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Association of Crohn's Disease, Thiopurines and Primary EBV Infection with Hemophagocytic Lymphohistiocytosis

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Abstract

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.

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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—20 cases of HLH occurred among children in Wisconsin during the study period. Five cases occurred in children with IBD. Common characteristics include: Crohn disease (CD), thiopurine administration, fever >5 days, lymphadenopathy, splenomegaly, anemia, lymphopenia and elevated serum triglycerides and ferritin. Four patients had primary Epstein Bar Virus (EBV) infections. The incidence of HLH among all children in Wisconsin was 1.5/100,000 per year. The risk was more than 100 fold greater for children with CD ($p < 0.00001$).

CONCLUSIONS—Pediatric patients with CD are at increased risk for developing HLH; primary EBV infection and thiopurine administration may be risk factors.

Keywords

inflammatory bowel disease; pediatrics; Epstein Bar Virus

Hemophagocytic lymphohistiocytosis (HLH) is a rare, often 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 with a reported yearly incidence of 1.2:1,000,000.(2, 7, 8) Secondary HLH can present at 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 with significant morbidity and mortality.(7, 15) Consequently, identifying screening factors to promote early diagnosis and 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 which includes either a molecular diagnosis consistent with HLH or diagnostic clinical criteria of which the patient must fulfill 5 out of the 8 clinical criteria in order to establish the diagnosis of HLH (Table I).(4)

Patients with IBD are at risk of developing HLH due to chronic systemic inflammation condition as well as exposure to immunosuppressive medications.(9, 11, 12) Despite the increased risk, efforts to alert treating physicians of this potential danger have been limited. (12). Over the last decade we observed a number of HLH cases in patients with IBD. In this report, we describe our single center series of patients with IBD 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. HLH cases were identified through a database maintained by Children's Hospital of Wisconsin Hematology/Oncology and Bone Marrow Transplant Department (4, 16, 17). Patients with HLH and a diagnosis of IBD were extracted for analysis.

The data extracted included patient demographics, presenting symptoms, past medical and surgical records, examination findings, duration of symptoms, means of diagnosis, treatment and outcome. Laboratory and diagnostic investigations including: complete blood counts, viral serologies, ferritin levels, complete metabolic profiles including triglyceride levels, as well as, fibrinogen levels, NK cell levels, soluble CD-25 levels (Interlukin-2 receptor) and

thiopurine s-methyltransferase (TPMT) genotype with any 6-thioguanine (6-TG)/6-methylmercaptopurine (6-MMP) levels.

To estimate the incidence of HLH in our study population of 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 has captured data covering the entire time period for this study (January 1, 2000 to June 30 2008). The incidence of HLH was calculated for the total pediatric population of Wisconsin and the pediatric IBD population. This study was approved by our institutional review board.

To compare the incidence of HLH in Wisconsin pediatric IBD population to 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 estimated to be 1 in 100,000. A Sign test was used to evaluate the probability of a pediatric patient being positive for a primary Epstein-Barr virus (EBV) infection and developing HLH.

RESULTS

Twenty cases of HLH were identified between January 2000 to June 2008 and 5 cases had IBD. According to the 2005–2007 U.S. Census Bureau data, the Wisconsin population was 5.5 million persons, and 24% were less than 19 years of age (1.3 million).(19) Based on these figures, the overall incidence rate is 1.5 cases of HLH per 100,000 children. From the multicenter IBD epidemiology project, 992 children were documented to have IBD with 5 cases of HLH occurring in this population.(18) The calculated incidence rate of HLH in the Wisconsin pediatric IBD population is 5/992 or 1/200 which is significantly higher than in the general population ($p < 0.00001$).

Of the 5 patients identified with IBD, all met diagnostic criteria for HLH (Table I) (4); all 5 patients had fever greater than 5 days at presentation, ferritin $> 500 \mu\text{g/L}$ and hemophagocytosis on bone marrow biopsy without evidence of malignancy. Three of the 5 individuals also had hypertriglyceridemia with fasting triglyceride $> 265 \text{ mg/dL}$ and splenomegaly on physical exam, and, for the remaining 2 individuals, one patient had splenomegaly with thrombocytopenia (platelets $< 100 \times 10^9/\text{L}$) and neutropenia (neutrophils $< 1.0 \times 10^9/\text{L}$) and the other patient had anemia (hemoglobin $< 90 \text{ g/L}$), neutropenia (neutrophils $< 1.0 \times 10^9/\text{L}$) and hypertriglyceridemia with fasting triglyceride $> 265 \text{ mg/dL}$, as well as low NK-cell activity.

All individuals were Caucasian, had CD, and ranged in age from 16.3 to 18.4 years old (mean 17.3). From the multicenter IBD epidemiology project, approximately one-half of the children with Crohn's disease were less than 14 years old. Based on this, the probability of all 5 patients who developed HLH being over 14 years old was < 0.03 . All patients receiving on thiopurine monotherapy with treatment dosages ranging between 0.9–3.15 mg/kg/day for azathioprine and 1.4–1.76 mg/kg/day for 6-mercaptopurine. In our Wisconsin CD population there were 353/689 patients receiving immunomodulators. Consequently, the probability of all five patients with CD who developed HLH receiving immunomodulators was < 0.03 . No individuals had ever received any other immune-modulating agents with the exception of prednisone (e.g. methotrexate, infliximab etc.) and only one patient received prednisone within 12 weeks of developing HLH. Four of the 5 patients had lymphopenia or borderline lymphopenia (800–1500 cells/ μL , mean 1000 cells/ μL) within 6 months of onset of symptoms and 2 patients were also anemic (hemoglobin ranging from 9.5–10 g/dL for the female patient and 11.4–12.6 g/dL for the male patient). Three of the 5 patients were

EBV IgM positive with 2 of these 3 patients also Epstein-Barr nuclear antigen (EBNA) negative. An additional patient was EBV IgM negative, IgG positive but also EBNA negative suggesting a primary EBV infection in 4 of 5 patients. Absence of Epstein-Barr nuclear antigen has been associated with acute primary infection or recent infection (ie. < 6 months).(20) Although we are unable to identify the exact number of Wisconsin pediatric patients IBD or Crohn's disease 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 ~ 0.1. (21) Thus, assuming that the susceptibility to EBV infection is similar for patient with CD compared with the general population, the probability of 4 out of 5 of our patients with HLH being positive for a primary EBV infection is < 0.001.

Four patients were treated based on recommendations published by the Histiocyte Society (2, 4). One patient, whose disease was considered mild, received infliximab. Three patients were discharged from the hospital within 17 days, and two required prolonged ICU admissions. One of these patients died from an intra-cerebral hemorrhage. Of those patients with significant morbidity or mortality, the median number of days with fever until diagnosis was 19 days, and in those patients without significant morbidity, the median number of days with fever until diagnosis was 10 days. The remainder of the common clinical elements are shown in (Table II).

DISCUSSION

The incidence of HLH treated at our institution appears similar to that reported in the general population which ranges from 1:50,000 to 1.2:1,000,000.(2, 7, 15, 22) Results from our pediatric IBD population suggests more than a 100 fold greater risk for developing HLH compared to the general population. Possible explanations for this discrepancy are that the data assume that all pediatric patients diagnosed with IBD were included in our retrospective statewide collaborative project, and that the vast majority of pediatric patients with HLH were treated at our tertiary pediatric referral center. Our incidence rate is also limited in that only individuals diagnosed in Wisconsin were included. Regardless of these limitations, our results demonstrate that pediatric patients with IBD are at increased risk compared with the general population.

Children with IBD may have unique characteristics which significantly increase their risk for developing this potentially lethal disorder. One characteristic may be a primary EBV infection. Although EBV has been associated with HLH, so have many other infections including CMV, mycobacterium tuberculosis, and histoplasmosis.(1, 9, 10, 23–25) Review of the literature identified 11 additional cases of HLH in patients with IBD (Table III). Only 3 of the 11 cases reported were associated with an EBV infection. Two of the 3 individuals were less than 20 years of age.(11, 29, 31) Eighty percent of our patients had a documented EBV infection with high viral loads suggesting EBV infection may have a greater role in the development of HLH than previously reported. In all of our EBV associated cases, the infection was primary. Using EBV prevalence data for this age group, the likelihood that a primary EBV infection was not associated with the development of HLH is low ($P < 0.001$). This association may explain the higher incidence of HLH in the pediatric IBD population since few adults are EBV naïve.

All of the patients with IBD we report were receiving thiopurine monotherapy. This is not surprising considering thiopurines are frequently the first immune-modulator utilized in pediatric patients.(32–35) However, our statewide collaborative data shows a sizable fraction of our IBD population were receiving alternative immune-altering medications.(18) Of the 11 previously reported cases of HLH in patients with IBD, the majority of individuals were receiving an immunosuppressive regimen which included thiopurines (Table III).

Whether thiopurines confer a unique risk in comparison with other immune modulating therapies remains uncertain. One possible connection suggesting thiopurines may interfere with the host's response to a primary EBV infection is that the majority of our patients had borderline lymphopenia well before the onset of symptoms. Routine clinical practice in monitoring for thiopurine toxicity focuses on total white blood cell count and absolute neutrophil count.(36) Prolonged courses of thiopurines have been documented to result in decreased circulating lymphocytes.(37, 38) Lymphopenia may be significant because, early in EBV infection, the virus is rapidly incorporated into epithelial cells and B lymphocytes. In response, there is extensive proliferation of suppressor T lymphocytes which are cytotoxic against EBV infected B lymphocytes.(20, 39–41) One could hypothesize that a decrease in circulating lymphocytes from thiopurine treatment impairs the host's cellular-mediated immune response against EBV infection. This mechanism of altered host cellular-mediated immunity is not unlike the development of post transplant lymphoproliferative disease (PTLD) seen in pediatric solid organ transplant. PTLD typically occurs in EBV naïve children whose chronic immune-suppression results in decreased circulating T lymphocytes.(20, 42, 43)

All of the patients in our cohort had CD, which may not be unique because the incidence of CD is twice that of ulcerative colitis (UC) in the pediatric population.(44) Only two of the 11 cases in the literature had UC and both were adults (Table III). It is unclear whether there is something unique about the pediatric CD population that increases the risk for developing HLH.

There are a number of limitations to consider. First, the accuracy of the diagnosis of HLH is vital. Patients with features of HLH (but milder disease) are frequently described in the rheumatology literature using the term Macrophage Activation Syndrome (MAS).(45) Some suggest that this sub-group do not require etoposide based therapies. There are no established guidelines for the diagnosis of MAS and all of our patients met the diagnostic criteria for HLH. Second, our series was retrospective. Consequently, we do not know whether any of our patients had an underlying primary immunodeficiency or genetic mutation which may have contributed to the development of HLH. Finally, our case series is fairly homogeneous, the published data shows greater variation in the etiology, epidemiology, and treatment of HLH (Table III). Despite these limitations, our data suggests an increased risk of HLH for children with IBD. It is important that general pediatricians and pediatric gastroenterologists be aware of this risk and have a low threshold for evaluation of HLH in pediatric patients with IBD because the majority of patients will present to these care providers for evaluation of their presumed "viral illness." Early intervention is known to improve survival.(46)

Pediatric patients with IBD, particularly patients with CD receiving thiopurine therapy, who present with five days of fever and cervical lymphadenopathy or previous evidence of lymphopenia should be screened for HLH. Demonstration of either lymphopenia, serum ferritin >500 µg/L or evidence of EBV infection should prompt discontinuation of all immunosuppressive medications and additional evaluation for HLH. Early referral to hematology/oncology for bone marrow biopsy is imperative giving the increased mortality associated with delay in diagnosis.

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List of Abbreviations

HLH	Hemophagocytic lymphohistiocytosis
IBD	Inflammatory bowel disease
CD	Crohn disease
EBV	Epstein Bar Virus
TPMT	Thiopurine s-methyltransferase
6-TG	6-thioguanine
6-MMP	6-methylmercaptopurine
PTLD	Post transplant lymphoproliferative disease
UC	Ulcerative colitis

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Table 1**Diagnostic Guidelines for HLH-2004**

The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled	
1	A molecular diagnosis consistent with HLH
2	Diagnostic criteria for HLH fulfilled (five out of the eight 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):
	Hemoglobin $< 90\text{g/L}$
	Platelets $< 100 \times 10^9/\text{L}$
	Neutrophils $< 1.0 \times 10^9/\text{L}$
	Hypertriglyceridemia and/or hypofibrinogenemia:
	Fasting triglycerides $\geq 3.0\text{ mmol/L}$ (ie., $\geq 265\text{mg/dl}$)
	Fibrinogen $\leq 1.5\text{g/L}$
	Hemophagocytosis in bone marrow or spleen or lymph node
b.	No evidence of malignancy
c.	New diagnostic criteria
	Low or absent NK-cell activity (according to local laboratory references)
	Ferritin $\geq 500\text{ }\mu\text{g/L}$
	Soluble CD25 (ie., soluble IL-2 receptor) $\geq 2,400\text{U/ml}$

Adopted from: Henter JJ, 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(2):124–31.

TABLE 2

Patient Clinical and Laboratory Characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean
Type of IBD	Crohn	Crohn	Crohn	Crohn	Crohn	
Months of IBD prior to HLH	13	19	84	52	4	34
Type of Immunosuppression	6-MP	AZA	AZA	AZA	6-MP	
TPMT genetics	Normal	Intermediate	Normal	Normal	Normal	
Months of Immunosuppression	13	4	78	52	6	31
6-TGN level prior to HLH	72	Unknown	210	171	Unknown	151
6-MMP level prior to HLH	875	Unknown	4066	1952	Unknown	2298
EBV IgM status	+	+	-	+	-	
EBV IgG status	+	+	+	-	-	
EBNA	-	Unknown	-	-	-	
EBV viral load at Diagnosis (copies/meg PBMC DNA)	81499	39421	70319	17655	0	41779
CMV PCR	-	-	-	-	-	
Hemoglobin (g/L)	93	87	94	98	77	90
Platelets ($\times 10^9/L$)	136	52	97	127	114	105
WBC ($\times 10^9/L$)	1.4	2.0	1.1	0.8	1.6	1.4
ANC ($\times 10^9/L$)	0.7	1.5	0.6	0.4	1.0	0.8
ALC ($\times 10^9/L$)	0.5	0.4	0.3	0.3	0.4	0.4
Ferritin ($\mu g/L$)	1870	4559	3300	3410	10500	4728
Triglycerides (mg/dL)	424	500	212	339	280	351
Fibrinogen (g/L)	1.82	1.24	1.74	1.42	3.23	1.89
NK cell activity	Normal	Unknown	Unknown	Low	Low	
sCD-25 level	Unknown	22035	Unknown	5941	Unknown	
Bone marrow biopsy	+	+	+	+	+	
ICU admission	-	+	+	-	-	
Death	-	-	+	-	-	

TABLE 3

Overview of Published Case Reports of IBD and HLH

	Age	Disease	Therapy	Pathogen	Treatment	Fatal
Serrate <i>et al</i> ²⁸	53	CD	T	EBV	S, IVIG Medications Held	N
Francolla <i>et al</i> ¹⁰	15	CD	T, I	EBV	S	N
Babu <i>et al</i> ^p	12	CD	T	CMV	Medications Held	N
Kanaji <i>et al</i> ²³	25	UC	S	unknown	γ-globulin, S, G-CSF	N
Koketsu <i>et al</i> ²⁴	35	UC	S	CMV	Anti-viral	N
Posthuma <i>et al</i> ²⁵	19	CD	T, S	EBV	Anti-viral	Y
Sijpkens <i>et al</i> ²⁷	20	CD	T, S	CMV	Anti-viral	N
Reiner <i>et al</i> ²⁶	44	CD	S	Histoplasmosis	Not Documented	N
Kohara <i>et al</i> ²⁰	22	CD	T, I	CMV	Splenectomy	N
Chauveau <i>et al</i> ²²	37	CD	I	CMV	Not Documented	N
James <i>et al</i> ¹¹	26	CD	T, I, S	MTb	Not Documented	Y

Therapy: T= Thiopurine, I= Infliximab, S= Steroids.