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Approval Sheet

Vitamin D and the Risk of Hospital-Acquired Infections in Adults Admitted to the Intensive Care Unit

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Abstract Cover Page

Vitamin D and the Risk of Hospital-Acquired Infections in Adults Admitted to the Intensive Care Unit

By

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M.D., University of Florida 2007

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An abstract of

A thesis submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Master of Science

in Clinical Research

2013

Abstract

Vitamin D and the Risk of Hospital-Acquired Infections in Adults Admitted to the Intensive Care Unit

By Jordan Anthony Kempker

Introduction: There is evidence that vitamin D is integral to the function of the innate immune response and that low serum 25-hydroxyvitamin D (25(OH)D) concentrations may be a risk factor for infection. There have been no prospective studies examining the relationship between serum 25(OH)D levels and the risk for hospital-acquired infections (HAI) in patients admitted to the intensive care unit (ICU).

Methods: This is a prospective observational cohort of adult patients admitted to the Emory University medical ICU at Grady Memorial Hospital, Atlanta, Georgia from November 1, 2011 through October 31, 2012. Patients were included in the study if they were anticipated to have an ICU stay ≥ 1 day and did not refuse enrollment, and excluded if they were not able to undergo study phlebotomy within 5 days of ICU admission.

Results: The cohort consisted of 314 subjects, with 136 (43%) of subjects deficient in vitamin D, as evidenced by serum 25(OH)D concentrations < 15 ng/mL. The patient characteristics significantly associated with low 25(OH)D levels included admission during winter months (28% vs. 18%, p = 0.04), higher PaO_2/FiO_2 (275 ± 142 vs. 226 ± 243 torr, p = 0.03) and longer time from ICU admission to study phlebotomy (1.8 vs. 1.5 days, p = 0.02). A total of 36 (11%) patients developed an HAI prior to discharge, death or within 30 days from ICU admission. In multivariate analysis adjusting for gender, APACHE II score, time to study phlebotomy, ICU length of stay and net volume status, serum 25(OH)D levels < 15 ng/mL were not associated with risk for HAIs (HR 0.94, 95% CI 0.44 – 2.00).

Conclusions: In this prospective, observational cohort of adults admitted to a single-center medical ICU, there was no significant association between 25(OH)D deficiency and the risk for HAI.

Cover Page

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Table of Contents

Introduction

Hospital-acquired infections (HAI) continue to be a significant burden on individuals and society. A recent report estimated that 400,000 children and adults in the United States were affected by an HAI in 2002, resulting in 100,000 deaths (1). This presents a significant burden on the healthcare system, with all HAIs in the United States costing an estimated $28 – 40 billion in 2009 (2). In response to this problem, the National Healthcare Safety Network of the Centers for Disease Control maintains standardized definitions for HAIs to facilitate national surveillance and guidelines for HAI prevention (3). Research continues to search for effective and efficient strategies for curtailing the problem of HAIs.

One promising avenue is in the anti-infective properties of vitamin D, a fat-soluble secosteroid hormone obtained through dietary intake and cutaneous synthesis. While Vitamin D's roles in the regulation of serum calcium for bone formation have long been understood, research in the last decade has begun to uncover that this steroid hormone has important roles in the optimal functioning of many organ systems. Vitamin D receptors and 1α-hydroxylase have been discovered in many extraskeletal tissues and the vitamin D response element (VDRE) found in over 900 genes (4,5). Furthermore, recent epidemiologic and clinical trials have suggested that optimal vitamin D status may be protective against several chronic illnesses, including risk of cardiovascular disease, lung disease, diabetes and systemic infection (6-10).

With these new discoveries in mind, the objective of this study was to determine if vitamin D levels assessed in patients at admission to the intensive care unit (ICU) were associated with the risk for HAI.

Background

Vitamin D and the Innate Immune System

Basic Science Research

The innate immune system acts to rapidly identify invading organisms and respond with humoral and cellular defense mechanisms to contain, neutralize and remove offending pathogens. These pathogens are identified by highly conserved pathogen-associated molecular patterns (PAMP) that bind to pathogen-recognition receptors (PRR) on immune cells. The cells that participate in these innate immune responses include neutrophils and monocytes as well as epithelial cells that not only provide barrier function but also have anti-pathogen activity (11,12). A vital role for vitamin D in this system was initially indicated by the discovery of vitamin D receptors (VDR) in nearly all types of immune cells, including activated CD4+ and CD8+ T cells, B cells, neutrophils, macrophages and dendritic cells (12). While these cells span the body's innate and adaptive immune responses to pathogens, vitamin D's roles in the optimal functioning of the innate immune system have been more clearly elucidated.

Vitamin D is integral to the innate immune system's production of antimicrobial peptides (AMP) in response to various pathogens (11). The most studied AMP is cathelicidin and its activated form, LL-37. This peptide is produced by phagocytic leukocytes, mucosal epithelium and keratinocytes, and is present in mucosal secretions and plasma (13). LL-37 has been shown to have direct microbicidal effects on various bacterial pathogens in vitro, including *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli, Listeria monocytogenes, Staphylococcus epidermidis*, *Staphylococcus aureus*, and vancomycin-resistant enterococci (14). Its immune functions

also include disruption of bacterial biofilms, promotion of phagocytosis and reactive oxygen species and chemotaxis of other immune cells to sites of infection (13).

Supporting vitamin D's role as a component of local cellular defense, in bovine mammary tissue infected in vivo, CD14+ cells demonstrated an increased expression of 1α-hydroxylase, which converts 25(OH)D to its activated form (15). Likewise, in women treated with vitamin D supplementation *in vivo* for 3 months, their urinary bladder cells demonstrated increased expression of cathelicidin and enhanced bacterial killing of *Escherechia coli* (16). In one study of human airway epithelial cells, vitamin D induced the production of cathelicidin and improved killing of the bacteria, *Pseudomonas aeuroginosa* and *Bordetella bronchiseptica* (17). However, not all studies have shown a positive effect of vitamin D in pathogen clearance. In mice with bacterial colitis, pretreatment with 1,25-α-dihydroxyvitamin D ($1,25(OH)_2D$) showed impaired T-cell immunity and antibacterial peptide production (18). Likewise, in oropharyngeal immune cells, incubation with $1,25(OH)_2D$ or LL-37 enhanced Group A streptococcal resistance (19). In another study of human airway epithelial cells infected with respiratory syncytial virus, vitamin D reduced the inflammatory response but not viral clearance (20). These data begin to construct a conceptual framework for the role of vitamin D and LL-37 in infection that is complex, likely having varying levels of action in different tissues, cell types, pathogens and degrees of systemic spread of infection.

Clinical Research

In a review of the observational literature regarding vitamin D and respiratory infections, much of the work has been performed in pediatric populations with 4 studies showing a positive association between respiratory infections and low 25(OH)D and 2

studies showing no association (21-26). Two other studies of children admitted with severe pneumonia showed associations between low vitamin D and increased severity or worse outcomes from pneumonia (27,28). This may suggest that vitamin D modulates the severity of respiratory infections or rather that it is a marker for poorer health status. Further complicating the picture, another study found that vitamin D receptor polymorphisms might be associated with the risk of acute lower respiratory tract infection in children (29). This suggests that there may be heterogeneity in the way different individuals utilize vitamin D, perhaps accounting for some of the heterogeneity of results among these non-randomized studies.

In the adult observational literature, a cross-sectional analysis of the over 18,000 patients in the NHANES III cohort, found an independent, inverse relationship between 25(OH)D and self-reported respiratory infection (30). A prospective cohort of adults with serial measurements of serum vitamin D in the fall and winter showed that higher concentrations were associated with a decreased risk of pneumonia (31). Similar to the pediatric literature, other studies have shown a relationship between severity of pneumonia and vitamin D. One prospective cohort of patients hospitalized with pneumonia showed that low 25(OH)D was an independent predictor of mortality and improved the prognostic accuracy of the pneumonia severity index score (32). Another prospective cohort of community acquired pneumonia patients revealed that the lowest vitamin D group was associated with a higher mortality (33).

Despite the provocative results from the basic sciences and observational literature, the results from clinical trials regarding vitamin D and respiratory infections have been predominantly null. In two trials of vitamin D in children hospitalized with

pneumonia, neither showed that treatment reduced duration of illness while one showed a decrease in pneumonia recurrence (34,35). The two trials of children in the community revealed conflicting results. One showed no effect on the incidence of pneumonia while the other demonstrated a reduction in the incidence of influenza A infection (36,37). In adults, all three randomized trials examining respiratory infections have all produced null results (38-40). The fact that all three of these trials demonstrated a control group with relatively normal 25(OH)D concentrations demonstrates that in studying vitamin D in the community, there may be no true placebo group since vitamin D is freely available from the sun, making it difficult to show positive effects.

While the relationship between vitamin D and the risk for HAI in critically ill patients has not been well studied, this subpopulation may have higher baseline rates of low vitamin D and infections that may be well suited for studying this relationship.

Vitamin D in the Critically Ill

While vitamin D's association with the risk of HAI in ICU patients has not been well studied, there is literature documenting a high prevalence of low vitamin D in the ICU and a potential effect on mortality. The latter is particularly important in studying the risk of HAIs since death is a competing event that will need to be accounted for in analysis.

Several retrospective studies have revealed a high prevalence of vitamin D insufficiency among the critically ill, with associations to outcomes less clear. A single center prospective observational study examined all ICU patients admitted in a spring-summer season and found serum 25(OH)D concentrations < 24 ng/mL in 79% (41). Spring admission, low albumin and high Simplified Acute Physiology II score were all

independently associated with low serum 25(OH)D concentrations (41). They did not find associations between vitamin D, mortality or HAI (41). McKinney et al. conducted a retrospective study of 136 veterans admitted to the ICU who had a serum 25(OH)D drawn within a month before or after admission to the ICU, revealing 98% of the veterans to have low serum 25(OH)D concentrations (42). The study also demonstrated a significantly increased survival rate (69% vs. 44%) among those with serum 25(OH)D concentrations greater than 20ng/mL (42). A retrospective study by Venkatram et al. revealed an association between mortality and vitamin D deficiency (25(OH)D < 20 ng/mL) in 437 patients at a single center ICU (43).

Other studies in critical care settings have provided more specific data on the relationship between vitamin D and infections. Jeng et al. showed that vitamin D insufficiency was present in 100% of critically ill patients with sepsis, 92% of critically ill patients without sepsis and 66.5% in healthy controls (44). A prospective study of 66 surgical ICU patients showed a non-significant trend towards an increased rate of infections and sepsis among the vitamin D deficient (<20 ng/mL) (45). Braun et al. conducted two retrospective studies on the same source population investigating this subject. One was a retrospective analysis of 2,399 patients admitted to medical and surgical ICUs with a 25(OH)D drawn within the year prior to admission. The data showed a 1.3 and 1.7 fold increase in all-cause mortality among vitamin D insufficient and deficient groups (<30ng/mL and <15ng/mL) respectively and a significant increase in blood culture positivity (46).

Background Conclusion

The multiple functions of vitamin D in the immune system's response to infection suggest it may be an integral component in combating infections. The basic science data point toward vitamin D's role in the optimal functioning of the innate immune system, in part by producing AMPs such as LL-37. The early clinical data on its role in preventing and attenuating infections has suggested a link but there have been no prospective studies investigating the relationship between low vitamin D and the risk for HAIs in patients in the ICU. Data suggest that this vulnerable population has a high prevalence of low vitamin D and with the risk for HAI in this group, it is study group amenable to the examination of the relationship between vitamin D and infection.

Methods

The main objective of this study was to conduct a prospective observational cohort study to assess vitamin D status, as measured by serum 25(OH)D concentrations, at admission to the medical ICU as a risk factor for subsequent HAI. The secondary objective was to describe the patient characteristics and outcomes associated with low vitamin D on admission to the medical ICU.

Null Hypothesis

In adults admitted to the Emory Medical ICU at Grady Memorial Hospital, a serum 25(OH)D concentration < 15 ng/mL, measured within 5 days of ICU admission, will not be independently associated with an increased risk of HAI at hospital discharge or within 30 days from ICU admission.

Alternative Hypothesis

In adults admitted to the Emory Medical ICU at Grady Memorial Hospital, a serum 25(OH)D < 15 ng/mL, measured within 5 days of ICU admission, will be independently associated with an increased risk of HAI at hospital discharge or within 30 days from ICU admission.

Study Design

Setting and Patient Selection

A prospective observational cohort design was utilized for this study. Patients admitted to the medical ICU at Grady Memorial Hospital in Atlanta, Georgia from November 1, 2011 through October 31, 2012 were screened for enrollment. Subjects were screened using an online ICU census and were eligible if they were ≥ 18 years of age and were anticipated to have an ICU stay ≥ 1 day. Subjects were enrolled if they or a

surrogate gave informed consent or they qualified for a waiver of consent. Subjects were excluded if they were previously enrolled in this study or the study staff was unable to perform phlebotomy within 5 days after ICU admission. The Institutional Review Board of Emory University and the Grady Research Oversight Committee approved the study.

Data and Sample Collection

The primary outcome was the development of an HAI, which was defined as an infection during the index hospitalization that was not present within the first 48 hours of admission to the ICU and qualified for an infection of the lower respiratory tract, urinary tract, blood stream or gastrointestinal tract using the 2008 criteria from the Centers for Disease Control and National Healthcare Safety Network (3). Infections were captured up to 30 days from ICU admission, determined from the laboratory data and clinical documentation in the electronic medical record and adjudicated by an Infectious Diseases specialist. The primary exposure of vitamin D was determined from serum drawn within 5 days of admission to the ICU by trained study staff, preferentially from a central venous catheter or arterial line or by peripheral phlebotomy if these were unavailable. Assays for 25(OH)D were performed using an automated chemiluminescent technique. Quality assurance of the serum 25(OH)D determinations was provided by participation in the vitamin D external quality assessment scheme (DEQAS) and the NIST/NIH Vitamin D Metabolites Quality Assurance Program. Vitamin D deficiency was defined as a concentration < 15 ng/mL. As there is no known physiologic cutoff for the vitamin D's effects on the immune system, this cutoff was chosen as it has some precedence in the critical care literature and from an extrapolation of the optimal vitamin D levels for bone health by the 2011 Institute of Medicine Report on calcium and vitamin D dietary

references (46-49). This report identifies a serum 25(OH)D < 12 ng/mL as carrying a significant risk of bone disease for the individual while setting a population target at 20 ng/mL (49).

The other information collected from the electronic medical record included patient demographics, ICU admitting diagnoses, past medical and social histories, physiologic data, hospital and ICU dates and vital status at hospital discharge. The presence of medical comorbidities was recorded if they were documented in the past medical history of the hospital record. Physiologic data was gathered from the 6 hours before through the 18 hours after the time of the ICU admit orders and selected as the worse value by the Acute Physiology and Chronic Health Evaluation (APACHE) II scoring system (50). Culture results were recorded as the final result listed in the electronic medical record. Winter season was defined as ICU admission within the months of December through February and race was dichotomized into white and non-White since the frequency of races other than Black and White was relatively small (Hispanic and Asian at 4% and 1% of entire cohort, respectively).

All data was initially collected into a paper form and then entered into a REDCap electronic database hosted at Emory University.

Sample Size Calculation

Initial sample size calculations were performed using a chi-squared test to look for the relative change in frequency of HAI between the two vitamin D groups. A target sample size of 400 gave an 85% power to detect a 30% relative reduction in HAI from a baseline HAI frequency of 50% and a 90% power to detect a 40% relative reduction in HAI from a baseline HAI frequency of 40%.

Data Analysis

All statistical analyses were performed using SAS 9.3 (SAS Institute: Cary, NC) with a p value < 0.05 considered statistically significant. Bivariate analyses were performed using pooled t-tests for continuous variables of normal distribution and equal variances, unpooled t-tests for those with unequal variances and Wilcoxon rank sum tests for those with non-normal distributions. Chi-squared tests were used for all categorical variables. The multivariate analysis was performed using a Cox Proportional Hazards model for the time to hospital-acquired infection with subjects censored for death, discharge or at 30 days from ICU admission, whichever came first. As he primary outcome was defined as an infection that was not present within the first 48 hours of admission to the ICU, the time zero for the analysis was defined as 2 days after ICU admission. The model was built using the purposeful selection of covariates described by Hosmer and Lemeshow and briefly described here (51). Covariates were initially selected for model inclusion if they were the primary exposure or associated with both the primary outcome and exposure at a $p < 0.25$ in the bivariate analyses. Covariates were then retained in the model if the $p < 0.10$ or upon removal, the vitamin D parameter estimate changed by $\geq 20\%$. Then each covariate from the entire dataset was entered into this reduced model and retained if they changed the parameter estimate of the primary exposure by $\geq 20\%$. Final p-values of <0.05 in the model were considered statistically significant.

Kaplan-Meier survival curves were also generated for in-hospital mortality, with subjects censored at discharge or at 30 days of hospitalization, whichever came first.

Kaplan-Meier method was also used to estimate mean time to infection in both vitamin D groups, with subjects censored for death, discharge or at 30 days of hospitalization, whichever came first. Cumulative incidence function curves for the incidence of HAI, accounting for the competing risk of death were constructed using a macro developed by SAS (SAS Institute: Cary, NC) (52).

There was a significant amount of missing data for the serum lactate (130 subjects, 41% of entire cohort) and the arterial blood gas (108 subjects, 34% of entire cohort). As the ratio of arterial oxygen tension to fraction of inspired oxygen (PaO_2/FiO_2), was associated with the outcome in the Cox Proportional Hazards model, the missing values for this covariate were imputed using a rule-based strategy. Since mechanical ventilation therapy is largely based on lung function that is in part determined by arterial blood gas measurements, receipt of this therapy was used to divide the entire cohort into two groups: those who received mechanical ventilation and those who did not. The mean PaO_2/FiO_2 from the mechanically ventilated subgroup with arterial blood gas data was imputed to all those who received mechanical ventilation but did not have an arterial blood gas. This same step was then performed for the group who did not receive mechanical ventilation. The effects of imputation are shown in Appendix A.

Ethics

This study was approved by the Emory University Institutional Review Board; study number 51263 and by the Grady Memorial Hospital Research Oversight Committee.

Results

The study enrollment results are described in Figure 1. A total of 798 subjects were screened, with 388 enrolled; 72 subjects were withdrawn for inability to draw blood within 5 days and 2 subjects were withdrawn given inadequate blood collection volume, leaving a final study cohort of 314 subjects. The entire cohort was predominantly male (57%) and black (83%), with 55 to 64 years old as the most represented age group (Table 1). The overall mean APACHE II score was 27.8 ± 6.9 with an in-hospital mortality of 16%.

Forty-three percent of the entire population had a serum 25(OH)D < 15 ng/mL with the overall histogram of serum 25(OH)D revealing a rightward skewed distribution (Figure 2). Graphical analysis of the seasonal variation of vitamin D over the course of the year revealed an attenuated sinusoidal pattern with a general peak in fall months and nadir the winter months (Figure 3).

Subject characteristics and clinical outcomes by vitamin D status are outlined in Table 1. Characteristics significantly associated with vitamin D deficiency were admission during the winter season (28 vs. 18%, p = 0.04), higher PaO_2/FiO_2 (275 ± 142 vs. 226 ± 243 torr, p = 0.03) signifying worse pulmonary function, and longer time from ICU admission to study phlebotomy (1.8 ± 1.2 vs. 1.5 ± 1.1 days, p = 0.02). In the vitamin D deficient group there were trends towards younger mean age, lower prevalence of documented hypertension, higher mean serum total bilirubin and longer median ICU length of stay.

A total of 36 adjudicated HAI were documented. The most common infection site was the respiratory tract (44%) followed by the genito-urinary tract (25%), blood stream

(22%), and gastrointestinal tract (8%). By the Kaplan-Meier method, mean time to infection in the high and low vitamin D groups was 24.8 and 23.4 days, respectively. While the infectious microorganism was not identified in 19% of the HAIs and 14% of the HAIs were polymicrobial, the single most commonly identified organism was *Enterococci* spp. (Table 2).

In the unadjusted Cox Proportional Hazards model, serum 25(OH)D < 15 ng/mL was not associated with an increased risk for HAI (HR 1.03, 95% CI 0.53 – 2.02). For the multivariate analysis, while values were imputed for PaO_2/FiO_2, there were still 37 subjects left out of the analysis due to missing values for other covariates. In the final multivariate model adjusting for gender, alcohol use history, APACHE II score, days from ICU admission to study phlebotomy, ICU length of stay and net volume status, serum 25(OH)D levels < 15 ng/mL were not associated with an increased risk for HAI (HR 0.94, 95% CI 0.44 – 2.00) (Figure 4). The only other covariate that remained significantly associated with the risk of HAI in this model was ICU length of stay (HR 1.05, 95% CI 1.01 – 1.10). The multivariate Cox Proportional Hazards model without imputation for PaO_2/FiO_2, included 192 subjects in the analysis and revealed similar results (Appendix A).

In addition to the primary outcome, in bivariate analyses vitamin D showed no associations with differences in the hospital length of stay, ICU length of stay or duration of mechanical ventilation (Table 1). Kaplan-Meier survival curves did not demonstrate an association between vitamin D deficiency and hospital mortality (Figure 5). Cumulative incidence curves were generated to examine the cumulative incidence of HAI

by serum 25(OH)D status accounting for the competing risk of death and did not reveal a significant association (Gray's Test p = 1.0) (Figure 6).

Discussion

In this prospective observational study, there was a high prevalence of vitamin D deficiency and a low rate of HAIs among patients admitted to a single center medical ICU. A total of 43% of patients had a serum 25(OH)D < 15 ng/ml and 11% of all subjects went on to develop a HAI within 30 days of ICU admission. There was no association between low vitamin D levels and the development of a HAI. While vitamin D has important immunological functions, this study's results show that low Vitamin D levels alone do not lead to a high risk of HAI among severely ill medical patients.

The findings did not support the *a priori* hypothesis that low vitamin D status upon admission to the medical ICU would be a risk factor for HAI. While this hypothesis is based on a large body of basic science that supports a role for vitamin D in the innate immune system's response to infection, the results are consistent with the parallel body of clinical research that has demonstrated mixed results for the role of vitamin D alone in predicting and preventing infections (53). The results are also consistent with extrapolations from the vitamin D literature in ICU patients, with one retrospective study showing an association between blood culture positivity and low vitamin D, while two others did not show significant associations between low vitamin D and infection (45,47,54).

The discrepancies between basic and clinical sciences may be explained by some of the difficulties in studying vitamin D in clinical studies. These may include small clinical effect sizes, lack of a true null vitamin D group, lack of measurement of vitamin D storage and utilization, and genetic variation in vitamin D action (55).

More specific to this study, serum 25(OH)D concentrations might have been influenced by the hemodilute or acute inflammatory state of our ICU patients rather than reflecting the vitamin D nutriture *per se*. Regarding hemodilution, one small, well-designed study has shown that intravenous fluid administration temporarily lowers the concentration of 25(OH)D (56). This is important to the study population as early in the ICU stay, when 25(OH)D was measured, many patients received large boluses of intravenous fluids. While the study did not find net fluid balance in the first 24 hours to be significantly associated with vitamin D status (Table 1), this number may not be indicative of the true clinical situation as the fluid administration by paramedics and in the emergency department was often not in the medical record. In regards to the effects of inflammation on the vitamin D axis, several studies have suggested that serum 25(OH)D levels decrease during acute inflammation only to recover a few days later, likely in part due to a decrease in vitamin D binding protein during acute inflammation (57-59). Therefore, this study's low 25(OH)D levels may not be indicative of a true vitamin D deficiency that would pose as a risk factor for future infection but rather the clinical state of the patient on ICU admission. While measuring vitamin D levels before clinical illness may solve this problem, this was not feasible in the design for this study.

In addition to the above measurement issues, serum levels of 25(OH)D may not be indicative of the body's utilization of vitamin D by the innate immune system. There is an emerging scientific literature in the area of tuberculosis and vitamin D that has identified genetic variations in the vitamin D receptor, introducing variation in the immune system's utilization of this nutrient (60-62). While the data are not conclusive, in tuberculosis these vitamin D receptor polymorphisms have been associated with

25(OH)D levels, the presence of multidrug resistance and response to vitamin D adjunctive therapy (61,62). In addition, one small study has shown associations between these polymorphisms and acute lower respiratory infections in children (29). As this study did not test the receptor polymorphisms in the study cohort, this could account for significant confounding of our results. Along the lines of determining the body's true vitamin D status and its effects on immune function, this study did not measure the other components of the vitamin D axis or the antimicrobial peptides that may be regulated by vitamin D stores. The measurement of free serum or local intracellular cathelicidin levels or the other metabolites of vitamin D, including 1,25α-dihydroxyvitamin D, 24,25-dihydroxyvitman D, blood concentrations of parathyroid hormone, or calcium, or serum and urinary concentrations of vitamin D binding protein may have given a better understanding of an interaction between vitamin D and our patients' immune function and risk for infection.

Despite the above issues, this study has several strengths. Its prospective design allowed for the determination of vitamin D status upon admission to the ICU and followed patients forward for a sufficient time after to determine 25(OH)D's association with the risk of HAI. The study also included a diverse subject population reflective of the pathology seen in this urban hospital medical ICU.

This study has important implications for the future directions of vitamin D research in infection and critical illness. While vitamin D's therapeutic role in the prevention and treatment of infections is best approached through randomized controlled trials, there is still observational work to be done to inform the design of these trials. Further studies measuring the important mediators in the vitamin D-immune axis may

help to differentiate a truly deficient state in terms of immune function from other inflammatory or hemodilute states, allowing identification of the at-risk population likely to benefit in a clinical trial. Furthermore, a better understanding of how receptor polymorphisms mediate the interaction between vitamin D and immune function may help us to not only identify target populations but also create more potent vitamin D analogues that may help larger populations (63).

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Tables

Table 1. Summary of Demographic Characteristics and Severity of Illness in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314 [a]

Variable	N(%)
Race	
Black	261 (83)
White	39 (12)
Hispanic	12 (4)
Asian	2 (1)
Age Categories in years	
18 – 44	63 (20)
45 – 54	81 (26)
55 – 64	87 (28)
65 – 74	52 (17)
≥ 75	31 (10)
Female	131 (42)
Mean APACHE II (SD)	27.8 (6.9)
In-hospital Mortality	49 (16)

[a]APACHE II = acute physiology and chronic health evaluation 2.

Table 2. Summary of Hospital-Acquired Infections at 30 days from Admission to the Intensive Care Unit by Infective Site and Organism in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314

	N (%)
Overall Hospital-Acquired Infections	**36 (11)**
Site of Hospital-Acquired Infections	
Respiratory	16 (44)
Genito-urinary	9 (25)
Blood Stream	8 (22)
Gastroenterological	3 (8)
Organism	
Unknown	**7 (19)**
Gram-positive	
Enterococci	4 (11)
Staphylococcus aureus	3 (8)
Clostridium dificile	2 (6)
Coagulase-negative staphylococci	1 (3)
Gram-negative	
Pseudomonas aeruginosa	2 (6)
Escherichia coli	1 (3)
Fungal	
Candida albicans	3 (8)
Polymicrobial	**5 (14)**
Other Organism	**8 (22)**

Table 3. Patient Characteristics and Hospital Outcomes by Vitamin D Status in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314[a]

Variable	25(OH)D ≥ 15 ng/mL N = 178	25(OH)D < 15 ng/mL N = 136	p
Demographics			
Age, mean (SD)	57.2 (15.5)	54.4 (14.6)	0.1
Age Categories, n (%)			0.2
18 – 44	25 (18)	38 (21)	
45 – 54	30 (22)	51 (29)	
55 – 64	41 (30)	46 (26)	
65 – 74	21 (15)	31 (17)	
≥ 75	19 (14)	12 (7)	
Female, n (%)	55 (40)	76 (43)	0.7
Weight, kg, mean (SD)	80.8 (22.9)	85.2 (33.2)	0.2
Race, n (%)			
White	20 (15)	19 (11)	0.3
History of Tobacco Use, n (%)	62 (46)	82 (46)	0.8
History of Alcohol Abuse, n (%)	26 (19)	45 (25)	0.4
Past Medical History			
Liver disease, n (%)	7 (5)	16 (9)	0.2
Pulmonary disease, n (%)	37 (27)	36 (20)	0.2
Heart disease, n (%)	98 (72)	129 (72)	0.9
Renal Disease, n (%)	32 (24)	37 (21)	0.6
Immunosuppression, n (%)	25 (18)	26 (15)	0.4
Diabetes Mellitus, n (%)	41 (30)	55 (31)	0.9
Hypertension, n (%)	90 (66)	102 (57)	0.1
Cerebrovascular disease, n (%)	20 (15)	20 (11)	0.4
Admission Data			
Winter	24 (18)	49 (28)	0.04
Primary Admission Diagnoses, n (%)			0.2
Respiratory	47 (35)	45 (25)	
Cardiac	49 (36)	75 (42)	
Neurological	17 (13)	22 (12)	
Gastroenterological	12 (9)	12 (7)	
Other	11 (8)	24 (13)	
Sepsis at Admission, n (%)	72 (53)	98 (55)	0.7

Table 3 Continued. Patient Characteristics and Hospital Outcomes by Vitamin D Status in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314[a]

Variable	25(OH)D ≥ 15 ng/mL N = 178	25(OH)D < 15 ng/mL N = 136	p
Worst Physiologic parameters within 24 hours of ICU Admission, Mean (SD)			
PaO_2/FiO_2 , torr	226(142)	275(243)	0.03
Creatinine, mg/dL	2.3 (2.5)	2.5 (2.7)	0.5
Mean Arterial Pressure, mm Hg	77 (37)	81 (40)	0.4
Patient on vasopressor therapy, n(%)	35 (26)	48 (27)	0.8
Total Bilirubin, mg/dL	1.0 (2.3)	1.6 (3.1)	0.1
Platelet count, x 10^9/L	193 (105)	189 (159)	0.8
White Blood Cells, x10^9/L	12.3 (10.4)	11.9 (7.7)	0.7
Hematocrit %	30.8 (8.7)	31.1 (8.1)	0.7
Lactate, mmol/L	3.1 (2.4)	3.4 (2.4)	0.4
Glasgow-Coma Scale score	10 (7-15)	12.5 (7-15)	0.2
Net Fluid Balance, L	-0.05 (2.1)	-0.08 (2.4)	0.9
SOFA score	7.1 (3.7)	7.1 (3.8)	1.0
APACHE II score	27.5 (7.0)	28.0 (6.9)	0.6
Days from Hospital Admission to serum 25(OH)D draw			
Mean (SD)	1.5 (1.1)	1.8 (1.2)	0.02
Clinical Outcomes			
Mechanically Ventilated, n (%)	76 (56)	100 (56)	1.0
Hospital Length of Stay, median (IQR)	9.5 (4-18)	11 (5-18)	0.3
ICU Length of Stay, median (IQR)	3 (1-8)	4 (2-7)	0.1
Days of Mechanical Ventilation, median (IQR)	4 (1-9)	4 (2-7.5)	1.0
Hospital-Acquired Infection, n (%)	16 (12)	28 (16)	0.3

[a]25(OH)D = serum 25α-hydroxyvitamin D; APACHE II = acute physiology and chronic health evaluation 2; PaO_2/FiO_2 = (partial pressure in torr of O_2 in arterial blood/the fraction of inspired oxygen); SOFA = Sequential Organ Function Assessment

Table 4. Results from An Adjusted Cox-Proportional Hazards Model For the Risk of Hospital-Acquired Infection in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 277. Global Likelihood Ratio p = 0.06[a]

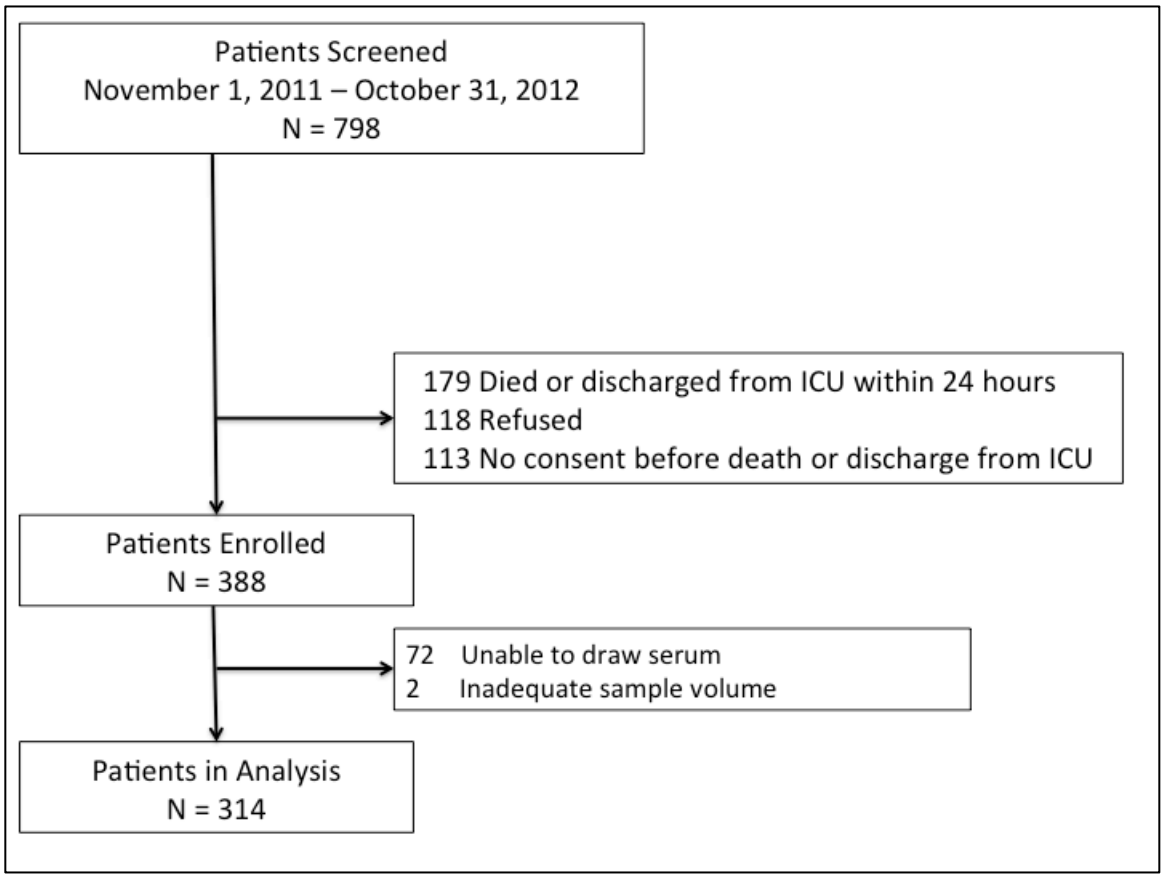
Variable	Adjusted HR (95%CI)
25(OH)D < 15 ng/mL	0.94 (0.44 – 2.00)
ICU Length of Stay, days	1.05 (1.01 – 1.10)
PaO_2/FiO_2 , torr	1.00 (0.99 – 1.00)
Days from ICU Admission to Phlebotomy	1.00 (0.77 – 1.39)
Male Gender	0.77 (0.36 – 1.64)
APACHE II Score	1.00 (0.94 – 1.05)
Net Fluid Balance, L	0.85 (0.71 – 1.01)

[a] In this analysis, imputed values were used for missing data for the PaO_2/FiO_2 covariates since 34% of the data were missing. Despite this, 37 observations were not used in the final model due to missing data in other covariates.

25(OH)D = serum 25α-hydroxyvitamin D; APACHE II = acute physiology and chronic health evaluation 2; ICU = Intensive Care Unit; PaO_2/FiO_2 = (partial pressure in torr of O_2 in arterial blood/the fraction of inspired oxygen).

Figures

Figure 1. Flowchart of Study Enrollment Process and Results[a]



[a] ICU = intensive care unit.

Figure 2. Distribution of Vitamin D Concentrations in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314

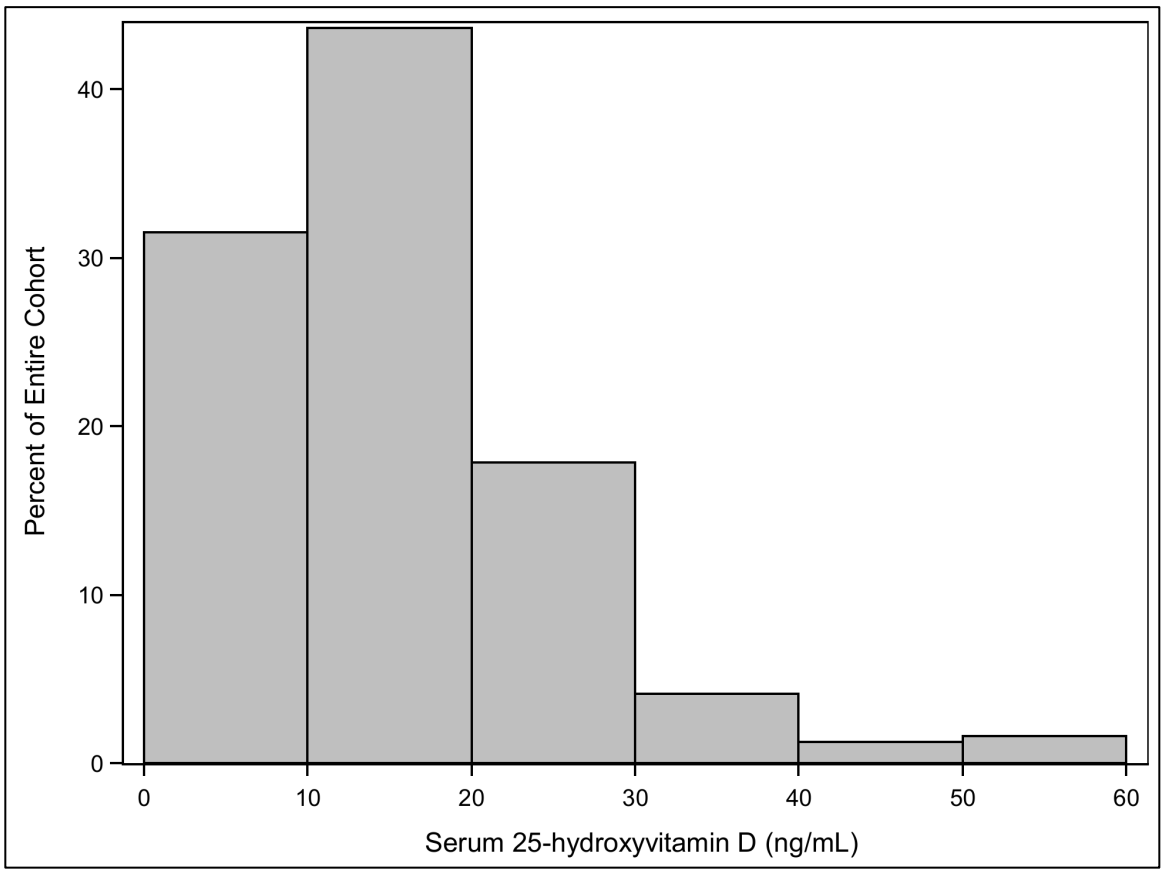
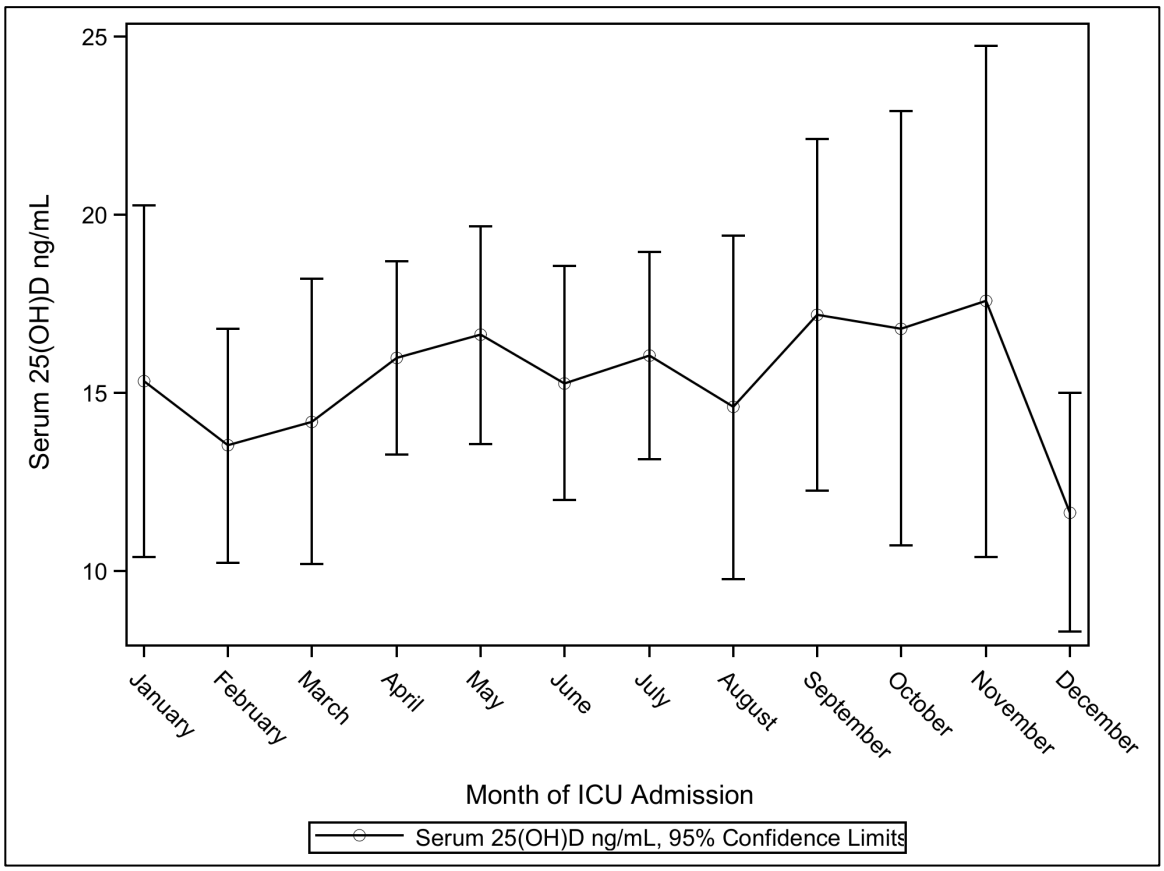
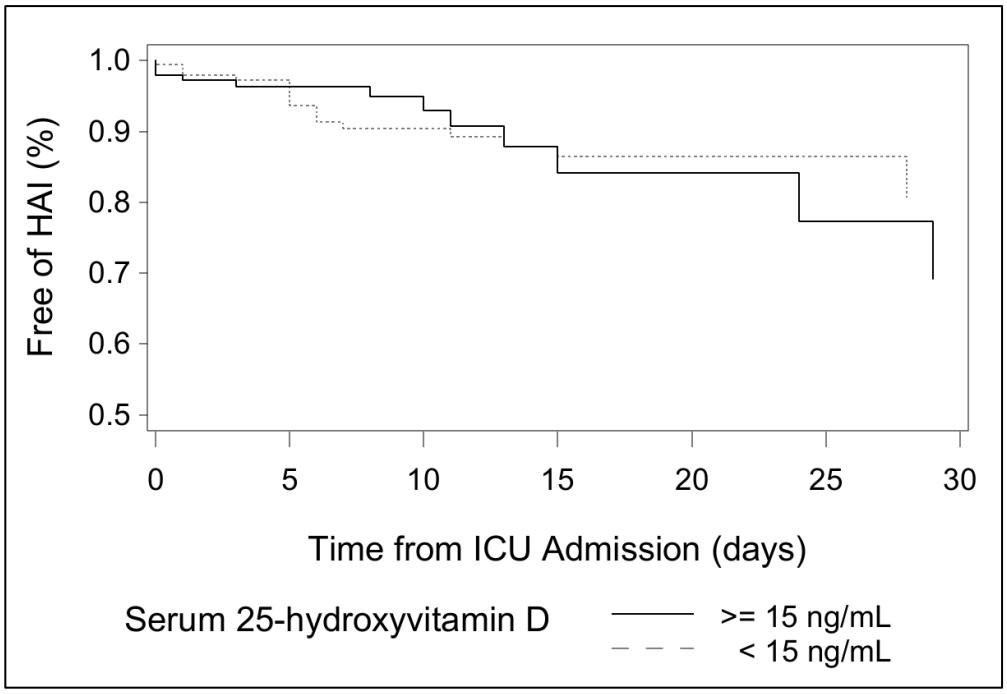


Figure 3. Mean Vitamin D by Month in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314[a]



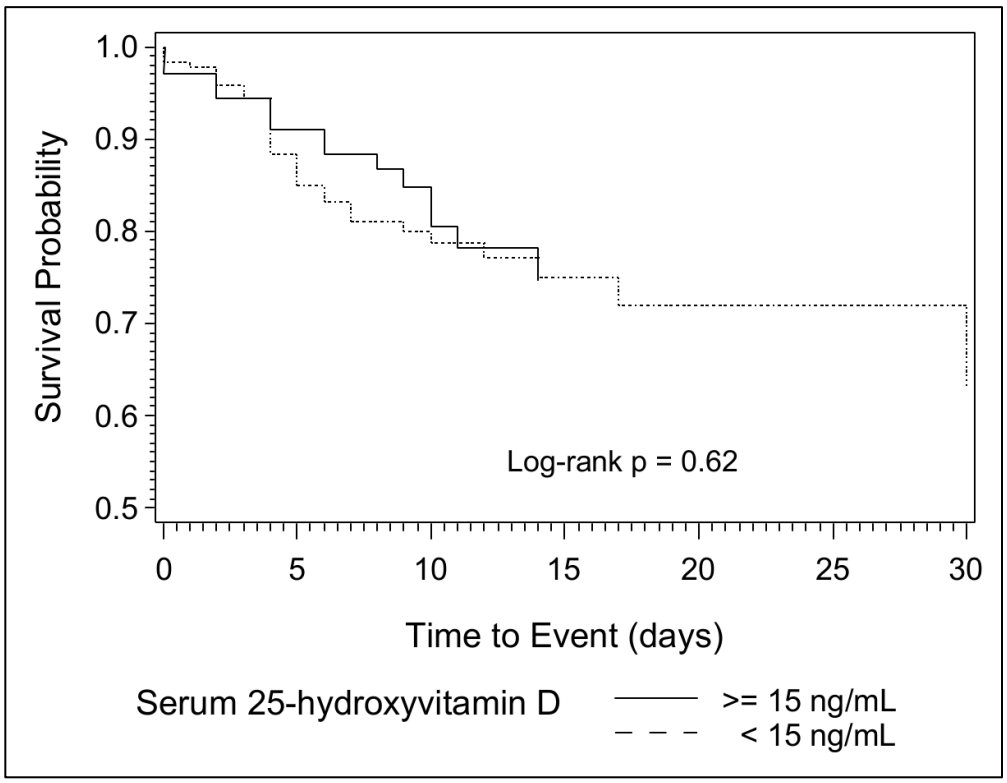
[a] 25(OH)D = serum 25α-hydroxyvitamin D

Figure 4. Adjusted Cox Proportional Hazards Curves for Hospital-Acquired Infection in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 277[a]



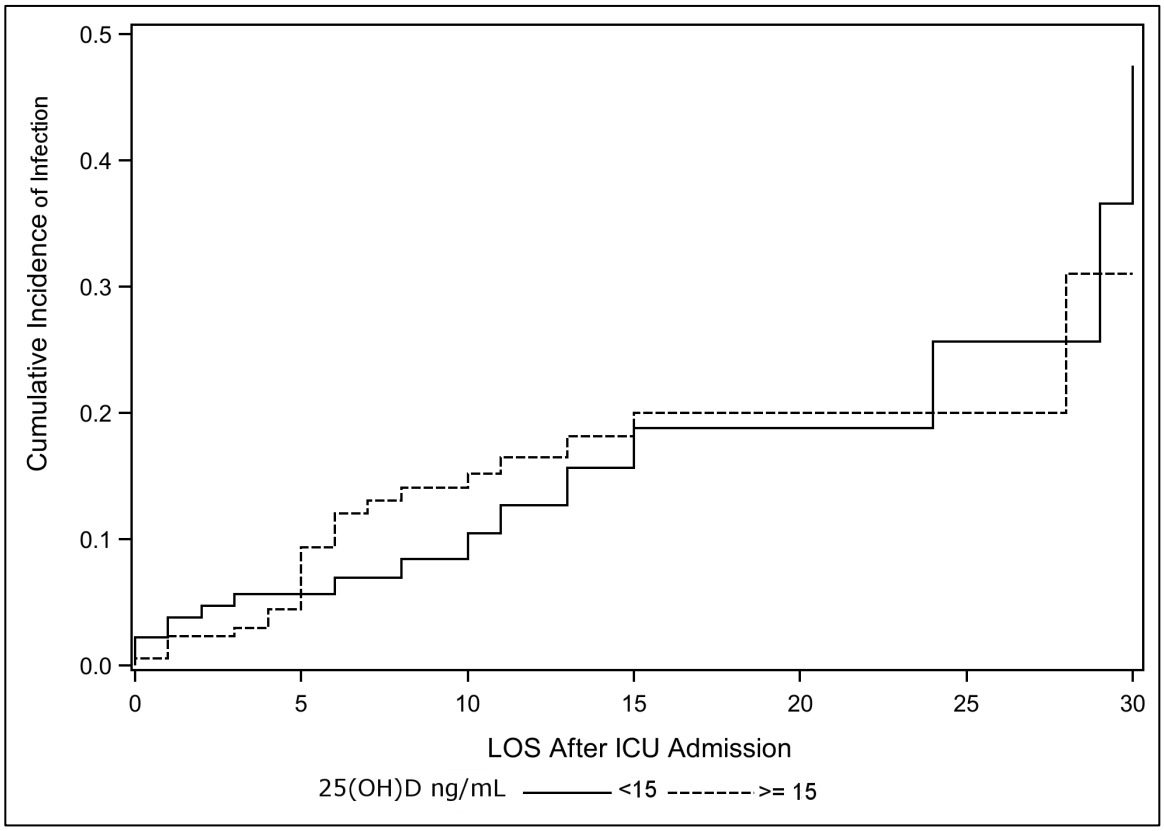
[a] Curves are adjusted for gender, alcohol use history, APACHE II score, days from ICU admission to study phlebotomy, ICU length of stay and net volume status. Subjects were censored at death, discharge or 30 days of hospitalization, whichever came first.
APACHE II = acute physiology and chronic health evaluation 2; HAI = hospital-acquired infection; ICU = intensive care unit.

Figure 5. Kaplan-Meier Survival Curves by Vitamin D Status Infection in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314[a]



[a] Subjects were censored at discharge or 30 days of hospitalization, whichever came first.

Figure 6. Cumulative Incidence Functions For Hospital-Acquired Infection by Vitamin D Status in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 314[a]



[a] The cumulative incidence function accounts for the competing risk of death while in the hospital.

25(OH)D = serum 25α-hydroxyvitamin D; ICU = Intensive Care Unit; LOS = length of stay

Appendix A. Effects of Imputation of Missing Values

Figure 1B. Effects of Imputation on the PaO_2/FiO_2 Mean and Standard Deviation by Vitamin D Status.

Variable	Imputation	25(OH)D ≥ 15 ng/mL N = 178	25(OH)D < 15 ng/mL N = 136	p
PaO_2/FiO_2 torr, mean (SD)	No	225.8 (141.8)	274.5 (173.1)	0.03
	Yes	251.0 (123.1)	280.8 (143.7)	0.05

Figure 2B. Effects of Imputation on the PaO_2/FiO_2 Mean and Standard Deviation by Hospital-Acquired Infection Status.

Variable	Imputation	Hospital-Acquired Infection N= 36	No Hospital-Acquired Infection N=278	p
PaO_2/FiO_2 torr, mean (SD)	No	202.1(116.7)	263.3(167.5)	0.01
	Yes	210.9(105.3)	277.2(138.1)	0.0004

Table 1A. Results from An Adjusted Cox-Proportional Hazards Model For the Risk of Hospital-Acquired Infection in Patients Admitted to the Medical Intensive Care Unit at Grady Memorial Hospital, Atlanta, GA November 1, 2011 – October 31, 2012. N = 192. Global Likelihood Ratio p = 0.41[a]

Variable	Adjusted OR (95%CI)
25(OH)D < 15 ng/mL	1.07 (0.48 – 2.41)
ICU Length of Stay, days	1.04 (0.99 – 1.09)
PaO_2/FiO_2 , torr	1.00 (1.00 – 1.00)
Days from ICU Admission to Phlebotomy	0.96 (0.70 – 1.32)
Male Gender	0.81 (0.36 – 1.82)
APACHE II Score	1.001 (0.96 – 1.07)
Net Fluid Balance, L	0.86 (0.71 – 1.04)

[a] In this analysis, 122 observations were not used in the final model due to missing data in other covariates.

25(OH)D = serum 25α-hydroxyvitamin D; APACHE II = acute physiology and chronic health evaluation 2; ICU = Intensive Care Unit; PaO_2/FiO_2 = (partial pressure in torr of O_2 in arterial blood/the fraction of inspired oxygen).