From www.bloodjournal.org by guest on August 18, 2018. For personal use only.
Blood First Edition Paper, prepublished online August 13, 2018; DOI 10.1182/blood-2018-01-827485

Treatment dilemmas in asymptomatic children with primary haemophagocytic lymphohistiocytosis

Short title:

HSCT for asymptomatic children with primary HLH.

Authors:

Giovanna Lucchini[1], Rebecca Marsh[2], Kimberly Gilmour[1], Austen Worth[1], Zohreh Nademi[3], Anupama Rao[4], Claire Booth[1], Persis Amrolia[1], Juliana Silva[1], Robert Chiesa[1], Robert Wynn[5], Kai Lehmberg[6], Itziar Astigarraga[7], Tayfun Gungor[8], Jan Stary[9], Despina Moshous[10], Marianne Ifversen[11], Daniel Zinn[12], Michael Jordan[2], Ashish Kumar[2] Takahiro Yasumi[13], Paul Veys[1], Kanchan Rao[1].

Affiliations:

1) Department of Immunology/Stem Cell Transplant, Great Ormond Street Hospital, London, United Kingdom
2) Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital, Cincinnati, USA
3) Division of Bone Marrow Transplant, Great North Children Hospital, Newcastle, United Kingdom
4) Department of Haematology, Great Ormond Street Hospital, London, UK
5) Department of Stem Cell Transplant, Royal Manchester Children Hospital, Manchester, United Kingdom
6) Division of Pediatric Stem Cell Transplantation and Immunology, University Medical Center Hamburg Eppendorf, Hamburg, Germany
7) Department of Pediatrics. Hospital Universitario Cruces. BioCruces. Health Research Institute. University of the Basque Country. UPV/EHU. Barakaldo. Bizkaia. Spain.
8) Division of Stem Cell Transplantation, University Children Hospital, Zurich, Switzerland

Copyright © 2018 American Society of Hematology

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

9) Department of Pediatric Hematology and Oncology, Motol University Hospital, Prague, Czech Republic
10) Department of Pediatric Immunology and Hematology, APHP, Hôpital Necker-Enfants Malades, Paris Descartes University Paris, France
11) Department for Children and Adolescents, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
12) Department of Pediatrics, Section Hematology Oncology, Baylor College of Medicine, Houston, USA
13) Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

Corresponding author

Kanchan Rao, MD

Department of Immunology and Bone Marrow Transplantation,

Great Ormond Street Hospital,

London,UK

Email: kanchan.rao@gosh.nhs.uk

Phone: 0044 (0)20 78138434

Fax: 0044 (0)20 78298640

Abstract world count: 247

Word count: 2813

Figure count: 4

Table count: 1

Reference count: 33

Supplemental material: 1 Table

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

Scientific category: Transplantation

Key Words

Primary haemophagocytic lymphohistiocytosis, stem cell transplantation, familial hemophagocytic lymphohistiocytosis

Key points

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

Abstract*:*

Asymptomatic carriers of pathogenic bilallelic mutations in causative genes for primary haemophagocytic lymphohistiocytosis (HLH) are at high-risk of developing life-threatening HLH, which requires allogeneic haematopoietic stem cell transplantation (HSCT) to be cured. There are no guidelines on the management of these asymptomatic patients. We analysed the outcomes of pairs of index cases (ICs) and subsequently diagnosed asymptomatic family members (ACs) carrying the same genetic defect. We collected data from 22 HSCT Centres worldwide. Thirty-two pairs (64 children) were evaluable. ICs presented with HLH at a median age of 16 months. Seven of 32 ICs died during frontline therapy, 2 are alive after chemotherapy only. 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. 10/32 ACs activated HLH whilst on observation and all underwent HSCT: 6/10 are alive and in complete remission (CR). 22/32 ACs remained asymptomatic, 6/22 have received no treatment and are in CR at a median follow up of 39 months. 16/22 underwent pre-emptive HSCT: 15/16 are alive and in CR. 8 yrs pOS in ACs who did not activate HLH was significantly higher than 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*). Pre-emptive HSCT in ACs prior to the development of HLH proved to be safe and should be considered.

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

Introduction

Primary haemophagocytic lymphohistiocytosis (HLH) is a rare disease with an estimated incidence of 1.8 per 100.000 live birth 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 further 5 genes are responsible for immunodeficiencies in which HLH is a prominent clinical feature (ie PRF1, STXBP2, UNC13D, STX11, RAB27A, BIRC4, SH2D1A, LYST, AP3B1)[4-14]. The curative treatment in primary HLH is haematopoietic stem cell transplantation (HSCT)[15-21]. Despite advances in chemo/immunotherapy for the treatment of primary HLH, overall survival (OS) remains at only about 59%, with virtually no survivors reported among non-transplanted patients once the disease is fully active[22,23,24]. Data from the largest cooperative prospective international studies (HLH 94 and 2004 protocols) show that up to 20% of patients do not achieve a durable complete remission with front 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 75-92% for patients who do not have CNS disease and who achieve good disease control prior to HSCT[20].

Whilst HSCT is clearly the treatment of choice once HLH becomes clinically manifest, the question of whether to offer HSCT to asymptomatic siblings diagnosed genetically before the onset of symptoms remains unanswered.

To date there is 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 makes decision-making extremely challenging. In fact previous studies demonstrated that only a third of relatives affected by the same genetic mutation as 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 cases[25]. In this study we describe for the first time

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

the outcome of children who carry a genetic mutation predisposing to primary HLH and diagnosed while asymptomatic as compared to that of manifesting ICs in the same family.

Methods and data collection

Children with an age < 18 years, who carried the same genetic mutations of 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 was collected via a questionnaire distributed to centres through the Histiocyte Society. A key physician was identified to collect and report local data for each of the consulted paediatric haematopoietic stem cell transplant (HSCT) centres in Japan, Europe and North America. 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 centres about their current attitude towards the management of asymptomatic family members with primary HLH. Given the recognised variability of the disease course in patients with XLP1 and XIAP deficiency, these cases were not analysed in this study. Patients enrolled in the study had given prior consent to the use of their anonymised clinical data in the context of national or international studies. Individual Institutional Review Boards in North America gave approval for the present study.

Statistics

Descriptive analyses results are reported as medians and ranges. Kaplan Meier estimators and confidence intervals are used to estimate overall survival (OS) and the Log Rank test as well as Fisher test (discrete variables) have been used to compare data. Patients are censored at last follow up if no events occurred. Confidence intervals are reported at the 95% and statistical tests are performed at the 0·05 level (two sided).

Results

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

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

Patients characteristics and outcomes

Out of the 32 pairs/triad of ICs/ACs identified, 13 had PRF1 (perforin) deficiency, 6 STXBP2 (MUNC18-2) deficiency, 4 UNC13D (MUNC13-4) deficiency, 2 STX11 (Syntaxin) deficiency, 6 were diagnosed with Griscelli syndrome type II (RAB27A deficiency) and 1 with Chédiak 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) and median follow up from diagnosis was 36 months (range 1-144).

Of the total 64 evaluable cases, 45 were alive, thus the probability of overall survival (pOS) in the whole cohort of patients was 56% (95%CI;35-70) (Fig 1A). Forty-nine patients underwent HSCT, 8/49 died from TRM and 3 from post-HSCT HLH progression. One patient had an unexplained sudden death while in CR 12 months post HSCT. 37/49 are alive at a median follow up of 36 months from diagnosis (8 yrs pOS for HSCT patients 63% 95%CI; 38-77). Fifteen patients did not receive HSCT, 1 died from front-line treatment related complications and 6 from HLH progression. Eight of them are alive at a median follow up of 12 months (pOS non-HSCT patients = 41% 95%CI; 7-74).

Index cases (n=32) (Figure 2)

As per inclusion criteria, all index cases had overt disease at the time of diagnosis. The median age of the ICs at disease presentation was 16 months (range 0 to 138). Median follow up for the ICs was 36 months from diagnosis (range 1-144). 14/32 had CNS involvement and 6 were refractory to front-line treatment.

Seven patients died during front line therapy (1 death from treatment related toxicity, 6 from HLH progression) and did not survive to HSCT. Twenty-three out of 32 patients

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

underwent HSCT. Six died due to transplant related mortality (1 chronic graft versus host disease, 1 lymphoproliferative disease and Gram negative sepsis, 1 infection following graft loss and 2ndHSCT, 1 Gram negative sepsis and 2 unspecified TRM) and one from disease progression post haploidentical HSCT. Sixteen children are alive and in complete remission (CR) after HSCT. Altogether, 18/32 evaluable ICs are alive at latest follow up (8 yrs pOS for ICs 45% 95%CI;21-62). Two patients affected by MUNC18-2 deficiency did not receive HSCT and are alive at a median follow up of 42 months from diagnosis (see Index case 2 and 6 in Supplemental Table 1 for details).

Asymptomatic cases (n=32)(Figure 3)

Among the ACs, 10/32 (31%) activated HLH whilst on observation (*n=4*) or waiting for HSCT (*n=6*); three of them were on 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 from disease progression and 2 from TRM).

Of the 22 patients who did not activate HLH while on observation, 5 were on prophylactic treatment while awaiting for HSCT. 16/22 underwent HSCT as a pre-emptive measure and 15 are alive in CR, 1 had a sudden death from unknown causes at 19 months of age and 12 months post HSCT, while in CR. Six of 22 patients (3 affected with MUNC 18-2 deficiency, 1 with LYST deficiency, 1 with RAB27A deficiency and 1 with MUNC13-4 deficiency) have received no specific treatment and are alive and in CR at a median follow up of 39 months from diagnosis. These 6 children are now older than their siblings at the time of disease presentation, in 2 cases the corresponding index cases have been treated with chemotherapy only achieving a good degree of disease remission and not requiring HSCT (see Table 1 AC 2,3,6,7,23,27 for details). Overall 21/22 ACs without HLH activation are alive with no survival difference between those who underwent SCT and those who are actively followed up (93 vs 100%, p =1).

Of 32 evaluable ACs, 27 are alive at last follow-up (8yrs pOS = 63% 95%CI;20-87). Twenty-one of 26 patients who underwent HSCT are alive. Importantly, 15/16 patients transplanted before the development of HLH are alive compared to 6/10 transplanted after development of HLH (pOS 64% vs 93%, *P= .032*, HR 0.13 95%CI;.02-.84).

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

Impact of genetic abnormality on overall survival

None of the genetic subgroups had a statistically significant difference in survival compared to the pOS of 56% for the whole group. Of evaluable patients, 19/26 with perforin deficiency were alive, 1/4 with syntaxin 11 deficiency, 7/9 with MUNC13-4 deficiency, 10/12 with MUNC 18-2 deficiency, 7/11 evaluable with Griscelli type 2 syndrome and 1/2 with Chédiak Higashi syndrome.

Concordance between sibling pairs activating HLH disease

Nine pairs where the AC activated HLH were analysed for concordance in presenting features. Seven pairs had perforin deficiency, one pair had syntaxin 11 deficiency, one had MUNC 18-2 deficiency. Age at disease activation was very variable with a difference between index case and AC from 2 weeks to 7 years. In 6 pairs, CNS status at diagnosis was discordant. EBV infection was a trigger for HLH in the index case 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 approximately 30% of patients die before HSCT due to 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) leading to the onset of HLH, incomplete genotype/phenotype correlation[27-31] and the ethical aspects of subjecting a young, asymptomatic child to a toxic procedure with a significant TRM.

Consistently with the findings from the HLH 94 and HLH2004 studies [22,23] – index cases in our study had an overall survival of approximately 50%: 7/32 ICs died before HSCT, while the incidence of TRM was 26%. Survival was significantly improved in ACs compared to ICs irrespective of treatment received (63% vs 45% *P=.02*) (Figure 1B). The majority of ACs were

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

transplanted pre-emptively and patients treated with this approach showed a survival of 15/16 (93%) ; the only death in this group was due to non-transplant related causes. Although numbers are small, it is interesting to note that survival in ACs who activated HLH prior to HSCT was not statistically different from ICs (33% vs 45%, *P=.9*; 95%CI .3-2.7, Fig 4). In contrast, ACs who were transplanted pre-emptively showed an improved survival compared to ACs transplanted once HLH had developed (pOS 93% vs 64%, *P=.03*, HR 0.13 95%CI; .02-.8).

The long-term outcome of 6/20 ACs who were not transplanted 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 correspondent ICs at the time of disease onset. Although Cetica *et al* [27] reported wide variation in the age of HLH onset of the index case and subsequent siblings in 9 of 26 familial cases, all developed eventually HLH, except one who remained asymptomatic until the age of 25 years. The mutation profile of these patients can only partially explain why they remain asymptomatic. Indeed while pt 3 and 6 had a MUNC18-2 mutation predicted to have a mild disease course [31], this cannot be documented for pt 2, moreover pt 7, 23 and 27 are affected respectively by LYST deficiency, MUNC13-4 and RAB27a deficiency, in which no genotype/phenotype correlation has been described. The prognosis for this group is at best guarded at present.

In our cohort of patients the tightest correlation between ICs and ACs in terms of disease onset was in the PRF deficient group. This group was also more likely to activate HLH early in life; 7/11 of the ACs with PRF deficiency activated full-blown disease at a median time of 1 month from their diagnosis. Although the number of patients is too small to make firm conclusions, most patients in this group had mutations that would predict for absent PRF expression. Three patients had a combination of del50T mutation with other mutations and 2 were documented to have absent perforin expression by flow cytometry[29], one patient was homozygous for c.1122G>A mutation which has been described as related to a severe and early phenotype of the disease (supplemental Table 1)[30]. Three other patients had no genetics available for consideration, but one of them had absent perforin expression on cytometric analysis. These observations match the reported data on patients with bi-allelic disruptive mutations in the PRF gene, who develop HLH at a significantly younger age

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

compared to patients with missense mutations only [28]. Pre emptive transplant might therefore be particularly indicated for this subgroups of patients.

At the other end of the spectrum, patients in our cohort with MUNC 18-2 deficiency exhibited a milder form of HLH. Only 1/5 asymptomatic cases activated HLH and had a successful HSCT. Among the other 5 MUNC 18-2 deficient ACs 2 received pre-emptive HSCTs and 3 are under active follow-up. Additionally, 2 MUNC 18-2 deficient ICs are alive without HSCT at a median of 44 months of follow up from diagnosis. Among MUNC 18-2 deficient patients who did not receive treatment and are under active observation with no signs of disease, 3 had a homozygous mutation 1247-1G>C previously described to be associated with a mild phenotype postulated to result from expression of an abnormal protein[31]. Ectopic expression of wild type STXBP2 overcoming the MUNC 18-2 deficiency, was also suggested as a possible explanation for the milder HLH phenotype in some MUNC 18-2 deficiency [32]. We could not prove this phenomena in our patients, but our report confirms a possible milder type of disease. The complexity of the clinical phenotype in MUNC 18-2 deficiency even in the presence of null mutations and the variability of multi organ involvement emphasises the need for further studies and long term follow-up in this and other HLH subtypes.

Transplant related mortality in our case series was acceptable with an incidence of 8/49 (16%) and we documented no significant difference in TRM between IC and ACs who had activated HLH (26% vs 20%, *P=1.00*). Significantly, no ACs died before HSCT, including in the group who activated HLH. We can speculate that ACs received HSCT earlier than ICs once diagnosed and it was therefore easier to control their disease, but our data are not detailed enough to confirm this finding. Despite small numbers, it is important to note that there was no TRM in the 16 ACs who were transplanted prior to the development of HLH. Whilst our study did not address the impact of donor choice or disease status on HSCT outcome, it would seem that the best HSCT results were seen in ACs transplanted pre-emptively. A pre-emptive HSCT approach has been documented to be efficacious in the context of other primary immunodeficiencies[33] allowing for significant improvement in overall outcome. Our data strongly suggest that most asymptomatic patients with HLH should be transplanted before the onset of symptoms if an adequate donor is available.

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

In summary, our data confirms 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 to that of index cases. Pre-emptive HSCT prior to the onset of HLH could offer a survival advantage, specifically in the subgroups of patients with a genotype predictive of severe disease, like children with complete lack of perforin expression[28]. On the other hand, MUNC18-2 deficient patients might require further consideration before HSCT, both because of the potential for milder forms of the disease and because of the possibility of multi organ involvement that may not be cured by HSCT. Based on our data, we would recommend pre-emptive HSCT in ACs in certain subtypes of primary HLH, ie perforin 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 MUNC 18-2 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.

Acknowledgements:

We thank the Histiocyte Society for supporting the present study and helping delivering the questionnaire for data collection to paediatric HSCT centres worldwide.

We thank Geneviève de Saint Basile for her diagnostic contribution to the French cohort of patients.

Authorship Contributions

Disclosure of Conflicts of Interest:

Authors have no relevant conflict of interest to disclose

References:

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

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

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. Arico' 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;25:173-176.

6. Barbosa MD, Nguyen QA, Tchernev VT et al. Identification of the homologous beige and Chédiak-Higashi syndrome genes. *Nature.* 1996; 382(6588):262-265.

7. Rigaud S, Fondaneche 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 11;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.

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

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, Arico' 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;90:4743–4748.

17. Durken 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;106:1052–1058.

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

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

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):S47–50.

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 AC, Arico' M, et al. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. *Blood.* 2011; 118(17): 4577–4584.

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

23 Bergsten E, Horne AC, Arico' M, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. *Blood.* 2017; 130: 2728-2738.

24. Mahlaoui N, Ouachee-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):e622-8.

25. Ouachee-Chardin M, Elie C, de Saint BG, 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, Arico M, et al. 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. Genotype-phenotype study of familial haemophagocytic lymphohistiocytosis due to perforin mutation. J Med Genet.2008; 45(1):15-21.

29. Molleran L, 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. Med Genet. 2004; 41(2): 137–144.

30. 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.

31. 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.

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

32. Cote M, Menager 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.

33. 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.

Figure 1: A Overall survival as per Kaplan Meyer estimate in the whole population of patients, B Overall survival as per Kaplan Meyer estimate in our population of patients, comparing Index cases (ICs) and Asymptomatic cases (ACs) .

Figure 2. Diagram of treatment and outcome for Index cases. SCT = stem cell transplantation, GVHD = graft versus host disease, TRM= transplant related mortality, CR= complete remission, LPD= lymphoproliferative disorder.

Figure 3. Diagram of treatment and outcome for asymptomatic cases. CR= complete remission GVHD = graft versus host disease, SCT = stem cell transplantation, TRM= transplant related mortality.

Figure 4. Overall survival as per Kaplan Meyer estimate in our population of patients comparing Asymptomatic cases who never activated HLH and those who activated HLH while on follow up.

Table 1. Genetic and clinic characteristics of the analysed patients.

ATG= anti-thymocyte globuline, CR= complete remission, CSA= cyclosporine, HLH04= treatment according to the HLH04 protocol from the International Histiocyte Society, Perforin=PRF 1, HSCT = haematopoietic stem cell transplantation, STX11= Syntaxin 11, TRM = transplant related mortality.

Pt	Protein deficit	Genetics	Age @ dx mts	Treatment	If AC at diagnosis, disease status	Outcome	Follow up in mts from diagnosis
Index 1	MUNC18-2	Compound heterozygous	3	ATG+CSA+steroid+HSCT		Died TRM	5
AC1	MUNC18-2	Compound heterozygous	1	HLH04+HSCT	Activated while on active follow up	Alive CR	22
Index 2	MUNC18-2	474-483delinsGA 1001C>T	12	steroid		Alive CR	48
AC 2	MUNC18-2	474_483delinsGA 1001C>T	24	none	Never activated	Alive CR	72
Index3	MUNC18-2	1247G>C	18	HLH04+Ritux+HSCT		Alive CR	108
AC 3	MUNC18-2	1247G>C	60	none	Never activated	Alive CR	36
Index4	MUNC18-2	unknown	16	HLH04+HSCT		Died TRM	72
AC 4	MUNC18-2	unknown	9	HSCT	Never activated	Alive CR	60
Index5	MUNC18-2	Compound heterozygous	NA	HLH04+HSCT		Alive CR	91
AC 5	MUNC18-2	Compound heterozygous	6	HSCT	Never activated	Alive CR	75
Index6	MUNC18-2	1247G>C	138	Steroids+IVIG		Alive CR	38

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

AC 6	MUNC18-2	1247G>C	108	none	Never activated	Alive CR	42
Index7	LYST	2749-50delAG	24	HLH04+HSCT		Died TRM post graft loss and 2nd HSCT	26
AC 7	LYST	2749-50delAG	102	none	Never activated	Alive CR	12
Index8	PRF1	50delT 853-855delAAG	5	Unspecified chemotherapy		Died HLH progression	1
AC 8	PRF1	50delT 853-855delAAG	5	HLH04+HSCT	Never activated on prophylactic treatment pre SCT	Alive CR	32
Index9	PRF1	445G>A 886T>C	60	steroid		Died from infection	72
AC 9	PRF1	445G>A 886T>C	1	HSCT	Never activated	Alive CR	24
Index10	PRF1	Not available	18	Steroid+CSA+Campath+HSCT		Alive CR	12
AC 10	PRF1	Not available	100	Steroid+CSA+Campath+HSCT	Activate while awaiting SCT	Alive CR	12
Index11	PRF1	254G>T;473C>T;	48	HLH94+HSCT		Alive CR	36

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

		390C>T					
AC 11	PRF1	254G>T;473C>T; 390C>T	9	HSCT	Never activated	Alive CR	38
Index12	PRF1	1122G>A	2	HLH04		Died HLH progression	2
AC 12	PRF1	1122G>A	birth	HLH04	Activated while on prophylactic treatment waiting for SCT	Lost to follow up	
Index13	PRF1	Not available	4	VP16+steroid+CSA+itMTX+ ATG+HSCT		Died HLH progression	8
AC 13	PRF1	Not available	prenatal	Steroid+CSA+ATG+ Campath+HSCT	Activated while on prophylactic treatment waiting for SCT	Died HLH progression	15
Index14	PRF1	Not available	54	Steroid+CSA+Campath+HSC T		Alive CR	18
AC 14	PRF1	Not available	75	Steroid+CSA+Rituximab+HS CT	Activated while on prophylactic treatment waiting for SCT	Alive CR	48
Index15	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
AC15b	PRF1	1376C>T	prenatal	HSCT	Never activated	Alive CR	17
Index16	PRF1	50delT;	8	HLH94+HSCT		Alive CR	144

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

		1034C					
AC 16	PRF1	50delT; 1034C	birth	HLH94+HSCT	Activated while on active follow up	Alive CR	48
Index17	PRF1	50delT; 1130G>A	1	Undefined induction+ HSCT		Alive CR	12
AC 17	PRF1	50delT;1130G>A	birth	HLH04+HSCT	Activated while on active follow up	Alive CR	62
Index18	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
Index19	PRF1	Not available	72	HLH04+HSCT		Alive CR	60
AC 19	PRF1	Not available	3	HLH04+HSCT	Activated while awaiting HSCT	Alive CR	72
Index 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
Index21	MUNC13-4	1389G>A;	1	HLH04+HSCT		Alive CR	36

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

		1620-1621delCA					
AC 21	MUNC13-4	1389G>A; 1620-1621delCA	Pre natal	HSCT	Never activated	Alive CR	60
Index22	MUNC13-4	118-308C>T	16	CSA+VP16 (withdrew)		Died HLH progression	2
AC 22	MUNC13-4	118-308C>T	13	HSCT, lost graft, HLH04+2nd HSCT	Never activated	Alive CR	89
Index23	MUNC13-4	Not available	2	HLH94+ATG+HSCT		Alive CR	144
AC 23	MUNC13-4	Not available	1	None		Alive CR	144
Index 24/a	MUNC13-4	118-308C>T; 1596+1G>C	1	HLH04+HSCT		Alive CR	45
Index 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
Index25	STX11	37+16>A	birth	HLH04		Died HLH progression	1
AC 25	STX11	37+16>A	pre natal	CSA+steroid+HSCT	Never activated on prophylactic treatment	Died from sudden death	17

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

						in CR	
Index26	STX11	Not available	36	HLH04+HSCT		Alive CR	120
AC 26	STX11	Not available	72	HLH94+HSCT	Activated while on active follow up	Died TRM	76
Index27	RAB27	281G>A	18	HLH04+HSCT		Alive CR	25
AC 27	RAB27	281G>	144	None	Never activated	Alive CR	2
Index28	RAB27	467+1G>A	12	HLH94		Lost to FU	NA
AC 28	RAB27	467+1G>A	birth	CSA+HSCT	Never activated on prophylactic treatment	Alive CR	12
Index29	RAB27	220G>C;335delA	9	Dexamethasone+VP16		Died from HLH progression	36
AC 29	RAB27	220G>C;335delA	1	HSCT	Never activated	Alive CR	91
Index30	RAB27	Not available	9	corticosteroids, CSA, IT MTX, HSCT x3		Alive CR	84
AC 30	RAB27	Not available	prenatal	corticosteroids, CSA,HSCT	Activated while waiting for HSCT	Died from HLH progression	18
Index31	RAB27	Not available	126	Campath, corticosteroids, CSA, MTX IT, Natalizumab, HSCT		Died from TRM	30

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

AC 31	RAB27	Not available	180	HSCT	Never activated on prophylactic treatment while waiting for HSCT	Alive in CR	36
Index32	RAB27	Not available	84	CSA+Campath+steroid		Died from HLH progression	12
AC 32	RAB27 RAB27	Not available	84	CSA+HSCT	Never activated on prophylactic treatment while waiting for HSCT	Alive in CR	26

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

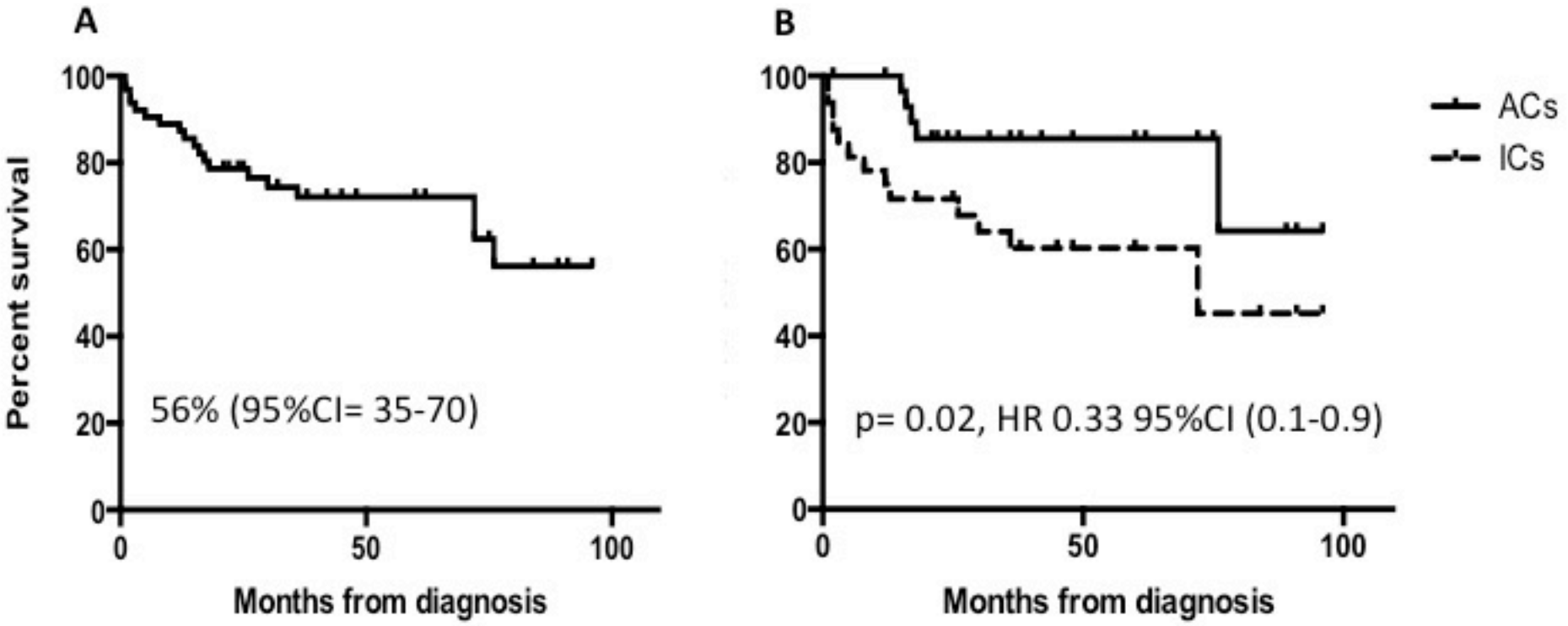
A
100
80
60
40
20
0
Percent survival
56% (95%CI= 35-70)
0
50
100
Months from diagnosis
B
100
80
60
40
20
0
p= 0.02, HR 0.33 95%CI (0.1-0.9)
0
50
100
Months from diagnosis
ACs
ICs

Figure 1

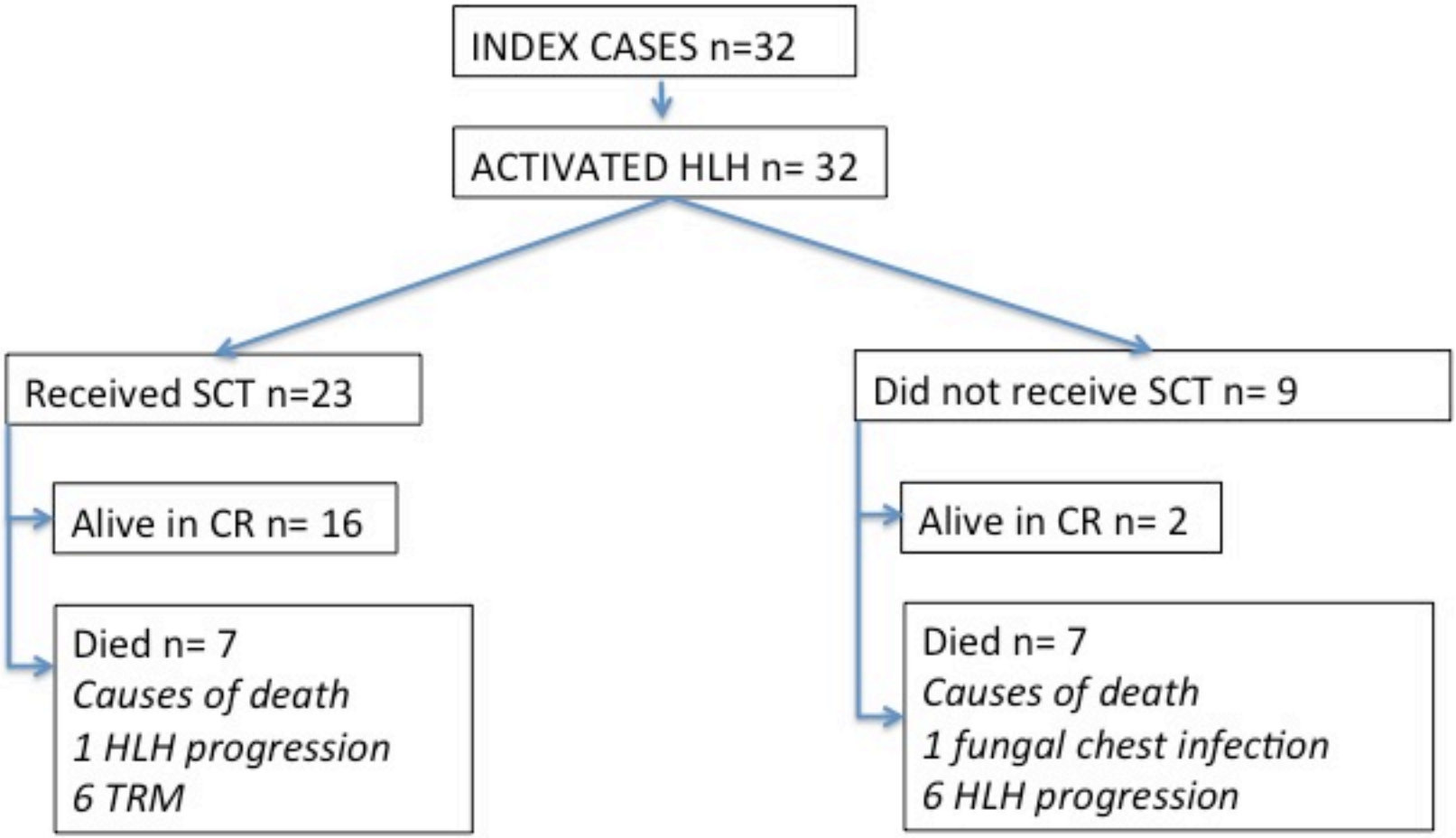
INDEX CASES n=32
ACTIVATED HLH n= 32
Received SCT n=23
Did not receive SCT n= 9
Alive in CR n= 16
Died n= 7
Causes of death
1 HLH progression
6 TRM
Alive in CR n= 2
Died n= 7
Causes of death
1 fungal chest infection
6 HLH progression

Figure 2

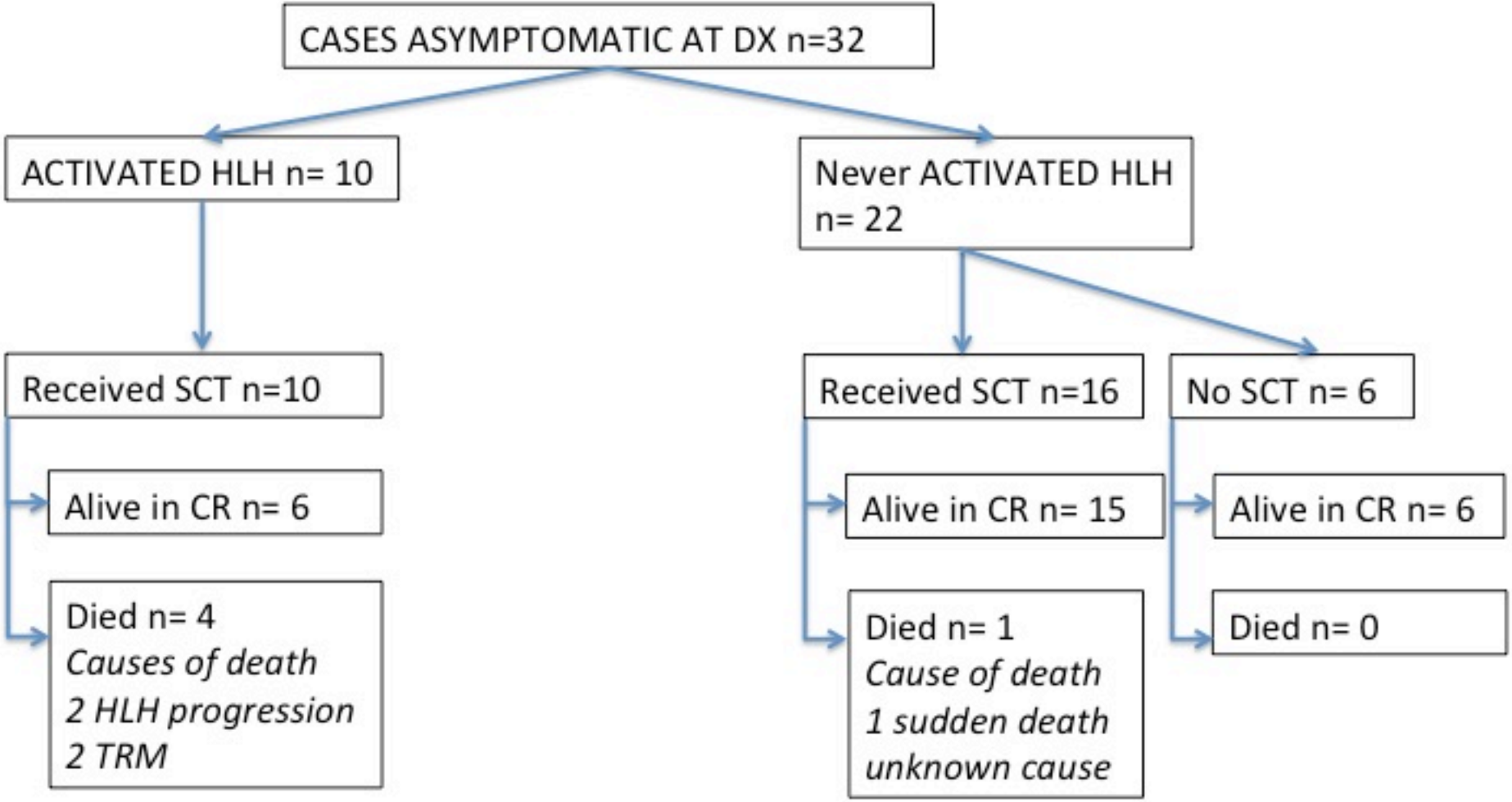
CASES ASYMPTOMATIC AT DX n=32
ACTIVATED HLH n= 10
Never ACTIVATED HLH n= 22
Received SCT n=10
Alive in CR n= 6
Died n= 4
Causes of death
2 HLH progression
2 TRM
Received SCT n=16
No SCT n= 6
Alive in CR n= 15
Alive in CR n= 6
Died n= 1
Cause of death
1 sudden death
unknown cause
Died n= 0

Figure 3

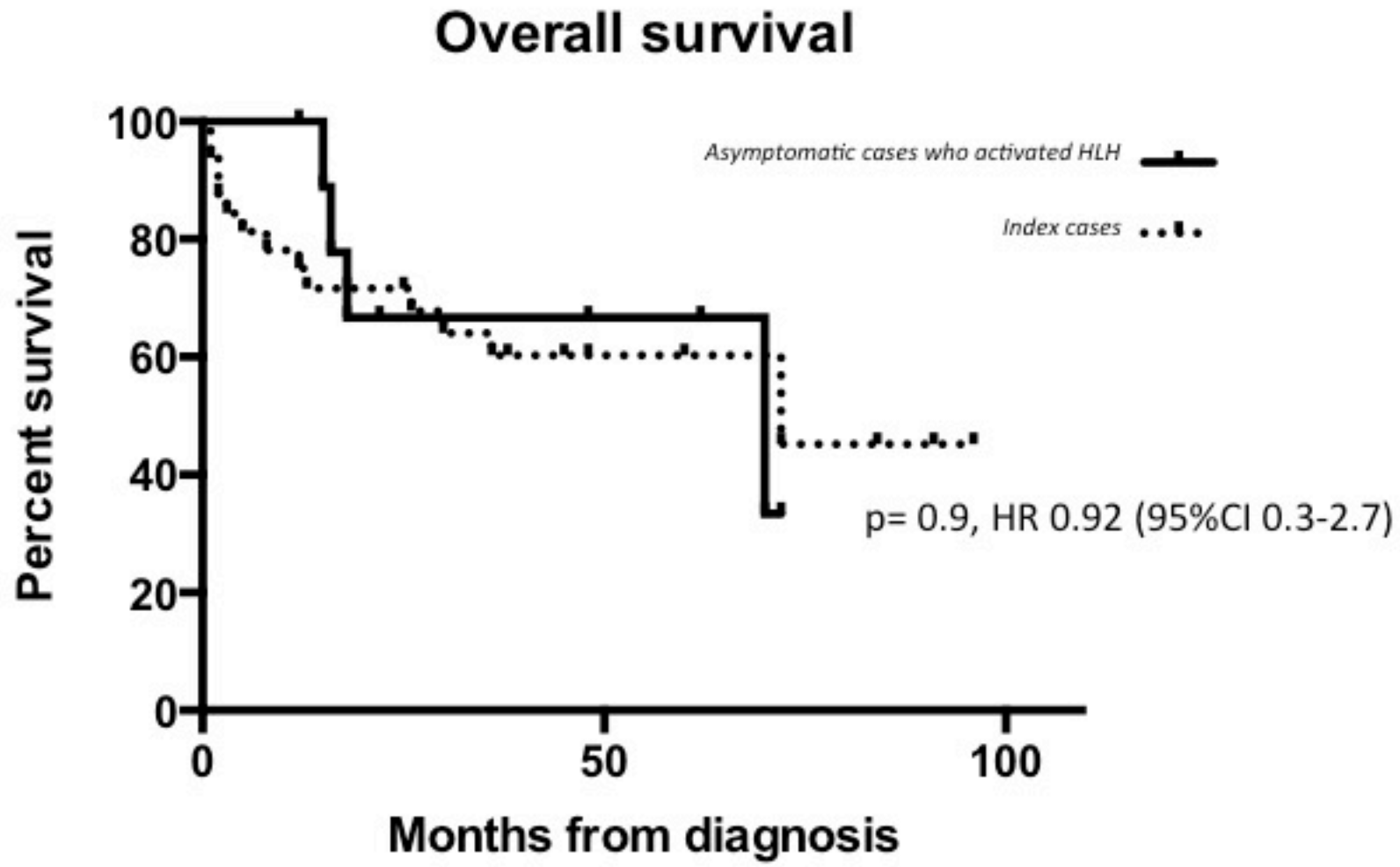
Overall survival
Asymptomatic cases who activated HLH
Index cases
Percent survival
100
80
60
40
20
0
0
50
100
Months from diagnosis
p= 0.9, HR 0.92 (95%CI 0.3-2.7)

Figure 4

From www.bloodjournal.org by guest on August 18, 2018. For personal use only.

Prepublished online August 13, 2018;
doi:10.1182/blood-2018-01-827485

Treatment dilemmas in asymptomatic children with primary haemophagocytic lymphohistiocytosis

Giovanna Lucchini, Rebecca Marsh, Kimberly Gilmour, Austen Worth, Zohreh Nademi, Anupama Rao, Claire Booth, Persis Amrolia, Juliana Silva, Robert Chiesa, Robert Wynn, Kai Lehmberg, Itziar Astigarraga, Tayfun Gungor, Jan Stary, Despina Moshous, Marianne Ifversen, Daniel Zinn, Michael Jordan, Ashish Kumar, Takahiro Yasumi, Paul Veys and Kanchan Rao

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.
Copyright 2011 by The American Society of Hematology; all rights reserved.