Post-transplant Hemophagocytic Lymphohistiocytosis in Benign Hematological Disorders: Experience of 4 Cases with Review of Literature

Akanksha Garg¹ · Sandip Shah¹ · Kinnari Patel¹ · Kamlesh Shah¹ ·
Asha Anand¹ · Harsha Panchal¹ · Apurva Patel¹ · Sonia Parikh¹

Received: 21 October 2019 / Accepted: 28 January 2020 / Published online: 24 February 2020
© Indian Society of Hematology and Blood Transfusion 2020

Abstract Post transplant Hemophagocytic lymphohistiocytosis (HLH) is a form of secondary HLH, which can be either early onset or late onset and is associated with significant morbidity and mortality. With the increasing popularity of post transplant cyclophosphamide based haploidentical stem cell transplantation (SCT), post transplant HLH is becoming a significant complication especially in benign hematological disorders. Methods: We present 4 cases of post transplant HLH occurring in 2 cases of severe aplastic anemia (post haploidentical SCT) and 2 cases of thalassemia major (post matched sibling SCT). All 4 cases had early onset variety with dismal prognosis. Conclusion: Post-transplant HLH is an important entity in benign hematological disorders, which needs to be identified early and treated promptly with steroids, monoclonal agents or immunosuppressive therapy. Serum ferritin levels are an important biomarker and help in monitoring response.

Keywords Post transplant HLH · Allogenic stem cell transplant · Haploidentical stem cell transplant · Benign hematological disorders

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a disorder characterized by fever, splenomegaly, cytopenias and hyperferritinemia and occurs due to uncontrolled activation

of cytotoxic T cells causing excessive immune activation [1]. It can be of two forms-primary and secondary HLH. Post transplant HLH is a form of secondary HLH that can be either early-onset (< 30 days post transplant) or late-onset ($\geq$ 30 days post transplant) [2]. The incidence of post-transplant HLH in previous case series has been estimated from 0.3 to 16.8% [3]. It is seen commonly post transplant in both benign and malignant conditions. Here, we report a series of 4 cases of HLH occurring post allogenic stem cell transplantation (SCT) for benign disorders; 2 cases of thalassemia major (following matched sibling SCT) and two cases of aplastic anemia (following haploidentical SCT).

Case 1

A 32-year old male was diagnosed with severe aplastic anemia (SAA) in 2017. There were no matched sibling donors available; hence, he underwent antithymocyte globulin (ATG) therapy. He achieved partial response and was subsequently started on tacrolimus in view of cyclosporine toxicity. He was transfusion dependent, hence was planned for haploidentical SCT from sister. He received fludarabine-cyclophosphamide-ATG (Flu-Cy-ATG) post Cy conditioning regimen. Patient engrafted on Day + 16, and was managed for febrile neutropenia according to unit protocol. Patient was discharged on Day + 19 but he again presented with falling counts and low-grade fever on Day + 24. Serology testing for CMV, parvovirus and EBV were negative. We suspected HLH and hence work up was done. Ferritin levels were 20,557 ng/ml and bone marrow examination was suggestive of extensive hemophagocytosis (Fig. 1a). He was managed with steroids and Intravenous immunoglobulin (IvIg) but clinical condition did

✉ Akanksha Garg
sadge85@gmail.com

¹ Department of Medical Oncology, Gujarat Cancer and Research Institute, Ahmedabad, Gujarat 380016, India

Fig. 1 **a** Bone marrow aspiration ($\times 100$) showing extensive hemophagocytosis. **b** Bone marrow biopsy ($\times 40$) showing hypocellular marrow with increased lymphocytes

not improve. Although ferritin levels decreased after treatment, he had graft rejection on Day + 32 and succumbed to gram-negative sepsis on Day + 56 post transplant.

Case 2

A 12-year old boy was diagnosed with SAA in July 2018, and taken up for upfront haploidentical SCT from brother. Patient received Flu-Cy-ATG-post Cy conditioning regimen. Neutrophil engraftment occurred on Day + 15. However, on Day + 17 TLC fell from 1.5×10^3 to $0.5 \times 10^3/\text{mm}^3$ with recurrence of fever. Bone marrow was suggestive of hypocellular marrow with increased lymphocytes and erythrophagocytosis (Fig. 1b) and ferritin was raised (36,697 ng/ml). Genetic evaluation for HLH was not done due to cost constraints. CMV, EBV and parvovirus serologies were negative. He was treated with IvIg and dexamethasone. Ferritin levels decreased to 7038 ng/ml. By Day + 19, counts started declining and on Day + 30 graft rejection was documented. Presently, he remains stable and transfusion independent.

Case 3

A 2-year old male diagnosed as thalassemia major at age of 6 months. He was taken up for matched sibling SCT from his elder sister in February 2016. He was given Busulfan-Cy-ATG conditioning regimen, and received peripheral blood stem cells. He achieved neutrophil engraftment on

Day + 13 but developed high-grade fever, loose stools along with the fall in counts on Day + 17. A suspicion of hemophagocytosis was raised and ferritin level was 44,000 ng/ml. Steroids were started promptly. Bone marrow could not be done as the patient succumbed to his illness on Day + 19.

Case 4

A 7 year-old female with thalassemia major underwent matched sibling SCT from elder brother. She received Busulfan-Cy-ATG conditioning regimen, and was given peripheral blood stem cells. On D + 2 post transplant she developed high-grade fever with loose stools, which subsided with empirical antibiotics. Leucocyte counts increased to $0.4 \times 10^3/\text{mm}^3$ on Day + 15 but again there was a fall in counts. Ferritin levels were raised to 33,500 ng/ml and bone marrow was suggestive of hemophagocytosis. She received high dose steroids and IvIg along with weekly etoposide (VP-16). Counts did not improve despite treatment and her condition deteriorated and she expired on Day + 41. Details of all cases are summarized in Tables 1 and 2.

Discussion

Hemphagocytic lymphohistiocytosis (HLH) occurs as a rare entity post allogenic SCT. However, now with the increasing number of transplants being done every year, the incidence is expected to rise. The first case of post

Table 1 Characteristics of the patients

S no.	Age/sex	Disease	Donor	CMV serostatus (IgG) (D/R)	Transplant	CD34 dose $\times 10^6$	Engraftment	Onset of HPS	Treatment	GVHD prophylaxis	Outcome
1.	32/M	SAA	Sister	+/+	HaploSCT	3.93	D + 16	D + 24	IvIg, dexa	CSA + MMF	Expired (D + 52)
2.	12/M	SAA	Brother	+/+	HaploSCT	6	D + 15	D + 17	IvIg, dexa	Tacrolimus + MMF	Alive
3.	2/M	TM	Sister	+/+	MSD	7	D + 13	D + 17	Dexa	CSA	Expired (D + 19)
4.	7/F	TM	Brother	+/+	MSD	4.54	D + 15	D + 19	Dexa, IvIg, VP-16	CSA, later changed to MMF	Expired (D + 41)

Table 2 Laboratory parameters

S no.	Ferritin (pre-treatment) (ng/ml)	Serial ferritin (post-treatment) (ng/ml)	Triglycerides (mg/dL)	Fibrinogen (mg/dL)	Chimerism	Bone marrow
1.	20,557	3678 (D + 28) 1354 (D + 35)	254	269	D + 18–94% donor D + 42–0% donor	D + 40-Increased histiocyte, hemophagocytosis
2.	36,697	7301 (D + 22) 3689 (D + 29) 1288 (D + 36)	277	299	D + 30–0% donor	D + 20-Hypocellular, increased histiocytic cells with erythrophagocytosis
3.	> 40,000	NA	284	255	D + 18–89% donor	NA
4.	33,409	21,611 (D + 20) 12,356 (D + 27) 8039 (D + 34)	261	188	D + 16–100% donor D + 28–0% donor	D + 20-Mildly hypercellular marrow, with hemophagocytosis

transplant HLH was described in 1987 [4]. The largest case series on HLH was published by Ishii et al., in which the incidence of post transplant HLH was 0.019% [5]. It can be either early-onset (≤ 30 days post transplant) or late-onset (> 30 days post transplant) [3]. All the above-illustrated cases had an early-onset post transplant HLH presenting within 30 days of transplant. While late onset post transplant HLH is caused by infective etiology, the causes for early onset variety are still obscure. The early onset variety overlaps with hyperacute GVHD, engraftment syndrome [2], sepsis, capillary leak syndrome, and cytokine release syndrome [3]. In a case series by Asano et al., on post-transplant HLH in children, it was shown that early onset HLH was commoner and presence of respiratory symptoms, elevated bilirubin and triglyceride levels were associated with poorer prognosis. The overall survival was 59% in patients with resolution of the HLH as compared to only 14% in those without HLH resolution [2].

The pathogenesis of post-transplant HLH involves hypercytokinemia precipitated by host tissue damage, interaction between host antigen presenting cells and donor lymphocytes or viral infections [2]. Recently, Yoshihara et al. depicted that post transplant HLH is precipitated by myeloid cytokines, T cell and macrophage activation [6]. The criteria for post transplant HLH is different from classical HLH and are defined by the Imashuku criteria comprising of (1) fever, (2) raised ferritin levels, (3) elevated LDH, (4) cytopenias and (5) presence of hemophagocytosis in the bone marrow [7]. Various other criterions have been proposed in the recent times. Sander et al., proposed a criteria for post HSCT HLH that was adapted from HLH-2004 protocol [8] (Table 3). Elkhany et al. suggested some fallacies to the criteria for post transplant HLH. Ferritin, triglyceride, sCD25 levels can be

variable during HSCT as they are influenced by pre transplant status, parenteral nutrition etc., respectively [9]. Early onset variety may not manifest as hemophagocytosis in the bone marrow and splenomegaly is usually not seen during transplant period [10]. Lehmberg et al., defined criteria for malignancy related HLH. More recently, the ‘H score’ has been invented to calculate the percentage probability of secondary HLH [11]. A remarkably high ferritin level most accurately reflects a possibility of post transplant HLH [10]. As per published recommendations, post HSCT patients with fever, serum ferritin $> 10,000$ ng/ml are likely to have hyperinflammation and hence warrant aggressive immunosuppression [12, 13]. Monitoring ferritin levels may help in estimating response to treatment. Soluble IL-2 receptor (sIL2R) is another promising biomarker as it indicates T-cell activation. All our 4 cases had fever, cytopenias, documented hemophagocytosis in bone marrow, hypertriglyceridemia and markedly raised ferritin levels, corroborating well with presence of HLH (Table 2).

Post transplant HLH is commoner in allogenic SCT when compared to autologous SCT [14]. It was first recognized to occur after unrelated cord blood transplants [15]. In a single centre study, the overall incidence of post transplant HLH was 4% and it increased to 8.8% when considering allogenic transplants alone. The authors suggested that post transplant HLH may be a form of GVHD where activation of host macrophages occurs in response to donor stem cells [14]. Case reports also suggest that post transplant HLH is an allogenic response to donor cells seen in the early post HSCT period with no underlying viral etiology [16]. Patients undergoing mismatched or haploidentical SCT are at higher risk of post transplant HLH as well as those receiving reduced intensity conditioning. It is seen more with T cell replete grafts as compared to T-cell

Table 3 Comparison of criteria for classical HLH and post SCT HLH

Criteria for classical HLH	Criteria for post SCT HLH
Fever	<i>Major criteria</i> —Engraftment delay, primary or secondary graft failure Histopathological evidence of haemophagocytosis
Splenomegaly	<i>Minor criteria</i> —Elevated ferritin
Cytopenia affecting > 2 lineages: haemoglobin < 9 g/L, platelets < 100×10^9 /L, neutrophils < 1.0×10^9 /L	Elevated LDH
Hypertriglyceridaemia and/or hypofibrinogenemia: triglycerides > 265 mg/dL, fibrinogen < 150 mg/dL	Fever
Haemophagocytosis in bone marrow, spleen or lymph node	Hepatosplenomegaly
Low or absent NK cell activity	
Ferritin > 500 µg/L	
Soluble cluster of differentiation (CD) 25 i.e. soluble IL-2 receptor > 2400 U/mL	

deplete grafts. Peripheral blood stem cell grafts are more prone to post-transplant HLH than bone marrow graft. The use of G-CSF causes increased content of T cells and monocytes in the graft leading to higher predisposition to HLH [17–19]. SCT for non-malignant conditions is associated with a higher predisposition to post transplant HLH than malignant conditions. The CD34 stem cell dose also correlates with the occurrence of post-transplant HLH. A dose of greater than 9×10^6 cells/kg is associated with higher incidence [20]. In our case series, the two cases with aplastic anemia underwent post transplant cyclophosphamide (PTCy) based T cell replete haploidentical SCT developed hemophagocytosis. All the cases were given PBSC grafts and stem cell dose was variable in the cases. Jaiswal et al. also reported 3 cases of SAA and 1 case of thalassemia major that developed post-transplant HLH after undergoing haploidentical SCT. They reported that younger age, SCT for non-malignant conditions, high CD34 dose as risk factors for hemophagocytosis [20].

Treatment of post-transplant HLH is complex and requires careful diagnosis and prompt management keeping in mind other complications and etiologies in the post transplant period. The usual modalities of therapy are steroids, high dose Ivig infusions, etoposide (VP-16) or salvage transplants for the HLH. The dose for etoposide as part of the immunosuppressive regimen during the transplant is 50 mg/m²/day [21]. HLH occurring secondary to infections is more amenable to treatment as compared to that occurring due to non-infective etiology. In our patients, infectious causes such as CMV, EBV, and Parvovirus infection were tested for and genetic testing was not performed due to cost constraints. Noguchi et al. treated a case of Behcet's syndrome with secondary myelodysplastic syndrome that developed post-transplant HLH following a matched-sibling transplant. They used anti-TNF agent, adalimumab for successfully treating the

HLH in the patient and preventing secondary graft failure. However, this patient succumbed to disseminated aspergillus infection, which was precipitated due to the use of adalimumab [22]. Carter et al. recommended first line treatment with IVIG and steroids. Patients with clinical deterioration can be administered Anakinra (IL-1 blocking antibody). For refractory patients, agents such as cyclosporine and etoposide may be considered [13]. Rituximab is useful in EBV-triggered post transplant HLH. Newer agents such as Epalamumab, an interferon gamma antagonist has been recently approved for treatment of familial HLH and is being investigated for secondary HLH [23].

In our cases, we gave steroids and IvIg for treatment in all 4 patients, and in addition one case received etoposide as well. Monoclonal antibodies could not be administered to any of the patients. Unfortunately, 3 of our patients succumbed and only one recovered but had subsequent graft rejection.

Conclusion

Post transplant HLH is a significant complication for benign hematological disorders and especially in the PTCy setting of haploidentical transplants. Very few such cases in non-malignant conditions have been reported in literature. It should be suspected in all patients with an unexplained, culture-negative febrile illness, ongoing coagulopathy and pancytopenia. Serum ferritin is a cheap and readily available biomarker for identifying patients at risk of post transplant HLH. Other potential causes for HLH such as CMV, EBV and parvovirus should also be tested for before starting steroids and managed promptly. Early onset post transplant HLH variety has a grim prognosis with poor response to treatment. Second-line agents like Anakinra, cyclosporine and etoposide are promising

agents in patients not responding to steroids and IVIG and should be initiated in a timely manner to prevent morbidity and mortality. Clinical studies and better diagnostic criteria are needed for early diagnosis and intensive treatment for this life-threatening entity. Newer modalities such as CAR-T cell and cellular therapy are promising and required in the setting of clinical trials.

Funding Nil.

Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest.

References

1. Brisse E, Wouters CH, Matthys P (2016) Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: differences and similarities. *Br J Haematol* 174:203–217
2. Asano T, Kogawa K, Morimoto A et al (2012) Hemophagocytic lymphohistiocytosis after hematopoietic stem cell transplantation in children: a nationwide survey in Japan. *Pediatr Blood Cancer* 59:110–114
3. Vatsayan A, Pateva I, Cabral L, Dalal J, Abu-Arja R (2018) Post-hematopoietic stem cell transplant hemophagocytic lymphohistiocytosis or an impostor: case report and review of literature. *Pediatr Transplant* 22(4):e13174
4. Sokal E, Michel M, Ninane J, Latinne D, de Bruyere M, Cornu G (1987) Bone marrow transplantation from an unrelated donor for Fanconi's anaemia: two unusual complications. *Bone Marrow Transplant* 2:99–102
5. Ishii E, Ohga S, Imashuku S, Yasukawa M, Tsuda H, Miura I et al (2007) Nationwide survey of hemophagocytic lymphohistiocytosis in Japan. *Int J Hematol* 86:58–65
6. Yoshihara S, Li Y, Xia J, Danzl N, Sykes M, Yang YG (2019) Posttransplant hemophagocytic lymphohistiocytosis driven by myeloid cytokines and vicious cycles of T-cell and macrophage activation in humanized mice. *Front Immunol* 13(10):186
7. Imashuku S (1997) Differential diagnosis of hemophagocytic syndrome: underlying disorders and selection of the most effective treatment. *Int J Hematol* 66:135–151
8. Sandler RD, Carter S, Kaur H, Francis S, Tattersall RS, Snowden JA (2019) Haemophagocytic lymphohistiocytosis (HLH) following allogeneic haematopoietic stem cell transplantation (HSCT)—time to reappraise with modern diagnostic and treatment strategies? *Bone Marrow Transplant*. <https://doi.org/10.1038/s41409-019-0637-7>
9. Elkhanany A, Halawa M, Myers D, Ahmed I (2015) Evaluation of the prognostic factors associated with development of HLH in human stem cell transplant recipients: a comprehensive systematic review. *J Clin Oncol*. https://doi.org/10.1200/jco.2015.33.15_suppl.e18000
10. Lehmborg K, Nichols KE, Henter JI, Girschikofsky M, Greenwood T, Jordan M et al (2015) Consensus recommendations for the diagnosis and management of hemophagocytic lymphohistiocytosis associated with malignancies. *Haematologica* 100:997–1004
11. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D et al (2014) Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthr Rheumatol* 66:2613–2620
12. Allen CE, Yu X, Kozinetz CA, McClain KL (2008) Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 50:1227–1235
13. Carter SJ, Tattersall RS, Ramanan AV (2018) Macrophage activation syndrome in adults: recent advances in pathophysiology, diagnosis and treatment. *Rheumatology* 58:5–17
14. Abdelkefi A, Ben Jamil W, Torjman L, Ladeb S, Ksouri H, Lakhal A et al (2009) Hemophagocytic syndrome after hematopoietic stem cell transplantation: a prospective observational study. *Int J Hematol* 89:368–373
15. Takagi S, Masuoka K, Uchida N, Ishiwata K, Araoka H, Tsuji M et al (2009) High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. *Br J Haematol* 147:543–553
16. Ishikawa J, Maeda T, Miyazaki T, Manabe N, Honda S, Nishiura T et al (2000) Early onset of hemophagocytic syndrome following allogeneic bone marrow transplantation. *Int J Hematol* 72:243–246
17. Norris R, Paessler M, Bunin N (2009) Donor T-cell-mediated pancytopenia after haploidentical hematopoietic stem cell transplant for severe combined immunodeficiency. *J Pediatr Hematol Oncol* 31:148–150
18. Park M, Yun YJ, Woo SI, Lee JW, Chung NG, Cho B (2015) Rotavirus-associated hemophagocytic lymphohistiocytosis (HLH) after hematopoietic stem cell transplantation for familial HLH. *Pediatr Int* 57:E77–E80
19. Kobayashi R, Tanaka J, Hashino S, Ota S, Torimoto Y, Kakinoki Y et al (2014) Etoposide-containing conditioning regimen reduces the occurrence of hemophagocytic lymphohistiocytosis after SCT. *Bone Marrow Transplant* 49:254–257
20. Jaiswal SR, Chakrabarti A, Chatterjee S, Bhargava S, Ray K, Chakrabarti S (2016) Hemophagocytic syndrome following haploidentical peripheral blood stem cell transplantation with post-transplant cyclophosphamide. *Int J Hematol* 103:234–242
21. Minoia F, Davi S, Horne A, Demirkaya E, Bovis F, Li C et al (2014) Clinical features, treatment, and outcome of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: a multinational, multicenter study of 362 patients. *Arthr Rheumatol* 66:3160–3169
22. Noguchi M, Moritake H, Kamimura S, Sonoda M, Ishimura M, Inagaki J (2018) Adalimumab for treatment of hemophagocytic syndrome following unrelated bone marrow transplantation in a boy with Behcet's disease and secondary myelodysplastic syndrome. *Bone Marrow Transplant* 53(9):1214–1217
23. Lounder DT, Bin Q, de Min C, Jordan MB (2019) Treatment of refractory hemophagocytic lymphohistiocytosis with emapalumab despite severe concurrent infections. *Blood Adv* 3:47–50

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.