Harbingers for *Clostridium difficile*–Associated Diarrhea

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Purpose: Recent research has recognized surrogate markers for *Clostridium difficile*–associated diarrhea (CDAD). Among the most consistently identified markers are the leukocyte count, platelet count, and albumin level. Previous investigators failed to exclude patients with hematologic disorders that may have confounded their results. Therefore, the exclusion of this subset from our study lends it a unique perspective.

Methods: We undertook a retrospective review of inpatients at our institution that were diagnosed with nosocomial diarrhea and subsequently had a stool sample sent for *C. difficile* toxins A and B. Patients with major hematologic disorders were excluded.

Results: A total of 77 *C. difficile*–positive patients and 91 *C. difficile*–negative patients were studied. Patients with CDAD had a significantly higher leukocyte and platelet count but a lower albumin level compared with patients without CDAD.

Conclusion: Our results support the conclusion of preceding studies that leukocytosis, thrombocytosis, and hypoalbuminemia are reliable clinical predictors for CDAD even after careful exclusion of confounding factors.

Key Words: *Clostridium difficile*, associated diarrhea, hypoalbuminemia, leukocytosis, thrombocytosis

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Clostridium difficile is a gram-positive spore-forming pathogen that has emerged as a major cause of antibiotic-associated diarrhea and pseudomembranous colitis.[1] The severity of this disease can range from an asymptomatic carrier state, to a mild diarrhea, to a fulminant toxic megacolon requiring surgery.[2] Although the bacterium was first isolated as early as 1935, it was not until the late 1970s that *C. difficile* was recognized as an important causative factor for nosocomial diarrhea.[3,4]

Today, *C. difficile* infection is the commonest cause of nosocomial diarrhea in the developed world, and its incidence is rapidly increasing.[4,5] The National Nosocomial Infections Surveillance System reported on this trend, showing that the hospital-wide incidence of *C. difficile*–associated diarrhea (CDAD) from 2000 to 2003 was 7.4/1000 admissions; this figure was almost 6 times greater than it was from 1987 to 2001.[6] *Clostridium difficile* is also responsible for nearly 25% of all antibiotic-associated diarrheas.[2] There are an estimated 3 million cases of CDAD each year in the United States; this increases the hospital length of stay by 3.6 days.[3] The burden of this disease translates into approximately 1.1 billion dollars in health care costs,[7] with a mean adjusted cost of $3600 per patient.[8] The disease has also progressed in severity, with the mortality rate increasing from 5.7/million in 1999 to 21.7/million in 2004.[9]

The most recognized risk factor in the causation of CDAD is prior antibiotic usage; notable culprits are clindamycin, the beta-lactams, and fluoroquinolones.[7,10–13] The gold standard test for the diagnosis of *C. difficile* infection is the cytotoxin assay.[7,11] However, the assay is difficult to perform and has a turnover time of almost 2 days.[3] The recently introduced and more widely prevalent enzyme-linked immunosorbent assay (ELISA) test requires only 6 hours to complete and has a specificity of 93% to 99% and a sensitivity of 63% to 99%.[7] Treatment of CDAD is most commonly achieved with either oral metronidazole or oral vancomycin.[7] Although studies have shown both drugs to be effective, there are some recent reports of metronidazole failure as a first-line treatment.[12]

Despite efficient diagnostic tests and effective medical treatment, the incidence and severity of CDAD continues to rapidly increase.[11] Recent research has focused on identifying surrogate markers of the disease, with the goal of more accurate, precise, and timely diagnosis leading to better clinical outcomes. For example, in previous retrospective studies, patients with CDAD were found to have higher mean white blood cell (WBC) and platelet counts and lower levels of albumin when compared with patients without CDAD.[2,7,10,12,14–20] Our study revisits some of these variables and their association with CDAD with an important difference in methodology. In this study, we have accounted for the confounding factor of patients with preexisting hematologic disorders, because this may have significant implications on the host's ability to mount a reactive thrombocytosis and/or leukocytosis in response to *C. difficile* infection. The importance of this confounding factor has been stressed by earlier investigators;[14] however, to our knowledge, previous studies have never accounted for this factor.

METHODS

We retrospectively reviewed the records of all inpatients at the Louisiana State University Health Science Center (LSU-HSC), Shreveport, who were diagnosed with nosocomial diarrhea and subsequently had a stool sample sent to test for *C. difficile* toxin from May 2005 to May 2007. The test used by our facility is an ELISA assay that tests for both *C. difficile* toxin A and toxin B. Over the 2-year span, there were 656 total tests performed at LSU-HSC. Of these, 216 tested positive for *C. difficile*, whereas 440 tested negative for *C. difficile*.

Our exclusion criteria were as follows: all patients with a major hematologic disorder that would be expected to affect the leukocyte or platelet count were excluded from our study. This included but was not limited to patients with leukemia, lymphoma, human immunodeficiency virus/acquired immunodeficiency syndrome, or with aplastic crises. We were also forced to exclude a small number of patients who had missing or incomplete laboratory data. In patients who tested positive

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more than once for the *C. difficile* toxin, only the initial test results were included. Meeting the above criteria, our final population included 77 *C. difficile*–positive patients and 91 *C. difficile*–negative patients.

Patient charts were thoroughly reviewed with attention to the clinical history, hospital course, diagnoses, and laboratory results. The age, sex, medical diagnoses, and date of *C. difficile* test were recorded. The laboratory values of WBC, platelets and albumin, blood urea nitrogen (BUN), and creatinine up to 3 days before the date of the *C. difficile* test and up to 5 days after the test were reviewed. Only the values closest to the test date were recorded for analysis. The distribution of the data was assessed using the Shapiro-Wilk test. Analysis of the data was done with the Student *t* test (normal distribution) and the Mann-Whitney *U* test (non-normal distribution). A Pearson χ^2 analysis was used for the sex of the patients.

We did not review the antibiotic usage of our subjects because this aspect is already an extensively studied and established risk factor for *C. difficile* diarrhea.

The institutional review board at LSU-HSC, Shreveport, approved our study.

RESULTS

A total of 168 patients were included in this study (Table 1). Our patient groups consist of 77 *C. difficile*–positive patients compared with 91 *C. difficile*–negative patients as determined by ELISA testing. There was no significant difference between the mean ages of the 2 populations; the mean age of the CDAD-positive group was 49.9 years, and the mean age of the CDAD-negative group was 54.4 years ($P = 0.06$).

There were 46 female patients and 31 male patients in the CDAD-positive group ($\chi^2_1 = 2.922$, $P = 0.0874$) compared with 56 women and 35 men in the CDAD-negative group ($\chi^2_1 = 4.846$, $P = 0.0277$).

Of the patients that were diagnosed with CDAD, 6 died during the course of the same hospital stay. After chart review, we identified chronic renal failure as a common factor in 5 of the 6 fatalities (mean BUN, 96 mg/dL [SD, 40 mg/dL]; mean creatinine level, 4.6 mg/dL [SD, 1.4 mg/dL]). The 1 patient who did not have renal failure died because of an intraparenchymal bleed secondary to cerebral mycotic aneurysms. Renal failure has been previously noted to be a predictor of severe CDAD.[21]

There was no significant difference in the BUN or creatinine levels among the *C. difficile*–positive and –negative groups. The median value of the BUN for the *C. difficile*–positive patients was 14 mg/dL versus a median value of 16 mg/dL for those patients with a negative test ($P = 0.99$). The median creatinine value for CDAD-positive and CDAD-negative patients was 0.9 mg/dL and 1.1 mg/dL, respectively ($P = 0.0533$).

The median WBC count was greater in patients who tested positive for *C. difficile*, with a value of 12.2 K/μL versus a median WBC count of 8.54 K/μL in the *C. difficile*–negative population ($P < 0.0001$). Further analysis of the data revealed that 26 (34%) of the 77 CDAD-positive patients had a leukocyte count greater than 15.0 K/μL, whereas only 11 (12%) of the 91 CDAD-negative patients had a white count greater than 15.0 K/μL.

The median platelet count in the *C. difficile*–positive patients was also noted to be greater (335,000/μL) compared with that in the patients who tested *C. difficile* negative (273,000/μL; $P < 0.01$). A platelet count greater than 400,000/μL was present in 31% of CDAD-positive group compared with 22% of the CDAD-negative group (24/77 and 20/91, respectively).

TABLE 1. Results of the Study

	Case (n = 77)	Control (n = 91)	*P*
Age, yr			
Mean (SD)	49.9 (16.6)	54.4 (14.9)	0.066*
Range	20–96	23–93	
Sex, n			
Male	31	35	
Female	46	56	
WBC count, K/μL			
Mean (SD)	13.9 (7.75)	9.66 (4.42)	
Range	34–3.4	23–3.4	
Median	12.2	8.54	<0.0001†
WBC count, >15 K/μL			
Percentage of patients	34	12	
Platelet count, K/μL			
Mean (SD)	353 (141)	309 (171)	
Range	714–112	1036–83	
Median	335	273	<0.01†
Platelet count >400 K/μL			
Percentage of patients	31	22	
Albumin level, g/dL			
Mean (SD)	2.41 (0.625)	2.86 (0.644)	
Range	4.0–1.5	4.4–0.9	
Median	2.3	2.9	<0.0001†
BUN level, mg/dL			
Mean (SD)	28.5 (32)	20.5 (16)	
Range	155–2.0	108–2.0	
Median	14	16	0.99†
Creatinine level, mg/dL			
Mean (SD)	1.69 (1.97)	1.58 (1.28)	
Range	10.7–0.5	7.4–0.4	
Median	0.9	1.1	0.0533†

**P* value calculated using the Student *t* test.

†*P* value calculated using the Mann-Whitney *U* test.

Lower albumin levels were observed in the CDAD-positive patients compared with the CDAD-negative patients (median, 2.3 g/dL vs 2.9 g/dL, $P < 0.0001$). However, it should be noted that both patient populations have hypoalbuminemia.

DISCUSSION

In our study, we have attempted to reanalyze previously identified surrogate markers for *C. difficile*–associated diarrhea. However, our methodology differs from previous investigators in an essential aspect, namely we eliminated patients from our study that had underlying hematologic disorders. This was done to avoid the introduction of a confounding factor while evaluating thrombocytosis and leukocytosis. For instance, patients with preexisting thrombocytopenia and leukocytopenia due to a previous disorder such as human immunodeficiency virus would not be expected to respond appropriately to *C. difficile* infection with a reactive increase in their platelet and WBC counts.

Our results revealed that patients who tested positive for *C. difficile* toxin had significantly higher WBC and platelet counts than those who tested negative for the toxin. Furthermore, a larger percentage of patients with CDAD had leukocyte

counts in excess of 15.0 K/μL and platelet counts in excess of 400.0 K/μL when compared with patients without CDAD. These findings have been consistently observed in previous similar studies.[2,7,10,12,14–20] However, this is the first instance that a study was controlled for the presence of patients with preexisting hematologic disorders as previously stated.

As observed in previous studies, we also demonstrated that patients with CDAD had significantly lower levels of albumin when compared with patients without CDAD. It remains an unresolved matter whether hypoalbuminemia is actually a surrogate marker for CDAD or is simply a result of the diarrheal disease process or is otherwise a nonspecific marker for extremely sick patients.

We are of the opinion that our control for confounding factors in our study enabled us to make a more accurate assessment of the increase in WBC and platelet counts as compared with earlier efforts. Our results suggest that leukocytosis and thrombocytosis along with hypoalbuminemia may serve as reliable clinical predictors for CDAD.

A shortcoming of our data collection is its retrospective nature as compared with a prospective study. Also, we lacked a diarrhea-free control group in our study. We recorded only patients with nosocomial diarrhea and assigned them to *C. difficile* toxin–positive and toxin–negative subcategories.

We were unable to include inflammatory markers such as C-reactive protein or erythrocyte sedimentation rate in our study, which have been shown by previous investigators as independent predictors for severe CDAD.[21] The LSU-HSC laboratory does not routinely test for any specific strains of *C. difficile*, and therefore, we were unable to determine how many cases of CDAD were due to hypervirulent strains such as the recently identified NAP1 strain.[22]

Despite good diagnostic tests and adequate treatment modalities, the incidence and severity of *C. difficile*–associated diarrhea seems to be a worsening problem in hospitalized patients. We hope that our efforts will help to further clarify the identification of harbingers for CDAD, possibly leading to the more prompt institution of preventive and therapeutic measures in these high-risk patients and ultimately a better outcome.[22]

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