DOI：10.6314/JIMT.202312_34(6).07 內科學誌 2023：34：474-482

Tuberculosis and Aspergillosis-Associated Hemophagocytic Lymphohistiocytosis: A Case Report

Yih-Shiaw Chen[1], Yu-Min Lin[1,2,3], Ying-Chu Lin[1,4]

[1]Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan;
[2]Division of Hepatology and Gastroenterology, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan;
[3]School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan;
[4]Division of Hematology and Oncology, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan

Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory response characterized by symptoms of fever, hepatosplenomegaly, and cytopenias. It may be a primary disorder (genetic or familial, mainly observed in children) or secondary to infection, malignancy, or rheumatologic disease (more frequently observed in adults). Most diagnostic and therapeutic guidelines for HLH are focused on pediatric patients, and standard treatment for adult HLH has not yet been developed. Here, we describe the case of an 80-year-old man with fever, hepatosplenomegaly, thrombocytopenia, and anemia who was given the diagnosis of HLH associated with tuberculosis and aspergillosis.

Key Words: ***Aspergillosis*, Hemophagocytic lymphohistiocytosis, Tuberculosis**

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory syndrome resulting from the aberrant proliferation and activation of benign macrophages[1,2]. It may be a primary disorder (genetic or familial) or secondary to infection, malignancy, rheumatologic disorders, and immune deficiency syndromes[3]. HLH typically presents as fever, hepatosplenomegaly, and cytopenias. Laboratory findings when HLH is present often reveal hypertriglyceridemia, hypofibrinogenemia, liver dysfunction, and elevated ferritin levels[4]. However, given that the initial presentation of HLH can be similar to that of common infections and that triglycerides, fibrinogen, and ferritin are not routinely checked in patients with fever, the diagnosis of HLH can be delayed, or the syndrome can be overlooked[5].

Here, we describe the case of a patient with fever and liver dysfunction who was diagnosed with hemophagocytic lymphohistiocytosis secondary to *Mycobacterium tuberculosis* and *Aspergillus* infec-

Reprint requests and correspondence：Yu-Min Lin
Address：Division of Hepatology and Gastroenterology, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan.

tion.

Case report

An 80-year-old man presented to the emergency room (ER) in April 2020 after accidentally falling down because of dizziness and chronic lower limb weakness. He had a past history of type A aortic dissection and underwent ascending aortic replacement 3 months ago. At ER, no loss of consciousness, fever, cough, chest pain, abdominal pain, nausea, vomiting, recent body weight loss, or allergic history was reported. He reported having visited the cemetery for the tomb sweeping ceremony approximately 1 week prior to the ER visit.

Upon arrival at the ER, his body temperature was 36.6 °C, heart rate was 78 beats per minute, respiratory rate was 18 breaths per minute, and blood pressure was 124/86 mmHg. He was alert and not acutely ill-appearing. A head and neck examination revealed pink conjunctiva and nonicteric sclera. A cardiopulmonary examination revealed clear breathing sounds over bilateral lung fields and regular heartbeats without murmurs. On palpation, the abdomen was soft, flat, and nontender, without splenomegaly or hepatomegaly. No skin lesions were noted. The neurological examination results were also unremarkable.

The initial complete blood count and serum biochemistry blood tests were summarized in the Table 1. Contrast-enhanced computed tomography (CT) from the chest to pelvis revealed heterogeneously early hepatic parenchymal enhancement and gallbladder wall edema. Compared with the CT images taken 1 month previously, those of the current CT revealed enlargement of the liver (from 14.34 cm to 17.43 cm) and the spleen (from 9.05 cm to 11.95 cm; Figure. 1**)**. Abdominal sonography revealed a thickened gallbladder wall. The patient was admitted under the tentative diagnosis of acute hepatitis and cholecystopathy.

After admission, an intermittent fever of up to 39.5 °C was noted. Because of the history of having been to the cemetery, leptospirosis, scrub typhus, Hanta virus, and severe fever with thrombocytopenia syndrome were suspected and were reported to the Taiwan Centers for Disease Control.

Table 1. The blood tests on admission of the patient

Blood Test	Value	Reference Range
White Cell Count	3,300	3,800-10,000 cells/μL
Hemoglobin	11.9	13.0-18.0 g/dL
Mean Corpuscular Volume (MCV)	89.1	81.0-98.0 fL
Platelet	79,000	140,000-450,000 cells/μL
Aspartate Transaminase (AST)	548	8-38 U/L
Alanine Transaminase (ALT)	582	4-44 U/L
Alkaline Phosphatase (ALP)	446	39-117 U/L
Gamma-Glutamyl Transferase (GGT)	682	16-73 U/L
Total Bilirubin	1.74	0.20-1.20 mg/dL
Direct Bilirubin	1.23	0.00-0.40 mg/dL
Creatinine	2.97	0.65-1.07 mg/dL
C-Reactive Protein (CRP)	5.88	<0.3 mg/dL

Penicillin and doxycycline were empirically prescribed. Further blood tests revealed a prolonged prothrombin time with INR (international normalized ratio)1.35, elevated triglyceride level (290 mg/dL), and low fibrinogen level (104 mg/dL). Tests for hepatitis B virus surface antigen, hepatitis C virus antibody, hepatitis A virus IgM antibody, and cytomegalovirus IgM; a human immunodeficiency virus screening test; a serological test for syphilis-rapid plasma; and a test for herpes simplex virus 1+2

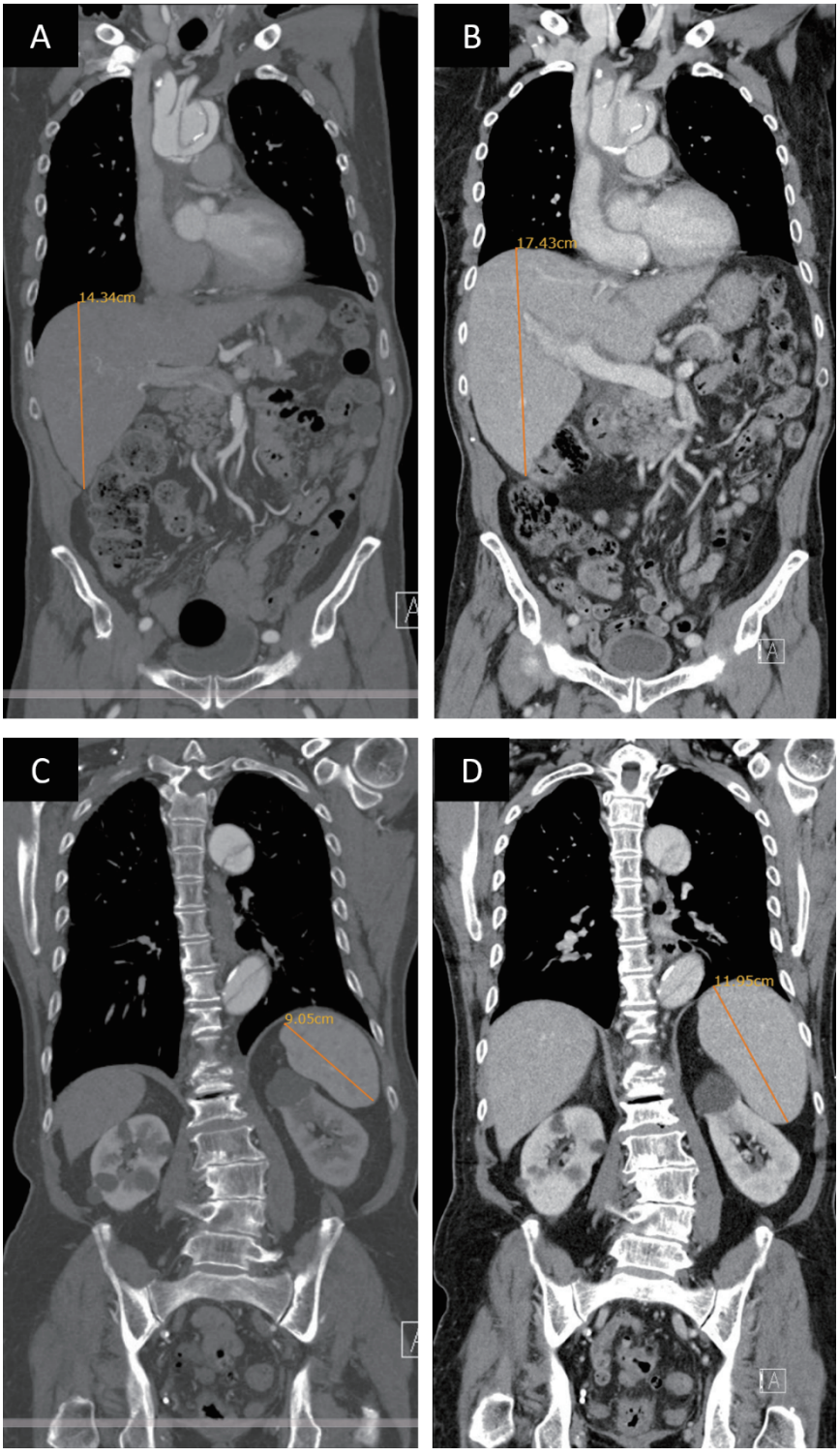

Fig. 1. Contrast-enhanced computed tomographic images showing enlarged liver and spleen. (A) Liver (14.34 cm) 1 month prior to the ER visit. (B) Liver (17.43 cm) at the time of the ER visit. (C) Spleen (9.05 cm) 1 month prior to the ER visit. (D) Spleen (11.95 cm) at the time of the ER visit.

IgM were all negative. His ceruloplasmin level was also within the normal limits (38 mg/dL). Tests for antinuclear antibodies, anti-smooth muscle antibodies, and antimitochondrial antibodies were negative. A test for Epstein-Barr virus IgG antibody was positive, and a test for IgM antibody was negative, which is compatible with prior infection. Later, the results for tests of leptospirosis, scrub typhus, hantavirus, and severe fever with thrombocytopenia syndrome were all negative.

At 3 days after admission, the patient developed melena and shock along with acute kidney injury. Hemorrhagic or septic shock was suspected. Meropenem was added to control suspected sepsis. Upper gastrointestinal endoscopy revealed some actively bleeding ulcers over the esophagogastric junction and duodenum, which were treated with epinephrine injection and endoscopic hemoclips. With unstable hemodynamics he was intubated and transferred to the intensive care unit. Multiple sets of blood cultures were negative, and one set of sputum culture indicated few mold fungus. Sputum acid-fast staining revealed 1-2 acid-fast bacilli/300 fields (scanty). The sputum polymerase chain reaction for

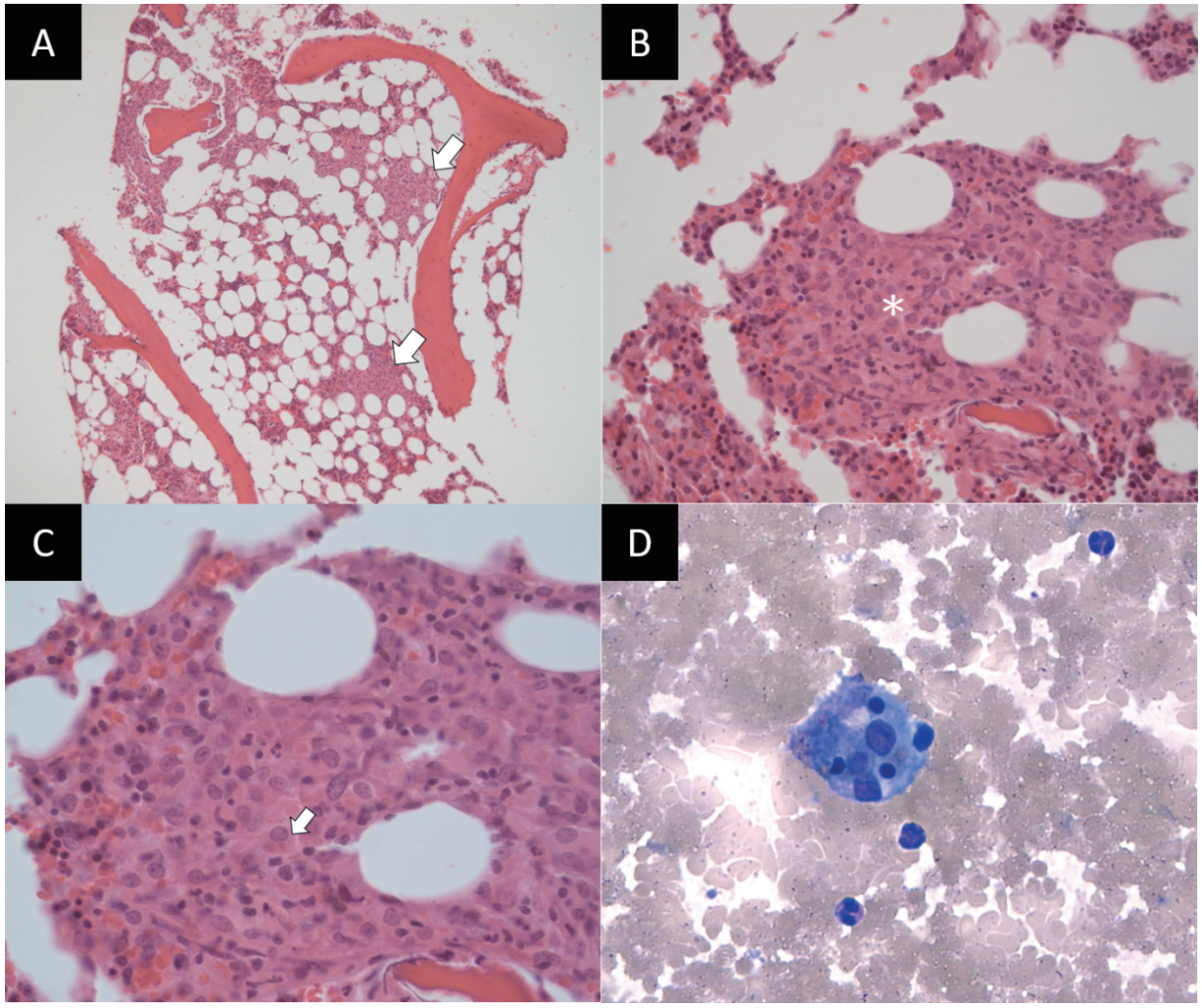

Fig. 2. Bone marrow biopsy (A, B, C) and bone marrow aspirate (D). (A) Bone marrow granulomas (white arrows), hematoxylin-eosin, magnification: 100×. (B) Aggregates of histiocytes (white asterisk), hematoxylin-eosin, magnification: 200×. (C) Phagocytosis of red blood cells (white arrow), hematoxylin-eosin, magnification: 400×. (D) Hemophagocyte with ingested red blood cells, white blood cells, and platelets (Wright's stain, magnification: 400×).

the *Mycobacterium tuberculosis* complex was positive. Bronchoalveolar lavage using fiberoptic bronchoscopy was positive for *Aspergillus* antigen, and acid-fast staining revealed 1-9 acid-fast bacilli/100 fields (1+). Amphotericin B liposome was added for pulmonary aspergillosis, whereas antituberculous treatment was not given because of the patient's impaired liver function. Fungal culture of the bronchoalveolar lavage fluid yielded *Aspergillus fumigatus* 4 days later.

During hospitalization, refractory thrombocytopenia and anemia persisted despite multiple transfusions. The lowest platelet count was 25,000 cells/μL, and the lowest hemoglobin concentration was 7.0 g/dL. Bone marrow aspiration and biopsy showed increased histiocytes with hemophagocytosis, while no evidence of malignancy **(**Figure. 2**)**. His blood tests at 10 days after admission revealed hyperferritinemia (11864.7 ng/mL). Hemophagocytic lymphohistiocytosis associated with *M. tuberculosis* and *Aspergillus* infection was diagnosed. However, sepsis rapidly progressed, and the acute kidney injury could not be corrected with continuous venovenous hemofiltration. The patient died the day after the bone marrow examination before HLH treatment could be initiated. One month later, sputum and bronchoalveolar lavage fluid cultures confirmed the infection of *M. tuberculosis* complex susceptible to ethambutol, isoniazid, rifampin, and streptomycin. The tests for determining the trigger factors of HLH were summarized in Table 2.

Table 2. The results of possible trigger factors of HLH

Possible Trigger Factor	Result
Hepatitis B Virus Surface Antigen	Negative
Hepatitis C Virus Antibody	Negative
Hepatitis A Virus IgM Antibody	Negative
Cytomegalovirus IgM	Negative
Human Immunodeficiency Virus Screening Test	Negative
Serological Test for Syphilis - Rapid Plasma	Negative
Herpes Simplex Virus 1+2 IgM	Negative
Ceruloplasmin	38 mg/dL
Antinuclear Antibodies	Negative
Anti-Smooth Muscle Antibodies	Negative
Antimitochondrial Antibodies	Negative
Epstein-Barr Virus IgG Antibody	Positive
Epstein-Barr Virus IgM Antibody	Negative
Leptospirosis	Negative
Scrub Typhus	Negative
Hantavirus	Negative
Severe Fever with Thrombocytopenia Syndrome	Negative
Fungal Test	*Aspergillus fumigatus*
Tuberculosis Test	*Mycobacterium tuberculosis*

Discussion

HLH is a life-threatening condition that was first described by Scott and Robb-Smith in 1939[6]. It can be classified as having primary and secondary (acquired or reactive) forms on the basis of its underlying etiology. Primary HLH primarily affects children and is characterized by genetic mutations that impact immune cell function. In this form, viral infections are often implicated as triggers for HLH development. On the other hand, secondary HLH can occur in individuals of any age and is associated with various underlying conditions. Secondary HLH can be associated with infections, malignancies (especially hematological malignancies like lymphomas or leukemias), rheumatologic disorders, or immune deficiency syndromes, with infections being the most prevalent triggers. In a study reviewing 2,197 cases of adult HLH, 50% of the cases were associated with viral, bacterial, parasite, or fungal infections. Viral infections, especially Epstein-Barr virus, were the most frequent triggers of adult HLH. Bacterial infections were reported in 9% of the cases (206/2,197 patients), of which 38% were related to *M. tuberculosis* infection[7]. HLH secondary to fungal infections is less common (37/2,197 patients) and is usually related to opportunistic infections in immunocompromised patients[7].

Diagnosing HLH is challenging because of no unique clinical or laboratory indicators have been identified for the syndrome. The diagnostic guidelines for HLH were proposed by the Histiocyte Society in 1991 and updated in 2004 (Table 3)[4]. The HScore, another diagnostic system developed by Fardet et al. in 2014 (Table 4)[8], is used to estimate a patient's risk of having secondary HLH on the basis of clinical, biologic, and cytologic variables. Our patient met six of the eight criteria for HLH: fever, splenomegaly, cytopenias (anemia and thrombocytopenia), hypertriglyceridemia and hypofibrinogenemia, hemophagocytosis in bone marrow without evidence of malignancy, and hyperferritinemia. The

Table 3. Diagnostic Guidelines for HLH

HLH-2004 Diagnostic Criteria
The diagnosis of HLH is established if either (1) or (2) is fulfilled.
(1) A molecular diagnosis consistent with HLH
(2) The presence of five of eight of the following diagnostic criteria for HLH
(A) Initial diagnostic criteria (to be evaluated in all patients with HLH)
Fever
Splenomegaly
Cytopenias (affecting ≥2 of 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
No evidence of malignancy
(B) New diagnostic criteria
Low or absent NK cell activity (according to local laboratory reference)
Ferritin ≥ 500 mg/L
Soluble CD25 (i.e., soluble IL-2 receptor) ≥ 2,400 U/mL

Adapted from reference 4.

Table 4. HScore

Parameter	No. of points (criteria for scoring)
Known underlying immunosuppresion*	0 (no) or 18 (yes)
Temperature (°C)	0 (<38.4), 33 (38.4-39.4), 49 (>39.4)
Organomegaly	0 (no), 23(hepatomegaly or splenomegaly), 38 (hepatomegaly and splenomegaly)
No. of cytopenias†	0 (1 lineage), 24 (2 lineages), 34 (3 lineages)
Ferritin (ng/mL)	0 (<2,000), 35 (2,000-6,000), 50 (>6,000)
Triglyceride (mmol/L)	0 (<1.5), 44 (1.5-4), 64 (>4)
Fibrinogen (g/L)	0 (>2.5) or 30 (≤2.5)
Serum glutamine oxaloacetic transaminase (IU/L)	0 (<30) or 19 (≥30)
Hemophagocytosis features on bone marrow aspirate	0 (no) or 35 (yes)

*HIV positive or receiving long-term immunosuppressive therapy (i.e., glucocorticoids, cyclosporine, and azathioprine).

†Defined as a hemoglobin level of ≤9.2 g/dL and/or a leukocyte count of ≤5,000/mm^3 and/or a platelet count of ≤110,000/mm^3.
Adapted from reference 8.

CD25 level could not be determined in our hospital. The HScore was 289, indicating a >99% probability of secondary HLH and supporting the diagnosis of HLH secondary to *M. tuberculosis* and *Aspergillus* infection.

Because HLH is a rare condition, the actual incidence is difficult to assess, and no controlled trials have been performed to determine its standardized treatment. Treatment goals for secondary HLH include eliminating triggers (mainly infection and malignancy) and suppressing inflammatory responses and cell proliferation[8]. Treating the inciting pathogen is important for infection-related HLH but might not be sufficient by itself, and immunosuppressive and cytotoxic agents are often necessary in severe cases[7,10]. Chemotherapy using dexamethasone, etoposide, and cyclosporine A, as proposed in the HLH-94 and HLH-2004 protocols[4], has been effective in both children and adults with HLH[7,11]. Some studies have reported successful treatment with intravenous immunoglobulin (IVIG)[12-14]. Biological agents inhibiting different targets in the hyperinflammatory response, such as anakinra (interleukin-1 receptor antagonist), tocilizumab (interleukin-6 receptor antagonist), rituximab (anti-CD20 antibody), alemtuzumab (anti-CD52 antibody), and ruxolitinib (JAK1/2 inhibitor), have been reported to be effective in some case reports and uncontrolled studies[11].

Most cases with *M. tuberculosis*-associated HLH have been managed with antituberculous treatment with or without steroids instead of etoposide or cyclosporine A. Brastianos et al. reviewed 37 cases of *M. tuberculosis*-associated HLH[15]; of them, 29 received antituberculous treatment alone (9 patients, with 7 survivors) or with immunomodulatory treatment (20 patients, with 12 survivors), and 8 received no treatment (no survivors). The immunomodulatory treatment mostly consisted of high-dose steroids although some patients received splenectomy, plasma exchange, intravenous immunoglobulin, and epipodophyllotoxin. The overall mortality rate was 48.6% (18/37), and the mortality rate of the patients who received antituberculous treatment was 34.5% (10/29). While the overall mortality rate in *M. tuberculosis*-associated HLH remains high, the findings from this study suggest that early diagnosis and prompt treatment of both HLH and tuberculosis

may improve patient outcomes. Our case raises an important point regarding the potential challenges in managing HLH secondary to infection when the patient has impaired liver function and cannot tolerate standard anti-TB treatment. In such cases, alternative treatment options might be considered. According to the Taiwan Guidelines for TB Diagnosis & Treatment, a combination of a second-line injectable agent, ethambutol, and a fluoroquinolone may be used in patients with impaired hepatic function. This approach could have potentially targeted the tuberculosis infection while considering the patient's liver function. Future research and clinical experience should continue to explore optimal treatment strategies, taking into account the specific characteristics of each individual case and considering alternative treatments when standard options are contraindicated.

HLH associated with fungal infections is less frequent and often occurs in the setting of AIDS, lymphoma, chronic steroid use, and transplants[7]. Seven cases of HLH secondary to aspergillosis have recently been reported[16]. All seven patients received antifungal therapy for aspergillosis: two received IVIG; two received intravenous steroids; one received anakinra; one received a combination of etoposide, steroids, and tocilizumab; and one received no specific HLH treatment. Four of the seven patients survived, including the patient treated with only voriconazole. One of the patients was a 47-year-old liver transplant recipient recovering from severe COVID-19 and was given a diagnosis of HLH secondary to invasive pulmonary aspergillosis and tuberculosis[17]. He was successfully treated with IVIG, liposomal amphotericin B, and antituberculous therapy. Our patient, however, was only treated with amphotericin B liposome for aspergillosis and did not receive antituberculous therapy or immunosuppressive or cytotoxic agents for HLH, which may explain the rapid deterioration and poor outcome.

In conclusion, HLH is a highly fatal disease if left untreated, and its diagnosis can be delayed because of its nonspecific presentations. HLH should be considered as a differential diagnosis in patients presenting with fever, cytopenias, and organomegaly. Detection of hyperferritinemia, hypofibrinogenemia, and hypertriglyceridemia may serve as a crucial hint for an HLH diagnosis, whereas an absence of bone marrow hemophagocytosis should not be sufficient to rule out the possibility of HLH. In the management of infection-associated HLH, although pathogen-directed treatments alone may not be sufficient, identification of suspicious pathogens followed by timely antimicrobial therapy is crucial and might improve the outcome of some patients.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

This manuscript was edited by Wallace Academic Editing

References

1. Larroche C, Mouthon L. Pathogenesis of hemophagocytic syndrome (HPS). Autoimmunity reviews 2004;3(2):69-75.
2. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. Hematology Am Soc Hematol Educ Program 2009;2009:127-31.
3. George MR. Hemophagocytic lymphohistiocytosis: review of etiologies and management. J Blood Med 2014;5:69-86.
4. Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48(2):124-31.
5. Janka GE. Hemophagocytic syndromes. Blood Rev 2007;21(5):245-53.
6. Scott R, Robb-Smith AH. Histiocytic medullary reticulosis. Lancet 1939;234(6047):194-8.
7. Ramos-Casals M, Brito-Zerón P, López-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. Lancet 2014;383(9927):1503-16.
8. Fardet L, Galicier L, Lambotte O, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol 2014;66(9):2613-20.
9. Verbsky JW, Grossman WJ. Hemophagocytic

lymphohistiocytosis: diagnosis, pathophysiology, treatment, and future perspectives. Ann Med 2006;38(1):20-31.
10. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infections associated with haemophagocytic syndrome. Lancet Infect Dis 2007;7(12):814-22.
11. La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. Blood 2019;133(23):2465-77.
12. Freeman B, Rathore MH, Salman E, Joyce MJ, Pitel P. Intravenously administered immune globulin for the treatment of infection-associated hemophagocytic syndrome. J Pediatr 1993;123(3):479-81.
13. Larroche C, Bruneel F, Andre MH, et al. [Intravenously administered gamma-globulins in reactive hemaphagocytic syndrome. Multicenter study to assess their importance, by the immunoglobulins group of experts of CEDIT of the AP-HP]. Ann Med Interne (Paris) 2000;151(7):533-9.
14. Emmenegger U, Spaeth PJ, Neftel KA. Intravenous immunoglobulin for hemophagocytic lymphohistiocytosis? J Clin Oncol 2002;20(2):599-601.
15. Brastianos PK, Swanson JW, Torbenson M, Sperati J, Karakousis PC. Tuberculosis-associated haemophagocytic syndrome. Lancet Infect Dis 2006;6(7):447-54.
16. Ali RS, Sen M, Tan IJ. Pulmonary aspergillosis complicated by hemophagocytic lymphohistiocytosis: a case report and literature review. Cureus 2022;14(10):e30908.
17. Gandotra A, Mehtani R, Premkumar M, et al. Invasive pulmonary aspergillosis and tuberculosis complicated by hemophagocytic lymphohistiocytosis-sequelae of COVID-19 in a liver transplant recipient. J Clin Exp Hepatol 2022;12(3):1007-11.

結核菌與麴菌感染相關噬血球症候群之案例報告

陳奕孝[1] 林裕民[1,2,3] 林瑛珠[1,4]

1新光吳火獅紀念醫院 內科;
2新光吳火獅紀念醫院 胃腸肝膽科;
3輔仁大學 醫學系;
4新光吳火獅紀念醫院 血液腫瘤科

摘 要

噬血球症候群是一種致命性的過度發炎反應，其臨床特徵包括發燒、肝脾腫大、血球低下。噬血球症候群依病因可分為原發性(與基因缺陷相關)或次發性(肇因於感染、惡性腫瘤、自體免疫疾病等)，前者多發生於兒童，後者則以成人較常見。至今大部分的診斷及治療指引以兒童病患為主，目前尚缺乏針對成人噬血球症候群的標準治療。此案例報告是一位80歲男性，表現發燒、肝脾腫大、血小板低下及貧血，診斷為結核菌與麴菌感染相關之噬血球症候群。