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Vitamin D Supplementation in Allo-HSCT Patients

Bailey Nelson
University of Nebraska Medical Center

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By

Bailey Nelson RD, LMNT

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Dr. Corrine Hanson

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Advisory Committee: Ann Anderson-Berry, Nicole Spurgeon, Glenda Woscyna

DEDICATION

I dedicate my thesis work to my parents Kim and Brad, my sister Alex and brother-in-law Matt, and my fiancé Sam. Thank you for the laughter, support, and truly knowing what this means to me.

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Thank you to my committee members, Corri, Nikki, Ann, and Glenda. Your patience, guidance, expertise, and valuable time was an integral part in helping me achieve my goal.

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ABSTRACT

BACKGROUND: Bone health is a serious concern for long-term survivors of Allo-HSCT due to multiple risk factors including steroids, chemotherapy, immunosuppressive drugs, and poor nutrition status[1]. Steroids have been long proven to have negative impact on bone health and the National Osteoporosis Foundation (NOF) lists steroids as a contributing factor to osteoporosis and fractures. NOF guidelines recommend providing adequate daily vitamin D as a safe and inexpensive way to help reduce fracture risk[1]. Therefore, proper supplementation of vitamin D may increase the quality of life for patients post Allo-HSCT.

PURPOSE: The purpose of this study is to determine if Allo-HSCT patients who receive steroids as part of their treatment at Nebraska Medicine are being supplemented with vitamin D.

METHODS: A retrospective review of electronic medical records was used to determine if vitamin D supplementation was prescribed to patients who received steroids after undergoing Allo-HSCT and whether this had an impact on their bone health, including serum 25(OH)D levels, incidence of fractures/falls, DEXA scan results, and GVHD

diagnosis. Inclusion criteria included adults who received Allo-HSCT from January 1, 2013 to December 31, 2014.

RESULTS: Out of 99 patients who underwent Allo-HSCT, 59 percent were prescribed steroids of ≥ 5 mg/day of prednisone or equivalent for ≥ 3 months, compared to 41 percent who were not. Vitamin D supplementation was significantly different between the two groups with 71 percent of the steroid group receiving vitamin D supplementation compared to 40 percent of the non-steroid group (p=0.004). Diagnosis of GVHD was also found to be statistically significant between groups with 90 percent of the steroid group compared to 71 percent of the non-steroid group (p=0.016). Mortality was found to be significantly higher in the non-steroid group compared to the steroid group (p=0.005).

CONCLUSION: This study found 71 percent of patients who received steroids of ≥ 5 mg/day of prednisone or equivalent for ≥ 3 months, received vitamin D supplementation. However, adequacy of vitamin D supplementation is still uncertain in this patient population.

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LIST OF ABBREVIATIONS

ACR: American College of Rheumatology

Allo-HSCT: Allogenic Hematopoietic Stem Cell Transplant

BMD: Bone mineral density

cGVHD: Chronic graft-versus-host-disease

DEXA: Dual-energy X-ray absorptiometry

GVHD: Graft-versus-host-disease

HR: Hazard ratio

LASA: Longitudinal Aging Study Amsterdam

NOF: National Osteoporosis Foundation

OR: Odds ratio

PTH: Parathyroid hormone

RR: Risk ratio

Serum 25(OH)D: Serum 25-hydroxyvitamin D

VDD: Vitamin D deficiency

CHAPTER 1: INTRODUCTION

Allogeneic hematopoietic stem-cell transplantation (Allo-HSCT) is a proven treatment for those suffering from malignant hematologic diseases of the blood or bone marrow such as acute myeloid leukemia or myelodysplastic syndrome. Allo-HSCT involves the transfer of stem cells from the donor to recipient, after their immune systems have been wiped out by chemotherapy, radiation, or a combination of both. The most significant risk of undergoing Allo-HSCT is the development of graft-versus-host disease (GVHD). Studies have been conducted to evaluate supplementation of vitamin D and improved survival rate of certain cancers and decreased risk of developing GVHD[2,3]. However, there are few studies which evaluate supplementation of vitamin D related to the use of steroids during Allo-HSCT. Steroids are proven to have negative effects on the metabolism vitamin D, including inhibition of osteoblast function, enhancement of bone resorption, inhibition of gastrointestinal calcium absorption, and increase in urine calcium loss. To treat vitamin D deficiency, NOF recommends adults be treated with 50,000 IU of vitamin D once a week or the equivalent daily dose (7,000 IU vitamin D_2 or vitamin D_3 daily) for 8-12 weeks to achieve normal ($\geq$ 30 ng/mL) serum 25(OH)D concentrations[1]. Overall, the nutrition status of patients who undergo Allo-HSCT is extremely complex and unstable. For those patients who are treated with steroids, it is important to prevent further deterioration of their health and functional status by adequately supplementing vitamin D to ensure adequate 25(OH)D levels.

CHAPTER 2: LITERATURE REVIEW

Vitamin D

Vitamin D is often categorized as a fat-soluble vitamin; however, it is not an essential dietary factor as humans can produce it endogenously[4]. More accurately, vitamin D is closely related to a steroid hormone due to its molecular structure. Vitamin D consists of two bioequivalent forms (ergocalciferol and cholecalciferol) both of which are biologically inert until metabolized in the body[5]. Ergocalciferol (vitamin D2) is obtained from dietary vegetable sources and oral supplements. Cholecalciferol (vitamin D3) is obtained in fortified foods and oral supplements, but primarily through skin exposure to ultraviolet B radiation in sunlight[5]. The skin produces vitamin D3 photochemically from 7-dehydrocholesterol, which is then metabolized in the liver to produce 25-hydroxycholecalciferol (25[OH]D). Within the kidneys, 25(OH)D serves as a substrate for 1-alpha-hydroxylase to make 1,25-dihydroxycholecalciferaol ($1,25[OH]_2D$), the biologically active form[4]. The biologically active form then has a significant role in bone health. $1,25(OH)_2D$ stimulates the absorption of calcium and phosphate from the gut by opening calcium channels and stimulating the formation of calcium binding proteins[6]**.** If $1,25(OH)_2D$ concentrations drop due to deficiencies, less calcium is available for bone mineralization. Parathyroid hormone (PTH) will then increase, stimulating the hydroxylation of 25(OH)D in the kidney to produce more $1,25(OH)_2D$. This increase in PTH stimulates bone turnover and loss[6]. Therefore, vitamin D deficiency is known to cause high bone turnover leading to osteoporosis, osteomalacia, falls and fractures. (Figure 1)

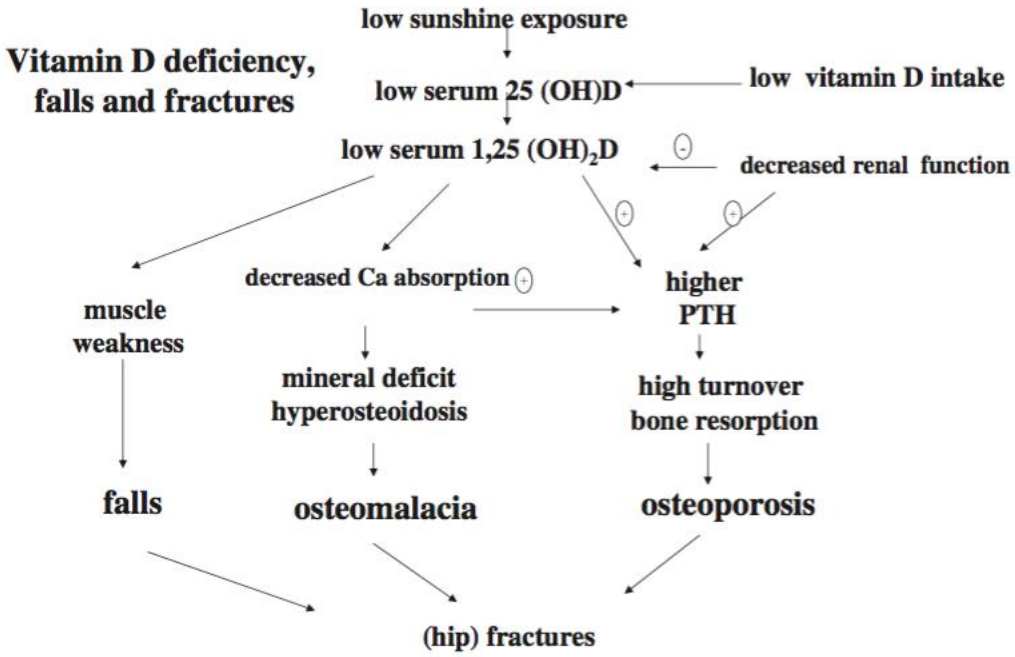

Figure 1: Pathophysiologic pathways from vitamin D deficiency to osteoporosis, osteomalacia, falls and fractures.[6]

In the Longitudinal Aging Study Amsterdam (LASA) study which looked at serum 25(OH)D influences on serum PTH and parameters of bone health, serum osteocalcin (maker for bone formation) and urinary deoxypyridinolin excretion (marker of bone resorption), were shown to be elevated when serum 25(OHD) was low. Both markers were shown to decrease with a rise in serum 25(OH)D levels up to 40 nmol/L (16.03 ng/dL)[7]. A placebo-controlled trial of 330 older women found a serum 25(OH)D level of 10 nmol/l (4 ng/mL) resulted in a risk ratio (RR) of hip fracture of 1.8[8]. In a global study of 7,441 postmenopausal women with osteoporosis there was a significant positive relationship between serum 25(OH)D and bone mineral density (BMD) in the trochanteric area of the hip, threshold below 50 nmol/L[9].

High bone turnover can then lead to increased risk of falls and fractures. Epidemiological studies show a relationship between vitamin D deficiency and fractures. In the LASA study incidence of fractures was higher when serum 25(OH)D was lower than 30 nmol/L (12.01 ng/mL), and the lowest percentage of fractures was found with serum 25(OH)D above 75 nmol/L (30 ng/mL)[10]. In the Women's Health Initiative study, all women were treated with 1500mg/day of calcium citrate and 1000 IU/day of vitamin D3 for 6 weeks[11]. The purpose of this study was to examine the effect of short term calcium and vitamin D supplementation on biochemical markers of bone turnover. Their analysis showed a significant decrease of hip fracture incidence and hip bone density was found to be 1.06 percent higher in the calcium plus vitamin D group than in the placebo ($p<0.01$)[11].

Assessing Vitamin D

To determine vitamin D levels within the body, serum 25(OH)D concentrations are assessed as the active form of vitamin D is physiologically regulated. 25(OH)D is the key substrate for 1-alpha-hydroxylase to produce $1,25(OH)_2D$. Therefore, production of $1,25(OH)_2D$ is directly dependent on the absolute concentrations of 25(OH)D. Currently there is debate among research regarding adequate levels of serum 25(OH)D levels. According to the Institute of Medicine, serum 25(OH)D levels of 20 ng/mL is considered adequate for most individuals[9]. However, the Endocrine Society, International Osteoporosis Foundation, American Geriatric Society, and NOF suggest a minimum level of 30 ng/mL[1,13-15]. The debate regarding adequate serum 25(OH)D concentrations is

based on adequate suppression of PTH concentrations to ensure intestinal absorption of calcium does not lead to a decrease in serum calcium. In a prospective study of serum 25(OH)D level it was found baseline 25(OH)D levels were inversely associated with all-cause mortality risk (adjusted hazard ratio [HR] = 0.95, 95% CI=0.92-0.98, per 10 nmol/L [4 ng/mL] of 25[0H]D)[16].

Vitamin D Supplementation

Vitamin D supplementation comes in two forms, ergocalciferol (vitamin D2) and cholecalciferol (Vitamin D3). Vitamin D3 is the most efficient form of supplementation as it has greater affinity for the vitamin D-binding protein and has a longer half-life (~3 weeks) compared to that of vitamin D2, therefore less frequent dosing may be required[18]. In a meta-analysis of seven randomized control trials evaluating serum 25(OH)D concentrations after supplementation with vitamin D3 versus vitamin D2, vitamin D3 was shown to increase serum 25(OH)D levels more efficiently (p=0.001)[19]. However, at this time the NOF recommends either form of supplementation to reach adequate serum 25(OH)D levels. The NOF recommends vitamin D intake for adults age 50 and older of 800-1000 IU per day, including supplementation if necessary[1]. If a patient is diagnosed with vitamin D deficiency (VDD) the NOF recommends adults be treated with 50,000 IU of vitamin D once a week or the equivalent daily dose (7,000 IU vitamin D_2 or vitamin D_3 daily) for 8-12 weeks to achieve serum 25(OH)D level of 30 ng/mL[1]. After this level is reached NOF recommends maintenance therapy to preserve those concentrations[1]. However, determining adequate dosing should be individualized based on a multitude of factors such as baseline serum 25(OH)D, absorptive capacity,

and ability of liver to convert metabolites[20]. Therefore, Allo-HSCT patients likely require higher dosages due to their multiple risk factors and complex health status. Regardless of specific units of vitamin D supplementation, NOF and ACR guidelines, recommend supplementing enough vitamin D to maintain adequate serum 25(OH)D levels, whatever amount that may be[1,17]. Although severe vitamin D deficiencies are uncommon, the 2005-2006 NHANES study reported subclinical vitamin D deficiencies (defined as $\leq$ 20 ng/mL) in 41.6% of their adult participants[21]. For generalized healthy adults, VDD may not have any severe signs and symptoms, but for those who are undergoing treatment for cancer, VDD may have more of an effect on their overall outcomes.

Vitamin D and Cancer

Research regarding vitamin D status and cancer has become of more interest in recent years, with the potential role of vitamin D as an easily modifiable mediator of a broad array of pathologic conditions, including cancer[22]. In a prospective study which looked at vitamin D status and cancer incidence in men, an increment of 25nmol/L (10 ng/mL) in predicted 25(OH)D level was associated with a 17% reduction in total cancer incident (RR=0.83, 95% CI 0.74-0.92), a 29% reduction in total cancer mortality (RR=0.71, 95% CI 0.60-0.83), and a 45% reduction in digestive-system cancer mortality (RR=0.55, 95% CI 0.41-0.74)[23]. Another study looked at association between total average annual sunlight and age-adjust breast cancer mortality rates in 87 regions of the U.S. Risk of fatal breast cancer in the major urban areas of the U.S. was inversely proportional to intensity of local sunlight (r=0.82, p=0.0001)[24]. In addition, a study which looked to determine how many types of cancer are affected by solar radiation found the annual

number of premature deaths (1970-1994) from cancer due to lower UV-B exposures was 21,7000 (95% CI 20,400-23,400) for white Americans, 1400 (95% CI 1100-1600) for black Americans and 500 (95% CI, 400-600) for Asian Americans and other mortalities[25].

Vitamin D and GVHD

Significant research has also been completed looking at vitamin D supplementation and its effect on GHVD, as GVHD is considered one of the most significant complications of Allo-HSCT with detrimental impact on transplant related mortality, overall survival, and patient quality of life[25]. According to research, Grade II-IV acute GVHD in patients range from 20-50% and chronic GVHD from 40-50% after Allo-HSCT[26,27]. In a retrospective cohort analysis, fifty-three patients' serum 25(OH)D levels were looked at prior to Allo-HSCT to determine correlation with risk of GVHD[27]. They found the cumulative incidence of chronic GVHD at 2 years in patients with serum 25(OH)D levels <25 ng/mL was 63.8% compared to 23.8% in patients with levels $\geq$25 ng/mL (p=0.009)[27]. Additionally, in another retrospective analysis of 166 patients which evaluated 25(OH)D levels before allo-HSCT found through multivariate analysis, vitamin D level before transplant to be a significant independent risk factor for development of chronic GVHD (cGVHD) (p=0.04)[28]. The RR of cGVHD at two years after transplant was measure at 2.66 (95% CI 1.03-6.87) for 25(OH)D below 60 nmol/L compared with above 60 nmol/L (24 ng/mL) before transplant[28].

Steroids and Allo-HSCT

Steroids, specifically glucocorticoids are often prescribed to patients undergoing Allo-HSCT as they have an inhibitory effect on specific immune responses as well as

suppressive effects which allow for management of inflammation or autoimmune disorders[29]. While they have many benefits for those undergoing Allo-HSCT, they have been proven to negatively impact bone health, causing decreased bone formation and bone loss, reduce bone quality, and disrupt microarchitectural integrity[1]. (Figure 2)

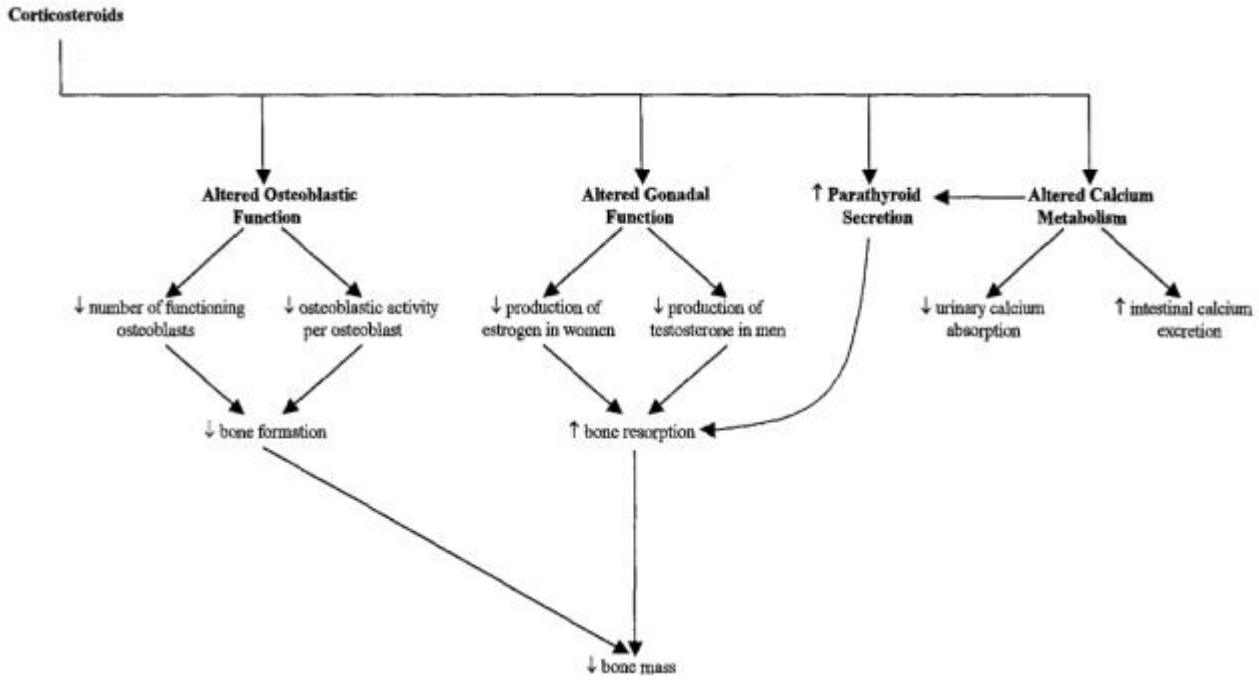

Figure 2. Pathophysiology of corticosteroid-induced osteoporosis[31].

Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis, and needs to be considered in patients who receive Allo-HSCT due to their fragile and complex health status[30]. Bone density declines rapidly at the beginning stages of using steroids and then more slowly and steadily as steroid use continues[30]. Therefore, appropriate supplementation at the beginning of steroid use is critical. The NOF lists glucocorticoids use of $\geq$ 5 mg/d of prednisone or equivalent for $\geq$ 3 months as a risk factor for developing osteoporosis and fractures, as well as the point to which supplementation of vitamin D should be initiated[1]. The American College of

Rheumatology (ACR) recommends stricter guidelines, recommending patients taking glucocorticoids at any dose or duration should receive vitamin D supplementation[17]. In this patient population in which steroids are often a standard form of treatment, it would be important to take swift action in providing proper supplementation early on in Allo-HSCT treatment. A prospective study of 146 patients who received Allo-HSCT found that at two months after transplant, more than 50% of the patients had DEXA detected osteoporosis, showing that subclinical bone abnormalities appear early post-HSCT[32]. The same study found that duration of steroid use was a significant risk factor for developing clinical bone complications as well (Hazards Ration [HR]: 1.06/month; 95% CI 1.00-1.14; p=0.038)[32].

Bone Mineral Density Assessment

To better assess patient's bone mineral density and risk of fracture, dual energy x-ray absorptiometry (DEXA) is considered the gold standard when determining bone mineral density of the lumbar spine and hip. DEXA measurements of the hip and spine is most often used to confirm a diagnosis of osteoporosis or predict future fracture risk[1]. Peak bone mass is achieved in early adulthood, followed by a declined which is accelerated in women at menopause[1]. NOF recommends BMD testing be performed in women age 65 and older and men age 70 and older. NOF continues to recommend BMD testing by DEXA scan in post-menopausal women and men $\geq$ 50 years of age if prescribed short or long term glucocorticoid treatment[1]. Although there is no consensus for optimal frequency of monitoring BMD, NOF guidelines suggest one to two year BMD testing after initiating medical therapy for treatment of osteoporosis and

every two years thereafter[1]. A study in 2001 assessed bone mineral density of 67 adult patients who received stem cell transplants. Of those patients 49% were found to have osteopenia and osteoporosis prior to transplant. There was also significant decrease in T-score when comparing bone mineral density before transplant and at six months post-HSCT (-1.23± 1.08 compared to -1.87± 1.28) ($p<0.001$)[3]. This study proposed the demand for vitamin D is increased after Allo-HSCT, likely due to the multiple risk factors this patient population carries, and supplementation of both calcium and vitamin D not being prescribed outpatient[3]. As a result, this study encourages measurement of BMD prior to transplant for baseline measurements.

CHAPTER 3: METHODS

Participants and Study Design

This study was a retrospective chart review of data collect from the electronic medical record. Inclusion criteria included age of 19 years or older and receiving an Allo-HSCT at Nebraska Medicine from January 1, 2013 to December 31, 2014. Exclusion criteria included children (less than 19 years of age) and those who received Auto-HSCT. The Study protocol was approved by the Institutional Review Board at the University of Nebraska Medical Center in Omaha, Nebraska. Ninety-nine patients were eligible for this study.

Data Collection

Patients were identified with help of the Oncology/Hematology Transplant Data Office at the University of Nebraska Medical Center. All data was collected between two time points. The first time point, time of transplant, was defined as the day the patient underwent Allo-HSCT (this included up to 30 days prior to transplant if labs or information was gathered prior to the exact date of transplant). The second time point was at the one year follow up appointment after receiving their transplant (this included up to 15 months after their transplant as scheduling conflicts could have impacted their "one year" follow-up). Baseline characteristics were gathered at time of transplant and included age, sex, and race. Additional data was gathered between the time of transplant and one year follow-up, this included: steroids prescribed, vitamin D supplementation, serum 25(OH)D levels, documented falls/fractures, DEXA results, LOS, and, mortality.

Steroids

Prescribed steroids were gathered by review of the medications tab and a keyword search of the term steroid within the medical record. The first classification of steroid use was based on the NOF criterial for vitamin D supplementation which specifies ≥ 5 mg of prednisone or equivalent for ≥3 months for inclusion in the steroid group. Patients did not have to be prescribed steroids consecutively to meet requirements for duration of ≥ 3 months. For example, if the patient was prescribed ≥ 5 mg for six non-consecutive two week periods, they met criteria to be included in the steroid group. All patients not meeting this criteria were placed in the non-steroid groups. Therefore, if a patient received high dose steroids for one month they did not meet criteria and were placed in the non-steroid group.

Steroid groups were later re-categorized based on ACR recommendations for when vitamin D supplementation should be started. The steroid group was re-defined as those who received any dose/duration of steroid during the first year following transplant. Therefore, the patient who received high dose steroids for one month would be included in the steroid group. The non-steroid group consisted of those who never received any steroid between time of transplant and one year follow-up.

Vitamin D Supplementation

The primary outcome of the study was vitamin D supplementation. Supplementation was found via the medications tab along with keyword search for vitamin D in the medical chart. Use of a multivitamin was not considered as supplemental vitamin D. Vitamin D supplementation was categorized into yes/no

groups. Any dose or duration of vitamin D supplementation was placed in the yes group. The no group consisted of those who did not receive any vitamin D supplementation during time of transplant and one year follow-up.

Serum 25(OH)D

Serum 25(OH)D levels were collected from the "results review tab" within the medical record, which is the location of all laboratory results in the electronic medical record. All serum 25(OH)D levels were collected that occurred between date of transplant and one year follow-up. Therefore, if a patient had five serum 25(OH)D levels drawn during our study time points, all were recorded. However only the first serum 25(OH)D level was used in the analysis. Serum 25(OH)D levels were collected as continuous variables and also categorized as "adequate" and "inadequate". Adequate was defined as $\geq$ 30 ng/mL and inadequate was defined as < 30 ng/mL.

GVHD

Diagnosis of GVHD was found through keyword search in the medical chart. Criteria for a diagnosis of GVHD was the documentation of diagnosis within a physician's note. GVHD diagnosis was categorized into yes/no groups. The yes group included acute and chronic GVHD and any specified type (i.e. skin, liver, gut). Those without diagnosis of GVHD within a physician's note were placed in the no group.

Falls/Fractures

Documented falls and fractures were found through key word search in the medical record. Only falls/fractures documented in physician or nursing notes during the defined time period were gathered. Severity of fall/fracture was not collected and

there was no differentiation if a patient had more than one fall/fracture. To meet criteria for the yes group there needed to be at least one documentation in a physician or nurse note stating the patient had a fall/fracture. Those who did not have documentation of either were placed in the no group.

DEXA scan Results

DEXA results were collected between date of transplant and one year follow-up. Results were found within the "results review tab" where non-laboratory tests are reported. All DEXA results were collected between our two time-points. However, only initial DEXA scans were used for analysis. DEXA results were categorized based on the stated report within each scan. DEXA results at Nebraska Medicine describe bone health in two ways: *"increased risk of fracture"* and *"low risk for fracture"*. Therefore, these were the two categories used for the classification of patients in this study. Risk of fracture was only categorized if patient underwent DEXA scanning. Therefore, if the patient had documentation in the medical record of osteoporosis or osteopenia but did not have a DEXA scan during our time of data collection, they were categorized as missing data from our analysis.

Mortality

Mortality was collected between time of transplant and one year follow-up and categorized a yes/no.

Length of Stay

Length of stay was defined as the length in days during the hospitalization to undergo Allo-HSCT. This number was taken from day of admittance to day of discharge.

This information was provided by the Oncology/Hematology Transplant Data Office.

Data Analysis

Descriptive statistics were calculated for all variables. Continuous variables were represented by means and standard deviations and categorical variables were represented as counts and percentages. The difference in means of the steroid vs. the non-steroid groups were tested using Independent Sample T-Test. The difference in proportion between steroid vs. the non-steroid group were evaluated with the Chi-Square Test or the Fischer's Exact Test, as appropriate. Multivariate analysis was preformed using Logistic Regression for those variables found to be significant through the univariate analysis. Logistic regression established an odds ratio (OR) for select variables of interest to determine if statistical significance remained independent of all other variables. A Chi-squared test was used to compare the proportion of vitamin D supplementation in both groups after re-categorizing steroid groups based on the ACR guidelines. Data analysis was preformed using IBM SPSS Statistics 23 software and p-values of < 0.05 were considered significant.

CHAPTER 4: RESULTS

Ninety-nine patients were included in the study. Forty-five were female and 54 were male, ranging in age from 19 to 71 years of age, with a mean age of 49.7 years. The majority of patients were Caucasian, compiling 94.5% of the population. Fifty-eight met criteria for the steroid group while 41 were placed in the non-steroid group as seen in Table 1.

Table 1: Steroid Groups

Steroid vs. Non-Steroid	N(%)
Steroid ($\geq$5 mg for $\geq$ 3 months)	58 (59)
Non-Steroid	41 (41)
Total Patients	99

Baseline and clinical characteristics can be seen in Table 3 and 4 based. There was no significant difference between steroid groups in age, sex, and race.

When assessing clinical characteristics based on steroid groups there was a statistically larger percentage of vitamin D supplementation in the steroid group compared to the non-steroid group (71% vs 41% respectively, p=0.04). GVHD diagnosis was significantly different between the groups as well; with 90% in the steroid group and 71% in the non-steroid group (p=0.016). Mortality was found to be statistically significant between groups with 51% of the non-steroid group compared to only 24% of those in the steroid group (p=0.005). Documented falls/fractures had a close trend to significance with a p-value of 0.08, with 41% of the steroid group compared to 24% of the non-steroid group having a documented falls or fracture. Serum 25(OH)D levels and DEXA results did not have p-values of significance.

Table 3: Continuous Variables Comparing Steroid Groups

Variables (N)	Groups				
	Steroid (N=58)		Non-Steroid (N=41)		
	N	Mean (S.D.)	N	Mean (S. D.)	P value
Age, years	58	49.3 (13.26)	41	50.3 (13.69)	0.708
Serum 25(OH)D, ng/mL	45	31.89 (16.32)	23	30.78 (18.05)	0.799
Length of Stay, days	58	24.48 (5.66)	41	23.90 (6.47)	0.637

Table 4: Categorical Variables Comparing Steroid Groups

	Groups				
	Steroid (N=58)		Non-steroid (N= 41)		
Variable	N	%	N	%	P Value
Gender					
Male	30	52	24	59	
Female	28	48	17	41	0.503
Race					
Caucasian	55	95	39	95	
Other	3	5	2	5	1.00
Vitamin D Supplementation					
Yes	41	71	17	41	
No	17	29	24	59	**0.004**
25(OH)D					
Adequate (≥ 30 ng/mL)	22	38	11	27	
Inadequate (< 30 ng/mL)	23	40	11	27	0.932
GVHD Diagnosis					
Yes	52	90	29	71	
No	6	10	12	29	**0.016**
Falls/Fractures					
Yes	24	41	10	24	
No	34	59	31	76	*0.08*
DEXA Results					
Increased Risk for Fracture	23	40	6	15	
Low Risk for Fracture	19	33	11	27	0.176
Mortality					
Yes	14	24	21	51	
No	44	76	20	49	**0.005**

Logistic regression was used to compare the two steroid groups and the variables that were found to be significant through univariate analysis. Those found to be significant include vitamin D supplementation, GVHD, and mortality. When controlling for GVHD and mortality the odds of patients in the steroid group receiving vitamin D supplementation are 2.952 times higher than the non-steroid group (p=0.019; 95% CI: 1.196-7.288). The odds of individuals being diagnosed with GVHD are 4.26 times higher in the steroid group (p=0.014; 95% CI (1.333-13.651) when vitamin D supplementation and mortality are controlled. Mortality was 3.004 times more likely to occur in the non-steroid group comparatively when controlling for vitamin D supplementation and GVHD (p=0.020; 95% CI (1.187-7.606) (Table 5).

Table 5: Results of the Logistic Regression Model

Variable(s)	p-value	OR	95% Confidence Interval (CI)
Vitamin D Supplementation	0.019	2.952	1.196-7.288
GVHD	0.014	4.267	1.333-13.651
Mortality	0.020	3.004	1.187-7.606

Additionally, an analysis was done to compare serum 25(OH)D levels between patients who were supplemented vitamin D and those who were not supplemented vitamin D. Out of 99 patients, 40 received vitamin D supplementation compared to 18 who did not. Overall there was no significant difference found between mean serum 25(OH)D levels between those who were supplemented and those who were not supplemented.

Table 6: Serum 25(OH)D Levels in Supplemented vs. Not Supplemented Patients

Variables (N)	Groups				
	Supplemented (N=45)		Non-Supplemented (N=23)		
	N	Mean (S.D.)	N	Mean (S. D.)	P value
Serum 25(OH)D (ng/mL)	45	31.84 (17.06)	23	31.13 (16.56)	0.927

One final analysis was done in which steroid groups were re-categorized based upon ACR recommendations for vitamin D supplementation. Using these redefined groups, 93 individuals met criteria for the steroid group, leaving 6 in the non-steroid group. Of those 93 patients in the steroid group, 60% received vitamin D supplementation while 40% did not. Using a Fischer's Exact test due to large discrepancy in group sizes, no statistical significance was found between groups (p=0.228).

Table 7: Steroid groups categorized according to the ACR guidelines

	Groups				
	Steroid (N=93)		Non-steroid (N=6)		
Variable (Total N)	N	%	N	%	P Value
Vitamin D Supplementation					
Yes	56	60	2	33.5	
No	37	40	4	66.5	0.228

CHAPTER 5: DISCUSSION

The primary outcome of this research was to determine if Allo-HSCT patients who received steroids ≥ 5mg of prednisone or equivalent for ≥ 3 months were supplemented with vitamin D. We found that a significantly higher proportion of those in the steroid group were supplemented with vitamin D compared to the non-steroid group. Based on this, we can speculate that clinicians at Nebraska Medicine are aware of the implications of steroid use in the Allo-HSCT population and are starting a significant proportion of those patients on vitamin D supplementation as a result. However, nearly a quarter of patients remained unsupplemented. This study did not quantify the dose or duration of vitamin D supplementation which would have been beneficial to determine if patients were receiving adequate amounts during and after their transplant, which may be of relevance for future studies in this patient population.

As discussed in the literature review, standard prophylactic measurements for bone health may not be enough to protect this patient population[3]. To gather more information on vitamin D supplementation according to steroid regimen in our population, we re-categorized groups based on ACR guidelines. Using these redefined groups, which recommend vitamin D supplementation with any dose/duration of steroid, it was found there was no statistical significance in vitamin D supplementation between the steroid and non-steroid groups. Therefore, the same proportion of patients were supplemented regardless of steroid use. The sample sizes of these two groups were highly skewed with 93 patients in the steroid group and only 6 in the non-steroid group. Therefore, 94% of our population received some dose/duration of steroid

between our defined time points, further demonstrating the need for vitamin D supplementation. Allo-HSCT have increased need for vitamin D supplementation based on multiple factors including steroid use, previous chemotherapy, lack of sun exposure, and poor nutrition status[1,20]. While those in the non-steroid group were likely appropriate for supplementation, it shows that patients who did not meet criteria for $\geq$ 5mg of prednisone or equivalent for $\geq$ 3 months were not being supplemented with vitamin D. Therefore, if a patient received high dose steroids for one month they would have likely not been prescribed vitamin D supplementation. A study by Petropoulou, et al. found that at two months' post-transplant more than 50% of patients had DEXA detected osteoporosis, showing how critical early intervention is[32].

Our secondary aim was to determine what clinical outcomes were associated with steroid use of $\geq$ 5mg of prednisone or equivalent for $\geq$ 3 months. Based on review of literature, we hypothesized that a significant proportion Allo-HSCT patients who received steroids would also have inadequate serum 25(OH)D levels, documented falls/fractures, DEXA results demonstrating increased risk for fracture, and diagnosis of GVHD.

There was no statistical difference in proportions of patients with inadequate serum 25(OH)D levels compared between steroid groups. There was also no significant difference in mean serum 25(OH)D levels when compared between steroid and non-steroid groups (31.89 ± 16.32 vs 30.78 ± 18.05, $p=0.799$). This could be explained by the fact there was a significant portion of patients in the steroid group who were supplemented with vitamin D, likely maintaining serum 25(OH)D levels or at least

preventing a decrease. Both of our mean serum 25(OH)D levels were very similar and met criteria for ≥ 30 ng/mL. With that being said, out of 99 patients only 68 had initial serum 25(OH)D levels drawn. Of those assessed, 50% had levels categorized as inadequate. Overall, 33% of our patient population were never assessed for serum 25(OH)D levels, making it difficult to accurately evaluate differences between steroid groups. Knowing the extensive risk factors Allo-HSCT patients carry, in the future it would beneficial to have more consistent testing of serum 25(OH)D levels.

We also assessed mean serum 25(OH)D compared between supplemented and non-supplemented groups, as supplementation of vitamin D is a better indicator of serum 25(OH)D. Through this analysis, we found there was no significant difference between supplemented and non-supplemented groups (31.84±17.06 vs 31.13±15.56, p=0.927). Urbain, et al. found baseline 25(OH)D levels were 16.4±8.9 ng/mL in HSCT patients[33]. Although our levels were not collected at baseline, they do show adequate serum 25(OH)D levels between our two time points. It should also be noted the study population of Allo-HSCT patients was predominately Caucasian, thus potentially impacting our results as Caucasian individuals are at less risk for vitamin D deficiency when compared to those with darker skin tones, such as African American or Hispanic individuals[1].

Falls and fractures between steroid groups were not found significant, however did trend in that direction (p=0.08). This finding is of interest, given our small sample size and the self-reported nature of this variable. Of the 34 documented falls or fractures, 71% were in the steroid group compared to only 39% in the non-steroid

group. The LASA study found incidence of fracture was higher when serum 25(OH)D was lower than 30 nmol/L (12.01 ng/mL)[10]. They also found the lowest percentage of fractures occurred with serum 25(OH)D levels were above 75 nmol/L (30 ng/mL)[10]. Knowing our mean serum 25(OH)D level for the steroid group was above 30 ng/mL (31.89±16.32) and there was still a trend towards significance for falls and fractures, we could propose our patient population may benefit from having serum 25(OH)D levels above adequate.

When assessing DEXA screening between groups, 72% of those in the steroid group underwent DEXA screening compared to 41% of the non-steroid group. Of those patient's in the steroid group, 55% were deemed to be at increased risk for fracture compared to only 15% in the non-steroid group (p=0.176). This finding is similar to Massenkeil, et al. which found 49% of HSCT patients to have osteopenia and osteoporosis prior to transplant[3]. Out of the total 99 patients who received Allo-HSCT, only 60% underwent DEXA scanning, leaving 40% of the population without an accurate look at their bone density health. These findings suggest there is need for more consistency when evaluating patients' bone health. Massenkeil, et al. states it may be beneficial for Allo-HSCT patients to receive DEXA scans prior to transplant and one year after treatment has been started[3]. While NOF recommends BMD follow-up one year after initiating treatment for osteoporosis[1]. In this patient population, we have room for improvement with assessing and treating risk factors for decreased bone health. Although there was a significant portion of those in the steroid group being supplemented with vitamin D, there was also a trend towards significant for

falls/fractures suggesting supplementation was not adequate to raise their 25(OH)D levels to sufficient amounts. The lack of consistency in testing 25(OH)D levels and DEXA scans as well made it difficult to find meaningful results.

GVHD diagnosis was statistically significant between steroid groups with 90% of the steroid group being diagnosed with GVHD compared to 71% of the non-steroid group ($p=0.016$). This can be explained as steroids are a common treatment for GHVD disease. Therefore, these patients were likely diagnosed with GVHD and then started on steroids. As we know pre-transplant 25(OH)D, specifically levels under <25 ng/mL, are associate with increased risk of chronic GVHD[27,28]. Therefore, it may be beneficial to start assessing serum 25(OH)D levels prior to transplant and have their levels adequate before starting transplant[27].

Mortality was not a variable we had predicted to have statistical significance when beginning our research. However, mortality was found to be significantly higher in the non-steroid group compared to the steroid group, with 51% of the non-steroid group compared to 24% of the steroid group ($p=0.005$). Initially, this finding seems inconsistent from the information gathered from the literature review. It would have seemed more likely that those in the steroid group would have a higher proportion of mortality as they were likely being treated for complications from Allo-HSCT such as GVHD. However, this finding could be explained by the fact these patients who died may not have lived long enough to meet our requirements of the steroid group.

Limitations

Inherently, this study has limitations. As it is a retrospective chart review, bias may have occurred due to misclassification errors. The small sample size could have shown less strength in association compared with a broader sample size. Another large limitation to the study was the lack of consistency in drawing 25(OH)D levels along with DEXA scanning. More consistent information regarding these outcomes could have given us more detailed information to draw conclusions.

Applications for Clinical Practice

Development of this study came from clinical judgement regarding the risk factors for decreased bone health in Allo-HSCT patients. It became apparent through daily observation and clinical experience that these patients had multiple factors putting their bone health at risk. Therefore, this study was developed in hopes to encourage use of vitamin D supplementation. The results from this study are to show that while many Allo-HSCT patients are being supplemented with vitamin D, there are inconsistencies in dosage and objective testing to determine if supplementation is enough to prevent a decline in bone health.

CHAPTER 6: CONCLUSION

In conclusion, our study reports that 71% of patients who received steroids according to NOF guidelines received vitamin D supplementation. Overall, it is encouraging that over half of the patient population received vitamin D supplementation. However, we do not know if supplementation was adequate for this complex patient population due to the lack of consistency when treating and assessing bone health. Further research should be completed to identify adequate amounts of vitamin D supplementation in Allo-HSCT to maintain adequate serum 25(OH)D levels and prevent deteriorating bone health in this complex patient population.

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