Hemophagocytic lymphohistiocytosis: a rare complication of autologous stem cell transplantation

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Abstract
Hemophagocytic lymphohistiocytosis (HLH) is a very severe and rare syndrome of pathologic immune activation characterized by cytopenia and clinical signs and symptoms of extreme inflammation. HLH is usually fatal without treatment so that accurate and timely diagnosis is very important. The syndrome occurs as a familial disorder (familial HLH – FLH) or as an acquired condition (secondary – sHLH) in association with a variety of pathological states: infections, rheumatologic, malignant or metabolic diseases. Malignancy associated HLH is primarily reported in T/NK (natural killer)-cell malignancies but also in B-cell neoplasms and other types of cancer. HLH has also been reported in rare cases as a highly fatal and difficult to diagnose complication of stem cell transplantation (SCT). In this paper, we present the case of a young male patient who underwent autologous SCT as consolidation therapy for a T/NK-cell lymphoma, complicated with graft failure due to HLH. The patient was successfully treated with corticosteroids, Etoposide, Cyclosporine and immunoglobulins. As a particularity, he developed a second B-cell neoplasia a few months after SCT. 

Keywords: hemophagocytic lymphohistiocytosis, autologous stem cell transplantation, graft failure.

Introduction
Hemophagocytic lymphohistiocytosis (HLH) is a rare syndrome of pathological immune activation characterized by clinical signs and symptoms of extreme inflammation, occurring either as a familial disorder (familial HLH – FLH) or as a sporadic, acquired condition (secondary – sHLH) in association with a variety of pathological states: infections, rheumatologic, malignant or metabolic diseases [1, 2]. Initially described in children as FLH, in the last decades, it has been increasingly described in adults as well, as sHLH. The delimitation between these two forms is often difficult because of significant clinical and probably genetic common features, making these two forms almost indistinguishable at presentation. HLH is usually fatal without treatment so that accurate and fast diagnosis is of the greatest importance. Unfortunately, the diagnosis of HLH is often delayed, due to a variety of factors, including the rarity of HLH, the complexity of clinical and laboratory manifestation that are also common to underlying illness complexity of diagnostic criteria, and not least, concern about the specificity of current diagnostic criteria [1, 2].

Typical clinical findings include hepatosplenomegaly and prolonged fever that is usually unresponsive to antibiotic therapy. Lymphadenopathy, different kinds of rash, edema and jaundice are less frequent manifestations. Laboratory findings include cytopenias (usually beginning with thrombocytopenia and evolving into severe pancytopenia), hyperferritinemia, elevated transaminases, hypofibrinogenemia, hypertriglyceridemia, hypoalbuminemia and hyponatremia [3]. Additional immunological findings include elevated soluble IL-2 receptor and reduced natural killer (NK) cell cytotoxicity. Many patients with HLH show signs of disseminated intravascular coagulation. The pathology hallmark of HLH is the phenomenon of hemophagocytosis described as the presence in different tissues of activated, histologically benign macrophages engulfing erythrocytes, leukocytes, platelets and their precursor cells. It is important to note that the diagnosis of HLH does not fundamentally depend on this morphological finding, because hemophagocytosis can be absent in the early stages of the disease, although serial bone marrow (BM) aspirations may reveal hemophagocytosis later in the course [4, 5].

In order to facilitate diagnosis, the Histiocyte Society proposed in 2007 a revised set of criteria [6], presented in Table 1.

Beside manifestations listed as diagnosis criteria, other findings like coagulopathy, hyponatremia, edema, rash, hypoalbuminemia, elevated lactate dehydrogenase (LDH), C-reactive protein, and D-dimer, increased very low-density lipoprotein, decreased high-density lipoprotein, elevated cerebrospinal fluid protein and cells, and neurological symptoms (focal deficits, altered mental status) could be present [2, 6–10]. As a diagnosis challenge, all of these features may not be present on initial presentation. It is important to remember that these criteria were initially developed for pediatric FLH, but have been thereafter applied to patients with sHLH, including adults. Currently, there still is not a widely accepted set of criteria for diagnosing HLH in the adult population [2].
Table 1 – Diagnostic guidelines for HLH [6]

| 1°. Molecular diagnosis consistent with HLH | or |
| 2°. Five of eight of the following criteria: |

| 1. Fever |
| 2. Splenomegaly |
| 3. Cytopenias affecting ≥2 lineages: |
| a. Hemoglobin ≤9 g/dL |
| b. Platelets ≤100×10³/µL |
| c. Neutrophils ≤1×10³/µL |
| 4. Hypertriglyceridemia and/or hypo-fibrinogenemia: |
| a. Triglycerides ≥265 mg/dL |
| b. Fibrinogen ≤150 mg/dL |
| 5. Hemophagocytosis in bone marrow, spleen, or lymph nodes |
| 6. Low or absent NK cell activity |
| 7. Ferritin ≥500 mg/L |
| 8. sCD25 (i.e., sIL2R) ≥2400 U/mL |

Malignancy associated HLH has been reported in patients with lymphomas or leukemias of the T- or NK-cell lineages, anaplastic large cell lymphoma, early B-lineage lymphoblastic leukemia, myeloid leukemia, mediastinal germ cell tumors and other solid tumors. In many of these patients, HLH is triggered by bacterial, viral or fungal infection in the context of immune dysfunctions caused by chemotherapy for the malignancy or cytokine production by the tumor cells [1].

HLH occurring after stem cell transplantation (SCT) (SCT-HLH) is a very rare complication and particularly difficult to diagnose. It is characterized by severe clinical manifestations and high mortality [11]. Takagi et al. proposed a specific, separate set of criteria for HLH after SCT [12]. The diagnosis of SCT-HLH requires both major criteria, or one major and all four minor criteria. The major criteria are (1) engraftment failure, delayed engraftment or secondary engraftment failure after SCT, and (2) histopathological evidence of hemophagocytosis. The four minor criteria are high-grade fever, hepatosplenomegaly, elevated ferritin and elevated serum LDH. Despite current therapeutic approaches, outcomes remain poor.

In this paper, we present the case of a young male patient who underwent autologous stem cell transplantation (ASCT) as consolidation therapy for a T/NK-cell lymphoma, complicated with graft failure due to severe HLH.

**Case presentation**

We used the criteria for HLH after SCT that were described by Takagi et al. [12] requiring both major criteria, or one major and all four minor criteria. Our patient fulfilled the two major criteria: engraftment failure, histopathological evidence of hemophagocytosis and one of the minor criteria – elevated ferritin.

Engraftment is defined as the sustained increase of count >0.5×10⁹/µL for at least three days.

Primary graft failure (no engraftment) defines cases in which neutrophils never reach 0.5×10⁹/µL.

Classical causes of graft failure are viral infections: cytomegalovirus (CMV), human herpes virus type 6 (HHV6), parvovirus, drug toxicity and sepsisemia so that an extensive work-up for infections was performed using polymerase chain reaction (PCR) assay for CMV, HHV6, Epstein–Barr virus (EBV) and parvovirus as well as cultures from blood, urine, stool for bacteria and fungi.

We present the case of a 29-year-old male, without known toxic exposure that reports the apparent onset of current disease about five years ago with chronic nasal obstruction, associated during the last six months with mucopurulent, fetid rhinorrhea with bloody streaks and right hemicrania.

At the first presentation in our Hospital, in December 2013, clinical examination as well as usual blood tests was normal. ENT (ear, nose & throat) examination revealed mucopurulent rhinorrhea, bloody ribbed rear wall of the oropharynx, nasal septum deviation with anterior perforation sized about 2/2 cm, with mucopurulent secretions in the middle and lower meatus, ulcerated and hyperemic pituitary mucosa. Rhinosinusual endoscopy was performed with ablation of a cyst in the left maxillary sinus and multiple biopsies at the edges of septum perforation, nasal septum mucosa, cavum.

Pathology showed nasal mucosa fragments with areas covered by squamous epithelium; malignant lymphoid tumor cell proliferation of medium and large size, polymorphic, with fine chromatin, nucleoli hardly visible; angiotropic character of proliferation; large areas of necrosis (Figure 1).

Immunohistochemistry analysis revealed T-cell proliferation, positive for CD3 (Figure 2), which express CD56 (NK-cell marker) (Figure 3) and Granzyme B (activated cytotoxic cell marker) (Figure 4), negative for CD20 (B-cell marker) and TdT (terminal deoxynucleotidyl transferase). Conclusions: extranodal T/NK-cell NHL (non-Hodgkin’s lymphoma) – nasal type.

The patient was referred to the Department of Hematology and staging work-up was performed (computed tomography (CT) scan of head, neck, thorax, abdomen, cerebral magnetic resonance imaging (MRI), bone marrow biopsy) and considered with IA, localized disease. The patient received local radiotherapy during January 2014.

In March 2014, he developed cervical lymph node enlargement (bilateral laterocervical nodes, submandibular lymphadenopathy) and we initiated systemic chemotherapy (March–June 2014) followed by positron emission tomography (PET) negative remission. We decided to consolidate therapeutic response with high dose chemotherapy and autologous stem cell transplantation. In July 2014, he received as mobilization treatment Etoposide 1.6 g/m² and Filgrastim 10 mcg/kg, followed by the harvest of peripheral stem cells (10.34×10⁶ CD34 cells/kg). In October 2014, we performed autologous stem cell transplantation using BEAM conditioning [BCNU (Carmustine), Etoposide, Cytarabine, Melphalan], followed by administration of the entire graft on day 0 (October 15, 2014) and initiation of Filgrastim from day +5.

Initially, the patient had a favorable evolution with Hb (hemoglobin) 7.7 g/dL, WBC (white blood cells) count 0.19×10⁹/µL and platelets (PLT) 33×10³/µL on day +9 (24/10/2014) after a minimum count of 0.02×10³/µL on day +4.

Starting with day +10 (25/10/2014), the WBC count abruptly fell to 0.04×10³/µL. Despite the lack of fever or other symptoms, we suspected CMV reactivation and we initiated treatment with Ganciclovir for eight days, discontinued after confirmation of CMV negative PCR. PCR testing for parvovirus, EBV and HHV6 were negative as well.
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Figure 1 – Nasal mucosa biopsy – malignant polymorphic medium and large-sized cells lymphoid infiltrate with angiotropic character and extensive necrosis (HE staining, ×400).

Figure 2 – Idem – malignant cells expressed CD3 (IHC staining for CD3, ×200).

Figure 3 – Idem – CD56 is positive in malignant cells (IHC staining for CD56, ×200).

Figure 4 – Idem – malignant cells are Granzyme B positive (IHC staining for Granzyme B, ×200).

The bone marrow aspiration performed on day +22, showed hypocellular marrow smears without megakaryocytes, with rare mature and immature neutrophils, extremely rare erythroblasts but relatively frequent small and medium sized plasma cells (approximately 15%) and numerous atypical macrophages (cca. 10%) with intense granulation and erythrophagocytosis (Figure 5, a and b).

Mean time we noted increased levels of ferritin (peak value 9810 ng/mL), triglycerides (max. 831 mg/dL), liver enzymes [max. AST (aspartate aminotransferase) 145 u/L, max. ALT (alanine aminotransferase) 150 u/L], LDH (max. 1295 U/L), C-reactive protein (15.1 mg/dL) and progressive drop of fibrinogen levels (155 mg/dL).

Considering the hemophagocytic syndrome with engraftment failure, we started a donor search in family and Registry in order to perform rescue by allogeneic transplantation, but no donor was identified.

On day +58 (12/12/2014) (Hb 7.9 g/dL, WBC 0.51×10^3/μL, PLT 33×10^3/μL), he received treatment with intravenous Immunoglobulin G (1 g/kg) followed by a temporary hematological response (values of WBC that reached 1.92×10^3/μL on day +62), subsequently decreasing to the values of 0.22×10^3/μL on day +68. On day +68 and respectively +97, he received two more administrations of immunoglobulins leading to stable growth of WBC over 3×10^3/μL, but with severe thrombocytopenia that persisted three more weeks.

On 22/01/2015, the patient was released from the BMT Unit to the Department of Hematology. One month later, he presented transitory lymphocytosis (48% on differential blood count) leading to the suspicion of relapse. Immunophenotyping showed an abnormal population of monoclonal B-cells as well as increased NK/T-cells. Bone
marrow examination reveal a monotypic plasma cells infiltration with kappa light chain restriction (~14–15% of the marrow cellularity) (Figures 7, 8a and 8b), without malignant T/NK-cells infiltration. Blood chemistry showed hyperproteinemia with elevated levels of IgG (4930 mg/dL) with monoclonal pattern on immunofixation.

The PET examination revealed multiple significant lesions: hypercaptant foci located on the left side of the nasal septum with max. SUV (standardized uptake value) 4.8; multiple images of metabolically active lymph nodes situated bilateral retroangulomandibulary (max. SUV 3.5), lateral-cervical bilateral (max. SUV 3.5), latero-tracheal bilateral (max. SUV 3.3), lung hilar bilateral (SUV 5.6 L), infracarinal (max. SUV 6.4), paracaval (max. SUV 4.3), retrocaval (max. SUV 3.9), interaortocaval, lumbo-aortic and right inguinal (lipoidic necrosis).

Figure 5 – (a and b) First BMB after autologous SCT – aplastic bone marrow; macrophages with hemophagocytosis (HE staining, ×400).

Figure 6 – Bone marrow aspiration after autologous SCT – macrophages with hemophagocytosis (May–Grünewald–Giemsa staining, ×1000).

Figure 7 – Second BMB after autologous SCT – mild hypercellular marrow with interstitial plasma cell infiltrate (HE staining, ×400).

Figure 8 – (a and b) Idem – monotypic plasma cell marrow infiltration with kappa light chain restriction. IHC staining: (a) for kappa light chain, ×200; (b) for lambda light chain, ×200.
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The biopsy performed from lateral-cervical lymph node showed partially deleted nodal structure by the presence of parafollicular frequent plasma cell clusters; parafollicular diffuse hyperplasia; rare reactive germinal centers; areas of sinus histiocytosis. Immunohistochemistry (IHC) tests: plasma cells have clonal character, with IgG kappa chain restriction (compared kappa/lambda ~10/1) and are negative for Cyclin D1, suggesting the development of multiple myeloma as a second malignancy (Figures 9, 10a and 10b). Thus, the patient presents another very rare outcome by developing a second neoplasia after aggressive treatment of lymphoma.

The patient is alive at eight months after SCT without clinical or biological signs of HLH and receiving high-dose Dexamethasone and Bortezomib for the plasma cell disorder.

Figure 9 – Lymph node biopsy – clusters of parafollicular plasma cells infiltration (HE staining, ×400).

Figure 10 – (a and b) Idem – monotypic plasma cell lymph node infiltration with kappa light chain restriction. IHC staining: (a) for kappa light chain, ×200; (b) for lambda light chain, ×200.

Discussion

HLH occurring after SCT (SCT-HLH) is a very rare complication and difficult to diagnose. It can complicate the course of both allogeneic and autologous SCT causing graft failure and is associated with high mortality. Onset of HLH could be related to infections, mostly viral, or independently of any infection or any other apparent cause [13–15].

Fukuno et al. (2001) described a female patient with B-cell non-Hodgkin’s lymphoma (NHL) with graft failure due to HLH 12 days after autologous peripheral blood stem cell transplantation (PBSCT). The patient developed a high fever on day 2 post-PBSCT. Her WBC count rose to 0.9×10⁹/L on day 10 post-PBSCT, but then began to decrease. A bone marrow aspirate on day 12 post-SCT revealed an increase in the number of benign histiocytes with hemophagocytosis and the patient was diagnosed with HLH. Although high-dose Methylprednisolone therapy was continued, her WBC count further decreased to 0.3×10⁹/L, and the patient died of multiple organ failure on day 29 post-PBSCT. A CT scan did not identify recurrent NHL, and necropsy specimens from the bone marrow, liver, and kidney revealed no neoplastic infiltration. Bone marrow necropsy showed marked hypocellularity with active histiocyte hemophagocytosis [16].

The group of Abe et al. (2002) reported two cases of HLH associated with allogeneic non-myeloablative SCT for malignant lymphoma who presented HLH diagnosed at day 15 and 56, respectively. Both patients had fever, skin eruption, neurological symptoms and cytopenia and both died in spite of therapy (Methylprednisolone in association with Etoposide and monotherapy with Methylprednisolone, respectively). Both had hemophagocytosis.
in bone marrow, one of them having also activated macrophages in peripheral blood and cerebrospinal fluid [17].

Abdelkefi et al. [15] performed a prospective observational study in order to evaluate the incidence of HLH after hematopoietic HSCT in a single institution over 18 months. They reported six cases of HLH out of 68 patients who received allogeneic SCT (8.8%) (one case of EBV-related HLH, two cases of CMV-related HLH and three cases with no evidence of bacterial, fungal or viral infections) and only one case of CMV-related HLH (1/103; 0.9%) after autologous stem cell transplantation. Four patients died despite aggressive supportive care.

A very interesting study was published by Kobayashi et al. analyzing a single center five-year experience. Out of 554 cases receiving SCT (219 – bone marrow, 232 – peripheral stem cells, 103 – cord blood), 24 were diagnosed with HLH (4.3%). The age of patients ranged from four to 64 years (median, 51 years). The patients who developed HLH after SCT had the following diagnoses: seven patients with non-Hodgkin’s lymphoma, four with acute myelogenous leukemia (AML), four with multiple myeloma, three with myelodysplastic syndrome, two with acute lymphoblastic leukemia (ALL), two with adult T-cell leukemia and one with chronic myelogenous leukemia (CML) and aplastic anemia (AA). The origin of stem cell was bone marrow in 10 patients, peripheral stem cells in eight patients and cord blood transplantation (CBT) in six patients. The median time from the date of transplant to onset of HLH was 21 days (range: 10–267 days). None of the patient had proven viral infection. Most patients (18 cases) developed HLH in the first 30 days after SCT, but there was no impact on outcome related to the moment of HLH diagnosis. Statistical analysis showed that Etoposide-containing conditioning regimens reduce the occurrence of HLH after SCT. Occurrence of HLH had a detrimental effect on survival and outcome on patients [18].

In the experience of the Bone Marrow Transplantation Unit, “Colţea” Hospital, Bucharest, Romania, this is the only case of HLH out of the 22 patients receiving auto-SCT in 18 months since the start of our activity – incidence 4.5%. The HLH in our patient was not related to any viral infection as proven by the negative PCR tests for CMV, EBV, HHV6, and parvovirus. The conditioning regimen was BEAM, containing Etoposide, but with no protective effect in our patient.

As showed in various studies and case reports, HLH has a dismal outcome despite therapy. The treatment for HLH covers a broad range of therapies from corticosteroids, low-dose Etoposide, Cyclosporine A to second allogeneic SCT. Ostromoff et al. [16] reported the successful treatment of a case with HLH after autologous SCT for myeloma with high dose intravenous immunoglobulins [19]. Our patient received initially corticosteroids, Etoposide and Cyclosporine without any sustained improvement. The association of high dose intravenous immunoglobulins led to efficient hematological recovery.

Conclusions

HLH is a rare and severe syndrome of uncontrolled immune activation that has become increasingly recognized over the past decade especially in adult patients in the setting of malignancy, autoimmune disorders and stem cell transplantation. The most important aspect in HLH is represented by the early diagnosis because without fast and prompt treatment the syndrome leads to high mortality. Treatment includes targeting the underlying disorder and controlling the immune dysregulation. Etoposide, Dexamethasone and SCT are the principal treatment methods for the majority of patients with HLH. As shown in our case and also reported in literature, the use of intravenous immunoglobulins is effective in this cases.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Andrei Coliţă and Anca Coliţă share equal contributions as first author.

References

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