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MAJOR ARTICLE

Humoral Immune Response to *Clostridioides difficile* Toxins A and B in Hospitalized Immunocompromised Patients With *C difficile* Infection

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Background. The humoral immune response to *Clostridioides difficile* toxins in *C difficile* infection (CDI) is incompletely characterized in immunocompromised hosts (ICHs).

Methods. We conducted a prospective study of hospitalized adults with CDI, with and without immunosuppression (hematologic malignancy, active solid tumor, solid organ or stem cell transplant, inflammatory bowel disease, autoimmune disease, congenital or acquired immunodeficiency, asplenia, chronic receipt of high-dose steroids, or receipt of immunosuppressing medications within 12 months). Serum and stool antibody concentrations of immunoglobulin (Ig)M, IgG, and IgA to *C difficile* toxins A and B at treatment days 0, 3, and 10–14 were compared.

Results. Ninety-eight subjects (47 ICH; 51 non-ICH) were enrolled. Baseline serum antitoxin A and B antibody levels were similar. At day 3, ICHs demonstrated lower serum levels of antitoxin A IgG, antitoxin A IgA, and antitoxin B IgA (all $P < .05$). At day 10–14, lower antitoxin A IgG concentrations were observed in ICHs (ICH, 21 enzyme-linked immunosorbent assay [ELISA] units; interquartile range [IQR], 16.4–44.6) compared with non-ICH subjects (49.0 ELISA units; IQR, 21.5–103; $P = .045$). In stool, we observed lower concentrations of antitoxin B IgA antibodies at baseline and at day 3 for ICH subjects, with a notable difference in concentrations of antitoxin B IgA at day 3 (ICH, 6.7 ELISA units [IQR, 1.9–13.9] compared with non-ICH, 18.1 ELISA units [IQR, 4.9–31.7]; $P = .003$).

Conclusions. The ICHs with CDI demonstrated lower levels of *C difficile* antitoxin antibodies in serum and stool during early CDI therapy compared with non-ICHs. These data provide insight into the humoral response to CDI in ICHs.

Keywords. *C difficile* toxins; *Clostridioides difficile* infection; humoral immunity; immunosuppression.

Clostridioides difficile is the leading cause of healthcare-associated infectious diarrhea. More than 450 000 cases and 20 000 associated deaths have been reported in the United States annually [1–3]. *Clostridioides difficile* infection (CDI) presents with a spectrum of clinical disease ranging from mild, self-limited diarrhea to a fulminant colitis. Infection may occur repeatedly in some patients leading to recurrent hospitalizations, high healthcare utilization, and poor quality of life [4]. Certain patient populations such as the elderly and patients with weakened immune systems appear to be at an enhanced risk for CDI and its complications [5–11]. The increased risk for CDI in immunocompromised hosts (ICHs) may be multifactorial and due to external clinical factors, such as antibiotic exposure and immunosuppressing agents, as well as intrinsic host factors including impaired specific humoral responses to *C difficile* toxins A and B.

Prior research in non-immunocompromised host populations (non-ICH) has suggested that the magnitude of antibody response *to C difficile* toxin A may protect against symptomatic CDI and recurrence [12]. In addition, serum antitoxin B antibody response has been associated with protection from recurrent CDI (rCDI) [13]. Although it is possible that these immunologic markers may also be of utility in ICH patient populations, data are lacking due to the exclusion of ICH patients from many studies.

The aim of this research was to evaluate the humoral immune response to *C difficile* toxins A and B in a cohort of immunocompromised patients. Our goal was to better understand whether impaired humoral immunity specific to *C difficile*

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toxins influences clinical symptoms and risk of rCDI. Our central hypothesis was that impairment in *C difficile*-specific antibody response to *C difficile* toxins A and B may drive host risk for CDI and influence clinical outcomes in immunocompromised patients. The importance of this research is 2-fold. First, a more complete understanding of the immune response to *C difficile* toxins is necessary to help predict whether future therapies such as a *C difficile* vaccine might work to prevent disease or recurrence in this population. Second, the data will help to inform future passive immunization strategies targeting this patient population.

METHODS

Patient Cohorts

Inpatients at Beth Israel Deaconess Medical Center ([BIDMC] Boston, MA) and Texas Medical Center ([TMC] Houston, TX) were prospectively enrolled between June 2016 and February 2020. Eligible subjects were ≥18 years old with positive stool *C difficile* nucleic acid amplification test (NAAT) result, initiating CDI therapy, and had acute diarrhea, defined as follows: (1) ≥3 unformed bowel movements (UBMs) during any 24 hours in the 48 hours before or the 24 hours after the time of stool collection; (2) persistent diarrhea in the same time window, per multiple provider notes; or (3) pseudomembranous colitis or (4) in patients with chronic diarrhea, a clear change in stool consistency or frequency. In most cases definition "1" was applied. Patients were excluded for the following: history of chronic diarrhea without acute exacerbation, presence of colostomy, receipt of bezlotoxumab, intravenous immunoglobulin (Ig) or fresh frozen plasma within 30 days, enrollment in any *C difficile* vaccine study, >48 hours of CDI therapy, insufficient stool specimen, or stool sample older than 72 hours.

The *C difficile* testing method at BIDMC was NAAT only (before July 2018) (GeneXpert real-time polymerase chain reaction; Cepheid) and NAAT with a reflex EIA (ImmunoCard Toxins A&B; Meridian Bioscience) if NAAT positive (after July 2018); TMC used 2 methods (BDMax Cdiff Assay, BD and BioFire FilmArray Gastrointestinal (GI) Panel [bioMérieux]). A subset had stool tested for *C difficile* toxins A and B with an ultrasensitive quantitative single molecule array immunoassay (Simoa; bioMérieux), which can separately detect and quantify *C difficile* toxins A and B over a 5-log range of concentrations with a clinical cutoff of 20 pg/mL in diluted stool samples [14]. A discarded serum sample from within 1 day of the stool sample was captured. Samples were collected prospectively under written informed consent.

Stool and serum samples were collected at baseline (day of CDI diagnosis), at day 3 (±day), and day 10–14 (±2 days) relative to CDI treatment initiation. Home stool collection kits were provided for patients who left the hospital before day 10–14. Every effort was made to collect follow-up serum samples, utilizing clinical follow-up visits where possible. Weekly phone calls assessed clinical response and CDI recurrence through 100 days. If symptoms returned, patients were encouraged to collect a stool sample for CDI testing; where possible, a paired serum sample was also collected. *Clostridioides difficile* treatment was determined by the subject's treating physician. For the purposes of analysis, treatment modalities were stratified into 1 of 3 categories: vancomycin-containing regimens, regimens containing metronidazole alone, and fidaxomicin-containing regimens.

Data Collection

Clinical outcomes and laboratory findings were gathered through chart review and patient phone calls. Outcomes included the following: time to resolution of diarrhea (defined as the time elapsed from the first dose of drug treatment for *C difficile* to the last UBM, followed by 2 consecutive days of ≤3 UBMs per day) and outcomes including death, intensive care unit (ICU) stay, and colectomy. Recurrent CDI was defined as resolution of diarrhea for ≥48 hours off CDI antibiotics, followed by new diarrhea and characterized by the patient's physician as having rCDI. Recurrences were classified as either "clinical diagnosis only" (no stool testing) or "clinical and laboratory diagnosis" (confirmatory stool testing performed). Retreatment with CDI agents was required. If CDI testing was negative, or the patient did not meet the diarrhea definition, or whether the diarrhea was not attributed to CDI by the patient's provider, the subject was not considered to have a recurrence. Definitions of severe CDI, CDI severity scores (Infectious Diseases Society of America [IDSA]-Society for Healthcare Epidemiology of America [SHEA] [15], European Society of Microbiology and Infectious Diseases [16], Zar [17], and Belmares [18]), and immunocompromised status were used in accordance with our prior work [19]. Immunocompromised status definitions are outlined in Supplementary Figure 1. Major categories included the following: active hematologic malignancy, solid tumor with cytotoxic chemotherapy in the last 3 months, receipt of stem cell transplant, chronic (>14 days total) receipt of high-dose steroids (mean prednisone ≥20 mg/day, or equivalent), inflammatory bowel disease on immunomodulating agents, receipt of a medication known to suppress the immune system within 12 months, congenital or acquired immunodeficiency, or asplenia. Laboratory characteristics including peak and nadir white blood cell count (WBC), absolute neutrophil count (ANC), and absolute lymphocyte count (ALC) nadirs, peak creatinine, and albumin nadir were recorded within 5 days preceding and 2 days after stool collection. Colonoscopy or sigmoidoscopy reports were reviewed for pseudomembranes (within 1 week of CDI). Colitis or ileus on abdominal imaging were noted if obtained within 48 hours of CDI diagnosis. Temperature ≥38.0°C, systolic blood pressure <100 mm Hg, and peak lactate values were recorded within

24 hours of CDI diagnosis. Abdominal tenderness required documentation in a physician physical exam ±1 day of specimen collection. *Clostridioides difficile* infection clinical resolution was defined as resolution of diarrhea (<3 UBMs/24 hours for 2 days) after completion of standard-of-care CDI therapy. Receipt of concomitant non-CDI antibiotics was documented through day 100.

Laboratory Analytes

Antibodies (IgA, IgG, or IgM) to toxin A and to toxin B were measured in serum and in stool by semiquantitative enzyme-linked immunosorbent assay (ELISA). Results are expressed as arbitrary ELISA units as previously described [12, 14, 20–24]. Stool toxin A and toxin B concentrations were measured by Simoa for 81 of 98 subjects according to methods previously described [14, 25].

Objectives

Our primary objective was to describe the humoral immune response to *C difficile* toxins A and B in hospitalized subjects with and without immunosuppression. Our main endpoints were the serum levels of IgG to toxins A and B at treatment day 10–14. Our secondary endpoints were the serum levels of IgM to toxins A and B at treatment day 3. We also aimed to compare CDI clinical outcomes and CDI recurrence at day 100 between immunocompromised and non-immunocompromised subjects.

Statistical Methods

Descriptive statistics included (1) median and interquartile range for continuous variables and (2) frequency and percentages for categorical variables. Continuous and discrete variables were compared between groups using the Mann-Whitney *U* test and the χ^2 or Fisher's exact test, respectively. Results were considered statistically significant when $P < .05$. All statistical analyses were performed using SPSS 23.0 software (SPSS, Chicago, IL). Figures were generated using GraphPad Prism 5 Software (GraphPad Software Inc., San Diego, CA).

Patient Consent Statement

Written informed consent was obtained for all participants before enrollment. The design of the work has been approved by the local ethical committees. At BIDMC this was the Committee on Clinical Investigations and at TMC this was the Institutional Review Board.

RESULTS

Between June 2016 and February 2020, 114 subjects consented (Figure 1). After exclusions, 98 subjects were available for analysis. Of these, 47 subjects (48%) met our study definition of ICH; 51 subjects (52%) were non-ICH. Patient characteristics are shown in Table 1. Groups had similar baseline sex, age, race, and ethnicity. Patients with active hematologic malignancy made up the largest proportion of immunocompromised subjects (11 patients, 23.4%), followed by receipt of high-dose steroids (8 patients, 17.0%), and malignancy requiring recent cytotoxic chemotherapy (8 patients, 17.0%).

Clinical and laboratory features at CDI diagnosis are presented in Table 2. Groups had similar baseline clinical features. Forty-nine percent of non-ICH and 51.1% of ICH subjects met criteria for severe CDI according to the IDSA guidelines. A substantial proportion (72.5% of non-ICH and 68.1% of ICH) met criteria for severe CDI by at least 1 of the 4 severity scores examined. On average, the ICH population had a lower median peak

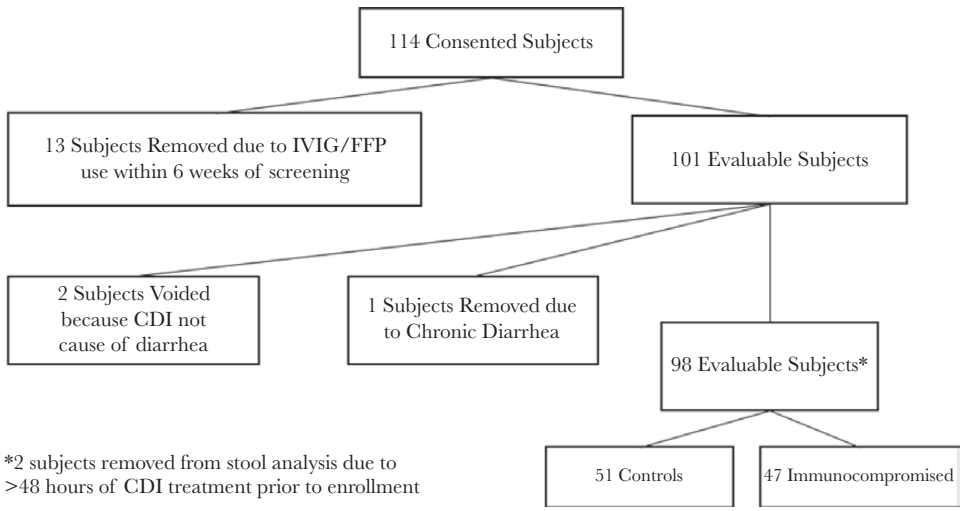

Figure 1. Results of screening, exclusion, and enrollment among study subjects. There were 114 consented subjects and 101 evaluable subjects after excluding 13 subjects who had received fresh frozen plasma (FFP) or intravenous immunoglobulin (IVIG). Three additional subjects were excluded from the analysis due to diarrhea determined to be of alternative cause (2) and 1 subject who had chronic diarrhea. Of the 98 evaluable subjects, there were 2 subjects whose stool was excluded from the stool antitoxin analyses due to receipt of >48 hours of *Clostridioides difficile* infection (CDI) antibiotics before sample collection.

Table 1. Demographic Characteristics of 98 Enrolled Subjects With CDI

Characteristics	Not Immunocompromised (N = 51, 52%)	Immunocompromised (N = 47, 48%)	*P* Value
Male (%)	20 (39.2)	23 (48.9)	.416
Age (median years, IQR)	62 (52–75)	66 (53–73)	.991
Race			.126
White (N, %)	46 (90.2)	39 (83.0)	
African American (N, %)	2 (3.9)	7 (14.9)	
Asian (N, %)	2 (3.9)	0	
Unknown (N, %)	1 (2.0)	0	
Mixed origin (N, %)	0	1 (2.1)	
Ethnicity			.934
Hispanic (N, %)	3 (5.9)	2 (4.3)	
Not Hispanic (N, %)	47 (92.2)	44 (93.6)	
Not reported (N, %)	1 (2.0)	1 (2.1)	
Immunocompromising Conditions			
Active hematologic malignancy (N, %)		11 (23.4)	
Solid tumor with recent chemotherapy (N, %)		8 (17.0)	
HSCT (N, %)		2 (4.3)	
SOT (N, %)		6 (12.8)	
Chronic administration of high-dose steroids (N, %)		8 (17.0)	
IBD (N, %)		6 (12.8)	
Autoimmune Conditions (N, %)		6 (12.8)	
History of prior CDI	13 (25.5)	13 (27.7)	.823

Abbreviations: CDI, *Clostridioides difficile* infection; HSCT, hematopoietic stem cell transplant; IBD, inflammatory bowel disease; IQR, interquartile range; SOT, solid organ transplant.

NOTES: HSCT included 1 allogeneic stem cell recipient. Inflammatory bowel disease included Crohn's disease (5 subjects) and ulcerative colitis (1 subject). Autoimmune conditions included rheumatoid arthritis (3 subjects), lupus (1 subject), mixed connective tissue disease (1 subject), and seronegative inflammatory arthropathy (1 subject).

WBC count compared with the non-ICH population; however, other laboratory parameters such as ANC nadir, ALC nadir, and renal dysfunction were not significantly different.

Serum Antitoxin Antibody Enzyme-Linked Immunosorbent Assay Results

Serum concentrations of IgA, IgG, and IgM antibodies to *C difficile* toxins A and B were measured by semiquantitative ELISA at treatment days 0, 3, and 10–14 (Supplementary Table 1).

Baseline

At treatment day 0, there was no difference in median baseline antitoxin A IgM levels between non-ICH and ICH subjects (P = .850). Similarly, no difference was detected in baseline antitoxin B IgM levels between non-ICH and ICH subjects (P = .532). Immunocompromised hosts had marginally lower median antitoxin A IgG (30.6 ELISA units; range, 14.2–63.8) levels compared with non-ICH subjects (50.4 ELISA units; range, 22.9–102.5; P = .061). There were no differences in baseline antitoxin B IgG levels (P = .674), baseline antitoxin A IgA levels (P = .294), or baseline antitoxin B IgA levels (P = .336) between non-ICH and ICH subjects.

Day 3

At treatment day 3, we examined the serum levels of IgM to toxins A and B (Figure 2a). We observed overall lower antitoxin B IgM levels in the ICH population. In the non-ICH population, serum IgM level was 6.5 ELISA units (range, 3.8–13.2). In the ICH population, we observed a serum IgM level of 4.8 ELISA units (range, 2.3–8.2; P = .051). Similar antitoxin A IgM levels were noted between ICH and non-ICH groups (P = .132). At this time point, ICH subjects had lower anti-A IgG values (non-ICH 59.9 ELISA units, range 22.6–101 versus ICH 25.2 ELISA units, range 12.4–51.4; P = .004) but no difference in anti-B IgG levels (Figure 2b). Statistically significantly lower anti-A IgA levels (non-ICH 68.8 ELISA units, range 21.2–105 compared with ICH 25.9 ELISA units, range 10.1–82.2; P = .012) and lower anti-B IgA levels were observed in ICH subjects (non-ICH 14.9 ELISA units, range 7.9–102 versus ICH 8.7 ELISA units, range 4.4–22.3; P = .008) (Figure 2c).

Day 10–14

Our main outcomes were the serum levels of IgG to toxins A and B at treatment day 10–14. Immunocompromised host subjects demonstrated lower levels of anti-A IgG than non-ICH subjects. In the non-ICH subjects, we observed an anti-A IgG level of 49.0 ELISA units (range, 21.5–103). In ICH subjects, we observed and anti-A IgG level of 21 ELISA units (range, 16.4–44.6; P = .045). However, there were no significant differences in day 10–14 anti-B IgG levels (P = .484). Day 10–14 anti-B IgA levels were also lower for ICH at this time point (P = .029).

Stool Results

Most subjects 81 of 98 (82.6%) had stool tested for ultrasensitive toxin by Simoa. Median values of Simoa Toxin A and B values did

Table 2. Clinical and Laboratory Features of Non-Immunocompromised and Immunocompromised Subjects at CDI Diagnosis

Clinical Characteristics	Not Immunocompromised (N = 51, 52%)	Immunocompromised (N = 47, 48%)	*P* Value
Abdominal tenderness	17 (33.3%)	8 (17.0%)	.103
Temperature ≥38.0°C[a] (n = 95)	6/49 (12.2%)	8/46 (17.4%)	.568
Systolic BP <100 mm Hg[a]	23 (45.1%)	23 (48.9%)	.840
Colitis on imaging	12 (23.5%)	7 (14.9%)	.316
CDI Severity Scores			
IDSA-SHEA	25 (49.0%)	24 (51.1%)	1.000
ESCMID	32 (62.7%)	26 (55.3%)	.539
Zar et al [17]	19 (37.2%)	12 (25.5%)	.278
Belmares et al [18]	8 (15.7%)	5 (10.6%)	.558
Any severe	37 (72.5%)	32 (68.1%)	.663
WBC peak*, $\times 10^3$/mL median (IQR)	13 (8.9–19.1)	9.6 (4.4–14.8)	.012
WBC nadir*, $\times 10^3$/mL median (IQR)	6.2 (3.7–8.7)	6.9 (2.8–8.7)	.991
WBC ≥15 $\times 10^3$/mL	19 (37.2%)	11 (23.4%)	.188
ANC nadir* median (IQR)	5635 (3235–9630)	5480 (1515–10595)	.713
ALC nadir* median (IQR)	740 (407–1365)	755 (365–1545)	.871
Cr >1.5 (not on renal replacement therapy)	11/48 (22.9%)	13/40 (32.5%)	.345
Renal replacement therapy at baseline	3 (5.9%)	7 (14.9%)	.188
Albumin nadir* g/dL, median (IQR)	2.9 (2.5–3.6)	3.1 (2.8–3.5)	.173
Lactate peak[a], mmol/L median (IQR)	1.5 (1.3–2.1)	1.8 (1.3–2.3)	.602
Death* (N, %)	1 (2.0)	2 (4.3)	.606
ICU stay* (N, %)	3 (5.9)	4 (8.5)	.707
Colectomy* (due to CDI) (N, %)	0	1 (2.1)	.480
Any severe CDI outcome (N, %)	4 (7.8)	6 (12.8)	.513
Time to resolution of diarrhea (median, IQR)	5.2 (2.7–16.9)	5.6 (1.9–10.9)	.418
Length of stay, median (IQR)	7 (4–15)	8 (4–19)	.441
CDI recurrence in 100 days (N, %)	9 (17.6)	6 (12.8)	.582

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; BP, blood pressure; CDI, *Clostridioides difficile* infection; Cr, serum creatinine; ESCMID, European Society of Microbiology and Infectious Diseases; ICU, intensive care unit; IQR, interquartile range; IDSA, Infectious Diseases Society of America; SHEA, Society for Healthcare Epidemiology of America; WBC, white blood cell count.

[a]Indicates within 24 hours of diagnosis.

*Indicates within –5 days to +2 days of diagnosis.

NOTES: There were no findings of pseudomembranes on colonoscopy or flexible sigmoidoscopy in either groups. Any severe CDI outcomes included a composite of severe outcomes: ICU admission, colectomy, or death *within 40 days of diagnosis.

not differ significantly between groups (non-ICH 1147.9 pg/mL, range 57.2–14 599 pg/mL versus ICH 56.6 pg/mL, range 0–5359.8 pg/mL; $P = .065$). Groups also had similar rates of NAP/ribotype 027 strain infection (10 of 51 subjects [19.6%] in non-ICH compared with 4 of 46 subjects [8.7%] in ICH group; $P = .155$).

An exploratory analysis evaluated stool IgA and IgG antitoxin antibody levels (Supplementary Table 2). This demonstrated that ICH subjects had lower antitoxin A and antitoxin B IgA levels at baseline compared with non-ICH subjects ($P = .005$ and $P = .002$, respectively). Baseline median stool levels of antitoxin B IgG were also lower for ICH subjects ($P = .016$); there was no statistically significant difference in median antitoxin A IgG levels between groups. By treatment day 3, the finding of lower median stool immunoglobulin levels persisted for stool antitoxin B IgA (Figure 3a). There was no statistically significant difference in stool IgG antibody levels to either toxin A or B at day 3 (Figure 3b).

Patient Clinical Outcomes

Major CDI clinical outcomes are presented in Table 2. Serious CDI outcomes including death, ICU stay, and colectomy were infrequently observed. Time to resolution of diarrhea and length of stay were also similar. Concomitant non-CDI antibiotic use occurred in 33 (64.7%) and 31 (66.0%) of the of the non-ICH and ICH subjects, respectively. For CDI therapy, most subjects received a vancomycin-containing regimen (90.2% non-ICH vs 93.6% ICH). Metronidazole alone (9.8% non-ICH vs 2.1% ICH) or a fidaxomicin-containing regimen (0% non-ICH vs 4.3% ICH) were used infrequently. Treatment duration was slightly longer in the ICH subjects (13 days vs 9 days; $P = .034$). There were 15 recurrences. One was classified as a clinical diagnosis only; the remainder had confirmatory testing. Nine subjects in the non-ICH group (17.6%) and 6 subjects in the ICH group (12.8%) developed rCDI ($P = .582$). There was no difference in time to recurrence between groups.

DISCUSSION

Clostridioides difficile infection is common among patients with a weakened immune system from underlying severe illness or iatrogenic immunosuppression [9]. In non-ICHs, the humoral

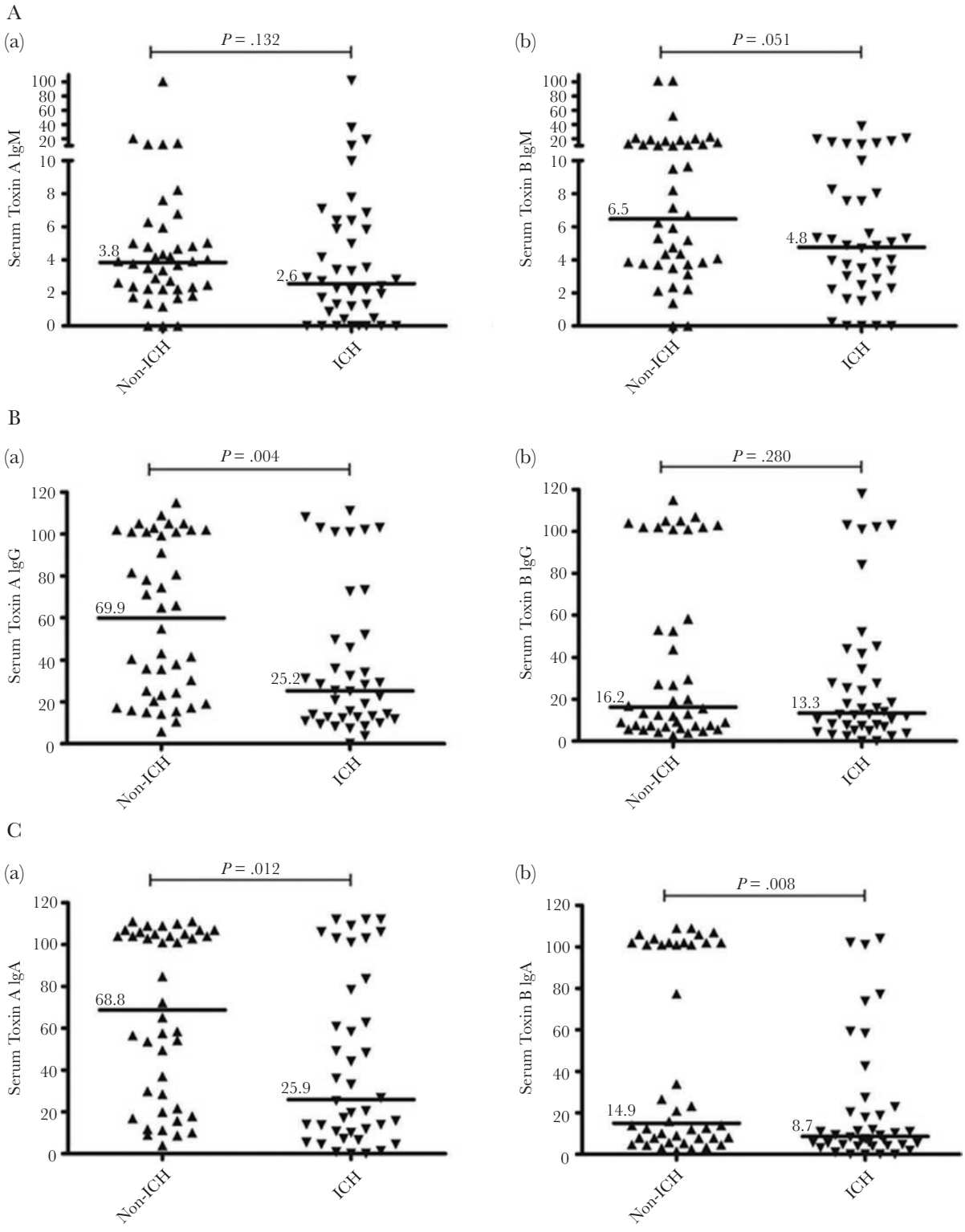

Figure 2. A panel indicates serum immunoglobulin (Ig) M levels at treatment day 3. A (a) demonstrates scatterplots for antitoxin A IgM concentration and A (b) demonstrates scatterplots for antitoxin B IgM for non-immunocompromised host (non-ICH) and immunocompromised host (ICH) subjects at *Clostridioides difficile* infection (CDI) treatment day 3. Parallel lines indicate median antitoxin levels. B panel indicates serum IgG levels at treatment day 3. B (a) demonstrates scatterplots for antitoxin A IgG concentration and B (b) demonstrates scatterplots for antitoxin B IgG for non-ICH and ICH subjects at treatment day 3. Parallel lines indicate median antitoxin levels. C panel indicates serum IgA levels at treatment day 3. C (a) demonstrates scatterplots for serum antitoxin A IgA for non-ICH and ICH subjects at treatment day 3. C (b) demonstrates serum antitoxin B IgA at treatment day 3. Parallel lines indicate median antitoxin levels.

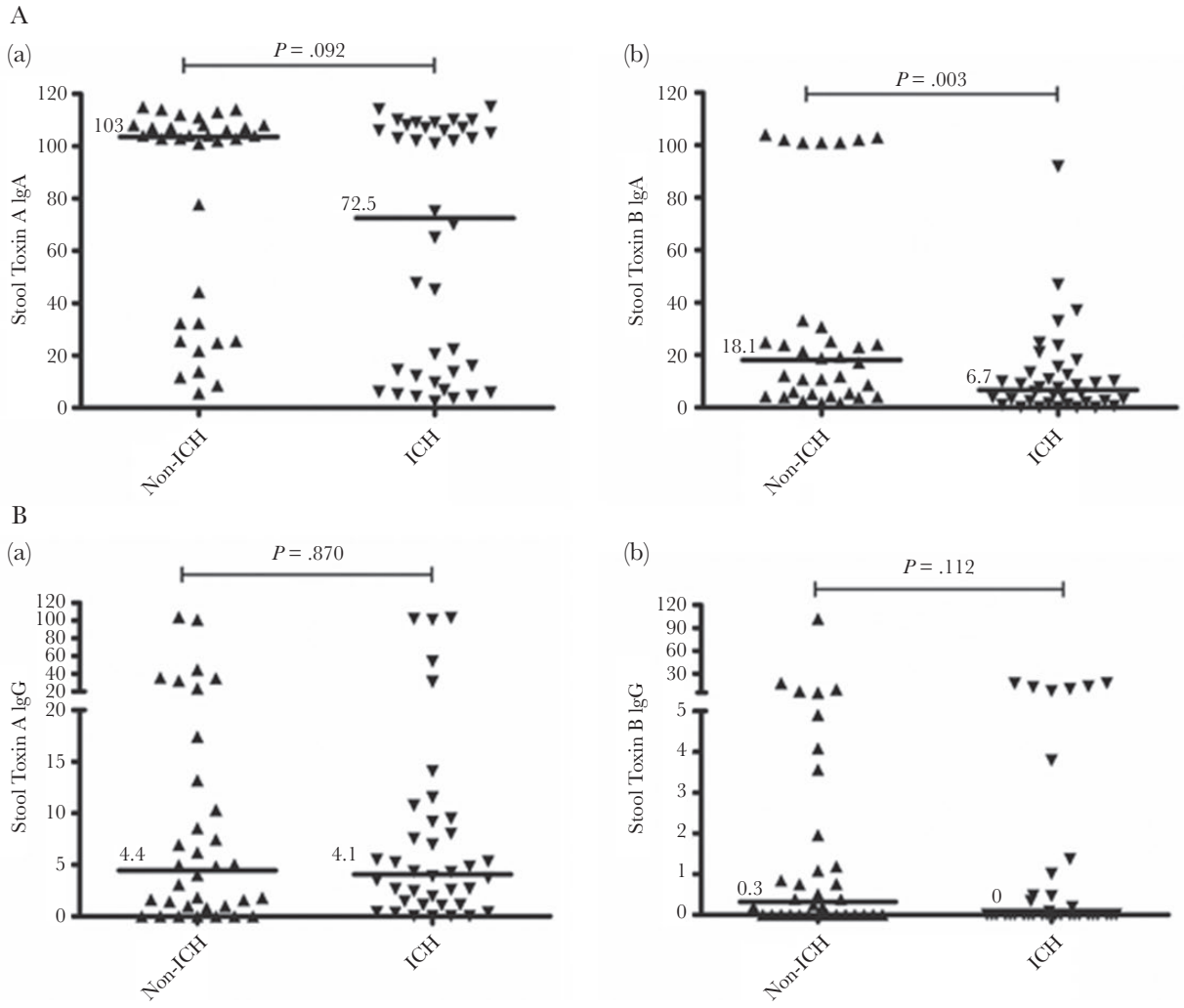

Figure 3. Panel A represents stool immunoglobulin (Ig)A levels at treatment day 3. A (a) demonstrates scatterplots for antitoxin A IgA concentration and A (b) demonstrates scatterplots for antitoxin B IgA concentrations in non-ICH and ICH subjects at *Clostridioides difficile* infection (CDI) treatment day 3. Parallel lines indicate median antitoxin levels. Panel B demonstrates stool IgG levels at treatment day 3. B (a) shows the concentration of stool antitoxin A IgG and B (a) shows the concentration of stool antitoxin B IgG at treamtent day 3. Parallel lines indicate median antitoxin levels.

immune response to *C difficile* toxins A and B has been linked to protection against CDI and prevention of rCDI [26]. Due to exclusion of ICHs from many of the prior studies, it is not known whether immunocompromised patients elaborate similar levels of antitoxin antibodies in the setting of CDI. We hypothesized that ICHs might have lower levels of serum antitoxin antibodies when compared with hospitalized non-ICHs with CDI. Identification of defects in the adaptive immune response, as described in this article, may provide the foundation for future CDI studies, which in turn may set the stage for development of CDI therapeutics for immunocompromised hosts.

In this prospective, multicenter, observational study, we measured serum antitoxin antibody levels from CDI treatment initiation through treatment day 10–14 and found that baseline IgM, IgG, and IgA serum antibodies to *C difficile* toxins A and B were not different in immunocompromised patients compared with non-immunocompromised control subjects. However, as patients progressed in their treatment courses, differences in serum antibody levels were noted. By CDI treatment day 3, ICHs had lower overall anti-A IgG and IgA and anti-B IgM and IgA serum levels. Although less pronounced at treatment day 10–14, these differences persisted for serum anti-A IgG and anti-B IgA levels, which remained consistently lower in the ICH group.

The humoral immune response to *C difficile* infection remains incompletely understood, and, in some cases, there are conflicting reports as to the relative importance of IgM, IgG, and IgA responses during CDI [12, 13, 20, 27]. Antitoxin IgM is generally considered to be an early marker of infection. Higher levels of serum IgM against toxin A have been associated with protection against rCDI [20, 28], whereas lower levels of IgM have been detected in symptomatic patients

compared with asymptomatic carriers [29]. In the present study, we found lower levels of IgM to Tox B at day 3 of treatment in the ICH population but similar levels of IgM for other time points. A notable finding was the stagnant IgM response in the ICH subjects over time. The clinical significance of this is not clear; however, it may represent a blunted early immune response as a reflection of the immunosuppressing disease states examined in this study. With the inclusion of subjects undergoing active chemotherapy and transplantation, we anticipated that some subjects may have had impaired B-cell function related to receipt of drugs such as antithymocyte globulin (used for solid organ transplant induction) or anti-CD20 agents such as rituximab (used in the setting of cancer chemotherapy). The impact of each of these agents directly on risk for CDI is incompletely understood.

We expected to find lower IgG levels in our ICH subjects as a possible reflection of underlying poor B-cell responses related to endogenous and exogenous immunosuppression. Prior literature has demonstrated that antitoxin A IgG levels are higher in asymptomatic *C difficile* carriers than in patients with symptomatic disease, suggesting that the magnitude of IgG response may play a role in prevention of CDI [12, 29]. Furthermore, the magnitude of IgG response to toxin A has also been associated with protection against CDI recurrence [20]. In our study, we confirmed the hypothesis that ICHs are likely to have lower antitoxin IgG levels and observed significantly lower levels of IgG to toxin A at days 3 and 10–14. These 2 time points may be important because they represent the timing in the disease course at which a rise in serum IgG levels might be expected, corresponding with disease response and recovery. In addition, we discovered significantly lower serum levels of antitoxin A and B IgA levels in immunocompromised subjects at treatment day 3. Immunoglobulin A is typically considered to be most important in its luminal protection against microbial pathogens. Low concentrations of intraluminal IgA to *C difficile* toxins have been associated with rCDI [30]. Among the ICH cohort, levels of serum IgA remained similar at the 3 observed time points. This finding raises the possibility that ICHs are unable to mount an adequate IgA response to replace IgA secreted into the lumen during CDI.

In addition to the serum serological findings, this study also contributes valuable information regarding CDI clinical outcomes among immunocompromised subjects. Most notably, CDI clinical severity, severe CDI outcomes, and recurrence within 100 days were not different between ICH and non-ICH subjects. These were unexpected findings because we had anticipated to observe worse clinical outcomes in the ICH population. As a possible explanation, it is important to note that 72.5% of the control subjects met criteria for severe CDI by 1 of the 4 severity scores we evaluated. The ill control group reflects the case mix of many tertiary medical centers. One hypothesis is that these individuals may have other factors such as age and medical comorbidities (including diabetes, cirrhosis, and malnutrition) that may have impacted humoral immune response, thus obscuring major differences between the ICH and non-ICH groups.

This study has several limitations. Our immunocompromised patient population had a heterogenous assortment of underlying disease states. Thus, planned subset analyses were unable to be performed for the stem cell transplant and solid organ transplant populations. Our planned recurrence analysis was also limited by low rates of recurrence within 100 days in the cohort (15%). Our sample size was informed by prior studies, including one from our center, that had reported higher rates of rCDI [12]. Thus, with the lower-than-expected rates of rCDI in the immunocompromised subset, we may have been underpowered to detect a difference in clinical outcomes. However, one of the strengths was the close telephonic follow-up, thereby reducing the likelihood that there were additional cases of recurrence that might have been missed after discharge. Overall, although the ICH population was heterogenous, the study reflects a real-world dataset, prospectively collected, and focused on a population that has traditionally been excluded from clinical trials in this area. A larger cohort would be needed to further refine our findings.

CONCLUSIONS

In summary, our study found lower serum levels of toxin A and B IgA and lower concentrations of toxin A IgG among immunocompromised subjects with CDI during the early course of CDI therapy. These data suggest possible targetable defects in the host immune system among ICH that may be leveraged for future passive and active CDI immunotherapies.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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