Bone Marrow Transplantation (2008) 42, 433–437

www.nature.com/bmt

REVIEW

Hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis: a journey of a thousand miles begins with a single (big) step

MB Jordan and AH Filipovich

Divisions of Immunobiology and Hematology/Oncology/BMT, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH, USA

Hemophagocytic lymphohistiocytosis (HLH) is a rare, highly fatal disorder of uncontrolled inflammation, usually affecting infants. Significant progress in the treatment of this disorder has been achieved during the last decade, and outcomes for larger series of patients have been reported in recent years. Although medical therapy has advanced, hematopoietic cell transplantation remains the only curative therapy for patients with the familial form of this disorder. Unfortunately, these patients have demonstrated relatively poor post-transplant outcomes for a nonmalignant disorder, with approximately 30% mortality in the first 100 days. Early deaths were attributable to infection, GVHD, and unusually high rates of primary nonengraftment, venoocclusive disease and pneumonitis. In addition, a significant number of deaths were due to HLH reactivation, a unique complication seen in this patient group. In contrast, late complications were relatively infrequent and essentially all patients with durable engraftment remained in remission indefinitely. In this review, we will discuss recent progress in the transplant management of patients with HLH and potential future strategies, including the use of reduced intensity conditioning regimens.
Bone Marrow Transplantation (2008) **42,** 433–437; doi:10.1038/bmt.2008.232; published online 4 August 2008
Keywords: hemophagocytic lymphohistiocytosis; transplantation; outcomes

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an unusual immunological disorder first recognized almost 70 years ago.[1] Genetic and animal studies have indicated that the familial form of HLH is clearly due to a deficiency of cytotoxic killing.[2,3] However, unlike other individuals with primary immune deficiencies, who most often suffer from unusual, severe or recurrent infections, patients with HLH present with a potentially fatal syndrome of 'hyperimmunity.' These patients have severe inflammation, associated with cytopenias and variably severe liver or central nervous system damage. Specific diagnostic criteria for HLH have recently been revised by the Histiocyte Society (see Table 1).[4]

Unlike other inborn errors of immune regulation, such as X-linked immunodysregulation, polyendocrinopathy and enteropathy syndrome or autoimmune lymphoproliferative syndrome, these patients do not have overt autoimmunity. Rather, tissue damage and mortality appear to be due to immune hyperactivation. An animal model and correlative human studies suggest that excessive cytokine secretion and abnormal, acute activation of T cells and macrophages drives the pathophysiology of this disorder.[3–6] Notably, HLH can occur as either a primary familial (autosomal recessive) disorder or as a secondary disorder in association with severe infection, malignancy or rheumatologic disorders. In these latter situations, immune hyperactivation is clearly associated with a specific immunologic trigger and findings of cytotoxic dysfunction are variable. In patients with familial HLH, a trigger (usually infectious) may or may not be apparent.[7] Although HLH is a rare disorder in childhood, it is exceedingly rare in adults. Most familial cases present in infancy, although they can occur in older children and even young adults.[8,9] HLH affecting adults is mostly described in case reports consisting of one or two patients. Typically, these patients have secondary HLH associated with viral infection (EBV, HIV, and so on), lymphoma or adult-onset Still's disease. Notably, some of these infection-associated cases of HLH occur in patients who have received either solid organ or BM transplants.

Historically, the survival of patients with familial HLH has been very poor.[10,11] However, in 2002, Henter *et al.*[12] reported the results of the first prospective multicenter therapeutic trial for these patients ($n = 119$). This therapy consisted of combination chemo/immunotherapy to achieve remission, followed by hematopoietic cell transplantation (HCT) to achieve a definitive cure. Overall survival (follow-up >3 years) was reported as 55%, with most fatalities occurring either early after diagnosis or early

Correspondence: Dr MB Jordan or Dr AH Filipovich, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, ML 7038, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA.
E-mails: michael.jordan@cchmc.org or lisa.filipovich@cchmc.org
Received 2 July 2008; accepted 2 July 2008; published online 4 August 2008

after HCT. Mahlaoui *et al.*[13] recently reported similar results with an immunotherapeutic approach (followed by HCT) in a series ($n = 38$) of patients from a single center, treated over a period of 14 years.

HCT as therapy for HLH

For individuals with familial HLH, HCT remains the only long-term curative therapy. Although therapy with chemo and/or immunotherapy is effective in achieving clinical remission of symptoms, HLH will inexorably (and fatally) recur in individuals with intrinsic, severe deficiencies of cytotoxic function.[7] In recent years, HCT treatment results have been reported in two multicenter,[14,15] and two single institution,[16,17] series of patients (Table 2). Although limited, these four series are the largest ones reported to date for this rare disorder. Each of these reports represents a somewhat unique group of patients. Horne *et al.*[14] described patients treated as part of the international HLH-94 protocol, who mostly received grafts from matched unrelated and matched related donors. Baker *et al.*[15] described a retrospective analysis of patients receiving unrelated donor transplants (mostly matched or one locus mismatched) in the United States through the National Marrow Donor Program. Ouachee-Chardin *et al.*[16] described a series of patients treated at a single institution, many of whom received marrow from haploidentical donors. Most patients in these three series received similar conditioning regimens: (standard) BU, cytoxan and etoposide, with or without ATG (antithymocyte globulin). Cooper *et al.*[17] described a small series of patients (with a variety of donor types) from a single institution, who received a campath/fludarabine/melphalan-based reduced intensity conditioning (RIC) regimen. In all these series (except Baker *et al.* who did not specify) at least half of the patients had primary (or familial) HLH, with the remainder suffering from secondary HLH. Unfortunately, outcomes were not reported for these subsets.

The outcomes reported in these series are summarized in Table 2. The most significant pattern to emerge from the three larger (myeloablative) studies is that TRM is quite high for patients with HLH. In these series, more than 30% of patients died within the first 100 days following HCT. The causes of death were multifactorial, including infection, hemorrhage, organ failure and GVHD. As expected, HLA-matched donors were associated with higher survival rates (70% for matched versus 50–54% for mismatched donors in one study[14]). However, it is notable that venoocclusive disease (VOD) and (noninfectious) pneumonitis were a prominent part of TRM in all the series, with all donor types. VOD and pneumonitis contributed to TRM in all four series and accounted for half of all early deaths in one series.[14] VOD was seen in 18%[15] and 28%[16] in the two series that reported the frequency of both fatal and nonfatal VOD. This incidence is surprisingly high and we suspect that it may reflect pre-existing, subclinical (or overt) liver damage due to HLH. In addition, HLH reactivation or persistence was reported to be a cause of death (or least contributory) in all four series. In fact, Ouachee-Chardin *et al.* concluded that HLH disease activity was the primary cause of death in 50% cases, when one considers primary nonengraftment due to active HLH. One can also speculate that HLH disease reactivation may be an unrecognized contributor to mortality, which was attributed to other causes, such as organ failure, hemorrhage, engraftment syndrome or VOD.

Other significant complications of HCT were also reported. Primary nonengraftment occurred 10%,[14] 9%[15] and 22%[16] of the time, in the myeloablative series.

Table 1 Current diagnostic criteria for HLH[a]

The diagnosis of HLH may be established by[b]
1. A molecular diagnosis consistent with HLH
   (for example, pathologic mutations of PRF1, UNC13D or STX11 are identified)

OR

2. Fulfillment of five out of the eight criteria listed below:
   Fever
   Splenomegaly
   Cytopenias (affecting at least two of three lineages in the peripheral blood):
      Hemoglobin <9 g/100 ml (in infants <4 weeks: hemoglobin <10 g/100 ml)
      Platelets $<100 \times 10^3$/ml
      Neutrophils $<1 \times 10^3$/ml
   Hypertriglyceridemia (fasting, $\geqslant 265$ mg/100 ml) and/or hypofibrinogenemia ($\leqslant 150$ mg/100 ml)
   Hemophagocytosis in BM, spleen or lymph nodes
   Low or absent NK cell activity
   Ferritin $\geqslant 500$ ng/ml
   Soluble CD25 (that is, soluble IL-2 receptor) >2400 U/ml (or per local reference laboratory)

Abbreviations: HLH = hemophagocytic lymphohistiocytosis; NK cell = natural killer cell.
[a]Adapted from Henter *et al.*[4]
[b]In addition, in the case of familial HLH, no evidence of malignancy should be apparent.

Table 2 HCT outcomes in patients with HLH

Series	n	*Primary nonengraftment (%)*	*aGVHD (II–IV) (%)*	*OS, day 100 (%)*	*OS, long term*	*Conditioning regimen*
Horne *et al.*[14]	86	10	32	70	64% (3 years)	Bu/Cy/Et, +/− ATG (mostly)
Baker *et al.*[15]	91	9	41	65	53% (5 years)	Bu/Cy/Et, +/− ATG (mostly)
Ouachee-Chardin *et al.*[16]	48	22	17	~70	58.5% (5.8 years)	Bu/Cy/Et, +/− ATG
Cooper *et al.*[17]	12	0	33	NS	75% (2.5 years)	Campath/Flu/Mel (mostly)

Abbreviations: aGVHD = acute GVHD; Et = etoposide; Flu = fludarabine; HCT = hematopoietic cell transplantation; HLH = hemophagocytic lymphohistiocytosis; Mel = melphalan; OS = overall survival; +/− ATG = with or without antithymocyte globulin.

Interestingly, 12 of 12 patients treated with a reduced intensity regimen achieved full donor chimerism.[17] Secondary or late graft rejection occurred in 4%,[14] 0%,[15] and approximately 14%[16] of patients in the three myeloablative series. The high rate of secondary graft rejection reported by Ouachee-Chardin *et al.* may reflect a reliance on haploidentical donors in their series (60% donors were haploidentical). The only series with a RIC regimen noted that mixed chimerism with less than 50% donor engraftment developed in 1 out of 12 patients.[17] Most patients in these reports received CYA and MTX for GVH prophylaxis. The incidence of grades II–IV GVHD was reported as 32%,[14] 41%,[15] 17%[16] and 33%.[17] The incidence of chronic GVHD was reported to be 9%[16] and 25%.[15]

Data from these series also suggest that both HCT complications and outcomes are affected by the HLH disease status. The three larger series found that active disease at the time of transplant was associated with decreased overall survival. Horne *et al.* correlated active disease at the time of transplant with primary graft failure. They also reported that, independent of the clinical status at the time of transplantation, patients whose disease was more refractory to initial medical therapy had decreased overall survival following HCT. Ouachee-Chardin *et al.* also noted that there was a trend toward decreased survival in patients who had HLH involvement of the central nervous system.

Another notable finding in these series was that HLH disease recurrence or persistence was reported in patients, regardless of whether they experienced primary non-engraftment, graft rejection or solid donor engraftment. Horne *et al.* reported that 3% of patients developed HLH disease recurrence despite adequate donor engraftment. Although the other series noted HLH recurrence, they did not clarify the context for all patients (that is, graft rejection versus successful donor engraftment). Most HLH persistence/recurrence-related mortality occurred before day 100, although some cases were observed as late as day 160.[14] In our experience, we have observed HLH disease recurrence (particularly in the central nervous system) as late as day 180 following HCT in some individuals with good donor engraftment. Despite this short-term recurrence risk, however, Ouachee-Chardin *et al.* have noted that stable donor chimerism of more than 10–20% has been associated with CR for up to 20 years in some patients. Cooper *et al.* also noted long-term protection from disease recurrence with donor chimerism limited to the T-cell compartment. The pathophysiologic basis for early HLH recurrence versus long-term protection in the presence of donor engraftment remains to be clarified, but may be related to persistent host chimerism of tissue macrophages and viral reactivation while immune competency is still being established, early after transplantation.

Future directions

The prognosis for patients with HLH has improved remarkably since the early 1980s when the median survival was approximately 1–2 months.[11] However, on the basis of the data described above, it appears that we still have a long way to go to achieve optimal outcomes for these patients. Two lessons are immediately apparent from the worldwide experience of HLH patients undergoing HCT. First, HLH that is not in remission before HCT should be a cause for significant concern. Every reasonable effort must be made to ensure that these patients proceed to HCT with optimal control of their underlying disease process. Systemic and central nervous system manifestations of HLH should be carefully monitored and treated while preparing for HCT. Monitoring of the complete blood count, soluble CD25, ferritin, spinal fluid and organ function should be routinely assessed. It is our impression that serial monitoring of soluble CD25 is one of the most useful clinical markers of disease activity (in the pre-transplant period only). Data from the literature also suggest that serial monitoring of soluble CD163 may be particularly useful.[18] Although HCT should not be unduly delayed, we would suggest that some delays to optimally treat residual active HLH are well justified. Therapy with dexamethasone/etoposide,[4] or prednisone/ATG[13] should be aggressively pursued, if indicated. If patients fail frontline immuno/chemotherapy, salvage therapies should be attempted. The second lesson we should draw from the worldwide HCT experience of patients with HLH is that new HCT approaches are clearly needed. Although some TRM may be due to poor control of HLH before transplant, significant excess mortality is also seen in patients with apparently good control of their underlying disease process.

We can speculate that poor survival in patients with HLH may be due to at least two factors. First, it may reflect occult liver or lung damage from HLH, which may predispose these patients to high rates of VOD or pneumonitis when treated with a BU-based conditioning regimen. Liver involvement is well described in patients with HLH, and aberrant activation of hepatic Kupfer cells (which persist for months post transplant) may contribute to the development of VOD. Although some patients have obvious organ dysfunction at the start of the transplant procedure, the clinical experience described above, as well as our own, suggests that many patients may have more organ damage than is apparent. Perhaps BU should be used in these individuals as cautiously as in those individuals who have previously received gemtuzumab ozogamicin (Mylotarg) or significant doses of hepatic irradiation. Second, traditional myeloablative conditioning regimens may not be adequately immunosuppressive to maintain control of the aberrant host immune system during the transplant process. Although this may sound like an odd claim, it is consistent with the well-documented recurrence of HLH after supposedly 'ablative' conditioning regimens. A small portion of patients in the series described above developed clinically recognizable recurrent HLH in the post-transplant period, whereas a larger portion of patients developed other complications, including primary graft failure, VOD, pneumonitis or engraftment syndrome, which may be attributable (at least in part) to abnormal host immune activation. Graft rejection rates are surprisingly high in the myeloablative series described above: approximately 10%, if one excludes the data from

Ouachee-Chardin *et al*., who relied heavily on haploidentical donors. As most donors in the first two series were well matched, the reasons for this high failure rate are unclear. Because marrow aplasia is a well-described result of uncontrolled HLH, we postulate that persistent HLH disease activity underlies this high complication rate, even when reactivation is not clinically obvious. Overall, it seems likely to us that the excess morbidity and mortality seen in these patients is due to either pre-existing (perhaps subclinical) organ damage and/or abnormal host immune activation (which may not be clinically recognized).

Notably, in their limited series, Cooper *et al*. observed no primary graft failure or VOD. Our own experience at the Cincinnati Children's Hospital mirrors this. With a similar campath/fludarabine/melphalan-based conditioning regimen, we have seen no primary graft failures, VOD, early HLH reactivation or hyperinflammatory engraftment syndrome, and have observed minimal primary GVHD in 15 patients transplanted thus far with HLH. With a median follow-up of greater than 1 year, 14 patients are surviving, which appears to be a substantial improvement over our previous HLH cohort treated with a conventional myeloablative conditioning regimen. Although the basis for this improvement is not understood, we can speculate that it may be due not only to the decreased intensity of the regimen, but also to the superior immunosuppressive effects of fludarabine and campath. Although fludarabine has not been used in other contexts for the treatment of HLH, it is known to be a profoundly immunosuppressive chemotherapeutic drug. Likewise, campath is a uniquely immunosuppressive drug. Although its mechanism of action is similar to that of ATG, there may be important clinical differences. HCT preparative regimens that incorporate it have been reported to be associated with lower rates of GVHD. It is also known to deplete circulating DC and DC precursors (unlike ATG).[19] Finally, in addition to the conditioning regimen employed, GVHD prophylaxis may be a significant variable. Most patients described in the series above received standard MTX and CYA for GVHD prophylaxis. We have used prednisone and CYA for GVHD prophylaxis for our patients. It is possible that the inclusion of post-transplant steroids may have an especially beneficial effect on this patient population.

Although our own unpublished experience and the series by Cooper *et al*. are encouraging, it is not yet clear as to what the optimal HCT approach should be, or whether RIC regimens will achieve superior, durable outcomes. Uncertainty remains because this patient population is unique, our follow-up is still quite short and this approach has not been tested in a multicenter trial. In addition, RIC regimens have their own drawbacks. Slower immune reconstitution may lead to increased difficulties with secondary graft loss and/or viral reactivation/persistence. In this context, waning engraftment can be combated with withdrawal of immune suppression and/or donor lymphocyte infusions, but this approach carries significant risk for the development of GVHD. Donor lymphocyte infusions are also not an option if one uses an umbilical cord blood donor.

To determine the optimal HCT regimen for patients with this rare disorder, multicenter trials will ultimately need to be conducted to prospectively test new approaches, such as RIC regimens. Others and we are actively investigating such collaborative trials. Finally, because patients with HLH represent a unique HCT population, with high morbidity/mortality and disease-specific complications, consideration should also be given to referring these patients to centers with significant experience in caring for them. Although excellent progress has been made, it is clear that there are many more steps in the journey toward an optimal cure for HLH.

References

1 Scott R, Robb-Smith A. Histiocytic medullary reticulosis. *Lancet* 1939; **2**: 194–198.
2 Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA *et al*. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science* 1999; **286**: 1957–1959.
3 Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): $CD8^+$ T cells and interferon gamma are essential for the disorder. *Blood* 2004; **104**: 735–743.
4 Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S *et al*. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007; **48**: 124–131.
5 Billiau AD, Roskams T, Van Damme-Lombaerts R, Matthys P, Wouters C. Macrophage activation syndrome: characteristic findings on liver biopsy illustrating the key role of activated, IFN-gamma-producing lymphocytes and IL-6- and TNF-alpha-producing macrophages. *Blood* 2005; **105**: 1648–1651.
6 Henter JI, Elinder G, Soder O, Hansson M, Andersson B, Andersson U. Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. *Blood* 1991; **78**: 2918–2922.
7 Henter JI, Arico M, Elinder G, Imashuku S, Janka G. Familial hemophagocytic lymphohistiocytosis. Primary hemophagocytic lymphohistiocytosis. *Hematol Oncol Clin North Am* 1998; **12**: 417–433.
8 Clementi R, Emmi L, Maccario R, Liotta F, Moretta L, Danesino C *et al*. Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying PRF1 mutations. *Blood* 2002; **100**: 2266–2267.
9 Santoro A, Cannella S, Bossi G, Gallo F, Trizzino A, Pende D *et al*. Novel Munc13-4 mutations in children and young adult patients with haemophagocytic lymphohistiocytosis. *J Med Genet* 2006; **43**: 953–960.
10 Henter JI, Elinder G, Soder O, Ost A. Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. *Acta Paediatr Scand* 1991; **80**: 428–435.
11 Janka GE. Familial hemophagocytic lymphohistiocytosis. *Eur J Pediatr* 1983; **140**: 221–230.
12 Henter JI, Samuelsson-Horne A, Arico M, Egeler RM, Elinder G, Filipovich AH *et al*. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood* 2002; **100**: 2367–2373.
13 Mahlaoui N, Ouachee-Chardin M, de Saint Basile G, Neven B, Picard C, Blanche S *et al*. Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: a single-center retrospective report of 38 patients. *Pediatrics* 2007; **120**: e622–e628.
14 Horne A, Janka G, Maarten Egeler R, Gadner H, Imashuku S, Ladisch S *et al*. Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis. *Br J Haematol* 2005; **129**: 622–630.

15 Baker KS, Filipovich AH, Gross TG, Grossman WJ, Hale GA, Hayashi RJ *et al*. Unrelated donor hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Bone Marrow Transplant* 2008; **42**: 175–180.
16 Ouachee-Chardin M, Elie C, de Saint Basile G, Le Deist F, Mahlaoui N, Picard C *et al*. Hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis: a single-center report of 48 patients. *Pediatrics* 2006; **117**: e743–e750.
17 Cooper N, Rao K, Gilmour K, Hadad L, Adams S, Cale C *et al*. Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis. *Blood* 2006; **107**: 1233–1236.
18 Schaer DJ, Schleiffenbaum B, Kurrer M, Imhof A, Bachli E, Fehr J *et al*. Soluble hemoglobin–haptoglobin scavenger receptor CD163 as a lineage-specific marker in the reactive hemophagocytic syndrome. *Eur J Haematol* 2005; **74**: 6–10.
19 Klangsinsirikul P, Carter GI, Byrne JL, Hale G, Russell NH. Campath-1G causes rapid depletion of circulating host dendritic cells (DCs) before allogeneic transplantation but does not delay donor DC reconstitution. *Blood* 2002; **99**: 2586–2591.