TRANSPLANTATION

Treatment dilemmas in asymptomatic children with primary hemophagocytic lymphohistiocytosis

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KEY POINTS

- Preemptive allogeneic stem cell transplantation improves prognosis of asymptomatic children genetically predisposed to primary HLH.

Asymptomatic carriers (ACs) of pathogenic biallelic mutations in causative genes for primary hemophagocytic lymphohistiocytosis (HLH) are at high risk of developing life-threatening HLH, which requires allogeneic hematopoietic stem cell transplantation (HSCT) to be cured. There are no guidelines on the management of these asymptomatic patients. We analyzed the outcomes of pairs of index cases (ICs) and subsequently diagnosed asymptomatic family members carrying the same genetic defect. We collected data from 22 HSCT centers worldwide. Sixty-four children were evaluable. ICs presented with HLH at a median age of 16 months. Seven of 32 ICs died during first-line therapy, and 2 are alive after chemotherapy only. In all, 23/32 underwent HSCT, and 16 of them are alive. At a median follow-up of 36 months from diagnosis, 18/32 ICs are alive. Median age of ACs at diagnosis was 5 months. Ten of 32 ACs activated HLH while being observed, and all underwent HSCT: 6/10 are alive and in complete remission (CR). 22/32 ACs remained asymptomatic, and 6/22 have received no treatment and are in CR at a median follow-up of 39 months. Sixteen of 22 underwent preemptive HSCT: 15/16 are alive and in CR. Eight-year probability of overall survival (pOS) in ACs who did not have activated HLH was significantly higher than that in ICs (95% vs 45%; $P = .02$), and pOS in ACs receiving HSCT before disease activation was significantly higher than in ACs receiving HSCT after HLH activation (93% vs 64%; $P = .03$). Preemptive HSCT in ACs proved to be safe and should be considered. (*Blood*. 2018;132(19):2088-2096)

Introduction

Primary hemophagocytic lymphohistiocytosis (HLH) is a rare disease with an estimated incidence of 1.8 per 100 000 live births per year.[1,2] It is caused by a number of genetic mutations that affect the exocytosis of cytotoxic granules in T and NK cells, thus hampering their killing function.[3] To date, 4 different genes have been identified as causative for primary HLH and an additional 5 genes are responsible for immunodeficiencies in which HLH is a prominent clinical feature (*PRF1*, *STXBP2*, *UNC13D*, *STX11*, *RAB27A*, *BIRC4*, *SH2D1A*, *LYST*, *AP3B1*).[4-14] The curative treatment in primary HLH is hematopoietic stem cell transplantation (HSCT).[15-21] Despite advances in chemoimmunotherapy for the treatment of primary HLH, overall survival (OS) remains at only about 59%, with virtually no survivors reported among patients who did not receive a transplant once the disease was fully active.[22-24] Data from the largest cooperative prospective international studies (HLH-94 and HLH-2004 protocols) show that up to 20% of patients do not achieve a durable complete remission (CR) with first-line chemotherapy, and disease progression represents the overriding cause of death for patients not receiving HSCT within 12 months from disease onset.[22] The initial HSCT experience with the use of myeloablative conditioning regimens was unsatisfactory.[18,25,26] Since the introduction of reduced intensity conditioning (RIC), better results have been achieved with limited toxicities and OS of 75% to 92% for patients who do not have central nervous system (CNS) disease and who achieve good disease control before HSCT.[20]

Although HSCT is clearly the treatment of choice once HLH becomes clinically manifest, the question of whether to offer HSCT to asymptomatic siblings who have been diagnosed

genetically before the onset of symptoms remains unanswered. To date there are no data on the natural history of asymptomatic siblings to guide clinicians or families in making this decision. The heterogeneity of clinical presentation in these autosomal recessive diseases renders decision-making extremely challenging. In fact, previous studies demonstrated that only one-third of relatives affected by the same genetic mutation as index cases (ICs) have a similar age at presentation.[27] The risks of HSCT, often performed at a very young age, need to be balanced with the risk of waiting for the first HLH episode to manifest, which can be fatal in up to 20% of the patients.[25] In this study, we describe for the first time the outcome of children who carry a genetic mutation predisposing to primary HLH and who are diagnosed while asymptomatic compared with that of ICs in the same family who have HLH that has activated.

Methods and data collection

Children younger than age 18 years who carried the same genetic mutations as a sibling affected by primary HLH and who were asymptomatic at the time of genetic diagnosis were eligible for this study. Only children diagnosed between 2005 and 2016 were eligible. Data were collected via a questionnaire distributed to centers through the Histiocyte Society. A key physician was identified to collect and report local data for each of the pediatric HSCT centers in Japan, Europe, and North America that participated. In brief, the questionnaire captured data on the clinical characteristics, management, and outcome of the ICs and of a subsequent asymptomatic family member with primary HLH (asymptomatic case [AC]). The questionnaire also asked participating centers about their current attitude toward the management of asymptomatic family members with primary HLH. Given the recognized variability of the disease course in patients with XLP1 and XIAP deficiency, these patients were not analyzed in this study. Patients enrolled in the study had given prior consent to the use of their anonymized clinical data in the context of national or international studies. Individual institutional review boards in North America approved this study.

Statistics

The results of descriptive analyses are reported as medians and ranges. Kaplan-Meier estimators and confidence intervals (CIs) are used to estimate OS, and the log-rank test and Fisher's exact test (discrete variables) have been used to compare data. Patients were censored at last follow-up if no events occurred. CIs were reported at the 95% level, and statistical tests were performed at the 0.05 level (two sided).

Results

Eleven of 22 contacted centers submitted a total of 32 pairs or triplets of ICs and ACs corresponding to a total number of 33 ICs and 33 ACs. Because 2 patients were lost to follow-up, only 32 ICs and 32 ACs were evaluable for analysis. Seven centers had no patients fulfilling the inclusion criteria, and 4 centers did not reply to the questionnaire.

Patients' characteristics and outcomes

Of the 32 pairs and triad of ICs and ACs identified, 13 had perforin (PRF1) deficiency, 6 had MUNC18-2 (STXBP2) deficiency, 4 had

MUNC13-4 (UNC13D) deficiency, 2 had syntaxin (STX11) deficiency, 6 were diagnosed with Griscelli syndrome type II (RAB27A deficiency), and 1 was diagnosed with Chediak Higashi syndrome (LYST deficiency).

Their genetic and clinical characteristics are summarized in Table 1. For the whole cohort of patients, median age at HLH diagnosis was 9 months (range, 0 to 142 months), and median follow-up from diagnosis was 36 months (range, 1 to 144 months).

Of the 64 total evaluable patients, 45 were alive; thus the probability of OS (pOS) in the whole cohort of patients was 56% (95% CI, 35%-70%) (Figure 1A). Forty-nine patients underwent HSCT, 8 of 49 died as a result of transplantation-related mortality (TRM), and 3 died as a result of post-HSCT HLH progression. One patient had an unexplained sudden death while in CR at 12 months after HSCT. Thirty-seven of 49 are alive at a median follow-up of 36 months from diagnosis (8-year pOS for HSCT patients, 63%; 95% CI, 38%-77%). Fifteen patients did not receive HSCT, 1 died as a result of complications related to first-line treatment, and 6 died as a result of HLH progression. Eight of those patients are alive at a median follow-up of 12 months (pOS for non-HSCT patients, 41%; 95% CI, 7%-74%).

ICs

Per inclusion criteria, all ICs (n = 32) (Figure 2) had overt disease at the time of diagnosis. The median age of the ICs at disease presentation was 16 months (range, 0 to 138 months). Median follow-up for the ICs was 36 months from diagnosis (range, 1-144 months). Fourteen of 32 had CNS involvement, and 6 were refractory to first-line treatment.

Seven patients died during first-line therapy (1 as a result of treatment-related toxicity, 6 as a result of HLH progression) and did not survive to HSCT. Twenty-three of 32 patients underwent HSCT. Six died as a result of TRM (1 as a result of chronic graft-versus-host disease, 1 as a result of lymphoproliferative disease and gram-negative sepsis, 1 as a result of infection following graft loss and second HSCT, 1 as a result of gram-negative sepsis, and 2 as a result of unspecified TRM) and 1 died as a result of disease progression after haploidentical HSCT. Sixteen children are alive and in CR after HSCT. Altogether, 18 of 32 evaluable ICs were alive at the latest follow-up (8-year pOS for ICs, 45%; 95% CI, 21%-62%). Two patients affected by MUNC18-2 deficiency did not receive HSCT and were alive at a median follow-up of 42 months from diagnosis (for details, see ICs 2 and 6 in supplemental Table 1 available on the Blood Web site).

ACs

Among the ACs (n = 32) (Figure 3), 10 (31%) of 32 had activated HLH during observation (n = 4) or while waiting for HSCT (n = 6); 3 were receiving cyclosporine at the time of HLH activation. Of these 10 patients, 1 had CNS disease, and 3 required multiple lines of treatment to achieve remission. All 10 patients underwent HSCT: 6 are alive in CR and 4 died (2 as a result of disease progression and 2 as a result of TRM).

Of the 22 patients who did not experience activated HLH during observation, 5 were receiving prophylactic treatment while waiting for HSCT. Sixteen of 22 underwent HSCT as a

Table 1. Genetic and clinical characteristics of the analyzed patients

Patient	Protein deficit	Genetics	Age at diagnosis (mo)	Treatment	Disease status if AC at diagnosis	Outcome	Follow-up from diagnosis (mo)
IC 1	MUNC18-2	Compound heterozygous	3	Antithymocyte globulin + cyclosporine + steroids + HSCT		Died TRM	5
AC 1	MUNC18-2	Compound heterozygous	1	HLH04 + HSCT	Activated during active follow-up	Alive CR	22
IC 2	MUNC18-2	474-483delinsGA 1001C>T	12	Steroids		Alive CR	48
AC 2	MUNC18-2	474_483delinsGA 1001C>T	24	None	Never activated	Alive CR	72
IC 3	MUNC18-2	1247G>C	18	HLH04 + rituximab + HSCT		Alive CR	108
AC 3	MUNC18-2	1247G>C	60	None	Never activated	Alive CR	36
IC 4	MUNC18-2	Unknown	16	HLH04 + HSCT		Died TRM	72
AC 4	MUNC18-2	Unknown	9	HSCT	Never activated	Alive CR	60
IC 5	MUNC18-2	Compound heterozygous	NA	HLH04 + HSCT		Alive CR	91
AC 5	MUNC18-2	Compound heterozygous	6	HSCT	Never activated	Alive CR	75
IC 6	MUNC18-2	1247G>C	138	Steroids + intravenous immunoglobulin		Alive CR	38
AC 6	MUNC18-2	1247G>C	108	None	Never activated	Alive CR	42
IC 7	LYST	2749-50delAG	24	HLH04 + HSCT		Died TRM after graft loss and second HSCT	26
AC 7	LYST	2749-50delAG	102	None	Never activated	Alive CR	12
IC 8	PRF1	50delT 853-855delAAG	5	Unspecified chemotherapy		Died HLH progression	1
AC 8	PRF1	50delT 853-855delAAG	5	HLH04 + HSCT	Never activated; received prophylactic treatment pre-SCT	Alive CR	32
IC 9	PRF1	445G>A 886T>C	60	Steroids		Died as a result of infection	72
AC 9	PRF1	445G>A 886T>C	1	HSCT	Never activated	Alive CR	24
IC 10	PRF1	Not available	18	Steroids + cyclosporine + alemtuzumab + HSCT		Alive CR	12
AC 10	PRF1	Not available	100	Steroids + cyclosporine + alemtuzumab + HSCT	Activated while waiting for SCT	Alive CR	12

HLH04, treatment according to the HLH04 protocol from the International Histiocyte Society; SCT, stem cell transplantation.

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Table 1. (continued)

Patient	Protein deficit	Genetics	Age at diagnosis (mo)	Treatment	Disease status if AC at diagnosis	Outcome	Follow-up from diagnosis (mo)
IC 11	PRF1	254G>T;473C>T; 390C>T	48	HLH94 + HSCT		Alive CR	36
AC 11	PRF1	254G>T;473C>T; 390C>T	9	HSCT	Never activated	Alive CR	38
IC 12	PRF1	1122G>A	2	HLH04		Died HLH progression	2
AC 12	PRF1	1122G>A	Birth	HLH04	Activated during prophylactic treatment while waiting for SCT	Lost to follow-up	
IC 13	PRF1	Not available	4	Etoposide + steroids + cyclosporine + intrathecal methotrexate + antithymocyte globulin + HSCT		Died HLH progression	8
AC 13	PRF1	Not available	Prenatal	Steroids + cyclosporine + antithymocyte globulin + alemtuzumab + HSCT	Activated during prophylactic treatment while waiting for SCT	Died HLH progression	15
IC 14	PRF1	Not available	54	Steroids + cyclosporine + alemtuzumab + HSCT		Alive CR	18
AC 14	PRF1	Not available	75	Steroids + cyclosporine + rituximab + HSCT	Activated during prophylactic treatment while waiting for SCT	Alive CR	48
IC 15	PRF1	1376C>T	36	HLH04 + HSCT		Alive CR	36
AC 15a	PRF1	1376C>T	5	HLH04 + HSCT	Activated while waiting for HSCT	Died TRM	16
AC 15b	PRF1	1376C>T	Prenatal	HSCT	Never activated	Alive CR	17
IC 16	PRF1	50delT; 1034C	8	HLH94 + HSCT		Alive CR	144
AC 16	PRF1	50delT; 1034C	Birth	HLH94 + HSCT	Activated during active follow-up	Alive CR	48
IC 17	PRF1	50delT; 1130G>A	1	Undefined induction + HSCT		Alive CR	12
AC 17	PRF1	50delT;1130G>A	Birth	HLH04 + HSCT	Activated during active follow-up	Alive CR	62
IC 18	PRF1	1081A>T; 1081A>T	60	HLH04 + HSCT		Died TRM	3
AC 18	PRF1	1081A>T; 1081A>T	30	HSCT	Never activated	Alive CR	21
IC 19	PRF1	Not available	72	HLH04 + HSCT		Alive CR	60

HLH04, treatment according to the HLH04 protocol from the International Histiocyte Society; SCT, stem cell transplantation.

Table 1. (continued)

Patient	Protein deficit	Genetics	Age at diagnosis (mo)	Treatment	Disease status if AC at diagnosis	Outcome	Follow-up from diagnosis (mo)
AC 19	*PRF1*	Not available	3	HLH04 + HSCT	Activated while waiting for HSCT	Alive CR	72
IC 20	PRF1	G5759C>T G5897A>C	8	HLH04 + HSCT		Alive CR	36
AC 20	PRF1	G5759C>T G5897A>C	Prenatal	HSCT	Never activated	Alive CR	18
IC 21	MUNC13-4	1389G>A; 1620-1621delCA	1	HLH04 + HSCT		Alive CR	36
AC 21	MUNC13-4	1389G>A; 1620-1621delCA	Prenatal	HSCT	Never activated	Alive CR	60
IC 22	MUNC13-4	118-308C>T	16	Cyclosporine + etoposide (withdrew)		Died HLH progression	2
AC 22	MUNC13-4	118-308C>T	13	HSCT, lost graft, HLH04 + second HSCT	Never activated	Alive CR	89
IC 23	MUNC13-4	Not available	2	HLH94 + antithymocyte globulin + HSCT		Alive CR	144
AC 23	MUNC13-4	Not available	1	None		Alive CR	144
IC 24/a	MUNC13-4	118-308C>T; 1596+1G>C	1	HLH04 + HSCT		Alive CR	45
IC 24/b	MUNC13-4	118-308C>T; 1596+1G>C	1	HLH04 + HSCT		Died TRM	13
AC 24	MUNC13-4	118-308C>T; 1596+1G>C	Birth	HSCT	Never activated	Alive CR	26
IC 25	STX11	37+16>A	Birth	HLH04		Died HLH progression	1
AC 25	STX11	37+16>A	Prenatal	Cyclosporine + steroids + HSCT	Never activated; receiving prophylactic treatment	Died; sudden death in CR	17
IC 26	STX11	Not available	36	HLH04 + HSCT		Alive CR	120
AC 26	STX11	Not available	72	HLH94 + HSCT	Activated during active follow up	Died TRM	76
IC 27	RAB27A	281G>A	18	HLH04 + HSCT		Alive CR	25
AC 27	RAB27A	281G>	144	None	Never activated	Alive CR	2
IC 28	RAB27A	467+1G>A	12	HLH94		Lost to follow-up	NA
AC 28	RAB27A	467+1G>A	Birth	Cyclosporine + HSCT	Never activated; receiving prophylactic treatment	Alive CR	12
IC 29	RAB27A	220G>C;335delA	9	Dexamethasone + etoposide		Died HLH progression	36

HLH04, treatment according to the HLH04 protocol from the International Histiocyte Society; SCT, stem cell transplantation.

Table 1. (continued)

Patient	Protein deficit	Genetics	Age at diagnosis (mo)	Treatment	Disease status if AC at diagnosis	Outcome	Follow-up from diagnosis (mo)
AC 29	*RAB27A*	220G>C;335delA	1	HSCT	Never activated	Alive CR	91
IC 30	*RAB27A*	Not available	9	Corticosteroids, cyclosporine, intrathecal methotrexate, HSCT × 3		Alive CR	84
AC 30	*RAB27A*	Not available	Prenatal	Corticosteroids, cyclosporine, HSCT	Activated while waiting for HSCT	Died HLH progression	18
IC 31	*RAB27A*	Not available	126	Alemtuzumab, corticosteroids, cyclosporine, intrathecal methotrexate, natalizumab, HSCT		Died TRM	30
AC 31	*RAB27A*	Not available	180	HSCT	Never activated; receiving prophylactic treatment while waiting for HSCT	Alive in CR	36
IC 32	*RAB27A*	Not available	84	Cyclosporine + alemtuzumab + steroids		Died HLH progression	12
AC 32	*RAB27A* *RAB27A*	Not available	84	Cyclosporine + HSCT	Never activated; receiving prophylactic treatment while waiting for HSCT	Alive in CR	26

HLH04, treatment according to the HLH04 protocol from the International Histiocyte Society; SCT, stem cell transplantation.

preemptive measure; 15 are alive in CR, 1 had a sudden death as a result of unknown causes at 19 months of age and at 12 months after HSCT while in CR. Six of 22 patients (3 with MUNC18-2 deficiency, 1 with *LYST* deficiency, 1 with *RAB27A* deficiency, and 1 with MUNC13-4 deficiency) have received no specific treatment and are alive and are in CR at a median follow-up of 39 months from diagnosis. These 6 children are now older than their siblings were at the time of disease presentation; in 2 cases the corresponding ICs have been treated with chemotherapy only, and they achieved a good degree of disease remission and did not require HSCT (AC2, -3, -6, -7, -23, and -27; Table 1). Overall 21 of 22 ACs without HLH activation are alive with no survival difference between those who underwent SCT and those who were actively followed up (93% vs 100%; *P* = 1).
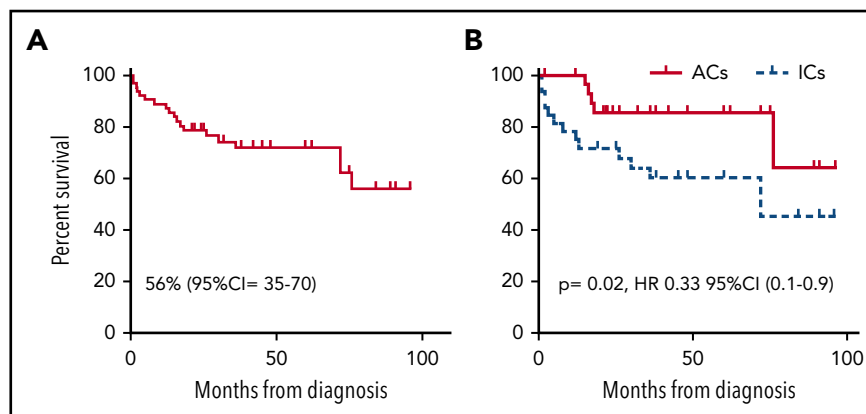

Figure 1. OS per Kaplan-Meier estimate comparing ICs and ACs. (A) The whole population of patients; (B) our population of patients. HR, hazard ratio.
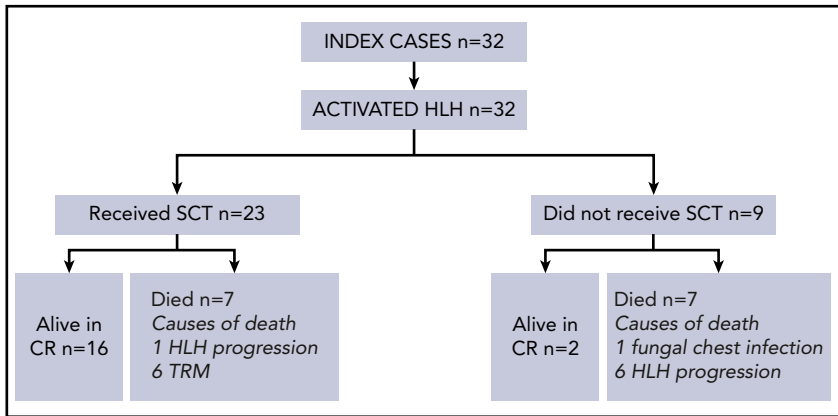
Figure 2. Diagram of treatment and outcome for ICs.
SCT, stem cell transplantation.

Of 32 evaluable ACs, 27 were alive at last follow-up (8-year pOS, 63%; 95% CI, 20%-87%). Twenty-one of 26 patients who underwent HSCT are alive. Importantly, 15 of 16 patients who received a transplant before the development of HLH are alive compared with 6 of 10 who received a transplant after the development of HLH (pOS, 64% vs 93%; *P* = .032; hazard ratio [HR], 0.13; 95% CI, 0.02-0.84).

Impact of genetic abnormality on OS

None of the genetic subgroups had a statistically significant difference in survival compared with the pOS of 56% for the whole group. Of evaluable patients, 19 of 26 with perforin deficiency were alive as well as 1 of 4 with syntaxin 11 deficiency, 7 of 9 with MUNC13-4 deficiency, 10 of 12 with MUNC18-2 deficiency, 7 of 11 with Griscelli type 2 syndrome, and 1 of 2 with Chédiak-Higashi syndrome.

Concordance between sibling pairs with activated HLH disease

Nine pairs in which the AC experienced activated HLH were analyzed for concordance in presenting features. Seven pairs had perforin deficiency, 1 pair had syntaxin 11 deficiency, and 1 pair had MUNC18-2 deficiency. Age at disease activation was variable, with a difference between ICs and ACs of 2 weeks to 7 years. In 6 pairs, CNS status at diagnosis was discordant. Epstein-Barr virus (EBV) infection was a trigger for HLH in the IC with syntaxin 11 deficiency but was not involved in the disease activation of her sibling.

Discussion

HSCT is the standard treatment for primary HLH. However, ~30% of patients die before HSCT because of uncontrolled HLH or treatment-related toxicity; hence, survival in children presenting with primary HLH is poor at 59%.[22,24] Children who have well-controlled disease and survive HSCT have a superior survival (pOS, 71%).[20,22,24] However, the decision to offer HSCT to ACs before the onset of HLH is complicated by the interplay between genetic and environmental factors (typically infectious triggers) that lead to the onset of HLH, incomplete genotype-phenotype correlation,[10,27-30] and the ethical aspects of subjecting a young asymptomatic child to a toxic procedure with a significant TRM.

Consistent with the findings from the HLH-94 and HLH-2004 studies,[22,23] ICs in our study had an OS of ~50%: 7 of 32 ICs died before HSCT, whereas the incidence of TRM was 26%. Survival was significantly improved in ACs compared with ICs, irrespective of treatment received (63% vs 45%; *P* = .02) (Figure 1B). The majority of ACs received a preemptive transplant, and patients treated with this approach showed a survival of 15 (93%) of 16; the only death in this group was a result of causes not related to transplantation. Although numbers are small, it is interesting to note that survival in ACs who experienced HLH activation before HSCT was not statistically different from that of ICs (33% vs 45%; *P* = .9; 95% CI, 0.3-2.7; Figure 4). In contrast, ACs who received a preemptive transplant showed an improved survival compared with ACs who received a transplant
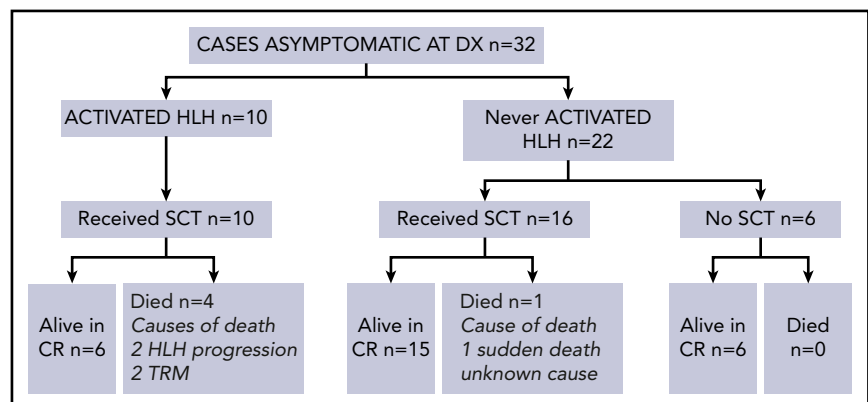

Figure 3. Diagram of treatment and outcome for ACs.
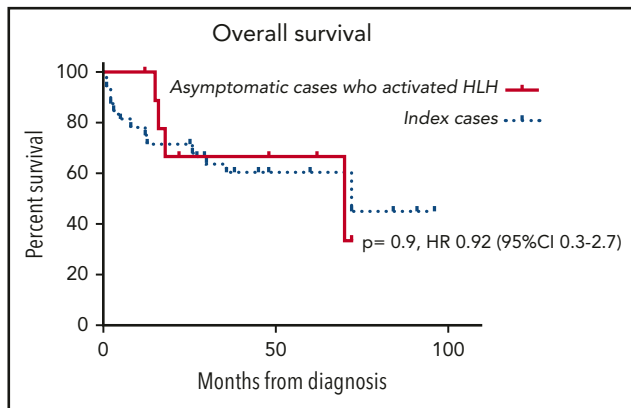DX, diagnosis.

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Figure 4. OS per Kaplan-Meier estimate in our population of patients comparing ACs who did not experience activated HLH with those who experienced activated HLH while on follow-up.

once HLH had developed (pOS, 93% vs 64%; $P = .03$; HR, 0.13; 95% CI, 0.02-0.8).

The long-term outcome of 6 of 20 ACs who did not receive a transplant remains to be determined. Median follow-up for this subgroup was 39 months, and at the latest follow-up, they were all older than their corresponding ICs at the time of disease onset. Although Cetica et al[27] reported wide variation in the age of the IC at HLH onset and the ages of subsequent siblings in 9 of 26 familial cases, all eventually developed HLH except 1 who remained asymptomatic until the age of 25 years. The mutation profile of these patients can only partially explain why they remained asymptomatic. Indeed, although AC 3 and 6 had an MUNC18-2 mutation predicted to have a mild disease course,[30] this cannot be documented for AC 2; moreover, AC 7, 23, and 27 were affected by LYST, MUNC13-4, and RAB27A deficiency, respectively, in which no genotype-phenotype correlation has been described. At present, the prognosis for this group is guarded at best.

In our cohort of patients, the tightest correlation between ICs and ACs in terms of disease onset was in the PRF1-deficient group. This group was also more likely to experience activated HLH early in life; 7 of 11 of the ACs with PRF1 deficiency experienced activated full-blown disease at a median time of 1 month from their diagnosis. Although it is not possible to make firm conclusions because the number of patients is too small, most patients in this group had mutations that would predict for absent perforin expression. Three patients had a combination of del50T mutation with other mutations, 2 were documented to have absent perforin expression by flow cytometry,[29] and 1 patient was homozygous for c.1122G>A mutation, which has been described as being related to a severe and early phenotype of the disease (supplemental Table 1).[10] Three other patients had no genetic data available for consideration, but one of them had absent perforin expression on cytometric analysis. These observations match the reported data on patients with biallelic disruptive mutations in the PRF1 gene who develop HLH at a significantly younger age compared with patients with missense mutations only.[28] Preemptive transplantation might therefore be particularly indicated for this subgroups of patients.

At the other end of the spectrum, patients in our cohort with MUNC18-2 deficiency exhibited a milder form of HLH. Only 1 of

5 ACs experienced activated HLH and had a successful HSCT. Among the other 5 ACs with MUNC18-2 deficiency, 2 received preemptive HSCTs, and 3 are being actively followed up. In addition, 2 IVs with MUNC18-2 deficiency are alive without HSCT at a median of 44 months of follow-up from diagnosis. Among patients with MUNC18-2 deficiency who did not receive treatment and who are being actively observed with no signs of disease, 3 had homozygous mutation 1247-1G>C previously described as being associated with a mild phenotype postulated to result from expression of an abnormal protein.[30] Ectopic expression of wild-type MUNC18-2 overcoming the MUNC18-2 deficiency was also suggested as a possible explanation for the milder HLH phenotype in some patients with MUNC18-2 deficiencies.[13] We could not prove this phenomenon in our patients, but our report confirms a possible milder type of disease. The complexity of the clinical phenotype in MUNC18-2 deficiency, even in the presence of null mutations and the variability of multiorgan involvement, emphasizes the need for further studies and long-term follow-up in this and other HLH subtypes.

TRM in our case series was acceptable, with an incidence of 8 (16%) of 49, and we documented no significant difference in TRM between ICs and ACs who had activated HLH (26% vs 20%; $P = 1.00$). Significantly, no ACs died before HSCT, including those in the group who experienced activated HLH. We can speculate that ACs received HSCT earlier than ICs once they were diagnosed, and it was therefore easier to control their disease, but our data are not detailed enough to confirm this finding. Despite small numbers of patients, it is important to note that there was no TRM in the 16 ACs who received a transplant before they developed HLH. Although our study did not address the impact of donor choice or disease status on HSCT outcome, it seems that the best HSCT results were seen in ACs who received a preemptive transplant. A preemptive HSCT approach has been documented to be efficacious in the context of other primary immunodeficiencies,[31] which allows for significant improvement in overall outcome. Our data strongly suggest that most ACs with HLH should receive a transplant before the onset of symptoms if an adequate donor is available.

In summary, our data confirm that primary HLH can have an unpredictable and severe course, even in ACs who are being carefully monitored. The initial presentation may involve the CNS and be refractory to primary therapy. Once HLH develops, the OS is not different from that of ICs. Preemptive HSCT before the onset of HLH could offer a survival advantage, especially in the subgroups of patients with a genotype predictive of severe disease, such as in children with a complete lack of perforin expression.[28] Conversely, patients with an MUNC18-2 deficiency might require further consideration before HSCT because of the potential for milder forms of the disease and because of the possibility of multiorgan involvement that may not be cured by HSCT. On the basis of our data, we would recommend preemptive HSCT in ACs with certain subtypes of primary HLH (ie, PRF1 mutations with absent perforin expression and RAB27A mutation). In those ACs who have other genetically confirmed primary HLH mutations with unclear genotype-phenotype correlation, we would encourage considering HSCT in the presence of a well-matched donor after adequate counseling of families. A watch-and-wait policy could be suggested for patients affected with MUNC18-2 deficiency with mutations associated with milder phenotypes. Longer follow-up and larger patient numbers are needed to make more accurate genotype-phenotype

correlations, which may in turn allow for more tailored treatment guidance on specific subgroups of patients.

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REFERENCES

1. Henter JI, Elinder G, Söder O, Ost A. Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. *Acta Paediatr Scand.* 1991;80(4):428-435.

2. Meeths M, Horne A, Sabel M, Bryceson YT, Henter JI. Incidence and clinical presentation of primary hemophagocytic lymphohistiocytosis in Sweden. *Pediatr Blood Cancer.* 2015;62(2):346-352.

3. Aricò M, Danesino C, Pende D, Moretta L. Pathogenesis of haemophagocytic lymphohistiocytosis. *Br J Haematol.* 2001;114(4):761-769.

4. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB. Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. *Clin Exp Immunol.* 2011;163(3):271-283.

5. Ménasché G, Pastural E, Feldmann J, et al. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nat Genet.* 2000;25(2):173-176.

6. Barbosa MD, Nguyen QA, Tchernev VT, et al. Identification of the homologous beige and Chediak-Higashi syndrome genes [published correction appears in *Nature.* 1997;385(6611):97]. *Nature.* 1996;382(6588):262-265.

7. Rigaud S, Fondanèche MC, Lambert N, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature.* 2006; 444(7115):110-114.

8. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science.* 1999;286(5446):1957-1959.

9. Feldmann J, Callebaut I, Raposo G, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell.* 2003;115(4):461-473.

10. Zur Stadt U, Beutel K, Kolberg S, et al. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: molecular and functional analyses of PRF1, UNC13D, STX11, and RAB27A. *Hum Mutat.* 2006;27(1):62-68.

11. Jessen B, Bode SF, Ammann S, et al. The risk of hemophagocytic lymphohistiocytosis in Hermansky-Pudlak syndrome type 2. *Blood.* 2013;121(15):2943-2951.

12. zur Stadt U, Rohr J, Seifert W, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. *Am J Hum Genet.* 2009;85(4):482-492.

13. Côte M, Ménager MM, Burgess A, et al. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest.* 2009;119(12):3765-3773.

14. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet.* 1998;20(2):129-135.

15. Henter JI, Horne A, Aricò M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2007;48(2):124-131.

16. Jabado N, de Graeff-Meeder ER, Cavazzana-Calvo M, et al. Treatment of familial hemophagocytic lymphohistiocytosis with bone marrow transplantation from HLA genetically nonidentical donors. *Blood.* 1997;90(12):4743-4748.

17. Dürken M, Horstmann M, Bieling P, et al. Improved outcome in haemophagocytic lymphohistiocytosis after bone marrow transplantation from related and unrelated donors: a single-centre experience of 12 patients. *Br J Haematol.* 1999;106(4):1052-1058.

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

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

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

21. Cesaro S, Locatelli F, Lanino E, et al. Hematopoietic stem cell transplantation for hemophagocytic lymphohistiocytosis: a retrospective analysis of data from the Italian Association of Pediatric Hematology Oncology (AIEOP). *Haematologica.* 2008;93(11):1694-1701.

22. Trottestam H, Horne A, Aricò M, et al; Histiocyte Society. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. *Blood.* 2011;118(17):4577-4584.

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

24. Mahlaoui N, Ouachée-Chardin M, de Saint Basile G, et al. Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: a single-center retrospective report of 38 patients. *Pediatrics.* 2007;120(3):e622-e628.

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

26. Henter JI, Samuelsson-Horne A, Aricò M, et al; Histiocyte Society. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood.* 2002;100(7):2367-2373.

27. Cetica V, Sieni E, Pende D, et al. Genetic predisposition to hemophagocytic lymphohistiocytosis: Report on 500 patients from the Italian registry. *J Allergy Clin Immunol.* 2016;137(1):188-196.e4.

28. Trizzino A, zur Stadt U, Ueda I, et al; Histiocyte Society HLH Study group. Genotype-phenotype study of familial haemophagocytic lymphohistiocytosis due to perforin mutations. *J Med Genet.* 2008;45(1):15-21.

29. Molleran Lee S, Villanueva J, Sumegi J, et al. Characterisation of diverse PRF1 mutations leading to decreased natural killer cell activity in North American families with haemophagocytic lymphohistiocytosis. *J Med Genet.* 2004;41(2):137-144.

30. Pagel J, Beutel K, Lehmberg K, et al. Distinct mutations in STXBP2 are associated with variable clinical presentations in patients with familial hemophagocytic lymphohistiocytosis type 5 (FHL5). *Blood.* 2012;119(25):6016-6024.

31. Brown L, Xu-Bayford J, Allwood Z, et al. Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. *Blood.* 2011;117(11):3243-3246.