**Vitamin D levels and risk of acute exacerbations of chronic obstructive pulmonary disease: a prospective cohort study**

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Manuscript Title:
Vitamin D levels and risk of acute exacerbations of chronic obstructive pulmonary disease: a prospective cohort study

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KMK drafted the protocol, obtained the funding, directed several of the statistical analyses, wrote the manuscript, and provided approval of the final manuscript.
DEN revised the protocol for critical intellectual content, directed several of the statistical analyses, and reviewed and approved the final manuscript.
JEC revised the protocol for critical intellectual content, performed the statistical analyses, and reviewed and approved the final manuscript.

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At a Glance Commentary

Scientific Knowledge on the Subject:
- Low vitamin D levels are common in COPD
- Low vitamin D levels are associated with higher risk of respiratory infections in the general population, and higher risk of exacerbations in asthmatics.
- Studies examining relationships between vitamin D levels and acute exacerbations of COPD are lacking

What This Study Adds to the Field
- In this large cohort study of 973 exacerbation-prone COPD patients, we found no association between baseline vitamin D levels and subsequent risk of acute exacerbations of COPD.

This article has an online data supplement, which is accessible from this issue’s table of contents online at www.atsjournals.org
Abstract

Rationale: Low blood levels of 25-hydroxyvitamin D (25[OH]D) have been associated with a higher risk of respiratory infections in general populations and higher risk of exacerbations of lung disease in asthmatics. We hypothesized that low blood levels of 25(OH)D in patients with chronic obstructive pulmonary disease (COPD) would be associated with an increased risk of acute exacerbations of COPD (AECOPD).

Objectives: To determine if baseline 25(OH)D levels relate to subsequent AECOPD in a cohort of patients at high risk for AECOPD.

Methods and Measurements: Plasma 25(OH)D was measured at baseline in 973 participants on entry to a one-year study designed to determine if daily azithromycin decreased the incidence of AECOPD. Relationships between baseline 25(OH)D and AECOPD over one year were analyzed with time to first AECOPD as the primary outcome and exacerbation rate as the secondary outcome.

Main Results: In this largely Caucasian (85%) sample of North American patients with severe COPD (mean FEV₁ 1.12L, 40% of predicted), mean 25(OH)D was 25.7 ± 12.8 ng/mL. 33.1% of participants were Vitamin D insufficient (≥20 ng/mL but <30 ng/mL), 32.0% were Vitamin D deficient (<20 ng/mL) and 8.4% had severe vitamin D deficiency (<10 ng/mL). Baseline 25(OH)D levels had no relationship to time to first AECOPD nor AECOPD rates.

Conclusions: In patients with severe COPD, baseline 25(OH)D levels are not predictive of subsequent AECOPD.
Key Words:
Pulmonary Disease, Chronic Obstructive
Vitamin D

Abbreviations
25(OH)D = 25-hydroxyvitamin D
AECOPD = acute exacerbation of chronic obstructive pulmonary disease
ANOVA = analysis of variance
CI = confidence interval
COPD = chronic obstructive pulmonary disease
FEV\textsubscript{1} = forced expiratory volume in one second
FVC = forced vital capacity
LHS 3 = Lung Health Study 3
RCT = Randomized controlled trial
SD = standard deviation
SF-36 = Medical Outcomes Study 36-item Short Form
SGRQ = St. George’s Respiratory Questionnaire
Introduction

Acute exacerbations of chronic obstructive pulmonary disease (AECOPDs) are associated with increased risk of short-term mortality\textsuperscript{1, 2} and impaired respiratory health status.\textsuperscript{3} Exacerbations are also costly,\textsuperscript{4-7} making prevention of AECOPDs an important goal of COPD management. Proven therapies to reduce AECOPDs include long-acting inhaled bronchodilators, inhaled corticosteroids, and macrolide antibiotics, but they are only modestly effective, and additional interventions are needed.

Viral and bacterial respiratory infections are thought to trigger most COPD exacerbations.\textsuperscript{8} As such, therapies that reduce respiratory infection risk might reduce the frequency of AECOPDs. A growing body of evidence suggests an important role for Vitamin D in mounting appropriate innate and adaptive immune responses to infections.\textsuperscript{9-11} Cross-sectional\textsuperscript{12} and prospective cohort\textsuperscript{13} studies show that lower 25(OH)D levels are associated with a higher frequency of respiratory infections in healthy adults and more frequent asthma exacerbations in children.\textsuperscript{14, 15} A randomized trial of 430 Japanese schoolchildren during influenza season showed that, compared to placebo, vitamin D supplementation lowered the frequency of influenza A, although a secondary outcome of influenza B infections showed a non-statistically-significant trend towards higher frequency in the supplemented children.\textsuperscript{16}
Vitamin D insufficiency and deficiency are highly prevalent in COPD patients, with the lowest levels being associated with the most severe airflow obstruction.\textsuperscript{17} Since patients with the most severe COPD are also at highest risk for exacerbations\textsuperscript{18} it is reasonable to posit that low levels of vitamin D might be an independent risk factor for AECOPDs. We therefore conducted a large observational cohort study to test this hypothesis in exacerbation-prone patients with COPD.

**Methods**

This study was a secondary analysis of data collected in a randomized, controlled trial testing the hypothesis that daily azithromycin decreases the frequency of AECOPD (ClinicalTrials.gov NCT00119860).\textsuperscript{19}

**Study participants**

Inclusion criteria included a ratio of forced expiratory volume in one second (FEV\textsubscript{1}) / forced vital capacity (FVC) <70\%, FEV\textsubscript{1} <80\% of predicted, $\geq$10 pack-year smoking history, and an increased risk of AECOPD in the subsequent year (requiring oxygen, using systemic corticosteroids or having an Emergency Department visit or hospitalization for COPD within 1 year of study entry). Exclusion criteria included a diagnosis of asthma or a disease resulting in the patient being either medically unstable or having a predicted life expectancy <3
years, macrolide hypersensitivity, taking medications with azithromycin interactions, electrocardiographic QTc interval >440 msec, resting heart rate >100 beats/minute, hepatic or renal insufficiency (creatinine >1.5 mg/dL and estimated creatinine clearance <20 mL/min), bronchiectasis, or hearing impairment. Participants were required to be free of AECOPD for ≥ 4 weeks prior to enrollment.

Participants were randomly assigned in a 1:1 ratio to either daily azithromycin or placebo for one year given in addition to their usual treatment. Participants were seen in clinic or contacted by phone on alternate months and detailed exacerbation information was collected over one year. AECOPDs were defined as a complex of respiratory symptoms (increased or new-onset) of >1 of the following: cough, sputum, wheezing, dyspnea, or chest tightness with a duration of at least three days and requiring treatment with an antibiotic or systemic corticosteroid.

All participants provided written informed consent to study participation and to storage of blood specimens for future research, such as this secondary analysis of vitamin D levels. Each participating institution’s institutional review board approved the protocol.

Plasma was collected at baseline, centrifuged, frozen at -80 °C, and shipped to the data coordinating center (University of Minnesota, Minneapolis, MN, USA).
Plasma was subsequently delivered to the Mayo Clinic Immunochemical Core Laboratory (Mayo Clinic, Rochester, MN, USA) for determination of 25-hydroxyvitamin D [25(OH)D] concentrations using liquid chromatography tandem mass spectroscopy. 25(OH)D has been shown to be stable in stored blood samples. 20, 21 25(OH)D measurement was not a pre-planned analysis prior to initiation of the trial.

**Statistical methods**

The pre-specified primary outcome of this study was the relationship between baseline 25(OH)D and time to first AECOPD as determined by multivariate Cox proportional hazards analysis. Covariates included characteristics potentially associated with both 25(OH)D status and AECOPD risk such as age, gender, ethnicity, FEV1 %predicted, baseline smoking status, season (Winter=January-March, Spring=April-June, Summer=July-September, Winter=October-December) and clinical center. Treatment assignment in the trial was also included as a covariate to account for the differential AECOPD risk among patients randomized to the two arms of the original trial.

A pre-specified alternate analysis using annualized rates of AECOPDs was also conducted. Rates of AECOPDs were determined by dividing the number of AECOPDs by person-years of follow-up and allowed for use of data from patients with multiple exacerbations over the one year of follow-up time. While the optimal analytic method of such data are not fully agreed upon22, 23 we analyzed
the relationship between baseline 25(OH)D and AECOPD rates using both Poisson and negative binomial analyses.

We also explored relationships between five 25(OH)D strata and AECOPD data, analyzing both time to first AECOPD using log-rank testing and annualized rates of AECOPDs using analysis of variance (ANOVA).

Our sample size of 973 patients (of whom 63% experienced an AECOPD) provided 84% power with a two-tailed alpha error rate of 0.05 to detect a hazard ratio of 1.10 for a 10-unit change in 25(OH)D and time to first AECOPD.

Statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

Among 1142 participants in the trial, 25 (2.2%) had no follow-up after the baseline visit and were not included in this analysis. AECOPD data were therefore available for 1117 participants. Of these participants, 973 (87%) had viable plasma samples available for this secondary analysis of the relationship between baseline 25(OH)D and subsequent AECOPDs.

The demographic data of the trial participants are provided in Table 1, both for those with and without plasma samples available for 25(OH)D analysis.
Mean 25(OH)D was 25.7 ± 12.8 ng/mL. 33.1% were Vitamin D insufficient (defined as ≥20 ng/mL but <30 ng/mL by widely used criteria\textsuperscript{24}), 32.0% of participants were Vitamin D deficient (<20 ng/mL) and 8.4% of participants had severe vitamin D deficiency (<10 ng/mL). 25(OH)D levels were related to the season of the year in which the blood was drawn (ANOVA \( p = 0.009 \)), with summer levels averaging the highest (27.3 ± 10.7 ng/ml) and winter levels the lowest (24.1 ± 15.7 ng/ml) (Figure 1). There was a significant ethnic difference in 25(OH)D levels, with lower mean levels in African-Americans (17.6 ± 10.2) and Hispanics (19.1 ± 9.7), compared to Caucasians (27.2 ± 12.6) and other ethnicity patients (25.1 ± 14.7) (ANOVA \( p<0.001 \); Figure 1). There was no significant association between clinical center and 25(OH)D levels (ANOVA \( p=0.254 \); Figure 1). Low 25(OH)D levels were associated with worse baseline health status as assessed by both Medical Outcomes Study 36-item Short Form (SF-36) and St. George’s Respiratory Questionnaire (SGRQ) data (see online data supplement).

These 973 participants experienced 1415 AECOPDs over one year. 360 (37%) remained AECOPD-free over one year, 278 (29%) had one AECOPD, 133 (14%) had two AECOPDs, and 202 (21%) had 3 or more AECOPDs.

In the primary analysis, 25(OH)D had no relationship to time to first AECOPD; for a 10 ng/mL increment in 25(OH)D, the estimated hazard ratio was 1.04, 95% confidence interval: 0.97-1.12. In the secondary statistical analyses, 25(OH)D
had no relationship to annualized rates of AECOPDs in either Poisson (p=0.82) or negative binomial analyses (p=0.87).

An analysis of AECOPD rates stratified by 25(OH)D levels showed that participants with severe vitamin D deficiency (<10 ng/mL) had a higher mean rate of AECOPDs, but this difference was not statistically significant (Table 2). When using time-to-event analysis, patients with severe vitamin D deficiency did not exhibit any faster time to first AECOPD. In fact, patients in the highest 25(OH)D stratum had the shortest time to first AECOPD, but this difference did not reach statistical significance.

There was no evidence in the data of interactions between the randomized treatment assignment (azithromycin vs. placebo) and baseline 25(OH)D levels as predictors of time to first exacerbation or rates exacerbations per person-year (p-values for interaction >0.50 for both time to first exacerbation and rates of exacerbations; analyses not shown).

**Discussion**

While some data suggest that low 25(OH)D levels are associated with an increased risk of respiratory infections, we found no relationship between baseline 25(OH)D levels and time to first AECOPD. The primary outcome used in this analysis was time to first AECOPD, but when we analyzed the data using
exacerbation rates, the consistent result was that baseline 25(OH)D had no relationship to AECOPD.

Based on these main results, we sought to explore a secondary hypothesis that the subgroup with severe vitamin D deficiency (25(OH)D <10 ng/mL) might be particularly vulnerable to AECOPD. In this post-hoc analysis, there was no statistically significant difference across the strata. Mean AECOPD rate was highest in the low 25(OH)D group, but median time to AECOPD was shortest in the high 25(OH)D group. Neither of these differences were statistically significant, and we stress the post-hoc nature of these stratified analyses.

The main strengths of our study include its large sample size (the largest to date regarding 25(OH)D levels in a COPD-specific cohort), use of an experienced laboratory to perform the 25(OH)D assays, a wide distribution of 25(OH)D levels, and the standardized, prospective collection of AECOPD data in the context of a clinical trial using AECOPD as its primary outcome. However, there are certain limitations to our data.

Our study was restricted to a single baseline assessment of 25(OH)D levels. Therefore, we can not exclude the possibility that 25(OH)D levels may have changed after our baseline measures. While we feel it is unlikely that many patients began Vitamin D supplementation during the trial, we certainly cannot
exclude such a possibility, especially in light of the popular media's recent attention on Vitamin D.

25(OH)D levels are also known to vary by season, so we can not exclude the possibility that individual subjects experienced subsequent seasonal changes in 25(OH)D levels after our baseline measurement. While our analysis showed a statistically significant difference between mean 25(OH)D levels drawn in summer versus winter, the magnitude of the summer versus winter seasonal difference was only 4.1 ng/mL. This difference is much smaller than the 14.2 ng/mL seasonal difference reported in a sample of 24 UK COPD patients\(^ {25} \) and the 13.4 ng/mL seasonal difference we reported in a sample of 198 Lung Health Study 3 (LHS 3) participants.\(^ {26} \) We used the same laboratory and the same liquid chromatography tandem mass spectroscopy methodology for 25(OH)D assays in both the previous LHS 3 study and the current study, so differences in analytic techniques would not explain the difference in seasonal effects. One potential explanation is that our previous LHS 3 sample was a group of patients with relatively mild COPD (mean FEV\(_1\) of 72% of predicted), while patients in this current study had much more severe COPD (mean FEV\(_1\) of 40% of predicted). FEV\(_1\) % predicted was not provided for the 24 patients in the UK study, but a mean FEV\(_1\) of approximately 1.2 L suggests they also had severe COPD. One important note is that participants in the current study were selected for particularly high risk of AECOPDs (more than 80% experienced an AECOPD in the year prior to enrollment and there were 1415 exacerbations during follow-up
in these 973 patients), so they represent the most severely ill COPD patients. As such, these participants may have had more ambulatory limitation than the participants in the LHS 3 or UK studies. Ambulatory limitation can limit sunlight exposure, leading to blunting of typical seasonal effects on 25(OH)D levels.

Study participants had a wide distribution of 25(OH)D levels across the spectrum of “normal”, “insufficient”, and “deficient”, but we note that these definitions have largely been defined on the basis of bone disease and suppression of parathyroid hormone levels.\textsuperscript{24} For non-skeletal effects of 25(OH)D such as prevention of respiratory infections or AECOPDs, we acknowledge that the threshold levels for benefits are unknown. Hypothetically, if the threshold 25(OH)D level to prevent AECOPDs is >50 ng/mL, we only had 23 patients in this supra-normal range so our study was not powered to address relationships between supra-normal 25(OH)D and AECOPDs. Due to the rare finding of naturally-occurring supra-normal 25(OH)D levels, observational studies to explore this question would require very large cohorts to achieve adequate power. Patients with levels this high also tend to achieve such levels through vitamin D supplementation, thus also introducing confounders such as health behaviors associated with vitamin use. Therefore, randomized, controlled trials would likely be the only way to test the hypothesis that supra-normal 25(OH)D levels reduce AECOPD risk.
Our primary results contrast with similar studies conducted in patients with asthma, another obstructive lung disease associated with episodic exacerbations. A cross-sectional study of 616 asthmatic children in Costa Rica showed that low 25(OH)D levels were associated with a higher number of self-reported asthma hospitalizations in the previous year. A larger study of 1024 children with asthma used similar methodology to our study by measuring a single baseline 25(OH)D level and then assessing relationships between baseline 25(OH)D and subsequent severe asthma exacerbation data collected as part of a large randomized trial. In that study, lower baseline 25(OH)D was related to a higher hazard of time to first severe asthma exacerbation and higher odds of ever having a severe exacerbation over the course of the study. A threshold 25(OH)D level of >30 ng/mL also appeared to be associated with a reduction in the observed risk of exacerbations, suggesting that at least in asthmatics, supra-normal 25(OH)D levels may not be necessary to potentially reduce exacerbation risk.

While 25(OH)D may have an effect on respiratory exacerbation risk in patients with asthma, complex effects in COPD such as bacterial colonization, airway inflammation, systemic inflammation, oxidative stress, and impaired mucociliary clearance may have stronger effects on AECOPD risk than any effects of 25(OH)D. We must stress that our data remain observational in nature and only a randomized controlled trial (RCT) can definitively answer the question of
whether or not modification of 25(OH)D levels (easily achievable through vitamin D supplementation) can alter the risk of AECOPD.

Published results from randomized trials of vitamin D supplementation in COPD patients are currently lacking, but recent RCT data in healthy individuals together with our current observational data suggest such trials are not likely to show an effect of vitamin D supplementation on AECOPD prevention. We also note that our current data are consistent with our previous findings that 25(OH)D levels in COPD are not related to either longitudinal lung function decline nor FEV₁ responses to inhaled corticosteroids.

Conclusion

Among COPD patients at high risk of AECOPD, baseline blood 25(OH)D levels are not related to the risk of subsequent AECOPDs. The notion that vitamin D supplementation might reduce AECOPD risk is not supported by our data.

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The views expressed in this article are those of the authors and do not necessarily represent the views of the U.S. Department of Veterans Affairs, The U.S. Government, the University of Minnesota, the Mayo Clinic, nor the National Institutes of Health.
**FIGURE 1: Distribution of Vitamin D levels by season and ethnicity (upper panel) and clinical center (lower panel)**

- **25(OH)D**: 25 hydroxyvitamin D
- A. Arbor: Ann Arbor, MI
- Balt: Baltimore, MD
- Birm: Birmingham, AL
- Bost: Boston, MA
- Denv: Denver, CO
- Minn: Minnesota
- Phil: Philadelphia, PA
- Pitt: Pittsburgh, PA
- UCLA: University of California, Los Angeles
- UCSF: University of California, San Francisco
Table 1: Baseline characteristics of trial study participants, for patients included and not included in this analysis of 25(OH)D.

<table>
<thead>
<tr>
<th></th>
<th>Included in 25(OH)D analysis (n=973)</th>
<th>Not included in 25(OH)D analysis (n=144)</th>
<th>p-value*</th>
</tr>
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<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>65.4 ± 8.6</td>
<td>63.6 ± 8.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Female gender (%)</td>
<td>40.0%</td>
<td>46.5%</td>
<td>0.14</td>
</tr>
<tr>
<td>Race/Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>85.2%</td>
<td>72.2%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>African-American</td>
<td>13.8%</td>
<td>27.1%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2.5%</td>
<td>0.7%</td>
<td>0.18</td>
</tr>
<tr>
<td>Asian</td>
<td>1.0%</td>
<td>0.7%</td>
<td>0.71</td>
</tr>
<tr>
<td>Native American</td>
<td>3.1%</td>
<td>4.2%</td>
<td>0.49</td>
</tr>
<tr>
<td>More than one</td>
<td>3.0%</td>
<td>4.2%</td>
<td></td>
</tr>
<tr>
<td>Post-bronchodilator FEV\textsubscript{1}, L (mean ± SD)</td>
<td>1.12 ± 0.51</td>
<td>1.08 ± 0.49</td>
<td>0.45</td>
</tr>
<tr>
<td>Post-bronchodilator FEV\textsubscript{1}, % predicted (mean ± SD)</td>
<td>39.6 ± 15.6</td>
<td>39.3 ± 15.8</td>
<td>0.80</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC, % (mean ± SD)</td>
<td>42.7 ± 12.8</td>
<td>41.7 ± 12.5</td>
<td>0.41</td>
</tr>
<tr>
<td>GOLD category, (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.1%</td>
<td>0.0%</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>26.0%</td>
<td>27.8%</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>41.9%</td>
<td>31.3%</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>32.1%</td>
<td>41.0%</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking history, pack-years (mean ± SD)</td>
<td>58.1 ± 31.7</td>
<td>60.6 ± 34.7</td>
<td>0.39</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>20.8%</td>
<td>30.6%</td>
<td>0.01</td>
</tr>
<tr>
<td>Medication use (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhaled corticosteroids only</td>
<td>5.2%</td>
<td>4.2%</td>
<td>0.58</td>
</tr>
<tr>
<td>Long-acting muscarinic antagonists only</td>
<td>6.7%</td>
<td>7.6%</td>
<td>0.71</td>
</tr>
<tr>
<td>Long-acting β2-agonists only</td>
<td>1.6%</td>
<td>3.5%</td>
<td>0.13</td>
</tr>
<tr>
<td>Inhaled corticosteroids + Long-acting β2-agonists</td>
<td>20.8%</td>
<td>18.8%</td>
<td>0.58</td>
</tr>
<tr>
<td>Inhaled corticosteroids + Long-acting muscarinic antagonists</td>
<td>4.9%</td>
<td>2.1%</td>
<td>0.13</td>
</tr>
<tr>
<td>Long-acting β2-agonists + Long-acting muscarinic antagonists</td>
<td>4.5%</td>
<td>6.3%</td>
<td>0.36</td>
</tr>
<tr>
<td>Inhaled corticosteroids + Long-acting β2-agonists + Long-acting muscarinic antagonists</td>
<td>47.3%</td>
<td>47.2%</td>
<td>0.99</td>
</tr>
<tr>
<td>None</td>
<td>8.8%</td>
<td>10.4%</td>
<td>0.54</td>
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25(OH)D: 25 hydroxyvitamin D  
FEV\textsubscript{1}: Forced expiratory volume in one second  
FVC: Forced vital capacity  
GOLD: Global Initiative for Chronic Obstructive Lung Disease  
SD: Standard deviation  
* p-values derived from t-test for continuous data or chi-square test for categorical data; not adjusted for multiple comparisons
Table 2: Comparison of AECOPD event data, stratified by baseline 25(OH)D status. p-value for AECOPD rate derived from ANOVA testing; p-value for time to first exacerbation derived from log-rank statistic.

<table>
<thead>
<tr>
<th>25(OH)D category</th>
<th>AECOPD metric</th>
<th>Mean exacerbation rate, exacerbations/year ± SD</th>
<th>Median time to first exacerbation, days (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-9.99 ng/mL (n=82)</td>
<td>2.20 ± 5.27</td>
<td>215 (145,361)</td>
</tr>
<tr>
<td></td>
<td>10-19.99 ng/mL (n=229)</td>
<td>1.57 ± 2.02</td>
<td>235 (196,298)</td>
</tr>
<tr>
<td></td>
<td>20-29.99 ng/mL (n=322)</td>
<td>1.57 ± 1.93</td>
<td>225 (188,269)</td>
</tr>
<tr>
<td></td>
<td>30-39.99 ng/mL (n=233)</td>
<td>1.47 ± 1.80</td>
<td>242 (188,294)</td>
</tr>
<tr>
<td></td>
<td>≥ 40 ng/mL (n=107)</td>
<td>1.79 ± 1.96</td>
<td>166 (124,299)</td>
</tr>
</tbody>
</table>

25(OH)D: 25 hydroxyvitamin D
AECOPD: acute exacerbation of chronic obstructive pulmonary disease
CI: confidence interval
SD: standard deviation
References

Online data supplement for:

Vitamin D levels and risk of acute exacerbations of chronic obstructive pulmonary disease: a prospective cohort study

Ken M. Kunisaki, Dennis E. Niewoehner, John E. Connett, for the COPD Clinical Research Network

Additional Methods
We performed an exploratory cross-sectional analysis of baseline characteristics across five 25(OH)D strata, using analysis of variance (ANOVA).

Additional Results
Results are shown in Table E1. Age and ethnicity were different among the different 25(OH)D strata, with the lowest stratum (severe vitamin D deficiency) having younger patients and more non-Caucasians than the higher 25(OH)D strata. Gender, smoking, and FVC showed non-monotonic relationships.

Health status scores measured by the Medical Outcomes Study 36-item Short Form (SF-36) and St. George's Respiratory Questionnaire (SGRQ) showed significantly worse overall scores in the severe vitamin D deficiency stratum (ANOVA p<0.001 for both). A minimal clinically important difference (MCID) of SF-36 scores has not been established in patients with lung disease, but the 9.3-point difference in total SGRQ score between the highest and lowest 25(OH)D strata exceeds the widely accepted MCID for SGRQ scores of 4 points.¹ Many of the subdomain scores were also worse in study participants with low 25(OH)D. When total SF-36 and SGRQ scores were adjusted for age, gender, FEV₁ % predicted, and smoking status, statistically significant differences in total health status scores persisted across 25(OH)D strata (p=0.001 for SF-36 and p=0.002 for SGRQ).

Reference:
Table E1: Baseline patient characteristics, stratified by baseline 25(OH)D levels. p-values derived from ANOVA testing.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>25(OH)D category</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-9.99 ng/mL (n=82)</td>
<td>10-19.99 ng/mL (n=229)</td>
</tr>
<tr>
<td>Age ± SD</td>
<td>62.9 ± 8.4</td>
<td>63.6 ± 8.0</td>
</tr>
<tr>
<td>Caucasian (%)*</td>
<td>56%</td>
<td>77%</td>
</tr>
<tr>
<td>African-American (%)*</td>
<td>45%</td>
<td>22%</td>
</tr>
<tr>
<td>Hispanic (%)*</td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Gender (%male)</td>
<td>29%</td>
<td>26%</td>
</tr>
<tr>
<td>Oxygen use (%)</td>
<td>68%</td>
<td>58%</td>
</tr>
<tr>
<td>FEV₁, post-bronchodilator, liters ± SD</td>
<td>1.06 ± 0.54</td>
<td>1.09 ± 0.51</td>
</tr>
<tr>
<td>FEV₁, post-bronchodilator, %predicted ± SD</td>
<td>38.5 ± 16.1</td>
<td>37.9 ± 15.2</td>
</tr>
<tr>
<td>FVC post-bronchodilator, liters ± SD</td>
<td>2.40 ± 0.93</td>
<td>2.61 ± 0.87</td>
</tr>
<tr>
<td>FEV₁/FVC ratio ± SD</td>
<td>44.4 ± 13.3</td>
<td>41.9 ± 13.5</td>
</tr>
<tr>
<td>SF36 total score ± SD</td>
<td>390.8 ± 140.1</td>
<td>417.4 ± 143.3</td>
</tr>
<tr>
<td>SF36 vitality ± SD</td>
<td>42.1 ± 19.5</td>
<td>43.7 ± 21.2</td>
</tr>
<tr>
<td>SF36 physical function ± SD</td>
<td>28.8 ± 20.8</td>
<td>34.0 ± 23.2</td>
</tr>
<tr>
<td>SF36 bodily pain ± SD</td>
<td>67.8 ± 27.4</td>
<td>67.8 ± 27.5</td>
</tr>
<tr>
<td>SF36 general health perception ± SD</td>
<td>34.6 ± 19.4</td>
<td>36.0 ± 20.1</td>
</tr>
<tr>
<td>SF36 physical role functioning ± SD</td>
<td>26.5 ± 30.6</td>
<td>30.6 ± 37.7</td>
</tr>
<tr>
<td>SF36 emotional role functioning ± SD</td>
<td>56.5 ± 46.2</td>
<td>66.1 ± 41.4</td>
</tr>
<tr>
<td>SF36 social role functioning ± SD</td>
<td>61.6 ± 28.7</td>
<td>66.7 ± 26.5</td>
</tr>
<tr>
<td>SF36 mental health ± SD</td>
<td>72.8 ± 17.9</td>
<td>73.3 ± 17.7</td>
</tr>
<tr>
<td>SGRQ total ± SD</td>
<td>55.1 ± 14.9</td>
<td>53.7 ± 15.5</td>
</tr>
<tr>
<td>SGRQ symptom domain ± SD</td>
<td>61.0 ± 19.3</td>
<td>62.9 ± 20.7</td>
</tr>
<tr>
<td>SGRQ activity domain ± SD</td>
<td>77.2 ± 14.7</td>
<td>73.3 ± 17.2</td>
</tr>
<tr>
<td>SGRQ impact domain ± SD</td>
<td>40.5 ± 19.2</td>
<td>39.4 ± 18.7</td>
</tr>
</tbody>
</table>

25(OH)D: 25-hydroxyvitamin D
FEV₁: forced expiratory volume in one second
FVC: forced vital capacity
SD: standard deviation
SF36: Medical Outcomes Study 36-item Short Form
SGRQ: St. George's Respiratory Questionnaire
*: Ethnicity categories not mutually exclusive; participants were allowed to indicate more than one ethnicity