Leuconostoc pseudomesenteroides-associated hemophagocytic syndrome: A case report

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Abstract. Hemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory syndrome characterized by fever, pancytopenia and splenomegaly. The underlying hemophagocytosis occurs primarily in the bone marrow, liver and lymph nodes. Multiple microbiological agents, including cytomegalovirus, Epstein-Barr virus and Mycobacterium tuberculosis, have been implicated in the pathogenesis of HLH. The present study presents a case of HLH associated with Leuconostoc pseudomesenteroides infection treated successfully with clindamycin. A 33-year-old man presented with recurrent episodes of fever and diarrhea. Upon initial treatment at another hospital (the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China), blood chemistry analysis demonstrated moderate anemia (hemoglobin 88 g/l; reference range, 120.0-160.0), elevated ferritin (1,068.47 mg/l; reference range, 4.0-5.5x10⁶), conjugated bilirubin (215.7 mmol/l; reference range, 0-10.0), and y-glutamyl transpeptidase (150 U/l; reference range, 5.1-28.0), total bilirubin (392.4 mmol/l; reference range, 0-10.0), and y-glutamyl transpeptidase (150 U/l; reference range, 10-60). The patient was treated with antibiotics for suspected pneumonia and cholecystitis, but new symptoms (including diarrhea and inflammatory colitis) started to emerge. The patient was subsequently treated with ganciclovir for suspected cytomegalovirus infection (4) and bacterial infection (6). Hemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory syndrome that is typically characterized by fever, pancytopenia, and splenomegaly (1). Other features include hypertriglyceridemia, hypofibrinogenemia, liver dysfunction, and elevated ferritin (1). A major underlying pathology is hemophagocytosis, which mainly occurs in the bone marrow, spleen and lymph nodes (1). Hemophagocytic syndrome may be associated with malignancies (malignancy-associated hemophagocytic), hereditary diseases and autoimmune diseases (2). It is also linked with Epstein-Barr virus (EBV) infection (2), parainfluenza virus infection (3), cytomegalovirus (CMV) infection (4) and bacterial infection caused by Mycobacterium tuberculosis (M. tuberculosis) (5) and Mycoplasma pneumonia (6). Hemophagocytic syndrome that is associated with infections has been suggested to be a result of immunological activation of the immune system, and is sometimes referred to as secondary HLH (sHLH) (1). Pathologically, hemophagocytosis occurs in the bone marrow and other tissues, where activated macrophages engulf erythrocytes, leukocytes, platelets and their precursors (2). An improved understanding of the pathophysiology of HLH may aid to clarify the interactions between the immune system and infectious agents (2).

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory syndrome that is typically characterized by fever, pancytopenia, and splenomegaly (1). Other features include hypertriglyceridemia, hypofibrinogenemia, liver dysfunction, and elevated ferritin (1). A major underlying pathology is hemophagocytosis, which mainly occurs in the bone marrow, spleen and lymph nodes (1). Hemophagocytic syndrome may be associated with malignancies (malignancy-associated hemophagocytic), hereditary diseases and autoimmune diseases (2). It is also linked with Epstein-Barr virus (EBV) infection (2), parainfluenza virus infection (3), cytomegalovirus (CMV) infection (4) and bacterial infection caused by Mycobacterium tuberculosis (M. tuberculosis) (5) and Mycoplasma pneumonia (6). Hemophagocytic syndrome that is associated with infections has been suggested to be a result of immunological activation of the immune system, and is sometimes referred to as secondary HLH (sHLH) (1). Pathologically, hemophagocytosis occurs in the bone marrow and other tissues, where activated macrophages engulf erythrocytes, leukocytes, platelets and their precursors (2). An improved understanding of the pathophysiology of HLH may aid to clarify the interactions between the immune system and infectious agents (2).

Leuconostoc pseudomesenteroides infection typically occurs in patients with underlying diseases or a history of vancomycin use (7-9). It was first reported in humans in 2 immuno-compromised patients in 1985 (7), and later observed in patients receiving solid organ transplants (8). An outbreak of Leuconostoc mesenteroides (a subspecies of Leuconostoc) involving 6 patients receiving parenteral nutrition in northwest Spain has also been reported (9). However, there has been no report of acquired HLH secondary to...
**Leucnostoc pseudomesenteroides** infection to the best of our knowledge.

In the present report, we present a case of sHLH with *L. pseudomesenteroides* bacteremia. The patient presented with recurrent episodes of fever, diarrhea, severe anemia, liver dysfunction and elevated levels of ferritin. HLH associated with non-viral pathogens often respond to treatment of the underlying infection (2). The present case was successfully managed with clindamycin. The present findings expand the spectrum of possible microbiological agents implicated in hemophagocytic syndrome.

**Case report**

A 33-year old man presented with recurrent episodes of fever and diarrhea for 3 months. The patient was treated at, and discharged from another hospital (the First Affiliated Hospital of Guangzhou University, Guangzhou, China) before presenting to us (the First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China). Previous medical history included unexplained anemia and splenomegaly. The patient denied previous blood transfusion. The patient was married and had 2 children and there was no family history of familial disease except for thalassemia in a sister. The patient provided written informed consent for the presentation of his case as a report.

The fever (>40°C) started 3 months prior to admission to our hospital, and the patient had received medical attention at the First Affiliated Hospital of Sun Yat-sen University 7 days after the first presentation of symptoms. A blood chemistry panel conducted at the First Affiliated Hospital of Sun Yat-sen University included hemoglobin (88 g/l reference range, 120.0-160.0), ferritin (1.068.47 mg/l; reference range, 21.81-274.66), total bilirubin (392.4 mmol/l; reference range, 5.1-28.0), conjugated bilirubin (335.7 mmol/l; reference range, 0-10.0), γ-glutamyl transpeptidase (γ-GT; 150 U/l; reference range, 10-60) and fibrinogen (1.53 g/l; reference range, 2.0-4.0). The medical record revealed that the patient received mechanical ventilation from day 1 to day 16 after admission for respiratory failure. The patient received antibiotic treatment for suspected pneumonia and cholecystitis. After 10 days, the patient developed diarrhea with watery stool (4-6 l/day) but no mucus or blood. He received treatment with itraconazole and norvancomycin, (details not available) but did not improve. Colonoscopy 6 days later identified inflammatory colitis. Immunohistochemical analysis of colon biopsy specimen identified CMV. Treatment with ganciclovir was initiated at a dose of 5 mg/kg/day for 1 month and the patient persistently (40°C) and the patient was transferred to the intensive care unit 10 days after admission. CMV-immunoglobulin M testing (cat. no. 315A; DIESSE Diagnostica Senese S.p.A., Siena, Italy) of the peripheral blood yielded a negative result. T lymphocyte count was measured, including cluster of differentiation (CD)3+ (79.2.2%; reference range, 50-84%), CD4 (38.2%; reference range, 27-51%), CD8 (35.3%; reference range, 15-44%), T helper/suppressor cell ratio (1.08; reference range, 0.71-2.78). The patient's hemoglobin was 39 g/l. Red blood cell count was 1.61x10^12/l (reference range, 4.0-5.5x10^12/l); leukocyte count was 2.67x10^9/l (reference range, 2.0-7.5x10^9/l); and platelet count was 57.1x10^9/l (reference range, 100-400x10^9/l). Ferritin was substantially elevated (7,931.21 mg/l). Blood chemistry analysis identified a total bilirubin value of 295.5 mmol/l and conjugated bilirubin value of 215.7 mmol/l. Urinalysis was conducted according to the AVE 764+752 (Fully Automated Urinalysis system; AVE Science & Technology Co., Ltd., Changsha, China); The dipstick test for occult blood detected the peroxidase activity of RBCs. The following scoring system was used: +, indicated ≥3 RBCs was present and therefore this suggested the urine was positive for blood; ++, indicated high blood content. The normal reference value of urinary occult blood was 0 (-, negative) and the normal reference value of urinary bilirubin was 0 (-, negative). Furthermore, normal reference value of erythrocytes: 0-1 RBCs/high power lens field (HPF).

Urinalysis identified occult blood (+), urinary bilirubin (+++) and 10 erythrocytes/HPF. An abdominal computed tomography (CT) scan identified splenomegaly, multiple gall bladder stones and inflammatory changes in the gall bladder (gall bladder was enlarged, the wall was thickened). Wright-Giemsa staining was performed at room temperature for 10 min on bone marrow smears using Wright stain (cat. no. MKBH5619; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Samples were viewed using a light microscope (magnification, x1,000). A bilateral iliac crest bone marrow biopsy identified active hemophagocytosis and phagocytosis of nucleated red blood cells and platelets (Fig. 1). A whole-body positron emission tomography/CT scan revealed no tumor mass. Peripheral blood and bone marrow aspiration sample specimens were obtained using a strict aseptic technique and injected into culture agar (blood agar, cat. no. 1004144770; bioMerieux, Craponne, France). The positive cells (1.5x10^6 cells per plate) were cultured in a Colombian blood plate and the bacteria were grown for 48 h. *L. pseudomesenteroides* was identified using a VITEK 2 GP Test kit (cat. no. 242347540, microbial identification platform, VITEK2 Compact system; BioMerieux). Results from a stool culture for *Clostridium difficile* (C. difficile) was negative. A blood and bone marrow aspirate culture
were obtained on day 10 after admission, and results came back positive on day 14 after admission for *L. pseudomesenteroides*. The Kirby-Bauer disc method was used to conduct a drug sensitivity test using Mueller-Hinton blood agar plates and disks filled with specific antibiotics, including penicillin (10 units), xefotaxime (30 µg), cefepime (30 µg), clindamycin (2 µg), levofloxacin (5 µg), chloramphenicol (30 µg) vancomycin (30 µg), linezolid (30 µg), ampicillin (10 µg) and ceftriaxone (30 µg). Plates were incubated at 37˚C for 24 h. Subsequently, the diameters of the zone of inhibition (ZOI) were calculated. The drug sensitivity test indicated that *L. pseudomesenteroides* was vancomycin-resistant and clindamycin-sensitive.

In addition to clindamycin (150 mg, taken orally every 12 h for 1 week), the patient received 4 units of red blood cell suspension and 350 ml frozen plasma and partial parenteral nutrition. The fever subsided rapidly (37.0˚C) after 1 day of clindamycin therapy and the patient became afebrile after 7 days of clindamycin therapy.

A repeat blood culture 10 days later identified *L. pseudomesenteroides*. Blood chemistry 5 days later revealed improvement in hemoglobin (77 g/l), red blood cell count (3.05x10¹²/l), platelet count (139x10⁹/l), and total (71.5 mmol/l) and conjugated bilirubin (45.2 mmol/l). Body temperature returned to normal (36.5˚C) and the volume of stool was reduced to 350 ml/day. A repeat blood culture 2 weeks later was negative for *L. pseudomesenteroides*. One week later, the patient requested discharge due to financial concern.

A telephone follow-up 2 weeks later revealed that the patient continued treatment (details not available) at Pengpai Memorial Hospital (Shanwei, China). Blood culture for *L. pseudomesenteroides* was negative based on patient disclosure. The patient visited our hospital 8 months later and a complete blood count test was performed; he continued to be anemic (hemoglobin at 89 g/l) but had no other complaints.

**Discussion**

Hemophagocytic lymphohistiocytosis is a hyperinflammatory syndrome that may become fatal if not treated appropriately. Multiple microbiological agents, including CMV, EBV and *M. tuberculosis* have been implicated in the pathogenesis of HLH (3-6). HLH has predominantly been reported in
children (10), but may occur in people of all ages. The current report indicates that *L. pseudomesenteroides* infection may also be associated with HLH.

To the best of our knowledge, fewer than 10 cases of *L. pseudomesenteroides* infections have been reported so far (11-14), all of which occurred in adults. Patients who have received vancomycin are particularly vulnerable (7-9). The patient in the present case received multiple antibiotics for suspected pneumonia and choledochitis, and subsequently norvancomycin for suspected *C difficile* pseudomembranous colitis. The patient exhibited clinical, laboratory and histopathological abnormalities (Table I) indicative of hemorrhagic lymphohistiocytosis, according to previously described diagnostic guidelines (1).

A number of cases of *Leuconostoc* spp. infection have previously been reported (15-19). In contrast, few cases of *L. pseudomesenteroides* infection have been documented. *L. pseudomesenteroides* infection may occur in patients with underlying diseases and/or a history of vancomycin use (11,12). Cappelli *et al* (11) reported 5 clinical isolates of *L. pseudomesenteroides* in nosocomially acquired urinary tract infections; all 5 isolates were sensitive to clindamycin. Furthermore, a drug sensitivity test in our hospital indicated resistance to vancomycin. Tholpady *et al* (12) observed that *Leuconostoc* sepsis in a 64-year-old liver transplant recipient following extended exposure to vancomycin.

*L. pseudomesenteroides* infections are typically treated with clindamycin, as in the current case and other reported cases (8,12). The patient in the present case responded well to clindamycin. The fever subsided (37.0°C) after 1 day of clindamycin therapy and the patient became afebrile after 7 days of clindamycin therapy. Although the patient remained positive for *L. pseudomesenteroides* 10 days later, clinical features (as reflected by liver function and hemoglobin) improved substantially. A blood culture 2 weeks later was negative for *L. pseudomesenteroides*.

*L. pseudomesenteroides* is intrinsically resistant to vancomycin and the majority of reports indicate that it is sensitive to clindamycin (8,12). Therefore, appropriate antibiotic therapy based on drug sensitivity results should be administered, and in the absence of drug sensitivity data, clindamycin may be preferred. Supportive therapy and treatment of the underlying diseases should also be initiated.

In conclusion, HLH may be associated with *L. pseudomesenteroides* infection. The present case expanded the spectrum of microbiological agents implicated in HLH and suggested that such patients may respond to treatment with clindamycin.

References

1. Henter JI, Horne A, Aričo M, Egerer RM, Filipovich AH, Imashuku S, Ladds S, McClain K, Webb D, Winiarski J and Janka G: HLH-2004: Diagnostic and therapeutic guidelines for hemorrhagic lymphohistiocytosis. Pediatr Blood Cancer 48: 124-131, 2007.

2. Fisman DN: Hemophagocytic syndromes and infection. Emerg Infect Dis 6: 601-608, 2000.

3. Beffermann N, Pilocante J and Sarmiento M: Acquired hemophagocytic syndrome related to parainfluenza virus infection: Case report. J Med Case Rep 9: 78, 2015.

4. Halfon P, Retornaz F, Mathieu D, Helbert T, Philibert P and Pégislasso H: Virus-associated hemophagocytic syndrome related to acute CMV and HBV sexual co-infection: A case report. J Clin Virol 46: 189-191, 2009.

5. Hui YM, Pillinger T, Luqmani A and Cooper H: Haemophagocytic lymphohistiocytosis associated with *Mycobacterium tuberculosis* infection. BMJ Case Rep 2015: bcr2014208220, 2015.

6. Hibino M, Sato S, Shimizu T, Yamamoto S, Ohe M and Kondo T: Hemophagocytic lymphohistiocytosis secondary to *Mycoplasma pneumoniae* infection without pneumonia. Intern Med 53: 1679-1683, 2014.

7. Bunt-Hoi A, Branger C and Acar JF: Vancomycin-resistant streptococci or *Leuconostoc* spp. Antimicrob Agents Chemother 28: 458-460, 1985.

8. Espinosa R, Kusne S, Pascale AW, Wada S, Fung J and Rakela J: *Leuconostoc pseudomesenteroides* after liver transplantation: another cause of vancomycin resistant gram-positive infection. Clin Transplant 11: 322-324, 1997.

9. Bou G, Salet J, Sáez Nieto JA, Tomás M, Valdezate S, Sousa D, Lueto F, Villanueva R, Jose Pereira M and Llinares P: Nosocomial outbreaks caused by *Leuconostoc pseudomesenteroides* subsp mesenteroides. Emerg Infect Dis 14: 968-971, 2008.

10. Arico M, Janka G, Fischer A, Henter JI, Blanche S, Elinder G, Martinetti M and Rusca MP: Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. FHL Study Group of the Histioyte Society. Leukemia 10: 197-203, 1996.

11. Cappelli EA, Barros RR, Camello TC, Teixeira LM and Merquior VL: *Leuconostoc pseudomesenteroides* as a cause of nosocomial urinary tract infections. J Clin Microbiol 37: 4124-4126, 1999.

12. Tholpady SS, Sifri CD, Sawyer RG, Hazen KC, Pruett TL and Bonatti H: *Leuconostoc pseudomesenteroides* blood stream infection following liver transplantation. Ann Transplant 15: 61-66, 2010.

13. Rodríguez J, Saavedra J, Fernández-Jurado A and Prados D: *Leuconostoc pseudomesenteroides* bacteremia. Sangre 44: 82-83, 1999 (In Spanish).

14. Azendon H, Lahlo J, Massou S, Bakhri H and Haimeur C: *Leuconostoc mesenteroides* bacteremia. Ann Fr Anesth Reanim 27: 457-458, 2008 (In French).

15. Friedland IR, Snipeliski M and Khoosal M: Meningitis in a neonate caused by *Leuconostoc* sp. J Clin Microbiol 28: 2125-2126, 1990.

16. Giaconetti A, Ranaldi R, Siquini FM and Scalise G: *Leuconostoc citreum* isolated from lung in AIDS patient. Lancet: 342 622, 1993.

17. Gollede CL: Infection due to *Leuconostoc* species. Rev Infect Dis 13: 184-185, 1991.

18. Green M, Wadowsky RM and Barbadora K: Recovery of *Leuconostoc citreum* isolated from lung in AIDS patient. Lancet: 342 622, 1993.

19. Handwerger S, Horowitz H, Coburn K, Kolokathis A and Merquior VL: *Leuconostoc pseudomesenteroides* bacteremia. J Med Case Rep 9: 78, 2015.