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ORIGINAL REPORT

Vitamin D Insufficiency and Prognosis in Non-Hodgkin's Lymphoma

Matthew T. Drake, Matthew J. Maurer, Brian K. Link, Thomas M. Habermann, Stephen M. Ansell, Ivana N. Micallef, Jennifer L. Kelly, William R. Macon, Grzegorz S. Nowakowski, David J. Inwards, Patrick B. Johnston, Ravinder J. Singh, Cristine Allmer, Susan L. Slager, George J. Weiner, Thomas E. Witzig, and James R. Cerhan

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From the College of Medicine, Mayo Clinic, Rochester, MN; the College of Medicine, University of Iowa, IA City, IA; and the University of Rochester, Rochester, NY.

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Corresponding author: James R. Cerhan, MD, PhD, Division of Epidemiology, College of Medicine, Mayo Clinic, 200 1st ST SW, Rochester, MN 55905; e-mail: cerhan.james@mayo.edu.

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ABSTRACT

Purpose
Vitamin D insufficiency is common in the United States, with low levels linked in some studies to higher cancer incidence, including non-Hodgkin's lymphoma (NHL). Recent data also suggest that vitamin D insufficiency is related to inferior prognosis in some cancers, although there are no data for NHL.

Patients and Methods
We tested the hypothesis that circulating 25-hydroxyvitamin D [25(OH)D] levels are predictive of event-free survival (EFS) and overall survival (OS) in a prospective cohort of 983 newly diagnosed patients with NHL. 25(OH)D and 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] levels were measured by liquid chromatography-tandem mass spectrometry.

Results
Mean age at diagnosis was 62 years (range, 19 to 94 years); 44% of patients had insufficient 25(OH)D levels (< 25 ng/mL) within 120 days of diagnosis. Median follow-up was 34.8 months; 404 events and 193 deaths (168 from lymphoma) occurred. After adjusting for known prognostic factors and treatment, 25(OH)D insufficient patients with diffuse large B-cell lymphoma (DLBCL) had inferior EFS (hazard ratio [HR], 1.41; 95% CI, 0.98 to 2.04) and OS (HR, 1.99; 95% CI, 1.27 to 3.13); 25(OH)D insufficient patients with T-cell lymphoma also had inferior EFS (HR, 1.94; 95% CI, 1.04 to 3.61) and OS (HR, 2.38; 95% CI, 1.04 to 5.41). There were no associations with EFS for the other NHL subtypes. Among patients with DLBCL and T-cell lymphoma, higher $1,25(OH)_2D$ levels were associated with better EFS and OS, suggesting that any putative tumor 1-α-hydroxylase activity did not explain the 25(OH)D associations.

Conclusion
25(OH)D insufficiency was associated with inferior EFS and OS in DLBCL and T-cell lymphoma. Whether normalizing vitamin D levels in these patients improves outcomes will require testing in future trials.

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INTRODUCTION

Vitamin D insufficiency is common in the United States. Multiple studies have demonstrated that 25% to 50% of patients seen in clinical practice have vitamin D levels below the optimal range.[1-3] Vitamin D is obtained either from skin exposure to ultraviolet B radiation in the form of sunlight, or through dietary sources including supplementation. Serum levels of 25-hydroxyvitamin D [25(OH)D] reflect whole body vitamin D stores, and are used to assess individual adequacy or insufficiency. 25(OH)D is converted to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], considered the physiologically active form of vitamin D, via the action of 1-α-hydroxylase. While much of this conversion occurs in the kidney, multiple other tissues (including lymphoma tumor cells) also have 1-α-hydroxylase activity, and can thus regulate $1,25(OH)_2D$ levels at the local tissue level. Once formed, $1,25(OH)_2D$ exerts its effects through binding to the vitamin D nuclear transcription factor receptor, where it may regulate the expression of nearly 200 genes.[4]

Although the central role of vitamin D in maintaining serum calcium and skeletal homeostasis has long been appreciated, much recent work has demonstrated that vitamin D also has pleiotropic effects on cellular differentiation, proliferation, apoptosis,

and angiogenesis.[5] Intriguingly, several reports now suggest low serum 25(OH)D levels may be associated with increased cancer incidence. At present, the strongest data for an inverse association between circulating vitamin D levels and malignancy exists for studies that have examined patients with colorectal[6,7] and breast[8,9] cancer. While there is little evidence from case-control studies that dietary intake of vitamin D is associated with non-Hodgkin's lymphoma (NHL) risk,[10] a pooled analysis of 10 such studies found that higher levels of recreational sun exposure were associated with lower risk of NHL.[11] Furthermore, data from two prospective cohort studies[12,13] provide suggestive evidence that low serum 25(OH)D levels are associated with increased risk of NHL incidence.

In addition to risk of developing malignancy, there are recent data that suggest low 25(OH)D levels at diagnosis may be associated with poorer prognosis in colorectal[14] and breast[15] cancer, as well as multiple myeloma.[16] To our knowledge, however, there are no data on NHL prognosis except for a small study of 24 patients which reported that alfacalcidol (a synthetic analog of $1,25(OH)_2D$) induced regression in follicular, small cleaved cell lymphoma.[17] To test the hypothesis that vitamin D levels are predictive of event-free and overall survival in patients with newly diagnosed NHL, we examined the prognostic effects of circulating 25(OH)D levels in a prospective cohort of consecutively enrolled patients with newly diagnosed NHL.

PATIENTS AND METHODS

Study Population

This study was reviewed and approved by the human subjects institutional review board at the Mayo Clinic and the University of Iowa, and written informed consent was obtained from all participants. All subjects in this analysis were from the Molecular Epidemiology Resource of the University of Iowa/Mayo Clinic Lymphoma Specialized Program of Research Excellence.[18] Since September 2002, we offered enrollment to consecutive, newly diagnosed patients with NHL (within 9 months) who were evaluated at Mayo Clinic Rochester and the University of Iowa, were age 18 years or older, and were a resident of the United States. Exclusion criteria included known HIV infection and unwillingness or inability to provide written informed consent.

All pathology was reviewed by a lymphoma hematopathologist to verify the diagnosis and to classify each case according to the WHO classification.[19] We grouped the subtypes for analysis into diffuse large B-cell (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL), post-FL (consisting of marginal zone and lymphoplasmacytic lymphoma), and T-cell lymphoma (TCL; consisting of peripheral TCL, anaplastic large cell lymphoma, cutaneous TCL, and TCL not otherwise specified [NOS]), as well as all other NHL.

Baseline clinical, laboratory, and treatment data were abstracted from medical records using a standard protocol. Participants provided a peripheral blood sample for serum and DNA banking. Timing of the sample collection with respect to treatment (ie, pretreatment, during treatment, or post-treatment) was recorded. All patients were systematically observed every 6 months for the first 3 years, and then annually thereafter. Disease progression, retreatment, and deaths were verified through medical record review. We also verified patients' reports of no disease progression on an annual basis against their physician's report. For decedents, we obtained a copy of the death certificate as well as medical records associated with death. Study physicians assigned a cause of death using definitions developed for the Eastern Cooperative Oncology Group (ECOG) Intergroup trial 4494.[20]

Vitamin D Measurements

We defined vitamin D insufficiency as a serum 25(OH)D level lower than 25 ng/mL (62.5 nmol/L). Although consensus guidelines for the diagnosis of vitamin D insufficiency have not been established, this is an accepted level for the establishment of hypovitaminosis D, and is the current threshold used

Table 1. Demographic and Clinical Correlates of Vitamin D Levels and Prevalence of 25-Hydroxyvitamin D Insufficiency

Covariate	No.	25-Hydroxyvitamin D Levels (ng/mL) Mean	SD	25-Hydroxyvitamin D Insufficient No.	%	P*
Timing of serum draw, < 120 days of diagnosis						
Pretreatment	649	27.4	10.4	265	40.8	.002
During or post-treatment	334	24.8	11.1	171	51.4	
Sex						
Male	540	26.3	10.2	242	44.8	.75
Female	443	26.7	11.3	194	43.8	
Age, years						
≤ 60	458	26.4	10.7	204	44.5	.91
61+	525	26.5	10.8	232	44.2	
Residence at diagnosis						
MN, IA, IL, WI, ND, SD	874	26.2	10.6	399	45.7	.02
Outside six-state region	109	29.0	11.3	37	33.9	
Month of diagnosis						
March-May	212	25.0	10.1	110	51.9	.02
June-August	249	28.3	10.1	94	37.8	
September-November	265	27.0	11.5	114	43.0	
December-February	257	25.5	10.8	118	45.9	
Performance status						
0 or 1	845	27.7	10.3	336	39.8	< .001
> 1	137	18.8	10.3	100	73.0	
Subtype†						
DLBCL	370	24.7	10.7	192	51.9	.02
TCL	70	23.2	11.8	40	57.1	
MCL	71	27.0	9.1	26	36.6	
FL	285	28.2	10.3	110	38.6	
Post-FL	109	28.8	11.0	41	37.6	
All other	78	28.1	10.5	27	34.6	
IPI for DLBCL only						
0-1	135	25.9	9.7	62	45.9	.07
2	97	25.6	11.0	50	51.6	
3	81	24.9	11.2	42	51.9	
4 or 5	57	19.9	10.7	38	66.7	
IPI for TCL only						
0-1	23	30.2	10.7	9	39.1	.17
2	18	20.5	10.5	11	61.1	
3	14	19.9	10.3	9	64.3	
4 or 5	15	18.9	12.5	11	73.3	
FLIPI for FL only						
0-1	127	28.5	10.2	48	37.8	.03
2	88	27.7	10.9	38	43.2	
3	50	30.3	9.5	12	24.0	
4-5	20	23.7	9.5	12	60.0	
MIPI for MCL only						
0-3 (low risk)	58	27.7	8.5	18	31.0	.04
4-12 (interm/high risk)	13	23.9	11.2	8	61.5	

Abbreviations: DLBCL, diffuse large B-cell lymphoma; TCL, T-cell lymphoma; MCL, mantle cell lymphoma; FL, follicular lymphoma; IPI, International Prognostic Index; FLIPI, Follicular Lymphoma International Prognostic Index; MIPI, Mantle Cell International Prognostic Index; PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; CTCL, cutaneous T-cell lymphoma; ALCL, anaplastic large-cell lymphoma; MZL, marginal-zone lymphoma; LPL, lymphoplasmacytic lymphoma; NHL, non-Hodgkin's lymphoma; BL, Burtkitt's lymphoma.

*P value from χ^2 test of vitamin D sufficient v insufficient.

†TCL includes PTCL NOS (n = 25), CTCL (n = 9), ALCL (n = 7), and miscellaneous (n = 29); Post-FL includes splenic MZL (n = 13), extranodal MZL (n = 73), nodal MZL (n = 6), and LPL (n = 17); and other NHL includes BL (n = 8), composite NHL (n = 10), other B-cell NOS (n = 51), and miscellaneous (n = 9).

by Mayo Medical Laboratories (http://www.mayomedicallaboratories.com). In order to avoid assay variability, which can significantly confound vitamin D determinations made using radioimmunoassay methods,[21] all vitamin D measurements were made by liquid chromatography-tandem mass spectrometry (LC-MS/MS). A single vitamin D measurement has been shown to be highly reproducible.[22,23]

Measurements of 25(OH)D were made by deuterated stable isotope [d_6-25(OH)D]-dilution LC-MS/MS on an API 4000 instrument (Applied Biosystems, Forest City, CA), with sample introduction performed by a cohesive four-channel multiplexed system (Thermo-Fisher, Waltham, MA). Calibration utilized a 6-point standard curve over a concentration range of 0 to 200 ng/mL. Each subject's total 25(OH)D was assessed as the additive sum of the $25(OH)D_2$ and $25(OH)D_3$ components. Intra- and interassay coefficients of variation were all lower than 7% (Appendix Table A1, online only). All 983 samples were successfully assayed. For patients with DLBCL and TCL, we also measured $1,25(OH)_2D$, which was the sum of each subject's $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ as determined by isotope-dilution LC-MS/MS on an API 5000 instrument (Applied Biosystems) using deuterated internal standards for each analyte. Intra- and interassay coefficients of variation are reported in Appendix Table A1.

Statistical Analysis

χ^2 and Fisher's exact tests, where appropriate, were used to assess the association of 25(OH)D insufficiency and clinical and demographic factors. All survival analyses were analyzed within the following grouped subtypes: DLBCL, FL, post-FL, MCL, TCL, and all other NHL. Event-free survival (EFS) was defined as the time from diagnosis to disease progression, re-treatment, or death due to any cause. Lymphoma-specific survival (LSS) was defined as the time from diagnosis to death due to disease, and overall survival (OS) was defined as the time from diagnosis to death due to any cause. Patients without an event or death were censored at time of last known follow-up. Kaplan-Meier[24] curves and Cox proportional hazards regression models[25] were used to assess the association of vitamin D levels and outcome. Cox models were adjusted for subtype specific prognostic factors. For DLBCL, this included the International Prognostic Index (IPI)[26] and treatment (defined as immunochemotherapy *v* other); for FL, this included the FLIPI score[27] and grade 3 FL; for TCL, this included the IPI[26]; for MCL, this included the mantle cell IPI[28]; for post-FL, this included stage and performance status (PS); and for all other NHL, this included stage and PS. We also analyzed the associations using the actual, continuously distributed 25(OH)D levels values via penalized smoothing splines, or P-splines.[29] Briefly, this is a nonparametric modeling approach that is a generalization of polynomial splines. It allowed us to examine the unrestricted association of the 25(OH)D levels with EFS and OS, without regard to functional form. For DLBCL and TCL, we further evaluated $1,25(OH)_2D$ levels (categorized into quartiles based on histology-specific distribution) with the three outcomes, overall and stratified by 25(OH)D levels (insufficient, sufficient). Analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC) and R (http://www.r-project.org/).

RESULTS

From September 2002 through February 2008, 1,691 patients with NHL were enrolled onto the study; this does not include chronic lymphocyte leukemia/small lymphocyte leukemia, which is being reported elsewhere. Of the 1,691 patients, 701 were excluded because they did not have a serum sample available and/or drawn within 120 days of diagnosis and seven were excluded for missing clinical or outcome data, leaving a total of 983 subjects available for analysis.

Of the 983 patients with NHL, the mean age at diagnosis was 62 years (range, 19 to 94 years) and 55% were male. Overall, 56 patients (5.7%) had severe insufficiency (< 10 ng/mL), 380

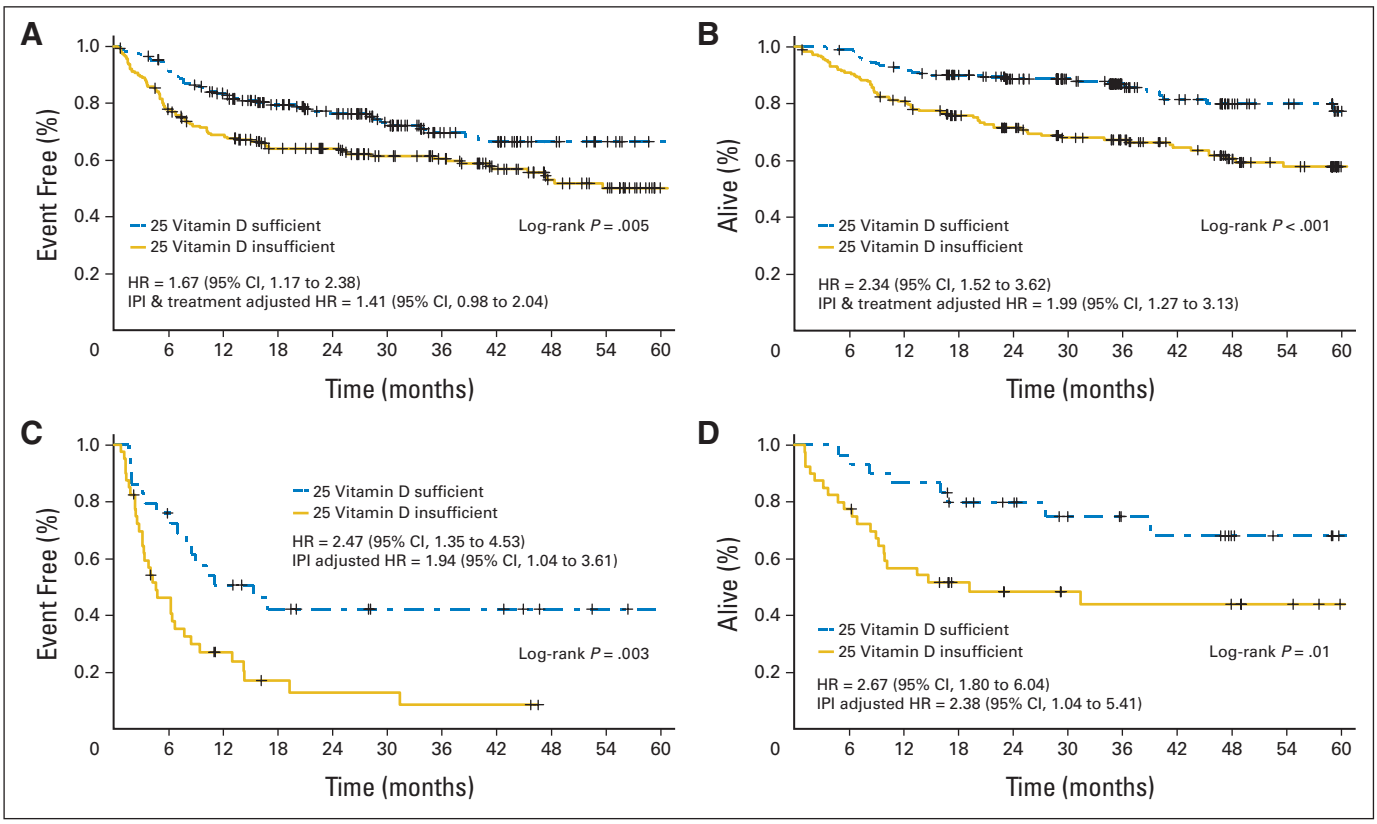

Fig 1. Kaplan-Meier curves for 25-hydroxyvitamin D deficiency and (A) diffuse large B-cell (DLBCL) event-free survival (EFS), (B) DLBCL overall survival (OS), (C) T-cell lymphoma (TCL) EFS and (D) TCL OS. HR, hazard ratio; IPI, International Prognostic Index.

Table 2. Multivariable-Adjusted Hazard Ratios for 25-Hydroxyvitamin Insufficiency and Event-Free, Lymphoma-Specific, and Overall Survival

Variable	No.	Distribution (%)	Adjustment Factor(s)	Event-Free Survival				Lymphoma-Specific Survival				Overall Survival			
				No. of Events	% Events	HR	95% CI	No. of Events	% Events	HR	95% CI	No. of Events	% Events	HR	95% CI
DLBCL			IPI, treatment*												
Sufficient	178	48.1		50	28.1	1.00	Reference	27	15.2	1.00	Reference	31	17.4	1.00	Reference
Insufficient	192	51.9		82	42.7	1.41	0.98 to 2.04	63	32.8	2.16	1.33 to 3.51	69	35.9	1.99	1.27 to 3.13
P						.07				.002				.003	
TCL			IPI												
Sufficient	30	42.9		16	53.3	1.00	Reference	8	26.7	1.00	Reference	8	26.7	1.00	Reference
Insufficient	40	57.1		33	82.5	1.94	1.04 to 3.61	20	50.0	2.26	0.99 to 5.17	21	52.5	2.38	1.04 to 5.41
P						.04				.05				.04	
MCL			MIPI												
Sufficient	45	63.4		27	60.0	1.00	Reference	11	24.4	1.00	Reference	11	24.4	1.00	Reference
Insufficient	26	36.6		18	69.2	1.09	0.59 to 2.01	8	30.8	1.35	0.53 to 3.39	8	30.8	1.35	0.53 to 3.39
P						.78				.53				.53	
FL			FLIPI, FLIII, treatment†												
Sufficient	175	61.4		65	37.1	1.00	Reference	5	2.9	1.00	Reference	10	5.7	1.00	Reference
Insufficient	110	38.6		39	35.5	1.07	0.71 to 1.62	4	3.6	0.90	0.23 to 3.49	9	8.2	1.52	0.60 to 3.88
P						.75				.88				.38	
Post-FL			Stage, PS												
Sufficient	68	62.4		25	36.8	1.00	Reference	4	5.9	1.00	Reference	4	5.9	1.00	Reference
Insufficient	41	37.6		14	34.2	0.98	0.51 to 1.89	4	9.8	2.76	0.58 to 13.1	4	9.8	2.76	0.58 to 13.1
P						.95				.20				.20	
All other			Stage, PS												
Sufficient	51	63.0		21	41.2	1.00	Reference	7	13.7	1.00	Reference	8	15.7	1.00	Reference
Insufficient	27	27.0		14	51.9	1.15	0.57 to 2.32	7	25.9	1.73	0.58 to 5.17	10	37.0	2.08	0.79 to 5.49
P						.71				.33				.14	

Abbreviations: HR, hazard ratio; DLBCL, diffuse large B-cell lymphoma; IPI, International Prognostic Index; TCL, T-cell lymphoma; MCL, mantle cell lymphoma; MIPI, Mantle Cell International Prognostic Index; FL, follicular lymphoma; FLIPI, Follicular Lymphoma International Prognostic Index; FLIII, follicular lymphoma grade 3; PS, performance status.

*Immunochemotherapy *v* all other therapy.

†Rituximab-based therapy, other chemotherapy *v* observation.

(38.7%) had mild to moderate insufficiency (10 to 24 ng/mL), and 547 (55.6%) were in the optimal range (25 to 80 ng/mL) for 25(OH)D; 436 (44%) were classified as 25(OH)D insufficient (combination of severe and mild to moderate insufficiency). 25(OH)D insufficiency was not correlated with age or sex, but was positively correlated with having a serum drawn during or post-treatment; residence in the six state region of the upper Midwest; diagnosis from March through May; PS higher than 1; and DLBCL or TCL subtypes (Table 1). Subtype-specific prognostic indices were also positively correlated with 25(OH)D insufficiency (Table 1), and this was most strongly driven by performance status within each of the subtypes (Appendix Table A2, online only). Disease stage was not correlated with 25(OH)D insufficiency for DLBCL, FL, or post-FL, but higher disease stage was correlated with 25(OH)D insufficiency in TCL (Appendix Table A2).

During a median follow-up of 34.8 months (range, 0.5 to 77 months), there were 404 events and 193 deaths, of which 168 were attributed to lymphoma. For DLBCL, 52% of the patients were 25(OH)D insufficient, and as shown in Figure 1, insufficient patients had inferior EFS (log-rank $P = .005$) and OS (log-rank $P < .001$); details on univariate HRs for all outcomes are available in Appendix Table A3 (online only). After adjusting for the IPI and treatment (Table 2), 25(OH)D insufficient patients continued to have inferior EFS (HR, 1.41; 95% CI, 0.98 to 2.04), LSS (HR, 2.16; 95% CI, 1.33 to 3.51) and OS (HR, 1.99; 95% CI, 1.27 to 3.13). The association of 25(OH)D levels with EFS (Fig 2A) and OS (Fig 2B) was mainly observed over the range of 15 to 25 ng/mL, and was relatively flat above 30 ng/mL.

For TCL (which included PTCL and CTCL, see Table 1 for details), 57% of the patients were 25(OH)D insufficient, and insufficient patients had inferior EFS (log-rank $P = .003$) and OS (log-rank $P = .01$). After adjustment for IPI (Table 2), 25(OH)D insufficient patients continued to have inferior EFS (HR, 1.94; 95% CI, 1.04 to 3.61), LSS (HR, 2.26; 95% CI, 0.99 to 5.17) and OS (HR, 2.38; 95% CI, 1.04 to 5.41). Inferior EFS (Fig 2C) and OS (Fig 2D) was observed among TCL patients in both the insufficient (< 25 ng/mL) and the lower end of the optimal range (25 to 80 ng/mL) of 25(OH)D levels.

The prevalence of 25(OH)D insufficiency for the remaining subtypes in Table 2 ranged from 27% to 39%, and there were no associations of 25(OH)D insufficiency with EFS. However, with the exception of LSS in FL, HRs for LSS and OS for the remaining subtypes were all above 1, although the confidence intervals were wide, reflecting the small number of deaths in these subtypes.

Further adjustment of results in Table 1 for season of diagnosis or residence in the upper six Midwest states did not materially alter the associations ($< 10\%$ change in HRs) reported in Table 2 (data not shown).

All serum samples tested were drawn within 120 days of diagnosis, and from a physiologic perspective we would expect little

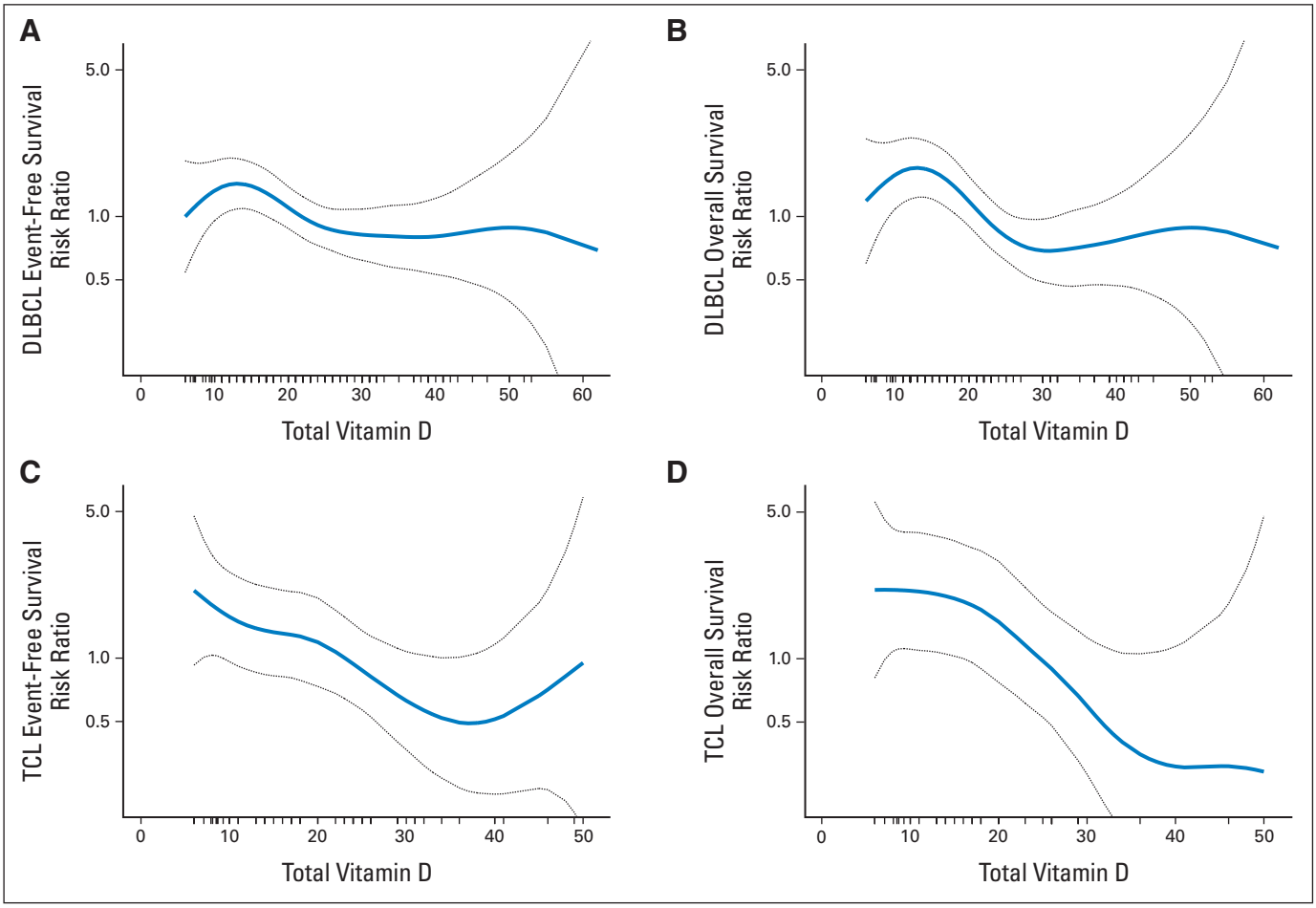

Fig 2. Estimated hazard ratios (solid line) and 95% CI (dotted lines) from multivariate models for 25-hydroxyvitamin D level (ng/mL) and (A) diffuse large B-cell (DLBCL) event-free survival, (B) DLBCL overall survival, (C) T-cell lymphoma (TCL) event-free survival, and (D) TCL overall survival.

change of 25(OH)D levels from chemotherapy over this short interval. Nevertheless, 34% of samples were drawn during or after initial therapy, and mean 25(OH)D levels and the prevalence of 25(OH)D insufficiency were modestly associated with timing of blood draw overall (Table 1). This overall association varied by subtype, and was seen only for TCL, MCL, and all other NHL, but not for DLBCL, FL, and post-FL (Appendix Table A2). Adjustment for timing of serum draw did not materially alter the associations in Table 2 (data not shown). Further, when restricted to pretreatment serum samples (Appendix Table A4, online only), our basic findings held, although the CIs around the point estimates became unstable due to small numbers for subset analyses in the lymphoma subtypes.

One potential interpretation of the association of low 25(OH)D levels with inferior DLBCL and TCL prognosis is that patients with a larger tumor burden might have increased conversion of 25(OH)D to $1,25(OH)_2D$ due to increased 1-α-hydroxylase activity from the tumor, leading to artificially low serum 25(OH)D levels.[30] As such, tumor size or aggressiveness might be a confounding factor. Although adjustment for IPI should remove most of the potential confounding, residual confounding remains a concern. Calcium levels were similar between 25(OH)D sufficient and insufficient patients with DLBCL and TCL (Appendix Table A5, online only). In addition, further adjustment of the results in Table 1 for albumin-corrected-calcium (as a surrogate for tumor activity, since high 1-α-hydroxylase activity would be expected to also increase serum calcium levels) did not materially change the DLBCL results (Appendix Table A5). The results for TCL attenuated for EFS (HR, 1.35; 95% CI, 0.63 to 2.88) and to a lesser extent for LSS (HR, 2.00; 95% CI, 0.74 to 5.37), while OS remained similar (HR, 2.17; 95% CI, 0.82 to 5.76).

To further address this issue, we measured $1,25(OH)_2D$ levels in patients with DLBCL and TCL. For DLBCL, patients in the lowest three quartiles of $1,25(OH)_2D$ had inferior EFS, LSS, and OS (Fig 3 and Table 3); this association was maintained when stratified by 25(OH)D sufficient versus insufficient. For TCL, patients below the median (too few patients to use quartiles) also had inferior outcomes in a pattern similar to that seen for DLBCL, although all estimates were imprecise and not statistically significant, likely due to small subject numbers (Fig 3 and Table 3). Overall, these data do not support the hypothesis that the association of lower 25(OH)D levels with poor prognosis in DLBCL and TCL is confounded by tumor production of 1-α-hydroxylase. Further, these data also indicate that there appears to be a direct association of lower $1,25(OH)_2D$ levels with inferior outcome.

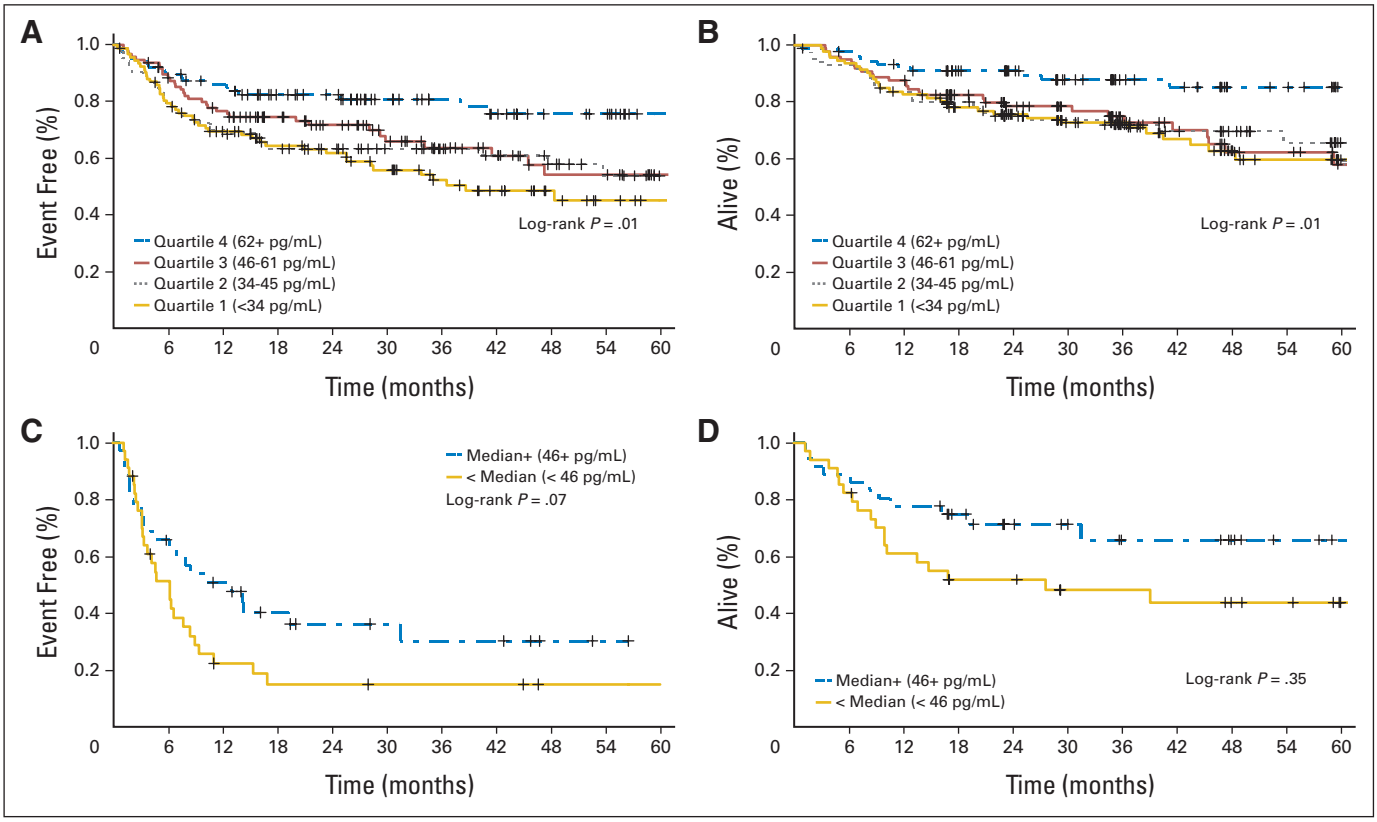

Fig 3. Kaplan-Meier curves for 1,25-dihydroxyvitamin D levels and (A) diffuse large B-cell (DLBCL) event-free survival (EFS), (B) DLBCL overall survival (OS), (C) T-cell lymphoma (TCL) EFS, and (D) TCL OS. 1,25-dihydroxyvitamin D levels in pg/mL.

DISCUSSION

Over 40% of patients with NHL in this cohort had insufficient 25(OH)D levels within 120 days of diagnosis, and low levels were associated with inferior EFS, LSS, and OS for DLBCL and TCL, two of the most aggressive NHL subtypes in this study. These associations remained after adjustment for clinical factors, including IPI, timing of blood draw (pretreatment *v* not), and season of diagnosis. While there was no association of vitamin D insufficiency and EFS for MCL, FL, post-FL or all other NHL, HRs were elevated for OS. These estimates were not statistically significant, most likely due to either the small number of patients with these types of NHL included in the cohort and/or few deaths during the relatively short follow-up time. Further studies in larger groups of patients should be performed to learn the longer-term implications of vitamin D insufficiency in these less aggressive NHL types.

Strengths of our study include the large, prospective cohort study design of consecutively enrolled patients with newly diagnosed NHL; availability of key baseline clinical and treatment data; and nearly complete follow-up of patients to define EFS, LSS, and OS. In addition, we used LC-MS/MS methods for the measurement of serum 25(OH)D and $1,25(OH)_2D$ levels, a technique which is considered to be the most reliable and accurate for 25(OH)D determination.[21] The major limitation of the study is the use of an observational study design, which is susceptible to confounding, although we were able to adjust for key clinical prognostic factors. Also, our study does not answer the question of whether replacing vitamin D would lead to a better prognosis.

Our finding that lower levels of both 25(OH)D and $1,25(OH)_2D$ were associated with inferior EFS, LSS, and OS in two aggressive NHL subtypes suggests that the prognostic effect of vitamin D may be directly related to its impact on the lymphoma and not simply a general host effect. Biologically, this is plausible, as vitamin D has been well-documented to be capable of modulating several critical cellular processes, including inhibition of carcinogenesis by induction of cellular differentiation, inhibition of proliferation and angiogenesis, and promotion of apoptosis.[5] Notably, in vitro vitamin D has been shown to inhibit proliferation and induce differentiation of both lymphocytes[31] and lymphoma cell lines.[32]

The associations with OS, but not EFS, which were hinted at in the other NHL subtypes, raise the hypothesis that vitamin D also likely impacts other health outcomes in these diseases with a long natural history, and thereby improves OS.[33] This will need to be evaluated in larger datasets with sufficient power.

In our study, we measured both the storage form of vitamin D [25(OH)D] and its biologically active metabolite [$1,25(OH)_2D$]. This examination was prompted by our concern that the low levels of 25(OH)D found in subjects with worse prognosis might be a function of greater tumor burden with resultant increased 1-α-hydroxylase activity, leading to increased conversion of 25(OH)D to $1,25(OH)_2D$. However, this was not the case. Rather, both lower 25(OH)D and $1,25(OH)_2D$ levels were associated with inferior EFS and OS. Whether

Table 3. Multivariable-Adjusted HRs for 1,25-Dihydroxyvitamin D Levels (overall, and stratified by 25-hydroxyvitamin D insufficiency) and Event-Free, Lymphoma-Specific, and Overall Survival

		Event-Free Survival				Lymphoma-Specific Survival				Overall Survival			
1,25-Dihydroxyvitamin D level (pg/mL)	No.	No. of Events	% Events	HR	95% CI	No. of Events	% Events	HR	95% CI	No. of Events	% Events	HR	95% CI
DLBCL, all													
Q4 (62+)	91	19	20.9	1.00	Reference	11	12.1	1.00	Reference	12	13.2	1.00	Reference
Q3 (46-61)	98	35	35.7	1.86	1.06 to 3.26	26	26.5	2.42	1.19 to 4.92	30	30.6	2.61	1.33 to 5.11
Q2 (34-45)	86	35	40.7	2.22	1.27 to 3.90	25	29.1	2.63	1.29 to 5.37	28	32.6	2.73	1.38 to 5.40
Q1 (<34)	92	43	46.7	2.18	1.26 to 3.76	28	30.4	2.16	1.07 to 4.37	30	32.6	2.14	1.09 to 4.21
P for trend				.01				.10				.11	
DLBCL, insufficient 25-hydroxyvitamin D													
Q4 (62+)	40	11	27.5	1.00	Reference	7	17.5	1.00	Reference	8	20.0	1.00	Reference
Q3 (46-61)	46	19	41.3	1.74	0.83 to 3.65	15	32.6	2.44	0.99 to 6.01	17	37.0	2.42	1.04 to 5.63
Q2 (34-45)	50	23	46.0	2.00	0.97 to 4.10	19	38.0	2.50	1.05 to 5.96	21	42.0	2.44	1.08 to 5.53
Q1 (<34)	54	29	53.7	1.95	0.96 to 3.97	22	40.7	1.99	0.84 to 4.71	23	42.6	1.88	0.83 to 4.26
P for trend				.11				.33				.36	
DLBCL, sufficient 25-hydroxyvitamin D													
Q4 (62+)	51	8	15.7	1.00	Reference	4	7.8	1.00	Reference	4	7.8	1.00	Reference
Q3 (46-61)	52	16	30.8	2.07	0.88 to 4.91	11	21.1	2.81	0.88 to 9.02	13	25.0	3.40	1.09 to 10.59
Q2 (34-45)	36	12	33.3	2.10	0.84 to 5.24	6	16.7	1.77	0.47 to 6.64	7	19.4	2.23	0.62 to 7.95
Q1 (<34)	38	14	36.8	2.32	0.93 to 5.34	6	15.8	1.66	0.47 to 5.93	7	18.4	1.92	0.56 to 6.58
P for trend				.17				.73				.62	
TCL, all													
Median+ (46+)	36	22	61.1	1.00	Reference	11	30.6	1.00	Reference	11	30.6	1.00	Reference
< median (< 46)	34	27	79.4	1.62	0.92 to 2.88	17	50.0	1.83	0.86 to 3.93	18	52.9	1.94	0.91 to 4.12
P				.09				.12				.08	
TCL, insufficient 25-hydroxyvitamin D													
Median+ (46+)	16	13	81.3	1.00	Reference	7	43.8	1.00	Reference	7	43.8	1.00	Reference
< median (< 46)	24	20	83.3	2.07	0.93 to 4.62	13	54.2	1.84	0.70 to 4.85	14	58.3	1.95	0.75 to 5.05
P				.08				.22				.17	
TCL, sufficient 25-hydroxyvitamin D													
Median+ (46+)	20	9	45.0	1.00	Reference	4	20.0	1.00	Reference	4	20.0	1.00	Reference
< median (< 46)	10	7	70.0	1.10	0.40 to 3.01	4	40.0	1.82	0.45 to 7.36	4	40.0	1.82	0.45 to 7.36
P				.86				.40				.40	

NOTE. Adjusted for International Prognostic Index. Quartile distribution based on histology specific cut points.
Abbreviations: HR, hazard ratio; DLBCL, diffuse large B-cell lymphoma; Q, quartile; TCL, T-cell lymphoma.

25(OH)D exerts biologic effects independent of $1,25(OH)_2D$, or serves merely as a substrate for conversion to $1,25(OH)_2D$ is unclear, although the affinity of $1,25(OH)_2D$ relative to 25(OH)D for the vitamin D receptor has been shown to be approximately 650-fold greater.[34]

Whether vitamin D supplementation in patients with newly diagnosed lymphoma and 25(OH)D levels below the optimal range will lead to improved outcomes is unknown, but warrants further investigation. The results for TCL, but not DLBCL, suggest supplementation even within the optimal range might be useful. None of the patients were above the optimal range, and there is no justification from our study to consider supraphysiologic levels. Finally, the role of vitamin D supplementation to maintain 25(OH)D levels within the optimal range for the primary prevention of NHL, which our study did not address, is not known.

In summary, our study provides strong data on the relationship between vitamin D and prognosis in NHL, and suggests a need for additional studies both to confirm our findings and to prospectively assess the role of vitamin D supplementation in NHL progression and survival. At this time, there is no definitive evidence for a causative relationship between lower vitamin D levels and poorer outcomes in lymphoma, and our study did not answer the question of whether normalizing vitamin D levels in these patients improves outcome. We do note that there are general clinical recommendations for vitamin D testing and replacement in patients with serum 25(OH)D levels below the optimal range.[35] Our data may provide additional incentive to follow these general guidelines particularly closely in DLBCL and TCL patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Matthew T. Drake, James R. Cerhan
Financial support: Matthew T. Drake, James R. Cerhan
Administrative support: James R. Cerhan
Provision of study materials or patients: Brian K. Link, Thomas M. Habermann, Stephen M. Ansell, Ivana N. Micallef, William R. Macon,

Grzegorz S. Nowakowski, David J. Inwards, Patrick B. Johnston, George J. Weiner, Thomas E. Witzig
Collection and assembly of data: Matthew T. Drake, Matthew J. Maurer, Brian K. Link, Thomas M. Habermann, Ravinder J. Singh, Cristine Allmer, James R. Cerhan
Data analysis and interpretation: Matthew T. Drake, Matthew J. Maurer, Cristine Allmer, Susan L. Slager, James R. Cerhan
Manuscript writing: Matthew T. Drake, James R. Cerhan
Final approval of manuscript: Matthew T. Drake, Matthew J. Maurer, Brian K. Link, Thomas M. Habermann, Stephen M. Ansell, Ivana N. Micallef, Jennifer L. Kelly, William R. Macon, Grzegorz S. Nowakowski, David J. Inwards, Patrick B. Johnston, Ravinder J. Singh, Cristine Allmer, Susan L. Slager, George J. Weiner, Thomas E. Witzig, James R. Cerhan

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