Association of Crohn's Disease, Thiopurines, and Primary Epstein-Barr Virus Infection with Hemophagocytic Lymphohistiocytosis

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Objective To assess the incidence of hemophagocytic lymphohistiocytosis (HLH) in a well-defined population of children with inflammatory bowel disease (IBD) and evaluate the common clinical and laboratory characteristics of individuals with IBD who developed HLH.

Study design We conducted a retrospective study of all children who developed HLH over an 8-year period. The incidence of HLH in patients with IBD was calculated using US census data and a statewide project examining the epidemiology of pediatric IBD.

Results Among children in Wisconsin, 20 cases of HLH occurred during the study period; 5 cases occurred in children with IBD. Common characteristics include: Crohn's disease (CD), thiopurine administration, fever lasting more than 5 days, lymphadenopathy, splenomegaly, anemia, lymphopenia, and elevated serum triglycerides and ferritin. Of the patients, 4 had primary Epstein-Barr virus infections. The incidence of HLH among all children in Wisconsin was 1.5 per 100 000 per year. The risk was more than 100-fold greater for children with CD ($P < .00001$).

Conclusions Pediatric patients with CD are at increased risk for developing HLH; primary Epstein-Barr virus infection and thiopurine administration may be risk factors. *(J Pediatr 2011;159:808-12)*.

Hemophagocytic lymphohistiocytosis (HLH) is a rare, commonly fatal disease in which macrophages are inappropriately activated, resulting in phagocytosis of all bone marrow-derived cells.[1-7] Primary HLH is a rare autosomal-recessive disorder of the immune system that has a reported yearly incidence of 1.2 in 1 000 000.[2,7,8] Secondary HLH can present in a person of any age and has been documented in association with a variety of infections and systemic conditions.[6,9-14] Both primary and secondary HLH result in significant histiocytosis with evidence of hemophagocytic activity in the bone marrow. Without early diagnosis, the resulting cytopenias render the host susceptible to opportunistic infections involving significant morbidity and mortality.[7,15] Consequently, identifying screening factors that promote early diagnosis so as to avoid adverse outcomes is imperative.

In 1991, the Histiocyte Society presented the first set of diagnostic guidelines for HLH. Based on cumulative experience and additional studies, revised diagnostic criteria were established in 2004; they include either a molecular diagnosis consistent with HLH or diagnostic clinical criteria of which the patient must fulfill 5 of the 8 clinical criteria in order to establish the diagnosis of HLH (Table I).[4,16]

Patients with inflammatory bowel disease (IBD) are at risk for developing HLH because of chronic systemic inflammation as well as exposure to immunosuppressive medications.[9,11,12] Despite the increased risk for these patients, efforts to alert physicians of this potential danger have been limited.[12] Over the past decade we observed a number of cases of HLH in patients with IBD. In this report, we describe our single-center series of patients with IBD who were diagnosed with HLH.

Methods

We conducted a retrospective chart review of all individuals with IBD who developed HLH at Children's Hospital of Wisconsin between January 2000 and June 2008. Cases of HLH were identified through a database maintained by the Children's Hospital of Wisconsin Hematology/Oncology and Bone

CD	Crohn's disease
EBNA	Epstein-Barr nuclear antigen
EBV	Epstein-Barr Virus
HLH	Hemophagocytic lymphohistiocytosis
IBD	Inflammatory bowel disease

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Table I. Diagnostic guidelines for HLH: 2004

The diagnosis of HLH can be established if either 1 or 2 below is fulfilled:
1. A molecular diagnosis consistent with HLH is present.
2. Diagnostic criteria for HLH are fulfilled (5 of the 8 criteria below):
   - A. Initial diagnostic criteria (to be evaluated in all patients with HLH):
     - Fever
     - Splenomegaly
     - Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood):
       - Hemaglobin < 90 g/L
       - Platelets < 100×10^9/L
       - Neutrophils < 1.0×10^9/L
     - Hypertriglyceridemia and/or hypofibrinogenemia:
       - Fasting triglycerides ≥ 3 mmol/L (ie., ≥265 mg/d L)
       - Fibrinogen ≤ 1.5 g/L
     - Hemophagocytosis in bone marrow or spleen or lymph node
     - No evidence of malignancy
   - B. New diagnostic criteria
     - Low or absent NK-cell activity (according to local laboratory references)
     - Ferritin ≥ 500 μg/L
     - Soluble CD-25 (ie., soluble interleukin-2 receptor) ≥ 2400U/mL

Adapted from Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31.

Marrow Transplant Department. Patients with HLH and a diagnosis of IBD were extracted for analysis.

The data extracted included patients' demographics, presenting symptoms, medical and surgical records, examination findings, duration of symptoms, means of diagnosis, treatment, and outcome. Laboratory and diagnostic investigations included complete blood counts, viral serologies, ferritin levels, complete metabolic profiles (including triglyceride levels as well as fibrinogen levels), natural killer cell levels, soluble CD-25 levels (interlukin-2 receptor), and the thiopurine s-methyltransferase genotype with any 6-thioguanine/6-methylmercaptopurine levels.

To estimate the incidence of HLH in our study population of children with IBD, we used an existing multicenter project to examine the epidemiology of IBD in the state of Wisconsin as well as the 2005 US Census data.[18,19] The multicenter IBD epidemiology project involved all pediatric gastroenterologists treating patients in the Wisconsin catchment area and captured data covering the entire time period of this study (January 1, 2000, to June 30, 2008). The incidence of HLH was calculated for the total pediatric population of Wisconsin and for the pediatric population with IBD. This study was approved by our institutional review board.

To compare the incidence of HLH in the pediatric population with IBD in Wisconsin to that in the general population we used a Fisher exact test with a normal approximation to a binomial distribution and the incidence of HLH in the general population, which is estimated to be 1 in 100 000. A sign test was used to evaluate the probability that a pediatric patient would be positive for a primary Epstein-Barr virus (EBV) infection and developing HLH.

Results

Between January 2000 and June 2008, 20 cases of HLH were identified and 5 of those patients had IBD. According to the 2005-2007 US Census Bureau data, the Wisconsin population was 5.5 million persons, and 24% were younger than 19 years of age (1.3 million).[19] Based on these figures, the overall incidence rate was 1.5 cases of HLH per 100 000 children. In the multicenter IBD epidemiology project, 992 children were documented to have IBD, and 5 cases of HLH occurred in this population.[18] The calculated incidence rate of HLH in the Wisconsin pediatric population with IBD is 5 of 992, or 1 of 200, which is significantly higher than the rate in the general population ($P < .00001$).

Of the 5 patients identified with IBD, all met the diagnostic criteria for HLH (Table I)[4]; all 5 patients had fever for longer than 5 days at presentation, ferritin levels above 500 μg/L, and hemophagocytosis on bone marrow biopsy without evidence of malignancy. Of the 5 individuals, 3 also had hypertriglyceridemia, with fasting triglyceride above 265 mg/dL and splenomegaly on physical exam; of the 2 remaining individuals, 1 patient had splenomegaly with thrombocytopenia (platelets < 100×10^9/L) and neutropenia (neutrophils < 1.0×10^9/L), and the other patient had anemia (hemoglobin < 90 g/L); neutropenia (neutrophils < 1.0×10^9/L); and hypertriglyceridemia with fasting triglyceride levels above 265 mg/dL, as well as low NK-cell activity.

All individuals were Caucasian, had Crohn's disease (CD), and ranged in age from 16.3 to 18.4 years (mean 17.3). Based on the multicenter IBD epidemiology project, approximately one-half of the children with CD were younger than 14 years of age, so the probability that all 5 patients who developed HLH were older than 14 years of age was less than 0.03. All patients received thiopurine monotherapy in treatment dosages ranging between 0.9 and 3.15 mg/kg/day of azathioprine and 1.4 to 1.76 mg/kg/day of 6-mercaptopurine. In our Wisconsin population with CD, 353 of 689 patients received immunomodulators. Consequently, the probability that all 5 patients with CD who developed HLH received immunomodulators was less than 0.03. No individuals had ever received any other immune-modulating agents except prednisone (eg, methotrexate, infliximab, etc), and only 1 patient had received prednisone within 12 weeks of developing HLH. Of the 5 patients, 4 had lymphopenia or borderline lymphopenia (800 to 1500 cells/μL, mean 1000 cells/μL) within 6 months of onset of symptoms, and 2 patients were also anemic (hemoglobin ranging from 9.5 to 10 g/dL for the female patient and 11.4 to 12.6 g/dL for the male patient). Of the 5 patients, 3 were EBV immunoglobulin M-positive, and 2 of these 3 patients also were Epstein-Barr nuclear antigen (EBNA)-negative. An additional patient was EBV immunoglobulin M-negative and immunoglobulin G-positive but also EBNA-negative, suggesting a primary EBV infection in 4 of 5 patients. The absence of EBNA has been associated with acute primary infection or recent infection (<6 months).[20] Although we were unable to identify the exact number of Wisconsin pediatric patients who had IBD or CD and who have been previously exposed to EBV, based on the literature we can estimate that the probability of not having been exposed to EBV by age 16 is approximately 0.1.[21] Thus, assuming that the susceptibility to EBV infection is similar for patients with CD and the general