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Vitamin D Levels Affect Outcome in Pediatric Hematopoietic Stem Cell Transplantation

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A B S T R A C T

The importance of vitamin D in immunologic processes has recently emerged, but whether it has any impact on the course of allogeneic hematopoietic stem cell transplantation (HSCT) has not been determined. Reports indicate that HSCT recipients, particularly children, often suffer from vitamin D deficiency. This study investigated the role of vitamin D in 123 children undergoing HSCT from 2004 to 2011. Vitamin D (ie, serum calcidiol) was analyzed in collected cryostored samples. Patients were grouped according to pre-HSCT calcidiol level: insufficient (<50 nm/L, n = 38) and sufficient (≥50 nm/L, n = 85). Older children who underwent transplants from January through June and children of Middle Eastern or African origin were more commonly found in the insufficient group. Acute grades II to IV graft-versus-host disease occurred more frequently in the vitamin D sufficient group (47% versus 30%, $P = .05$), whereas no difference was demonstrated for chronic graft-versus-host disease. The neutrophil granulocytes rose significantly faster in the vitamin D sufficient group. No difference in lymphocyte counts, immunoglobulin levels, or infectious disease burden during the first year post-HSCT were observed. Among children with malignancies, overall survival was significantly better in the sufficient group (87% versus 50%, $P = .01$). In addition, rejection (0% versus 11%, $P = .06$) and relapse (4% versus 33%, $P = .03$) rates were lower in patients with sufficient vitamin D levels. To conclude, vitamin D may have an important impact on the outcome of pediatric HSCT, particularly in patients with malignant disease. Further studies investigating whether vitamin D acts as an immunomodulator or is merely a surrogate marker of patient health or nutritional status are warranted.

INTRODUCTION

The importance of micronutrients, including vitamin D, in immunologic processes has emerged in recent years. An association between vitamin D insufficiency and a variety of infections (eg, tuberculosis and respiratory tract infections) has been reported [1,2], along with the biologic effects of vitamin D beyond its hormonal activity in calcium homeostasis, including a role in both innate and adaptive immune responses [3-5].

Whether vitamin D status affects the course or outcome of allogeneic hematopoietic stem cell transplantation (HSCT) has not been fully established. Previous data suggest that vitamin D might prevent graft-versus-host disease (GVHD). Vitamin D exposure also resulted in immature dendritic cell populations with bias toward tolerizing rather than stimulatory T-lymphocyte populations, which could provide a possible mechanism for its beneficial effects against GVHD [6,7]. Humans attain vitamin D mainly from exposure to sunlight and to a lesser extent from dietary sources. After HSCT, children's exposure to direct sunlight is restricted, and they might also suffer from poor nutrition and malabsorption due to HSCT-related side effects. An increased catabolism because of the use of glucocorticoids and other immunesuppressants could also affect vitamin D levels in serum [8]. Low vitamin D levels in children undergoing HSCT have been reported, but these data were not related to clinical outcome [9].

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The optimal serum levels of vitamin D have not been precisely defined given uncertainties regarding the relationship between vitamin D levels and various clinical endpoints. A sufficient level of vitamin D can be defined as serum calcidiol ≥50 nmol/L, because this level is believed to cover the needs of 97.5% of the population [10]. The role of vitamin D in the context of pediatric HSCT remains elusive, and therefore the main objective of this study was to evaluate whether vitamin D levels were associated with short- and long-term outcome parameters in pediatric HSCT.

METHODS

Patients

Between June 2004 and December 2011, 163 pediatric patients underwent HSCT at our center at the Karolinska University Hospital in Huddinge, Sweden. Of these, 123 were included in this study (40 patients were excluded because of a lack of follow-up of study-specific samples). Patients were followed for up to 8 years after HSCT. Patients with hematologic malignancies as well as nonmalignant conditions (ie, benign hematologic, metabolic, and primary immunodeficiency disorders) were included (Table 1). The population was 67% male, and the ethnic composition was 75% European, 7% African, 15% Middle Eastern, and 3% of other ethnic groups. The study was approved by the Regional Ethical Review Board in Stockholm.

Table 1
Patient Characteristics (N = 123)

Characteristic	Calcidiol at baseline		*P*
	<50 nM (n = 85)	≥50 nM (n = 38)	
Age, yr, median (range)	10 (0-19)	5 (0-15)	.03
Sex (male/female), n	57 (67%)/28 (33%)	26 (68%)/12 (32%)	
BMI, median (range)	18.5 (12.0-45.1)	17.0 (13.1-26.7)	<.01
Ethnic origin, n			
European	57 (67%)	34 (89%)	.008
African	9 (11%)	0	<.0001
Middle Eastern	16 (19%)	3 (8%)	
Other	3 (4%)	1 (3%)	
Diagnosis, n			
Nonmalignant	42 (49%)	15 (39%)	
AML/ALL	8/22 (9%/26%)	2/6 (5%/16%)	
CML	3 (4%)	0	
Lymphoma	3 (4%)	0	
MDS	7 (8%)	13 (34%)	<.001
Other	0	2 (5%)	
Disease stage Early/late,* n	58 (68%)/27 (32%)	20 (53%)/18 (47%)	.10
Conditioning, n			
fTBI + Cy	26 (31%)	7 (18%)	
Bu + Cy	30 (35%)	19 (50%)	
Flu + Bu	2 (2%)	1 (3%)	
Flu + Cy	7 (8%)	7 (18%)	
Flu + fTBI + Cy	4 (5%)	1 (3%)	
Flu + Treo	16 (19%)	3 (8%)	
ATG	67 (79%)	29 (76%)	
Donor			
Age, yr (range)	22 (0-55)	22 (0-46)	
Female to male, n	20 (24%)	8 (21%)	
MRD, n	29 (34%)	14 (37%)	
MUD, n	38 (45%)	19 (50%)	
MM, n	18 (21%)	5 (13%)	
Stem cell source, n			
Bone marrow	55 (65%)	29 (76%)	
PBSC	16 (19%)	5 (13%)	
Cord blood	14 (16%)	4 (11%)	
Cell dose			
Nucleated cells, × 10^8/kg	4.1 (.3-37)	5.2 (.4-34)	
CD34 cells, × 10^6/kg	4.8 (.1-44)	5.5 (.1-15)	
GVHD prophylaxis, n			
CsA + MTX	52 (61%)	29 (76%)	
CsA + prednisolone	12 (14%)	5 (13%)	
Tacrolimus + sirolimus	18 (21%)	3 (8%)	
Other	3 (3.5%)	1 (3%)	
aGVHD, n			
None	46 (54%)	15 (39%)	
Grade I	14 (16%)	5 (13%)	
Grades II-IV	25 (29%)	18 (47%)	
cGvHD, yes/no	11/73	6/32	
IgG replacement, n	48 (56%)	29 (76%)	
Vitamin D substitution, n	25 (29%)	16 (42%)	
Lansky/Karnofsky score,† mean (range)	86 (60-100)	88 (50-100)	.04

BMI indicates body mass index; AML, acute myeloid leukemia; ALL, acute lymphatic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; fTBI, fractionated total body irradiation; Cy, cyclophosphamide; Bu, busulfan; Flu, fludarabine; Treo, treosulfan; ATG, antithymocyte globuline serotherapy; MRD, HLA-matched related donor; MUD, HLA-matched unrelated donor; MM, HLA-mismatched donor; PBSC, peripheral blood stem cells; CsA, cyclosporine A; MTX, methotrexate.

* Early = first complete remission/first chronic phase and nonmalignant.

† Lansky scale were used for children <16 and Karnofsky scale for those ≥16 years.

HSCT Procedure

The HSCT procedure and conditioning regimens were published previously [11], and treatment data are presented in Table 1. Myeloablative conditioning was applied in 82 transplants (67%). Mainly, cyclosporine A and methotrexate (n = 81, 66%) were used as GVHD prophylaxis. Other patients received cyclosporine A and prednisolone (n = 17, 14%), tacrolimus and sirolimus (n = 21, 17%), or other combinations (n = 4, 3%). Antithymocyte globulin serotherapy was given to patients (n = 96, 78%) with unrelated donors or nonmalignant disorders. Cotrimoxazole was used as *Pneumocystis jiroveci* prophylaxis. In patients who developed hypogammaglobulinemia (ie, plasma IgG < 4 g/L) post-HSCT, IgG substitution was applied as previously described [12].

Deficiencies in vitamin D levels were neither assessed nor treated routinely. However, some children received vitamin D substitution because of the following conditions: age <2 years, seizures, nephrologic disease, or deteriorated calcium levels.

Data Collection

Data on each patient were obtained from medical records and patient databases. The collected data included basic parameters (eg, sex, age at transplantation, ethnicity), transplant characteristics (eg, diagnosis, conditioning, GVHD prophylaxis, donor source, cell dose), and outcome parameters (eg, survival, rejection, relapse, number of infections, GVHD). Data on vitamin D substitution (daily doses and length of treatment), immunoglobulin substitution (number of treatments), and total parenteral nutrition (length of treatment) were extracted from medication administration records. To verify the reproducibility of the abstracted data, 10 cases were selected randomly and reabstracted by different researchers. Complete agreement between the abstractions was confirmed.

Outcome Data

Concentration of calcidiol (25-OH vitamin D3) in serum is widely regarded as the optimal indicator of vitamin D status. In this study vitamin D insufficiency, also referred to as hypovitaminosis D, was defined as a calcidiol level <50 nmol/L at baseline (ie, the first sample, before the start of conditioning regimen) [10].

Chosen endpoints in this study were as follows: *presence of acute GVHD* (aGVHD; grades I to IV following international criteria [13]), *presence of chronic GVHD* (cGVHD; defined by international criteria [14]), *immune recovery* (lymphocyte and neutrophil granulocyte counts and measurements of immunoglobulins), *presence of infections* (as explained below), *overall survival* (OS), *rejection* (defined as progressive mixed chimerism reaching >95% recipient-derived cells in the lineage of interest), and *relapse* (defined by criteria in current protocols from the Nordic Society of Pediatric Hematology and Oncology [www.nopho.org]).

Infections were recorded if they were registered in the patient's medical record by a physician. Bacterial and fungal infections required that positive cultures were recorded. Viral infections required positive diagnostics unless the clinical presentation was typical (eg, seasonal viral gastroenteritis). Documented infections that could not be classified as bacterial, viral, or fungal were denominated as other infections. Reactivation of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) was distinguished from other viral infections and defined as 1000 viral DNA copies/mL in serum and in whole blood. Treatment was initiated when viral DNA copies reached 2000 and 10,000 DNA copies/mL for CMV and EBV, respectively.

Clinical Chemistry

Full blood counts and immunoglobulin levels were obtained from the medical records. Serum samples were collected prospectively before the start of the conditioning regimen and post-HSCT (+3, 6, and 12 months), and these were cryostored at −20°C for analysis of total calcidiol by a competitive chemiluminescence assay (Liaison XL; DiaSorin Inc., Stillwater, MN). All laboratory procedures were carried out using accredited methodology at the Department of Clinical Chemistry in the Karolinska University Laboratory, Karolinska University Hospital. The accrediting authority was the Swedish Board for Accreditation and Conformity Assessment (Swedac).

Statistics

Continuous variables and proportions were compared using the Mann-Whitney U-test and the chi-square test, respectively. GVHD, relapse, and rejection were estimated using an estimator of cumulative incidence curves, taking competing events into consideration [15,16]. The Cox regression method was used in the predictive analysis for OS and relapse-free survival, whereas the method outlined by Fine and Gray [17] was used for predictive analysis regarding GVHD and relapse. Factors included were donor and recipient age, donor type, HLA match, disease stage (ie, early represents first complete remission/first chronic phase and nonmalignant, whereas late represents other cases), GVHD prophylaxis, nucleated cell dose, $CD34^+$ cell dose, stem cell source, reduced-intensity conditioning versus myeloablative conditioning, total body irradiation versus no total body irradiation, antithymocyte globulin versus no antithymocyte globulin, European versus non-European origin, and calcidiol level. Statistical significance was set at $P < .05$. Analysis was performed using the cmprsk software package (The R Project for Statistical Computing, http://www.r-project.org), S-Plus 6.2 software (TIBCO Software, Inc., Boston, MA), and Statistica software (StatSoft, Inc., Tulsa, OK).

RESULTS

Vitamin D Levels and Demographics

Patients were divided into 2 cohorts based on calcidiol level at baseline (ie, pre-HSCT): low level (<50 nmol/L) and sufficient level (≥50 nmol/L). The mean calcidiol levels at baseline were 33 nmol/L (range, 13 to 49) in the low level group and 63 nmol/L (range, 50 to 97) in the sufficient level group. Calcidiol levels remained significantly higher in the sufficient level group at least until the 6-month post-HSCT (Figure 1A). No significant association could be found between sample storage time and calcidiol levels (data not shown).

Patients with low levels were older than patients with sufficient levels (mean, 10 versus 5 years; $P = .025$). More patients also had myelodysplastic syndrome with sufficient calcidiol levels ($P < .001$). No differences in other characteristics were seen between the groups (Table 1). The season of

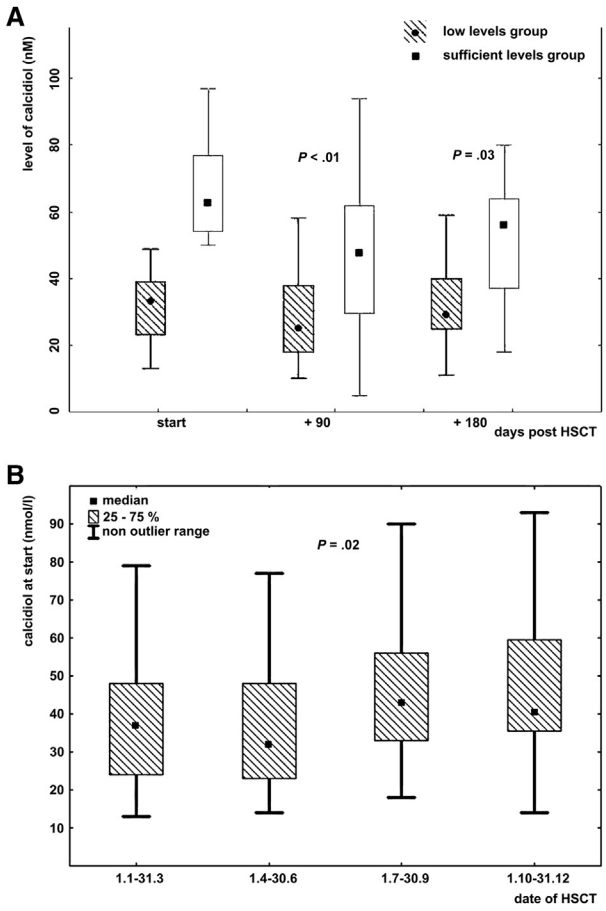

Figure 1. (A) Levels of calcidiol remained significantly higher in the group that had sufficient levels before HSCT (baseline >50 nmol/L) during the first 6 months of post-HSCT follow-up. (B) Time of year at transplantation had a significant impact on levels of calcidiol at baseline: patients who underwent transplants in the first 2 quarters of the year had significantly lower levels than those in the 2 last quarters.

transplantation had a significant impact on calcidiol levels: patients who underwent transplants from January through June had significantly lower levels than patients who had transplants in July through December ($P = .002$; Figure 1B).

The distribution of ethnic origins differed between the groups (Table 1). Patients of African descent all had low calcidiol levels (11% versus 0%, $P < .0001$), whereas patients with European descent were less prone to be deficient (67% versus 89%, $P = .008$).

Vitamin D Levels, GVHD, and Immune Recovery

aGVHD occurred in 62 patients, corresponding to 50% of the patients, of which 43 patients (35%) suffered from moderate to severe aGVHD (grades II to IV). In the sufficient calcidiol baseline level group, moderate to severe aGVHD occurred more frequently than in the low baseline group (47% versus 30%, $P = .05$; Figure 2A). Multivariate analysis revealed that late disease stage was associated with grades II to IV aGVHD in malignancies, whereas sufficient calcidiol levels at baseline displayed a trend toward statistical significance (Table 2). cGVHD occurred in 17 patients (14%), with no significant difference between the low and sufficient calcidiol levels groups. However, comparing patients with moderate to severe cGVHD with those without cGVHD revealed a significant difference in calcidiol levels at 6 months post-HSCT (23 nmol/L [range, 18 to 24] versus 37 nmol/L [range, 10 to 80], $P = .004$).

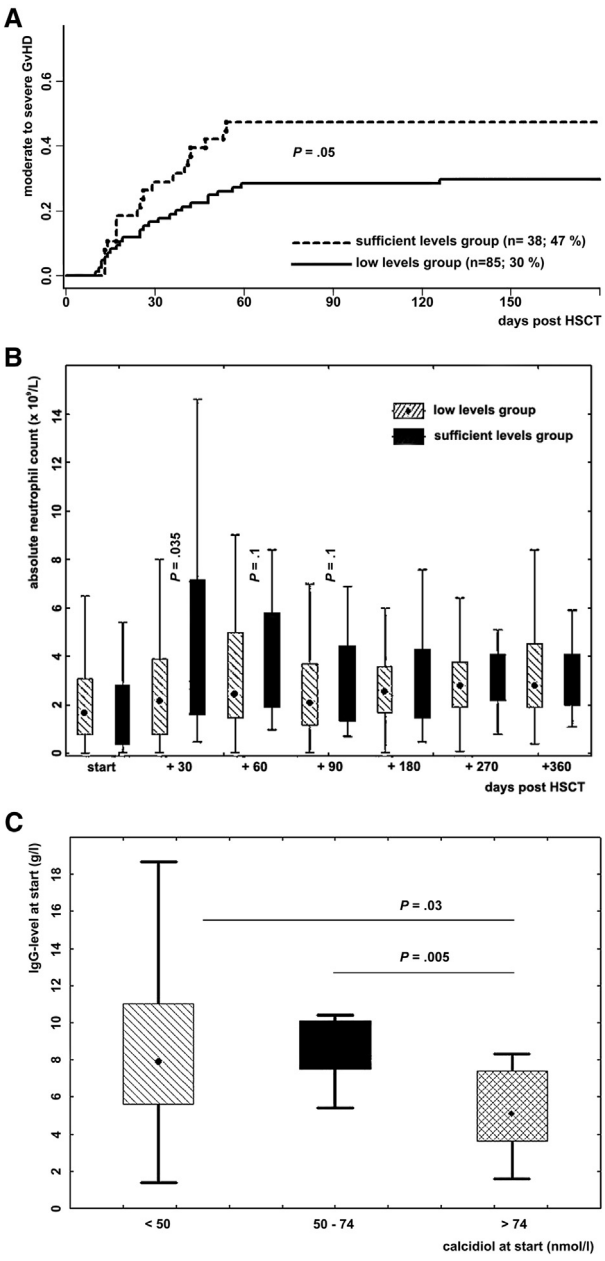

Figure 2. (A) Moderate to severe aGVHD (grades II to IV) occurred more commonly among patients with sufficient levels of calcidiol at baseline (>50 nmol/L). (B) Absolute neutrophil count rose faster during the first 3 months post-HSCT among patients with sufficient levels of calcidiol at baseline. (C) Levels of IgG were significantly lower in a group with calcidiol above 75 nmol/L at baseline than in other groups.

Neutrophil granulocytes (measured as absolute neutrophil count) rose significantly faster during the first 3 months post-HSCT among patients with sufficient calcidiol levels at baseline. During the next months no significant difference was observed (Figure 2B). Baseline calcidiol levels had no significant impact on lymphocytes or immunoglobulin levels (IgG, IgM, and IgA) post-HSCT. Only when comparing a group of patients with the highest baseline levels of calcidiol (>75 nmol/L, n = 11) with the insufficient group or a middle group (with baseline calcidiol levels 50 to 74 nmol/L, n = 27) were significantly lower IgG levels at HSCT observed (Figure 2C). However, this difference in IgG levels did not remain over time post-HSCT. IgG substitution was more common in the calcidiol sufficient group (76% versus 56%, $P = .04$).

Vitamin D and Infections

The number of observed clinical infections did not differ significantly between groups. In both groups most patients suffered from 1 to 4 infections during the first year post-HSCT. Data on specific viral infections did not show significant differences other than lower incidence of herpes simplex virus and varicella zoster virus at 3 months post-HSCT in the sufficient level group (35% versus 27%, $P = .05$ and 37% versus 25%, $P = .04$, respectively).

The number of patients reactivating CMV or requiring treatment against CMV did not differ between the low level and the sufficient level group (29% versus 34% and 22% versus 26%, respectively). Likewise, the number of patients reactivating EBV or requiring treatment against EBV did not differ between the groups (21% versus 18% and 9% versus 8%,

Table 2
Risk Factor Analysis* for aGVHD, Mortality, Relapse, and Transplant Failure

	HR	95% CI	*P*
aGvHD grades II-IV, all			
Calcidiol > 50 nmol/L	1.72	.96-3.13	.065
Late disease	2.59	1.42-4.71	.002
Mortality, malignancies			
Calcidiol > 50 nmol/L	.15	.04-.57	.005
URD	3.77	1.11-12.8	.03
MDS	2.93	1.09-7.88	.03
Relapse, malignancies			
Calcidiol > 50 nmol/L	.08	.01-.63	.02
Transplant failure,† malignancies			
Calcidiol > 50 nmol/L	.14	.04-.50	.002
URD	3.58	1.23-10.4	.02

HR indicates hazards ratio; 95% CI, 95% confidence interval; Late disease, beyond first complete remission/first chronic phase; URD, unrelated donor.

* Corrected for differences in the characteristics, such as age, stage and myelodysplastic syndrome.

† That is, relapse or death.

respectively). This was true also for patients in whom the serologic EBV status of donor and recipient were mismatched.

Vitamin D and Survival

OS in all patients did not differ significantly in the low level and sufficient level groups (69% versus 87%). Also, OS did not differ between patients with nonmalignant and malignant diseases (87% in both groups). However, in patients with malignant diseases, sufficient calcidiol levels were associated with better OS (87% versus 50%, $P = .01$; Figure 3A).

There was no difference in treatment-related mortality between the groups. In multivariate analysis of patients with malignancies, a baseline calcidiol level >50 nmol/L was associated with better OS, whereas unrelated donor and myelodysplastic syndrome carried an increased risk for mortality (Table 2). Although graft rejection did not occur among patients in the sufficient level group, 11% of patients in the low level group rejected their grafts ($P = .06$).

Relapse was more common among patients in the low level group (33% versus 4%, $P = .03$; Figure 3B). The multivariate regression model found that only the baseline calcidiol level was significantly associated with relapse (protective) (Table 2). Relapse-free survival was significantly better in patients with sufficient levels of calcidiol than among patients with insufficient levels (87% versus 41%, $P = .002$). Factors affecting transplant failure (ie, relapse or death) in multivariate analysis were calcidiol level >50 nmol/L at baseline (protective) and the use of an unrelated donor (risk) (Table 2).

DISCUSSION

The steroid hormone vitamin D is known to affect immunologic processes [18], and, more recently, novel mechanisms of action have been uncovered [3,4,18,19]. A few studies suggest that sufficient vitamin D levels may benefit patients undergoing HSCT [6,7,9]. In the present study we followed 123 pediatric patients for up to 8 years post-HSCT and correlated their baseline vitamin D status with a number of clinical outcomes. This is one of the first reports in which such associations are analyzed in detail.

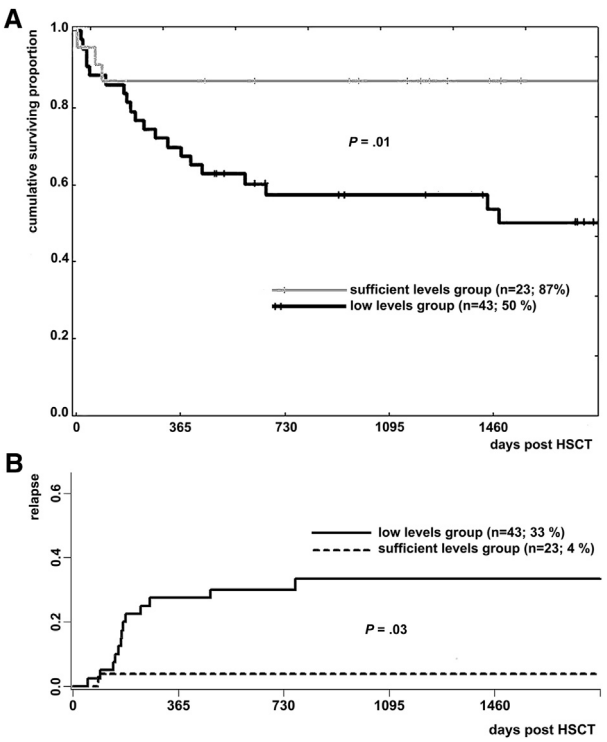

Figure 3. (A) In patients with malignant diseases, sufficient calcidiol levels at baseline (<50 nmol/L) were associated with greater OS. (B) Relapse was less common among patients with sufficient levels of calcidiol at baseline.

We chose to define hypovitaminosis D as baseline calcidiol (25[OH]-vitamin D) levels in serum below 50 nmol/L, based on previous reports [12-14]. To date, consensus regarding sufficient levels is not established, but 75 nmol/L or higher is commonly referred to as sufficient in both children and adults. Most recommendations are based on studies of vitamin D and bone health; however, levels for optimal influence on immunologic processes might be even higher [20].

Young children, institutionalized patients, and non-Western immigrants are usually regarded as risk groups for hypovitaminosis D [21], but up to one-third of American children and adolescents are also vitamin D deficient with seasonal variation [20,22]. In adult HSCT patients, numbers are even higher; 70% to 89% of adult HSCT patients are vitamin D deficient before transplantation [23,24]. Duncan et al. [9] followed 67 pediatric HSCT patients and found that 37% were deficient at baseline, but in the subgroup of patients ≥11 years old, 67% were deficient, a figure that accords with our findings (69%). HSCT patients are more prone to develop hypovitaminosis D as a result of sun avoidance, hospitalization, corticosteroid treatment, and diminished intestinal uptake affected by GVHD and bacterial overgrowth [9,25-27]. The lowest baseline levels in our study were found in patients who underwent transplants during winter, spring, and early summer—that is, before opportunities for sun exposure in northern latitudes become plentiful enough to raise serum levels. In brief, our patients with insufficient baseline levels were older, more often of non-European origin, and underwent transplants during winter and spring, when vitamin D levels tend to be low. The groups did not differ significantly regarding HSCT parameters (eg, conditioning, donor, stem cell source). Comparable and diverging results were reported by others [9,24,25,28] and might be explained by study population differences. However, it is clear that certain patients are at risk for developing hypovitaminosis D that easily can be treated.

Because GVHD is the major obstacle to successful HSCT, great efforts are undertaken to understand and modify this complication. T-lymphocyte responses, which can be modified by vitamin D, are central in the GVHD pathogenesis. Pakkala et al. [29] showed that a vitamin D analog decreased signs of aGVHD in mice, probably by down-regulation of both T-lymphocyte activation and inflammatory effector mechanisms. In vitro studies have shown that an active vitamin D dose dependently inhibits mixed lymphocyte cultures [30] and affects dendritic cell maturation, resulting in a T helper cell type 2 polarized T-lymphocyte population [6]. Active vitamin D also alters dendritic cell surface phenotype and morphology, which may compromise contacts between dendritic cell and T lymphocytes and thereby diminish interaction and T-lymphocyte cytokine secretion [31]. Together, such conditions would lead to tolerizing rather than to stimulated T-lymphocyte populations.

These findings serve as a rationale for treating or preventing GVHD by upholding normal levels of vitamin D. Rosenblatt et al. reported 2 cases of adult patients suffering from corticosteroid-refractory GVHD in which improvement

occurred when vitamin D levels were corrected [6]. Contradicting this, the frequency of aGVHD was higher and that of cGVHD lower in patients with sufficient calcidiol levels in our material. Our finding emphasizes the different pathogenesis of the 2 forms of GVHD but may also underline the fact that vitamin D has dual roles—both immunostimulatory and immunoinhibitory. The linkage between vitamin D levels and cGVHD is supported by observations in adult HSCT, becasue low pre-HSCT vitamin D levels were a significant factor associated with cGVHD. Patients receiving vitamin D substitution post-HSCT suffered fewer cGVHD relapses and could more often be taken off immunosuppressive treatment [7,32]. We found that calcidiol levels were significantly lower in patients with moderate to severe cGVHD at 6 months post-HSCT than they were in patients without cGVHD or with mild disease ($P = .004$ and $P = .005$, respectively). The difference can partially be explained by an impaired nutritional status and lower sun exposure among the severely ill patients, but because low levels of calcidiol in this context are unfavorable, substitution with cholecalciferol may be considered.

Systemic or locally produced calcitriol ($1,25[OH]_2$-vitamin D) affects several types of immune cells. In addition to acting as an inhibitor on parts of the adaptive immune system, active vitamin D can stimulate monocyte proliferation and enhance synthesis of the antibacterial peptide LL-37 (human cathelicidin) [3,4,33]. The effect of vitamin D on immune reconstitution after HSCT is not well described. In our material, neutrophil counts were significantly higher during the first 3 months post-HSCT in the sufficient level group. This might be interpreted as a vitamin D–mediated stimulatory effect in the early phases of immune recovery. Moreover, aGVHD appears during this period, and, as described above, higher calcidiol levels were associated with an increased frequency of aGVHD. The difference in neutrophil was, however, not sustained during the later phase of follow-up. Calcitriol has been shown to suppress B-lymphocyte proliferation, plasma cell differentiation, and IgG secretion [3,4], a finding that aligns with our own. Patients with the highest levels of calcidiol at baseline had the lowest IgG levels at follow-up and more commonly needed IgG replacement. However, these patients tended to be younger, which may be why the physiologic variation in IgG levels might influence the finding. A faster normalization of immune cell counts might have implications for infection susceptibility, but no significant impact was discerned in our material. One reason for this could be the overall relatively low baseline calcidiol levels, because protective effects on infection susceptibility might not be seen unless levels of calcidiol are well above 75 nmol/L [7,19].

In the patient group as a whole, OS did not differ between groups, probably because of the favorable survival rate in patients with nonmalignant disease. However, in patients with malignancies, OS was significantly higher in the group that had sufficient baseline calcidiol levels. This finding is at odds with data from another recent study, however, considering adult patients and no significant difference related to vitamin D levels was observed [32]. The difference in OS between the 2 groups in our study was mainly due to a significantly increased relapse rate in the insufficient level group, suggesting that vitamin D played a beneficial role. Thus, the combination of better OS and less frequent relapse may suggest that vitamin D exerts immunostimulatory effects, mediating the expansion of alloreactive cells, including those with a capacity to kill malignant cells (ie, graft-versus-leukemia reaction). An alternative explanation concerning the apparent effect on OS might be that calcidiol levels constitute a surrogate marker for patient well-being and overall health status immediately before HSCT. However, there was no difference in pre-HSCT Lansky/Karnofsky sores, and in the multivariate analysis, sufficient calcidiol levels remained significantly associated with better OS, which supports a true association. Rejection occurred only among patients in the insufficient level group in our material. This also suggests a beneficial role for vitamin D in allograft survival. In analogy with the hypothesis of OS and relapse, higher vitamin D levels might stimulate the expansion of alloreactive cells killing recipient cells, giving rise to full donor chimerism development. These findings merit further experimental studies, as well as clinical trials in larger patient cohorts.

As in all retrospective studies, our data also have to be interpreted with caution. There are a restricted number of patients with uneven distributions between the groups, and, as mentioned above, there is no general consensus regarding the definition of hypovitaminosis D. However, there was consistency in how the patients were cared for (ie, single intuition with a solid medical team), and the distribution of hypovitaminos D cases are interesting per se.

We conclude that vitamin D levels seem to affect the clinical course of children undergoing HSCT. Hypovitaminosis D was associated with slower recovery of neutrophil granulocyte counts and increased risk of death, relapse, and cGVHD. aGVHD, however, occurred more frequently among patients with sufficient baseline levels of vitamin D, whereas treatment-related mortality was not affected. Effects on infection rates were not obvious. However, because observational studies cannot indicate causality, further studies—preferably with an interventional design—are needed to establish the ways that vitamin D, as well as other micronutrients, influence immunologic processes in HSCT and subsequent outcomes.

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