

## Laboratory Examination in Hemophagocytic Lymphohistiocytosis

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### ABSTRACT

Hemophagocytic Lymphohistiocytosis (HLH) is derived from the word hemophagocytosis, in which macrophages infiltrate tissue extensively, and unspecifically phagocyte blood and bone marrow cells. The deviant activation of cytotoxic CD8<sup>+</sup> T-cells causing the release of inflammatory cytokines is the core pathogenesis of HLH. Hemophagocytic lymphohistiocytosis is a regulatory disorder of the immune system, with clinical signs and symptoms of extreme inflammation and cytopenia, hepatitis, and severe and life-threatening central nervous system dysfunction. The name of the HLH disorder was recently proposed to be "Hyperinflammatory Lymphohistiocytosis" (also known as HLH). Enforcement of HLH diagnosis by the Histiocyte Society based on HLH 2004 updated diagnostic criteria consists of five of the following eight diagnostic criteria: fever, splenomegaly, cytopenia (two or more of three lineages in peripheral blood), hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, hemophagocytes in the bone marrow/lien/lymph, the low or non-existent activity of Natural Killer (NK) cells, increased sCD25. H-score, MH-score, and systemic Juvenile Idiopathic Arthritis (sJIA)/Macrophage Activated Syndrome (MAS) classification criteria are also used to enforce HLH diagnoses. Hemophagocytic lymphohistiocytosis is challenging to recognize and has a high mortality rate, especially in adults, ranging from 42 to 88%. Therefore, immediate diagnosis and therapy are essential. The introduction of HLH triggers is critical because treatment is based on the underlying trigger. Cytokine storms due to Coronavirus Disease 19 (COVID-19) infection have significant similarities to the clinical and laboratory findings of HLH. Secondary HLH (sHLH) is suspected in severe COVID-19 patients, so early diagnosis is potentially made based on the H-score.

**Keywords:** Hemophagocytic lymphohistiocytosis, hemophagocytosis

### INTRODUCTION

Hemophagocytic Lymphohistiocytosis (HLH) was first described as a familial disease by Farquhar and Claireux in 1952, called familial hemophagocytic reticulosis. The term HLH was formally adopted by the International Histiocyte Society in 1998 who established criteria for its diagnosis, which was updated in 2004.<sup>1,2</sup>

The North American Consortium for Histiocytosis (NACH) recommends a new classification system, which is: family-related HLH (familial HLH/FHLH), malignancy-related HLH, HLH associated with rheumatic conditions (rheumatic associated HLH), HLH in immunocompromised conditions, either primary or acquired (immune-compromised HLH), HLH not related to other specific conditions (HLH NOS).<sup>3</sup>

Other literature suggested two divisions of HLH: primary HLH and secondary HLH. FHLH is a form of primary HLH. Immune deficiencies such as Chediak Higashi Syndrome (CHS), Griscelli Syndrome (GS), and X-Linked Lymphoproliferative Syndrome (XLPs)

are also included in primary HLH (pHLH).<sup>4</sup> Malignancy-related HLH, rheumatism-related HLH, immune-compromised HLH and HLH NOS are classified into secondary HLH (sHLH).<sup>5-7</sup> It is important to differentiate the etiology of primary HLH and secondary HLH, because treatment based on underlying conditions is administered in secondary cases.<sup>8</sup>

The differences between familial (primary) HLH and secondary hemophagocytic syndrome are shown in Table 1. Perforin is absent, and mutations occurred in perforin and other genes with normal NK cell counts and non-functioning NK cells in FHLH. In secondary hemophagocytic syndrome, normal perforin and gene expression with normal or reduced NK cell counts are associated with normal or reduced function.<sup>9</sup>

Timely diagnosis of HLH is important because late diagnosis contributes to poor outcomes. Diagnosis is based primarily on clinical and laboratory results. No single laboratory test or clinical finding is pathognomonic.<sup>10</sup> The diagnosis of HLH, was confirmed by the Histiocyte Society with HLH 2004

**Table 1.** Differences between familial (primary) hemophagocytic lymphohistiocytosis and secondary hemophagocytic syndrome<sup>9</sup>

<b>Familial (primary) Hemophagocytic Lymphohistiocytosis</b>	<b>Secondary Hemophagocytic syndrome</b>
Familial involvement (autosomal recessive)	Not familial
Perforin absent and mutations in perforin and other genes (30%)	Perforin expression and other genes normal
Normal NK cell numbers; missing NK cell function	Normal or reduced NK cell number, corresponding to normal or reduced NK cell function

**Table 2.** Diagnostic criteria of hemophagocytosis lymphocytosis enforced based on updated HLH 2004 criteria<sup>3,11</sup>

<b>Hemophagocytic Lymphohistiocytosis (HLH) 2004 Clinical Trial Diagnostic Guidelines</b>
The diagnosis of HLH is established in the presence of five of the eight following clinical criteria:
Fever
Splenomegaly
<b>Cytopenia affecting <math>\geq 2</math> of 3 lineages</b>
Hemoglobin $< 9$ g/dL (in infants $< 4$ weeks: $< 10$ g/dL)
Platelets $< 100 \times 10^3/\mu\text{L}$
Neutrophils $< 1 \times 10^3/\mu\text{L}$
<b>Hypertriglyceridemia or hypofibrinogenemia</b>
Fasting triglycerides $\geq 3.0$ mmol/L (i.e., 265 mg/dL)
Fibrinogen $\leq 1.5$ g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
Low or absent natural killer cell activity (according to local laboratory reference)
Elevated ferritin $\geq 500$ $\mu\text{g/L}$
Elevated soluble CD25 (i.e., soluble interleukin-2 receptor) $\geq 2400$ U/mL

Comments: Familial HLH should have no evidence of malignancy

diagnostic criteria listed in Table 2.<sup>3,11</sup>

Table 3 describes the diagnostic guidelines for HLH, clinical and laboratory-based on HLH-2004, H score, MH score, sJIA/MAS.<sup>3</sup>

The H-score estimates the best risk of HLH, whereas the MH score differentiates between MAS and pHLH in children. H-score is more accurate at the onset of disease in adult patients, but the recommended cut-off value for H-score varies depending on the underlying condition.<sup>3</sup> The website that provides this online calculator for H-scores is <http://saintantoine.aphp.fr/score>.<sup>12</sup>

### COVID-19-related HLH

The clinical characteristics of COVID-19 are similar to sHLH. Respiratory symptoms such as dyspnoea and cough, or ARDS can also occur in patients with sHLH. The clinical similarity between severe COVID-19 and sHLH leads to the hypothesis that SARS-CoV-2 cytokine storm syndrome triggered, such as in sHLH. SARS-CoV-2 can also start sHLH in some patients.<sup>2,13</sup>

Severe COVID-19 patients show signs of rapid multiorgan damage, such as in sHLH. The pathogenesis of COVID-19 is similar to sHLH. Early diagnosis and rapid immunosuppression are crucial before multiorgan failure occurs. sHLH should be suspected in patients with worsening or severe COVID-19. Early diagnosis can potentially be made using a diagnostic test panel based on H-score (see Figure 1).<sup>2,13</sup>

### BMA Hemophagocytic lymphohistiocytosis

Hemophagocytosis is a self-destructive phenomenon consisting of the phagocytosis of red blood cells, WBC, platelets, and their precursors in the bone marrow and other tissues by activating histiocytes. Hemophagocytosis is found in patients with HLH, but it is neither sensitive nor specific for the diagnosis of HLH. Hemophagocytosis is not pathognomonic for the diagnosis of HLH but rather a component of diagnostic criteria. Hemophagocytosis in bone marrow may not be seen during the early phase of the disease, and therefore

**Table 3.** Diagnostic guidelines for HLH<sup>6</sup>

Clinical/Laboratory Findings	HLH-2004	H Score <sup>e</sup>	MH Score <sup>s,d</sup>	sJIA/MAS
Fever (°C)	≥ 38.5	< 38.4: 0 points 38.4-39.4: 33 points	-	+
Organomegaly	Splenomegaly	Absent: 0 points Hepatomegaly or splenomegaly: 23 points hepatomegaly and splenomegaly: 38 points	Splenomegaly: Absent: 0 points Presents: 12 points	-
Cytopenia	Cytopenia of ≥ 2 series Haemoglobin < 9 g/dL Platelet count < 100 x 10 <sup>9</sup> /L Absolute neutrophil count < 1x10 <sup>9</sup> /L	Single series: 0 points Two series: 24 points Three series: 34 points	Neutrophil count > 1.4: 0 points ≤ 1.4x 10 <sup>9</sup> /L: 37 points Platelet: > 78: 0 points ≥ 78x10 <sup>9</sup> /L: 11 points Haemoglobin > 8.3: 0 points ≤ 8.3.g/dL: 11 points	Platelet count ≤ 181x10 <sup>9</sup> /L
Triglycerides (mmol/L)	Hypertriglycemia and/or hypofibrinogenemia Tryglycerides ≥ 3.0 (65mg/dL)	< 1.5: 0 points 1.5-4.0: 44 points		> 156 mg/dL
Fibrinogen (mg/dL) <sup>2</sup>	Fibrinogen ≤ 150 mg/dL (1.5 g/l)	> 2.5: 0 points ≤ 2.5: 30 points	> 131: 0 points ≤ 131: mg/dL: 15 points (1.31 g/L)	≤ 360 mg/dL
Ferritin (ng/L)	≥ 500 µg/L	< 2000: 0 points 2000-6000: 35 points > 6000: 50 points		> 684 ng/mL
Hemophagocytosis in the bone marrow	Hemophagocytosis in the bone marrow, spleen, or the lymph nodes	Absent: 0 points Present: 35 points		> 48 U/L
Immunosuppression	-	Absent: 0 points Present: 18 points		
NK cell activity	Low or absent	-		
sCD25 (sIL2ra)	≥ 2400 U/mL	-	> 1.6: 0 points	-
Age at onset (years)	-	-	≤ 1.6: 37 points	

<sup>a</sup> re-used as single parameters for HLH-2004 scoring

<sup>b</sup> The presence of Human Immune Deficiency Virus (HIV) infection or long-term treatment with immunosuppressive drugs such as glucocorticoids, cyclosporine, or azathioprine

<sup>c</sup> A scoring is applied. An H-score of ≥ 169 has 93% sensitivity and 86% specificity for HLH. A cut-off value of >60 for MH score discriminated best between pHLH and MAS

<sup>d</sup> In a sJIA patient with fever and ferritin >684 ng/mL, the presence of two additional parameters has a sensitivity of 73% and specificity of 99% for this score

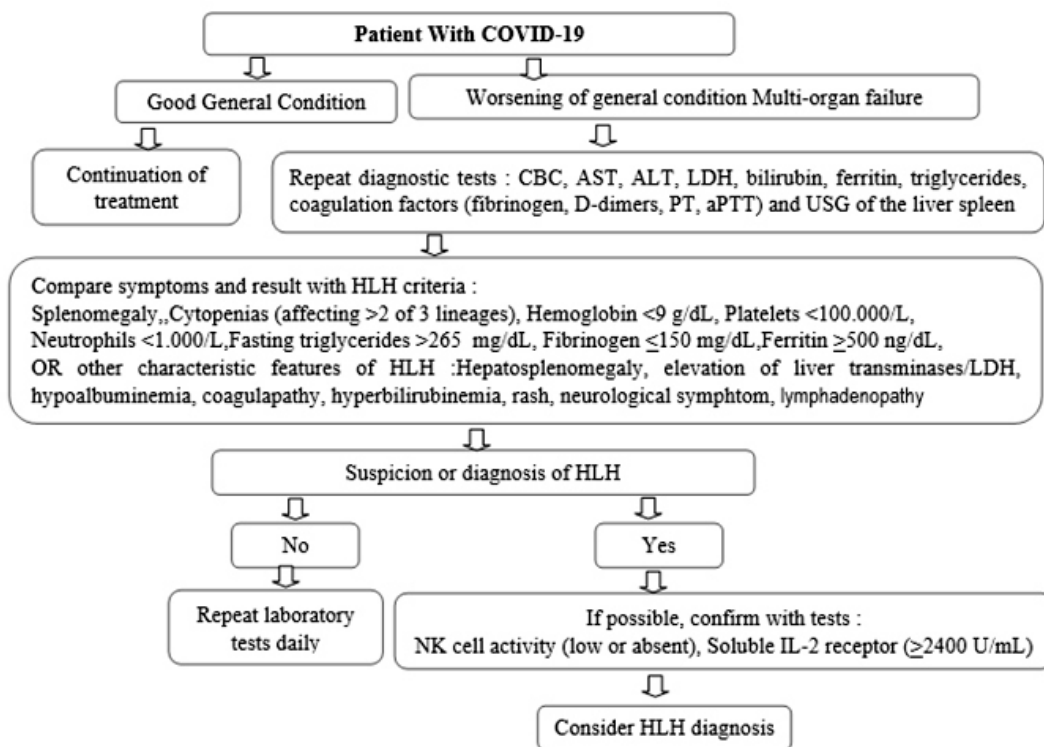
the absence of hemophagocytosis does not exclude the diagnosis of HLH.<sup>9,14</sup> Infiltration of CD163<sup>+</sup> macrophages to bone marrow or liver, together with a global clinical evaluation, can differentiate HLH from other causes of hemophagocytosis. Bone marrow examination is mandatory, not only to observe hemophagocytosis but also to differentiate HLH from hematological malignancies.<sup>9,14</sup>

The prevalence of hemophagocytosis in HLH varies from 25% to 100%. Hemophagocytosis in bone marrow aspiration in HLH patients can be a marker of excessive macrophage activation. Still, morphological features are also present in several diseases, including infection, autoimmune disease,

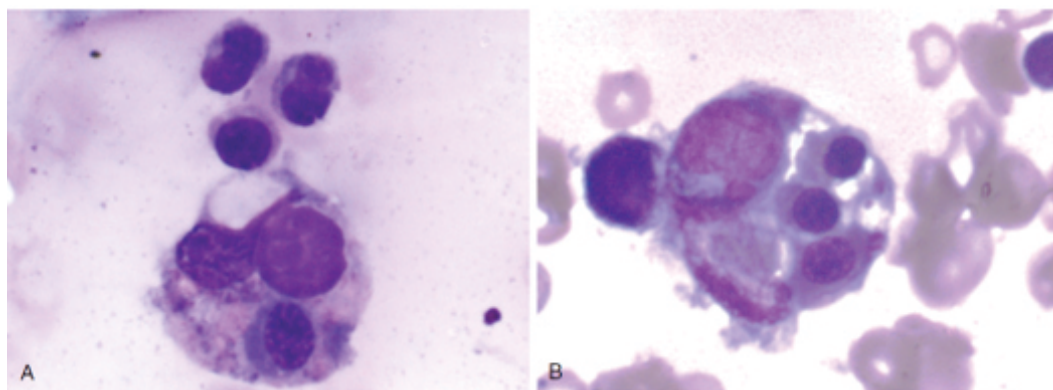
and other forms of bone marrow failure or etiology of red blood cell destruction.<sup>1</sup> Hemophagocytosis can also be seen in inflammatory conditions such as sepsis, influenza, leishmaniasis, malaria, rheumatological disorders/diseases.<sup>15</sup>

Bone marrow culture is performed to find the organisms that caused the infection, evidence of malignancy, and whether it is secondary or the etiology. Sixty-four percent of adult patients with hemophagocytosis in bone marrow aspiration are lymphomas, namely T/NK and B cell lymphomas.<sup>9</sup>

Hemophagocytic lymphohistiocytosis morphological criteria in bone marrow include: Non-nucleated erythrophagocytosis alone is not



**Figure 1.** Diagnostic scheme for COVID-19 patients<sup>2</sup>



**Figure 2.** Bone marrow aspiration shows macrophages lymphocytosis of red blood cells and debris in patients with pHLH<sup>9</sup>

specific for HLH; Hemophagocytosis of granulocyte (1:1000 cells), nucleated erythrocyte hemophagocytosis (4:1000 cells), at least one hemophagocytosis consisting of multiple nucleated cells: Hemophagocytosis of granulocytes is the most important predictor, followed by NRBC and lymphocytes.<sup>16</sup>

The presence of 1 hemophagocytosis of a granulocyte, 2 or more NRBC hemophagocytosis, 1 lymphocyte hemophagocytosis. Fulfillment of these diagnostic criteria increased the likelihood of HLH diagnosis to be 100%.<sup>16</sup>

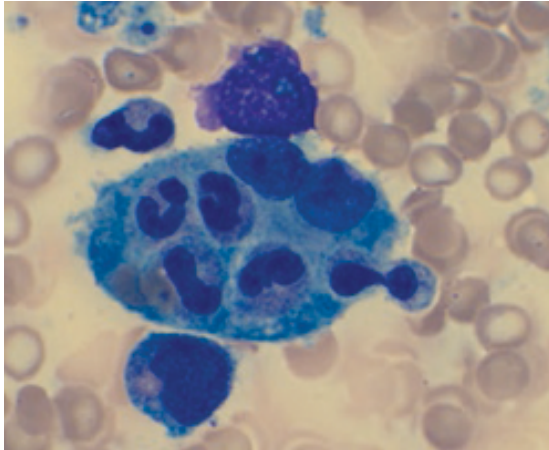
Figure 2 shows that macrophages lymphocytosis of red blood cells and debris in patients with pHLH in BMA. Figure 3 shows hemofagocytosis in bone marrow aspiration.

Perforin deficiency examination is performed with flow cytometry CD107a examination and perforin staining.

#### Flow Cytometry Examination of CD107a

CD107a is a protein present in cytotoxic granules. CD107a on the surface of NK cells is evidence of degranulation. CD107a is used to determine the





**Figure 3.** Hemofagocytosis in bone marrow aspiration<sup>5</sup>

presence or absence of decreased activity of NK cells and cytotoxic T-cells.<sup>10,12</sup> CD1-07a expression can be quantified by flow cytometry.<sup>17</sup> This test has 96% sensitivity and 88% specificity for genetic degranulation disorders. Absence or decreased CD107a signal intensity is a genetic cause of degranulation failure.<sup>18,19</sup> The presence of genetic defects affecting the cytotoxic route of degranulation leads to functional impairment in transferring glycoprotein membrane-bound lysosomal CD107a to the cell surface, thereby reducing CD107a expression.<sup>20</sup> CD107a screening and perforin protein expression were performed in all HLH patients.<sup>10</sup>

### Perforin Staining

Perforin, a protein that forms pores, is encoded by the PRF1 gene, the primary key to FHLH. Perforin is stored in cytotoxic granules. Perforin oligomerizes on the surface of target cells to form pores after secretion by cytotoxic lymphocyte granules.<sup>18</sup> In individuals with mutated perforin genes, intracellular perforin staining targeting cytotoxic lymphocytes can accurately detect reduction or absent protein levels.<sup>9</sup> Perforin is easily stained in NK cells using conjugated monoclonal antibodies. Perforin is greatly reduced or absent in a person with a biallelic mutation of the PRF1 gene. The diagnostic test for perforin expression in NK cells to detect PRF1 mutations has 96.6% sensitivity and 89.5% specificity.<sup>18</sup> Hemophagocytic lymphohistiocytosis monitoring is done by CD163, serum ferritin, and sCD25 tests.

### CD163 Test

CD163 serves as a scavenger receptor for macrophages that take up the haptoglobin

-hemoglobin (Hp-Hb) complex.<sup>21</sup> Soluble CD163 (sCD163) plasma levels in HLH are much higher than in infections, autoimmune diseases, and cancer. The combination of sCD25 and sCD163 is useful in the diagnosis and follow-up of HLH disease activity.<sup>10</sup> Cut-off value of sCD163 is 359.08 µg/L, with 83.3% diagnostic sensitivity and 83.9% specificity. sCD163 values in HLH patients before, two weeks, and eight weeks after treatment showed a statistically significant downward trend.<sup>22</sup>

### Serum Ferritin and sCD25

Serum ferritin is significantly associated with sHLH disease activity. Serial ferritin measurements are helpful for monitoring treatment response. Ferritin measurement is repeated 1-2 times per week for monitoring HLH.<sup>15</sup>

Soluble CD25 is used as a marker of disease activity, estimated to decrease in remission, and has been shown to help differentiate HLH from mimic HLH. sCD25 tests are performed weekly for HLH monitoring. sCD25 reflects T-cell activation, whereas ferritin reflects macrophage activation.<sup>23</sup> sCD25 values in HLH patients before treatment, two weeks, and eight weeks after treatment showed a statistically significant downward trend.<sup>22</sup>

### Prognosis

The prognosis of HLH in adults is still poor. The mortality rate ranged from 42% to 88%. High sCD25 was associated with a poorer prognosis of HLH. A <50% reduction in ferritin after treatment was associated with higher mortality.<sup>23</sup>

### DISCUSSION

Diagnostic criteria of hemophagocytic lymphohistiocytosis are based on updated HLH 2004 criteria presence of 5 of the 8 following clinical criteria fever, splenomegaly, cytopenia affecting  $\geq 2$  of 3 lineages (hemoglobin < 9 g/dL, platelets <  $100 \times 10^3/\mu\text{L}$ , neutrophils <  $1 \times 10^3/\mu\text{L}$ ), hypertriglyceridemia or hypofibrinogenemia (fasting triglycerides  $\geq 3.0$  mmol/l (i.e., 265 mg/dL), fibrinogen  $\leq 1.5$  g/l), hemophagocytosis in bone marrow or spleen or lymph nodes, low or absent natural killer cell activity, elevated ferritin  $\geq 500$  µg/l, elevated soluble cd25 (i.e., soluble interleukin-2 receptor)  $\geq 2400$  U/mL.<sup>3,11</sup>

Ferritin value greater than 500 ng/mL in HLH-2004 criteria has 84% sensitivity in diagnosing HLH patients with genetic disorders.<sup>4</sup> sIL-2r threshold of 2400 U/mL has 100% sensitivity, and 63% specificity in diagnosing HLH.<sup>24</sup> Hemophagocytic lymphohistiocytosis

morphological criteria in bone marrow include: Non-nucleated erythrophagocytosis alone is not specific for HLH; hemophagocytosis of granulocyte (1:1000 cells), nucleated erythrocyte hemophagocytosis (4:1000 cells), at least one hemophagocytosis consisting of multiple nucleated cells: Hemophagocytosis of granulocytes was the most important predictor, followed by NRBC and lymphocytes. The presence of 1 hemophagocytosis of granulocyte, 2 or more NRBC hemophagocytosis, 1 lymphocyte hemophagocytosis. Fulfillment of these diagnostic criteria increases the likelihood of HLH diagnosis to be 100 percent.<sup>16</sup>

Besides HLH-2004, diagnostic guidelines for HLH clinically and laboratory are based on H score, MH score, sJIA/MAS. The HLH 2004 criteria are reliable in the diagnosis of HLH. H-score is the best at estimating HLH. H-score is more accurate at the onset of disease in adult patients. The probability of developing HLH was less than 1% for H-score  $\leq 90$  and 99% for H-score  $\geq 250$ . A cut-off value of 169 has 93% sensitivity and 86% specificity for HLH.<sup>3</sup> Online calculator for H-score is available on <http://saintantoine.aphp.fr/score>.<sup>12</sup>

MH-score differentiates between MAS and pHLH in children. The probability of a pHLH diagnosis is less than 1% for score  $< 1$  and 99% for score  $\geq 123$ . A cut-off value  $\geq 60$  is the best score to differentiate between pHLH and MAS.<sup>3</sup>

sJIA-MAS classification criteria are used to overcome difficulties in establishing the diagnosis of MAS in cases of sJIA. The difference between the classification of sJIA-MAS and HLH 2004 is no measurement of sCD25. The sensitivity and specificity of this classification are 75% and 99%.<sup>3</sup>

## CONCLUSION

The histiocyte society established a diagnosis of HLH based on updated 2004 HLH diagnostic criteria. The updated 2004 HLH diagnostic criteria consist of 5 of following eight diagnostic criteria: fever, splenomegaly, cytopenia (involving  $\geq 2$  lineages in peripheral blood), hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, hemophagocytosis of bone marrow/spleen/lymph, low or absent NK cells activity, and increased sCD25. In addition, H-score, MH-score, and sJIA/MAS classification criteria are also used in diagnosis in addition to the HLH 2004 diagnostic criteria.

The immunological markers used to diagnose FHLH are cytotoxicity, CD107a degranulation, and perforin and granzyme tests.

Serum ferritin is significantly associated with

disease activity during sHLH. Therefore, serial ferritin measurements are helpful for monitoring response to treatment. Reduction of ferritin to baseline reflects successful treatment. sCD25, a marker of disease activity, is thought to decrease in remission and has been shown to help differentiate HLH from mimic HLH. sCD163 is also useful in the diagnosis and follow-up of HLH disease activity.

## REFERENCES

1. Hoffman R, Benz EJ. Haematology, basic principles and practice. Philadelphia, Elsevier, 2018; 731.
2. Opoka-Winiarska V, Grywalska E, Roliński J. Could hemophagocytic lymphohistiocytosis be the core issue of severe COVID-19 cases?. BMC Medicine, 2020; 18(1): 1–11.
3. Griffin G, Susan Sheno S, Hughes GC. Hemophagocytic lymphohistiocytosis: An update on pathogenesis, diagnosis, and therapy. Best Practice and Research Clinical Rheumatology, 2020; 34(4): 101515.
4. Machowicz R, Janka G, Wiktor-Jedrzejczak W. Similar but not the same: Differential diagnosis of HLH and sepsis. Critical Reviews in Oncology/Hematology, 2017; 114: 1–12.
5. Brisse E, Wouters CH, Matthys P. Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: Differences and similarities. British Journal of Haematology, 2016; 174(2): 203–17.
6. Eiichi I. Hemophagocytic lymphohistiocytosis in children: Pathogenesis and treatment. Frontiers in Pediatrics, 2016; 4: 1–9.
7. Hsi ED. Hematopathology. Philadelphia, Elsevier, 2018; chapter 19: 567–583.
8. Kwak A, Jung N, Shim YJ, Kim HS, Lim HJ, *et al.* A retrospective analysis of etiology and outcomes of hemophagocytic lymphohistiocytosis in children and adults. Yeungnam University Journal of Medicine, 2021; 38(3): 208–218.
9. Jaffe ES, Harris NL. Hematopathology. Philadelphia, Saunders Elsevier, 2011; 960.
10. Soy Mehmet, Pamir Atagündüz, Işı k Atagündüz, Gülsan Türköz Sucak. Hemophagocytic lymphohistiocytosis: A review inspired by the COVID-19 pandemic. Rheumatology International, 2020; 0123456789.
11. Hines M, Bhatt N, Julie-An M. Talano. Critical care of the pediatric immunocompromised hematology/oncology patient. Critical Care of the Pediatric Immunocompromised Hematology/Oncology Patient, 2019; 159–182.
12. La Rosée P, Horne AC, Hines M, Von Bahr Greenwood T, Machowicz R, *et al.* Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. Blood, 2019; 133(23): 2465–77.
13. Lima R, Célio Cortinhas Filho, Carlos Martins Ferreira Filho, Vaisman M, Cossenza A, *et al.* Hemophagocytic

- syndrome and COVID-19. Respiratory Medicine Case Reports, 2020; 31: 101162.
14. Stephen NL, Nicholas D. Cytotoxic T-lymphocytes and natural killer cells. Fifth Ed., Huntington, Elsevier Ltd, 2019; 247-259
15. Skinner J, Yankey B, Shelton BK. Hemophagocytic lymphohistiocytosis. AACN Advanced Critical Care, 2019; 30(2): 151-64.
16. Gars E, Purington N, Scott G, Chisholm K, Gratzinger D, *et al.* Bone marrow histomorphological criteria can accurately diagnose hemophagocytic lymphohistiocytosis. Haematologica, 2018; 103(10): 1635-1641.
17. Lorenzo-Herrero, S. Sordo-Bahamonde C, Gonzales S, López-Soto A. CD107a degranulation assay to evaluate immune cell anti-tumor activity. Methods in Molecular Biology, 2019; 119-130.
18. Chiang SCC, Blessing JJ, Marsh RA. Current flow cytometric assays for the screening and diagnosis of primary HLH. Frontiers in Immunology, 2019; 10: 1740.
19. Rubin TS, Zhang K, Gifford K, Lane A, Choo S, *et al.* Perforin and CD107a testing is superior to NK cell function testing for screening patients for genetic HLH. Blood, 2017; 129(22): 2993-2999.
20. Jin Z, Wang Y, Wang J, Zhang J, Wu L, *et al.* Primary hemophagocytic lymphohistiocytosis in adults: The utility of family surveys in a single-center study from China. Orphanet Journal of Rare Diseases, 2018; 13(1): 1-11.
21. Skytté MK, Graversen JH, Moestrup SK. Targeting of cd163<sup>+</sup> macrophages in inflammatory and malignant diseases. International Journal of Molecular Sciences, 2020; 21(15): 1-31.
22. Wang Y, Liu D, Zhu G, Yin C, Sheng G, Zhao X. Significance of soluble CD163 and soluble CD25 in diagnosis and treatment of children with hemophagocytic lymphohistiocytosis. Zhonghua Er Ke Za Zhi, 2015; 53(11): 824-829.
23. Sandler RD, Carter S, Kaur H, Francis S, Tattersall RS, Snowden JA. Haemophagocytic Lymphohistiocytosis (HLH) following allogeneic Haematopoietic Stem Cell Transplantation (HSCT)-time to reappraise with modern diagnostic and treatment strategies. Bone Marrow Transplantation, 2020; 55(2): 307-16.
24. Hayden A, Lin M, Park S, Pudek M, Schneider M, *et al.* Soluble interleukin-2 receptor is a sensitive diagnostic test in adult HLH. Blood Advances, 2017; 1(26): 2529-2534.