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Vitamin D Deficiency and Survival in Children after Hematopoietic Stem Cell Transplant

Gregory Wallace [1], Sonata Jodele [1], Jonathan Howell [2], Kasiani C. Myers [1], Ashley Teusink [3], Xueheng Zhao [4], Kenneth Setchell [4], Catherine Holtzapfel [1], Adam Lane [1], Cynthia Taggart [1], Benjamin L. Laskin [5], Stella M. Davies [1,*]

[1] *Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[2] *Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[3] *Department of Pharmacy, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[4] *Department of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[5] *Division of Nephrology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania*

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Vitamin D has endocrine function as a key regulator of calcium absorption and bone homeostasis and also has intracrine function as an immunomodulator. Vitamin D deficiency before hematopoietic stem cell transplantation (HSCT) has been variably associated with higher risks of graft-versus-host disease (GVHD) and mortality. Children are at particular risk of growth impairment and bony abnormalities in the face of prolonged deficiency. There are few longitudinal studies of vitamin D deficient children receiving HSCT, and the prevalence and consequences of vitamin D deficiency 100 days after transplant has been poorly studied. Serum samples from 134 consecutive HSCT patients prospectively enrolled into an HSCT sample repository were tested for 25-hydroxy (25 OH) vitamin D levels before starting HSCT (baseline) and at 100 days after transplantation. Ninety-four of 134 patients (70%) had a vitamin D level $<$ 30 ng/mL before HSCT, despite supplemental therapy in 16% of subjects. Post-transplant samples were available in 129 patients who survived to day 100 post-transplant. Vitamin D deficiency persisted in 66 of 87 patients (76%) who were already deficient before HSCT. Moreover, 24 patients with normal vitamin D levels before HSCT were vitamin D deficient by day 100. Overall, 68% of patients were vitamin D deficient ($<$30 ng/mL) at day 100, and one third of these cases had severe vitamin D deficiency ($<$20 ng/mL). Low vitamin D levels before HSCT were not associated with subsequent acute or chronic GVHD, contrary to some prior reports. However, severe vitamin D deficiency ($<$20 ng/mL) at 100 days post-HSCT was associated with decreased overall survival after transplantation ($P = .044$, 1-year rate of overall survival: 70% versus 84.1%). We conclude that all pediatric transplant recipients should be screened for vitamin D deficiency before HSCT and at day 100 post-transplant and that aggressive supplementation is needed to maintain sufficient levels.

INTRODUCTION

Vitamin D has important endocrine functions, such as modifying absorption of dietary calcium and regulating bone homeostasis. Severe dietary vitamin D deficiency manifests as rickets in children. In children, the bone matrix is poorly mineralized, and abnormal chondrocyte maturation leads to the classical bowing of the legs described in rachitic children, together with widened epiphyseal plates at the end of long bones and costochondral junctions, frontal bossing of the skull, and delayed tooth eruption [1]. In contrast, adults have sufficient mineral in the long bones and epiphyseal plates are closed, so there are no visible skeletal abnormalities but rather manifestations of osteomalacia. The unmineralized matrix beneath the periosteal membrane, which is heavily innervated with sensory fibers, is hydrated and pushed upward, often causing throbbing, aching bone pain. These data support possible important differences in clinical manifestations of vitamin D deficiency between children and adults.

Studies have indicated widespread vitamin D deficiency or insufficiency in the US population, perhaps because of inadequate dietary intake, sedentary lifestyles, and reduced

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* Correspondence and reprint requests: Stella M. Davies, MBBS, PhD, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229.
E-mail address: stella.davies@cchmc.org (S.M. Davies).

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sun exposure [1,2]. Understanding the true prevalence of clinically significant vitamin D deficiency is complicated by changing views on optimal vitamin D levels. There is broad agreement that levels less than 20 ng/mL indicate deficiency, and many consider levels below 30 ng/mL insufficient [1,3,4]. However, some other authors argue that levels above 50 or 75 ng/mL are optimal, based at least in part on the serum vitamin D level above which no further suppression of parathyroid hormone occurs [2,4,5].

Vitamin D is stored in the body as 25-(OH)-vitamin D, an inactive form that is activated enzymatically to 1,25 (OH) vitamin D in the kidneys, an activity that is impaired in significant renal dysfunction. The same enzyme (25-hydroxyvitamin D-1-hydroxylase) activity that activates vitamin D in the kidney is present in other tissues, including breast, prostate, brain, activated T cells, and antigen-presenting cells such as activated macrophages. The binding of 1,25 (OH) vitamin D to the nuclear vitamin D receptor in macrophages, dendritic cells, and T cells increases expression of cathelicidin, promoting innate immune responses and destruction of infective agents [6,7]. There is also likely secretion of 1,25 (OH) vitamin D, acting locally on T and B lymphocytes. Vitamin D receptor stimulation leads to modification of cytokine secretion, through direct interaction with vitamin D responsive promoter elements and perhaps indirectly through interaction with other transcription factors [8]. These data support potential important effects of vitamin D deficiency on immune recovery and perhaps on graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation (HSCT).

Previous studies of vitamin D deficiency after HSCT have produced conflicting results, with some indicating reduced survival and increased risk of acute or chronic GVHD and some finding no impact on some or all of these endpoints. Few studies have studied vitamin D levels patients longitudinally, and most studies examined adults and not children. Our center focuses on children with genetic disorders who generally have not had prior chemotherapy, so we hypothesized that the frequency of vitamin D deficiency might be lower in our patient population. Moreover, approximately half of our children receive reduced-intensity conditioning regimens that we hypothesized would be less likely to be associated with vitamin D deficiency. In contrast to these hypotheses, we found a very high frequency of vitamin D deficiency before transplant and found that many children remain or become vitamin D deficient during the first 100 days after transplant.

METHODS

Patients and Transplant Procedures

Patient and transplant characteristics are summarized in Table 1. Children in this study were enrolled in 1 of 2 prospective cohort studies, the first a biomarkers study for thrombotic microangiopathy and the second the Cincinnati Children's Hospital and Medical Center BMT repository. All consecutive participants in these 2 studies were eligible to participate and had signed consent for sample collection for biological studies of complications of transplantation. The institutional review board of Cincinnati Children's Hospital Medical Center approved the study.

Median patient age was 7.1 years, and most patients were transplanted for a nonmalignant disease. Most recipients were white, and almost half received a reduced-intensity preparative regimen. Most donors were unrelated adult donors, and most grafts were bone marrow. Clinical data were abstracted from the transplant database and verified by chart review if needed. Registered dieticians with experience in HSCT oversaw dietary issues in all children, but routine measurements of vitamin D were not made without clinical indications. If a clinical indication arose and vitamin D deficiency or insufficiency were identified, supplementation with recommended daily doses were prescribed.

Table 1
Patient (N = 134) and Transplant Demographics

Characteristic	Value
Male/female	87/47
Mean age, yr (range)	7.1 (2.7-14.9)
Diagnosis	
Bone marrow failure	36 (27%)
Immune deficiency	52 (38.8%)
Malignancy	38 (28.2%)
Genetic/metabolic	8 (6%)
Race	
White	116 (86.6%)
Nonwhite	18 (13.4%)
Conditioning regimen	
Myeloablative	73 (54.4%)
Reduced intensity	61 (45.5%)
Donor type	
Related	40 (29.8%)
Unrelated	94 (70.2%)
Stem cell source	
Bone marrow	108 (80.6%)
PBSC	16 (11.9%)
Cord blood	10 (7.5%)
HLA matching	
8/8 Allele matched	108 (80.6%)
Mismatched	26 (19.4%)

PBSC indicates peripheral blood stem cell.

Dietary Management and Supportive Care during HSCT

A registered dietician assessed and followed all patients undergoing HSCT. Patients were allowed to eat normally while following the dietary restrictions of the low bacteria diet. If unable to consume adequate calories, patients were started on enteral feeding using an age-appropriate formula. Parenteral nutrition was provided to children unable to tolerate enteral nutrition. Vitamin and mineral supplementation were provided on an as-needed basis. Antifungal prophylaxis was primarily voriconazole, with pharmacogenetically assigned drug dosing, adjusted pharmacokinetically to maintain therapeutic levels. Children with contraindication or intolerance to voriconazole were treated with micafungin.

Vitamin D Analysis by Ultra-High-Performance Liquid Chromatography Coupled to Electrospray Tandem Mass Spectrometry

Serum samples were collected prospectively on consenting HSCT recipients less than 18 years old receiving their first HSCT, and samples were stored at −80°C until analyzed. Human serum concentrations of vitamin D (vitamin D_2 and D_3) and 25-hydroxyvitamin D (25-OH D_2 and 25-OH-D_3) were determined by ultra-high-performance liquid chromatography coupled to electrospray tandem mass spectrometry (Waters, Milford, MA) [9]. Serum samples were extracted by liquid–liquid extraction with methyl tert-butyl ether/ethyl acetate/hexane (5:4:1). Combined extracts were dried and derived by 4-phenyl-1,2,4-thriazoline-3,5-dione before transfer to sample vials. Quantification was conducted with multiple reaction monitoring and with a stable isotope dilution ultra-high-performance liquid chromatography coupled to electrospray tandem mass spectrometry method on a Supelcosil LC-18-DB column (33 × 3 mm, 3 μm; Sigma, St. Louis, MO). A vitamin D level < 30 ng/mL was defined as vitamin D insufficiency, and a vitamin D level < 20 ng/mL was defined as vitamin D deficiency.

Statistical Analysis

Continuous and categorical variables were compared using Wilcoxon rank sum test and Fisher's exact test, respectively. Survival and cumulative incidence curves, percentages, and standard errors were computed using the Kaplan-Meier method. Survival between groups was assessed using log rank tests. For other time to event data, Gray's method for competing risks was used. Death and relapse (for GVHD) were treated as competing risks. Cox proportional hazards was used for the multivariable survival models. A backward selection model was used, including all variables mentioned in the demographic table, but only significant variables were retained in the final model. All statistical computations were performed using R software (version 3.0.1; www.r-project.org). Statistical significance was determined at the .05 level.