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# Elevated 1, 25-dihydroxyvitamin D levels are associated with protracted treatment in sarcoidosis

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**KEYWORDS**
Sarcoidosis;
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**Summary**
*Background:* Active vitamin D metabolite, 1, 25-dihydroxyvitamin D, has pleomorphic effects on both innate and acquired immunity. Sarcoid granuloma derived 1, 25-dihydroxyvitamin D leads to hypercalcemia, but the association of 1, 25-dihydroxyvitamin D with the clinical phenotype of the disease is currently unknown.
*Objective:* To determine the relationship between serum 1, 25-dihydroxyvitamin D levels and the degree of sarcoidosis disease chronicity.
*Design:* Serum 1, 25-dihydroxyvitamin D levels were measured and associated with sarcoidosis activity and phenotypes as assessed by Sarcoidosis Severity Score and Sarcoidosis Clinical Activity Classification respectively.
*Results:* Fifty nine patients were recruited with 44% having a sub-acute onset, and the chronic disease phenotype. There was no significant difference in serum 1, 25-dihydroxyvitamin D levels by chest radiograph stage ($p = 0.092$) nor did the levels correlate with the Sarcoidosis Severity Score ($r = -0.16$; $p = 0.216$). Serum 1, 25-dihydroxyvitamin D levels were associated with patients requiring repeated regimens of systemic immunosuppressive therapy or >1 year of therapy (SCAC Class 6). Increasing quartiles of serum 1, 25-dihydroxyvitamin D level was associated increased odds of the chronic phenotype (OR 1.82, 95% CI, 1.11, 2.99, $p = 0.019$). The majority (71%) of the patients with levels >51 pg/mL required chronic immunosuppressive therapy as defined by SCAC class 6.

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*Conclusions:* In patients with sarcoidosis, elevated 1, 25-dihydroxyvitamin D levels are associated with chronic treatment needs.


## Introduction

Sarcoidosis is a systemic inflammatory disease that primarily affects the lung and lymphatic system of the body. The clinical expression, natural history, and prognosis of sarcoidosis are highly variable but depend upon a Th1 lymphocytic immune reaction due to an unknown antigen(s).[1,2] The subsequent granulomatous reaction can coalesce resulting in progressive organ dysfunction.

The role of vitamin D, more specifically of its active metabolite 1, 25-dihydroxyvitamin D, has been investigated rather extensively in sarcoidosis, but mainly as an explanation for hypercalcemia seen in 2–5% patients.[3] Seasonal sun exposure, iatrogenic ultraviolet irradiation, and vitamin D supplementation provide excess 25-hydroxyvitamin D through the actions of 25-hydroxylase in the liver.[4,5] CD4+ derived interferon-gamma has been shown to stimulate macrophage 1$\alpha$-hydroxylase enzyme resulting in increased production of 1, 25-dihydroxyvitamin D, the metabolically active form of vitamin D.[6–9] Without the usual product inhibition loop to reduce activated alveolar macrophage 1$\alpha$-hydroxylase activity, excessive 1, 25-dihydroxyvitamin D production leads to increased intestinal calcium absorption (hyperabsorptive hypercalcemia), and to a lesser extent increased bone resorption, although the mechanism for the latter is less clear.[9] In addition, reduction in serum 1, 25-dihydroxyvitamin D levels by corticosteroid therapy promptly reduces serum calcium levels.[5]

Increasingly, 1, 25-dihydroxyvitamin D has been investigated as a potential immunomodulating hormone. There is a reduction of T-cell proliferation and interleukin-2 and interferon-$\gamma$ synthesis upon exposure to 1, 25-dihydroxyvitamin D3.[10–14] In addition, antigen-presenting cells (APC) become more tolerogenic with exposure to 1, 25-dihydroxyvitamin D.[15,16] Contrary to the tolerogenic effect of 1, 25-dihydroxyvitamin D on the adaptive immune system, Hansdottir et al. have shown increased local production of 1, 25-dihydroxyvitamin D by respiratory epithelial cells leading to expression of immunogenic proteins common to innate immunity.[17] The potential influence of these established immunomodulating properties of 1, 25-dihydroxyvitamin D have not been investigated in sarcoidosis. Two possibilities could exist in the relationship between 1, 25-dihydroxyvitamin D and sarcoidosis. First, 1, 25-dihydroxyvitamin D may be associated with increased sarcoidosis activity as a reflection of the overall granulomatous disease burden and subsequent 1, 25-dihydroxyvitamin D production. Alternatively, 1, 25-dihydroxyvitamin D produced by the granulomatous response may inhibit CD4+ inflammation by its immune modulating properties and thus may be associated with less severe disease. To the best of our knowledge, the relationship between serum 1, 25-dihydroxyvitamin D levels and sarcoidosis disease severity has not been investigated. Accordingly, the objectives our study was to (1) determine the correlation between serum 1, 25-dihydroxyvitamin D levels and sarcoidosis disease activity, and (2) to determine the association of serum 1, 25-dihydroxyvitamin D levels with specific sarcoidosis clinical phenotypes.

## Methods

All patients with a clinical-pathologic history of sarcoidosis according to the American Thoracic Society (ATS) consensus guidelines[1] were recruited from the pulmonary clinic at the Henry Ford Hospital between March 2004 and September 2007 a minimum of one year after diagnosis. Subjects were excluded if they were taking multivitamins or supplemental vitamin D. The consent and the study protocol were approved by the Institutional Review Board.

Enrolled patients were characterized using two rubrics. First, the Sarcoidosis Clinical Activity Classification (SCAC), which classifies patients into 6 separate phenotypes as determined by the following: a) acute versus non-acute disease onset, b) need for treatment, and c) need for >1 year of treatment[18] (Table 1).

To be included into the study and characterized by the SCAC scheme, each patient must have had at least 2 years of observed clinical care recorded in the electronic medical record. The second rubric used was the Sarcoidosis Severity Score (SSS), which summates race, percent predicted Forced Vital Capacity (FVC), need for non-corticosteroid immunosuppression, and the presence of cardiac and/or neurologic disease, to establish a continuous score of

**Table 1** Sarcoidosis Clinical Activity Classification.[a]

| SCAC Class | Phenotype[a] |
|---|---|
| 1 | Acute onset, no need for immunosuppressive therapy |
| 2 | Acute onset, one period of treatment, not longer lasting than one year |
| 3 | Acute onset, need of several periods of immunosuppressive therapy or long lasting treatment (>12 months) |
| 4 | Sub-acute onset, no need for immunosuppressive therapy |
| 5 | Sub-acute onset, one period of treatment, not longer lasting than one year |
| 6 | Sub-acute onset, need of several periods of immunosuppressive therapy or long lasting treatment (>12 months) |

[a] Sarcoidosis Clinical Activity Classification (SCAC). Prasse A, Katic C, Germann M et al. Phenotyping sarcoidosis from a pulmonary perspective. Am J Respir Crit Care Med 2008; 177:330–336.

**Table 2** Study Population.

| | African-American $n = 50$ | Caucasian $n = 9$ | |
|---|---|---|---|
| Female-no. (%) | 36 (72%) | 6 (67%) | $p = 0.708$[b] |
| Age-mean (± S.D.) | 45.8 (±9.7) | 54 (±10.8) | $p = 0.0232$[a] |
| Percent Predicted FVC-mean (S.D.) | 86.4% (±16%) | 76.0% (±15%) | $p = 0.076$[a] |
| Scadding Radiographic Class-no. (%) | | | |
| 0 | 15 (30%) | 0 (0%) | |
| 1 | 16 (32%) | 3 (33.3%) | |
| 2 | 15 (30%) | 2 (22.2%) | |
| 3 | 4 (8%) | 4 (44.4%) | $p = 0.016$[b] |
| SCAC Class-no.(%) | | | |
| 1 | 0 (0%) | 0 (0%) | |
| 2 | 0 (0%) | 0 (0%) | |
| 3 | 2 (4%) | 0 (0%) | |
| 4 | 18 (36%) | 3 (33.3%) | |
| 5 | 8 (16%) | 3 (33.3%) | |
| 6 | 22 (44%) | 3 (33.3%) | $p = 0.648$[b] |
| Lofgren's Syndrome | 3 | 0 | |
| Neurologic Disease-no. | 3 | 0 | |
| Cardiac Disease-no. | 3 | 0 | |

[a] *t*-test.
[b] fisher's test. This tests for the general association of Scadding radiographic stage or SCAC class with race.

disease severity.[19] NHANES III predicted reference equations were used to calculate the percent predicted FVC. The Scadding staging system was used for chest radiograph scoring using the chest radiograph taken closet to time of study enrollment.[20]

Serum 25-hydroxyvitamin D, the best available index of vitamin D nutrition was measured by a direct, competitive chemiluminiscent immunoassay (DiaSorin, Inc. Stillwater, MN, USA) with a notional clinical reference range of 17—80 ng/mL[21] Serum 1, 25-dihydroxyvitamin D was measured by radioimmunoassay (Cat.no. 65100E, DiaSorin, Inc. Stillwater, MN, USA) with a clinical reference range of 7—60 pg/mL using a blood sample taken at time of study enrollment. Serum angiotensin converting enzyme (ACE) level was measured by enzyme immunoassay using Olympus AU400 by spectrophotometric method. (Warde Medical Laboratory, Ann Arbor, MI, USA) with a clinical reference range of 8—52 U/L.

Statistical analysis was performed using R Statistical Package (r-project.org, Version R 2.8.1).

Descriptive statistics were used to report demographic and clinical characteristics with mean and standard deviation (S.D.) for normally distributed continuous variables median and inter-quartile (IQR) range for duration of disease at enrollment. Chi-square or Fisher's exact tests were used to determine if differences existed between race and radiographic stage, SCAC class, and gender distributions. Student's *t*-tests were used to test for the differences in continuous variables (age, percent predicted FVC, and SSS) by race. One-way analysis of variance (ANOVA) was used to test for the differences in serum 25-hydroxyvitamin D, 1, 25-dihydroxyvitamin D, and ACE levels by Scadding chest radiograph class and SCAC class. Pearson correlation coefficients were used to correlate serum 1, 25-dihydroxyvitamin D levels with the Sarcoidosis Severity Score. Logistic regression was used to investigate the association between 1, 25-dihydroxyvitamin D levels with medication treatment >1 year or repeated courses of treatment (SCAC 6). The initial analysis was a crude estimate of the association between serum 1, 25-dihydroxyvitamin D and SCAC class 6. For clinically useful interpretation, the serum 1, 25-dihydroxyvitamin D levels were further stratified into quartiles and the data was re-analyzed with an unadjusted model and a model adjusting for Scadding radiographic class, race, and current immunosuppressive medication use. This model was also utilized to test for the effect of disease duration at the time of vitamin D assessment. Scadding radiographic class was controlled for as a class variable. The results were expressed as adjusted odds ratios of the association between 1, 25-dihydroxyvitamin D quartiles with the SCAC class 6. The lowest quartile was used as the referent for significance testing. Angiotensin converting enzyme (ACE) levels were also tested for associations with SCAC class 6. The association of disease duration with continuous vitamin D levels, at the time of study enrollment, was also investigated utilizing correlation coefficients. This study was funded internally without industry support.

## Results

Sixty-two patients were recruited with 59 meeting the observation criteria of >2 year. Consistent with our clinical population in general, 85% of the study subjects were African—Americans. (Table 2) The median time since the diagnosis of sarcoidosis to the study enrollment was 35 months (IQR = 12—90 months). The small Caucasian sample had more advanced Scadding radiographic class ($p = 0.016$), but no difference in the percent predicted FVC was found when compared to African—Americans

**Table 3** A Comparison of 25-hydroxyvitamin D, 1, 25-dihydroxyvitamin D, and ACE level by Radiographic Class, Phenotypic Class, and Race.

| Comparison Groups | No of Subjects ($n=$) | 25-hydroxyvitamin D (ng/ml) (mean ± S.D.) | 1, 25-dihydroxyvitamin D (pg/ml) (mean ± S.D.) | ACE (units/L) (mean ± S.D.) |
|---|---|---|---|---|
| Scadding Radiographic Class | | | | |
| 0 | 15 | 10.53 (3.56) | 48.33 (14.71) | 45.07 (24.43) |
| 1 | 19 | 9.77 (6.84) | 36.95 (11.20) | 66.59 (52.70) |
| 2 | 17 | 11.64 (6.04) | 43.00 (9.59) | 89.53 (169.36) |
| 3 | 8 | 15.63 (9.87) | 41.00 (17.77) | 64.13 (108.26) |
| p-value[a] | | 0.190 | 0.092 | 0.702 |
| SCAC Class | | | | |
| 3 | 2 | 13.50 (7.78) | 38.00 (15.56) | 19.50 (27.58) |
| 4 | 21 | 9.90 (5.00) | 39.10 (11.27) | 66.42 (58.68) |
| 5 | 11 | 11.15 (6.82) | 38.55 (11.50) | 44.09 (38.10) |
| 6 | 25 | 12.36 (7.60) | 46.76 (14.52) | 82.28 (146.66) |
| p-value[a,b] | | 0.618 | 0.156 | 0.698 |
| Race | | | | |
| Caucasian | 9 | 21.67 (8.06) | 42.56 (16.09) | 32.89 (23.66) |
| African-American | 50 | 9.43 (4.11) | 42.14 (12.77) | 73.89 (112.27) |
| p-value[c] | | <0.001 | 0.932 | 0.283 |

[a] Analysis of Variance (ANOVA). Tests for significant differences in mean values of vitamin D levels or ACE by Scadding radiographic class or SCAC class.
[b] Additional *t*-test comparison of 1, 25-dihydroxyvitamin D3 levels for SCAC 6 (47.2 ± 14.7 pg/ml) vs. SCAC 3,4,5 (38.8 ± 11.0 pg/ml) ($p = 0.02$).
[c] *t*-test of mean vitamin D or ACE levels by race group.

($p = 0.07$). Neurologic ($n = 3$) and cardiac sarcoidosis ($n = 3$) was uncommon as was the acute onset disease as defined by the SCAC classes 1 through 3.

Association of serum vitamin D metabolite levels with race, Scadding radiographic class, and disease Severity. The mean (S.D.) serum 25-hydroxycholecalciferol level was 11.3 ± 6.6 ng/mL and the mean serum 1, 25-dihydroxyvitamin D level was 42.2 ± 13.2 pg/mL. Serum 1, 25-dihydroxyvitamin D levels were normally distributed without significant outliers as determined by visual inspection of the QQ plot and the upper extreme value (median + 3*IQR). African–Americans had a lower mean serum 25-hydroxyvitamin D level than Caucasians (9.4 vs. 21.7 ng/mL; $p < 0.001$) but similar serum 1, 25-dihydroxyvitamin D levels (42.1 vs. 42.6 pg/ml, $p = 0.93$). (Table 3).

There was no significant difference in serum levels of 1, 25-dihydroxyvitamin D when the study subjects were classified based on chest radiograph stage ($p = 0.092$) or overall SCAC class distribution ($p = 0.16$). In addition, serum 1, 25-dihydroxyvitamin D levels correlated with neither the Sarcoidosis Severity Score ($r = -0.16$; $p = 0.216$) nor serum ACE levels ($r = 0.04$, $p = 0.77$).

Association of serum 1, 25-dihydroxyvitamin D levels and SCAC 6. SCAC class 6 individuals had significantly higher mean (±S.D) serum 1, 25-dihydroxyvitamin D levels compared to those not classified as SCAC 6 (47.2 pg/ml ± 14.7 vs. 38.8 pg/ml ± 11.0 V; $p = 0.02$). Our initial model was aimed to determine if serum 1, 25-dihydroxyvitamin D levels are associated with SCAC class 6. In the unadjusted model, 1, 25-dihydroxyvitamin D was significantly associated with SCAC class 6 with every 1 unit increase in serum 1, 25-dihydroxyvitamin D level increased the odds of SCAC class 6 by 1.05 (95% CI 1.008, 1.104, $p = 0.02$).

Serum 1, 25-dihydroxyvitamin D Strata and >1 Year Treatment Needs. Given this general association and for ease of clinical interpretation, serum 1, 25-dihydroxyvitamin D levels were divided into quartiles with the resulting groups of <=33, 34 to ≤40, 41 to ≤51, and >52 pg/mL. For each 1, 25-dihydroxyvitamin D strata increase above the lowest quartile, the odds of requiring >1 year of treatment increased by 1.82 (95% CI, 1.11, 2.99; $p = 0.02$) (Fig. 1).

To control for potential confounders such as Scadding radiographic class, race, and current immunsuppressive medication an adjusted model was utilized. In the base model, serum 1, 25-dihydroxyvitamin D level was still

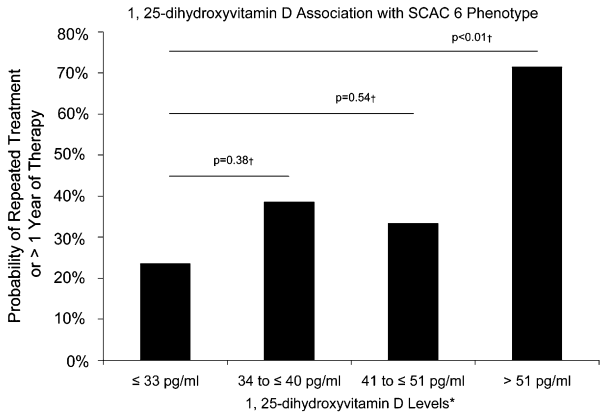


**Figure 1** 1, 25-dihydroxyvitamin D levels determined by quartiles based on the 25th percentile, median, and 75th percentile. Test for trend, *p*-value = 0.019. † Chi-square test of significance with reference level ≤ 33 pg/ml.

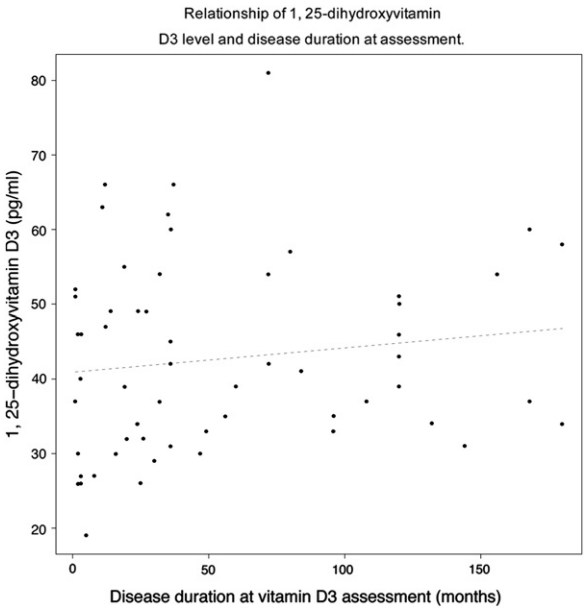


**Figure 2** Correlation of time since diagnosis and 1, 25-dihydroxyvitamin D levels.

associated with SCAC 6 after controlling for these confounders (OR 2.71, 95% CI 1.18, 6.19, $p = 0.02$). The duration of disease had no impact on the 1, 25-dihydroxyvitamin D adjusted association with SCAC 6 as the adjusted model (disease duration + base model covariates) odds ratio was not different from the base model (OR 2.81, 95% CI, 1.2, 6.59, $p = 0.02$). The highest quartile (>51 pg/mL) of 1, 25-dihydroxyvitamin D was strongly associated with SCAC class 6 (OR 8.12, 95% CI 1.62, 40.74, $p = 0.01$) when compared to the reference 1, 25-dihydroxyvitamin D level (the lowest quartile). In addition, there was no association between the duration of disease at recruitment and serum 1, 25-dihydroxyvitamin D levels ($r = 0.17$; $p = 0.21$; Fig. 2). Seventy one percent of the patients with >51 pg/mL required >1 year of therapy. Serum ACE levels were also tested for crude associations with SCAC class 6, however no significant association was found (OR 1.00, 95% CI 0.99, 1.01, 1.009, $p = 0.325$).

## Discussion

This study demonstrates that serum 1, 25-dihydroxyvitamin D level is positively associated with a more chronic, subacute onset sarcoidosis phenotype, independent of the established predictors such as race and Scadding radiographic class. This association is robust and not influenced by current medication use or duration of the disease at vitamin D assessment. In addition, we provide for the first time, descriptive disease related data utilizing the recently described Sarcoidosis Clinical Activity Classification and the Sarcoidosis Severity Score in a cohort of predominantly African–Americans.

The primary aim of our study was to determine if serum 1, 25-dihydroxyvitamin D levels are associated with sarcoidosis severity and long-term treatment needs. We have demonstrated that serum 1, 25-dihydroxyvitamin D levels are independently associated with the need for multiple treatment courses or >1 year of therapy. The highest quartile of 1, 25-dihydroxyvitamin D appears to be driving most of the association. (Fig. 1) Considering that the vitamin D metabolite levels were drawn at various time points of care and not necessarily at diagnosis, we cannot conclude with certainty that serum 1, 25-dihydroxyvitamin D levels are predictors of future disease chronicity. However, the strong association (OR 8.12, 95% CI 1.62, 40.74, $p = 0.01$) of a serum 1, 25-dihydroxyvitamin D levels >51 pg/ml with SCAC 6 suggests a discriminate ability to predict disease chronicity. Prediction of future disease chronicity would require a prospective study with assessment of serum 1, 25-dihydroxyvitamin D levels at diagnosis.

An ideal marker of sarcoidosis chronicity should be easily obtainable (such as serum or urine), not altered by current medication use, and could be assessed at any point in the clinical course. In this study, we have demonstrated that the association of serum 1, 25-dihydroxyvitamin D levels, particularly >51 pg/ml, with chronicity is not influenced by the current corticosteroid or immunosuppressant therapy (medication adjusted OR 2.71, 95% CI 1.18, 6.19, $p = 0.019$). In addition, the duration of the disease at the time of assessment did not influence the 1, 25-dihydroxyvitamin D levels. This is supported by regression modeling in which the adjusted odds ratio was not altered by the disease duration at enrollment (OR 2.71 vs. 2.81) and a lack of correlation between enrollment disease duration and serum 1, 25-dihydroxyvitamin D levels ($r = 0.17$, $p = 0.21$).

Vitamin D, more specifically 1, 25-dihydroxyvitamin D, has significant immunomodulatory properties, with known effects on antigen-presenting cells (APC) and regulatory T-cells.[22] Non-obese diabetic (NOD) mouse experiments have shown reduced expression of APC co-stimulatory receptors CD40, CD80, and CD86 and decreased production of the inflammatory IL-12 cytokine with exposure to 1, 25-dihydroxyvitamin D.[16,15] This, in addition to increased CD4+CD25+ regulatory T-cell levels, suggest 1, 25-dihydroxyvitamin D has tolerogenic effects on adaptive immunity.[16,15,23,24] Extra renal sources of 1$\alpha$-hydroxylase activity have now been described.[25] Respiratory epithelial cells have shown constitutive and inducible expression of 1$\alpha$-hydroxylase.[17] Through both active and inactive forms of vitamin D, the production of the toll like receptor (TLR) co-receptor CD14 and cathelicidin are increased, suggesting an accentuated innate immune response. At the level of global disease, our data suggest that elevated 1, 25-dihydroxyvitamin D levels are associated with a more chronic disease course, reflecting ongoing cytokine release, rather than the tolerogenic effects of vitamin D.

In our study population of predominantly African–Americans (85%), serum 25-hydroxyvitamin D levels were significantly lower in African–Americans compared to Caucasians (9.4 vs. 21.7 ng/mL, $p < 0.001$) consistent with the known higher prevalence of vitamin D deficiency in the United States.[26–28] In addition to a lower daily intake of vitamin D, dark skin, insufficient sunlight exposure in northern latitudes, and genetic variations in the vitamin D binding protein gene are reported explanations for these

findings.[29,30] Racial differences in vitamin supplementation are not likely explanation for differences in serum 25-hydroxyvitamin D levels between African—Americans and Caucasians since we excluded subjects who took vitamin D supplementation.

Angiotensin converting enzyme levels have been investigated as a simple serum measure of sarcoidosis activity. Our study is the first to determine the association of ACE level with a validated measure of sarcoidosis severity (SSS rubric). We found no association of ACE levels with global disease severity (SSS) or with a chronic phenotype (SCAC class 6).

We used the SSS described by Wasfi et al., which quantifies commonly available data by the use of model weighting to generate a continuous severity score. In our study, serum 1, 25-dihydroxyvitamin D levels did not correlate with SSS ($r = -0.16$, $p = 0.22$).[19] This may be explained by a lack of correlation between serum 1, 25-dihydroxyvitamin D levels or Sarcoidosis Severity Score, or both with granuloma burden. In addition, the SSS score is heavily weighted by the presence of central nervous system or cardiac disease involvement, both of which may not require a heavy granuloma burden to cause a chronic, symptomatic disease course or elevate the SSS.

Although our study suggests that elevated 1, 25-dihydroxyvitamin D levels are associated with a more chronic sarcoid phenotype, this may not be simply due to a higher granulomatous burden. We found no association of serum 1, 25-dihydroxyvitamin D levels with serum ACE levels, the radiographic presence of parenchymal lung disease, or the Sarcoidosis Severity Score. In addition, serum 1, 25-dihydroxyvitamin D levels were not different by Scadding radiography classification or by race, the two factors we have previously associated with increased lung granuloma density.[31] The influence of interferon-$\gamma$ in sarcoidosis and its effects on the conversion of 25-hydroxyvitamin D to 1, 25-dihydroxyvitamin D production, could provide a partial explanation for our observations. Serum 1, 25-dihydroxyvitamin D levels are tightly regulated by product inhibition of 1$\alpha$-hydroxylase enzyme activity and accelerated metabolic clearance rate of 1, 25-dihydroxyvitamin D by 24-hydroxylase.[32] Interferon-$\gamma$ has been shown to increase the production of 1, 25-dihydroxyvitamin D, induce resistance to 1, 25-dihydroxyvitamin D in the autocrine feedback loop, and reduce expression of 24-hydroxylase mRNA expression in activated macrophages.[32,9] The presence of interferon-$\gamma$ in the ongoing inflammatory milieu of patients with persistent sarcoidosis could explain the higher serum 1, 25-dihydroxyvitamin D levels in this group (SCAC 6).

Sarcoidosis severity at diagnosis and the disease course are different between race. African—Americans have more advanced disease at the time of diagnosis based on forced vital capacity, chest radiographic stage, extra-thoracic involvement, and lung granuloma density compared to Caucasians.[3,31,33,34] In addition, African—Americans are more likely to have a chronic disease course with a lower frequency of disease remission and are more likely to develop new organ involvement.[33,3] Understanding the mean values and distribution of the SSS and SCAC among a large cohort of African—Americans is crucial in the design and sample size estimates for future investigations. We provide these estimates as the earlier studies either had low enrollment of African—Americans or did not provide the ethnicity of the cohort.[18,19]

## Limitations

Considering the cross-sectional nature of our study, many subjects were on immunosuppressive therapy at the time of vitamin D metabolite measurements. Since corticosteroids, are known to reduce serum levels of 1, 25-dihydroxyvitamin D, our study design would have underestimated the association between 1, 25-dihydroxyvitamin D and SCAC 6.[9] Our analysis adjusted for medication use found a stronger association when controlled for current immunosuppressive therapy (OR 1.7 vs. 2.7).

Our study found that serum 1, 25-dihydroxyvitamin D level is positively associated with persistent or recurrent medication therapy for sarcoidosis. One possible explanation for this association is that the highest level of 1, 25-dihydroxyvitamin D leads to hypercalcemia, which is the driver of long-term medication use (SCAC 6) rather than chronic systemic or pulmonary disease. However, no enrolled patient with a serum 1, 25-dihydroxyvitamin $D > 51$ pg/mL and SCAC class 6 ($n = 10$) had a serum Ca $>10.3$ mg/dl at any time during the time of their observation nor had a previous treatment indication of hypercalcemia.

By the nature of our study design where a two year observation period was required for patient phenotyping,[18] patients with limited follow-up such as a Lofgren's sarcoidosis phenotype or asymptomatic Scadding stage 0 or I presentation were excluded. However, since our main study objective was to investigate whether serum 1, 25-dihydroxyvitamin D levels were associated with chronic sarcoidosis that requires more aggressive treatment, exclusion of self-limiting disease from our study population was not a limiting factor in testing our hypothesis.

## Conclusion

Patients with elevated serum 1, 25-dihydroxyvitamin D levels are likely to have more protracted treatment needs for their sarcoidosis, independent of conventional predictors such as race and Scadding radiographic patterns. Serum 1, 25-dihydroxyvitamin D should be assessed as a predictor of future disease chronicity in a prospective study.

## Conflict of interest statement

The authors have no competing relationships with regards to employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. The study was funded within the Division of Pulmonary and Critical Care Medicine, Henry Ford Health System.

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