-IV) and chronic GvHD (no *vs.* limited or extensive). The variables found to be significant in univariate analysis were included in a multivariate logistic regression analysis. All reported p values are two-sided, and a significance level of $\alpha = 0.05$ was used. Follow-up data are as of December 31st, 2007.

Results

Table 2 summarizes the main HSCT data. Of the 61 HSCT recorded over a 17-year period, 46 (75%) were performed after 1998 (i.e. in the last 9 years). The medi-

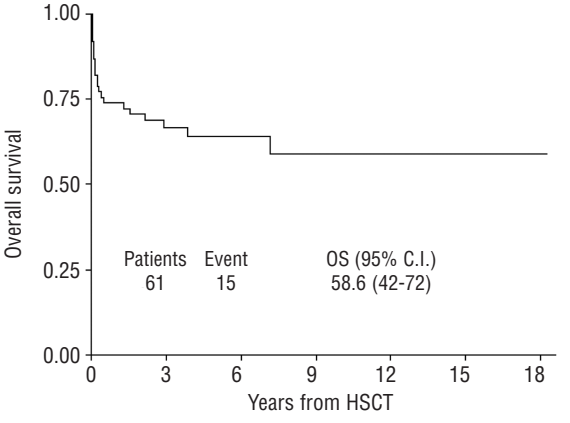

Figure 1. Overall survival (OS) of the 61 hemophagocytic lymphohistiocytosis patients.

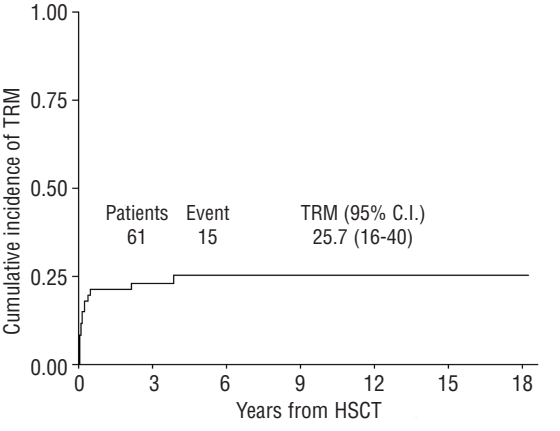

Figure 2. Cumulative incidence of transplant-related mortality (TRM) of the 61 hemophagocytic lymphohistiocytosis patients.

an time interval from diagnosis to HSCT was 0.6 years (range, 0.13–5). The donor was an unrelated volunteer in 35 cases (57%); of the 26 related donors (43%), 12 were HLA-identical siblings and 14 HLA-disparate relatives. The source of stem cells was bone marrow in 49 cases (80%), peripheral blood in six (10%), and cord blood in another six cases (10%).

A busulfan-based combination of myeloablative drugs was adopted in 45 HSCT (74%); one patient received myeloablative chemotherapy with etoposide and melphalan. A conditioning regimen with reduced extra-hematologic toxicity based on fludarabine was adopted in 11 HSCT (18%). Total body irradiation was used in four patients.

Cyclosporine alone (15 cases, 26%) or in combination with methotrexate (27 cases, 47%) or with prednisone (11 cases, 19%) was used for GvHD prophylaxis.

The median number of nucleated cells infused was 6.2×10^8/kg (range, 1-27.3) for bone marrow recipients, and 5.9×10^7/kg (range, 1.3-15) for cord blood recipients, while the median number of $CD34^+$ cells was 12×10^6/kg for patients transplanted with peripheral blood stem cells.

Engraftment

Four patients were not evaluable because they died within 10 days after HSCT and primary graft failure occurred in three patients (5%). In the remaining 54 patients neutrophil recovery was obtained at a median time of 15 days (range 8-31) after the allograft. Overall, the cumulative incidence of neutrophil engraftment was 89% (95%CI, 81-97).

Platelet engraftment was recorded in 47 of the 54 patients who had myeloid engraftment, at a median time of 25 days after HSCT (range, 11-122). Six of the remaining seven patients died early (five within 2 months and one at 3 months after HSCT); one patient achieved full donor engraftment after a second transplant. The cumulative incidence of platelet engraftment was 78% (95%CI, 68-89). Five patients received a second HSCT because of secondary graft failure (n=4) or HLH relapse (n=1) at a median time of 6.3 months (range, 0.8-12.2) after the first transplant. Four of them had both neutrophil and platelet engraftment while one patient needed a third HSCT.

Graft-versus-host disease

Data on GvHD occurrence were not available for two patients. Among the remaining 52 patients, grade II-III acute GvHD occurred in 16 (31%); no patient developed grade IV acute GvHD. Seven of 41 patients (17%) who survived more than 100 days developed chronic GvHD: two had limited disease and five had an extensive form of disease.

Transplantation-related mortality, overall survival and their risk factors

After a median follow-up of 5.5 years (range, 2.1-18.2) 39 patients were alive. Twenty-two patients died at a median time of 0.18 years (range, 0.02-7.1) after HSCT from the following causes: 15 (68%) of toxicity, six of progressive disease (27%) and one of sudden death not related to HSCT or its complication (5%). This last death occurred 2.9 years after HSCT, in a patient who was a full-donor chimera in complete remission.

The last deaths from TRM and HLH progression occurred 3.9 and 7.1 years after HSCT, respectively.

The probability of overall survival at 8 years after HSCT was 58.6% (CI, 42-72), while the overall cumulative incidence of TRM was 25.7% (95% CI, 16-40) (Figures 1 and 2).

Table 3 shows early organ toxicity according to Bearman's score. Eleven toxic deaths occurred within day +100, due to severe veno-occlusive disease (VOD, n=7) and infection (sepsis or pneumonia) (n=4), accounting for a TRM rate of 18.0% (96% CI, 11-31). Other causes of TRM beyond day +100 were hemorrhage (n=2), and severe lung disease due to chronic GvHD (n=1) A trend although not statistically significant, to better outcome was observed among the 49 patients transplanted with an unrelated or a family mismatched donor according to the period of HSCT, name-

Table 3. Overall organ toxicity in the first 100 days post-hematopoietic stem cell transplantation according to Bearman's score.

	Mild (%)	*Moderate/Severe (%)*	*Total (%)*
Heart	3	5	8
Bladder	8	0	8
Kidney	3	8	11
Liver	18	13	31
Central nervous system	5	2	7
Mucosa	52	2	54
Gastrointestinal system	26	3	29
Lung	7	13	20

Mild included grade I-II of Bearman's score; moderate/severe included grade III-IV of Bearman's score.

ly after *vs.* before 1998, the overall survival rate being 63.9% (95% CI, 45-78) *vs.* 45.5% (95% CI, 17-71), and the overall TRM rate being 21.1% (95% CI, 11-39) *vs.* 36.4% (95% CI, 17-79) (p=0.5, and p=0.3), respectively. The source of stem cells did not influence the outcome, survival probability being 83.3% (95% CI, 27-97) among cord blood recipients, 57.7% (95% CI, 39-72) among bone marrow recipients, and 50% (95% CI, 11-80) among patients (p=0.5) transplanted with peripheral blood stem cells.

None of the patient-, donor- or transplant-related characteristics analyzed was significantly associated with a better overall survival and/or lower TRM (*data not shown*). In detail, the TRM of patients transplanted in complete or partial remission was 33.3% (95% CI, 19-59) compared to 16.7% (95% CI, 8-35) in those transplanted with active disease (p=0.4). The corresponding figures for overall survival were 57.3% (95% CI, 35-74) *vs.* 64.2% (95% CI, 41-80) (p=0.2), respectively. Moreover, the outcome of patients with a known genetic defect was not different from that of patients with no genetic mutation or incomplete genetic studies: overall survival rate of 52.5% (95% CI, 25-74), *vs.* 62.0% (95% CI, 38-79) (p=1.0) and TRM 25.7% (95% CI, 15-44), *vs.* 24.4% (95% CI, 11-53), respectively (p=0.8).

Post-hematopoietic stem cell transplantation status of hemophagocytic lymphohistiocytosis and sequelae

Among the 39 patients alive, 31 (79%) were full donor chimera, while six (15%) had stable mixed chimerism, the percentage of donor cells ranging from 60% to 80% at the latest analysis. Two patients had autologous reconstitution and chemotherapy was resumed in one after disease reactivation.

Overall, the performance status was good, the median Lansky play score or Karnofsky score being 100 (range, 80-100). Fourteen patients (36%) had the following post-HSCT sequelae: neurological disease in seven [psychomotor delay (n=4), epilepsy (n=2), ataxia and paraparesis (n=1)], growth hormone deficiency in three, chronic GvHD in two (lung dysfunction, skin eczema, abnormal skin pigmentation), busulfan-related alopecia in one, and chronic lung disease with bronchiectasis and severe humoral immunodeficiency in one.

Twelve of 21 patients with central nervous system (CNS) involvement at diagnosis are alive after HSCT. Their median Karnofsky score at latest follow-up is 100 (range, 80–100). The initial CNS involvement of 12 patients was as follows: clinical-radiological signs of CNS disease in seven patients, pleocytosis in four patients and both abnormalities in one patient. Neurological complications after HSCT were observed in six of these patients, five of whom had had previous clinical-radiological signs of CNS disease and one patient with pleocytosis.

A patient with severe humoral immunodeficiency who was given a transplant of CD34 positively-selected cells from a mismatched family donor received 4 weekly doses of rituximab for Epstein-Barr virus-related lymphoproliferative disease (day +88).[27] A severe humoral immunodeficiency requiring monthly immunoglobulin administration was diagnosed after 17 months, followed by bronchiectasis at +59 months.

Discussion

HSCT, by replacing the patient's immune system with that of a healthy donor, is the only treatment option with the potential to cure FHL, irrespective of the genetic subtype. In Table 4, we summarize the main results of HSCT surveys published in the last 15 years (1995-2007) that data on more than ten HLH patients,[15-17,28-31] together with the results of our study.

Our data, representing the second largest series reported so far, show that more than half of patients with HLH treated in nine AIEOP centers over almost 20 years have been cured by HSCT.

The overall survival rate of 58.6% observed in our cohort is comparable to the 58% recently reported by Ouchèe-Chardin *et al.*, who analyzed their single center experience over the last two decades.[17] These patients received induction remission treatment with etoposide (31%) or steroids, cyclosporine A (15%) and anti-lymphocyte serum (54%). The differences between the two series for some transplant outcomes, such as rate of primary failure, incidence of GvHD and incidence of TRM, may be attributed to the more frequent use in the French series of family haploidentical donors (60%) and the consequent greater need for T-cell depletion of the graft.

Conversely, the slightly better overall survival rate of 64% reported by Horne *et al.* is probably related to more homogeneous pre-HSCT treatment of all patients, with more recent accrual.[15]

While in the past bone marrow and peripheral blood were the almost exclusive sources of stem cells,[32] transplantation of cord blood cells is now being increasingly employed. Indeed, cord blood cells were used in 10% of our patients. This percentage is in line with other reports,[15,30] and reflects the increasing access to cord blood units for patients who lack an HLA-matched bone marrow donor. Our results, indicating that the stem cell source did not influence the outcome of HSCT, find support in a previously published analysis showing that the outcome of children with acute

Table 4. Summary of the data regarding hematopoietic stem cell transplantation studies in hemophagocytic lymphohistiocytosis patients performed in the last 15 years.

Ref. N.	Author (Period of transplant)	Patients (n.)	Engraftment (%)	aGvHD (II-IV) (%)	cGvHD (%)	TRM (%)	Survival (%) (median follow-up)
28	Baker *et al.*, 1997 (1988-1995)	20[a]	90	44	28	40	45 (3-year OS)
29	Jabado *et al.*, 1997 (1991-1996)	14[b]	76*	0	9	14	64 (33 months)
30	Imashuku *et al.*, 1999 (1988-1998)	17[c]	94	24	10	12	54 (2-year OS)
31	Durken *et al.*, 1999 (1992-1998)	12[d]	100	17	8	0	100 (2 years)
15	Horne *et al.*, 2005 (1995-2000)	86[e]	90*	32	9	30	64 (3-year OS)
16	Cooper *et al.*, 2005 (1999-2004)	12[f]	100	33	25	25	75 (2.5 years)
17	Ouachée-Chardin *et al.*, 2007 (1982–2004)	48[g]	87.5*	17	9	21	58 (5.8 year OS)
/	The AIEOP–Study (1989-2006)	61[h]	95	31	17	25.7	58.6 (8- year OS)

*[a]Matched related donor (MRD) (n=4), unrelated donor (URD) (n=16). Conditioning: busulfan (Bu) 16 mg/kg + cyclophosphamide (Cy) 200 mg/kg + etoposide (VP16) 900-1500 mg/m² + antithymocyte serum (ATG); [b]Haplo (n=11), mismatched family donor (MMF) (≥ 1 Ag) (n=2), URD (n=1). *Three patients received two transplants for primary graft failure. Conditioning: Bu 16-20 mg/kg + Cy 200 mg/kg + VP16 900 mg/m² + T-cell depletion (Haplo patients); [c]MRD (n=24), URD (n=49), Haplo (n=16). Three patients received a second transplant for primary graft failure. Conditioning: Bu 16mg/kg + Cy 200 mg/kg + VP16 900 mg/m² ± ATG. T-cell depletion or CD34⁺ positive selection not reported for Haplo patients; [d]MRD (n=7), MMF (n=1), URD (n=7), Haplo (n=2). Conditioning: Bu 16 mg/kg + Cy 200 mg/kg + VP16 1200 mg/m² + ATG or total body irradiation (TBI) 12 Gy + Cy 120 mg/kg, + VP16 1200 mg/m² or TBI + Cy 120 mg/kg + ATG or TBI + Bu 16 mg/kg + melphalan 210 mg/m² . CD34⁺ positive selection was used for the Haplo cases. [e]MRD (n=4), URD (n=8). Conditioning: Bu 16-20 mg/kg + Cy 120 mg/kg + VP16 30-60 mg/kg + ATG; [f]MRD (n=1), MUD (n=8), Haplo (n=3). Conditioning: fludarabine 150 mg/m², melphalan 140 mg/m² + aletuzumab or fludarabine 150 mg/m², melphalan 125 mg/m² + Bu 8 mg/kg + ATG and CD34⁺ positive selection for Haplo patients; [g]MRD (n=24), URD (n=49), Haplo (n=16). Three patients received a second transplant for primary graft failure. Conditioning: Bu 16 mg/kg + Cy 200 mg/kg + VP16 900 mg/m² + ATG. T-cell depletion or CD34⁺ positive selection not reported for Haplo patients. [g]MRD (n=14), MUD (n=5), Haplo (n=29). *Seven patients received a second transplant for primary graft failure. Conditioning: Bu 16-20 mg/kg + Cy 200 mg/kg + VP16 30 mg/kg + ATG and/or T-cell depletion or CD34⁺ positive selection (Haplo patients). [h]see tables 1, 2 and text for details. Ref. no, reference number; aGvHD: acute graft-versus-host disease; cGVHD: chronic graft-versus-host disease; TRM, transplant related mortality; OS: overall survival.*

leukemia who underwent unrelated cord blood transplantation is similar to that of patients transplanted with bone marrow from an HLA-matched unrelated donor.[33]

We observed a trend, although not statistically significant, towards an improvement in survival and TRM among patients who underwent HSCT from an unrelated or family-mismatched donor after 1998. For children treated before that year, overall survival was 45.5%, a value comparable to that published by Baker *et al*. for a small series of 20 patients transplanted between 1988 and 1995.[28] Yet, for patients transplanted from 1998 onward, the overall survival rate was 63.9%, which is comparable to that reported by Horne *et al*. in 86 patients homogeneously treated according to the HLH-94 protocol and transplanted between 1995 and 2000.[15] The continuous improvement of transplant results over the years may reflect both improved management of HLH and better donor selection through the introduction of high-resolution molecular typing of both HLA class I and II loci.[34]

We could not document the beneficial effect of complete control of HLH at the time of HSCT, reported by Ouachèe-Chardin and Baker,[17,28] but not confirmed by others.[30,31] In view of the lack of evidence that disease control has a favorable impact on post-transplantation outcome, we suggest that the transplant option should not be postponed in patients with FHL not benefiting from complete control of the inflammatory syndrome. Indeed, the choice of continuing chemo-immunotherapy in patients with delayed or suboptimal response might be associated with an increased risk of both infectious complications (fatal or with high morbidity) and fulminant neurological complications, thus preventing a subsequent HSCT.

TRM remains the major obstacle to the cure of FHL by HSCT.[15-17,28] In our series, moderate to severe non-hematologic toxicity affected mainly the liver, lungs and kidneys, vaso-occlusive disease, sepsis and pneumonia being the main causes of death. The frequent use of a busulfan-based myeloablative conditioning regimen may be responsible for a higher risk of severe vaso-occlusive disease.[35] Recently, Cooper *et al*. reported that no case of vaso-occlusive disease occurred in eight patients who received an unrelated donor graft using a fludarabine/melphalan-based conditioning regimen, or in three patients who received a haploidentical donor graft using a conditioning regimen with fluda-

rabine/melphalan and a reduced dose of busulfan (8 mg/kg over 2 days).[16] The prophylactic use of defibrotide, a drug that has shown both a good safety profile and efficacy against vaso-occlusive disease, is still under preliminary evaluation due to the limited data available.

Mild to severe mucositis often occurs in patients treated with a standard myeloablative regimen based on busulfan (16-20 mg/kg), cyclophoshamide (120-200 mg/kg) and etoposide (50-60 mg/kg), and may necessitate elective intubation for airway protection.[28,31] In order to diminish early organ toxicity, conditioning regimens with reduced extra-hematologic toxicity have been used without compromising engraftment.[16,27,36,37] In our cohort, a fludarabine-based conditioning regimen was used in 18% of HSCT, but we were unable to document that this had any significant influence on overall survival or TRM. Although promising, especially in non-malignant diseases, the use of regimens with reduced extramedullary toxicity requires further investigations in the future.

CNS involvement occurs in 29% to 60% of patients with HLH,[15-17,38] with a progressive course if the disease is not appropriately treated. Despite the potential neurotoxicity of some drugs employed for HSCT and GvHD prevention/treatment, there is evidence that HSCT may stabilize or even revert HLH-associated CNS disease.[17,31,39] In this series, none of the patients who were free from CNS manifestations of HLH developed neurological sequelae after HSCT. Moreover, five of 12 patients with CNS disease at diagnosis showed an improvement or even reversal of neurological signs/symptoms of disease, the median Karnofsky score being 100.

In conclusion, we confirm that HSCT represents a curative treatment for HLH patients irrespective of remission status before HSCT. The major barrier to a successful HSCT outcome is still TRM, and further investigation needs to be done on this subject to reduce or prevent extra-hematologic toxicity.

Appendix

The AIEOP-HSCT centers and investigators contributing patients included in this analysis are as follows (the number of patients transplanted in each Center is given in parentheses): Oncoematologia Pediatrica, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Franco Locatelli, Marco Zecca, Giovanna Giorgiani, Maria Ester Bernardo (26 patients); Dipartimento di Ematologia e Oncologia, Ospedale Giannina Gaslini, Genova, Giorgio Dini, Edoardo Lanino, Giuseppe Morreale, Maura Faraci (8 patients); Clinica Pediatrica, Spedali Civili, Università di Brescia, Fulvio Porta, Alessandro Plebani (7 patients); Clinica Pediatrica, Università di Milano-Bicocca, Ospedale Nuovo San Gerardo, Monza, Cornelio Uderzo, Attilio Rovelli, Adriana Balduzzi, Giuseppe Masera (6 patients); Oncoematologia Pediatrica, Dipartimento di Pediatria, Università di Padova, Chiara Messina, Simone Cesaro, Marta Pillon, Elisabetta Calore, Modesto Carli (4 patients); Oncologia ed Ematologia Pediatrica "Lalla Seràgnoli", Ospedale S. Orsola Malpighi, Università di Bologna, Arcangelo Prete, Roberto Rondelli, Andrea Pession (3 patients); Oncoematologia Pediatrica, Ospedale Pausilipon, Napoli, Mimmo Ripaldi, Vincenzo Poggi (3 patients); Oncoematologia Pediatrica, Ospedale Infantile Regina Margherita, Torino, Franca Fagioli, Enrico Madon (2 patients); Centro Trapianti Trapianti di Midollo, IRCCS Burlo Garofolo, Trieste, Natasha Maximova, Marino Andolina (2 patients).

Authorship and Disclosures

SC designed the study and wrote the manuscript. RR and SC collected the data and performed the statistical analysis. MA performed the genetic study. SC, FL, EL, FP, LDM, CM, AP, MR, NM, GG, and FF performed HSCT. FL contributed to writing the manuscript and revised its definitive version. All authors read and approved the definitive version of the manuscript. The authors declared that they have no potential conflicts of interest.

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