CASE SERIES

INFECTION-ASSOCIATED HEMOPHAGOCYTIC SYNDROME AMONG PATIENTS WITH DENGUE SHOCK SYNDROME AND INVASIVE ASPERGILLOSIS: A CASE SERIES AND REVIEW OF THE LITERATURE

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Abstract. The authors report four autopsy cases of previously healthy children with dengue shock syndrome complicated with infection-associated hemophagocytosis and invasive aspergillosis. Hemophagocytosis is confirmed by histopathology of autopsied reticuloendothelial organs. All four children were identified to have invasive aspergillosis by histopathology and three cases were positive on fungal culture for *Aspergillus* spp. Regarding the cause of death among the four children without pre-existing underlying disease, three cases were directly ascribable to invasive aspergillosis and the remaining case was ascribed to dengue shock syndrome. The transmigration of preexisting fungi from the respiratory mucosa damaged by the dengue shock process is postulated as the pathogenesis of invasive aspergillosis. The main predisposing factor was found to be prolonged dengue shock syndrome. We reviewed the clinicopathologic features and therapeutic management of infection-associated hemophagocytic syndrome in patients with dengue shock syndrome and invasive aspergillosis.

Keywords: dengue shock syndrome, dengue hemorrhagic fever, infection-associated hemophagocytosis, invasive aspergillosis

INTRODUCTION

Dengue infection is one of the most important public health problems in tropical countries. Dengue fever is high on the list of mosquito-borne diseases that may worsen with global warming. The manifestations of dengue infection include non-specific febrile illness, dengue fever, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Hemophagocytic syndrome (HS) represents a severe hyperinflammatory condition and is characterized by histiocytic proliferation, hemophagocytosis, fever, hepato-

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splenomegaly, and hypofibrinogenemia. Dengue virus-associated with HS has been reported (Bhamarapravati *et al*, 1967; Wong *et al* 1991; Jain and Singh, 2008; Srichaikul *et al*, 2008; Lei, 2009). The degree of hemophagocytosis ranges from mild to severe. Systemic mycosis is usually encountered as an opportunistic infection in immunocompromised patients, but rarely occurs in dengue infection. Concurrent infection-associated hemophagocytosis (IAH) in patients with DSS and invasive aspergillosis has never been reported.

A complete autopsy was performed on the four patients. DSS was confirmed by clinical criteria and laboratory findings showing dengue viral capsid antigen-immunoglobulin M (IgM) on enzyme-linked immunosorbent assay and/or polymerase chain reaction (PCR) for dengue virus. Classification of the types of dengue infection followed World Health Organization guidelines (WHO, 1997).

This present study was approved by the committee on human research at the Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID08-51-12).

CASE SERIES

Case 1

A 2-year-old girl presented with fever for 3 days. On physical examination she had fever of 40.5°C and tachypnea. She was hypotensive and had tachycardia. A touniquet test was positive. The liver span was 12 cm. Laboratory examination showed thrombocytopenia and a hematocrit of 54%. The provisional diagnosis was DHF with impending shock. She was started on intravenous fluid resuscitation. She had capillary leak as evidenced by ascites and a right pleural effusion. Upper gastrointestinal bleeding, epistaxis, and bleeding per endotracheal tube were detected. Despite a high fluid infusion rate and administration of dopamine, packed red cells and fresh frozen plasma, hypotension persisted. Dobutamine was given and exchange transfusion was carried out. She had steady improvement until day 13 when she developed high-grade fever. Chest radiography showed bilateral alveolar infiltration. A complete blood count (CBC) revealed bicytopenia. Systemic antibiotics were given. A bronchoalveolar larvage (BAL) was performed and a culture of this specimen yielded *Aspergillus fumigatus*. She was started on intravenous amphotericin B. She succumbed to multiorgan failure twenty days after the diagnosis of dengue infection. The autopsy was performed and the results are showed in Table 1.

Case 2

An 8-year-old boy was referred to the intensive care unit (ICU) with fever for 6 days. On physical examination he had fever and a hepatomegaly without splenomegaly. The laboratory examination showed thrombocytopenia and a hematocrit of 33%. The chest radiography revealed bilateral pleural effusions. A provisional diagnosis of DHF with impending shock was made. He was treated with exchange transfusion, peritoneal dialysis and plasmapheresis. Over the next 24 hours, his condition worsened rapidly, and he developed upper gastrointestinal bleeding, epistaxis, hepatic failure, acute renal failure, coma, and acute respiratory distress syndrome. On the second day, cardiac arrest occurred and resuscitation efforts failed. Autopsy disclosed pulmonary aspergillosis (Table 1) and hemophagocytosis (Fig 1).

Case 3

A 13-year-old Thai boy was referred to the ICU in a comatose state with high

Table 1
Clinical, paraclinical, and pathologic presentations of the four reported autopsy cases.

	Case no. 1	Case no. 2	Case no. 3	Case no. 4
Age	2 years old	8 years old	13 years old	12 years old
Sex	Female	Male	Male	Female
Year of diagnosis	1997	1997	2005	2007
Clinical presentation	Fever for 3 days	Fever for 6 days	Fever for 5 days	Fever for 4 days
Duration of hospitalization	20 days	2 days	8 days	4 days
Platelet count on admission (cells/ml)	56,000	78,000	29,000	70,900
Method of dengue detection	Positive IgM antibody	Positive IgM antibody	Positive dengue viral type-IV-RNA PCR, positive 1:5,120 indirect hemagglutination assay	Positive IgM antibody, positive 1:1,280 indirect hemagglutination assay
Method of diagnosis of fungal infection	Histopathology and tissue culture grew *Aspergillus fumigatus*	Histopathology	Histopathology and tissue culture grew *Aspergillus* spp	Histopathology and tissue culture grew *Aspergillus niger*
Autopsy findings	Disseminated aspergillosis involving lungs, heart, diaphragm, liver, pancreas, adrenal glands, peritoneum, thyroid gland, and paratracheal lymph nodes Diffuse alveolar damage Massive centrilobular hepatic necrosis Adrenal hemorrhage Disseminated intravascular coagulopathy (DIC) Hemophagocytosis	Pulmonary aspergillosis Diffuse alveolar damage Hemorrhage of internal organs Centrilobular hepatic necrosis Acute renal tubular necrosis Adrenal hemorrhage DIC Hemophagocytosis	Invasive aspergillosis of the tracheobronchial mucosa and brain Diffuse alveolar damage Centrilobular hepatic necrosis Acute renal tubular necrosis Adrenal hemorrhage DIC Hemophagocytosis	Pulmonary aspergillosis Diffuse alveolar damage Hemorrhage of the internal organs Centrilobular hepatic necrosis Acute renal tubular necrosis Adrenal hemorrhage DIC Hemophagocytosis
CBC	Bicytopenia	Pancytopenia	Bicytopenia	Pancytopenia
Organs involved by hemophagocytosis	Bone marrow, lymph node, spleen and liver	Bone marrow, lymph node, spleen and liver	Bone marrow, lymph node, spleen and liver	Bone marrow, lymph node, spleen and liver
Weight of spleen (normal weight as a function of age and sex)	200 grams (30 grams)	200 grams (80 grams)	500 grams (120 grams)	350 grams (110 grams)
Cause of death	Disseminated aspergillosis	DSS	Invasive aspergillosis	Pulmonary aspergillosis

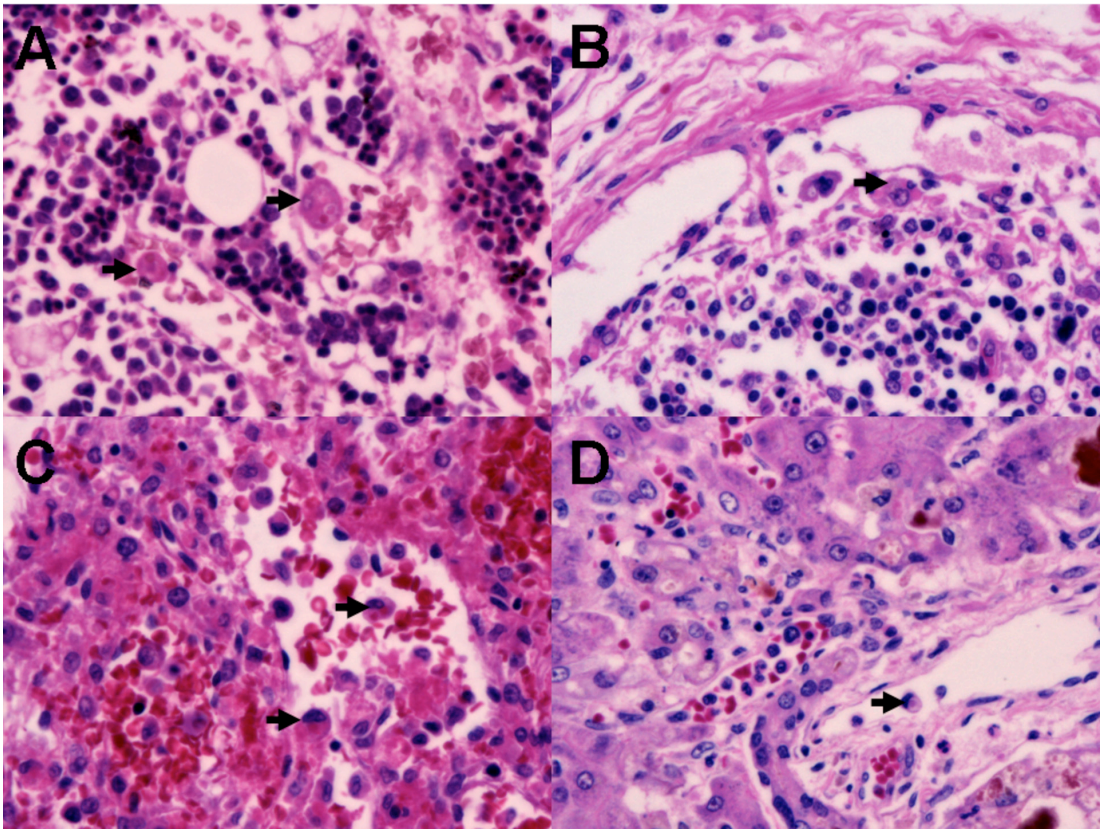

Fig 1–A section of the erythrophagocytic activity of macrophages in bone marrow (A), lymph node (B), spleen (C) and liver (D) H&E, x400.

grade fever, headache and multi-organ failure because of renal insufficiency, anuria, cholestatic hepatitis, leukocytosis, thrombocytopenia and anemia. Intubation and mechanical ventilation were performed. DSS was deemed probable, so the patient was initially treated with intravenous fluid therapy. Five days later, clinical findings showed suspected ventilator associated pneumonia. A chest X-ray showed numerous alveolar and interstitial ground glass-like infiltrates in association with pleural effusion. A culture from the tracheal secretion fluid grew *Aspergillus* spp. The patient was started with intravenous amphotericin B. The patient expired despite intensive treatment over 8 days. Autopsy was performed and the results are shown in Table 1.

Case 4

A 12-year-old girl was referred to the ICU with the presenting symptoms of fever for 4 days. On physical examination she had a fever of 38°C and tachypnea. She had hypotension and tachycardia. A touniquet test was positive. The liver span was 13 cm. Examination revealed no abnormalities. Laboratory examination showed thrombocytopenia and a hematocrit of 35.2%. A provisional diagnosis of DHF with impending shock was made. She was started on intravenous fluid resuscitation. She had an increase in

capillary leakage as evidenced by ascites and right pleural effusion. She developed an upper gastrointestinal bleed. Despite a high fluid infusion rate and administration of dopamine, pack red cells and fresh frozen plasma, hypotension persisted; dobutamine was subsequently given. She showed steady improvement until day 4, when she developed high-grade fever. Chest radiography showed bilateral alveolar infiltration. Systemic antibiotics were given. BAL was performed and culture yielded *Aspergillus niger*. She succumbed to the multiorgan failure four days after the diagnosis of DHF. Autopsy was performed and the results are shown in Table 1.

DISCUSSION

IAH was first described by Risdall *et al* (1979). Three reported cases had DHF, and one had DSS and developed hepatic and renal failure. Fungal infection is thought to be uncommon during dengue virus infection. There are a few reports of systemic mycosis superimposed on dengue viral infection (Kohli *et al*, 2007; Suzuki *et al*, 2007). Invasive aspergillosis usually occurs in patients with severe immunosuppression. None of the patients had a previous history of immunosuppression or significant health problems. All the patients had DSS with a short time from admission to development of hospital-acquired aspergillosis.

The pathogenesis of dengue-associated HS remains poorly understood. Hemophagocytosis may be virus induced or associated with multiple organ failure (Gauvin *et al*, 2000). Monocyte/macrophage infection is the core to the pathogenesis of dengue fever and to the origin of DSS. Excessive activation of monocytes/macrophages is the main pathogenesis with HS. Previous infection with heterogenous dengue-virus serotype may result in the production of nonprotective antiviral antibodies that bond to the virion surface and through interaction with the Fc receptor focus secondary dengue viruses on the target cell. Cross-reactivity of the second antibody response at the T-lymphocyte level results in a release of physiologically active cytokines, including tumor necrosis factor-alpha, interferon-gamma, interleukin-10, and other cytokines (Azeredo *et al*, 2001; Martina *et al*, 2009). These released cytokines are responsible for plasma leakage syndrome and the shock stage in DSS and may be possible pathogenetic mediators of hemophagocytosis in dengue infection (Fisman, 2000; Veerakul *et al*, 2002; Leong *et al*, 2007). Interferon-gamma is a potent activator of macrophages and may serve to amplify the T-lymphocyte response. The uncontrolled accumulation of activated T-lymphocytes and macrophages in multiple organs of the reticuloendothelial system may result in a cytokine storm, leading to virus-associated HS, similar to Epstein-Barr virus-associated T-cell lymphoproliferative disorder (Su *et al*, 1995).

DSS associated with reactive HS has a poor overall prognosis. Removing the etiologic agent of HS remains the cornerstone of management. However, no antiviral agent against dengue virus and/or direct immunoglobulin against dengue antibody is not available. Symptomatic and supportive treatment is required in these patients. Successful treatment of HS with pulse methylprednisolone and high dose intravenous immunoglobulin G during the convalescent phase of dengue infection has been reported (Srichaikul *et al*, 2008). Plasma exchange should be reserved for patients who fail to respond to corticosteroids and immunoglobulin.

Transient immunosuppression is commonly found in DSS. Suppressor/regulatory T-lymphocytes appear to play a cornerstone in this immunopathogenesis (Chaturvedi *et al*, 2007). The negative regulator of the Toll-like receptor-independent pathway resulting in interferon-beta suppression is a postulated mechanism (Ubol *et al*, 2010). This immune suppressed state is further amplified by interleukin-10, which leads to suppression of a secondary antiviral response (Green and Rothman, 2006; Ubol *et al*, 2010). These appear to be the immunopathogenesis of the transient immunosuppressive stage in DSS.

The postulated pathogenesis of invasive aspergillosis is the transmigration of preexisting colonized *Aspergillus* spp from the respiratory mucosa, which is damaged by the dengue shock process, in combination with impaired cellular and humoral immune responses in IAH. These may have promoted the growth of fungi from the respiratory tract, as suggested by the demonstration of *Aspergillus* spp in BAL fluid and/or tracheobronchial mucosa, in patients with dengue associated HS. DSS induces transient immunosuppression that may allow massive growth and overwhelming dissemination of preexisting fungi. Invasive aspergillosis should be looked for early in patients with unexplained worsening of severe DHF/DSS having pulmonary infiltration.

Risk factors for disseminated fungal infections have been identified with fulminant hepatic dysfunction, renal failure, and IAH. All these findings are typically found in patients with profound DSS. The main predisposing factor prolonged dengue shock state. Early detection of galactomannan (GM) antigen using an enzyme-linked immunosorbent assay is a non-invasive method and helpful for the management of high-risk patients. GM antigen should be obtained from the BAL fluid and serum of patients with DSS who have pulmonary infiltrations. Early use of antifungal agents may lead to successful treatment. However, the outcome of invasive aspergillosis, especially in DSS remains poor. Treatment with amphotericin B was not successful in the 2 patients reported here.

In conclusion, cases of concurrent IAH among patients with DSS and invasive aspergillosis have a more aggressive course and a worsen prognosis. Systemic evaluation of patients with DSS might help identify IAH and invasive aspergillosis. Recommendations in these patients include the use of pulse methylprednisolone and high dose intravenous immunoglobulin G with clinical signs and laboratory findings of HS and rapid initiation of antifungal therapy in patients with unexplained worsening of severe DHF/DSS having pulmonary infiltration.

ACKNOWLEDGEMENTS

The authors thank the pediatric staff and residents for managing the reported cases and the Thailand Research Fund-Senior Research Scholarship for 2006.

REFERENCES

Azeredo EL, Zagne SM, Santiago MA, *et al*. Characterisation of lymphocyte response and cytokine patterns in patients with dengue fever. *Immunobiology* 2001; 204: 494-507.

Bhamarapravati N, Tuchinda P, Boonyapaknavik V. Pathology of Thailand haemorrhagic fever: a study of 100 autopsy cases. *Ann Trop Med Parasitol* 1967; 61: 500-10.

Chaturvedi UC, Shrivastava R, Tripathi RK, Nagar R. Dengue virus-specific suppressor T cells: current perspectives. *FEMS Immunol Med Microbiol* 2007; 50: 285-99.

Fisman DN. Hemophagocytic syndromes and infection. *Emerg Infect Dis* 2000; 6: 601-8.

Gauvin F, Toledano B, Champagne J, Lacroix J. Reactive hemophagocytic syndrome presenting as a component of multiple organ dysfunction syndrome. *Crit Care Med* 2000; 28: 3341-5.

Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. *Curr Opin Infect Dis* 2006; 19: 429-36.

Jain D, Singh T. Dengue virus related hemophagocytosis: a rare case report. *Hematology* 2008; 13: 286-8.

Kohli U, Sahu J, Lodha R, Agarwal N, Ray R. Invasive nosocomial aspergillosis associated with heart failure and complete heart block following receovery from dengue shock syndrome. *Pediatr Crit Care Med* 2007; 8: 389-91.

Lei HY. Transient hemophagocytic activity in dengue immunopathogenesis. *J Formos Med Assoc* 2009; 108: 595-8.

Leong AS, Wong KT, Leong TY, Tan PH, Wannakrairot P. The pathology of dengue hemorrhagic fever. *Semin Diagn Pathol* 2007; 24: 227-36.

Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 2009; 22: 564-81.

Risdall RJ, McKenna RW, Nesbit ME, *et al*. Virus-associated hemophagocytic syndrome: a benign histiocytic proliferation distinct from malignant histiocytosis. *Cancer* 1979; 44: 993-1002.

Srichaikul T, Punyagupta S, Kanchanapoom T, Chanokovat C, Likittanasombat K, Leelasiri A. Hemophagocytic syndrome in dengue hemorrhagic fever with severe multiorgan complications. *J Med Assoc Thai* 2008; 91: 104-9.

Su IJ, Wang CH, Cheng AL, Chen RL. Hemophagocytic syndrome in Epstein-Barr virus-associated T-lymphoproliferative disorders: disease spectrum, pathogenesis, and management. *Leuk Lymphoma* 1995; 19: 401-6.

Suzuki S, Kitazawa T, Ota Y, *et al*. Dengue hemorrhagic shock and disseminated candidiasis. *Intern Med* 2007; 46: 1043-6.

Ubol S, Phuklia W, Kalayanarooj S, Modhiran N. Mechanisms of immune evasion induced by a complex of dengue virus and preexisting enhancing antibodies. *J Infect Dis* 2010; 201: 923-35.

Veerakul G, Sanpakit K, Tanphaichitr VS, Mahasandana C, Jirarattanasopa N. Secondary hemophagocytic lymphohistiocytosis in children: an analysis of etiology and outcome. *J Med Assoc Thai* 2002; 85 (suppl 2): S530-41.

Wong KF, Chan JK, Chan JC, Lim WW, Wong WK. Dengue virus infection-associated hemophagocytic syndrome. *Am J Hematol* 1991; 38: 339-40.

World Health Organization (WHO). Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: WHO, 1997: 12-23.