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Hyperinflammation, rather than hemophagocytosis, is the common link between Macrophage Activation Syndrome and Hemophagocytic Lymphohistiocytosis

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Abstract

Purpose of Review—Macrophage activation syndrome is the rheumatic disease-associated member of a group of hyperinflammatory syndromes characterized by uncontrolled cytokine storm. In this review, we highlight recent publications related to the pathoetiology of hyperinflammatory syndromes with an emphasis on how this new knowledge will guide our diagnosis, treatment, and future research efforts to better understand these deadly conditions.

Recent findings—The heterogeneity of clinical manifestations seen in patients with hyperinflammatory syndromes continues to grow as novel genetic and immunotherapeutic triggers of cytokine storm have been identified. Recent studies characterize unique cytokine and gene expression profiles from patients with different hyperinflammatory syndromes, while novel murine models begin to define networks of immune dysregulation thought to drive excessive inflammation in cytokine storm.

Summary—Emerging evidence suggests hypercytokinemia is the driving cause of pathology and morbidity/mortality in hyperinflammatory syndromes. Therefore, approaches to block cytokine function may be fruitful in treating hyperinflammatory syndromes with less toxicity than current therapies. However, not all hyperinflammatory syndromes result in the same pathogenic cytokine profile implying a personalized approach will be required for effective use of anti-cytokine therapies in the treatment of hyperinflammatory syndromes.

Keywords

Macrophage Activation Syndrome; cytokine storm; hemophagocytosis; Hemophagocytic Lymphohistiocytosis

Introduction

Macrophage Activation Syndrome (MAS) is a life-threatening complication of acute systemic inflammation arising in the context of a variety of autoimmune and auto-inflammatory conditions. More common rheumatic diseases associated with MAS include systemic juvenile idiopathic arthritis (SJIA), systemic lupus erythematosus, and Kawasaki

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disease, while MAS is less frequently reported to occur in patients with dermatomyositis, ankylosing spondylitis, polyarteritis nodosa, and adult-onset Still Disease. MAS is characterized by uncontrolled inflammation manifest as unremitting fevers, cytopenias, splenomegaly, hepatitis, coagulopathy, multisystem organ failure, and death in its most severe form. Evidence highlights the broad spectrum of manifestations seen in MAS from clinically unrecognized “occult” disease to the traditional fulminant and rapidly fatal disease (1).

The clinical features of MAS are shared by other cytokine storm syndromes, and are believed to represent the same end-stage pathophysiologic state reached by disparate initiating triggers of uncontrolled inflammation (2). These other cytokine storm syndromes are classified based on known underlying triggers or defects. These triggers or defects include: 1) genetic defects in cellular cytotoxicity resulting in Familial Hemophagocytic Lymphohistiocytosis (FHL), 2) immune response defects resulting in Immunodeficiency-Associated Hyperinflammatory Syndrome (IDAHS), 3) pathogenic microbial triggers resulting in Infection-Associated Hyperinflammatory Syndrome (IAHS) including severe sepsis, and 4) neoplasms resulting in Malignancy-Associated Hyperinflammatory Syndrome (MAHS) (Table 1)(3-15). However, the cellular and molecular mechanisms underpinning the pathogenesis of these disparate causes of cytokine storm are not fully understood.

In this review, we focus on recent advances in our understanding of hyperinflammatory syndromes with an emphasis on defining novel approaches to better inform the diagnosis and treatment of these conditions. We will discuss the latest advances describing cytotoxicity defects and cytokine networks thought to initiate and perpetuate systemic immunopathology in hyperinflammatory syndromes. These advances open the door to personalizing the diagnosis and treatment of hyperinflammatory syndromes, as we begin to uncover the unique pathways leading to the development of cytokine storm in individual patients.

Diagnosis

The diagnosis of hyperinflammatory syndromes is wrought with difficulty. These rapidly fatal conditions require immediate recognition to ensure urgent treatment. Secondly, tremendous clinical overlap occurs between different types of hyperinflammatory syndromes making the appropriate diagnosis challenging. Finally, appropriate diagnosis is essential, as current treatment recommendations for each individual hyperinflammatory syndrome are different.

Hemophagocytic Lymphohistiocytosis (HLH) is the only hyperinflammatory syndrome with formal diagnostic criteria, which relies on clinical criteria and testing of HLH-predisposing genetic defects to fulfill the diagnosis (Table 2)(16). Preliminary diagnostic guidelines are also available for MAS, but are restricted to patients with a known diagnosis of active SJIA (17). These guidelines may be difficult to apply to the diagnosis of MAS when it is the first manifestation of SJIA, which occurs in 0-67% of patients with SJIA complicated by MAS (17, 18). As HLH-related genetic testing can delay definitive diagnosis for days to weeks, a

physician is left with HLH clinical criteria to determine whether a patient warrants toxic HLH-related therapies or not in the setting of a possible hyperinflammatory syndrome.

The HLH clinical criteria include many non-specific findings that overlap with other diseases. For example, Ho *et al.* stratified 58 patients with a clinical suspicion for hyperinflammatory disease into HLH high-risk and low-risk groups, and demonstrated the amount of hemophagocytosis from bone marrow aspirates does not correlate with disease probability (19). This corroborates prior evidence showing the presence of hemophagocytosis is not sensitive or specific for hyperinflammatory syndromes (20, 21). Furthermore, Moore *et al.* published data on 627 patients showing a diverse range of conditions causing markedly elevated ferritin levels $> 1000 \mu\text{g/L}$ (22), meaning ferritin is another nonspecific feature of HLH. In SJIA patients, the 2004 HLH criteria were shown to be an insensitive tool for the diagnosis of SJIA-related MAS, as 33% of SJIA-related MAS patients did not meet HLH diagnostic criteria (18). Therefore, it is clear the HLH diagnostic criteria should not be used to diagnose SJIA-related MAS, and should be used with caution in the diagnosis of other cytokine storm syndromes.

Alternative methods to differentiate between hyperinflammatory syndromes are needed. To this end, Lehmborg *et al.* recently identified absolute neutrophil count $1.8 \times 10^9/\text{L}$, CRP 90 mg/L , and sCD25 7900 U/mL as cutoff values more specific for SJIA-related MAS than FHL or viral-associated HLH (18). Lehmborg *et al.* also demonstrated dynamic changes in standard laboratory tests, such as declining platelet and white blood cell counts, can differentiate between a flare in SJIA disease activity and full-blown MAS (18). However, they did not test whether a falling sedimentation rate or fibrinogen level would be predictive of MAS-related disease, which have been useful markers of MAS in our clinical experience. Sumegi *et al.* introduced another novel method for the diagnosis and differentiation of hyperinflammatory syndromes, whereby gene expression profiles of peripheral blood mononuclear cells from patients diagnosed with FHL type 2 demonstrated unique signatures compared to patients with relapsing FHL and rapidly-evolving FHL subtypes (23). It will be necessary to validate whether these cutoff values and gene expression profiles are useful in larger and more diverse cohorts of patients with cytokine storm syndromes before the full clinical benefit of these measures can be realized.

Prognostication

New insights into the basic mechanisms driving clinical heterogeneity in hyperinflammatory syndromes caused by defects in cellular cytotoxicity highlight how more informative prognoses and patient-specific treatment options may be the wave of the future. Three independent studies recently demonstrated the severity of FHL and IDAHS in genetically susceptible mice and humans correlates with the severity of the underlying cytotoxicity defect (24, 25). Jessen *et al.* showed patients with Syntaxin 11 and LYST deficiency, conditions harboring less severe cytotoxicity defects, had a later onset of hyperinflammatory disease compared with patients with Griscelli Syndrome and FHL2, diseases with severe cytotoxicity defects (24). In a separate paper, Jessen *et al.* describe a mild viral-induced hyperinflammatory syndrome in *pearl* mice harboring a mutation in AP-3, which causes a mild defect in cytotoxicity (26). This mutation is described in Hermansky-Pudlak syndrome

type 2 where the penetrance of full-blown hyperinflammatory disease is low and likely means pre-emptive bone marrow transplant is not warranted (26). Similarly, Sepulveda *et al.* showed the age of onset of hyperinflammatory disease in patients occurs later and a less severe viral-induced disease is seen in murine models of FHL4 compared to Griscelli Syndrome and FHL2, which correlates with the severity of the underlying cytotoxicity defects described in these diseases (25).

Recent advances in our understanding of the production, trafficking and release of cytolytic vesicles further highlight mechanisms of heterogeneity in disease severity. Interactions between Munc-18-2 and Syntaxin 11 are necessary for priming and release of cytolytic vesicles, and therefore lead to FHL4 and FHL5 when defective forms of these proteins are expressed (27). Interestingly, Hackman *et al.* now show how addition of interleukin (IL)-2 restores functional cytotoxicity to defective cytotoxic lymphocytes in patients with FHL4 and FHL5 by increasing levels of Syntaxin 3 and Munc 18-1, respectively (27). IL-2-induced expression of these alternative Munc18 and Syntaxin proteins complement the specific defects in Syntaxin 11 or Munc18-2 deficiencies, and highlight how IL-2-related therapies may lead to targeted interventions in patients with FHL4 and FHL5 (27).

Factors independent of defects in cytotoxicity may also regulate the severity of disease in patients with FHL. A recent study investigating a mouse model of FHL4 with a severe defect in cytotoxicity demonstrated nonfatal disease upon viral infection (28). In this model, immunopathogenic CD8 T cells had a phenotype consistent with CD8 T cell exhaustion manifest by decreased cellular activation and effector function leading to a reduction in global immunopathology (28). A better understanding of how defects in cytotoxicity affect T cell exhaustion may lead to novel therapeutic targets in hyperinflammatory diseases caused by immunopathologic CD8 T cells.

Pathogenesis

Cytokine storms syndromes are established during self-perpetuating, feed-forward inflammatory cycles (Figure 1)(29, 30). Evidence from cytokine profiles support the notion of dysregulated cytokine production as a central driver in the propagation of systemic inflammation. Unique cytokine profiles have been identified in patients with hyperinflammatory syndromes, including an IL-18 signature in patients with SJIA-related MAS (31). More recently, Shimizu *et al.* described the presence of two different cytokine profiles in SJIA with an IL-6 signature in patients with arthritis-related disease manifestations and an IL-18 predominate cytokine profile in patients with MAS (32). In contrast, Wada *et al.* recently demonstrated IL-6, IL-18, tumor necrosis factor (TNF) α , and interferon (IFN) γ elevations in patients with active FHL, Epstein-Barr Virus (EBV)-HLH, and IDAHS (33). In a patient with SLE-related MAS, Shimizu *et al.* demonstrated a more striking TNF α cytokine signature compared to the cytokine profiles from patients with other forms of hyperinflammatory disease (31, 32). These data implicate unique networks of cytokines in disease pathogenesis across a variety of hyperinflammatory syndromes, and will revolutionize the diagnosis and treatment of hyperinflammatory syndromes as we begin to understand how these networks uniquely perpetuate cytokine storm in individual hyperinflammatory syndromes.

Recent investigations using murine models of hyperinflammatory conditions have dissected the contribution of individual cytokines in the regulation and propagation of systemic inflammation. Previous reports demonstrate the central role of IL-10 as an anti-inflammatory regulator of hyperinflammatory disease (29). Interestingly, Ohyagi *et al.* now show an important source of IL-10 comes from hemophagocytes (34), cells once thought to be propagators of inflammation (35, 36). When hemophagocytosis is blocked or hemophagocytes are incapable of producing IL-10, viral-induced mortality is increased supporting a role for IL-10 and hemophagocytes as anti-inflammatory regulators of hyperinflammatory disease (34). Recent human and murine data support the notion that hemophagocytes are anti-inflammatory, as these cells display markers of alternatively activated macrophages, cells known to be important in the resolution phase of inflammation (37). The fact that hemophagocytosis may be reactive, not causal, may aid in understanding why its presence ultimately has a poor sensitivity and specificity for hyperinflammatory syndromes (19-21).

Previous studies have implicated IFN γ as the central mediator of the immunopathology in multiple hyperinflammatory diseases. However, two recent studies using chronic activation of innate immune cells to drive hyperinflammatory disease highlight how immunopathology can proceed in the absence of IFN γ . Canna *et al.* demonstrate how repeated injection of CpG, a Toll-like receptor 9 agonist, in combination with IL-10 receptor blockade leads to severe hyperinflammatory disease even in IFN γ -deficient mice (38). This study demonstrates IFN γ is dispensable for hyperinflammatory disease and implicates other inflammatory mediators as culprits of cytokine storm-related immunopathology (38). Avau *et al.* also describe an IFN γ -independent MAS-like disease following treatment of mice with complete Freund's adjuvant (39). This potent stimulator of innate immune cells leads to MAS-like disease in IFN γ -deficient mice with amelioration of disease following blockade of IL-12/23 or IL-17 implicating these cytokines as IFN γ -independent drivers of cytokine storm (39).

Clinically, known triggers of hyperinflammatory syndromes continue to expand with the most striking associations uncovered during the use of novel cancer immunotherapies in the treatment of specific cancers. A recent example of this is chimeric antigen receptor (CAR) T cell therapies, whereby engineered receptors graft the specificity of a monoclonal antibody onto a signaling scaffold capable of activating T cells (40). These artificial receptors can be expressed in T cells that are adoptively transferred into cancer patients to allow targeting and destruction of specific cancers (40). In patients with chemotherapy-resistant B cell acute lymphoblastic leukemia, treatment with CAR T cells has recently been shown to induce remission in patients with otherwise terminal disease (41, 42). However, this therapy has been associated with cytokine release syndrome mimicking many of the features of hyperinflammatory syndromes including massive hypercytokinemia (41, 42). Interestingly, blockade of IL-6 mitigated the effects of the cytokine release syndrome without affecting anti-leukemia efficacy (41, 42). Future studies will be necessary to determine if cytokine release syndromes are specific to the cytokine milieu produced by B cell tumor-targeted immunotherapy or whether this is specific to CAR T cell-mediated cytotoxicity. It is possible insights into the mechanism driving cytokine release syndrome may help us better

understand how other hyperinflammatory diseases are triggered and regulated by specific patterns of cytokine production.

Therapeutics

Great attention has been paid to the treatment outcomes of patients with hyperinflammatory syndromes. However, treatment of hyperinflammatory syndromes remains difficult and outcomes remain poor. The best-defined treatment occurs in patients with HLH as defined by the HLH-2004 therapeutic guidelines. HLH patients are often treated with one of two different induction therapies including etoposide and dexamethasone with or without cyclosporine A or anti-thymocyte globulin and prednisone (43). Response rates and survival to hematopoietic cell transplant (HCT) approach 80% with these regimens, but complete responses are only achieved in approximately 50% of HLH patients with the majority of deaths prior to transplant accruing from uncontrolled disease (43). Adjunctive therapy for patients with refractory HLH is much less well defined. Use of infliximab, anakinra, alemtuzumab, daclizumab, and vincristine have been reported in small cases for refractory HLH, and now Jordan *et al.* report the use of alemtuzumab as successful salvage therapy for refractory HLH in 22 patients with 77% survival to HCT (43). While this report offers some progress in the treatment of refractory disease, the risks of infection, severe side effects, treatment-related malignancies, and death prior to transplant remain real. Promising alternative approaches to treatment of refractory disease include anti-cytokine based therapies, which are currently under investigation using therapies to neutralize the effects of IL-6 or IFN γ .

For non-HLH related hyperinflammatory syndromes, therapy is based on treatment of the underlying trigger of cytokine storm and is much less well defined than HLH-related therapies. In SJIA-related MAS, the hallmark of therapy is pulse steroids and cyclosporine A with refractory disease being treated with IVIg, rituximab (in EBV-associated MAS), alemtuzumab, daclizumab and anti-cytokine therapies including anti-IL-1 and anti-IL-6 (44, 45). For patients with IAHS, specific therapies directed at controlling infection may be sufficient for disease control (44). However, many patients with IAHS require immunosuppressive therapies to manage the hyperimmune state without clear guidelines for use of any particular immunosuppressive regimen (44). In EBV-associated hyperinflammatory syndromes, rituximab can be used in conjunction with other immunosuppressive therapies to ameliorate disease by specifically targeting B cells, which are specific targets of EBV infection (44).

Two recent reports highlight the use of recombinant thrombomodulin in ameliorating symptoms of cytokine storm in 2 patients with disease complicated by disseminated intravascular coagulation (DIC), a common complication in hyperinflammatory syndromes (46, 47). Thrombomodulin is a molecule expressed on vascular endothelium and is thought to inactivate the coagulation cascade and reduce inflammation from endogenous products of tissue damage (46). Multiple cytokines known to be elevated in hyperinflammatory syndromes down-regulate the expression of thrombomodulin and may predispose these patients to the development of DIC (47). Thrombomodulin has also shown promising results in the treatment of sepsis patients with DIC adding to the potential use of this therapeutic in

the broader context of hyperinflammatory syndromes complicated by DIC (48). However, availability of this medicine may preclude its use as it is only approved in Japan.

Conclusion

New insights into the pathogenesis of hyperinflammatory syndromes continue to unravel the complex cytokine networks involved in end-organ damage and/or propagating severe inflammation. Further understanding of the immunopathogenic triggers and perpetrators of severe inflammation will likely uncover novel therapeutic targets to allow a personalized approach to the treatment and prevention of hyperinflammatory syndromes. The increasing recognition of cytokine-driven inflammation as the pathogenic mediator of disease and lack of hemophagocytes as a sensitive or specific disease marker make the name Hemophagocytic Lymphohistiocytosis obsolete. Perhaps the time is correct to rename HLH and MAS as “Hyperinflammatory” or “Hypercytokinemia” Syndromes to reflect our current understanding of the hyperinflammatory state underlying the pathogenesis of these conditions. This would provide a single term to link MAS, FHL, and other cytokine storm syndromes under a common theme, and focus clinicians on the hyperinflammatory nature of the pathology toward which therapy should be directed.

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Abbreviations

FHL	Familial Hemophagocytic Lymphohistiocytosis
MAS	Macrophage Activation Syndrome
IDAHS	Immunodeficiency-Associated Hyperinflammatory Syndrome
IAHS	Infection-Associated Hyperinflammatory Syndrome
MAHS	Malignancy-Associated Hyperinflammatory Syndrome
SJIA	systemic Juvenile Idiopathic Arthritis
CAR	Chimeric Antigen Receptor

HCT	Hematopoietic Cell Transplant
DIC	Disseminated Intravascular Coagulation
HLH	Hemophagocytic Lymphohistiocytosis
IL	Interluekin
IFN	Interferon
TNF	Tumor Necrosis Factor
EBV	Epstein Barr Virus
PRR	Pattern Recognition Receptor

Key Points

Clinical heterogeneity, overlap in clinical presentations, and lack of perfect diagnostic tests makes the diagnosis of hyperinflammatory syndromes difficult.

Hypercytokinemia is a common immunopathogenic state seen in hyperinflammatory syndromes.

Cytokine profiles from patients with hyperinflammatory syndromes have unique signatures and may lead to novel anti-cytokine directed therapeutic approaches.

Hemophagocytes are unlikely to be pathogenic cells, as they have poor predictive capability for disease state and appear to have regulatory roles in controlling inflammation during hyperinflammatory disease.

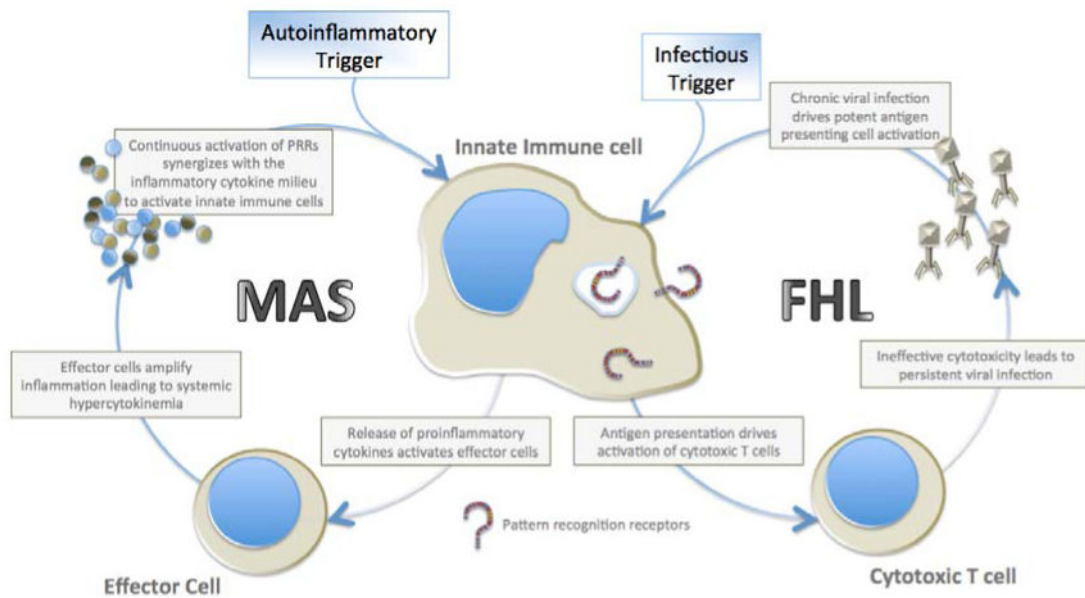


Figure 1. Self-perpetuating inflammatory cycles sustain enhanced systemic inflammation in hyperinflammatory syndromes

The working model of pathogenesis for hyperinflammatory diseases occurs from amplifying feed-forward inflammatory cycles leading to downstream immunopathology. In MAS, an autoinflammatory trigger likely mediated through pattern recognition receptor activation of innate immune cells leads to the release of proinflammatory mediators. Effector cells amplify this proinflammatory cytokine cascade leading to hypercytokinemia. The combination of proinflammatory cytokines and continued activation of pattern recognition receptors (PRRs) leads to synergistic activation of innate immune cells driving an enhanced immunopathologic cycle (29). In FHL, viral infections stimulate antigen-presenting cells to activate cytotoxic T cells to release tissue-damaging effector molecules. Due to defective cytotoxicity, uncontrolled viral replication propagates robust innate immune cell antigen presentation perpetuating a vicious cycle of self-sustaining and self-enhancing systemic inflammation (30).

Table 1

Classification of Hyperinflammatory Syndromes.

Disease	Gene	Protein	Defect	Trigger	Reference
<u>Familial Hemophagocytic Lymphohistiocytosis</u>					
FHL1	9q21-3-22	Unknown	Unknown	Infection	(3)
FHL2	PRF1	Perforin	Deficiency of cytotoxicity effector		(4)
FHL3	UNC13D	Munc13-4	Impaired cytolytic vesicle maturation and release		(5)
FHL4	STX11	Syntaxin 11	Abnormal priming and release of cytolytic vesicles		(6)
FHL5	STXBP2	Munc18-2	Abnormal priming and release of cytolytic vesicles		(7)
<u>Immunodeficiency-Associated Hyperinflammatory</u>					
Chédiak Higashi	LYST	LYST	Abnormal biogenesis of cytolytic vesicles	Infection	(8)
Griscelli 2	RAB27A	RAB27A	Abnormal cytolytic vesicle docking at the plasma membrane		(9)
Hermansky-Pudlak 2	AP3B1	AP-3	Impaired maturation and trafficking of cytotoxic granules		(10)
XLP1	SH2D1A	SAP	Defect in signaling pathway for cytotoxicity in T and NK cells		(11)
XLP2	XIAP	XIAP/Birc4	Defect in apoptosis and immune effector signaling pathways		(12)
ITK deficiency	ITK	ITK	Abnormal development, function, and/or survival of cytotoxic T cells		(13)
Macrophage Activation Syndrome	Unknown	Unknown	Unknown	Rheumatic Disease Flare OR Infection	(14, 15)
<u>Infection-Associated Hyperinflammatory Syndrome</u>					
Malignancy-Associated Hyperinflammatory Syndrome	N/A	N/A	N/A	Infection	(14)
				Malignancy	(14)

Table 2
2004 Diagnostic Guidelines for HLH (16)

The diagnosis of HLH can be established if one of the following criteria (A or B) is fulfilled.

(A) Molecular diagnosis consistent with known genetic susceptibility for the development of HLH (*PRF1*, *UNC13D*, *STX11*, *STXBP2*, *RAB27A*, *SH2D1A* or *XIAP*)

or

(B) Presence of 5 of 8 of the following clinical criteria:

Fever

Splenomegaly

Cytopenias affecting 2/3 lineages in the peripheral blood

Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)

Platelets <100 × 10⁹/L

Neutrophils <1.0 × 10⁹/L

Hypertriglyceridemia and/or hypofibrinogenemia

Fasting triglycerides 3.0 mmol/L (i.e., 265 mg/dL)

Fibrinogen 1.5 g/L

Hemophagocytosis in bone marrow, spleen, or lymph nodes

Low or absent natural killer cell activity (according to local laboratory reference)

Elevated ferritin 500 ug/L

Elevated soluble CD25 (i.e., soluble IL-2 receptor) 2400 U/mL

No evidence of malignancy

Comments:

1. Absence of hemophagocytosis does not exclude a diagnosis of HLH.
2. The following may provide strong supportive evidence for the diagnosis:
 - a. spinal fluid pleocytosis (mononuclear cells) and/or elevated spinal fluid protein
 - b. histological evidence resembling chronic persistent hepatitis by liver biopsy.
3. Other abnormal clinical and laboratory findings consistent with the diagnosis: Jaundice, hepatic enzyme abnormalities, coagulopathy, lymph node enlargement, edema, skin rash, hypoproteinemia, hyponatremia, VLDL increased, HDL decreased.