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JPEN J Parenter Enteral Nutr. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

JPEN J Parenter Enteral Nutr. 2015 January ; 39(1): 47–55. doi:10.1177/0148607113511991.

Association Between Prehospital Vitamin D Status and Hospital-Acquired *Clostridium difficile* Infections

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Abstract

Objective—To investigate whether preadmission 25-hydroxyvitamin D (25(OH)D) levels are associated with the risk of hospital-acquired *Clostridium difficile* infection (HACDI).

Materials and Methods—Our retrospective cohort study focused on 568 adult patients from 2 Boston teaching hospitals between August 1993 and November 2006. All patients had 25(OH)D levels measured before hospitalization and were at risk for HACDI (defined as the presence of *C difficile* toxin A or B in stool samples obtained >48 hours after hospitalization). We performed multivariable regression analyses to test the association of prehospital 25(OH)D levels with HACDI while adjusting for clinically relevant covariates.

Results—In these 568 patients, mean (SD) 25(OH)D level was 19 (12) ng/mL, and 11% of patients met criteria for incident HACDI. Following adjustment for age, sex, race (nonwhite vs white), patient type (medical vs surgical), and Deyo-Charlson index, patients with 25(OH)D levels <10 ng/mL had higher odds of HACDI (odds ratio [OR], 2.90; 95% confidence interval [CI], 1.01–8.34) compared with patients with 25(OH)D levels ≥30 ng/mL. When patients with HACDI were analyzed relative to a larger patient cohort without HACDI (n = 5047), those with 25(OH)D levels <10 ng/mL (OR, 4.96; 95% CI, 1.84–13.38) and 10–19.9 ng/mL (OR, 3.36; 95% CI, 1.28–

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8.85) had higher adjusted odds of HACDI compared with patients with 25(OH)D levels ≥30 ng/mL.

Conclusions—In our cohort of adult patients, vitamin D status before hospital admission was inversely associated with the risk of developing HACDI. These data support the need for randomized, controlled trials to test the role of vitamin D supplementation to prevent HACDI.

Keywords

vitamin D; 25-hydroxyvitamin D; Clostridium difficile; hospital-acquired infection; nosocomial infection

Introduction

In the United States, the incidence of hospital-acquired *Clostridium difficile* infections (HACDIs) has almost tripled over the past decade.[1] Approximately 350,000 new cases of nosocomial *C difficile* infections are associated with roughly 15,000 potentially avoidable fatalities each year.[1,2] Excess annual healthcare expenditures attributable to HACDIs range from $1.1 to $3.2 billion, and the average hospital length of stay is prolonged by 3–6 days among patients who develop *C difficile* infections during acute care hospitalizations.[3-5] Despite the existence of national guidelines, the adoption of recognized preventative strategies has not resulted in the eradication of HACDIs.[6,7]

C difficile is typically an opportunistic pathogen, resulting in disease if the normal gastrointestinal (GI) flora is perturbed and when host immune responses are suboptimal.[8-10] Risk factors for acquiring *C difficile*–associated infection include age, antibiotic exposure, and GI procedures.[11] Recent evidence suggests that vitamin D is a key regulator of the immune system,[12] and as such, it may play an important role in patient susceptibility to hospital-acquired infections,[13] including HACDIs.[14] Given that the prevalence of suboptimal levels of 25-hydroxyvitamin D (25(OH)D) has increased in the general population,[15-19] we performed a 2-center retrospective cohort study of hospitalized adult patients among whom 25(OH)D was measured within 1 year of hospitalization. Our objective was to test the hypothesis that vitamin D status before hospital admission is associated with the risk of developing HACDIs.

Materials and Methods

Source Population

We abstracted laboratory and administrative data from the electronic medical records of individuals admitted to 2 teaching hospitals in Boston, Massachusetts: Brigham and Women's Hospital (BWH), with 793 beds, and Massachusetts General Hospital (MGH), with 902 beds. These 2 hospitals provide primary and tertiary care to a diverse population within eastern Massachusetts and the surrounding region. BWH and MGH are both level 1 trauma centers, and both have 45,000–47,000 hospital admissions per year. BWH and MGH are members of Partners HealthCare, which is the largest healthcare provider in Massachusetts.

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Data Sources

We obtained data on all patients admitted to BWH or MGH between August 1993 and November 2006 through the Research Patient Data Registry (RPDR). RPDR is a computerized registry, which serves as a central data warehouse for all inpatient and outpatient records at Partners HealthCare facilities (including BWH and MGH). The registry has been used for multiple clinical research studies.[20-24] Institutional review board approval for the study was granted by the Partners Human Research Committee.

Study Population

We identified 5341 individual patient admissions (age ≥18 years) over the study period that were assigned a Diagnostic Related Group (DRG) and that had documented serum 25(OH) D measurements between 7 and 365 days before hospitalization. We then excluded 5 foreign patients without Social Security numbers since vital status in this study was determined by the Social Security Administration Death Master File, 44 patients who received high-dose vitamin D supplementation (ergocalciferol, 50,000 IU) between the 25(OH)D level draw and hospital admission, 153 patients who had evidence of prior *C difficile* infection, 92 patients who had *C difficile* toxin A or B detected within 48 hours of hospital admission, and 4479 patients who did not have stool sample testing for *C difficile* toxin >48 hours after hospital admission. We did not exclude patients with diarrhea at hospital presentation. The final study cohort was therefore composed of 568 patients.

Exposure of Interest and Comorbidities

The exposure of interest was preadmission serum 25(OH)D level obtained 7–365 days prior to the date of hospitalization. 25(OH)D levels were categorized a priori as <10 ng/mL, 10–19.9 ng/mL, 20–29.9 ng/mL, and ≥30 ng/mL (to convert from ng/mL to nmol/L, multiply by 2.496). All cut points were adapted from existing national clinical guidelines.[25]

We used the International Classification of Diseases, Ninth Revision coding algorithms, which are well studied and validated,[26,27] to derive the Deyo-Charlson index to assess the burden of chronic illness in our study cohort.[28] “Patient type” was defined as medical or surgical and incorporated the DRG method.[29] Inpatient antibiotic use was determined by pharmacy records with exclusion of antibiotics given following HACDI testing. Prior use of vitamin D supplementation in the year prior to hospitalization was determined by outpatient pharmacy records for cholecalciferol, calcitriol, and ergocalciferol (but excluding ergocalciferol ≥50,000 units given following 25(OH)D draw). Critical care services were determined by the assignment of *Current Procedural Terminology* (*CPT*) code 99291 (critical care, first 30–74 minutes) during hospital admission. The use of *CPT* code 99291 in this manner has been previously validated in the RPDR database.[24]

Assessment of Mortality

Information on vital status for the study cohort was obtained from the Social Security Administration Death Master File, which has a reported sensitivity for mortality up to 92% and a specificity of 99.9%.[30-33] Utilization of the Death Master File allows for long-term follow-up of patients following hospital discharge.

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Serum 25(OH)D Assay

Serum 25(OH)D in all cohort subjects was determined by radioimmunoassay (RIA). Between 1993 and 2006, at both hospitals, RIA was performed using the 25-Hydroxyvitamin D ^{125}I RIA kit (DiaSorin Corporation, Stillwater, MN).[34]

End Points

The primary end point was incident HACDI. Microbiology reports on stool samples for the study cohort were obtained from the computerized registry at the hospitals under study. All cohort patients had stool sample testing for *C difficile* toxin A and B by an enzyme-linked immunosorbent assay (ELISA). A positive toxin result was defined as the presence of toxin A or B in at least 1 stool sample. To be considered a HACDI, the first positive toxin result must have been on a stool sample obtained >48 hours after hospital admission in patients with no known history of *C difficile* infection. Thus, HACDI was defined as the presence of *C difficile* toxin A or B in a stool sample obtained >48 hours after hospital admission in patients without a record of previous *C difficile* toxin A or B positivity. The secondary outcome was an assessment of 30-day and 90-day mortality.

Power Calculations and Statistical Analysis

On the basis of our prior study of bloodstream infection susceptibility among hospitalized patients,[35] we assumed HACDI incidence would decrease from 15% in patients with a prehospital 25(OH)D <20 ng/mL to 7% in those with a prehospital 25(OH)D ≥20 ng/mL. With an α error level of 5% and a power of 80%, the minimum sample size required for our primary end point (HACDI) was 530 total patients.

Categorical variables were described by frequency distribution and compared across 25(OH)D groups using contingency tables and χ^2 testing. Continuous variables were examined graphically (eg, histogram, box plot) and in terms of summary statistics (ie, mean and standard deviation or median and interquartile range) and then compared across exposure groups using 1-way analysis of variance.

Unadjusted associations between 25(OH)D levels and HACDI were estimated by bivariable logistic regression models. Adjusted odds ratios (ORs) were estimated by multivariable logistic regression models with a priori inclusion of covariates thought to be linked with both 25(OH)D level and HACDI. In this manner, we sought to create a parsimonious model that did not unnecessarily adjust for variables that do not affect bias or the causal relation between exposure and outcome.[36] For the primary model (HACDI), specification of each continuous covariate (as a linear vs categorical term) was adjudicated by the empiric association with the primary outcome using the Akaike information criterion; overall model fit was assessed using the Hosmer-Lemeshow test. Models for secondary analyses were similar to the primary model. Unadjusted event rates were calculated with the use of the Kaplan-Meier methods and compared with the use of the log-rank test. Locally weighted scatter plot smoothing (LOWESS) was used to graphically represent[37,38] the relationship between prehospital 25(OH)D level and risk of HACDI. A secondary analysis was performed to investigate the 25(OH)D-HACDI association in the parent cohort of 5047 inpatients with serum 25(OH) D measured between 7 and 365 days before hospital

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admission (568 from the study cohort and the 4479 patients who did not have a *C difficile* toxin assay determined >48 hours after hospital admission). All *P* values are 2-tailed, with values <.05 considered statistically significant. All analyses were performed using Stata 12.0MP statistical software (StataCorp LP, College Station, TX).

Results

The mean (SD) age at hospital admission was 63 (18) years (Table 1). Most patients were female, were white, and had a medically related DRG. The mean (SD) 25(OH)D level was 19 (12) ng/mL. Approximately half (53%) of the 25(OH)D measurements occurred in the 3 months before hospital admission. Over the hospital stay, 11% of the cohort met criteria for HACDI (n = 64). There was no statistical difference between HACDI incidence and season of hospital admission ($\chi^2(3, N = 568) = 3.45$, $P = .33$) or year of hospital admission ($\chi^2(3, N = 568) = 7.62$, $P = .054$). Over the years of the study, there does not appear to be a significant difference in 25(OH)D serum levels ($\chi^2(9, N = 568) = 16.18$, $P = .063$), but there appears to be a pattern where fewer cases of 25(OH)D <10 ng/mL are found over time.

Patient characteristics were stratified according to pread- mission 25(OH)D levels (Table 2). Factors that significantly differed between stratified groups included sex and race. The most common admission diagnosis categories were ill-defined conditions (13%); circulatory system (13%); endocrine, nutrition, and metabolic (12%); digestive system (9%); genitourinary system (7%); neoplasms (6%); and respiratory system (6%). Age and 25(OH)D levels were significant predictors of HACDI (Table 3). Thirty-day and 90-day mortality rates were 10% and 17%, respectively.

Primary Outcome

Preadmission 25(OH)D level <10 ng/mL was a strong predictor of HACDI after adjustment for age, sex, race, patient type, and Deyo-Charlson index (Table 4). The adjusted odds of HACDI in patients with 25(OH)D levels <10 ng/mL was 3-fold higher than that of patients with levels ≥30 ng/mL. Additional adjustment for white blood cell count did not materially alter the point estimates for HACDI (fully adjusted OR, 2.88; 95% confidence interval [CI], 1.01–8.30). LOWESS plot (Figure 1) demonstrated a near-inverse linear association between 25(OH)D level and risk of HACDI up to 25(OH)D levels near 30 ng/mL. Beyond serum 25(OH)D levels of 50 ng/mL, the curve appears flat.

Secondary Analyses

In the parent cohort of 5047 inpatients with serum 25(OH)D measured between 7 and 365 days before hospital admission (568 from the study cohort and the 4479 patients who did not have *C difficile* toxin ELISA determined >48 hours after hospital admission), the proportion of patients with *C difficile* toxin measured was highest in those with the lowest prehospital 25(OH)D level: 15%, 13%, 10%, and 7% in patients with levels <10 ng/mL, 10–19.9ng/mL, 20–29.9 ng/mL, and ≥30 ng/ mL, respectively ($\chi^2(3, N = 5047) = 41$, $P < .001$).

Under the assumption that vitamin D status was not a factor in the decision to order a stool *C difficile* toxin assay and that those who did not have ELISA testing were likely to be true negatives and not false negatives, we performed a further analysis on the 5047 inpatients

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with 25(OH)D levels determined prior to hospital admission to determine the association between vitamin D status and HACDI. In this subanalysis, pre- hospital 25(OH)D levels <20 ng/mL were associated with increased odds of HACDI (Table 5). Compared with the patients with 25(OH)D levels ≥30 ng/mL, multivariable adjusted odds of HACDI in those with levels <10 ng/mL and those with levels 10–19.9 ng/mL were 4.96 (95% CI, 1.84–13.38) and 3.36 (95% CI, 1.28–8.85), respectively. Furthermore, using data from this larger cohort (n = 5047), the HACDI rate in patients with prehospital 25(OH)D <10 ng/mL, 10–19.9 ng/ mL, 20–29.9 ng/mL, and ≥30 ng/mL was 27, 18, 18, and 8 per 10,000 patient-days, respectively. Finally, we examined the association of HACDI with all-cause mortality in this 5047 inpatient sample (Figure 2). When HACDI was considered as the exposure and all-cause mortality was the outcome, patients with HACDI had a 2-fold increased risk of 90-day mortality (OR, 2.02; 95% CI, 1.04–3.92) relative to those without HACDI after adjustment for age, sex, race, patient type, and Deyo-Charlson index.

Effect Modification

The fully adjusted model for the 25(OH)D-HACDI association was further evaluated for the presence of effect modification. We individually tested creatinine, hematocrit, white blood cell count, and season of 25(OH)D draw by adding an interaction term to the multivariable model. None of these variables emerged as an effect modifier of the association between 25(OH)D and HACDI (*P* interaction was >.20 for all variables tested). Further effect modification analyses showed that the association between 25(OH)D and HACDI was not modified by serum calcium >10.5 mg/dL (*P* interaction = .99). In addition, an analysis was performed to evaluate the data related to the 25(OH)D draw being ≥90 or <90 days before hospital admission as well as the hospital where care was received. The effect modification analysis showed that the association between vitamin D status and HACDI was neither modified by the time between 25(OH)D draw and hospital admission (*P* interaction = .65) nor the hospital of care (*P* interaction = .48).

Discussion

In this cohort study, we investigated whether prehospital vitamin D status was associated with the risk of HACDI. We demonstrated that 25(OH)D <10 ng/mL before hospital admission was indeed associated with a significant increase in the odds of developing HACDI. While others have also hypothesized that vitamin D status may play an important protective role against HACDIs,[13,39] our work provides important evidence to suggest that vitamin D supplementation may provide a novel approach to lowering the risk of HACDI. However, due to the observational design of this study, causal inferences about the relationship between vitamin D status and HACDI are limited.

Clostridium difficile is a Gram-positive, anaerobic bacillus typically implicated in the progression to antibiotic-associated colitis.[8] The pathogen is primarily transmitted from patient to patient through shared medical equipment or on the hands of healthcare workers.[6] In the GI tract, *C difficile* causes disease by the production of toxins (toxins A and B), which induce characteristic inflammatory responses.[9] Toxin A directly attracts neutrophils and monocytes, while toxin B is associated with a more generalized inflammatory response due

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its effect on colonic epithelial cell integrity.[9] After alteration of the healthy colonic bacterial flora (which occurs with antibiotic usage), the immune response to *C difficile* toxin appears to play a major role in determining host susceptibility to *C difficile* infections.[9,10]

Recent studies have demonstrated that cells of the innate and adaptive immune system express the vitamin D receptor (VDR).[12] Macrophages activated through the VDR by 1,25-dihydroxyvitamin D (the most hormonally active vitamin D metabolite) upregulate expression of cathelicidin (LL-37).[40] LL-37 is an endogenous antimicrobial peptide that is active against a broad spectrum of infectious agents such as bacteria, viruses, fungi, and mycobacteria.[41] Moreover, LL-37 is highly expressed at natural barrier sites (eg, skin, lungs, gut) by epithelial cells and may represent an important first line of defense for the innate immune system.[42] In addition, vitamin D stimulates the expression of β-defensin in the intestinal epithelium, which has broad-spectrum antimicrobial activity.[43] Since it is evident that derangements in immune function and disruption of natural barrier sites predispose patients to HACDI and that vitamin D status is essential for both optimal immune function and natural barrier integrity, our study findings raise intriguing questions that merit further investigation.

The association between prehospital vitamin D status and HACDI may also be explained by antibiotic usage for infection. We have demonstrated in prior studies that patients with hypovitaminosis D prior to hospital admission may be at higher risk for bloodstream infection[35,44] and sepsis.[45] Since antimicrobial therapy with quinolones, cephalosporins, macrolides, clindamycin, and intravenous β-lactam/β-lactamase inhibitors have been shown to be associated with *C difficile*–associated diarrhea,[46] it is likely that antibiotic usage is a contributor to the prehospital vitamin D status–HACDI association.

Although cohort studies provide a high level of observational evidence and may direct future research by illustrating the existence or absence of a true effect,[47] they also have several potential limitations, such as confounding, reverse causation, and/or the lack of a randomly distributed exposure. Ascertainment bias may exist in our study since our patient cohort had both prehospital vitamin D status determined and toxin A or B measured in stool samples, both related to unknown reasons that may be absent in other hospitalized patients. Furthermore, the cohort under study had a particularly high 30-day mortality rate. These differences may decrease the generalizability of our results to all hospitalized patients. Despite adjustment for multiple potential covariates, there may still be residual confounding that contributed to the observed differences in outcomes. Specifically, low 25(OH)D levels may be a marker for the general condition of patients, for which we are unable to fully adjust. As patients received care from physicians outside of our health system, we are unable to determine with confidence the number of patients who were actively taking vitamin D supplements.

Another potential limitation is related to the fact that we used 25(OH)D measurements between 7 and 365 days prior to hospitalization as a reflection of preadmission vitamin D status. Others have shown that the intraperson Pearson correlation coefficient for 25(OH)D in outpatients following adjustments for age, race, and season is 0.70 at 3 years between blood draws.[48] There was no interaction of the 25(OH)D-HACDI association with regard to

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when 25(OH)D was obtained. Despite this observation, vitamin D status at the time of hospitalization may have been different than when prehospital values were drawn. Indeed, we know that inflammatory changes and IV fluid administration contribute significantly to the rapid drop (30%−40%) in circulating 25(OH)D levels during acute stress.[49] The assumption used in the study power estimate may not be valid since there are no prior studies available on incident HACDI and vitamin D status. Furthermore, the somewhat wide confidence intervals seen in our data (Tables 4 and 5) are likely due to relatively low sample numbers in the 25(OH)D groups and reflect lower precision and more statistical instability than in a study of larger size. These issues will need to be addressed by future studies as we and other groups try to replicate and extend the current finding.

In large published studies from Quebec, Canada, the *C difficile* infection incidence/1000 hospital admissions was 3–12 cases/1000 hospital admissions in 1991–2002 and 25–43 cases/1000 admissions in 2003–2004.[50] The incidence in our study sample (n = 568) was 113/1000 hospital admissions, and in the parent cohort of 5047 inpatients, the incidence of *C difficile* infection was 13/1000 hospital admissions. We are likely missing true-positive cases in the population of patients who did not have 25(OH)D measured.

The present study has several strengths. We have sufficient statistical power to detect a clinically relevant difference in HACDI. The Deyo-Charlson index accounts for chronic conditions that may alter immune function (including diabetes mellitus and chronic renal failure).[51] By our design of measuring 25(OH)D prior to hospitalization, we have attempted to uncouple the influence of illness and inflammation on vitamin D status. For example, since inflammation is associated with decreased vitamin D binding protein,[52] we used 25(OH)D levels that were measured prior to hospitalization and thus conceivably prior to the inflammation that may have altered 25(OH)D levels. We did not include 25(OH)D levels drawn in the 7 days prior to hospitalization to avoid any potential alterations of vitamin D status related to illness or inflammation.

Conclusion

Our results suggest that vitamin D status may be a modifiable risk for HACDI. We hypothesize that 25(OH)D levels ≥30 ng/ mL are associated with optimal expression of endogenous antimicrobial peptides. In turn, this may attenuate the effect of barrier site disruptions that are characteristic of HACDI. This is particularly important since the incidence of high virulence and antibiotic-resistant *C difficile* strains is increasing at an alarming rate.[53,54] As a consequence, the overall severity of infections, cases of recurrence, costs, and associated mortality are expected to continue to rise.[55,56] Prospective studies are needed to validate our findings, to assess the potential benefit of optimizing preadmission 25(OH)D levels, and to identify the mechanism by which vitamin D may confer protection against nosocomial infections such as *C difficile* infections.

Acknowledgment

This manuscript is dedicated to the memory of our dear friend and colleague, Nathan Edward Hellman, MD, PhD.

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Financial disclosure: This study was support by 5T32GM007592-33 and UL1 RR025758 (S.A.Q.), R01 AI093723 and U01 AI087881 (C.A.C.), and K08AI060881 (K.B.C.) from the National Institutes of Health.

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Clinical Relevancy Statement

Hospital-acquired *Clostridium difficile* infections are difficult to treat, require prolonged therapy, and may result in devastating consequences. The results of the current study provide novel insights on the potential immunomodulatory role of vitamin D in patients who develop nosocomial infections.

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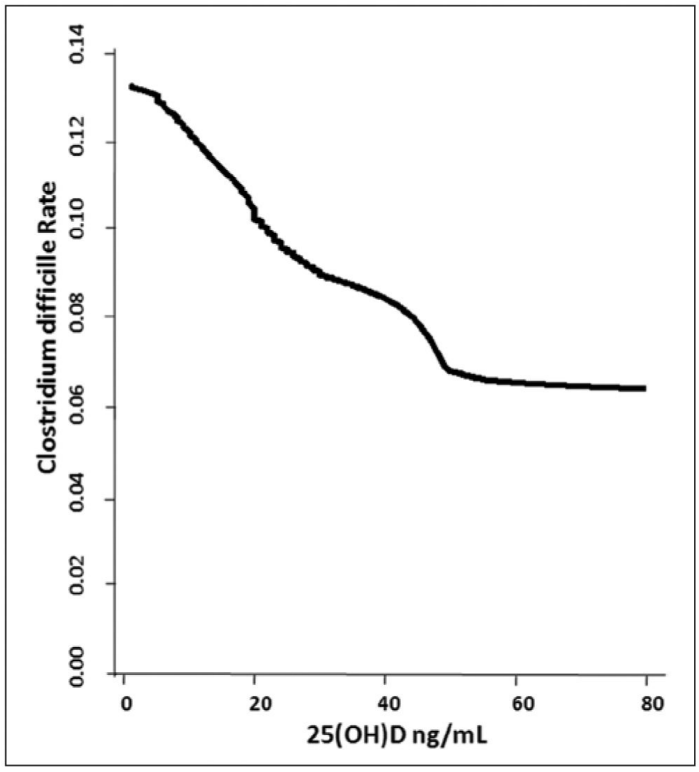

Figure 1.

Vitamin D status vs risk of hospital-acquired *Clostridium difficile* infection. Locally weighted scatter plot smoothing was used to represent the near-inverse linear association between prehospital 25(OH)D level and risk of HACDI. Plot constructed with data from inpatients with prehospital vitamin D status and toxin A or B measured in stool samples (n = 568). HACDI, hospital-acquired *C difficile* infection; 25(OH)D, 25-hydroxyvitamin D.

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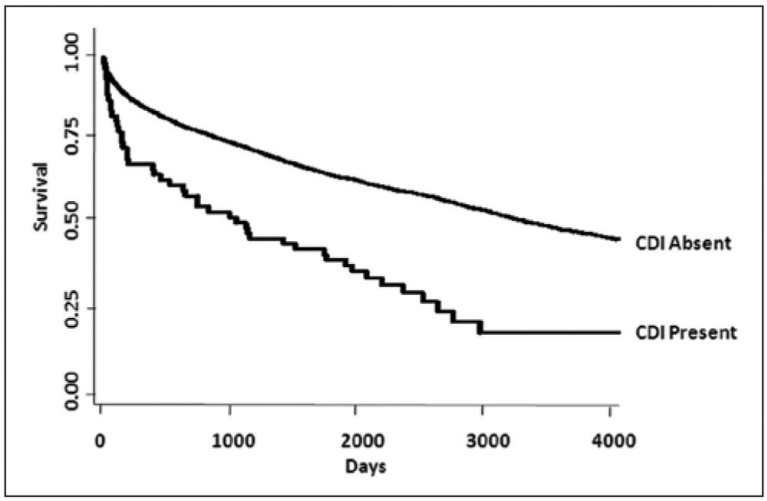

Figure 2.

Time-to-event curves for the secondary end point (mortality). Unadjusted event rates were calculated with the use of the Kaplan-Meier methods and compared with the use of the log-rank test. The global comparison log rank *P* value is <.0001. Curve constructed with data from all inpatients with prehospital vitamin D status determined (n = 5047). CDI, *Clostridium difficile* infection.

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Table 1

Baseline Demographic Characteristics of the Study Population.

Characteristic	Total	HACDI	Non-HACDI
Total number of cases	568	64	504
Age, mean (SD), years	63 (18)	71 (15)	62 (17)
Sex, No. (%)			
Female	353 (62)	44 (69)	309 (61)
Male	215 (38)	20 (31)	195 (39)
Race, No. (%)			
Nonwhite	88 (15)	9 (14)	79 (16)
White	480 (85)	55 (86)	425 (84)
Patient type, No. (%)			
Medical	401 (71)	50 (78)	351 (70)
Surgical	167 (29)	14 (22)	153 (30)
Deyo-Charlson index, No. (%)			
0–3	147 (26)	20 (31)	127 (25)
4–6	169 (30)	19 (30)	150 (30)
≥7	252 (44)	25 (39)	227 (45)
Antibiotic use, No. (%)	403 (71)	45 (70)	358 (71)
25(OH)D, mean (SD), ng/mL	19 (12)	17 (10)	19 (12)
25(OH)D, mean (SD) by season, ng/mL			
Fall (n = 151)	18 (9)	20 (9)	18 (10)
Spring (n = 147)	17 (10)	16 (8)	17 (10)
Summer (n = 132)	21 (11)	19 (10)	21 (11)
Winter (n = 138)	19 (9)	15 (12)	19 (15)
Vitamin D supplemental use, No. (%)	78 (14)	6 (9)	72 (14)

HACDI, hospital-acquired *Clostridium difficile* infection; 25(OH)D, 25-hydroxyvitamin D. Antibiotic use is exposure of antibiotics during the hospitalization prior to measurement of *Clostridium difficile* toxin A or B in stool samples. Vitamin D supplemental use is vitamin D supplementation in the year prior to hospitalization.

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Table 2

Patient Characteristics by Prehospital Vitamin D Status.

	Prehospital 25(OH)D				
Characteristic	**<10 ng/mL**	**10–19.9 ng/mL**	**20–29.9 ng/mL**	**≥30 ng/mL**	***P* Value**
Number of cases	135	232	137	74	
Age, mean (SD), y	63 (17)	61 (18)	64 (17)	65 (17)	.35[a]
Sex, No. (%)					<.0001
Female	82 (61)	118 (53)	94 (69)	59 (80)	
Male	53 (39)	104 (47)	43 (31)	15 (20)	
Race, No. (%)					.04
Nonwhite	30 (22)	31 (14)	21 (15)	6 (8)	
White	105 (78)	191 (86)	116 (85)	68 (92)	
Patient type, No. (%)					.94
Medical	96 (71)	157 (71)	98 (72)	50 (68)	
Surgical	39 (29)	65 (29)	39 (28)	24 (32)	
Deyo-Charlson index, No. (%)					.42
0–3	27 (20)	56 (25)	39 (28)	25 (34)	
4–6	40 (30)	68 (31)	40 (29)	21 (28)	
≥7	68 (50)	98 (44)	58 (42)	28 (49)	
Antibiotic use, No. (%)	89 (66)	166 (75)	101 (73)	47 (65)	.21
Vitamin D supplemental use, No. (%)	14 (10)	35 (16)	18 (13)	11 (15)	.52
White blood cells, × 10^3/μL, No. (%)					.81
0–3.9	9 (7)	10 (5)	9 (6)	4 (5)	
4.0–9.9	50 (37)	89 (40)	61 (45)	28 (38)	
>10	76 (56)	123 (55)	67 (49)	42 (57)	
Red blood cell distribution width, mean (SD)	16.7 (2.5)	15.7 (2.4)	15.2 (2.5)	15.1 (2.1)	<.0001
ICU admission, No. (%)	24 (18)	44 (20)	34 (25)	13 (18)	.45

ICU, intensive care unit; 25(OH)D, 25-hydroxyvitamin D. White blood cells and red cell distribution width measured at hospital admission. Antibiotic use is the exposure of antibiotics during the hospitalization prior to measurement of *Clostridium difficile* toxin A or B in stool samples. Vitamin D supplemental use is vitamin D supplementation in the year prior to hospitalization.

[a] *P* value was determined by Kruskal-Wallis (all other *P* values were determined by χ^2).

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Table 3

Multivariable-Adjusted Associations Between Covariates and Hospital-Acquired *Clostridium difficile* Infections.

Characteristic	Odds Ratio	95% CI	*P* Value
Age (per 1 year)	1.04	1.02–1.06	<.0001
Sex			
Female	1.00	Reference	.37
Male	0.76	0.42–1.38	
Race			
Nonwhite	1.00	Reference	.93
White	0.96	0.44–2.08	
Patient type			
Medical	1.00	Reference	.13
Surgical	0.61	0.32–1.15	
Deyo-Charlson index			
0–3	1.00	Reference	
4–6	0.62	0.31–1.25	.18
≥7	0.58	0.30–1.14	.11
Prehospital 25(OH)D, ng/mL			
<10	2.90	1.01–8.34	.048
10–19.9	2.14	0.76–5.98	.15
20–29.9	1.70	0.58–5.03	.34
≥30	1.00	Reference	

CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D. Adjusted odds ratios were estimated by a multivariable logistic regression model with inclusion of covariate terms thought to plausibly associate with vitamin D status and hospital-acquired *Clostridium difficile* infections. Estimates for each variable are adjusted for all other variables in the table.

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Table 4

Unadjusted and Adjusted Associations Between Prehospital Vitamin D Status and HACDI in Patients With Confirmed Toxin A or B in Stool Samples (n = 568).

	Prehospital 25(OH)D			
Association	**<10 ng/mL**	**10–19.9 ng/mL**	**20–29.9 ng/mL**	**≥30 ng/mL**
Unadjusted				
OR (95% CI)	2.40 (0.86–6.69)	1.75 (0.66–4.75)	1.57 (0.54–4.55)	1.00 (Reference)
P value	.094	.21	.84	
Adjusted				
OR (95% CI)	2.90 (1.01–8.34)	2.14 (0.76–5.98)	1.70 (0.58–5.03)	1.00 (Reference)
P value	.048	.15	.34	

CI, confidence interval; HACDI, hospital-acquired *Clostridium difficile* infection; OR, odds ratio; 25(OH)D, 25-hydroxyvitamin D. Unadjusted associations between 25(OH)D groups and HACDI were estimated by bivariable logistic regression models. Adjusted ORs were estimated by multivariable logistic regression models with inclusion of covariate terms thought to plausibly associate with both 25(OH)D concentrations and HACDI. Estimates adjusted for age, sex, race (nonwhite vs white), patient type (medical vs surgical), and Deyo-Charlson index.

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Table 5

Unadjusted and Adjusted Associations Between Prehospital Vitamin D Status and HACDI in Patients With Confirmed Toxin A or B in Stool Samples Relative to All Hospitalized Patients (n = 5047).

	Prehospital 25(OH)D			
Association	**<10 ng/mL**	**10–19.9 ng/mL**	**20–29.9 ng/mL**	**≥30 ng/mL**
Unadjusted				
OR (95% CI)	4.94 (1.85–13.22)	3.11 (1.19–8.15)	2.20 (0/79–6.12)	1.00 (Reference)
P value	.001	<.0001	.13	
Adjusted				
OR (95% CI)	4.96 (1.84–13.38)	3.36 (1.28–8.85)	2.30 (0.82–6.42)	1.00 (Reference)
P value	.002	.01	.11	

CI, confidence interval; HACDI, hospital-acquired *Clostridium difficile* infection; OR, odds ratio; 25(OH)D, 25-hydroxyvitamin D. Unadjusted associations between 25(OH)D groups and HACDI were estimated by bivariable logistic regression models. Adjusted odds ratios were estimated by multivariable logistic regression models with inclusion of covariate terms thought to plausibly associate with both 25(OH)D concentrations and HACDI. Estimates adjusted for age, sex, race (nonwhite vs white), patient type (medical vs surgical), and Deyo-Charlson index. Patients with HACDI were analyzed relative to all patients with either negative *C difficile* infections or those without stool samples analyzed for *C difficile*.