CLINICAL VIGNETTE

Late-Onset Hemophagocytic Lymphohistiocytosis Associated with STXBP2 Allele Variant

Juan M. Alcantar, MD, and David Dae-Young Kim, MD

Clinical Case

A 72-year-old female was hospitalized with persistent night sweats and unintended weight loss. Four years prior the patient was diagnosed with multifocal left breast cancer, pathological stage T1c N0 (i+) Mx invasive ductal carcinoma with lobular features, which was estrogen and progesterone receptor positive, HER2 non-amplified with a 21-gene expression assay recurrence score of 22. The patient was managed surgically with a simple mastectomy and sentinel lymph node sampling. At that time the patient was started on adjuvant endocrine therapy with anastrozole, and did not receive adjuvant chemotherapy or radiation therapy. On routine follow up with her medical oncologist prior to admission, she was noted to have new-onset anemia and also right axillary lymphadenopathy on physical exam. PET/CT scan showed hypermetabolic lymph nodes above and below the diaphragm including a conglomerate of lymph nodes in right axilla. The patient had an excisional lymph node biopsy from the right axilla showing reactive immunoblastic hyperplasia with no evidence of metastatic carcinoma or lymphoma.

On current admission the patient was noted to have a white blood cell count of 24.6 thousand per μL, hemoglobin of 8.6 gm/dL, and platelet count of 320 thousand per μL. Mean corpuscular volume was 78 FL and red cell distribution width 21 %. Automated cell count differential showed 91% neutrophils, 4% lymphocytes, and 6% monocytes. Kidney and liver function tests were within normal parameters aside from an albumin level of 1.8 gm/dL. Further biochemical evaluation revealed an elevated ferritin level of 45,590 ng/mL (normal range: 20 – 200 ng/mL). The patient proceeded to have a bone marrow aspiration and core biopsy as part of evaluation for possible hemophagocytic lymphohistiocytosis (HLH). Shortly thereafter, she developed altered mental status with hyperbilirubinemia and acute renal failure requiring hemodialysis support. The patient was empirically started on dexamethasone for a presumptive clinical diagnosis of HLH.

Additional evaluation included MRI of the brain, which did not identify an etiology for her alteration. Diagnostic lumbar puncture was unremarkable and did not show hemophagocytosis in cerebral spinal fluid.

Bone marrow biopsy results confirmed hemophagocytosis with no evidence of malignant cells and negative stains for AFB, GMS, and EBV-EBER. Serologic testing for CMV, hepatitis B and C, as well as HIV were negative. Soluble CD25 (Interleukin-2 Receptor) level was 15,840 pg/mL (reference range: < or = to 1033 pg/mL). D-dimer was 30,751 ng/mL, fibrinogen 252 mg/dL, and triglycerides 118 mg/dL. Patient had a comprehensive evaluation by infectious disease and rheumatology consultants, and a causative etiology for HLH could not be identified. She was started on induction therapy with weekly etoposide along with dexamethasone as per HLH-94 protocol. She received both anti-fungal and Pneumocystis jiroveci pneumonia prophylaxis. The patient had rapid improvement of clinical symptoms with subsequent normalization of kidney function and hyperbilirubinemia. She also had marked reduction in her ferritin level down to 3710 ng/mL.

A subsequent chest X-ray revealed an 8 mm nodular density in right mid lung. Further evaluation with a non-contrast CT of the chest revealed multiple bilateral lung nodules with the largest nodule in left lower lobe measuring up to 2 cm. The patient had a CT-guided biopsy of the left lower lobe nodule showing necrotic tissue and vessel wall with invasive fungal hyphae. The patient was started on voriconazole but developed respiratory insufficiency requiring continuous oxygen supplementation. Repeat CT of the chest showed increased size of the lung nodules with development of cavitary changes. At this point treatment with amphotericin B was instituted but there was progressive clinical deterioration and the patient was enrolled on a hospice program. Approximately six months later she returned to re-establish her medical care after dis-enrolling from hospice. She was fully ambulatory and semi-independent

in her activities of daily living without requiring oxygen supplementation. Biochemical evaluation revealed normal blood counts as well as normal kidney and liver function. In addition, ferritin level was down to 1796 ng/mL and soluble CD25 level was normal at 543 U/mL. She had Next Generation Sequencing testing which revealed the patient to be heterozygous for a point mutation in the HLH-associated gene STXBP2 (c.914A>G, p.Glu305Gly).

Discussion

Hemophagocytic lymphohistiocytosis (HLH) is a rare and often unrecognized fatal disease. It is usually associated with non-specific symptoms commonly seen in patients with systemic inflammatory response syndrome (SIRS). In essence, HLH is a syndrome of abnormal and exuberant immune activation resulting in an excessive and pathogenic inflammatory milieu. The disorder is characterized by defective signaling pathways of cytotoxic cells as well as unrestrained macrophage activity, resulting in hypersecretion of cytokines that mediate underlying inflammatory response.

Previously HLH was categorized as either primary or secondary. However with the understanding that genetic mutations can occur at any age, HLH is now broadly categorized as either genetic or acquired. In general, it is believed that all forms of HLH are due to functional impairment of cytotoxic T lymphocytes (CTLs) and NK cells.[1] The pathophysiology of genetic HLH is best understood in the context of an immunologic synapse between a cytotoxic lymphocyte (CTL or NK cell) and a target cell. In an otherwise healthy individual, antigens elicit an inflammatory response mediated by T helper 1 (Th1) lymphocyte cytokines including interferon-γ (IFN-γ), tumor necrosis factor α (TNF-α), and granulocyte-monocyte colony stimulating factor (GM-CSF). These cytokines lead to activation of macrophages, NK cells, and CTLs. Activated NK cells release perforin and granzyme from intracellular granules, as part of their normal cytotoxic function. Perforin inserts itself in the membrane of a target cell, leading to osmotic lysis and apoptosis. Typically under normal physiologic conditions, removal of antigenic stimulus terminates the inflammatory response. Inadequate levels of perforin or impaired release of granules, as seen in patients with HLH, can lead to loss of normal cytotoxic function of NK cells and CTLs. This leads to an inability to clear antigenic stimulus and hence turn off the inflammatory response. The end result is persistent overproduction and secretion of cytokines, resulting in a "cytokine storm", which is the pathophysiologic hallmark of HLH.

Acquired HLH can occur in both immunocompetent as well as in immunocompromised individuals. Most commonly it is associated with different types of infections, rheumatologic diseases, and malignancies. On the other hand, genetic HLH is most commonly associated with discrete genetic abnormalities. Genetic HLH itself is broadly categorized into two subgroups: familial HLH (FHL) and HLH associated with a primary immunodeficiency syndrome. The perforin gene mutation was the first genetic defect to be associated with HLH.[1] FHL subtype 2 is due to mutations involving perforin gene 1 (PRF1), and this accounts for 20% to 50% of familial cases of HLH. Other gene mutations in the perforin pathway account for the other subtypes of familial HLH : UNC13D (FHL subtype 3), STX11 (FHL subtype 4), and STXBP2 or UNC18B (FHL subtype 5). Familial HLH is usually diagnosed in the pediatric population. However, late-onset cases of familial HLH have now been described in adults as old as the sixth decade of life. Some types of primary immunodeficiency syndromes are associated with a predisposition to development of HLH. The underlying mechanism is thought to be also due to ineffective antigen clearance by genetically impaired and defective NK and T cells.

The diagnosis of HLH is currently based on consensus criteria from HLH-2004, an updated treatment protocol to the original HLH-94, developed by the Histiocyte Society.[2] The original diagnostic guideline included 5 criteria: (1) fever, (2) splenomegaly, (3) peripheral blood cytopenias affecting at least two cell lineages, (4) hypertriglyceridemia and/or hypofibrinogenemia, and (5) hemophagocytosis in the bone marrow, spleen, or lymph nodes. Three new criteria were added: (6) low or absent NK cell activity, (7) hyperferritinemia, and (8) high soluble IL-2 receptor (CD25) levels. To make the diagnosis of HLH, 5 of these 8 criteria need to be met. Patients with a genetic defect consistent with HLH do not have to meet these criteria for the diagnosis. It is important to note that HLH-2004 diagnostic criteria were developed in the pediatric population and, therefore, are not universally accepted for adults.[3]

The current treatment of HLH is based on protocol HLH-94.[4] The HLH-94 protocol consists of an initial 8-week course of therapy with etoposide and dexamethasone with the goal of achieving a clinical remission. This initial phase of therapy can be followed by continuation therapy with additional doses of etoposide and dexamethasone with the option of an allogeneic hematopoietic cell transplant for selected cases. The HLH-94 treatment protocol yielded a 3-year overall survival of 55%. The 3-year survival after a hematopoietic cell transplant was 62%. These outcomes were considered successful given that HLH was invariably fatal without treatment. The pro-apoptotic effect of etoposide is thought to be the reason for its benefit in HLH, where there is a defect in inducing apoptosis. Dexamethasone is well known for its anti-inflammatory effect. Moreover, in an effort to improve on success of HLH-94 protocol, a revised protocol was developed for the treatment of HLH. In the HLH-2004 protocol, cyclosporine was introduced earlier in the initial phase rather than in the continuation phase.[2] As of today, the HLH-2004 protocol is most commonly used within the research setting while awaiting definitive results.

Improved understanding of the pathophysiology of HLH has allowed for development of novel targeted therapies, which are currently being investigated in clinical trials. NI-0501 is a fully human anti-IFN-γ monoclonal antibody which was granted breakthrough therapy designation by the Food and Drug

Administration for patients with primary HLH with refractory disease, or those with recurrent or progressive disease during conventional therapy.[5] An open-label phase 2 trial, presented at the annual American Society of Hematology meeting in 2015, showed significant clinical efficacy and tolerable toxicity in thirteen children treated with NI-0501.[6] In this clinical trial, NI-0501 markedly improved parameters of HLH activity. In addition, nine patients showed clinically significant responses, and seven children were able to proceed to allogeneic stem cell transplantation.

Conclusion

As illustrated by our case, HLH is an insidious hematologic disorder that can result in significant morbidity and mortality. Clinicians must maintain a high index of suspicion when patients present with symptoms that appear to be consistent with otherwise non-specific systemic inflammatory response syndrome. Early recognition of biochemical abnormalities and involvement of a hematology consultant can expedite diagnostic workup and lead to rapid institution of therapy. In general, patients can experience a rapid improvement in clinical and biochemical parameters with prompt HLH-directed therapy. Newer investigational treatment options are in development, which may improve the adverse outcomes of this otherwise fatal disease. Although familial HLH is typically diagnosed at an early age, our patient is an example of late-onset HLH associated with an STXBP2 gene mutation and no apparent secondary HLH causes.

REFERENCES

1. **Rosado FG, Kim AS.** Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis. *Am J Clin Pathol*. 2013 Jun;139(6):713-27. doi:10.1309/AJCP4ZDKJ4ICOUAT. Review. PubMed PMID: 23690113.
2. **Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G.** HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007 Feb;48(2):124-31. PubMed PMID: 16937360.
3. **Schram AM, Berliner N.** How I treat hemophagocytic lymphohistiocytosis in the adult patient. *Blood*. 2015 May 7;125(19):2908-14. doi:10.1182/blood-2015-01-551622. Epub 2015 Mar 10. Review. PubMed PMID: 25758828.
4. **Henter JI, Samuelsson-Horne A, Aricò M, Egeler RM, Elinder G, Filipovich AH, Gadner H, Imashuku S, Komp D, Ladisch S, Webb D, Janka G; Histocyte Society.** Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood*. 2002 Oct 1;100(7):2367-73. PubMed PMID:12239144.
5. Nonimmune's NI-0501 Granted Breakthrough Therapy Designation by US FDA for Treatment of Patients with Primary Hemophagocytic Lymphohistiocytosis (HLH). http://novimmune.com/news/pr160316.html
6. **Jordan M et al.** A novel targeted approach to the treatment of hemophagocytic lymphohistiocytosis (HLH) with anti-interferon gamma (IFN-γ) monoclonal antibody (mAb), NI-0501: first results from a pilot phase 2 study in children with primary HLH. Blood 2015 126: LBA 3.

Submitted October 16, 2017