Temporal Changes in Serum Albumin and Total Protein in Patients with Hospital-acquired *Clostridium difficile* Infection

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**Abstract.** Studies have demonstrated low serum levels of total protein (TP) and albumin (ALB) in patients with *Clostridium difficile* infection (CDI), especially with refractory and recurrent disease. However, it is not known whether low TP and/or ALB levels are a risk factor for CDI or merely a result of diarrheal loss. The aim of this study is to determine if low TP and/or ALB level is an antecedent or sequela of CDI, which would be useful in risk stratification of hospitalized or nursing home patients. A retrospective cohort study was conducted in a 700-bed tertiary care teaching hospital. Records of all hospitalized patients with CDI from 2006-2011 were analyzed. The inclusion criteria for the final cohort (n=46) were: subjects not diagnosed with HIV; onset of CDI at least one week after hospitalization; serial values of TP and ALB available on three occasions (at onset of CDI, seven days prior, and post-onset of CDI). Seven days prior to the onset of CDI, 40/46 (87%) subjects had low ALB levels with a mean of 2.6±0.7 g/dL and 37/46 (80.4%) had low TP with a mean of 5.8±1.0 g/dL. At the onset of CDI, 45/46 (97.8%) subjects had low ALB (group: 2.1±0.6 g/dL) and 41/46 (89.1%) had low TP (group: 5.1±1.0). Seven days post-onset of CDI, 45/46 subjects continued to have decreased ALB (group: 2.0±0.6) and 39/46 (84.8%) had low TP (group: 5.2±1.2). The pre-onset data for ALB and TP were significantly different than the comparable data at onset and seven days post-onset (p<0.0001 for both ALB and TP). No significant difference was observed between onset and seven days post-onset. Most patients are hypoproteinemic prior to the onset of hospital-acquired CDI. Although some subjects lost protein after the onset of CDI, this was not statistically significant. This study suggests that antecedent low levels of ALB and TP may be a risk factor for the acquisition of CDI.

**Key words:** Serum albumin, total protein, *Clostridium difficile*, biomarkers, hospital epidemiology.

**Introduction**

*Clostridium difficile*, a spore-forming bacteria, is a major infectious cause of nosocomial diarrhea [1-3]. *C difficile* is estimated to colonize 3% of healthy adults [4] and 15-25% of hospitalized adults [5-7]. *C difficile* infection (CDI) is a major health care burden with increasing morbidity and mortality [8-14]. It is therefore important to identify all clinical and laboratory findings that may represent risk factors for CDI.

Known risk factors of CDI are antibiotics [15], cancer chemotherapy [16], use of proton pump inhibitors [17], advanced age [18,19], critical illness, and chronic kidney disease [20]. *C difficile* produces two major toxins, toxin A and toxin B, and the latter is largely responsible for CDI. The clinical spectrum of illness can range from an asymptomatic carrier state to a life-threatening colitis with toxic mega colon. The clinical presentation can be mirrored by some laboratory findings such as serum albumin and total protein. Studies have demonstrated low serum levels of total protein (TP) and albumin (ALB) in patients with CDI [21,22] especially with refractory and recurrent disease [23]. However, it is unclear whether low TP and/or ALB

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levels put patients at risk for CDI or are merely a result of diarrheal loss. The aim of this study is to determine if low TP and/or ALB levels are an antecedent or sequela of CDI, which would be useful in risk stratification of patients in acute care or long-term healthcare settings.

### Materials and Methods

**Setting.** The study was conducted at a 700-bed tertiary care teaching hospital in New Jersey, USA. Because of the retrospective nature of the study, the use of only existing medical records, and because the study posed no additional risk to the patients, the Institutional Review Board of St. Joseph’s Healthcare System designated the study exempt. All necessary procedures pertaining to patient confidentiality were maintained.

**Protocol.** This is a retrospective cohort study with a longitudinal component, in which we examined changes in serum albumin and total protein over a two-week period in a group of subjects who developed CDI during their hospital stay. A summary of the development of the cohort is provided in Figure 1. We required that the subjects have no evidence of diarrheal disease upon admission and had available data on albumin and total protein at three points during the two-week interval: at seven days prior to onset, at onset, and seven days post-onset. All patients were required to have a positive test for *C. difficile* toxins A and B with onset of CDI at least one week after hospitalization. These data were gathered over the period 2006-2011, inclusive. The characteristics of the study group are described in Table 1.

**Laboratory analyses.** Albumin and total protein were obtained from the patients’ charts. As described in a previous paper [22], the analyses were performed using the Beckman DXC® analyzer with all materials and reagents from the manufacturer (Beckman Coulter Inc., CA, USA). The reference intervals used for ALB and TP were 3.2-4.6 g/dl and 6.3-8.2 g/dl, respectively.

Fecal analysis for *C. difficile* toxins A and B was performed using an ELISA test kit (TechLab, Blacksburg, VA).

**Statistical Methods.** Two approaches were used to evaluate changes in albumin and total protein over the time periods studied. In the first approach, we examined the continuous data as repeated measures for the three times. The data were tested for fit-to-normality by the D’Agostino-Pearson omnibus normality test. Because most of the group-wise data were found to differ significantly from normal distributions, we used repeated measures, nonparametric ANOVA (Friedman’s test) with Dunn’s test used, *post hoc*, to examine differences between the groups.

In the second approach, we examined categorical changes from the first to the second time period and from the

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### Table 1. Characteristics of the study group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median and IQR</td>
<td>71 (61-81)</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>23/23</td>
</tr>
<tr>
<td>Nursing home resident</td>
<td>13 (28%)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>Chronic Kidney Disease</td>
<td>10 (22%)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>10 (22%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>10 (22%)</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>Other concurrent infec-</td>
<td>12 (26%)</td>
</tr>
<tr>
<td>tions</td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>3 (6.5%)</td>
</tr>
<tr>
<td>GI Bleed</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>
second to the third time period; changes were considered either decreases, increases, or no change. An increase or decrease required a minimum change of 5% from the previous value of albumin or total protein; if this criterion was not met, the variation was considered "no change". We then compared these observed changes with expected changes. For expected changes, we considered that pure chance would lead to one third of the changes being decreases, one third being increases, and one third being in the no change category. Because n=46 is not evenly divisible by three, we brought the expected values in each category to the nearest whole number, that being fifteen. Contingency tables were constructed to examine observed versus expected values: 2x3 contingency tables were tested for significance by chi-square tests; 2x2 tables by Fisher’s exact test, on a post hoc basis.

### Results

**Quantitative differences.** Longitudinal changes in serum albumin and total protein concentration are shown in Figure 2. For albumin, 40 of 46 subjects (87%) had concentrations below our RI; at 7 days prior to onset, albumin concentrations [median, (Interquartile range, IQR)] were 2.6 (2.1 to 3.1). By onset, albumin decreased to 2.1 (1.8 to 2.5); p<0.001. There was no significant change (p≥0.05) from day of onset to seven days post-onset, at which time albumin concentrations for the group were 1.95 (1.7 to 2.5).

For total protein, there were 37 of 46 subjects whose values were below the RI. Of the remaining subjects, one was hyperproteinemic and eight had total protein concentrations within the RI. Thus 80.4% of subjects were hypoproteinemic. Groupwise concentration of total protein fell from 5.9 (5.0 to 6.4) at seven days prior to onset to 5.1 (4.3 to 5.8) on day of onset (p<0.001); however, the
change from day of onset to seven days post-onset 5.0 (4.4 to 6.0), as was the case for albumin, was not statistically significant (p≥0.05).

Categorically-evaluated directional changes. Table 2 provides data on observed versus expected changes in albumin and total protein over the course of the observation period, evaluating significant increases, decreases, and those subjects for whom no change occurred from Day -7 to Day 1 and Day 1 to Day 7.

For albumin (Table 2A) and total protein (Table 2B), changes from Day -7 to Day 1 were significant: p=0.0002 overall for albumin and p=0.0031 for total protein. When evaluated on a pair-wise basis, albumin showed statistically significant differences for decrease versus increase (p=0.0011) and for decrease versus no change (p=0.0017). There was no significant difference between no change and increase (p=1.000). Total protein demonstrated a congruent result in terms of increase versus decrease (p=0.0031) and no change versus increase (p=0.3517); however, decrease versus no change, with p=0.0432, did not achieve our post hoc criterion for statistical significance (p≤0.0167). For both albumin and total protein, the data for Day 1 to Day 7 suggest that no differences are detectable.

Discussion

CDI is an increasingly common clinical problem with greater incidence in the elderly [24-26], hospitalized [27,28] and nursing home patients [29-31]. Antibiotic exposure is the major established risk factor for CDI [15]. However, exposure of antibiotic treated adults to C. difficile does not always lead to colonization and symptomatic infection [32]. Host factors are increasingly recognized as determinants of disease expression [33]. In our study, most patients are hypoproteinemic prior to the onset of hospital-acquired CDI. Regarding mean levels, further protein loss was not demonstrated in the cohort following the onset of infection.

In a recent case-control study [22], we compared differences in serum ALB and TP in patients with CDI (n=171) and a contemporaneous control group (n=332). For both ALB and TP, over 75% of CDI subjects had both ALB and TP concentrations...
below the RI in our laboratory. On the other hand, the control subjects had values of TP and ALB approximately at or above the median of the RI. These differences were highly significant \((p<0.0001\) for both ALB and TP). As we noted therein, a limitation of that study was the cross-sectional nature of the measurements. This naturally led to the longitudinal approach used in this paper. This study suggests that antecedent low levels of ALB and TP may be a risk factor for the acquisition of CDI.

The pathophysiologic role of serum ALB and TP in CDI may be explained as follows. Albumin is a plasma protein that accounts for a major part of colloid osmotic pressure. Hence, hypoalbuminemia results in decreased colloid osmotic pressure. This can be associated with intestinal mucosal edema, which impairs the colonic mucosal barrier of the host to \(C\) difficile [34-37]. Hypoalbuminemia could further cause fluid to shift into the third space. This can subsequently result in intravascular volume depletion and dehydration and thus worsen renal function. Of note, elevated serum creatinine is often used as a marker for grading the severity of CDI [33].

In addition, a compromised immune system in patients with severe hypoalbuminemia due to poor nutritional status could depress host barriers to CDI infection, and may also be responsible for delayed response to treatment and predisposition to relapses [38]. Low protein level could suggest both low albumin levels and low serum immunoglobulins, which usually neutralize \(C\) difficile toxin [21,22]. Low immunoglobulins may mirror poor serum antitoxin antibody response, which has provided the basis for giving passive immunization with intravenous immunoglobulins in severe and recurrent CDI, as demonstrated in some studies [39,40].

An unanswered question in several studies was whether the low TP and/or ALB level is a risk factor for CDI or merely a result of diarrheal loss. Exposure to antibiotics may not only increase the risk for CDI but may also cause antibiotic-associated diarrhea (AAD), which can lead to protein-losing enteropathy [33]. As seen in our study, most patients are hypoproteinemic prior to the onset of hospital-acquired CDI, but further protein loss was not demonstrated in the cohort following the onset of infection. It can be speculated that while AAD may cause protein-losing enteropathy, CDI may not. However, it should be noted that in this small, prospectively designed, cross-sectional study [41], nine of ten subjects with symptomatic CDI had elevated \(\alpha_1\)-antitrypsin levels when compared with five of ten subjects with diarrhea due to factors other than CDI. Thus, it is quite likely that, though depressed serum protein concentrations predispose hospitalized patients to CDI, in some subjects, a degree of protein loss may continue as a result rather than as a cause of CDI.

As with any retrospective model, this study clearly has certain limitations. The nutritional status of the patients could not be assessed. As only those patients with available serial lab values were included in the cohort, the study population is relatively small. Nevertheless, the findings of our study are consistent and important because an understanding of hypoproteinemia as a potential risk factor may help identify those hospitalized patients at risk for acquisition of CDI. In addition, to the best of our knowledge, there are no other studies that have examined temporal changes of serum ALB and TP prior, at onset, and post-onset of CDI.

In conclusion, low serum ALB and TP levels can serve as a useful marker in identifying the subset of hospitalized patients that are at high risk for acquisition of CDI. Any antibiotic use in these patients should be reassessed by the physician on a daily basis to further reduce their risk.

References