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*Original Article*

# Accuracy of the criteria for hemophagocytic lymphohistiocytosis

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**Abstract:** Hemophagocytic lymphohistiocytosis, also known as a hemophagocytic syndrome, is a life-threatening condition that can develop in critically ill patients with malignancies, severe infections, during chemotherapy, and may be associated with currently known or unknown genetic abnormalities; however, this list of potential causes can be extensive. The purpose of this study is to draw attention to the accuracy of its diagnostic criteria, association with a variety of clinical conditions, pathophysiological mechanisms, and outcomes of the diseases. From the medical records in our hospital, we retrospectively extracted 13 cases with hemophagocytosis over a 10-year period. Subsequently, we thoroughly analyzed medical records for the criteria used, the time required for making a diagnosis, adequacy of the criteria, management, and outcomes. We found that not all criteria were used for diagnosis, and the most sensitive and specific tests (genetic study, ferritin, and soluble IL-2r levels) were sometimes bypassed. Late diagnosis delayed management of some patients. Only a few treatment options were used for patient care. The hemophagocytic syndrome is a very rare and fatal entity requiring highly sensitive and specific diagnostic criteria for prompt diagnosis, targeted management, and thorough follow-up. Every patient admitted to the hospital with life-threatening conditions should be suspected and tested for the hemophagocytic syndrome as early as possible. The criteria for hemophagocytic lymphohistiocytosis should be revised, with the most sensitive and specific ones being done in all cases. Subsequently, each patient should be tested for the presence of genetic abnormalities that correlate with the syndrome.

**Keywords:** Hemophagocytosis, hemophagocytic lymphohistiocytosis, hemophagocytic syndrome

## Introduction

Regular assessment and analysis of the diagnostic criteria for any type of human pathology are necessary to ensure the highest level of patient care, especially in cases of life-threatening conditions, where timely diagnosis and proper treatment are required.

Hemophagocytosis (HPC) is a microscopic feature. It is one of the criteria for the diagnosis of hemophagocytic lymphohistiocytosis (HLH), a rare clinical syndrome with a high risk of unfavorable outcomes. Current literature reports that more than half of the deaths occurred within 30 days of starting therapy [1] with the overall median survival time ranging from 1.5 to 2.1 months [2-5]. The meaning of HPC is the ingestion by macrophages or histiocytes of cellular components of blood and their precursors, which can be manifested in cytopenias [4, 6].

Pathologists play an important role in the diagnosis of this condition. Using the state-of-the-art microscopes, we meticulously search for blood cells that have been ingested by macrophages, and then confirm the origin of the cells using immunohistochemistry. We identify and evaluate the clinical and pathological parameters required for the diagnosis, as well as perform molecular studies of genetic abnormalities if they are ordered or indicated. We see a clinical HLH problem with its complications, and raise the question of conducting an assessment of its diagnostic criteria.

In regular clinical practice, not every physician has experience with HLH, and the presence of hemophagocytosis in bone marrow aspirates or on biopsies of any other tissue is often considered as the gold standard for diagnosis. However, it can be observed after blood transfusion, in patients with advanced malignancies,

**Table 1.** Characteristics of the study group

| | | Age (y) | Gender | Diagnosis of hospitalization | HLH diagnosis, HD | | Outcome/follow up |
|---|---|---|---|---|---|---|---|
| | | | | | Preliminary | Final | |
| Patient No. | 1 | 29 | M | Fever, Pancytopenia, Diffuse Large B-cell lymphoma | 5 | 5 | Expired in 10 month |
| | 2 | 36 | F | Pneumonia, AIDS/HIV, Altered mental status | 9 | Not made | Alive, remission |
| | 3 | 52 | F | Pancytopenia, Possible TTP | 6 | 6 | Expired, HD 7 |
| | 4 | 35 | M | Cholangitis, SIRS, Transaminitis | 6 | 9 | Alive, remission |
| | 5 | 76 | M | Acute leukemia, Rheumatoid arthritis | 1* | 1* | Expired, HD 9 |
| | 6 | 24 | M | Sepsis, DIC | 2 | 5 | LFU |
| | 7 | 33 | M | Thrombocytopenia, Transaminitis, COPD exacerbation, Fever, Diabetes mellitus | 5 | 6 | LFU |
| | 8 | 0.25 | M | CMV infection, Sickle cell trait | 1 | 2 | Alive, remission |
| | 9 | 4 | F | Fever, Sickle cell trait | 11 | 12 | Alive, remission |
| | 10 | 31 | M | Cardiogenic shock, Lupus | 4 | 6 | Alive, remission |

*The diagnosis was made outside before the admission. Abbreviations: LFU - lost follow-up; HD - hospital day/day post admission.

or in critically ill patients with sepsis without connection with HLH [7, 8].

We carefully studied every case of established HPC in our department over a 10-year period. Subsequently, after reviewing medical records, we analyzed the clinical criteria used to make the diagnosis of HLH. To ensure confidentiality, patients were assigned numbers in chronological order according to the order in which cases were reported.

We found that HLH can be overdiagnosed or underdiagnosed due to the lack of defined diagnostic criteria or their incomplete use, which can significantly affect patient care [7].

## Materials and methods

### *Case selection*

After the study was approved by the hospital institutional review board, using the anatomic pathology information systems CoPath and Soft PathDx, we retrieved and retrospectively reviewed each relevant report on established HPC at our hospital from January 2009 to December 2019. We combined a list of 13 patients representing 11 bone marrow aspirates, 4 lymph node biopsies, 1 liver biopsy, and 1 spleen sample, with some samples being obtained from the same patient. Based on the presence of HLH diagnosis (preliminary and/or final) in the medical records, only 10 cases were selected for further analysis (**Table 1**). The other three cases were excluded from the study due to the absence of required laboratory testing and lack of preliminary or final clinical diagnosis of HLH in the chart. The cases prior to 2009 were not possible to restore because of the transition to another electronic medical record system in December 2019.

All slides related to the study were retrieved from the departmental archive and reexamined for HPC. The diagnostic accuracy was confirmed in all the cases. Subsequently using the medical records system EPIC, each case was carefully studied for the following aspects:

1) The demographic characteristics including age and gender.

2) The diagnosis of hospitalization.

3) The day post admission or hospital day (HD) when a preliminary and/or final diagnoses of HLH were made.

4) The outcomes and follow-ups.

5) The clinical criteria that determined the diagnosis of HLH in accordance with the recom-

mendations of the Histiocyte Society (**Table 2**) [8-11].

6) The used treatment options in accordance with the modified HLH-94/04 protocol [11].

7) The microscopic features on bone marrow aspirates, biopsies of lymph nodes and liver, and a spleen sample.

8) The probability of HLH based on the H-Score, which was calculated using the H-score calculator program (http://saintantoine.aphp.fr/score/) [4, 6-8].

*Laboratory data*

Most of the laboratory tests were conducted at the university hospital main laboratory. The complete blood count was performed on the Beckman Coulter DxH 800 Hematology Analyzer. The levels of ferritin, triglyceride, and serum glutamic oxaloacetate acid (SGOA) were measured on the Dimension Vista 1500 Intelligent Lab System with normal reference ranges for ferritin of 10-300 ng/ml, triglyceride of 0.3-1.5 mmol/L, and SGOA of 10-40 IU/L. The levels of fibrinogen were measured on the ACL TOP 550 CTS system with the normal reference range of 2.14-4.54 g/L. The natural killer (NK) cell activity and soluble CD25 concentration (soluble interleukin-2 receptor (IL-2r)) were measured at LabCorp Birmingham with the normal reference interval for IL-2r of 223-710 units/mL. The hemophagocytic hereditary lymphohistiocytosis gene panels were performed at LabCorp Birmingham (Birmingham, AL) and Fulgent Diagnostics (Temple City, CA). Laboratory results were obtained from the day of the bone marrow biopsy. In cases where the necessary blood test results were not available, we used the results of the period from the preliminary diagnosis of HLH to the day of the bone marrow biopsy.

*Immunophenotypic analysis*

All samples were formalin-fixed and paraffin-embedded at the time of diagnosis. In addition to routine staining with hematoxylin and eosin (H&E), tissue sections (4 microns thick) were treated and immunostained with Roche BenchMark Ultra Slide Stainer. A variety of stains were applied. Hemophagocytosis was observed on H&E stains, special stains (PAS and MPO) and immunostains (CD68, CD163, and CD14).

*Statistical analysis*

Results are presented as median, frequencies, or percentages. The fractions of the total number of cases are represented in percentage in parenthesis using GraphPad Prism 6.0 software.

## Results

*Demographic characteristics and clinical findings*

The mean age of manifestation of the syndrome was 32.0 ± 6.9 years, with a male to female ratio of 7:3. The median days for the preliminary and final diagnosis of HLH were day 5 and 6, respectively. 3 patients (30%) expired, 2 of whom (20%) expired while in the hospital. 7 patients (70%) survived with confirmed remission in 5 patients (50%), and 2 patients (20%) were lost for follow-ups (**Table 1**). Patient #1 was hospitalized twice for diffuse large B-cell lymphoma with subsequent negative bone marrow aspirates for HPC.

*Laboratory data and next generation sequencing results used for the diagnosis and treatment*

The required laboratory data was retrieved from electronic medical records. Levels of ferritin, triglyceride, SGOA, fibrinogen, and complete blood count were measured at our institution (the equipment with normal reference ranges are described above). The natural killer cell activity and soluble IL-2r were rendered at LabCorp Birmingham (**Table 2**). Based on the HLH-2004 diagnostic criteria, only 5 patients (50%) were tested for all the criteria necessary for diagnosis. It is not possible to identify the exact reason why the other criteria were not evaluated by the managing team. In addition, two patients were tested for HLH Genetic Sequencing after all diagnostic criteria were fulfilled and the final diagnosis of HLH was made. Patient #8 was found to have a pathogenic variant detected in the PRF1 gene, associated with HLH (familial, type 2) (OMIM: 170280) (performed at Fulgent Diagnostics, Temple City, CA). Patient #9 had a heterozygous missense variant of unknown significance in exon 16 of the AP3B1 gene, a heterozygous synonymous variant of unknown significance in exon 3 of the PRF1 gene, and a heterozygous intronic variant

4 bp before the start of exon 24 of the AP3B1 gene (performed at LabCorp Birmingham, Birmingham, AL). 9 patients (90%) received the treatment recommended for HLH syndrome; in 4 cases (40%), steroids were already on the list for comorbid clinical conditions (**Table 2**) [9].

*Assessment of the functional states of the bone marrows, lymph nodes, liver, and spleen*

The functional states of bone marrows were evaluated on a peripheral blood smears (Wright-Giemsa stain), bone marrow aspirate smears (Wright-Giemsa and iron stains), clot sections (H&E and iron stains), bone marrow touch preparations (Wright-Giemsa stain), and core biopsy recuts (H&E, PAS, reticulin, and iron stains). The peripheral blood smears revealed pancytopenia in 5 cases (50%), bi-lineage cytopenia in 1 case (10%), anisocytosis and poikilocytosis in 1 case (10%), and without any serious cellular abnormalities in 3 cases (30%). Analysis of bone marrow aspirates revealed 2 cases (20%) with hypocellular, 5 cases (50%) with normocellular, and 3 cases (30%) with hypercellular bone marrow (**Table 3**).

Reviewing bone marrow aspirates, hemophagocytosis was focally identified on H&E stains, special stains (PAS and MPO) and immunostains (CD68, CD163, and CD14) representing macrophages with engulfed red blood cells and other marrow elements (**Figure 1**).

In cases of blood malignancies, the flow cytometry results were available only for Patient #1. The study showed a mixed population of T and B lymphocytes and natural killer cells without light chain restriction in B cells. There was no flow cytometric evidence of bone marrow involvement by non-Hodgkin lymphoma, which suggests the appearance of HPC was due to another pathophysiological process, not directly because of lymphoma.

On the liver biopsy, within hepatic sinusoids, there were increased immature monocytic cells in single cells or in clusters, with slightly open chromatin, irregular nuclear contours, and relatively abundant cytoplasm. HPC was seen within macrophages or monocytes (**Figure 2**).

Analysis of the lymph nodes showed focal hemophagocytosis in the parafollicular region. In most of the cases, follicular hyperplasia and medullary lymphoid hyperplasia were present.

Microscopy of the spleen sample showed extramedullary hematopoiesis with focal HPC in the red pulp.

H&E slide reviews confirmed the presence of mature red blood cells within hemophagocytes in all the cases. However, it was difficult to confirm the identity of other blood cells based on pathomorphology with 100 percent certainty. Since the features of the HPC were observed focally, sometimes 1-2 cells per slide, it was easy to miss. In addition, it is difficult to assess its clinical significance and correlation with a particular clinical symptoms, since there were no differences in pathomorphology with correlation to tissue, the main clinical conditions, and the presence of other criteria, whether patient has HLH or not.

*Determination of H-Score*

Based on H-Score of 2014 Fardet et al., the probability of hemophagocytic syndrome in each patient was analyzed and calculated using the H-score calculator program (http://saintantoine.aphp.fr/score/), with no missing data revealed (**Table 4**) [3, 7, 8]. The median H-Score was 221.5 (range 96-288) with the probability of the disease ≥ 80% in 7 cases (70%).

## Discussion

HLH is a very rare and potentially lethal syndrome with an ineffective hyperinflammatory immune response and incidence of 1-2 cases per million in Europe and Japan [12]. HLH is divided into primary (genetic or familial) and secondary (acquired) types [2-4, 6, 7, 13]. Multiple genetic defects are associated with primary HLH, which are usually provoked by infections and mainly occur during childhood [2, 3, 6]. However, it can manifest from the intrauterine stage in the form of hydrops fetalis up to the age of 70's [13]. Acquired HLH can occur in critically ill patients at any age and is usually triggered by malignant neoplasms, autoimmune diseases, and viral infections [2-4]. Despite the existence of several hypotheses about the primary and secondary HLH, its actual pathogenesis is still not clear, especially for the secondary type [6, 13].

A certain role is assigned to cytokines, whose actions lead to fever, phagocytosis, coagulopathy, lipidic changes, and cytope-

**Table 2.** Completed HLH-2004 diagnostic criteria and treatment for HLH

| | | Patient No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Revised HLH-2004 criteria | Fever (≥ 38.5°C) | + | - | + | + | - | + | + | - | - | + |
| | Splenomegaly | + | - | + | - | + | + | + | + | - | + |
| | At least 2 of 3 cytopenias in peripheral blood (Hb < 90 g/L, Plt < 100 • $10^9$/L, Absolute neutrophils < 1.0 • $10^9$/L) | + | + | + | + | + | + | - | + | + | + |
| | Hypertriglyceridemia (≥ 3 mmol/L) and/or hypofibrinogenemia (≤ 1.5 g/L) | + | - | + | + | + | + | + | + | + | + |
| | Hemophagocytosis | + | + | + | + | + | + | + | + | + | + |
| | Low or absent Natural Killer (NK) cell activity | n/a | n/a | n/a | - | + | - | n/a | n/a | n/a | - |
| | Hyperferritinemia (≥ 500 ug/L) | - | + | + | + | + | + | - | + | + | + |
| | Soluble CD25 (soluble interleukin-2 receptor) (≥ 2400 U/mL) | n/a | n/a | n/a | + | + | - | n/a | n/a | + | + |
| Total No. of completed criteria | | 5 | 3 | 6 | 6 | 7 | 6 | 4 | 5 | 5 | 7 |
| Identified genes with genetic abnormalities correlated with HLH | | n/a | n/a | n/a | n/a | n/a | n/a | n/a | PRF1 gene | AP3B1, PRF1, and AP3B1 genes | n/a |
| Prescribed treatment | | Steroids, Etoposide, Cyclosporin | Steroids | Steroids | Steroids | Steroids | n/a | Steroids | Steroids, Cyclosporin | Steroids, Cyclosporin | Steroids |

Abbreviations: n/a - not applicable (i.e., not ordered); "+" - criterion is positive; "-" - criterion is negative.

**Table 3.** Microscopic findings on bone marrow aspirates with HPC features

| | | Bone marrow aspirate | | | | | | | Blood smear |
|---|---|---|---|---|---|---|---|---|---|
| | | Cellularity | | | Ratios | | Iron stores | Reticulin fibrosis | |
| | | hypo- | normo- | hyper- | Fat/cell | Myeloid/Erythroid | | | |
| Patient No. | 1 | | | • | 8:92 | 1:3 | ↓ | Normal | Pancytopenia |
| | 2 | | | • | 20:80 | 2:1 | ↓ | ↑ | Pancytopenia, schistocytes |
| | 3 | • | | | 65:35 | not evaluable | Absent | ↑ | Pancytopenia, atypical lymphocytes |
| | 4 | | • | | 35:65 | 1:3 | Normal | Normal | Normal |
| | 5 | | | • | 20:80 | 5:1 | ↑ | ↑ | Anemia, thrombocytopenia |
| | 6 | | • | | 50:50 | 4:1 | Absent | Normal | Pancytopenia |
| | 7 | | • | | 45:55 | 2:1 | Absent | ↑ | Leukopenia, thrombocytopenia |
| | 8 | | • | | 0:100 | 3:1 | Absent | Normal | Normal |
| | 9 | • | | | 50:50 | 5:1 | ↓ | Normal | Anisocytosis, poikilocytosis, thrombocytopenia |
| | 10 | | • | | 40:60 | 1:1 | ↓ | ↑ | Pancytopenia, poikilocytosis |

Abbreviations: ↓ - levels decrease, ↑ - levels increase.

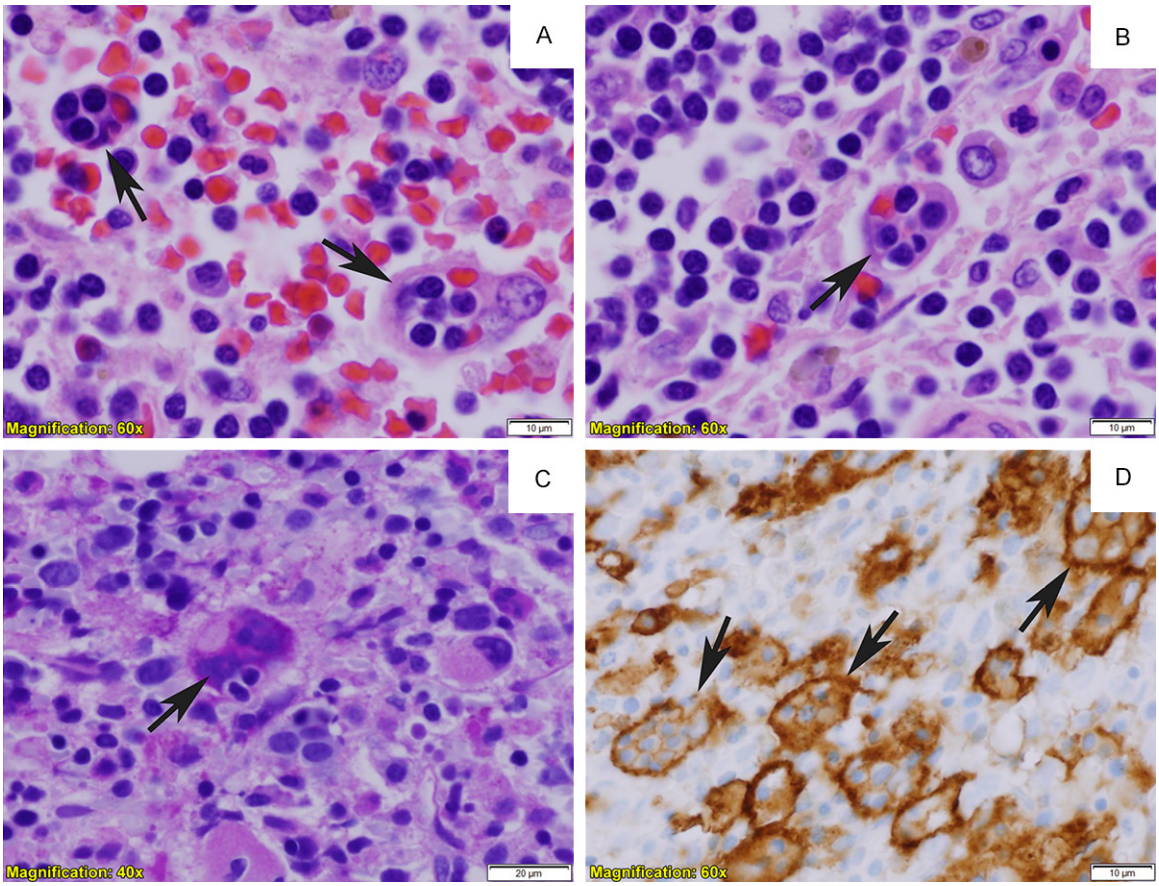


**Figure 1.** Hemophagocytosis. The colorful microphotographs demonstrate ingestion of mature and immature blood cells by macrophages (arrows) in the red pulp of spleen (A) and in the parafollicular zone of the lymph node (B) (H&E, 60×). Hemophagocytosis can be highlighted with PAS special stain (C) and CD163 (D) immunostain on bone marrow aspirates (40× and 60×, respectively).

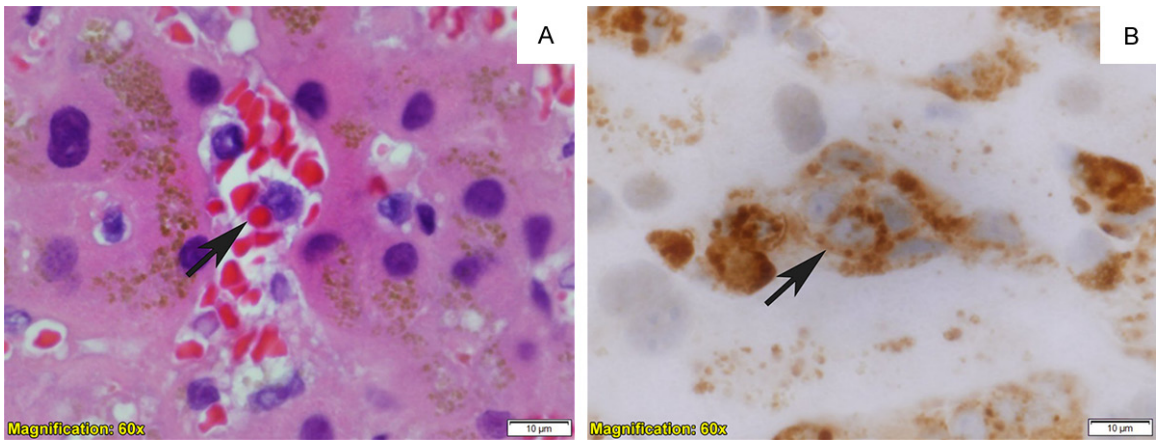


**Figure 2.** Hemophagocytosis on the liver biopsy. Microphotographs demonstrate the ingestion of mature red blood cells by macrophages (arrows) on H&E stain (A) and CD68 immunostain (B) within hepatic sinusoids (60×).

nia. Activation of macrophages leads to alterations of lipidic enzymes and hyperferritinemia [14].

As mentioned above, genetic abnormalities associated with familial HLH syndrome include mutations in the gene encoding perforin (PRF),

**Table 4.** H-Score and probability of HLH (modified)

| | | Patient No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Parameter | Known immunosupression | 18 | 18 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 |
| | • 0 (no) | | | | | | | | | | |
| | • 18 (yes) | | | | | | | | | | |
| | Temperature (°C) | 33 | 0 | 33 | 49 | 0 | 33 | 33 | 0 | 0 | 33 |
| | • 0 (< 38.4) | | | | | | | | | | |
| | • 33 (38.4-39.4) | | | | | | | | | | |
| | • 0 (> 39.4) | | | | | | | | | | |
| | Organomegaly | 23 | 0 | 23 | 0 | 23 | 23 | 23 | 23 | 0 | 23 |
| | • 0 (no) | | | | | | | | | | |
| | • 23 (hepato- or splenomegaly) | | | | | | | | | | |
| | • 38 (hepato- and splenomegaly) | | | | | | | | | | |
| | Cytopenias | 34 | 24 | 34 | 24 | 34 | 34 | 0 | 24 | 24 | 24 |
| | • 0 (1 lineage) | | | | | | | | | | |
| | • 24 (2 lineages) | | | | | | | | | | |
| | • 34 (3 lineages) | | | | | | | | | | |
| | Ferritin (ng/ml) | 0 | 0 | 50 | 50 | 35 | 0 | 0 | 50 | 0 | 50 |
| | • 0 (< 2000) | | | | | | | | | | |
| | • 35 (2000-6000) | | | | | | | | | | |
| | • 50 (> 6000) | | | | | | | | | | |
| | Triglyceride (mmol/L) | 0 | 0 | 64 | 64 | 44 | 44 | 44 | 44 | 0 | 44 |
| | • 0 (< 1.5) | | | | | | | | | | |
| | • 44 (1.5-4) | | | | | | | | | | |
| | • 64 (> 4) | | | | | | | | | | |
| | Fibrinogen (g/L) | 30 | 0 | 30 | 0 | 30 | 30 | 0 | 30 | 30 | 30 |
| | • 0 (> 2.5) | | | | | | | | | | |
| | • 30 (≤ 2.5) | | | | | | | | | | |
| | SGOT* (IU/L) | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 |
| | • 0 (< 30) | | | | | | | | | | |
| | • 19 (≥ 30) | | | | | | | | | | |
| | HBMA** | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 |
| | • 0 (no) | | | | | | | | | | |
| | • 35 (yes) | | | | | | | | | | |
| H score | | 192 | 96 | 288 | 241 | 238 | 218 | 154 | 225 | 108 | 258 |
| Probability (%) | | 80 | < 1 | > 99 | 99 | 98 | 93 | 25 | 96 | 1 | > 99 |

*Serum glutamic oxaloacetic transaminase. **HPC features on bone marrow aspirate.

UNC13D gene (17q25), STX11 gene (6q24), SAP gene (SLAM-associated protein) and others [4]. Also, during clinical courses of some syndromes, such as X-linked lymphoproliferative syndrome, Chediak-Higashi, and Griscelli syndrome type 2, true signs of HLH can be observed [2, 7].

In our study, 2 cases (20%) with unfavorable outcomes were associated with hematologic malignant neoplasms. In 5 cases (50%), the most frequent provoking diseases were certain types of infections, which was noted by other authors [15, 16]. Some researchers have shown a link to malignancies, especially lymphomas [5]. In addition, two children with HLH related genetic mutations had sickle cell traits.

Based on the revised HLH-2004 diagnostic criteria, to diagnose HLH, a molecular diagnosis consistent with HLH or 5 of the 8 diagnostic criteria for HLH should be fulfilled (**Table 2**) [2,

7-9]. At the same time, the clinical appearance of these criteria is not always associated with the actual occurrences of HLH, which puts the physician in a difficult situation of making the diagnosis, subsequently leading to unnecessary or incorrect medical procedures and delays in treatment [7, 17, 18].

Fever, cytopenia, splenomegaly, hypofibrinogenemia, and hypertriglyceridemia criteria are neither specific nor sensitive for HLH. HPC is not a common sign of secondary HLH when it occurs and can only be observed in the later stages of disease progression. On bone marrow examination, HPC can be appreciated in half to two-third of HLH patients [12]. Low or absent NK cell activity is detected in primary HLH, but its activity fluctuates over time in the secondary type. At the same time, ferritin levels ≥ 500 μg/L and soluble IL-2r level ≥ 2400 U/ml have a sensitivity of 0.84 and 0.93, respectively [9]. Some sources claim that ferritin level > 10,000 μg/L shows 90% sensitivity and 96% specificity for HLH [4]. Applying the 2014 criteria to our study, 2 out of 7 cases (29%) were not tested for soluble IL-2r, which is exclusive for HLH at high levels [8]. Marker of macrophage activation (sCD163) and marker of degranulation (CD107a) were not done in all the cases. Unfortunately, these sensitive tests are not available in all laboratories and usually cannot be obtained immediately, which delay the diagnosis and treatment [2].

It is very important to mention the process of emperipolesis, in which a cell penetrates into another living cell, can exist inside, and can exit at any time without any associated functional or structural abnormalities. In HPC, the absorbed cells are destroyed by lysosomal enzymes of macrophages [19]. Since macrophages and histiocytes originate from monocytoid precursor cells, they are allocated the same staining patterns described above for HPC, which makes the criterion of HPC more subjective and unreliable.

The cytopenia criterion is highly variable and can fluctuate depending on the states of the bone marrow, the patient's age, treatment, and more. In some cases, cytopenias based on the complete blood count (CBC) were used for preliminary diagnoses, but later on the bone marrow biopsy, some peripheral blood smears were completely normal. On the CBC, one or another degree of cytopenia was detected in 9 cases (90%). At the same time, cytopenia was detected in 6 cases (60%) on blood smears on the day of bone marrow biopsy. The difference was probably caused by a gap in the days of material collection, therapeutic measures, or changes in the patient's condition. In addition, when bone marrow biopsies were performed after the preliminary diagnosis was made, the patients' condition usually worsened and more prominent blood abnormalities were expected. This makes this criterion not entirely accurate and easily overlooked.

Analyzing cases after 2014, none of our patients were rated on the H-Score scale. Some researchers noted that the cut-off value of 169 corresponded to a sensitivity of 93%, a specificity of 86%, and an accurate classification of 90% of the patients [7]. Applying H-Score to our list, 7 cases (70%) crossed the level of 169.

There were no repeated bone marrow aspirates performed despite ongoing cytopenias. However, it is recommended to determine whether they are related to the secondary toxicity of treatment or the activity of HLH [3]. We found that the cellularity of bone marrow aspirates varied depending on major clinical conditions.

Since the liver function abnormalities are associated with HLH, hemophagocytosis on liver biopsies or aspirates should also be added to the main criteria [2].

## Conclusions

HLH is a rare, complex and life-threatening syndrome characterized by multiorgan failure due to immune system dysregulation [11, 20]. We encourage to test each severely ill patient against all the criteria at once since a delay in testing extends the time to reach the correct diagnosis and may lead to failure of patient management. Despite the presence of multiple cohort studies and reviews [1, 6, 15, 16, 21], the comprehensive study of the entity is still necessary for the better understanding of the mechanisms and stratification of diagnostic criteria, both major and minor, based on sensitivity and specificity. Current criteria are incomplete and partially used in clinical and pathological practices, which need to be reviewed and improved. Since the primary type of HLH

can manifest up to old age, and some genetic abnormalities can be detected in the secondary type, all patients with HLH should be checked for genetic abnormalities [16]. It is likely that early detection with targeted treatment might reduce fatality significantly.

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## Disclosure of conflict of interest

None.

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