

## BILIARY CRYPTOSPORIDIOSIS IN HIV-INFECTED PEOPLE AFTER THE WATERBORNE OUTBREAK OF CRYPTOSPORIDIOSIS IN MILWAUKEE

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**Abstract Background.** In March 1993, the municipal water supply in Milwaukee became contaminated with cryptosporidium and a widespread outbreak of cryptosporidiosis occurred.

**Methods.** We retrospectively studied the relations among the clinical presentation, CD4 count, and survival in a group of 82 patients infected with the human immunodeficiency virus (HIV) in whom cryptosporidiosis developed during the outbreak. Infection was documented by the finding of cryptosporidium in stool.

**Results.** There was a sharp increase in the number of HIV-infected patients with cryptosporidiosis after the period of water contamination. Four of 24 patients with biliary symptoms (17 percent) and 30 of 58 without biliary symptoms (52 percent) were alive one year after the outbreak ( $P=0.003$ ). Twenty-one of the patients with biliary

symptoms had CD4 counts  $\leq 50$  per cubic millimeter, as compared with 36 of 57 patients without biliary symptoms (88 percent vs. 63 percent,  $P=0.03$ ). The CD4 count was not available for one patient in the latter group. Within one year, 41 of the patients with CD4 counts  $\leq 50$  per cubic millimeter had died, as compared with 6 of those with higher counts (72 percent vs. 25 percent,  $P<0.001$ ). After adjustment for the CD4 count, independent predictors of death were older age and the presence of nausea and vomiting.

**Conclusions.** When HIV-infected patients are exposed to cryptosporidium, those with CD4 counts  $\leq 50$  per cubic millimeter are at increased risk for biliary symptoms and for death within one year after the infection. (N Engl J Med 1996;334:19-23.)

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IN the spring of 1993, a waterborne outbreak of cryptosporidiosis occurred in Milwaukee. The municipal water supply became contaminated with cryptosporidium, affecting more than 400,000 people.<sup>1</sup> Immunocompromised patients are susceptible to biliary infections with cryptosporidium,<sup>2</sup> and a number of such patients have been described with biliary disease caused by cryptosporidiosis.<sup>3-9</sup> However, many aspects of the disease in this population of patients, including its natural history, treatment, and outcome, remain uncertain.<sup>10</sup> The Milwaukee outbreak allowed us to study the impact of a waterborne epidemic of cryptosporidiosis on a large number of people infected with the human immunodeficiency virus (HIV). We examined the relations among the clinical presentation, CD4 counts, and survival in a group of HIV-infected patients in whom cryptosporidiosis developed during the outbreak.

### METHODS

We retrospectively studied HIV-infected patients in whom cryptosporidiosis developed after the contamination of the Milwaukee water supply with cryptosporidium between March 21 and April 5, 1993. Each patient had been followed by one of us for months or years before the outbreak of cryptosporidiosis. Historical data preceding the outbreak were obtained from the patients' medical records. Stool specimens from patients who presented with diarrhea after the period of water contamination were examined for cryptosporidium by modified acid-fast staining or direct fluorescent-antibody staining.<sup>1</sup> Stool specimens from 82 patients contained cryptosporidium. Fifty-eight of these patients had diarrhea and periumbilical cramping without pain in the right upper quadrant (the group without biliary symptoms).

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Twenty-four had constant or intermittent pain in the right upper quadrant (the group with biliary symptoms). Changes in liver-chemistry values were not required for inclusion in the group with biliary symptoms because infectious cholecystitis may not be associated with such changes.<sup>11</sup> All patients were studied and treated by the investigators at academic medical centers, community hospitals, private practices, or clinics. No attempt was made to modify the approach to the investigation and treatment of patients at individual sites. Paromomycin (Humatin, Parke-Davis, Morris Plains, N.J.) and azithromycin (Zithromax, Pfizer, New York) were used to treat the cryptosporidium infection.

The response to medical or surgical therapy was determined by the patients' subjective assessment of symptom improvement. In patients without biliary symptoms, a decrease in stool frequency and the incidence of abdominal cramping was considered a partial response, complete resolution of diarrhea and cramping a complete response, and no change in the frequency of diarrhea or cramping a lack of response. In patients with biliary symptoms, complete relief of pain was considered a complete response, incomplete relief a partial response, and no relief a lack of response. The study period continued until April 5, 1994, one year after the end of the period of water contamination. The study was approved by the Human Subjects Review Board of Sinai Samaritan Medical Center.

### Statistical Analysis

Results are presented as means  $\pm$  SE. The primary outcome variable was the number of days from the diagnosis of cryptosporidiosis (base line) until death. The primary statistical analyses used the Cox proportional-hazards regression model<sup>12</sup> to compare survival in the two groups after adjustment for clinically relevant base-line predictors such as the CD4 count ( $\leq 50$  cells per cubic millimeter or  $>50$  cells per cubic millimeter), age, and the presence of nausea and vomiting. Relative risks are described in terms of the hazard ratio, along with associated 95 percent confidence intervals. Survival curves were estimated according to the method of Kaplan and Meier, and differences in survival were determined by the log-rank test.<sup>13</sup> Two-way analysis of variance was used to compare the two groups after adjustment for the CD4 count ( $\leq 50$  cells per cubic millimeter or  $>50$  cells per cubic millimeter), with regard to the following variables: age and levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin. The laboratory values were compared between the groups both before and during the illness and were log-transformed for statistical analysis. Fisher's exact test was used for dichotomous variables, and other comparisons were performed with the Mann-Whitney test. All sta-

tistical tests were two-tailed, and a P value of 0.05 or less was considered to indicate statistical significance.

## RESULTS

### Clinical Course

In 1992 there were six cases of cryptosporidial diarrhea and one case of biliary cryptosporidiosis among HIV-infected patients at the participating centers. There was a sharp increase in the number of cases (Fig. 1) in April 1993, which corresponded with the period of water contamination in Milwaukee. The peak occurrence of biliary and nonbiliary symptoms was at approximately the same time (Fig. 1). Fourteen of the 24 patients with biliary symptoms (58 percent) and 19 of the 58 patients without biliary symptoms (33 percent) had nausea and vomiting. In 7 of the 82 patients (8.5 percent), cryptosporidiosis was the illness that defined the presence of the acquired immunodeficiency syndrome (AIDS). Liver-chemistry values were similar in both groups before the illness but were significantly higher in the patients with biliary symptoms after the onset of the disease (Table 1). Twenty-two patients with biliary symptoms had an increase in alkaline phosphatase after the onset of pain. The two patients in this group who did not have a significant change in alkaline phosphatase levels were found to have acute cryptosporidial cholecystitis at surgery. Four patients with biliary symptoms were alive at one year (17 percent), as compared with 30 patients without biliary symptoms (52 percent,  $P=0.003$ ).

### Predictors of Outcome

CD4 counts  $\leq 50$  per cubic millimeter were a risk factor for the development of biliary symptoms. Twenty-one of the 24 patients with biliary symptoms (88 percent) had CD4 counts  $\leq 50$  per cubic millimeter, as compared with 36 of the 57 patients without biliary symptoms (63 percent,  $P=0.03$ ). The CD4 count was not available for one patient with no biliary symptoms, who survived. Cox regression analysis (Table 2) showed that the strongest base-line predictor of mortality was a CD4 count  $\leq 50$  per cubic millimeter (hazard ratio, 4.10; 95 percent confidence interval, 1.72 to 9.76;

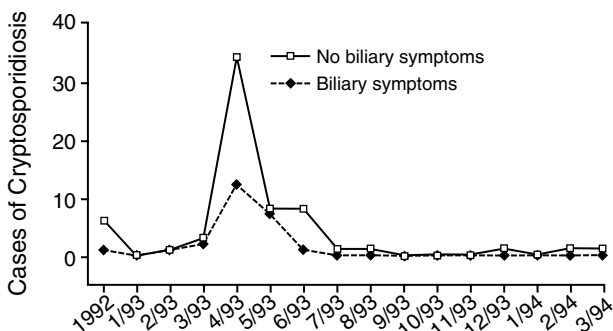


Figure 1. Cases of Cryptosporidiosis among HIV-Infected People in Relation to the Period of Water Contamination in Milwaukee (March 21 to April 5, 1993).

$P<0.001$ ). Other independent predictors included increasing age and the presence of nausea and vomiting. After adjustment for these factors, biliary disease was not associated with poorer survival. These findings were substantiated by the Kaplan–Meier survival curves, which demonstrated a significantly higher mortality rate among patients with CD4 counts  $\leq 50$  per cubic millimeter than among those with higher counts ( $P<0.001$ ) (Fig. 2A). When patients with CD4 counts below 50 per cubic millimeter were analyzed according to the presence or absence of biliary symptoms, there was no significant difference in survival ( $P=0.22$ ) (Fig. 2B).

### Diagnostic Studies and Therapeutic Interventions

Forty-one diagnostic studies were performed in the 24 patients with biliary symptoms: 17 abdominal ultrasound examinations, 7 biliary scintiscans, 7 computed tomographic scans of the abdomen, and 10 endoscopic retrograde cholangiopancreatograms. On ultrasonography, dilatation of the common bile duct was seen in 14, thickening of the gallbladder wall in 7, and dilatation of the intrahepatic ducts in 6. Of the three patients with normal ducts at ultrasonography, two had dilated ducts on subsequent computed tomographic scans and one had thickening of the gallbladder wall and pericholecystic accumulation of fluid that was later shown at cholecystectomy to be due to cryptosporidial cholecystitis. Eight diagnostic studies were performed in the 58 patients without biliary symptoms: five abdominal ultrasound examinations and three abdominal computed tomographic scans, which were done to evaluate other conditions related to AIDS.

Ten of the 24 patients with biliary symptoms (42 percent) underwent endoscopic retrograde cholangiopancreatography, and all were found to have a dilated common bile duct (range, 10 to 16 mm in diameter) and a narrowing in the distal duct consistent with the presence of papillitis or papillary stenosis. Three patients had intrahepatic ductal strictures, and one had a dilated pancreatic duct. Endoscopic ampullary biopsies or examination of cytologic samples from the biliary tract revealed cryptosporidium in 5 of these 10 patients. Two of the five patients with negative results on testing of endoscopic samples subsequently underwent cholecystectomy, and cryptosporidial cholecystitis was found in both. Cytomegalovirus was not found on biopsies or in analysis of brushings in any of the patients.

Nine of the 10 patients with biliary symptoms who underwent endoscopic retrograde cholangiopancreatography also underwent endoscopic sphincterotomy; sphincterotomy could not be completed in the 10th patient because he became agitated. Sphincterotomy did not completely relieve the pain in any of the patients: three had partial relief, and six had no significant change in the level of pain. Sphincterotomy did not significantly change the values for aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, or bilirubin.

Table 1. Clinical Characteristics of the 82 HIV-Infected Patients with Cryptosporidiosis, According to the Presence or Absence of Biliary Symptoms.

CHARACTERISTIC	BILIARY SYMPTOMS (N = 24)	NO BILIARY SYMPTOMS (N = 58)	P VALUE
	<i>mean ±SE</i>		
<b>Before the onset of cryptosporidiosis</b>			
CD4 count (cells/mm <sup>3</sup> )*	30±6	113±24	0.15
Age (yr)	36±1	35±1	0.81†
Aspartate aminotransferase (U/liter)	67±21	56±13	0.67‡
Alanine aminotransferase (U/liter)	81±30	45±4	0.39‡
Alkaline phosphatase (U/liter)	112±13	113±20	0.84‡
Total bilirubin (mg/dl)‡	0.65±0.05	0.68±0.07	0.86‡
<b>After the onset of cryptosporidiosis§</b>			
Aspartate aminotransferase (U/liter)	114±19	80±16	0.11‡
Alanine aminotransferase (U/liter)	123±22	83±17	0.03‡
Alkaline phosphatase (U/liter)	477±109	172±22	0.002‡
Total bilirubin (mg/dl)‡	1.0±0.03	0.79±0.13	0.33‡

\*The CD4 count was not available for one patient with no biliary symptoms, who survived.

†Adjusted for the CD4 count.

‡To convert values for total bilirubin to micromoles per liter, multiply by 17.1.

§These are the peak values.

bin ( $P>0.1$  for all values). There was no correlation between changes in laboratory values and the relief of pain.

In four patients with biliary symptoms, symptoms of acute cholecystitis developed, and cholecystectomy was performed. None of these four had gallstones, and histologic analysis revealed cryptosporidial cholecystitis in all of them. Three had complete relief of pain after cholecystectomy. None of the other patients with biliary symptoms underwent endoscopic retrograde cholangiopancreatography, because they either were too ill or refused. One patient had severe pain that was unresponsive to analgesics, and a celiac-ganglion block was performed, with partial relief of pain.

A definitive diagnosis was established in nine patients with biliary symptoms (38 percent) by the demonstration of cryptosporidium in the biliary tree on analysis of biliary brushings obtained by endoscopy, on biopsy, or on histologic examination of the gallbladder. In these nine patients, the CD4 count and levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin were not significantly different from those in the rest of the patients with biliary symptoms ( $P>0.25$  for all).

Twenty-two patients with biliary symptoms (92 percent) and 43 patients without such symptoms (74 percent) received paromomycin as treatment for cryptosporidiosis. Twelve (18 percent) had a complete response, all of whom had no biliary symptoms; 22 (34 percent) had a partial response (12 with biliary symptoms and 10 without such symptoms); and 31 (48 percent) had no response (10 with biliary symptoms and 21 with no biliary symptoms). In 19 of the patients who had a partial response or no response to paromomycin, azithromycin was administered. One of these patients, who had intestinal cryptosporidiosis, had a complete response with the addition of azithromycin; 8 had no response, and 10 had a partial response. The mean

CD4 count in the patients who had a complete response to therapy was  $157\pm66$  per cubic millimeter; in those with a partial response it was  $33\pm8$  per cubic millimeter; and in those with no response it was  $29\pm6$  per cubic millimeter ( $P=0.25$ ). Soma-tostatin was used in 8 patients with biliary symptoms (33 percent) and 16 patients without such symptoms (28 percent).

Fourteen patients with biliary symptoms (58 percent) and 10 without such symptoms (17 percent) were given total parenteral nutrition. Three patients with biliary symptoms (12 percent) and 26 without such symptoms (45 percent) did not need to be hospitalized ( $P=0.005$ ). Patients with CD4 counts  $\leq 50$  per cubic millimeter spent longer periods in the hospital than those with higher counts ( $22\pm 5$  vs.  $7\pm 3$  days,  $P=0.003$ ).

## DISCUSSION

Waterborne outbreaks of cryptosporidiosis have been described in the United States and other countries,<sup>14,15</sup> but there are few data on the effects of such outbreaks on people infected with HIV. Defining biliary cryptosporidiosis in clinical practice is difficult. The results of noninvasive studies and retrograde cholangiography in HIV-infected patients are nonspecific and similar to those in other disorders, such as cytomegalovirus infection<sup>5</sup> and microsporidiosis.<sup>16</sup> Furthermore, symptoms may not be related to the findings on imaging studies: asymptomatic patients may be found to have cholangiopathy on retrograde cholangiography.<sup>17</sup> The demonstration of cryptosporidium in the biliary tree of a symptomatic patient with an abnormal cholangiogram or histologic demonstration of cryptosporidial cholecystitis provides a definitive diagnosis, but such studies cannot be done in patients who are too ill to undergo invasive procedures.

We found that when HIV-infected persons were exposed to cryptosporidium, a CD4 count  $\leq 50$  per cubic millimeter was a significant risk factor for the development of biliary symptoms. Patients with biliary symptoms tended to have cholestasis with the onset of pain and abnormal findings on imaging studies of the biliary

Table 2. Effect of Prognostic Factors on Survival.

VARIABLE	HAZARD RATIO	95% CONFIDENCE INTERVAL	P VALUE
Age (change in risk per year of age)	1.04	1.01–1.08	0.02
Presence of nausea and vomiting	1.87	1.03–3.38	0.04
Presence of biliary disease	1.43	0.78–2.64	0.26
CD4 count $\leq 50/\text{mm}^3$	4.10	1.72–9.76	<0.001

tree. Patients with CD4 counts  $\leq 50$  per cubic millimeter had a long hospital stay and decreased survival (as compared with patients with CD4 counts  $> 50$  per cubic millimeter). The peaks in the onset of diarrhea and biliary symptoms occurred at similar times, which suggests that biliary disease develops soon after susceptible patients acquire cryptosporidia.

In a recent placebo-controlled study of patients with AIDS and intestinal cryptosporidiosis, treatment with paromomycin decreased stool frequency and oocyst excretion, but biliary disease progressed despite long-term suppressive therapy.<sup>18</sup> Our data support these observations. In some uncontrolled studies, sphincterotomy has been reported to result in rapid and sustained relief of right-upper-quadrant pain in the majority of

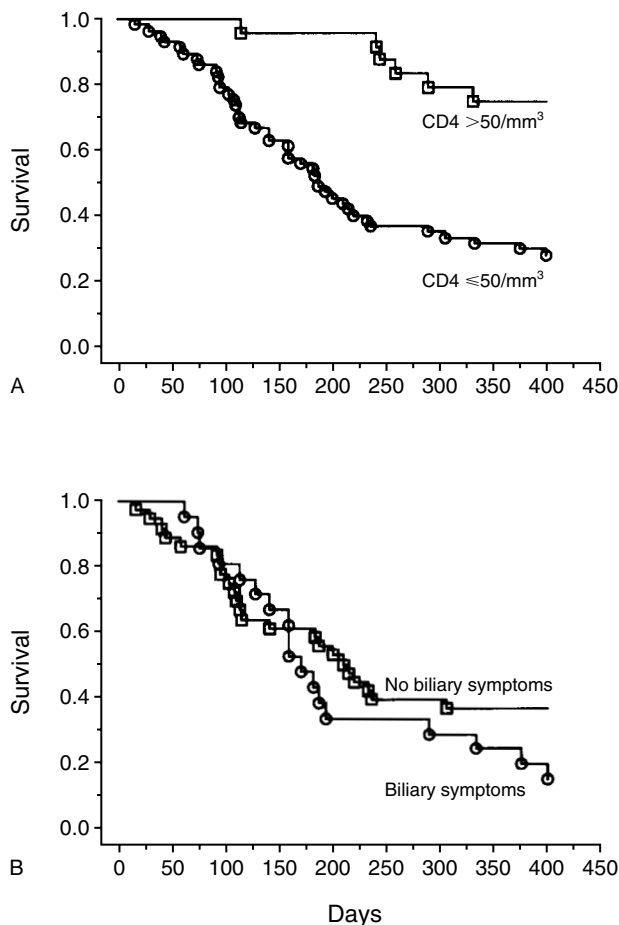


Figure 2. Kaplan-Meier Estimates of Survival According to the CD4 Count (Panel A) and, among Patients with CD4 Counts  $\leq 50$  per Cubic Millimeter, According to the Presence or Absence of Biliary Symptoms (Panel B).

In each panel "days" refers to the number of days after the onset of symptoms (base line), and symbols indicate deaths. In Panel A, patients with CD4 counts above 50 per cubic millimeter had significantly higher rates of survival than those with CD4 counts of 50 per cubic millimeter or less (6 deaths vs. 41 deaths,  $P < 0.001$  by the log-rank test). In Panel B, biliary-symptom status had no significant effect on survival ( $P = 0.22$ ). In both groups, most deaths occurred within 250 days of the onset of symptoms.

patients with AIDS and biliary cryptosporidiosis,<sup>19</sup> whereas in other studies it afforded only partial relief.<sup>20</sup> In anecdotal reports involving extended periods of follow-up, pain has been described as a persistent problem after sphincterotomy in patients with AIDS.<sup>9</sup> Some studies reported initial improvement in liver-chemistry values after sphincterotomy, but longer follow-up showed that the values subsequently worsened.<sup>20</sup> Comparisons of such studies are difficult because the duration of follow-up was not specified in some, and others did not distinguish between partial and complete relief of pain.

In our study, the results of sphincterotomy for relief of pain were disappointing. Most patients continued to have severe pain that required treatment with narcotics. The most dramatic relief of biliary pain was in patients presenting with acute cholecystitis. Three of four such patients were free of pain after cholecystectomy, but only one of these patients was alive one year later.

Patients with CD4 counts  $\leq 50$  per cubic millimeter had a significantly higher risk of death than those with higher counts. However, the survival rate among patients with CD4 counts  $\leq 50$  per cubic millimeter was similar in patients with and those without biliary symptoms, suggesting that the CD4 count, and not the presence of biliary disease, was the primary determinant of outcome. Flanigan et al.<sup>21</sup> have reported that HIV-infected patients with CD4 counts  $\leq 140$  per cubic millimeter tend to have persistent cryptosporidium infection — an association that would explain our finding of a poor outcome in patients with low CD4 counts. In our study, proportional-hazards analysis suggested that increasing age, the presence of nausea and vomiting, and CD4 counts  $\leq 50$  per cubic millimeter were independent predictors of mortality, but the presence or absence of biliary disease was not.

Infection with cryptosporidium is a reportable disease in only a few states, and there is no national surveillance for infections with this organism.<sup>22</sup> Public water supplies that meet current federal and state standards for drinking water may still become contaminated with cryptosporidial oocysts and cause large outbreaks,<sup>14</sup> as occurred in Milwaukee.<sup>1</sup>

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