Laboratory Markers as Predictors of Mortality in Patients With *Clostridium difficile* Infection

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Abstract: Previous studies have identified laboratory markers for severe *Clostridium difficile* infection (CDI). The most consistent of these markers is the presence of marked leukocytosis. We examined the validity of these markers as predictors of mortality in patients with CDI. We excluded patients with preexisting hematologic conditions that would be expected to impair their ability to demonstrate leukocytosis. On univariate analysis, marked leukocytosis ($P = 0.02$), thrombocytopenia ($P = 0.008$), and increased blood urea nitrogen ($P < 0.001$) and creatinine ($P = 0.001$) levels were found to be significantly associated with mortality in patients with CDI. However, on logistic regression analysis, only renal impairment was found to be an independent predictor (odds ratio, 5.07). Importantly, in our study, leukocytosis was not an independent predictor after adjustment for other variables, which may be due to our selection criteria when adjusting for confounding variables. We are therefore of the opinion that in immunocompromised hosts who are leukopenic at the time of CDI diagnosis, other laboratory markers should be identified to serve as indicators for severe disease.

Key Words: Leukocytosis, renal failure, laboratory markers, *Clostridium difficile*–associated diarrhea

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Clostridium difficile infection (CDI) is now recognized as the most common cause of nosocomial diarrhea,[1] and since the beginning of this millennium, North America and Europe have reported case statistics that have assumed epidemic proportions. The disease is associated with a significant mortality and morbidity,[2] and the associated health care cost is estimated at nearly $1 billion in the United States alone.[3] Recently identified epidemiological factors for the disease may include increased use of fluoroquinolones[4] and the emergence of a hypervirulent strain of the bacterium known as the North American pulsotype (NAP1) strain.

Significance

Previous studies have identified laboratory markers that may correlate with poor outcomes in patients with CDI. These include leukocytosis, increased creatinine levels, and hypoalbuminemia. Although there is some variability in the results of these studies, marked leukocytosis (generally defined by studies to be leukocytes >20,000/μL) has emerged as the single consistent predictor of severe disease.[5–9] This is important to note because, as our results indicate, we did not find leukocytosis to be an independent predictor for mortality in patients with CDI.

Difference in Methodology

We have previously shown that leukocytosis, thrombocytosis, and hypoalbuminemia are reliable predictors for the presence of CDI.[10] This is after careful exclusion of confounding factors, specifically patients with preexisting hematologic disorders who may be unable to sustain thrombocytosis and/or leukocytosis in response to infection. We therefore wished to determine whether the exclusion of these confounding factors might also be important when evaluating laboratory markers for mortality in patients with CDI. To our knowledge, no previous studies have addressed this important concern.

METHODS

We retrospectively reviewed the records of all inpatients at the Louisiana State University Health Science Center, Shreveport, who were diagnosed with nosocomial diarrhea and subsequently had a stool sample that tested positive for *C. difficile* over a 10-year period. The sole test used by our facility is an enzyme-linked immunosorbent assay that tests for both *C. difficile* toxins A and B. Over the 10-year study period, 550 patients had stool samples that tested positive for *C. difficile* toxin.

Exclusion Criteria

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 also excluded patients receiving active chemotherapy. We were also forced to exclude a small number of patients who had missing or incomplete laboratory data. In patients who tested positive more than once for the *C. difficile* toxin, only the initial test results were included. Meeting the above criteria, our final population included 184 *C. difficile*–positive patients. Of these 184 patients, 25 died during a 30-day period (13.6% mortality rate); this was our case group. On the other hand, 159 patients survived and were discharged from the hospital; this group of patients served as our control. The 30-day mortality of patients with CDI was 13.6%. It is very difficult to determine the specific cause of death from CDI and therefore the degree to which CDI contributes to death from seemingly unrelated causes.[11] For these reasons, the all-cause, 30-day mortality was chosen as the primary end point.

Statistical Methods

Patient charts were thoroughly reviewed with attention to the clinical history, hospital course, diagnoses, and laboratory

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results. The age (>75 years), sex, medical diagnoses, and date of the *C. difficile* test were recorded. The laboratory values of white blood cells (WBCs), 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. Patients were also assessed for the presence of nonhematologic malignancies. The distribution of the data was assessed using the Shapiro-Wilk test and was found to be nonparametric. Therefore, analysis of the data was done with the Mann-Whitney *U* test (nonnormal distribution). A Fisher exact test was used for the sex and age of the patients and also for the presence or absence nonhematologic malignancies.

Logistic Regression

A logistic regression model was then used to identify factors that were independently associated with mortality in patients with CDI, adjusting for other variables that may logically or historically influence each value. A logistic regression calculator was used to generate the coefficients of a prediction formula (and SEs of estimate and significance levels) and odds ratios (ORs) (with 95% confidence intervals [CIs]).

The institutional review board at Louisiana State University Health Science Center, Shreveport, approved our study.

RESULTS

A total of 184 patients who tested positive for *C. difficile* diarrhea were included in this study (Table 1). Our patient groups consist of 159 *C. difficile*–positive patients who survived their hospital stay and 25 *C. difficile*–positive patients who died during a 30-day period. Fisher exact test did not reveal a difference in age (older or younger than 75 years, $P = 1.00$), sex ($P = 0.08$), or the presence of nonhematologic malignancies ($P = 1.00$) between the 2 groups.

Renal Function

There was a significant difference in the BUN and creatinine levels among the survivor and nonsurvivor groups. The median value of the BUN for the surviving patients was 13 mg/dL versus a median value of 57 mg/dL for those patients with a negative test ($P < 0.001$). The median creatinine value for the surviving patients versus the nonsurvivors was 0.9 and 3.3 mg/dL, respectively ($P < 0.001$).

TABLE 1. Univariate Analysis Results for 184 Patients With CDI Who Either Survived or Died During the Course of Their Hospital Stay

Variable	Survivors (n = 159)	Nonsurvivors (n = 25)	*P*
Age >75 y, n (%)	17 (11)	2 (8)	1.00
Sex, n			0.08
Male	76	17	
Female	83	8	
Malignancy, n (%)	28 (18)	4 (16)	1.00
Median WBC count, ×1000/μL	11	13.7	0.09
WBC >20,000/μL, n (%)	24 (15)	9 (36)	0.02
Median BUN level, mg/dL	13	57	<0.001
Median creatinine level, mg/dL	0.9	3.3	<0.001
Median platelet count, ×1000/μL	301	222	<0.01
Platelet <150,000/μL, n (%)	17 (11)	8 (32)	0.008
Median albumin level, mg/dL	2.5	2.4	0.28
Albumin <2 g/dL, n (%)	28 (18)	9 (36)	0.06

TABLE 2. Logistic Regression Model to Identify Variables Independently Associated With Mortality in CDI

Variable	OR	95% CI	*P*
Age >75 y	0.6	0.1–3.0	0.60
Female	0.5	0.2–1.3	0.16
Malignancy	1.1	0.3–4.0	0.84
WBC >20,000/μL	2.0	0.7–6.0	0.21
Creatinine >2 mg/dL	5.07	1.8–13.9	0.001
Platelet <150,000/μL	2.6	0.8–8.0	0.10
Albumin <2 g/dL	1.6	0.5–4.6	0.40

Leukocytosis

There was no significant difference in the median WBC count between the 2 patient populations; for the survivors, it was 11,000/μL versus a median WBC count of 13,700/μL for the nonsurvivors ($P = 0.09$). Further analysis of the data revealed that 24 (15%) of the 159 survivors had a leukocyte count greater than 20,000/μL, whereas 9 (36%) of the nonsurviving patients had a WBC count greater than 20,000/μL. Using Fisher exact test, this difference was noted to be significant ($P = 0.02$).

Thrombocytopenia

The median platelet count in the surviving patients was also noted to be greater (301,000/μL) compared with that in the patients who did not survive (222,000/μL; $P < 0.01$). The number of survivors with thrombocytopenia was noted to be 17 (11%) as compared with the nonsurvivors with thrombocytopenia that was 8 (32%). Using Fisher exact test, this difference was noted to be significant ($P = 0.008$).

Hypoalbuminemia

Albumin levels were not noted to be significantly different between the surviving and nonsurviving groups of patients (median, 2.5 vs 2.4 g/dL, respectively; $P = 0.28$). Even when we tested for severe hypoalbuminemia (<2.0 g/dL), the difference was not statistically significant between the 2 groups ($P = 0.06$). However, it should be noted that both patient populations have hypoalbuminemia.

Independent Predictors

Next, we performed a logistic regression for a dichotomous outcome adjusted for factors that would logically or historically influence each value. These were either the presence or absence of renal failure (defined by a creatinine level >2.0 mg/dL), marked leukocytosis (defined by a leukocyte count >20,000/μL), thrombocytopenia (defined by a platelet count <150,000/μL), female sex, hypoalbuminemia, nonhematologic malignancies, and age older than 75 years.

Using the above logistic model, the only variable that was independently associated with mortality in CDI was renal failure (OR, 5.08; 95% CI, 1.8–13.9; $P = 0.001$) (Table 2). Surprisingly, our analysis did not find leukocytosis, a previously well-recognized marker for severe CDI, to be significantly associated with mortality in patients with CDI when adjusting for covariates ($P = 0.21$).

DISCUSSION

In our study, we have attempted to reanalyze previously identified laboratory markers for mortality in patients with CDI.

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 WBC and platelet counts. For instance, patients with preexisting thrombocytopenia and leukocytopenia due to a previous disorder such as human immunodeficiency virus or leukemia/lymphoma would not be expected to respond appropriately to CDI with an increase in their platelet and/or WBC counts.

We have previously shown that thrombocytosis is a surrogate marker for CDI even when controlling for the presence of preexisting hematologic disorders.[10] However, our results indicate that, on univariate analysis, instead, it was thrombocytopenia that was significantly associated with mortality in patients with CDI. This may be explained by the fact that thrombocytopenia is a predictor of mortality associated with intensive care unit patients and in severe sepsis and that the degree and duration of thrombocytopenia, as well as the net change in the platelet count, are important determinants of survival.[12] In any case, as the above logistic regression model results indicate, thrombocytopenia is not an independent predictor for mortality in patients with CDI.

Also, surprisingly, while univariate analysis did show marked leukocytosis to be a predictor of mortality in patients with CDI, it was not an independent predictor when we controlled for the presence of other variables. In fact, the only significantly associated independent factor for mortality in patients with CDI on logistic regression analysis was the presence of renal failure. As stated above, several studies have shown marked leukocytosis to be independently associated with severe CDI. We hypothesize that this difference in our results is because we were careful to exclude patients who had preexisting hematologic disorders that would impair their ability to respond to infection with leukocytosis/thrombocytosis. Based on our evaluation of data, more than half of diagnosed patients with CDI also have coexisting conditions such as HIV, leukemias, lymphomas, and other immunocompromised conditions. Again, preliminary analysis revealed that a significant number of these patients had a leukopenia (WBC count <3000/μL) and thrombocytopenia (platelet count <150,000/μL). Therefore, we believe that this is an important consideration when interpreting laboratory values to predict severe disease or mortality.

It may be mentioned in this regard that a recent study concluded that C-reactive protein is a much better indicator of severe CDI as compared with leukocytosis.[13] We believe that this may be especially true in patients who are already leukopenic at the time of CDI diagnosis and are thus unable to mount an adequate reactive leukocytosis in response to infection.

Our study was limited by its retrospective nature. Another differing aspect of our study was that our primary end point was limited to all-cause, 30-day inpatient mortality in patients with CDI. Therefore, our results cannot be strictly compared with many of the above cited studies,[5–9] because they deal with severe CDI, which is a broader term, defined as fulminant colitis, often requiring intensive care unit admission, mechanical ventilation, inotropic support, and colectomy.

We believe that using the above criteria will enable clinicians to identify patients who may be at a greater risk of mortality in CDI and improve outcomes for the disease by more aggressive and timely intervention. Ultimately, we hope to develop a comprehensive prediction tool for assessing the severity of CDI.

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