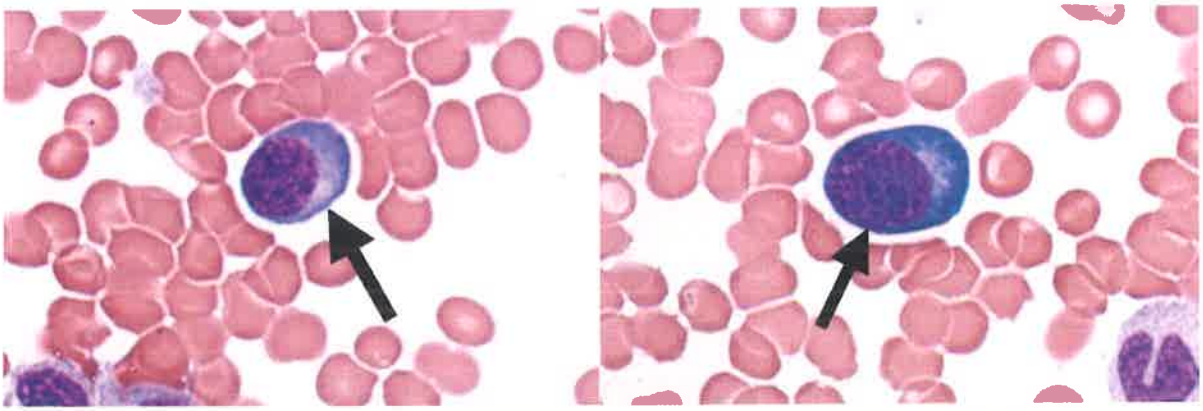
Committee Comments on the Bone Marrow Differential and Aspirate

This case is an example of a bone marrow exhibiting hemophagocytosis in a patient with hemophagocytic lymphohistiocytosis. There are several large macrophages containing phagocytized material including partially degenerated granulocytes, platelets and erythroid precursors. Phagocytic vacuoles often displace and indent the macrophage nucleus. Trilineage hematopoiesis is present, although erythropoiesis is relatively decreased.

Cell Identification

BMD-09

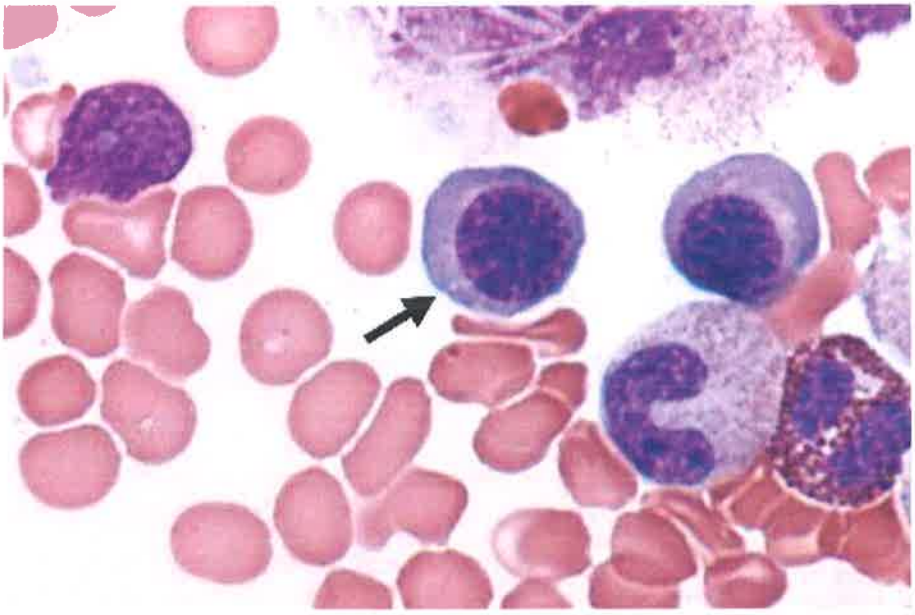


Identification	Participants No.	%	Evaluation
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	268	87.9	Educational
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	20	6.6	Educational
Lymphocyte	10	3.3	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	3	1.0	Educational
Basket cell/smudge cell	1	0.3	Educational
Erythrocyte precursor with megaloblastic changes/maturation	1	0.3	Educational
Lymphocyte, large granular	1	0.3	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.3	Educational

The arrowed objects are plasma cells, as correctly identified by 87.9% of participants. Plasma cells represent terminally differentiated B-cells, and function to produce immunoglobulins. While rare in the blood, they are present in the bone marrow in small numbers (usually less than 5%), although greater percentages can be seen in both reactive and neoplastic conditions. The cells are 10 - 20 µm in diameter, and are often oval in shape, in part due to the eccentric localization of the nucleus. Nuclei are generally round with coarsely condensed chromatin, sometimes described as having a clock-face pattern. The cytoplasm in usually bluish, and sometimes deeply basophilic. Plasma cells often have a prominent perinuclear clearing or hof. This represents an enlarged Golgi apparatus, consistent with a cell in which the main function is to produce proteins.

6.6% of participants incorrectly identified the arrowed objects as normal erythroid precursors. Description of normal erythroid precursors can be found under the BMD-10 image discussion. In these images the significant eccentricity of the nuclear placement, with one edge of the nucleus essentially present at the edge of the cell, and adjacent directional hof, rather than a perinuclear halo, distinguish these cells from erythroid precursors.

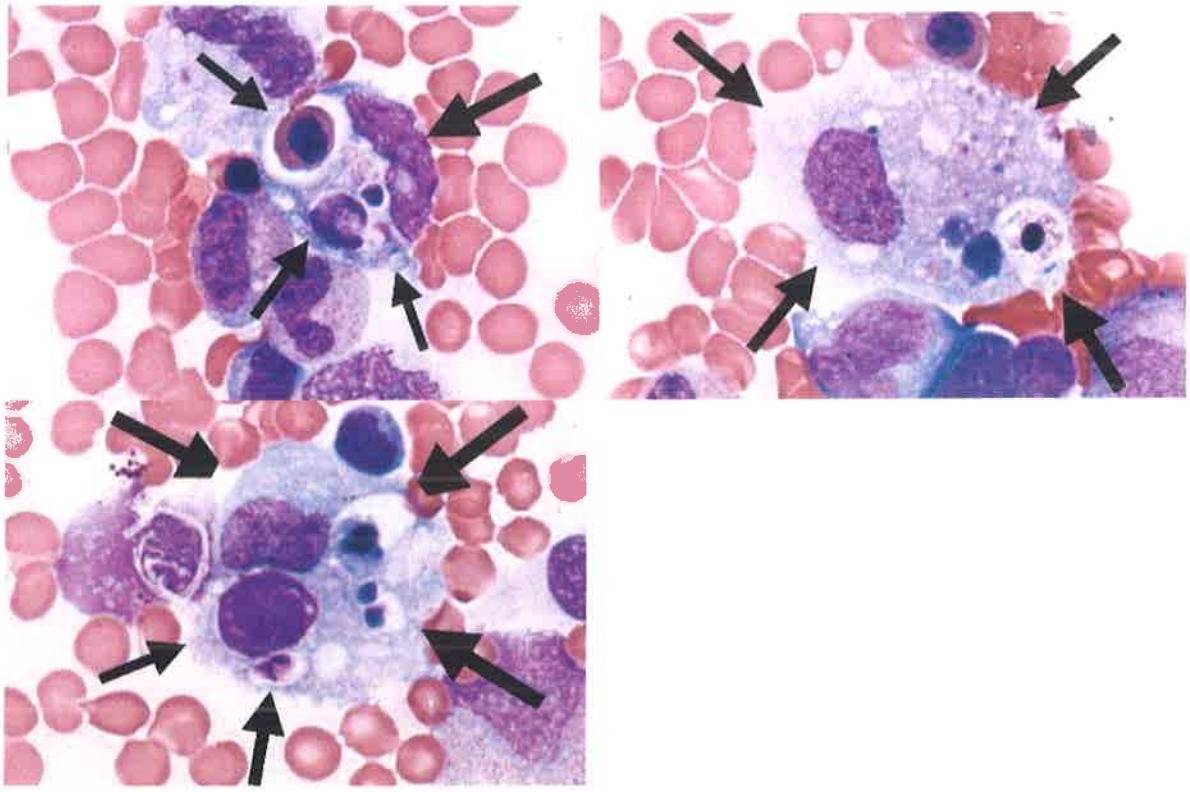
BMD-10



Identification	Participants No.	%	Evaluation
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	295	96.7	Educational
Erythrocyte precursor with megaloblastic changes/maturation	5	1.6	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	3	1.0	Educational
Basophil, any stage	1	0.3	Educational
Erythrocyte	1	0.3	Educational

The arrowed object is an erythroid precursor (normal), as correctly identified by 96.7% of participants. Erytrhoid precursors characteristically have very round nuclei. The chromatin characteristics vary from being fine and lacy in pronormoblasts, the earliest and largest cells, to being coarsely condensed with a checkerboard appearance. As the cells mature they decrease in size, from 17 - 24 µm in diameter for pronormoblasts to 10 to 15 µm in diameter for polychromatophilic normoblasts, and the cytoplasm changes from deeply basophilic to pink-grey as hemoglobin is produced and RNA decreases. A perinuclear halo is usually present, but should be circumferential, not be confused with a perinuclear hof. In addition the cytoplasm in these cells are more reddish than would be seen in a plasma cell.

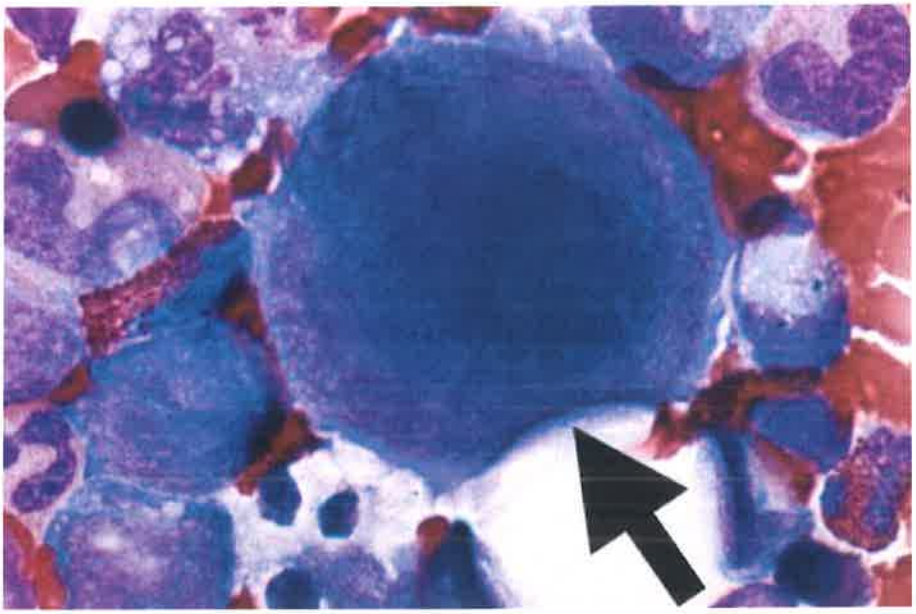
BMD-11



Identification	Participants No.	Participants %	Evaluation
Macrophage containing cell (hemophagocytosis)	298	97.7	Educational
Macrophage containing hemosiderin (siderophage)	3	1.0	Educational
Neutrophil/macrophage containing fungi, *Leishmania*, or *Toxoplasma*	2	0.7	Educational
Histiocyte, sea blue	1	0.3	Educational
Macrophage (histiocyte)	1	0.3	Educational

The arrowed objects are macrophages containing cells (hemophagocytosis), as correctly identified by 97.7% of participants. Macrophages are large (15 to 80 µm in diameter) phagocytic cells present in tissues. The cytoplasm is pale gray-blue and often has frayed edges or pseudopodia. Coarse azurophilic granules may be present. The nuclei are round or oval but may be indented, with reticular chromatin and occasionally small nucleoli. Macrophages can phagocytize cells including leukocytes, erythrocytes, platelets and nuclear remnants. These may be present and partially degenerated within vacuoles, and vacant vacuoles may also be present. In some of these examples the macrophage nuclei has been displaced and indented by the presence of vacuoles. Granulocytes, platelets, and erythroid precursors are present in various stages of degeneration.

BMD-12

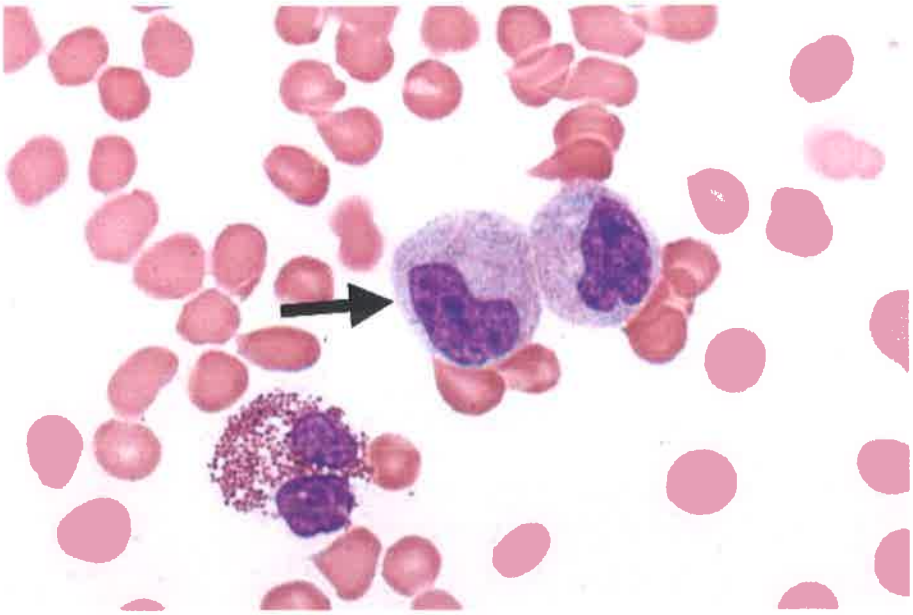


Identification	Participants No.	%	Evaluation
Megakaryocyte or precursor, normal	243	79.7	Educational
Megakaryocyte or precursor, abnormal	51	16.7	Educational
Megakaryocyte nucleus	7	2.3	Educational
Erythrocyte precursor with parvovirus infection	2	0.7	Educational
Malignant lymphoid cell (other than blast)	1	0.3	Educational
Metastatic tumor cell or tumor cell clump	1	0.3	Educational

The arrowed object is a megakaryocyte, as correctly identified by 79.7% of participants. Megakaryocytes are the largest cells in the bone marrow, at least 25 to 50 µm in diameter, and have a characteristic multilobulated nucleus formed during rounds of nuclear replication without cell division (endomitosis). The nuclear lobes are connected by bands or threads of chromatin, and the chromatin is coarsely condensed. Megakaryocytes have pinkish cytoplasm with fine azurophilic granules as seen in platelets. Platelets are produced when bits of megakaryocyte cytoplasm are pinched off from the cell.

16.7% of participants incorrected identified the arrowed object as an abnormal megakaryocyte. In a bone marrow aspirate smear, abnormal megakaryocytes can be identified by abnormalities in size or nuclear shape. The megakaryocyte in this image is approximately 3 times the diameter of an adjacent metamyelocyte (which should be 10 - 18 µm in diameter), indicating it is of normal size. The nucleus is somewhat dark but can clearly be seen to be multilobated with normal connections between the lobes. Particularly in the lobe closest to the surface of the cell, typical chromatin distribution can be seen, without pyknosis, and a normal amount of cytoplasm is present.

BMD-13



Identification	Participants No.	%	Evaluation
Neutrophil, metamyelocyte	283	92.8	Educational
Neutrophil, segmented or band	12	3.9	Educational
Monocyte	4	1.3	Educational
Neutrophil, myelocyte	3	1.0	Educational
Metastatic tumor cell or tumor cell clump	1	0.3	Educational
Neutrophil, giant band or giant metamyelocyte	1	0.3	Educational
Neutrophil with hypersegmented nucleus	1	0.3	Educational

The arrowed object is a metamyelocyte, as correctly identified by 92.8% of participants. Metamyelocytes are granulocytic precursors, and are the first of the lineage to be post-mitotic. They are seen in the bone marrow and occasionally in the blood in response to stress or in pathologic states. The cells are 10 to 18 µm in diameter and round to oval. The nuclei are indented to less than half the maximal nuclear diameter, with a nuclear-to-cytoplasmic ratio of 1.5:1 to 1:1. The cytoplasm has both specific (bluish) granules, as well as few primary (azurophilic) granules.

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BMD-B 2018: HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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None

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Identify the clinical features and diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH).
2. Describe the underlying causes of HLH.
3. Understand the importance of diagnosis and classification of HLH for clinical outcomes.

BMD-B 2018: HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Case Presentation:

This bone marrow aspirate smear is from a 14-year-old girl with a 2 week history of sore throat, fever, rash, generalized lymph node enlargement, worsening abdominal distension, and pain. Laboratory data includes: WBC = 16.0 x 10E9/L; RBC = 2.89 x 10E12/L; HGB = 8.4 g/dL; HCT = 25.5%; MCV = 88 fL; and PLT = 22 x 10E9/L.

(BONE MARROW, WRIGHT-GIEMSA)

EPIDEMIOLOGY AND CLINICAL PRESENTATIONS

Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome, is a rare disease caused by dysregulated immune activation which is almost universally fatal if untreated. Even with current treatment regimens mortality is up to 40%. Patients usually present severely ill, most often with fever, hepatosplenomegaly and cytopenias. The disease may also manifest with coagulopathy, hypertriglyceridemia, hepatic dysfunction, hyperferritinemia, and neurologic symptoms.

There are two forms of HLH, primary and secondary. Primary HLH is an autosomal recessively inherited disorder caused by mutations in one of several genes, including *PRF1*, *UNC13D*, *STXBP2*, and *STX11*. Patients with primary immune disorders such as X-linked lymphoproliferative disorder, Chédiak–Higashi syndrome, and Griscelli syndrome may also develop HLH. Most of these mutations affect perforin-mediated cytotoxicity. The disease is rare, with an estimated incidence of 1:50,000 to 1:100,000 children. Patients usually present in early infancy or childhood, and the first episode may be triggered after infection.

Secondary HLH (sHLH) is caused by an inappropriate response to immune stimulation, such as severe infection, autoimmune disease, or malignancy. sHLH is seen in all age groups, without preference for gender. Heterozygous mutations in some genes associated with primary HLH have been found in patients with sHLH, suggesting there may be a component of genetic predisposition in some patients.

When sHLH occurs in the setting of rheumatologic disease (juvenile idiopathic arthritis or adult-onset Still disease) it is known as macrophage activation syndrome (MAS). Hematologic malignancies are more likely to be associated with sHLH than solid organ tumors, with reports of NK/T-cell malignancies and rare forms of B-cell lymphoma having the highest association. Epstein-Barr virus (EBV) is the most common infectious agent associated with sHLH, although many different viral, bacterial, fungal, and protozoan pathogens have been implicated. Malignancy-associated sHLH is more common in adults than children, and may occur in up to 1% of patients with malignancy. In many cases of malignancy-associated HLH there is concurrent infection, most commonly with EBV.

PATHOPHYSIOLOGY AND DIAGNOSIS

HLH is a manifestation of an inappropriate immune response including dysfunction in NK/T-cell activity and hypercytokinemia resulting in activation of macrophages and cytotoxic T-cells, downregulation of regulatory T-cells, and ultimately organ damage. The cytokine storm includes elevated levels of IFN-γ, IL-1β, IL-6, IL-12, IL-18, and TNF. These elevated cytokines illicit some of the classic features of HLH, such as fever and cytopenias, and higher cytokine levels may be associated with worse outcomes.

HLH can be difficult to diagnose as no single sign, symptom, or laboratory value is in itself diagnostic. The namesake hemophagocytic macrophages can be seen in bone marrow, spleen, liver, enlarged lymph nodes, and cerebrospinal fluid, however this finding is neither specific nor entirely sensitive for the disease. Hemophagocytosis can also be seen in other inflammatory conditions including infection (sepsis) and systemic inflammatory response syndrome. Some authors have even proposed removing detection of hemophagocytic cells from diagnostic criteria, especially since this requires invasive sampling. However, given that hematologic malignancy should be ruled out in most cases, a bone marrow biopsy is usually prudent.

In 2004 the Histiocyte Society conducted a clinical trial for HLH therapy and revised its HLH-94 guidelines for diagnosis, adding additional criteria to help recognize atypical presentations, including low/absent NK cell activity, hyperferritinemia, and elevated soluble IL-2 receptor. These criteria are summarized in Table 1: Diagnosis requires either number 1 or 5 of the 8 criteria in number 2.

Table 1. HLH-2004 diagnostic criteria for hemophagocytic lymphohistiocytosis

1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria below)
    - Fever
    - Splenomegaly
    - Cytopenias affecting 2 or 3 lineages
        - Hemoglobin < 90g/L (< 100 g/L in infants younger than 4 weeks)
        - Platelets < 100 x 109/L
        - Neutrophils < 1.0 x 109/L
    - Hypertriglyceridemia and/or hypofibrinogenemia
        - Fasting triglycerides ≥ 3.0 mmol/L (≥ 265 mg/dL)
        - Fibrinogen ≤ 1.5 g/L
    - Hemophagocytosis in bone marrow, spleen, or lymph nodes
    - Low or absent NK cell activity (according to laboratory reference ranges)
    - Ferritin ≥ 500 µg/L
    - Soluble CD25 (soluble IL-2 receptor) ≥ 2,400 U/ml

Modified from Henter J-I et al, *Pediatr Blood Cancer.* 2007.

Although these are the most widely used criteria, there are limitations, including the fact that these were developed based on a pediatric population, and two of the tests (NK cell activity and soluble CD25) may not be widely available. There is some concern that the HLH-2004 criteria may lead to under-diagnosis, especially in adults with malignancy-associated HLH. Some authors have proposed additional diagnostic criteria be added, especially when considering sHLH in adults, such as evidence of renal failure, coagulopathy, elevated lactate dehydrogenase, and elevated liver enzymes.

Because rheumatologic disease can cause cytopenias and hypofibrinogenemia, the HLH-2004 criteria are also difficult to apply to patients with potential MAS. It has been proposed that measuring the decrease from baseline for these analytes, rather than absolute values, may be more helpful in these cases. Additional proposed

diagnostic criteria for MAS include elevated aspartate aminotransferase, hepatomegaly, and the presence of neurologic symptoms. For each of these proposed diagnostic guidelines, prospective validation data are currently lacking.

THERAPY AND OUTCOMES

The HLH-2004 treatment protocol remains the most widely used and includes chemotherapeutic drugs and supportive care. All patients, regardless of underlying etiology, start with 8 weeks of therapy with etoposide, dexamethasone, and cyclosporine A, as well as supportive therapy, including prophylactic antifungals, antiviral agents if viral infection is present, intravenous immunoglobulin , and broad spectrum antibiotics as necessary. Patients with severe central nervous system involvement also receive intrathecal methotrexate and corticosteroids.

Patients with sHLH who respond to this initial therapy may need no further treatment. Patients without a family history or molecular diagnosis who still have active disease after this time period are recommended to continue on therapy. Some patients may also experience reactivation, in which intensification of therapy is recommended, including potential hematopoietic stem cell transplant (HSCT). Patients with a family history or molecular diagnosis of disease are kept on continuing therapy until HSCT. Overall this regimen resulted in a 5-year probability of survival of 61% in all patients, and 66% in patients receiving HSCT. In the subgroup of patients with primary HLH, overall survival was 59% and 70% after HSCT.

Additional therapeutic strategies have also been attempted. Biologic agents against cytokines like TNF and IFN-γ may be helpful, especially in MAS cases, where these also treat the underlying rheumatologic disorder. The anti-CD20 antibody rituximab has also been used in some cases of EBV-associated HLH. Other potential treatment strategies include dampening of cytokine signalling pathways by inhibiting Janus kinases, and stimulating inhibitory T-cell receptors such as PD-1. Autologous stem cell transplantation with genetically modified cells is an attractive target for primary cases.

FURTHER WORKUP OF THIS CASE

In all cases of HLH, every effort should be made to identify the underlying condition. In children, distinguishing primary HLH from secondary forms is especially important; although initial treatment is generally the same, patients with primary forms need ongoing therapy and eventual HSCT. The patient in this case likely has EBV infection, based on the history, including fever, pharyngitis, and lymphadenopathy. This should be confirmed and other infectious agents ruled out. However, even if an infectious agent is found in children, it is still recommended to perform molecular testing to evaluate for primary HLH, since the onset of an HLH episode may be triggered by infection in these patients.

In secondary forms efforts should be made to identify the underlying cause since long-term therapy will also include management of the underlying disorder. Patients—both children and especially adults—should be thoroughly investigated for malignancy. In bone marrow cases ancillary testing should be performed, such as flow cytometry if increased lymphocytes, blasts, or atypical cells are seen. Given that adults may also have EBV reactivation with associated HLH, studies to detect EBV should also be performed (EBV PCR from blood, EBER staining in bone marrow).

CONCLUSION

HLH is disease caused by a severe state of inflammation with high mortality, even with current therapies. The morphologic finding of hemophagocytosis is neither specific nor entirely sensitive for the overall syndrome. Diagnosis may be challenging, and requires integration of multiple clinical and laboratory findings. It is important to not only recognize the syndrome, but also to identify the underlying cause, and specifically distinguish primary from secondary forms because the former require hematopoietic stem cell transplantation, and in the latter the underlying cause must also be treated.

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