

# Low plasma concentrations of retinol and $\alpha$ -tocopherol in hematopoietic stem cell transplant recipients: the effect of mucositis and the risk of infection<sup>1-4</sup>

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## ABSTRACT

**Background:** Although vitamin deficiencies are rare in the United States, acute reductions in concentrations of plasma retinol (vitamin A) or  $\alpha$ -tocopherol (vitamin E) have been associated with impaired immune responses in some clinical settings.

**Objective:** The objectives were to determine the plasma concentrations of retinol and  $\alpha$ -tocopherol in patients undergoing dose-intensive therapy and hematopoietic stem cell transplant and to examine the association of plasma concentrations with clinical outcomes reflecting immunity.

**Design:** This was an observational trial of 120 consecutive recipients of hematopoietic stem cell transplant and a multivariate analysis of plasma vitamin concentrations, mucositis, infections in the first 30 d, and herpes zoster infections in the first year after hematopoietic stem cell transplant.

**Results:** Plasma retinol and  $\alpha$ -tocopherol concentrations declined from baseline to day 7, typically recovering without specific replacement toward baseline by day 14. The severity of mucositis was a strong predictor of low plasma retinol on day 7 ( $P = 0.001$ ). Eighty-two patients (68%) had at least one plasma retinol concentration  $\leq 1.05 \mu\text{mol/L}$ , a concentration previously determined to be of immunologic significance, during the peritransplant period (day -8 to day 14). Men more frequently acquired herpes zoster than women, and men who developed hyporetinolemia ( $\leq 1.05 \mu\text{mol/L}$ ) had a significantly higher risk of herpes zoster (OR: 6.6; 95% CI: 1.5, 29.6). Plasma  $\alpha$ -tocopherol was not associated with any clinical event measured in this study.

**Conclusion:** Hyporetinolemia is common, particularly in subjects with severe mucositis, and is associated with an increased risk of herpes zoster infection in recipients of hematopoietic stem cell transplant. Additional investigations are required to determine whether these findings indicate a causal relation. *Am J Clin Nutr* 2002;76:1358-66.

**KEY WORDS** Retinol, tocopherol, hematopoietic stem cell transplantation, mucositis, infection, herpes zoster

## INTRODUCTION

Despite major advances in the prevention and treatment of cancer, aggressive chemotherapy and radiotherapy remain important treatment modalities for many patients with advanced cancer. These approaches have a narrow therapeutic index, creating significant morbidity and even mortality. The most extreme example is dose-intensive therapy (DIT), followed by hematopoietic stem cell

transplant [HSCT (ie, rescue by transfusion of bone marrow, peripheral blood stem cells, or both)]. DIT is associated with significant toxicity, including mucositis, pancytopenia, and infection (1-3). Some of these toxicities have been hypothesized to result from oxidative damage to normal tissues, perhaps in part because of the consumption of antioxidant compounds (4-6). Others investigated plasma antioxidant concentrations in patients undergoing DIT and HSCT and found significant declines in circulating  $\beta$ -carotene and  $\alpha$ -tocopherol (vitamin E), but no effect on retinol (vitamin A) concentrations (4, 7, 8). However, those studies included few patients, measured antioxidant concentrations only at baseline and on days 0 and 12, and did not attempt to correlate plasma vitamin concentrations with clinical outcomes.

Deficits in innate immunity (primarily mucositis and neutropenia) are present in essentially 100% of patients after DIT, but rapidly resolve within 10-30 d after HSCT. In contrast, adaptive immune deficits (T cell-mediated responses) occur after engraftment and persist for 1-2 y [often longer in the presence of graft versus host disease (GVHD)] (9,10). Retinol is particularly likely to contribute to the functional competence and maturation of the immune system after HSCT. Mucositis is a major risk factor for infection, increased length of stay in hospital, and mortality in HSCT recipients (11), and retinol is a critical component for epithelial cell regeneration (12). Furthermore, retinol is essential for appropriate development and maturation of many tissues, including hematopoietic and immune cells (13-15). Vitamin E ( $\alpha$ -tocopherol) is an antioxidant that may limit oxidative tissue

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damage from oxygen radicals and, thus, reduce the severity of mucositis (4–8).

To further explore these relations, we measured plasma retinol and  $\alpha$ -tocopherol concentrations in 120 patients who underwent DIT and HSCT at the Wake Forest University Baptist Medical Center and determined whether an association existed between plasma retinol or  $\alpha$ -tocopherol and a variety of clinical outcomes that indicate immune competence.

## SUBJECTS AND METHODS

### Subjects

Study subjects were recruited from the Bone Marrow Transplant Program of Wake Forest University Baptist Medical Center. Potential participants were contacted on admission to the bone marrow transplant (BMT) unit. All patients had an underlying diagnosis of leukemia, lymphoma, or breast cancer. A total of 120 patients were recruited over an 18-mo period, and they were followed until death or day 390 after BMT.

All procedures followed were in accord with the ethical standards of Wake Forest University Baptist Medical Center and approved by its Institutional Review Board. All subjects provided written informed consent.

### Preparative regimen, supportive care, and antiinfective prophylaxis

The bone marrow–ablative regimen and the type of transplant did not affect eligibility, but they were recorded as control variables. All subjects received conditioning regimens and supportive care outlined by institutional protocols (including GVHD prophylaxis for allogeneic transplant recipients). No antibacterial (eg, fluoroquinolone) prophylaxis was administered during the HSCT procedure or the period of neutropenia, but fever or neutropenia was treated with intravenous antibacterial medications according to institutional care maps. All patients received trimethoprim and sulfamethoxazole daily as part of the conditioning regimen and twice daily on Saturdays and Sundays for 6 mo after engraftment occurred to prevent *Pneumocystis carinii* infection. All subjects received fluconazole antifungal prophylaxis from the start of conditioning until engraftment (allogeneic transplant recipients continued to receive fluconazole until  $\geq 6$  mo after HSCT). Acyclovir antiviral prophylaxis was given to all herpes simplex virus–seropositive patients until engraftment, but not thereafter unless a clinical illness necessitated reinstitution of antiviral therapy. Cytomegalovirus prophylaxis was given only to recipients of allogeneic HSCT and consisted only of cytomegalovirus hyperimmunoglobulin (ie, no antiviral drugs) given every 3 wk. A daily oral vitamin supplement (of prenatal-vitamin strength) was prescribed for subjects not receiving parenteral nutrition. Total parenteral nutrition was provided to all patients who were unable to support their energy requirements by oral intake. It was supplemented daily with the US recommended dietary allowance for all vitamins, including vitamins A and E, until a national shortage of parenteral multivitamins necessitated thrice-weekly supplementation over the last 6 mo of the study.

### Blood sample collection and processing

Blood samples were collected before DIT (baseline), on the day of HSCT but before the transfusion (day 0), and on days 7 and 14 after HSCT. If patients were discharged before day 14, the day 14

sample was collected on the day of discharge (97% of samples were collected within 72 h of the scheduled day 14 blood sample collection). All blood samples were collected early in the morning, into heparin-containing (green top) tubes that were protected from light; subjects were in the fasted state.

### Measurements of plasma retinol and $\alpha$ -tocopherol concentrations

Plasma was promptly processed in light-protected conditions by centrifugation at  $1000 \times g$  for 10 min at ambient temperature, withdrawn, and stored in amber polypropylene tubes with O-ring-sealed caps at  $-70^\circ\text{C}$  for subsequent HPLC analysis. HPLC analysis took place in the Chromatography Core Laboratory of the Comprehensive Cancer Center of Wake Forest University. Reversed-phase HPLC assays to determine retinol and  $\alpha$ -tocopherol concentrations were performed by standard methods (16–18). All procedures were performed under light-protected conditions. Briefly, a 200- $\mu\text{L}$  aliquot of an ethanolic solution containing internal standards was placed into a 1.8-mL screw-cap glass vial. A 200- $\mu\text{L}$  aliquot of plasma was then added and the mixture was stirred by vortex mixing. One milliliter of hexane containing 50  $\mu\text{g}$  2,6-di-*t*-butyl-4-methylphenol/ $\mu\text{L}$  was added, and the mixture was purged with nitrogen and vigorously stirred by vortex mixing for 4 min. The mixture was centrifuged at  $2000 \times g$  for 5 min at ambient temperature, and the hexane layer was transferred to another screw-cap vial. After evaporation to dryness, the extract was reconstituted in 75  $\mu\text{L}$  of 1:1 ethanol:ethyl acetate (vol:vol) containing 30  $\mu\text{g}$  2,6-di-*t*-butyl-4-methylphenol/ $\mu\text{L}$  and purged with nitrogen. Samples were stored under light protection on ice until they were injected into the HPLC column (150  $\times$  4.6 mm, Supelcosil LC-18, 5- $\mu\text{m}$  particle size; Supelco Inc, Bellefonte, PA) isocratically eluted with methanol:acetonitrile:methylene chloride (75:20:5) containing 0.05% triethylamine at a flow rate of 1.5 mL/min. A dual ultraviolet–visual light detection system was used. Absorbance at 292 and 295 nm determined the concentration of  $\alpha$ -tocopherol and retinol, respectively. Duplicate samples of plasma used in the HPLC analysis typically varied by  $< 15\%$ , and the mean of the 2 determinations defined the plasma concentration at that time point.

### Clinical indicators of immune competence

Three clinical indicators of innate immune response were evaluated: severity of mucositis (representing integrity of mucous membranes), duration of neutropenia, and documented infection (clinical or microbiologic) in the first 30 d after HSCT. The clinical indicator of adaptive immunity chosen for this study was the reactivation of varicella zoster virus [herpes zoster (HZ)]. The definitions and methods for measuring these clinical outcomes are outlined below.

#### *Mucositis assessment*

Nurses on the BMT unit or the research study coordinator performed a daily assessment of mucosal surfaces to determine the extent of mucositis. The BMT nurses and the research coordinator were specifically trained in the use of the Oral Assessment Score (OAS) instrument of Eilers et al (19). This instrument assesses voice, swallow, lips, tongue, saliva, mucous membranes, gingiva, and teeth, and it has been shown to have excellent inter-rater reliability ( $r = 0.912$ ) (19–21): 1–3 points are given in each of the 8 categories, and total scores range from 8 (normal mucosa) to 24 (most severe mucositis). Severity of mucositis was estimated

by averaging the daily OAS from day 0 to day 7, the period of maximum mucositis in this patient group (19). Only 2 patients had <5 OASs during this time, and those 2 subjects were excluded from the analysis; 93% had >7 OASs.

#### Neutropenia and acute infections

Neutropenia was defined as the period from the first day the total white blood cell count fell below 1000 cells/mm<sup>3</sup> until the total white blood cell count returned to 1000 cells/mm<sup>3</sup> for 2 consecutive days. Documented infections were based on daily assessments by the primary care team and the primary investigator (KPH) who went on rounds weekly with the BMT service. Generally accepted standards and definitions were used for clinical and microbiologic documentation of infection in patients with neutropenia (22).

#### Herpes zoster

HZ is common in BMT recipients, occurring in 10–50% and usually within the first year after the transplant (1–3, 23–26). The illness is almost exclusively dependent on the absence of cell-mediated immunity; humoral immunity (antibody titer) does not correlate with risk. HZ is also characterized by an easily recognized clinical illness, and sophisticated diagnostic techniques are not required. HZ in this study was defined as a physician-confirmed clinical diagnosis that was made after day 14 but within the first 13 mo (day 390 follow-up visit) after HSCT.

#### Statistical analysis

The distribution of characteristics of enrolled patients and eligible patients who were not enrolled was compared with the use of a *t* test for age and chi-square tests for the other factors. General mixed linear models were used in modeling plasma retinol and  $\alpha$ -tocopherol (27). Because these data exhibited right-skewed distributions, they were log transformed for analyses and the estimates from analyses were transformed back to the original scale for reporting. Initial models included time, sex, age, underlying diagnosis, type of transplant, and all interactions with time as independent variables. Backward multivariate analysis was carried out. Interactions were tested first and kept in the models if they maintained a significance level of  $\leq 0.05$ . Main effects were tested similarly. A significance level of  $\leq 0.01$  was used for all pairwise comparisons because of the large number of comparisons.

Multivariate regression models with backward elimination were used to investigate the duration of neutropenia and the severity of mucositis. The presence or absence of infections and of HZ was described with the use of backward logistic regression and survival analysis, respectively. Initial models included sex, age, underlying diagnosis, type of transplant, and retinol and  $\alpha$ -tocopherol plasma concentrations. For modeling of the associations of plasma vitamin concentrations with the duration of neutropenia, the presence of infections, and the severity of mucositis, plasma retinol and  $\alpha$ -tocopherol on day 0 were used as the predictor variables, because mucositis, neutropenia, and infection rarely precede day 0, and the plasma concentration on day 0, as opposed to baseline (before DIT), reflects the influence of DIT. The preparative regimen was initially included as a control variable, but was covaried by the underlying diagnosis and was therefore removed from the model. Total body irradiation, which has been linked to a variety of outcomes in BMT recipients, was analyzed within the group of patients with hematologic malignancies (ie, leukemia or lymphoma). For analyses

**TABLE 1**

Demographic and clinical data for enrolled patients and those eligible but not enrolled<sup>1</sup>

	Enrolled (n = 120)	Not enrolled (n = 43)
Age (y) <sup>2</sup>	46.0 ± 9.7	45.8 ± 10.7
Men (n [%])	42 [35]	15 [35]
Race (n [%])		
White	114 [95]	37 [86] <sup>3</sup>
Nonwhite	6 [5]	6 [14]
Underlying diagnosis (n [%])		
Leukemia	25 [20]	12 [28]
Lymphoma	38 [32]	14 [33]
Breast cancer	57 [48]	17 [39]
Type of transplant (n [%])		
Allogeneic	23 [19]	11 [26]
Related donor	11 [9]	6 [14]
Unrelated donor	12 [10]	5 [12]
Autologous	97 [81]	32 [74]
Preparative regimens (n [%])		
Cyclophosphamide, TBI	31 [20]	NR
Cyclophosphamide, busulfan	13 [11]	NR
Cyclophosphamide, carboplatin, thiotepa	54 [45]	NR
Cyclophosphamide, BCNU, VP-16	2 [2]	NR
Cyclophosphamide, carboplatin, VP-16, TBI	8 [6]	NR
Other	12 [10]	NR

<sup>1</sup>TBI, total body irradiation; BCNU, carmustine; VP-16, etoposide; NR, not recorded.

<sup>2</sup> $\bar{x} \pm SD$ .

<sup>3</sup>Significantly different from enrolled group, *P* = 0.05.

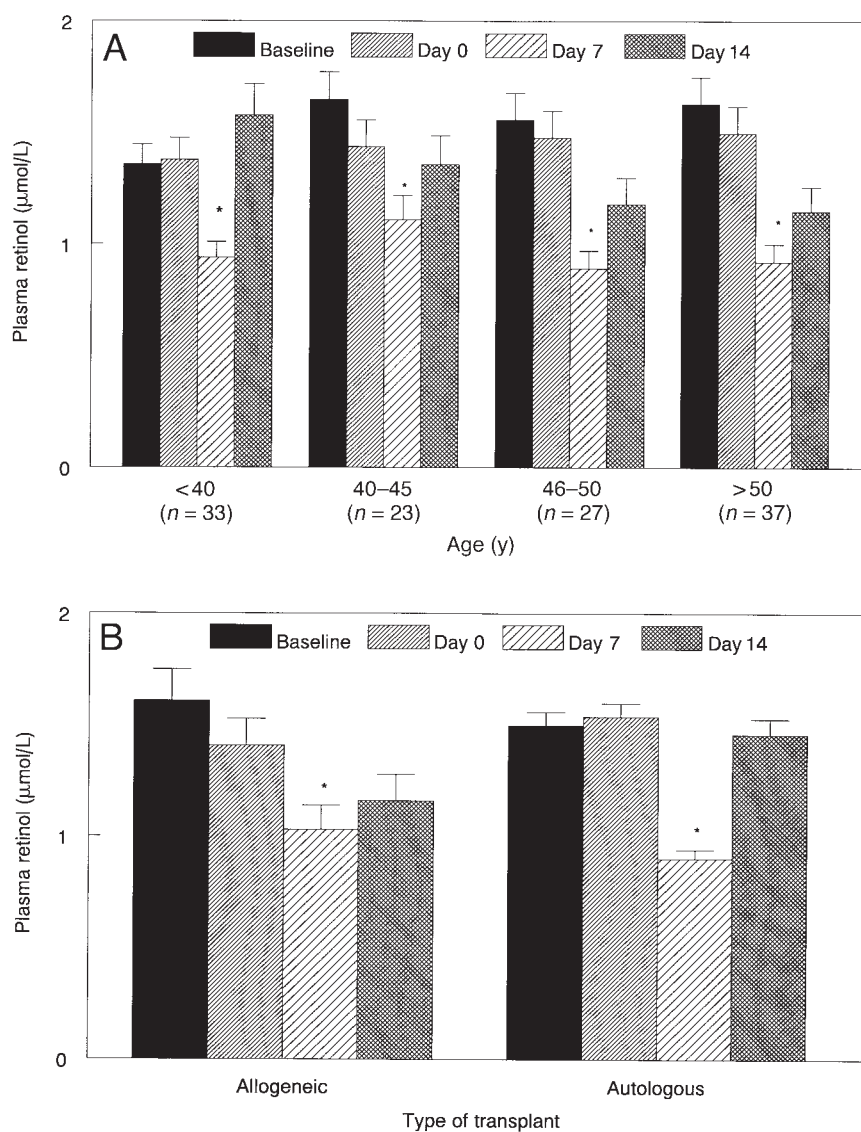
correlating clinical outcomes (mucositis, duration of neutropenia, infection risk in the first 30 d, and risk of HZ infection) with plasma vitamin concentrations, a *P* value < 0.05 was considered significant. All analyses were performed with SAS software, version 6.12 (SAS Institute, Inc, Cary, NC).

## RESULTS

One hundred sixty-three eligible patients underwent DIT and HSCT during the 18-mo recruitment period, and 120 (74%) agreed to enroll in the study. Demographic and clinical characteristics of subjects enrolled and of those eligible but not enrolled are shown in **Table 1**. The distributions of age, sex, underlying diagnoses, and type of transplant (autologous or allogeneic) did not differ significantly between enrolled and eligible but unenrolled subjects. Race did differ significantly, fewer nonwhite subjects than white subjects were enrolled.

### Plasma retinol

Baseline plasma retinol was within normal limits [men: 1.2–2.8  $\mu\text{mol/L}$ ; women: 1.1–2.6  $\mu\text{mol/L}$  (28, 29)] for all subjects. However, baseline concentrations differed by underlying diagnosis (1.67 ± 0.08  $\mu\text{mol/L}$  in 63 patients with hematologic malignancy and 1.42 ± 0.10  $\mu\text{mol/L}$  in 57 patients with breast cancer; *P* = 0.03). There was a significant interaction of age with change in plasma retinol over time (*P* = 0.002); therefore, the data in **Figure 1A** are stratified by age. All age groups, except the oldest (>50 y), experienced a significant drop in mean plasma retinol concentration on day 7 that recovered spontaneously to baseline concentrations by day 14. There was also a significant interaction



**FIGURE 1.** Mean ( $\pm$ SE) plasma retinol as a function of age (A) and type of transplant (allogeneic or autologous) (B) at baseline and on days 0, 7, and 14 in 120 recipients of hematopoietic stem cell transplantation after dose-intensive therapy. There was a significant interaction of age (A;  $P = 0.03$ ) and type of transplant (B;  $P = 0.002$ ) with change in plasma retinol over time. \*Significantly different from baseline,  $P < 0.01$ .

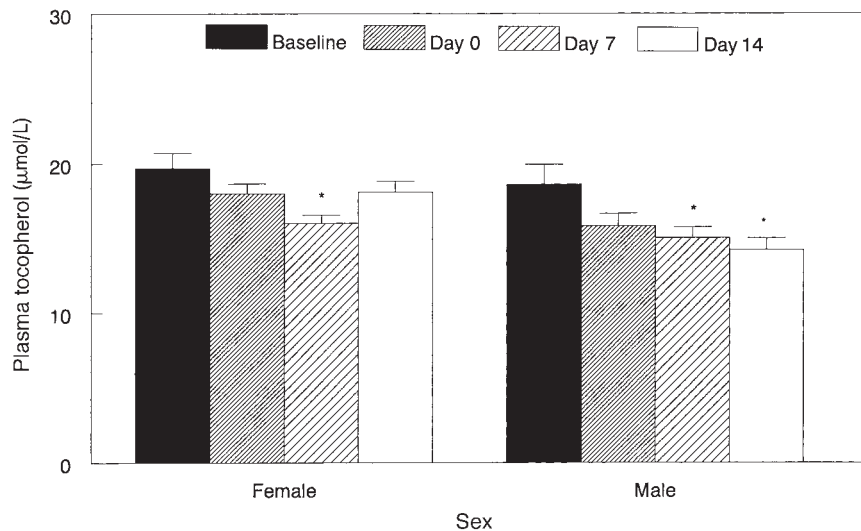
of change in plasma retinol over time with the type of transplant (Figure 1B, stratification by transplant type). Compared with baseline mean plasma retinol concentrations, both allogeneic and autologous transplant recipients experienced significant declines by day 7. A national shortage of injectable multivitamins necessitated a reduction in supplementation (from daily to 3 times/wk) in patients receiving total parenteral nutrition during the last 6 mo of therapy, but there was no effect of this change on plasma vitamin concentrations.

Overall, 82 of 120 patients (68%) had plasma retinol concentrations  $\leq 1.05$   $\mu\text{mol/L}$  at least once during the peritransplant period (baseline to day 14), and 37 (31%) had at least one plasma concentration below the World Health Organization definition of retinol deficiency ( $\leq 0.70$   $\mu\text{mol/L}$ ). In most subjects, the nadir of serum retinol concentrations occurred on day 7. Important predictor variables for day 7 plasma retinol were the plasma retinol concentration on day 0 ( $P = 0.0001$ ), the type of transplant

( $P = 0.0001$ ), and the severity of mucositis ( $P = 0.001$ ). The magnitude of the difference by type of transplant is shown in Figure 1B. The magnitude of difference in day 7 plasma retinol for the other 2 variables is as follows: for every increase of 1 point on the 24-point OAS, day 7 retinol declined by 0.05  $\mu\text{mol/L}$ , and for every decrease of 0.1  $\mu\text{mol/L}$  in plasma retinol on day 0, day 7 retinol declined by 0.20  $\mu\text{mol/L}$ .

#### Plasma tocopherol

Plasma tocopherol concentrations were within normal limits (albeit at the lower limit of normal) at baseline and did not differ by sex (women:  $19.7 \pm 1.04$   $\mu\text{mol/L}$ ; men:  $18.6 \pm 1.35$   $\mu\text{mol/L}$ ;  $P = 0.54$ ). Over the post-HSCT course, however, there were significant sex differences ( $P = 0.02$ , **Figure 2**). The men had declines in plasma vitamin E by day 7, and mean plasma tocopherol remained below baseline to day 14. The minimum plasma vitamin E concentration in men occurred on day 14 ( $14.2 \pm 0.8$   $\mu\text{mol/L}$ ). In women,



**FIGURE 2.** Mean ( $\pm$ SE) plasma  $\alpha$ -tocopherol at baseline and on days 0, 7, and 14 of the peritransplant period in the men ( $n = 42$ ) and women ( $n = 78$ ). There was a significant interaction of change in plasma  $\alpha$ -tocopherol by sex ( $P = 0.02$ ), and thus plasma  $\alpha$ -tocopherol is shown stratified by sex. \*Significantly different from baseline,  $P < 0.01$ .

mean day 7 plasma vitamin E concentrations were significantly lower than baseline, day 0, and day 14 values (Figure 2). Unlike plasma retinol,  $\alpha$ -tocopherol did not differ by age, primary diagnosis, or type of transplant.

#### Clinical indicators of innate immunity

The mean duration of neutropenia was associated with the type of transplant (allogeneic transplant:  $21.2 \pm 0.8$  d, autologous transplant:  $10.3 \pm 0.4$  d;  $P = 0.0001$ ), but not with any other variable measured, including plasma retinol or  $\alpha$ -tocopherol (5 patients had neutropenia for  $> 30$  d and were excluded from this analysis as outliers).

Plasma retinol on day 0 was also not predictive of mucositis severity. In a final model of mucositis, only the underlying diagnosis was significantly associated ( $P = 0.002$ ). However, as outlined above, the reverse association was true. The severity of mucositis predicted the day 7 plasma retinol concentrations. Patients with hematologic malignancies had a mean OAS of  $13.4 \pm 0.3$ , whereas

breast cancer patients had a mean OAS of  $11.9 \pm 0.3$ , which likely reflects the toxicity associated with DIT regimens used for leukemia or lymphoma. Some of the difference in OAS may have been due to total body irradiation ( $\bar{x} \pm$  SD:  $13.7 \pm 0.4$  for those who underwent total body irradiation and  $12.6 \pm 0.5$  for those who did not), but total body irradiation was not independently associated with severity of mucositis after control for other variables ( $P = 0.10$ ).

Sixty-five clinically or microbiologically confirmed infections occurred in the first 30 d after HSCT (bacteremia, 23; urinary tract infection, 18; catheter infection, 14; pneumonia, 4; sinus infection, 4; infectious diarrhea, 1; and pharyngitis, 1). Trends toward an increased risk of infection were noted in association with several variables in the univariate analysis, including day 0 plasma retinol (Table 2), but none of the trends were statistically significant. Although there was a trend toward increased risk of infection with longer duration of neutropenia after adjustment for retinol in the analysis (OR: 1.7; 95% CI: 0.8, 3.7 for neutropenia  $> 10$  d compared with  $\leq 10$  d), it was not statistically significant ( $P = 0.14$ ), probably because of the limited power in this study (see Discussion).

#### Plasma retinol concentrations and risk of herpes zoster

Twenty-nine patients developed HZ during the study. There was no association with plasma  $\alpha$ -tocopherol. The risk of HZ was compared between subjects who had  $\geq 1$  plasma retinol concentration  $\leq 1.05$   $\mu\text{mol/L}$  at one of the 4 time points measured (ie, peritransplant hyporetinolemia had occurred;  $n = 82$ ) and those who did not ( $n = 38$ ). Overall, 23 of 82 (28%) patients with hyporetinolemia developed HZ; only 6 of 38 (16%) without hyporetinolemia developed HZ. Because not all patients survived to be at risk of HZ, we used survival analysis to determine the association of HZ risk with hyporetinolemia and to control for other important variables. Age and type of transplant were not significantly associated with HZ risk. HZ risk differed significantly by sex ( $P = 0.05$ ; Figure 3); thus, subsequent analyses were determined separately in men and women. In women with a plasma retinol concentration  $\leq 1.05$   $\mu\text{mol/L}$  at any time from

**TABLE 2**

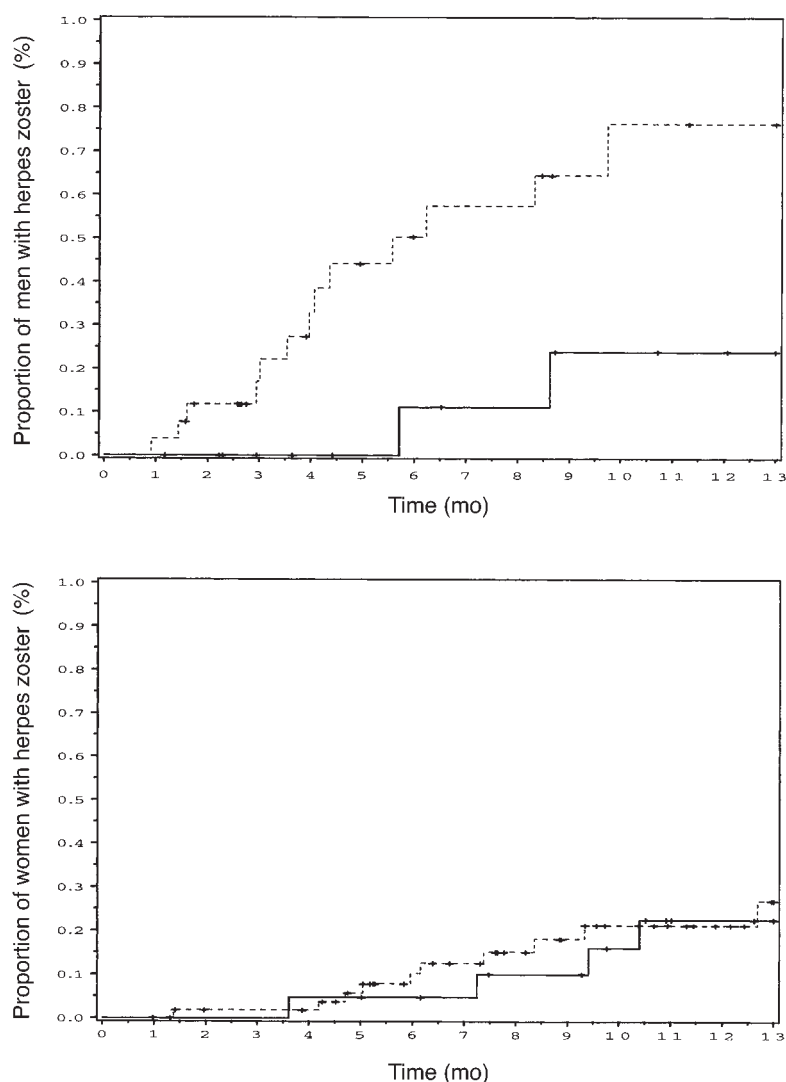
Risk of early infection within the first 30 d after hematopoietic stem cell transplant<sup>1</sup>

Risk factor	Risk of infection
Univariate analysis	
Age, per 10 y	0.9 (0.6, 1.3)
Male sex	0.8 (0.4, 1.6)
Underlying diagnosis <sup>2</sup>	1.3 (0.6, 2.7)
Allogeneic transplant	1.8 (0.7, 4.7)
Total body irradiation <sup>3</sup>	1.2 (0.4, 3.4)
Neutropenia for $> 10$ d	1.8 (0.9, 3.8)
Day 0 plasma retinol, per 0.5 $\mu\text{mol/L}$ decrease	1.4 (0.5, 3.7)

<sup>1</sup>The values are odds ratios; 95% CI in parentheses.

<sup>2</sup>For patients with hematologic malignancy (ie, leukemia or lymphoma) compared with patients with an underlying diagnosis of breast cancer.

<sup>3</sup>Analyzed only within the group of patients with leukemia or lymphoma ( $n = 63$ ).



**FIGURE 3.** Risk of herpes zoster in the first year after dose-intensive therapy followed by hematopoietic stem cell transplantation in the men and women. Men with at least one plasma retinol concentration  $\leq 1.05 \mu\text{mol/L}$  in the peritransplant period (baseline to day 14; dashed line;  $n = 26$ ) had a relative risk of herpes zoster of 6.6 (95% CI: 1.5, 29.6), whereas men in whom all retinol concentrations remained  $> 1.05 \mu\text{mol/L}$  (solid line;  $n = 16$ ) did not. Women with hyporetinolemia (dashed line;  $n = 56$ ) had a 1.2-fold greater risk of herpes zoster (95% CI: 0.4, 3.9) than did those without hyporetinolemia (solid line;  $n = 22$ ).

baseline to day 14, the risk of HZ was 1.2 (95% CI: 0.4, 3.9) times that in women with all plasma retinol levels  $> 1.05 \mu\text{mol/L}$ . In men with a plasma retinol  $\leq 1.05 \text{ mmol/L}$  in the peritransplant period, the risk of HZ was 6.6 (95% CI: 1.5, 29.6). Thus, the cumulative risk of HZ in men with hyporetinolemia was  $\approx 75\%$ , whereas that in all women or men without peritransplant hyporetinolemia was  $\approx 25\%$  (Figure 3). After the underlying diagnosis (ie, breast cancer as compared with all other diagnoses) was controlled for, the relative risks (RRs) for sex and retinol status did not change. Thus, breast cancer alone did not account for the sex differences in HZ risk.

#### Survival

The overall 1-, 6-, and 12-mo probabilities for survival in this study were 0.99, 0.80, and 0.71, respectively. Neither plasma retinol nor  $\alpha$ -tocopherol was associated with overall mortality. Only the type of transplant (RR: 3.3 for allogeneic transplant

compared with autologous transplant;  $P = 0.003$ ) and underlying diagnosis (RR: 2.6 for hematologic malignancy compared with breast cancer;  $P = 0.05$ ) were associated with mortality.

#### DISCUSSION

DIT followed by HSCT is associated with significant metabolic changes and toxicity. In this study, we showed that plasma concentrations of 2 vitamins important in immune function, retinol and  $\alpha$ -tocopherol, decline significantly in most patients who undergo DIT and HSCT, and those concentrations recover spontaneously in most patients by day 14 without specific supplementation. Peritransplant hyporetinolemia occurred in two-thirds of subjects to concentrations associated with altered immune function ( $\leq 1.05 \text{ mmol/L}$ ). Furthermore, low plasma retinol on day 7 was associated with severe mucositis, but  $\alpha$ -tocopherol was not, and the risk of HZ over the first 13 mo after HSCT was higher in men who had hyporetinolemia.

Previously published studies showed a decline in circulating  $\beta$ -carotene (a retinol precursor),  $\alpha$ -tocopherol, and overall antioxidant capacity in HSCT recipients after DIT (4–8). However, when retinol was examined in a few small studies, no significant change in plasma concentration was observed (4, 7, 8). In contrast, our study showed a decline in plasma retinol in most patients. Several factors made it likely that previously published studies missed the hyporetinolemia we found. Earlier studies measured plasma retinol concentrations in a small number of patients and obtained samples at only 3 time points (baseline, day 0, and day 12). The minimum plasma retinol value in patients enrolled in our study typically occurred on day 7, a time point not investigated in prior studies, and the concentration recovered spontaneously by day 14. Measures on day 12 may already reflect the spontaneous recovery of plasma retinol toward baseline concentrations. In addition, type II error may have affected the results of previous studies, all of which involved <30 patients.

Retinol deficiency is not usually defined only by plasma retinol in population-based studies, because the hepatic storage of retinyl esters is typically sufficient to supply normal bodily needs for months (28). However, acute hyporetinolemia, an abrupt lowering of plasma retinol even when total body stores are presumably adequate, may have clinical relevance. Several clinical syndromes of severe physiologic stress have been associated with transient hyporetinolemia (30–34). The mechanism of acute hyporetinolemia in severely ill patients is not known, but it may be consequent to decreased synthesis of retinal-binding protein, release of retinol-binding protein–retinol complex by the liver, or increased excretion of retinol in the urine (28, 30). These mechanisms appear to be linked to the acute-phase reaction of inflammation, because lower serum retinol is associated with markers of inflammation in population data (29, 35). A valid concern about our study is that plasma retinol could merely reflect the severity of the acute phase response, which may be the true risk factor for the events outlined. In fact, hyporetinolemia on day 7 was more likely in subjects with severe mucositis, which perhaps indicates a more robust acute phase response. However, there are situations of transient hyporetinolemia caused by an acute phase response in which retinol replacement is of significant benefit. The best-studied clinical syndrome of transient hyporetinolemia is rubeola infection (measles). In patients with measles, the plasma retinol concentration falls below the World Health Organization criteria for hyporetinolemia in  $\leq 70\%$  of patients, even in US children with extremely low rates of underlying malnutrition (36–41). Furthermore, the administration of a single oral dose of vitamin A (200 000–400 000 U as retinyl palmitate) to children with measles is associated with rapid normalization of serum retinol, enhanced immune responses, more rapid recovery from pneumonia and diarrhea, shorter hospital stays, and lower mortality (37–39). Although treatment of subclinical deficiency may underlie this effect, because many of the studies were in developing countries (37–39), the benefit may also be due to enhanced delivery of retinol to target tissues that were not receiving adequate retinol despite an absence of true deficiency, because hepatic stores are not mobilized efficiently during the acute phase response (28, 38).

Patients who undergo HSCT have immune defects that are qualitatively (if not quantitatively) similar to those associated with measles: acute destruction of mucous membranes, which is followed by secondary infections, and a profound deficiency of cell-mediated immunity. In this study, plasma retinol was associated


with several clinical measures of inadequate immune function in HSCT recipients, even after control for many other variables. Retinol is an essential nutrient for the regeneration and maturation of epithelial surfaces (10), and we found a strong predictive association between the severity of mucositis and the day 7 plasma retinol concentration. The same association was not found for the antioxidant vitamin,  $\alpha$ -tocopherol, which suggests that hyporetinolemia could be due to the acute phase response and the increased peripheral tissue demands of severe mucositis and is not likely a consequence of oxidative consumption.

Most infections during the first few weeks after DIT are caused by endogenous flora that enter damaged mucosa or breaks in skin integrity. In this study, hyporetinolemia on day 0 did not predict subsequent mucositis or risk of infection in the first 30 d. However, this may have been due to a type II error (underpowered study), because a positive association was found for risk of infection (RR: 1.4), but the CI included 1 (95% CI: 0.5, 3.7), perhaps as a result of the relatively small number of infections in this study. Retinol has also been shown to augment several other limbs of the innate immune system (13–15) that were not directly measured in our study. Most notably, macrophages (the only phagocytes present during the period of severe neutropenia) show enhanced activity (42–44) and more rapidly clear bacterial infection in some animal models after retinol supplementation (44). Thus, multiple mechanisms may affect the risk of infection in subjects with hyporetinolemia.

The relation of peritransplant hyporetinolemia to the post-BMT risk of HZ infection is not intuitively obvious. However, early events after HSCT, such as acute GVHD or circulating cytokine concentrations, can have long-term influences on adaptive immune recovery (9, 10, 45–47). In a recent multivariate examination of HZ risk in HSCT recipients, Offidani et al (25) found no clinical signs or symptoms that were predictive of HZ, but CD4+ and CD8+ cell counts on day 30 were highly correlated with HZ risk in the next year. We did not measure CD4+ or CD8+ cell counts in our study. Peritransplant hyporetinolemia may represent another marker of subsequent HZ risk, perhaps by influencing subsequent production or maturation of lymphocyte precursors (14). An alternative explanation is that incubating or subclinical HZ caused the hyporetinolemia, rather than the reverse. Acute varicella infection has been shown to be a cause of retinol deficiency in marginally nourished populations (48), but only 3 subjects in our study had an episode of previous HZ, none within 30 d of HSCT. In addition, only HZ occurring after day 14 was considered an event in this study, which excluded incubating HZ. Thus, it is unlikely that subclinical HZ caused the low plasma retinol concentrations. Finally, the relation of hyporetinolemia and HZ may certainly be confounded by an as yet unidentified risk factor, which would render hyporetinolemia an epiphenomenon.

Hyporetinolemia was not a risk factor for HZ in women. This was not due to breast cancer as an underlying diagnosis, because it was controlled in the analysis. The normal serum retinol concentration in women is lower than that in men (34), and thus a cutoff < 1.05  $\mu\text{mol/L}$  might show an association; however, the present study's sample size did not afford adequate power for such an analysis. Further research is needed to resolve these apparent differences in sex and risk of HZ.

The data shown here indicate that hyporetinolemia is common in HSCT recipients, that severe mucositis increases the risk of hyporetinolemia, and that peritransplant hyporetinolemia is associated with an increased risk of HZ. However, our study has

several limitations. First, the study was conducted in an era when breast cancer patients made up a large percentage of the HSCT recipients. Despite evidence that HZ and other infections may be less common in breast cancer patients than in other HSCT recipients (26), our study had enough events to allow analysis of the associations of hyporetinolemia with infection after we controlled for the underlying diagnosis as described. A second, and more important, limitation in our study—as in any observational study—is that the associations described did not prove causality because there likely were many confounding variables, both measured and unmeasured. In this complex patient group, only a randomized controlled trial of retinol supplementation is likely to prove causality. On the basis of these data, such a trial appears to be warranted. However, similar trials in developed countries in children with respiratory syncytial virus showed mixed results, with some even raising concerns for toxicity (49–52). Thus, any supplementation trial must be closely monitored for toxicity as well as efficacy. 

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