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Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis

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Summary

Haemophagocytic lymphohistiocytosis (HLH) poses major therapeutic challenges, and the primary inherited form, familial haemophagocytic lymphohistiocytosis (FHL), is usually fatal. We evaluated, including Cox regression analysis, survival in 86 children (29 familial) that received HLH-94-therapy (etoposide, dexamethasone, ciclosporin) followed by allogeneic stem cell transplantation (SCT) between 1995 and 2000. The overall estimated 3-year-survival post-SCT was 64% [confidence interval (CI) = ±10%] ($n = 86$); 71 ± 18% in those patients with a matched related donor (MRD, $n = 24$), 70 ± 16% with a matched unrelated donor (MUD, $n = 33$), 50 ± 24% with a family haploidentical donor (haploidentical, $n = 16$), and 54 ± 27% with a mismatched unrelated donor (MMUD, $n = 13$). After adjustment for potential confounding factors, estimated odds ratios (OR) for mortality were 1·93 (CI = 0·61–6·19) for MUD, 3·31 (1·02–10·76) for haploidentical, and 3·01 (0·91–9·97) for MMUD, compared with MRD. In children with active disease after 2-months of therapy ($n = 43$) the OR was 2·75 (1·26–5·99), compared with inactive disease ($n = 43$). In children with active disease at SCT ($n = 37$), the OR was 1·80 (0·80–4·06) compared with inactive disease ($n = 49$), after adjustment for disease activity at 2-months. Mortality was predominantly transplant-related. Most HLH patients survived SCT using MRD or MUD, and survival with partially mismatched donors was also acceptable. Patients that responded well to initial pretransplant-induction therapy fared best, but some persisting HLH activity should not automatically preclude performing SCT.

Keywords: stem cell transplantation, familial haemophagocytic lymphohistiocytosis, survival, treatment.

Haemophagocytic lymphohistiocytosis (HLH) poses major diagnostic and therapeutic challenges. The primary autosomal recessive form, familial haemophagocytic lymphohistiocytosis (FHL) with an incidence of around 1:50 000 live-born children, is fatal without adequate treatment (Janka, 1983; Henter *et al*, 1991a,b, 1998; Aricò *et al*, 1996; Janka *et al*, 1998). In addition, central nervous system (CNS) involvement may be severe and cause permanent CNS dysfunction (Henter & Elinder, 1992; Haddad *et al*, 1997; Henter & Nennesmo, 1997). Prompted by earlier dismal therapeutic results (Janka, 1983; Henter *et al*, 1991a; Aricò *et al*, 1996), in 1994 the Histiocyte Society developed a consensus study protocol: the HLH-94 protocol. HLH-94 comprises an induction/continuation phase, which aims to induce a remission in these often

dramatically sick patients, followed by haematopoietic stem cell transplantation (SCT) from the best available donor, which aims to replace the immune system and thereby induce a definitive cure, in patients with familial, persistent and recurrent disease (Henter *et al*, 1997, 2002). Recent studies have revealed perforin and hMunc 13–4 gene defects in FHL-families, providing a strong rationale to correct the disease with a substitution of haematopoietic stem cells (Stepp *et al*, 1999; Clementi *et al*, 2001; Göransdotter Ericson *et al*, 2001; Feldmann *et al*, 2002, 2003; Suga *et al*, 2002).

We have previously reported results of HLH-94, the first prospective international therapeutic study on HLH, with an overall estimated 3-year probability of survival of 55% (±9%) and 51% (±20%) in the familial cases. We also showed that the major risk of fatality now occurs after SCT, as opposed to in the pre-SCT phase (Henter *et al*, 2002). The present paper analysed the outcome of SCT of 86 children with HLH in more detail, all of whom were initially treated with the HLH-94 protocol. We focused on parameters associated with post-transplant disease-free survival, including multivariate analysis of donor- and treatment-related measures.

Material and methods

Patients

Information concerning the patients was submitted at regular intervals by the treating physicians on follow-up forms. As of June 2002, the HLH-94 data-base in Stockholm contained 107 patients aged <15 years at onset of HLH-94 therapy, who had not received any previous cytotoxic therapy, and had their first SCT performed between 1 January 1995 and 31 December 2000. Of these patients, 87 were eligible for the present study since they either fulfilled the diagnostic criteria (Henter *et al*, 1991a) (Table I) or had familial disease (as indicated by an affected sibling), whereas 19 remaining patients did not fulfil either of these criteria and there was missing data for one patient. We report on the 86 individuals that had complete information on the covariates included in the multivariate analysis (listed in 'Statistics' below). The patient excluded because of missing information was a boy below 1 year of age at onset of disease, who was transplanted with a matched unrelated donor and who is still alive (more than 4 years after transplantation). When comparing the 86 children studied and the 21 that were not eligible, there were no differences in age, consanguinity or CNS involvement, although significantly more girls did not fulfil the eligibility criteria, and there was no statistically significant difference in the estimated 3-year probability of survival between the two groups. Sixty-four of the patients were included in a previous report on HLH-94 outcome in which 65 patients underwent SCT (Henter *et al*, 2002); in that report, the term 'bone marrow transplant' also includes cord and peripheral stem cell transplants. Patient data and outcome were analysed as of 1 December 2003.

Table I. Diagnostic guidelines for haemophagocytic lymphohistiocytosis (HLH) in HLH-94* (data from Henter *et al* (1991a)).

- Fever: duration ≥7 days
- Splenomegaly ≥3 cm below the costal margin
- Cytopenia affecting two or all three lineages in the peripheral blood with haemoglobin <9·0 g/dl, platelets $<100 \times 10^9$/l, neutrophils $<1{\cdot}0 \times 10^9$/l
- Hypertriglyceridaemia and/or hypofibrinogenaemia (fasting triglycerides ≥2·0 mmol/l or ≥3 SD of the normal value of age, fibrinogen ≤1·5 g/l or ≤3 SD)
- Haemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy.

*All criteria are required for the diagnosis of HLH. In addition, the diagnosis of FHL is justified by a positive family history, and parental consanguinity is suggestive. SD, standard deviation.

HLH disease status

In the HLH-94 protocol, inactive disease was defined as having no clinical signs of disease; i.e. no fever except if infection-induced, no hepatosplenomegaly, no clinical signs of active CNS disease, and no cytopenias (except if drug-induced). CNS disease was defined as having an abnormal neurological examination and, in addition, cerebrospinal fluid (CSF) pleocytosis and/or elevated CSF protein. In the nine cases with a discrepancy between the report on active/inactive disease at 2 months and the separate specific reports on the clinical and laboratory data included in the definition for active disease, the reporting physicians rechecked the data prior to the final analysis.

Pre-SCT therapy

All 86 patients were initially treated according to the HLH-94 protocol (Henter *et al*, 1997, 2002), consisting of 8 weeks initial therapy with etoposide (VP-16) and dexamethasone (Fischer *et al*, 1985; Henter *et al*, 1986, 1993). In addition, intrathecal (IT) methotrexate (MTX) was administered to 11 of 24 patients with CNS disease at registration. Continuation therapy with alternating weekly pulses of VP-16 and dexamethasone together with daily ciclosporin A (CSA) (Stéphan *et al*, 1993; Imashuku *et al*, 1999a), followed for all but three patients who changed therapy after the initial treatment.

Donor typing, transplantation conditioning and GVHD prophylaxis

Human leucocyte antigen (HLA)-typing was performed using genomic techniques in 50 patients (58%) and serological techniques in 18 (21%); information regarding technique used was unavailable for 18 patients (21%). Both the conditioning regimen and graft-*versus*-host disease (GVHD) prophylaxis were determined by the treating transplant unit, although a suggestion was included in the HLH-94 protocol (Henter *et al*,

1997, 2002). The suggested preparative regimen consisted of oral busulphan at 4 mg/kg on days −9, −8, −7 and −6 (total dose 16 mg/kg), intravenous cyclophosphamide at 50 mg/kg on days −5, −4, −3 and −2 (total dose 200 mg/kg) and intravenous VP-16 at 300 mg/m^2 on days −5, −4 and −3 (total dose 900 mg/m^2). In practice, 80 patients (93%) were reported to have received treatment with busulphan, 78 (91%) were administered cyclophosphamide and 43 (50%) received VP-16; 41 of 86 (48%) received all three drugs. Additional immunosuppression was recommended for unrelated donor transplants, with intravenous antithymocyte globulin (ATG) on days −2, −1, +1 and +2. This treatment was reported to have been administered to 28 of 46 patients (61%) with SCT from unrelated donors.

The suggested GVHD prophylaxis included intravenous MTX 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +5, and +11, in combination with CSA beginning on day −3. In practice, 75/85 patients (87%) were reported to have received treatment with CSA and 43 of 84 patients (50%) with MTX (all of whom also had CSA therapy). Seven of the 10 children not receiving CSA were children with family haploidentical stem cell donors (Table II).

Statistics

Differences in distribution were compared by using the chi-square test or, where frequencies were small, the two-tailed Fisher's exact test. Survival rates were analysed using the Kaplan–Meier life table method and univariate comparison of survival using the log rank test. Multivariate analysis using Cox proportional hazards regression was performed with time to death as the endpoint and using the maximum follow-up time available. The covariates used were: sex, age at start of treatment, CNS involvement at start of therapy, disease activity at 2 months after start of treatment, disease activity at SCT, time to SCT, and donor type; matched related donors (MRD), matched unrelated donors (MUD), family haploidentical donors (HAPLO), and mismatched unrelated donors (MMUD).

All analyses were carried out using the Statistical Package for the Social Sciences (SPSS, version 11·5; Chicago, IL, USA). *P*-values <0·05 were considered significant. The study was approved by the Histiocyte Society and the Ethics Committee of the Karolinska Institute.

Results

Patients

Eighty-six patients from 18 countries were eligible for inclusion in the analyses. The characteristics and donor groups of these patients are detailed in Table II. Family history of the disease was present in 29 of 85 patients (34%) and parental consanguinity in 17 of 84 (20%). The diagnostic criteria were

Table II. Patient data and transplantation variables in relation to the donor groups.

	Donor group				
Variable	Matched related	Matched unrelated	Family haploidentical	Mismatched unrelated	Total
Number of evaluated patients	24	33	16	13	86
Males	14 (58)	21 (64)	13 (81)	8 (62)	56 (65)
Age <12 months at start*	18 (75)	19 (58)	12 (75)	10 (77)	59 (69)
Age 12–24 months at start*	4 (17)	8 (24)	2 (12)	2 (15)	16 (19)
Age >24 months at start*	2 (8)	6 (18)	2 (12)	1 (8)	11 (13)
Parental consanguinity	8/23 (35)	6/32 (19)	0 (0)	3 (23)	17/84 (20)
Familial disease	9 (38)	12/32 (38)	5 (31)	3 (23)	29/85 (34)
CNS-involvement at start*	8 (33)	8 (24)	6 (38)	3 (23)	25 (29)
Active disease 2 months after start*	15 (62)	15 (45)	8 (50)	5 (38)	43 (50)
≤180 days from start to SCT*	21 (88)	9 (27)	6 (38)	8 (62)	44 (51)
SCT 1995–96	10 (42)	11 (33)	2 (12)	3 (23)	26 (30)
SCT 1997–98	8 (33)	11 (33)	10 (62)	7 (54)	36 (42)
SCT 1999–2000	6 (25)	11 (33)	4 (25)	3 (23)	24 (28)
Active disease at time for SCT	14 (58)	10 (30)	8 (50)	5 (38)	37 (43)
Conditioning with VP-16	12 (50)	18 (55)	1 (6)	12 (92)	43 (50)
Conditioning with busulphan	24 (100)	27 (82)	16 (100)	13 (100)	80 (93)
Conditioning with cyclophosphamide	22 (92)	29 (88)	14 (88)	13 (100)	78 (91)
Conditioning with ATG	9/22 (41)	20 (61)	13/15 (87)	8 (62)	50/83 (60)
Conditioning with irradiation	1 (4)	6 (18)	0 (0)	1 (8)	8 (9)
GVHD prophylaxis with CSA	23 (96)	30/32 (94)	9 (56)	13 (100)	75/85 (88)
GVHD prophylaxis with MTX	13/23 (57)	21/32 (66)	3 (19)	6 (46)	43/84 (51)

Percentages are given in parentheses.
*At start of HLH-94 therapy.

completely fulfilled by 76 patients (88%). Of those who did not fulfil all criteria but were evaluated, all 10 had a positive family history. CNS involvement was found in 25 patients (29%) on disease diagnosis. Neurological alterations were reported in 12 of 83 patients (14%) at 2 months after start of treatment and in 13 of 80 (16%) at the time of SCT.

The median age at SCT was 13 months (range 4 months to 11 years) and the median time from start of therapy to SCT was 6 months (range 2 months to 10 years); being 8, 19, 13 and 11 months in the MRD, MUD, HAPLO, and MMUD groups, respectively. MRD were utilized in 24 transplantations (21 from siblings), MUD in 33 (including two umbilical cord transplants), HAPLO in 16, and MMUD in 13 (including five umbilical cord transplants).

Engraftment, graft failure, second transplantation and relapse

Information on engraftment was available for 83 patients, 75 of whom (90%) were reported to have achieved engraftment (for details on donor types see Table III). All three patients for whom information was missing died during the first 100 days after SCT. Data on the number of cells provided and the timing of engraftment is not available. Of the eight children who never engrafted, seven had active and one inactive disease at 2 months ($P = 0{\cdot}029$, Fisher's exact test, two-sided) whereas at the time of SCT, five had active and three had inactive disease (not significant). Of the eight children who did not achieve engraftment (none of whom had a cord transplant), two received a second transplant; one is alive at the last follow-up (32 months after the second transplant). Among the patients who engrafted, secondary graft failure was reported in three, all within the first 100 days (all died, one after a second transplant).

In total, there were seven patients with recurrent disease, four of whom had no engraftment (two MUD and two HAPLO), two of these children received a second transplant (see above). Two individuals had active disease at SCT, and two had inactive disease.

Among the engrafted patients without known graft failure, three (with MRD, MUD and MMUD, respectively) were reported to have suffered recurrent episodes of HLH. One of these died on day +160 (no information is available on whether graft failure occurred). The other two received additional HLH-therapy post-SCT and thereafter stabilized; they are now alive off therapy with inactive disease. They both had full donor haematopoiesis at the time of relapse, both received VP-16, and one had an EBV-induced post-SCT-HLH. The other patient, who experienced two bouts of HLH activity following cord transplant, became severely ill and needed artificial respiration for 58 days and VP-16 was administered for nine consecutive weeks (until day +144) (Schwinger *et al*, 1988).

GVHD

Acute GVHD (grade 2–4) was reported in 25 of 78 patients (32%), with 18% in the MRD group as compared with 30%, 36% and 58% in the MUD, HAPLO and MMUD groups, respectively (Table III). At 1 year after transplant, 57 patients were alive. Among these, chronic GVHD was reported in four of 44 patients (9%); one of 14 in the MRD group, as compared with one of 16 and two of six in the MUD and HAPLO groups, respectively.

Of the seven children with recurrent disease, two had acute GVHD. Both had an initial engraftment, and one had a sustained engraftment and was alive at follow-up, and for the other child, who died, no information was available on whether graft failure occurred. None of the three children with recurrent disease that were alive after 1 year had chronic GVHD reported at that time (missing data for one child).

Table III. Outcome after haematopoietic stem cell transplantation (SCT) with respect to donor groups.

	Donor group				
Variable	Matched related	Matched unrelated	Family haploidentical	Mismatched unrelated	Total
Number of evaluated patients	24	33	16	13	86
Engraftment	21/23 (91)	28/31 (90)	13/16 (81)	13 (100)	75/83 (90)
Acute GVHD	4/22 (18)	9/30 (30)	5/14 (36)	7/12 (58)	25/78 (32)
Disease relapse despite sustained engraftment*	0/20 (0)	1/27 (4)	0/12 (0)	1/12 (8)	2/71 (3)
Second transplantation	0/22 (0)	2/31 (6)	1/16 (6)	0/13 (0)	3/82 (4)
Alive 100 days after SCT	17 (71)	27 (82)	9 (56)	7 (54)	60 (70)
Alive 1 year after SCT	17 71)	24 (73)	9 (56)	7 (54)	57 (66)
Alive at last follow-up	17 (71)	23 (70)	8 (50)	7 (54)	55 (64)

*Includes two patients that responded to HLH therapy including VP-16 during a period of time post-SCT, and that are now alive without therapy and without signs of HLH. One patient, not included here, suffered disease relapse after an initial engraftment but no data are available on whether he had a sustained engraftment or a graft rejection.

Percentages are given in parentheses.

Survival

Overall, the 3-year probability of survival (with 95% confidence interval) was 64% (±10%) as shown in Fig 1A, and in the 29 familial cases 66% (±17%). At the time of analysis 55 patients were alive, with a median follow-up period of 4·1 years post-SCT (range 1·1–7·2 years), all reported as being without active disease. With regard to their quality of life, we used a measure of 'ability to play' as an indicator of quality of life; 44 children had a normal ability to play, seven had a mild to moderate inhibition to play and two had a moderate to severe inhibition to play, according to the last report (missing data in two cases) (Lansky *et al*, 1987). Of the 25 patients with CNS involvement at diagnosis, 15 (60%) were long-term survivors (nine died within the first year after transplant, and one died during the second post-transplant year).

Of the 55 patients alive, data on chimaerism at 1 year or more after SCT was available in 43 patients. Eight of these were reported to have mixed chimaerism, all with inactive disease, at a median time of 3 years after SCT.

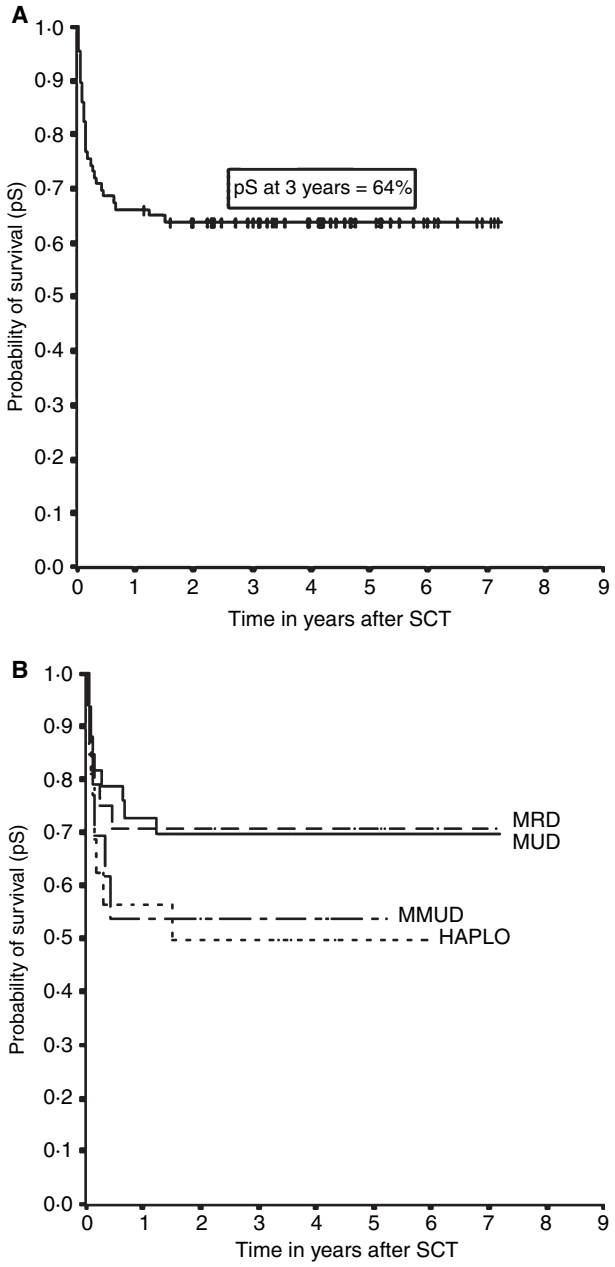

Fig 1. Kaplan–Meier survival curves for 86 children with HLH, starting at the time of SCT. (A) The estimated overall probability of survival at 3-years was 64% (±10%). (B) The estimated overall probability of survival according to the donor groups were for matched related donors (MRD) 71% (±18%) (3-year probability of survival, ±95% CI), matched unrelated donors (MUD) 70% (±16%), mismatched unrelated donors (MMUD) 54% (±27%) and for family haploidentical donors (HAPLO) 50% (±24%).

Causes of death

At the time of analysis 31 patients had died. Of these, 26 were reported to have had transplant-related mortality (TRM), two died after relapse of HLH (one had a graft failure, and the other patient is the one without information on whether graft failure occurred), one died of secondary acute myeloid leukaemia (AML) as previously reported (Henter *et al*, 2002), one died of a respiratory disease of unknown cause and one died during surgery (unrelated to HLH). The two children who died following relapse of HLH both had active disease at the time of SCT.

A total of 23 deaths occurred within 100 days after SCT. Causes of death were GVHD ($n = 3$); cytomegalovirus (CMV; $n = 3$); pneumonia, bronchiolitis obliterans organizing pneumonia, or respiratory failure of unknown origin ($n = 8$); veno-occlusive disease (VOD) ($n = 4$, including one with additional CMV and one with additional septicemia); septicemia ($n = 1$); interstitial pneumonia with CMV and adenovirus ($n = 1$). The further three cases were reported as SCT complications, but were not more closely defined. Thus, at least half of the early deaths were attributed to treatment-related complications of the lung and the liver.

Of the eight deaths that occurred more than 100 days after SCT, two occurred after more than 1-year (one following graft failure, relapse and subsequent AML at day SCT+ 450, and the other at day SCT+ 550 because of respiratory disease). The other six deaths were caused by relapse ($n = 2$); infections ($n = 1$); post-transplant lymphoproliferative disease ($n = 1$); GVHD and aspergillus infection ($n = 1$) and surgical haemorrhage ($n = 1$).

Donor groups

Univariate analyses of survival are shown in Table IV. For the donor groups, survival was 71% (±18%) for MRD ($n = 24$, three with non-sibling donors), 70% (±16%) for MUD ($n = 33$), 50% (±24%) for HAPLO ($n = 16$) and 54% (±27%) for MMUD ($n = 13$), at 3 years after SCT (Fig 1B).

Table IV. Probability of survival 3 years after haematopoietic stem cell transplantation (SCT) in HLH-94 (univariate analyses).*

Variable	*n*	Probability (95% CI)	*P*-value
All evaluated patients	86	64 (54–74)	
Sex			
Male	56	63 (50–76)	0·755
Female	30	67 (50–84)	
Age group at start of treatment			
<12 months	59	64 (52–76)	†
12–24 months	16	63 (39–87)	0·912
>24 months	11	64 (36–92)	0·888
Familial disease			
No	56	64 (51–77)	0·871
Yes	29	66 (49–83)	
Parental consanguinity			
No	67	63 (51–75)	0·325
Yes	17	76 (56–96)	
CNS-involvement at registration			
No	61	66 (54–78)	0·747
Yes	25	60 (41–79)	
Disease activity 2 months after start of treatment			
Non-active	43	77 (65–89)	0·009
Active	43	51 (36–66)	
Time from start of treatment to SCT			
≤180 days	44	59 (44–74)	0·362
>180 days	42	69 (55–83)	
Disease activity at time for SCT			
Non-active	49	71 (58–84)	0·065
Active	37	54 (38–70)	
Year of SCT			
1995–97	41	61 (46–76)	0·615
1998–2000	45	67 (53–81)	
Donor			
Matched related	24	71 (53–89)	†
Matched unrelated	33	70 (54–86)	0·972
Family haploidentical	16	50 (26–74)	0·202
Mismatched unrelated	13	54 (27–81)	0·323

*Missing data on familial disease in one patient and on consanguinity in two patients.
†Reference variable.

Multivariate analysis was performed and the results are shown in Table V. There was no statistically significant difference in survival between MRD and MUD. With regard to unadjusted OR there was no significant difference between HAPLO and MMUD. However, the adjusted OR for HAPLO compared with MRD was 3·31 (1·02–10·76) whereas the OR for MMUD compared with MRD was 3·01 (0·91–9·97).

Disease activity at 2 months after start of HLH-therapy

Our data indicated that children with active disease at 2 months after start of HLH-94 treatment had a significantly worse outcome after SCT (51 ± 15%) than those with inactive disease (77 ± 12%) ($P = 0·009$) (Table IV). This increased risk of mortality post-SCT for these patients remained statistically significant after adjustment for potentially confounding factors (OR = 2·75, 1·26–5·99, $P = 0·011$) (Table V).

Disease activity at time for transplantation

Active disease at the time of SCT was associated with a decreased survival indicated by an unadjusted odds ratio of 1·93 (0·95–3·91, $P = 0·070$) from Cox regression analysis reported in Table V. Adjustment for the potential confounding factors reduces these odds to 1·80 (0·80–4·06, $P = 0·154$).

Discussion

A number of conclusions with regard to SCT in HLH can be drawn from the HLH-94 study, the first prospective international study on HLH: (1) The probability of surviving 3 years after SCT is satisfactory (64%), in view of the mixed clinical and geographical backgrounds of the patients. (2) Survival for MUD transplant recipients is not significantly lower than that for MRD transplant recipients, and although the use of HAPLO and MMUD gives less favourable survival, the outcome is still acceptable, supporting the use of alternative donors where matched donors are unavailable. (3) The survival curve reaches a plateau 2-years after SCT, consistent with persistent control of underlying HLH. (4) Some degree of disease activity at time for transplantation should not automatically preclude performing SCT, but this topic deserves closer scrutiny in future studies. (5) Persistent disease activity at 2 months after start of HLH treatment appears to suggest a worse long-term prognosis.

Fischer *et al* (1986) were the first to show that a cure for FHL could be achieved by SCT, as later confirmed by others (Todo *et al*, 1990; Blanche *et al*, 1991; Nespoli *et al*, 1991; Bolme *et al*, 1995; Baker *et al*, 1997; Jabado *et al*, 1997; Dürken *et al*, 1999; Imashuku *et al*, 1999b). Initially, only matched donors were used but, since the outcome of FHL is invariably fatal without a successful SCT, HLA non-identical donors are now used more often and represent a third of the donors in this analysis. We have demonstrated that the use of MUD (6/6) provide results comparable with those achieved with MRD. Furthermore, 15 of 29 (52%) patients were alive at last follow-up following HLA non-identical transplantation, with a median follow-up time of 50 months in the survivors. These data support SCT with the use of alternative donors when matched donors are unavailable. We suggest these SCT are performed in experienced centres.

In FHL, two steps are essential for survival and cure; first, an effective initial and continuation therapy, and second, a successful SCT. HLH-94 is highly successful in achieving symptomatic remission, allowing most patients to be admitted to SCT (with a pre-SCT fatality rate of around 20%) (Henter *et al*, 2002). The overall estimated 3-year probability of survival has increased to around 50% when the protocol has been followed (Henter *et al*, 2002). This represents a

Table V. Results of Cox proportional hazards regression analysis of survival following haematopoietic stem cell transplantation (SCT) on 86 patients with HLH.

	Unadjusted			Adjusted		
Covariate*	OR of death	95% CI	*P*-value	OR of death	95% CI	*P*-value
Sex (male)	1·13	0·53–2·39	0·755	0·89	0·40–2·00	0·787
Age <12 months at start†	1			1		
Age 12–24 months at start†	1·04	0·42–2·59	0·925	0·68	0·25–1·89	0·464
Age >24 months at start†	1·08	0·37–3·16	0·882	1·26	0·41–3·91	0·685
CNS at start†	1·13	0·53–2·40	0·747	0·99	0·44–2·22	0·985
Active disease 2 months after start†	2·62	1·23–5·57	0·012	2·75	1·26–5·99	0·011
Active disease at SCT	1·93	0·95–3·91	0·070	1·80	0·80–4·06	0·154
>180 days from start to SCT†	0·72	0·35–1·47	0·365	0·57	0·24–1·37	0·209
Matched related donor	1			1		
Matched unrelated donor	1·02	0·39–2·68	0·420	1·93	0·61–6·19	0·265
Family haploidentical donor	1·93	0·70–5·32	0·205	3·31	1·02–10·76	0·047
Mismatched unrelated donor	1·75	0·59–5·22	0·314	3·01	0·91–9·97	0·071

*The covariates used were: sex, age at start of treatment, CNS involvement at start of therapy, disease activity at 2 months after start of treatment, disease activity at SCT, time to SCT, and donor type.
†At start of HLH-94 therapy.

remarkable improvement in disease control, particularly when compared with data from the early 1980s, when the cure rate in FHL was 0% and the median survival time after diagnosis was only 1–2 months (Janka, 1983). Moreover, the importance of SCT in the cure of HLH is highlighted by our previous report of the HLH-94 data, in which none of the patients with verified familial disease survived without having a SCT (Henter *et al*, 2002).

Opinions vary as to what degree of disease activity immediately prior to transplantation compromises long-term outcome. In this study, there was a tendency towards better survival in patients with inactive disease at SCT, but this association failed to achieve statistical significance in univariate analysis, and adjustment for potential confounding factors further eroded the risk associated with active disease at SCT. Our results suggest that some degree of disease activity, e.g. residual (but reduced) splenomegaly, at the time for transplantation should not automatically preclude performing SCT, which is worthy of consideration, particularly for patients in whom a state of inactive disease is difficult to achieve. This topic deserves closer scrutiny in future studies. Apart from donor type, the only independent statistically significant association with improved survival was inactive disease after 2 months of HLH-94 therapy. Whether or not this association is because of different underlying molecular abnormalities remains to be elucidated. It could be speculated that poor responders to chemotherapy may respond poorly to conditioning treatment and may only suffer toxicity and not benefits. The analyses on disease activity should, though, be interpreted with caution. To validate our findings, the answers to the question on active or inactive disease at 2 months have been compared with answers on specific clinical and laboratory data after 2 months of treatment that were reported separately. In the few cases with inconsistencies, the data have been rechecked by the reporting centres and amended according to the definitions set out in the study. One potential limitation with our study is the possibility of time censoring, since the time to SCT was different in the various groups (longest in MUD patients). However, in the HLH-94 study most of the deaths prior to SCT occurred during the first 3 months (Henter *et al*, 2002), suggesting that the risk of time censoring in the current study may not be that great.

A substantial proportion of early deaths post-SCT (<100 days) were attributed to regimen-related toxicity: non-infectious pulmonary toxicity and VOD. Post-transplant complications associated with fevers, capillary leak, vascular instability, respiratory distress and/or hepatic dysfunction, sometimes referred to as the 'hypercytokinaemia syndrome', occur more frequently in HLH patients than in SCT patients with other diagnoses (unpublished observations). This constellation of symptoms often occurs around the time of engraftment or with the onset of GVHD, and has been reversed with the use of very high doses of corticosteroids and/or anti-tumour necrosis factor agents. The hypercytokinaemia syndrome is usually observed in the context of complete or predominant donor type engraftment and differs from the recurrent HLH experienced later after SCT, particularly in patients who have waning engraftment or rejection. We have no data on how many of the patients reported here developed this syndrome, since we did not specifically ask for this information. Early recognition and aggressive management of the hypercytokinaemia syndrome may improve the overall success of SCT. Whether this syndrome is associated with the underlying biological deficiency in HLH remains to be elucidated.

This study confirms that successful transplantation will induce not only prolonged survival but also cure in HLH. The vast majority of deaths occurred within the first year after SCT, two during the second year, and then the survival curve was

flat, indicating a limited likelihood of late relapse (Fig 1A). Importantly, HLH may develop in the early post-SCT period despite apparent earlier engraftment, as reported in a few of our patients. There are at least two possible explanations in addition to graft rejection. One possibility is delayed and/or insufficient lymphocyte recovery, resulting in delayed normalization of NK cell function and relapse despite complete three-lineage engraftment (Schwinger *et al*, 1988). Another is that the immunocompromised child experiences an episode of secondary HLH after SCT, such as in connection with a viral infection, which may be treatable. In both situations, increasing immunosuppression with CSA and corticosteroids and, in addition, administration of VP-16 may result in ultimate remission.

To conclude, we report the largest collection of SCT data so far presented on this rare disease, the result of a broad international collaboration. Further, the study was prospective and, to our knowledge, reported the only multivariate analysis of survival following SCT in patients with HLH.

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