

Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation

Jan-Inge Henter, AnnaCarin Samuelsson-Horne, Maurizio Aricò, R. Maarten Egeler, Göran Elinder, Alexandra H. Filipovich, Helmut Gadner, Shinsaku Imashuku, Diane Komp, Stephan Ladisch, David Webb, and Gritta Janka, for the Histiocyte Society

Hemophagocytic lymphohistiocytosis (HLH) comprises familial (primary) hemophagocytic lymphohistiocytosis (FHL) and secondary HLH (SHLH), both clinically characterized by fever, hepatosplenomegaly, and cytopenia. FHL, an autosomal recessive disease invariably fatal when untreated, is associated with defective triggering of apoptosis and reduced cytotoxic activity, resulting in a widespread accumulation of T lymphocytes and activated macrophages. In 1994 the Histiocyte Society initiated a prospective international collaborative therapeutic study (HLH-94), aiming at improved

survival. It combined chemotherapy and immunotherapy (etoposide, corticosteroids, cyclosporin A, and, in selected patients, intrathecal methotrexate), followed by bone marrow transplantation (BMT) in persistent, recurring, and/or familial disease. Between July 1, 1994, and June 30, 1998, 113 eligible patients aged no more than 15 years from 21 countries started HLH-94. All had either an affected sibling (n = 25) and/or fulfilled the Histiocyte Society diagnostic criteria. At a median follow-up of 3.1 years, the estimated 3-year probability of survival overall was 55% (95% confidence interval \pm 9%), and

in the familial cases, 51% (\pm 20%). Twenty enrolled children were alive and off therapy for more than 12 months without BMT. For patients who received transplants (n = 65), died prior to BMT (n = 25), or were still on therapy (n = 3), the 3-year survival was 45% (\pm 10%). The 3-year probability of survival after BMT was 62% (\pm 12%). HLH-94 is very effective, allowing BMT in most patients. Survival of children with HLH has been greatly improved. (Blood. 2002;100:2367-2373)

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) represents a spectrum of inherited and acquired conditions with disturbed immune regulation of different severity, and it encompasses 2 main conditions that have common clinical and pathobiological characteristics: familial (primary) hemophagocytic lymphohistiocytosis (FHL) and secondary hemophagocytic lymphohistiocytosis (SHLH). In contrast to SHLH, which may affect any age and which may subside spontaneously, FHL is an invariably fatal inherited disease seen mostly in infancy and early childhood.¹⁻³ The annual childhood incidence of FHL has been estimated (in Sweden) at 1.2 cases per 1 000 000, corresponding to 1:50 000 births.⁴ Common findings include fever, hepatosplenomegaly, pancytopenia, and reduced cytotoxic T- and natural killer (NK)-cell activity, as well as a widespread accumulation of T lymphocytes and macrophages, some of which may engage in hemophagocytosis.¹⁻⁵ Central nervous system (CNS) involvement is frequent, ranging from irritability, bulging fontanel, and neck stiffness, to convulsions, cranial nerve palsies, ataxia, psychomotor retardation, and coma.⁶⁻⁹

A hypercytokinemia, mainly involving proinflammatory cytokines, mediates the clinical and laboratory findings.¹⁰⁻¹⁴ A defective triggering of apoptosis in FHL was recently suggested as the underlying pathophysiologic mechanism.¹⁵ In 1999, genetic studies showed linkage to the chromosome regions 9q21.3-22 and 10q21-22 for some, but not all, patients.¹⁶⁻¹⁸ Recent studies revealed perforin gene defects in 10q21-22-linked FHL-patients,¹⁹⁻²¹ supporting the hypothesis that FHL, at least in some patients, is caused by a deficiency in the triggering of apoptosis.^{15,19-23} Of differential diagnostic importance is that a few male patients with HLH recently have been shown to harbor germline mutations in SH2D1A/SAP, the gene causing X-linked lymphoproliferative syndrome (XLP).²⁴

Chemotherapy with epipodophyllotoxin derivatives, etoposide (VP-16) and teniposide (VM-26), combined with corticosteroids and intrathecal methotrexate (IT MTX) induce remission in FHL.²⁵⁻²⁷ Remission also can be achieved with immunotherapy, that is, antithymocyte globulin (ATG) and steroids followed by cyclosporin A (CSA).²⁸ Ultimately, all FHL patients relapsed and

From the Childhood Cancer Research Unit, Karolinska Institutet, Department of Pediatric Hematology and Oncology, Karolinska Hospital, Stockholm, Sweden; the Department of Pediatrics, Stockholm Söder Hospital, Karolinska Institutet, Stockholm, Sweden; the Onco Ematologia Pediatrica, Ospedale dei Bambini G di Cristina, Palermo, Italy; the Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands; the Children's Hospital Medical Center, Cincinnati, OH; the St Anna Children's Hospital, Vienna, Austria; the Children's Research Hospital, Kyoto Prefectural University of Medicine, Japan; the Department of Pediatrics, Yale University School of Medicine, New Haven, CT; the Children's Research Institute, Washington, DC; the Great Ormond Street Hospital, London, United Kingdom; the Department of Hematology and Oncology, Children's University Hospital, Hamburg, Germany.

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Reprints: Jan-Inge Henter, Childhood Cancer Research Unit Q6:05, Karolinska Hospital, S-171 76 Stockholm, Sweden; e-mail: Jan-Inge.Henter@kbh.ki.se.

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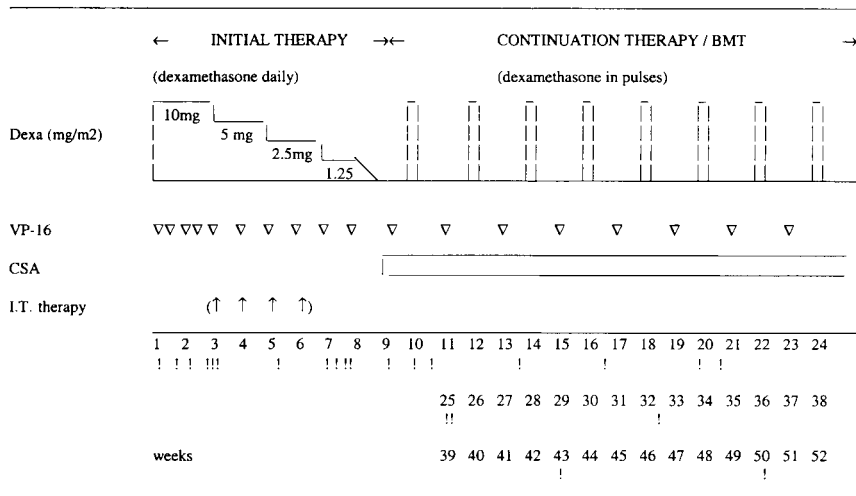


Figure 1. Overview of the treatment protocol HLH-94. Day of death for the 23 patients who died the first year of therapy, except those who underwent BMT, is marked with an exclamation point (!). (BMT = Go to BMT during continuation therapy as soon as an acceptable donor is available, preferably when the disease is nonactive. The patients without either familial or persistent disease were recommended to cease therapy after the initial therapy and restart in case of reactivation. Dexamethasone daily [pulses are 10 mg/m² for 3 days]; VP-16 = etoposide 150 mg/m² intravenously; CSA = cyclosporin A; I.T. therapy = intrathecal methotrexate [if progressive neurological symptoms or if an abnormal CSF has not improved].)

died until Fischer and coworkers showed that cure could be achieved through allogeneic bone marrow transplantation (BMT),²⁹ which was later confirmed by others.³⁰⁻³²

Despite these improvements in treatment, multiple problems remained, including death prior to or during remission.² Prompted by these therapeutic difficulties, in 1994 the Histiocyte Society developed a treatment strategy (HLH-94) that combines (rather than randomizes between) 2 previously reported regimens: chemotherapy³³ and immunotherapy.³⁰ HLH-94 is based on VP-16, corticosteroids, CSA, and, in selected patients, IT MTX, prior to intended BMT.³⁴ Herein we present results for 113 patients, recruited during a 4-year period, with a major focus on survival and outcome.

Patients, materials, and methods

Treatment protocol

The HLH-94 treatment protocol includes 8 weeks of initial therapy, aiming at achieving a clinical remission, followed by a continuation therapy aiming at keeping the children alive and stable until an acceptable BMT donor becomes available (Figure 1). The initial therapy consists of VP-16 (150 mg/m² twice weekly for 2 weeks and then weekly) and dexamethasone (initially 10 mg/m² for 2 weeks followed by 5 mg/m² for 2 weeks, 2.5 mg/m² for 2 weeks, 1.25 mg/m² for one week, and one week of tapering). After 8 weeks of initial treatment, it was recommended that children with known familial disease or persistent nonfamilial disease proceed to continuation therapy and BMT, whereas children with resolved nonfamilial disease ceased therapy and restarted HLH-94 only in case of reactivation, in order to avoid prolonged therapy and BMT for patients with presumably SHLH (Figure 2). The continuation therapy from week 9 and onward comprised dexamethasone pulses 10 mg/m² for 3 days every second week and VP-16 infusions 150 mg/m² every alternating second week in

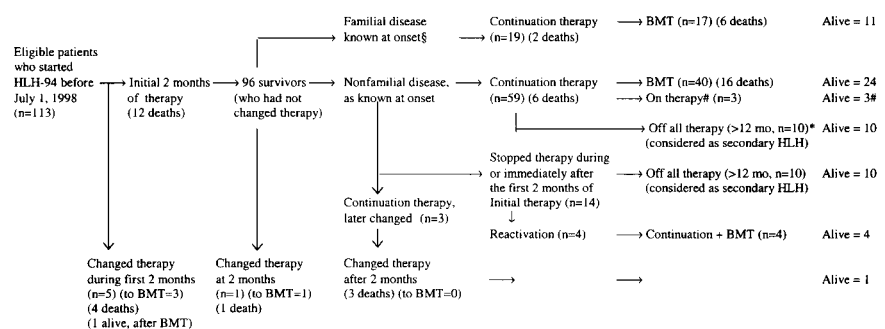
combination with daily oral CSA, aiming at trough levels of 200 µg/L. An important role for the continuation therapy is to keep the children alive and in a stable condition during the search of a marrow donor. IT MTX was administered at a maximum of 4 doses, but was recommended only if there were progressive neurological symptoms or if an abnormal CSF had not improved. Recommended supportive therapy included antimycotic treatment during the initial dexamethasone therapy and continuous cotrimoxazole treatment, equivalent to 5 mg/kg of trimethoprim 3 times weekly, as *Pneumocystis carinii* prophylaxis.

It is often not possible to differentiate between inherited HLH and SHLH at diagnosis, unless there is already an affected child in the family. Most children with the inherited form of the disease will appear as sporadic cases since the inheritance is autosomal recessive. Moreover, an association with a viral infection cannot be used to justify the diagnosis of SHLH, since such infections may be concomitant with the onset of FHL and even trigger the disease.^{4,35,36} Because of these difficulties, children with severe or persistent disease are recommended to start HLH therapy also if there is no evidence of familial disease. In patients without family history but with relapsing or nonresponding disease, an underlying inherited defect is most likely, as in the verified familial ones. On the contrary, in responding patients that do not relapse when treatment is stopped, a secondary cause is likely. Genetic analyses may provide a distinct diagnosis,¹⁶⁻²¹ but the results are rarely available at onset of the disease. Moreover, the genetic explanation is presently known only for a minority of the patients.²⁰

Bone marrow transplantation

The conditioning regimen and the graft-versus-host-disease (GVHD) prophylaxis were determined by the treating transplantation unit, but a suggested protocol was provided. The suggested conditioning consisted of busulfan 4 mg/kg on days -9, -8, -7, and -6; cyclophosphamide 50 mg/kg on days -5, -4, -3, and -2; and VP-16 300 mg/m² on days -5, -4, and -3. In case of transplants from unrelated donors, additional immunosuppression with horse ATG was suggested, 15 mg/kg twice daily on days -2 and -1, and once daily on

Figure 2. Flow of 113 patients in the HLH-94 treatment protocol. ‡: Of the 25 familial cases reported, 3 were not known at onset and another 3 changed therapy prior to onset of continuation therapy. #: Two were on cyclosporin A only, and one also received corticosteroids. *: Eight had received therapy for 2-12 months, and 2 for 12-24 months.



days +1 and +2. GVHD prophylaxis included intravenous methotrexate 15 mg/m² on day +1 and 10 mg/m² on days +3, +5, and +11, in combination with CSA beginning on day -3.

Patients

Altogether 119 children aged no more than 15 years who had not received any previous cytotoxic or CSA therapy and who were either familial cases or fulfilled the diagnostic criteria approved by the Histiocyte Society (Figure 3) started HLH-94 therapy between July 1, 1994, and June 30, 1998. Of the 25 patients with an affected sibling, 17 fulfilled all diagnostic criteria, 4 fulfilled all criteria but 1, and 4 were missing 2 criteria (the missing criteria being fever = 3, cytopenia = 3, splenomegaly = 0, hypertriglyceridemia/hypofibrinogenemia = 1, hemophagocytosis = 5). The cut-off times were set in order to obtain a minimum of one-year follow-up. Six children were subsequently found to have specific underlying disorders (non-Hodgkin lymphoma [n = 2]), T-cell acute lymphoblastic leukemia, large granular lymphocytic leukemia, juvenile rheumatoid arthritis, and a metabolic disease). Thus, 113 children (61 males [M]/52 females [F]) were eligible for complete analyses, with a median follow-up after onset of therapy in the surviving patients of 38 months (range, 15-69). The 113 patients were recruited from 21 countries: Argentina, Austria, Canada, Denmark, Finland, Germany, Hong Kong, Italy, Japan, Korea, The Netherlands, Norway, Saudi Arabia, South Africa, Spain, Sweden, Switzerland, Turkey, United Kingdom, United States of America, and Yugoslavia.

Since it may be difficult and sometimes impossible to distinguish FHL from SHLH, and since the mortality in SHLH is also high,³⁵ the HLH-94 protocol, though primarily designed for the treatment of FHL, was open for all HLH patients. Furthermore, bouts of FHL may be triggered by infections,³⁶ which is also true of SHLH, the latter commonly associated with a strong macrophage activation and often referred to as infection- (virus-) associated hemophagocytic syndrome (IAHS/VAHS) or malignancy-associated hemophagocytic syndrome (MAHS).³⁵ Acknowledging these diagnostic difficulties, the protocol recommended stopping therapy after 8 weeks for nonfamilial cases with resolved disease, and treatment was restarted only in cases with reactivation.

Statistical analysis

The comparisons of different variables, such as age and disease status, were performed by univariate analyses. Log-rank test comparing categories with respect to the cumulative survival were performed with SPSS 10.0 (Chicago, IL) and illustrated by the Kaplan-Meier method. The cut-off time for entering data was July 31, 2000, and the data reported refer to the last information obtained. The study was approved by the Histiocyte Society and the Ethics Committee of the Karolinska Institute.

Clinical criteria

- Fever

- Splenomegaly

Laboratory criteria

- Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)

Hemoglobin (< 90 g/L), platelets (< 100 × 10⁹/L), neutrophils (< 1.0 × 10⁹/L)

- Hypertriglyceridemia and/or hypofibrinogenemia

Fasting triglycerides ≥ 2.0 mmol/L or ≥ 3 SD of the normal value for age

Fibrinogen ≤ 1.5 g/L or ≤ 3 SD)

Histopathologic criteria

- Hemophagocytosis in bone marrow or spleen or lymph nodes

No evidence of malignancy

Comments:

1. If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.
2. The following findings may provide strong supportive evidence for the diagnosis: (1) Spinal fluid pleocytosis (mononuclear cells), (2) Histological picture in the liver resembling chronic persistent hepatitis (biopsy), or (2) Low natural killer cell activity.
3. Other abnormal clinical and laboratory findings consistent with the diagnosis are cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash, hepatic enzyme abnormalities, hyperferritinemia, hypoproteinemina, hyponatremia, spinal fluid protein ↑, VLDL ↑, HDL ↓, circulating soluble IL-2 receptor ↑.

Figure 3. Diagnostic guidelines for HLH (adapted from Henter et al⁴). All criteria required for the diagnosis of HLH. In addition, the diagnosis of FHL is justified by a positive family history, and parental consanguinity is suggestive.

Table 1. Overview of overall outcome in 113 patients with HLH treated according to the HLH-94 protocol

	All patients (n = 113)	Familial cases* (n = 25)
Alive after BMT	40	13
Dead after BMT	25†	7†
Alive without BMT	23‡	
Dead prior to BMT	25	5

*All these patients had an affected sibling with HLH.

†One child, with a positive family history, died of a surgical hemorrhage unrelated to HLH.

‡Three patients were still on therapy (2 on CSA alone; 1 also received corticosteroids); the others were off therapy for more than 12 months.

Results

Overall survival

Altogether, 63 (56%) of the 113 children were alive at latest follow-up (median follow-up, 37.5 months). Forty of these 63 patients (63%) had undergone BMT (Table 1). Fifty percent of the deceased children (25/50) had undergone BMT. The estimated 3-year probability of survival of all 113 children is 55% ± 9%, 95% confidence interval (Figure 4A), and if the 9 patients who changed therapy are excluded, the 3-year probability of survival of the remaining 104 children is 59% ± 10% (see Figure 2, bottom line). The 3-year survival in the 88 patients without an affected sibling is 56% ± 11%. If the 20 patients who are alive and off therapy without BMT are not included in the analysis, 43 of 93 (46%) were alive with a 3-year probability of survival of 45% ± 10% (n = 93).

Family history, gender, and age at onset

Of the 113 children, 25 (13 M/12 F) had a positive family history, that is, an affected sibling, either at diagnosis (n = 22) or later during the study (n = 3), the oldest being 6 years at onset. The 3-year probability of survival in these 25 patients was 51% ± 20% (Figure 4B). Twenty of these children had a BMT, 13 (65%) of whom are alive. None of the patients with verified familial disease survived without BMT (Table 1), resulting in death from disease at days 2, 64, 89 (after a varicella infection), 111 (changed protocol day 29), and 294, respectively. There was no difference in the 3-year overall survival with regard to gender (data not shown).

The mean age at onset was similar in the familial cases (13 months) and in the patients who received transplants (13 months), whereas the corresponding age was higher (47 months) in the 20 patients who are alive and off therapy without BMT (Table 2). Overall, the 3-year probability of survival was significantly better in children at least 1 year old at onset (72% ± 13%), compared to children younger than 1 year old (42% ± 12%) (P < .005). However, if the 20 patients who are alive and off therapy without BMT are not included in the analysis, there was no significant difference in survival comparing these ages at onset (P = .17).

Initial and continuation immunochemotherapy

The initial and continuation therapy was successful in altogether 88 of 113 (78%) children, in that they were either admitted for BMT (n = 65) or were still alive at last follow-up (n = 23) (Table 3). Similarly, 80% (20 of 25) of the patients with a positive family history received BMT. Of the 25 deaths during the initial and continuation therapy, 20 were reported as death from disease (at a median of 100 days after onset of therapy, range, 2-899 days),

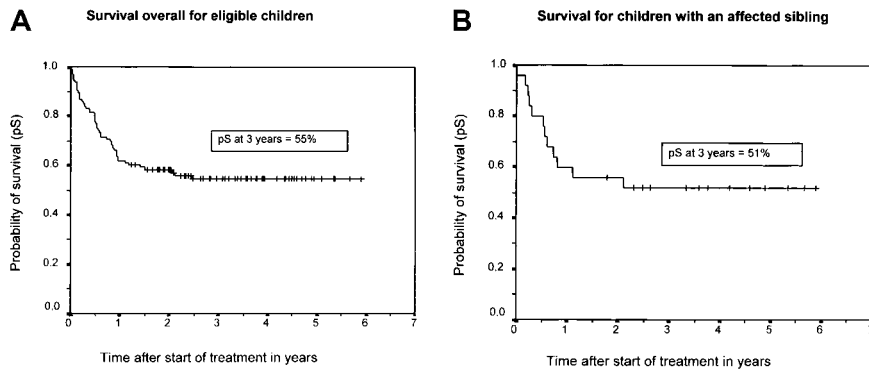


Figure 4. Kaplan-Meier survival curve. (A) All eligible study patients treated with HLH-94 ($n = 113$); (B) Patients with an affected sibling ($n = 25$).

4 died of toxicity (median, 16 days; range, 6-59), and 1 after a diagnostic biopsy. During the first 2 months of therapy (the initial therapy), 56 patients (53%) achieved a resolution (7 of whom had a reactivation), 34 (32%) improved but had no resolution, whereas 4 (4%) did not improve and 12 (11%) died (starting at day 2) (missing data in 7 patients). Eight children died during the subsequent 4 months, and 5 died more than 6 months after onset of therapy (days 221, 294, 345, 507, and 899) (Figure 1).

Results of BMT

Sixty-five children (41 M/24 F) underwent BMT, 40 (62%) (24 M/16 F) of whom are alive. The 3-year probability of survival after BMT is 62% ($\pm 12\%$) with a median follow-up after BMT in the survivors of 30 months (range, 10-63) (Figure 5) (estimated in 64 patients because of missing date of BMT in 1 child). The median time from onset of therapy to BMT was 187 days (range, 65-995), being 164 days (range, 65-995) in the familial and 217 days (range, 78-933) in the nonfamilial cases. There was no difference in survival when comparing the patients with BMT performed early ($n = 32$, 62% $\pm 17\%$, estimated 3-year-survival) versus late ($n = 32$, 61% $\pm 18\%$) after onset of therapy, using the median time to BMT as the cut-off time. Survival with regard to donor is presented in Table 4. Donor-cell engraftment was achieved in 51 of 57 patients, 2 of the nonengrafted died within 17 days (no data [nd] in 8 additional patients). All surviving BMT patients are free of disease. Among patients alive at BMT + 1 year ($n = 41$), only 1 of 26 patients (nd = 15) had a mixed chimerism.

In the 25 deceased BMT patients, death occurred prior to day +100 in 20 patients, caused by BMT complications ($n = 17$) or reactivation of HLH ($n = 3$). One child died at day +121 (261 days after onset of therapy) of a surgical hemorrhage due to a disease unrelated to HLH. The remaining 4 died of reactivation at day +112, Epstein-Barr virus (EBV)-associated lymphoproliferative disease at +152, relapse and subsequent AML at +433, and unclear respiratory disease at +550.

Surviving non-BMT patients

Altogether, 23 children (8 M/15 F), all without a positive family history, were alive without having received BMT, 20 of whom are off therapy and without evidence of disease for more than 12 months (follow-up range, 1.1-4.2 years; mean, 2.3 years), and 3 have been on therapy for more than 1 year (1.9-2.0 years; 2 on CSA alone, 1 also receives corticosteroids). Ten of the 20 patients off therapy received only initial therapy, and for the 10 patients who started continuation therapy, the mean duration of their total treatment was 359 days. Five of the latter 10 children had a nonactive disease at 2 months, whereas 4 had not received a resolution and 1 had experienced reactivation during the initial therapy. Almost all (19 of 20) of the patients off therapy were older than 12 months at onset (including 4 of the altogether 6 children 6 years and older), and the majority ($n = 12$; 60%) were reported from East Asia.

Neurological symptoms and intrathecal therapy

Neurological alterations at onset were reported in 35 of 109 (32%) patients (missing data [md] in 4 patients). At 2 months, with 101 alive patients, neurological alterations were reported in 13 of 95 (14%) patients (md = 6). At the time of BMT, 9 of 53 patients (17%) had neurological alterations (md = 12), and at BMT + 100 days 8 of 36 patients (22%) (md = 9). Of the 14 patients with neurological manifestations at onset who underwent BMT and were alive at BMT + 1 year, 3 had neurological alterations at that time and 10 had not (md = 1).

Patients with neurological alterations at onset ($n = 35$). At 2 months after onset, 21 of the surviving 31 patients did not have any neurological abnormalities. Intrathecal therapy during the first 2 months, administered to 15 of the 30 survivors (missing data on intrathecal therapy in 1 patient), was associated with normalization of symptoms at 2 months in 10 of 15 individuals. In the patients who did not receive intrathecal therapy, the neurological symptoms also normalized in 10 of 15 children.

Table 2. Survival at the latest follow-up with regard to age in 113 patients with HLH treated according to the HLH-94 protocol

Age at onset of therapy	All patients ($n = 113$)	Familial cases ($n = 25$)	BMT patients ($n = 65$)	Patients alive and off therapy without BMT ($n = 20$)
0 to younger than 3 mo	12 of 30 (40%)	6 of 13 (46%)	11 of 21 (52%)	
3 mo to younger than 12 mo	15 of 34 (44%)	2 of 4 (50%)	14 of 22 (64%)	1 of 1
12 mo to younger than 24 mo	19 of 25 (76%)	3 of 4 (75%)	10 of 14 (71%)	8 of 8
24 mo or older	17 of 24 (71%)	2 of 4 (50%)	5 of 8 (62%)	11 of 11
Totals	63 of 113 (56%)	13 of 25 (52%)	40 of 65 (62%)	20 of 20
Mean age	19 mo	13 mo	13 mo	47 mo
Age range	0-145 mo	0-82 mo	0-84 mo	10-145 mo

Table 3. Evaluation of the efficacy of immunochemotherapy during initial and continuation period with regard to survival and possibility to obtain BMT

	All patients (n = 113)	Familial cases (n = 25)
Patients admitted to BMT		
+ patients alive without BMT	65 + 23 (78%)	20 + 0 (80%)
Patients dead during initial/ continuation therapy*	25 (22%)	5 (20%)

*Five of these patients died more than 6 months after onset of therapy (one with a positive family history).

Patients without neurological alterations at onset (n = 74).

Altogether, 68 of these 74 patients survived 2 months, during which time 11 had and 48 had not received intrathecal therapy (md = 9). At 2 months after onset, 3 of these 68 children had neurological alterations (md = 6), 1 of whom had received intrathecal therapy.

Prognostic factors

The prognostic influence of 2 factors, age at onset and the interval between onset of therapy and BMT, were analyzed, but neither of these factors was associated with significant alterations in overall survival (data not shown).

Discussion

Untreated FHL is invariably fatal, with a median survival of 1 to 2 months.¹ In 1993, a study of 122 patients reported an estimated overall 5-year survival of 22%.² Thus, the present results with an estimated 3-year probability of survival of 51% ($\pm 20\%$) for the familial cases represents a great improvement. When comparing different HLH reports with regard to therapeutic results, it is important to be aware that the percentage of patients with SHLH may vary and, importantly, our data above refer to children with verified familial disease (Table 5). This figure also represents a reasonable estimate of the final cure rate, since few deaths occur later than 3 years after onset of therapy. In contrast to many reports, our study registered patients prospectively from diagnosis, not only patients recruited for BMT. The HLH-94 protocol was effective in a wide range of institutions internationally, each treating very few cases, and the present report includes patients from 21 countries.

In FHL, 2 steps are essential for survival: (1) effective initial and continuation therapy, and (2) a successful BMT. Altogether, 20 (80%) of the 25 familial cases, that is, children with an affected

Table 4. Survival at the latest follow-up with regard to BMT donor for patients treated according to the HLH-94 protocol (n = 65)

BMT donor	All cases (n = 65)	No. alive (%) (n = 40)	Median follow-up (range) after BMT
Matched related donor	15	10 (67)	43 (14-55) mo
Matched unrelated donor	25	17 (68)	28 (10-63) mo
Mismatched unrelated donor	4	1 (25)	40 (40) mo
Family haploidentical	14	6 (43)	22 (12-48) mo
Cord blood	5	4 (80)	25 (12-31) mo
Incomplete data*	2	2 (100)	

*Includes related donor with match not reported (n = 2, both alive).

sibling, survived the initial and continuation therapy and were admitted for BMT, with only 5 deaths prior to BMT, indicating a high success rate of the immunochemotherapy (Table 3). In the entire study population, a total of 88 of 113 (78%) were either admitted to BMT (n = 65) or still alive without BMT, with at least 1-year follow-up since onset (n = 23). The major toxicity of the pre-BMT therapy was neutropenia, in particular during the first 2 months, but since neutropenia is common also in untreated HLH, it is sometimes difficult to determine to what extent neutropenia was due to therapy or to active disease.

The 3-year probability of survival after BMT was 62% in our multi-institutional study (n = 65). It has to be considered that only a minority of the BMTs were performed with matched related donors (n = 15). Recent BMT series have reported data ranging from a 3-year probability of survival of 45% (n = 20) to an overall survival of 64% with HLA-nonidentical donors (n = 14) and 100% in a single-center material with matched sibling donors and unrelated donors (n = 12).³⁷⁻⁴⁰

High mortality rates have previously been described also in infection-associated HLH (52% in a review of all patients reported in the literature 1979-1996), in particular in EBV-associated HLH.³⁵ Treatment according to HLH-94, without BMT, appears beneficial also in secondary HLH.⁴¹ Lack of signs of disease activity during a prolonged period (longer than 12 months) after cessation of therapy, without previous BMT, will most likely suggest the diagnosis of SHLH.

The inflammatory meningoencephalopathy in HLH, which may cause severe and permanent CNS dysfunction,⁶⁻⁸ deserves prompt and adequate therapy considering the low regenerative potential of nervous tissue. This was an important rationale for the intensive initial systemic dexamethasone and etoposide therapy in HLH-94. Neurological alterations were reported in 35 of 109 (32%) of the patients at registration. In these 35 affected individuals, the symptoms normalized in 21 of the 31 survivors after 2 months of HLH-94 therapy. The rate of normalization was similar whether intrathecal therapy was used (67%) or not (67%) as an additional treatment to systemic corticosteroids, etoposide, and CSA, but the value of IT MTX was not studied in a randomized fashion. Additional data will be required to better evaluate the value of intrathecal therapy.

Table 5. Survival as reported in the 3 largest reports on HLH

Publication	Year of report	Number of patients	Survival
Janka (review) ¹	1983	121	5% (1 yr)*
Arico et al ²	1996	122	22% (5 yr)†
Present study	2002	113	55% (5 yr)†
Present study (familial cases)	2002	25	51% (5 yr)†

*Of 121 patients reviewed, 5 of 101 with follow-up data survived more than 12 months.

†Probability of survival according to Kaplan-Meier estimate.

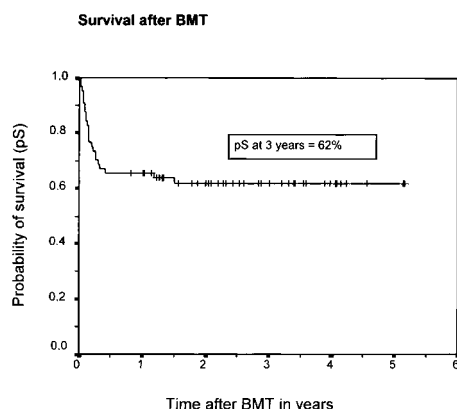


Figure 5. Kaplan-Meier survival curve for patients who underwent BMT, starting at the time of BMT (missing date of BMT in one of the 65 patients, leaving 64 patients for analysis).

We speculate that the biology of the remarkably beneficial effects of etoposide in HLH, previously not well understood, may be explained by the recent findings that FHL is associated with a defective triggering of apoptosis,^{15,19,22} at least in a subset of patients, since etoposide is known to be an excellent initiator of apoptosis.⁴² In contrast to autoimmune lymphoproliferative syndrome (ALPS) with defective Fas-induced apoptosis, FHL is characterized by lack of (lytic granule-dependent) apoptosis induction, that is, lack of cytotoxic and NK cell-mediated apoptosis but, interestingly, in ALPS as well as in FHL, the result is a lymphoproliferation/nonmalignant accumulation of immune cells.²² Similarly, the effect of dexamethasone might be explained by its anti-inflammatory and proapoptotic properties, particularly valuable since the drug also penetrates well into the CNS, and CSA is known to reduce T-cell activity, which is increased in HLH.

An increased incidence of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) has been reported following the use of epipodophyllotoxin derivatives.⁴³ Etoposide was included in the present protocol because it previously had shown to have a positive effect in FHL, a disease that without treatment is uniformly fatal. We are aware of 2 reports on AML/MDS in HLH following prolonged use of epipodophyllotoxins,^{44,45} and here we report a third patient, a child without sustained engraftment after BMT, who relapsed and died of AML on day 552 after onset of therapy (day +433 after BMT). An alternative pre-BMT approach based entirely on immunosuppression (ATG, corticosteroids, CSA, and IT MTX) can also be effective, but it may result in a lower rate of complete

remission at the time of BMT as compared to treatment including the highly apoptosis-inducing drug etoposide.^{38,42} We conclude that the risk of development of MDS/AML in HLH patients following etoposide treatment is limited and acceptable considering its positive therapeutic effects but that prolonged administration of epipodophyllotoxins should be avoided if possible.

Whereas HLH traditionally is separated in familial (primary) and secondary HLH, this distinction may not be possible in the initial clinical setting until improved molecular diagnosis is available, but the search for underlying gene mutations is encouraged.⁴⁶ Proving an acute infection at onset does not have any major therapeutic importance, since not only SHLH but also FHL often features a triggering infectious agent.³⁶ In less severe SHLH cases, either no treatment or a short duration of therapy might suffice, but future studies are necessary to define these subsets, possibly with additional genetic markers. If the disease is familial, relapsing, or severe and persistent even without family history, the BMT from the best available donor is strongly recommended.³⁸⁻⁴⁰ Finally, this report also demonstrates the value of international collaboration in conducting clinical studies of rare disorders.

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