Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study

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Background: We examined the association between vitamin D [25(OH)D] level and disease progression in HIV infection.

Methods: Within the EuroSIDA study, 2000 persons were randomly selected for 25(OH)D measurement in stored plasma samples closest to study entry. 25(OH)D results were stratified into tertiles. Factors associated with 25(OH)D levels and associations of 25(OH) levels with subsequent risk of all-cause mortality, AIDS and non-AIDS events were analyzed.

Results: Of 1985 persons with 25(OH)D levels available, 23.7% had 25(OH)D below 10, 65.3% between 10 and 30, and 11% above 30 ng/ml. At the time of 25(OH)D measurement, older persons, persons of black ethnic origin, living outside Southern Europe/Argentina, sampled during winter, and infected with HIV through nonhomosexual exposure were at higher odds of having low 25(OH)D levels, whereas persons receiving protease inhibitors were at lower odds. Compared to those in the lowest 25(OH)D tertile (<12 ng/ml), those in the middle (12–20) and higher (>20) tertiles had a significantly lower risk of clinical progression during subsequent follow-up. Adjusted incidence rate ratios for all-cause mortality were 0.68 (95% CI 0.47–0.99, $P = 0.045$) and 0.56 (95% CI 0.37–0.83, $P = 0.0039$), and for AIDS events were 0.58 (95% CI 0.39–0.87, $P = 0.0086$) and 0.61 (95% CI 0.40–0.93, $P = 0.020$), for the middle and higher tertiles, respectively. There was a similar, nonsignificant reduced incidence of non-AIDS events in the middle and higher tertiles.

Conclusion: 25(OH)D deficiency was frequent in HIV-infected persons (83% on combined antiretroviral therapy), and was independently associated with a higher risk of mortality and AIDS events. Causality relationships should be examined, because of potential public health consequences.

AIDS 2011, **25**:1305–1315

Keywords: AIDS-defining events, all-cause mortality, HIV infection, vitamin D

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Received: 4 December 2010; revised: 5 April 2011; accepted: 18 April 2011.

DOI:10.1097/QAD.0b013e328347f6f7

Introduction

In the general population, vitamin D insufficiency, assessed by the measurement of serum 25-hydroxyvitamin D [25(OH)D], is frequent. There is increasing agreement that 25(OH)D levels below 30 ng/ml reflect insufficiency [1–6]. This threshold was initially based on the relationship observed in apparently healthy populations between 25(OH)D serum levels and parathyroid hormone, whereas it now more consistently relies on the 25(OH)D concentrations obtained during randomized trials in which vitamin D has been shown to reduce the relative risk of peripheral fractures [7]. It is, however, probable that the extent to which low 25(OH)D levels are associated with various conditions differs. The cut-off of 30 ng/ml is not universally agreed upon as some consider that 20 ng/ml is more appropriate [8]. It is, however, consensual to consider that a 25(OH)D level below 10 ng/ml corresponds to vitamin D deficiency because such low levels are associated with rickets and osteomalacia, the recognized diseases caused by a lack of vitamin D [1–8]. In many observational studies, low 25(OH)D levels have been associated with osteopenia, cardiovascular disease, insulin resistance and diabetes, infections, cancer, renal abnormalities, inflammation and autoimmune diseases [1–7], and with mortality in elderly people [9], persons with high cardiovascular risk [10], renal failure [11], heart transplant [12], cancer [13] and diabetes [14]. Vitamin D deficiency could be viewed only as an end-marker of bad health, but the vitamin D receptor, a member of the nuclear receptor of steroid hormones family, is widely expressed, and active vitamin D, a steroid hormone, has been involved in the physiology of numerous cell types [2,5,6].

Vitamin D deficiency is frequent in HIV-infected persons [15–19], even on successful combined antiretroviral therapy (cART). Factors linked to HIV infection that could contribute to decreased 25(OH)D levels include low sun exposure and alimentary intake, poor absorption, altered storage in adipose tissue, abnormal vitamin D activation due to hepatic or renal impairment, and the interference of antiretroviral drugs with vitamin D metabolism [20]. In a group of untreated HIV-infected Tanzanian women, low 25(OH)D levels was associated with the risk of mother-to-child transmission, child death, and anemia, clinical disease progression and death in the mothers [21,22].

In the present study, the association of 25(OH)D level with clinical disease progression was examined in a large population of HIV-infected persons from the EuroSIDA cohort, the large majority of whom were on cART.

Methods

Study population

EuroSIDA is a prospective, observational, open cohort of 16599 HIV-1-infected persons in 102 centers across 31 European countries, Israel and Argentina. The study is described in detail at www.cphiv.dk. Persons were enrolled into eight cohorts from May 1994 onwards and median follow-up is to August 2008. Data, collected on a standardized form every 6 months, include all CD4 cell counts and viral loads measured since last follow-up, starting and stopping dates of all antiretroviral drugs, dates of all AIDS-defining diagnoses using the 1993 CDC clinical definition, death, and, since 1 January 2001, non-AIDS events (cardiovascular events, non-AIDS defining malignancies, pancreatitis, end-stage renal disease and liver disease) [23]. Clinicians report ethnicity as Asian, black, white or unknown. As part of the study protocol, plasma samples are stored every 6 months.

For the present study, in order to have more than 80% power to detect a 1.5-fold increased risk of all-cause mortality or AIDS over a 5-year follow-up period, we calculated that a sample size of 2000 vitamin D measurements was necessary. The first available sample for each patient was chosen (used as baseline). Persons had to have at least 1 month follow-up prior to the earliest of AIDS or death, to have both a CD4 and viral load measurement in the 6 months prior to the sample date, and to be over 16. We randomly extracted 2001 samples from the 5435 fulfilling all these criteria, after stratification by region, to maintain regional distribution as in the complete EuroSIDA population. Among these samples, 1985 25(OH)D results were available for analysis. Characteristics of persons who were included and excluded were compared and differences examined.

Vitamin D measurement

Plasma samples were sent on dry ice from the EuroSIDA repository in Copenhagen, to the Necker Hospital, Paris, where 25(OH)D was measured in all samples in a row, blinded to persons characteristics and outcomes, by the same laboratory technician. Plasmas were processed in duplicates using the DiaSorin radioimmunassay [24]. Intra-assay and inter-assay coefficients of variation are less than 6 and 8%, respectively, throughout the whole concentration range. In 18 samples, 25(OH)D level was below the functional detection limit of 3 ng/ml and was arbitrarily given a value of 2.9 ng/ml.

Statistical methods

The persons were divided into three groups based on well accepted threshold for abnormal low levels of 25(OH)D (<10, 10–30, >30 ng/ml) for descriptive purposes, and in tertiles for identifying factors associated with low levels of 25(OH)D and for studying the association between 25(OH)D levels and clinical outcomes. The season of the sample was defined as spring (months 3–5; Argentina: months 9–11), summer (months 6–8; Argentina: months 12, 1–2), autumn (months 9–11, Argentina: months 3–5) and winter (months 12, 1–2, Argentina: months 6–8). Characteristics of the persons in the 25(OH)D tertiles were compared using chi-squared tests for

categorical variables and Kruskal–Wallis test for continuous variables. Multinomial logistic regression, using forward selection with P less than 0.1 as entry criterion was used to identify the factors associated with having a 25(OH)D level in the lowest or middle tertile, compared to the higher 25(OH)D tertile (cross-sectional analysis). Incidence rates of a new AIDS event, non-AIDS event and death (three endpoints) were calculated from the time when 25(OH)D levels were determined (i.e. baseline) and Kaplan–Meier estimation was used to determine the probability of progression within the three 25(OH)D strata. For AIDS and death, persons were followed from baseline until the development of a new AIDS event (excluding recurrences) or death, or last visit. As non-AIDS events were not collected until January 2001, analyses for this endpoint were left-censored at 1 January 2001. Poisson regression, adjusting for baseline factors, were used to assess the relationship between 25(OH)D strata and clinical progression. A sensitivity analysis further stratified the deaths into AIDS and non-AIDS related [25]: here, non-AIDS-related deaths incorporated a wider range of non-AIDS events than those collected during routine follow-up (see www.cphiv.dk).

Results

Comparison of included and excluded persons

After adjustment for region, there were few differences between patients with available samples who were ($n = 1985$) and were not ($n = 3450$) selected for analysis. HCV antibody-positive persons were more likely to be included [adjusted odds ratio (aOR) 1.30; 95% confidence interval (CI) 1.11–1.52, $P = 0.0010$)], as were those with a higher viral load (aOR 1.07 per $\log_{10}$ higher; 95% CI 1.02–1.13, $P = 0.0056$). Older persons were less likely to be included (aOR 0.93 per 10 years; 95% CI 0.87–0.99, $P = 0.016$). Persons sampled during summer (aOR 0.78; 95% CI 0.66–0.92, $P = 0.0043$), autumn (aOR 0.81; 95% CI 0.67–0.98, $P = 0.029$) or winter (aOR 0.74; 95% CI 0.63–0.88, $P = 0.0005$) were less likely to be included, compared to those sampled during spring.

25(OH)D levels at date of sample

Four hundred and seventy (23.7%) persons had 25(OH)D levels below 10, 1296 (65.3%) between 10 and 30, and only 219 (11%) above 30 ng/ml at baseline. For statistical analyses, the population was stratified into tertiles, which defined the following groups: below 12, 12–20 and above 20 ng/ml (Table 1). Stratification according to tertiles will be used throughout the result section.

There was a strong influence of season, with the highest proportions of persons with a high and a low 25(OH)D level having been tested in summer and in winter, respectively. Persons infected via nonhomosexual HIV exposure, those from regions other than Southern Europe, and older persons were more likely to have a low 25(OH)D level (Table 2). Dividing the sample date into quartiles, there was an increasing trend for those with later sample dates to be more likely to have a low 25(OH)D level. Compared to persons sampled in spring, those sampled in summer and autumn were less likely, whereas those sampled in winter were more likely to have low 25(OH)D levels. After adjustment for the other factors in Table 2, compared to those of white ethnic origin, persons of a black ethnic origin ($n = 89$) had almost four times the odds of having a 25(OH)D level in the lowest tertile (aOR 3.95; 95% CI 2.06–7.57, $P < 0.0001$). There was no increased odds for Asians ($n = 55$, aOR 1.22; 95% CI 0.59–2.50, $P = 0.58$), or for those of unknown ethnic origin ($n = 122$, aOR 1.24; 95% CI 0.75–2.09, $P = 0.41$). Compared to persons receiving no antiretrovirals at baseline, persons on protease inhibitor-based cART were at lower risk of having low 25(OH)D levels. No significant association was found with the use of efavirenz, nucleoside reverse transcriptase inhibitors (NRTIs) as a whole, or tenofovir.

Progression to clinical events

The event rates within the 25(OH)D strata are shown in Table 3. Kaplan–Meier curves of progression to these endpoints are shown in Fig. 1a–c. At 5 years after baseline, 10.3% of persons had developed AIDS in the lowest 25(OH)D group (95% CI 7.8–12.8), 6.0% in the middle 25(OH)D group (95% CI 3.9–8.1) and 5.1% in the upper 25(OH)D group (95% CI 3.3–6.9). The corresponding figures for death were 11.1% (95% CI 8.5–13.7), 6.6% (95% CI 4.7–8.9) and 5.6% (95% CI 3.7–7.5) and, for non-AIDS events were 8.8% (95% CI 6.3–11.3), 6.7% (95% CI 4.4–8.8) and 6.5% (95% CI 4.4–8.7), respectively.

Figure 2 shows the results of the univariate and multivariate analyses. The lowest 25(OH)D tertile was the reference group for all analyses. After adjustment, persons with 25(OH)D levels in the middle and upper tertiles had a statistically significant lower risk of AIDS, with an incidence rate ratio (IRR) of 0.58 (95% CI 0.39–0.87, $P = 0.0086$) and 0.61 (95% CI 0.40–0.93, $P = 0.020$), respectively. Similarly, persons with a 25(OH)D level in the middle tertile had a significantly reduced incidence of death (IRR 0.68, 95% CI 0.47–0.99, $P = 0.045$), as did those with a 25(OH)D level in the higher tertile (IRR 0.56; 95% CI 0.37–0.83, $P = 0.0039$). There were no differences between persons with 25(OH)D levels in the middle and higher tertiles. A similar trend was observed for the incidence of non-AIDS events: persons with 25(OH)D levels in the middle and higher tertiles had a nonsignificantly reduced incidence of non-AIDS events (IRR 0.73, 95% CI 0.48–1.12, $P = 0.16$, and IRR 0.79, 95% CI 0.52–1.20, $P = 0.27$, respectively). After adjustment, there were no significant differences in the incidence of the most frequent

Table 1. Characteristics of included persons at sample date and 25(OH)D tertiles.

	Lowest 25(OH)D tertile (<12 ng/ml)		Middle 25(OH)D tertile (12.1–20 ng/ml)		Higher 25(OH)D tertile (>20 ng/ml)		
	Number	Percent	Number	Percent	Number	Percent	*P*
All	714	36.0	622	31.3	649	32.7	
Sex							
Male	524	73.4	483	77.7	492	75.8	0.19
Female	190	26.6	139	22.3	157	24.2	
Ethnic origin							
White	585	81.9	546	87.8	588	90.6	<0.0001
Other	129	18.1	76	12.2	61	9.4	
HIV risk							
Homosexual	252	35.3	276	44.4	298	45.9	0.0022
Heterosexual	164	23.0	130	20.9	141	21.7	
IDU	232	32.5	167	26.8	165	25.4	
Other	66	9.2	49	7.9	45	6.9	
Region							
South	175	24.5	171	27.5	219	33.7	<0.0001
Central	198	27.7	136	21.9	129	19.9	
North	180	25.2	134	21.5	181	27.9	
East	156	21.9	173	27.8	112	17.3	
Argentina	5	0.7	8	1.3	8	1.2	
Season (months)[a]							
Spring (3–5)	186	26.1	163	26.2	112	17.3	<0.0001
Summer (6–8)	137	19.2	168	27.0	254	39.1	
Autumn (9–11)	87	12.2	106	17.0	132	20.3	
Winter (12, 1–2)	304	42.6	185	29.7	151	23.3	
Hepatitis B status							
Negative	569	79.7	502	80.7	539	83.1	0.097
Positive	59	8.3	33	5.3	39	6.0	
Unknown	86	12.0	87	14.0	71	10.9	
Hepatitis C status							
Negative	425	59.5	378	60.8	372	57.3	0.32
Positive	184	25.8	140	22.5	159	24.5	
Unknown	105	14.7	104	16.7	118	18.2	
Prior AIDS							
Yes	212	29.7	160	25.7	185	28.5	0.26
Prior treatment							
None	66	9.2	70	11.2	44	6.8	0.043
ART	61	8.5	50	8.0	44	6.8	
cART	587	82.2	502	80.7	561	86.4	
	Median	IQR	Median	IQR	Median	IQR	*P*
Age (years)	39.3	33.2–46.1	38.1	32.4–45.2	38.0	33.4–44.2	0.19
CD4 cell count (cells/μl)	356	216–534	376	288–546	360	220–509	0.13
CD4 nadir (cells/μl)	164	60–280	180	80–286	159	62–271	0.11
Viral load (log_{10}copies/ml)	2.5	1.7–3.6	2.6	1.7–3.8	2.6	1.7–3.5	0.36
Sample (month/year)	2/02	12/98–12/05	11/01	11/98–7/04	9/99	1/98–10/02	<0.0001
Months (from enrolment)	9	0–23	7	0–23	12	3–29	<0.0001

cART, combined antiretroviral therapy; IDU, intravenous drug use; IQR, interquartile range.
[a]Except for Argentina, where seasons were defined as spring (months 9–11), summer (months 12, 1–2), autumn (months 3–5) and winter (months 6–8).

non-AIDS events, cardiovascular events and non-AIDS malignancies, according to 25(OH)D tertile (data not shown).

Deaths were stratified according to cause. Of 188 deaths, 160 were of known cause, of which 48 (30.0%) were judged to be AIDS-related and 112 (70.0%) non-AIDS related. Trends of IRR for both causes of deaths across 25(OH)D strata were consistent with those in Fig. 2, although the lower number of events reduced the power to detect differences. Persons in the middle and higher strata had a nonsignificantly reduced incidence of AIDS-related death (IRR 0.53, 95% CI 0.24–1.15, $P=0.11$, and IRR 0.61, 95% CI 0.28–1.32, $P=0.21$, respectively). The incidence of non-AIDS-related death was nonsignificantly reduced for persons with 25(OH)D values in the middle tertile (IRR 0.67, 95% CI 0.41–1.09, $P=0.10$), but significantly reduced for those with values in the higher tertile (IRR 0.60, 95% CI 0.37–0.98, $P=0.043$).

There was no evidence of an interaction between race and 25(OH)D tertile and any of the clinical events ($P>0.1$), although this analysis had limited power because

Table 2. Factors associated with having 25(OH)D levels in the lowest and middle tertiles, as compared to higher 25(OH)D tertile (>20 ng/ml), at baseline.

	Lowest 25(OH)D tertile (≤12 ng/ml)						Middle 25(OH)D tertile (12.1–20 ng/ml)					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Ethnic origin (global $P<0.0001$)												
White	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
Other	2.13	1.54–2.94	<0.0001	1.90	1.32–2.73	0.0006	1.34	0.94–1.92	0.10	1.38	0.94–2.03	0.10
HIV risk (global $P=0.08$)												
Homosexual	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
IDU	1.38	1.04–1.82	0.02	1.52	1.11–2.09	0.008	1.00	0.75–1.33	0.97	0.9	0.65–1.23	0.48
Heterosexual	1.66	1.28–2.16	0.0001	1.42	1.07–1.89	0.01	1.09	0.83–1.43	0.52	0.92	0.69–1.23	0.59
Other	1.73	1.15–2.63	0.009	1.25	0.80–1.96	0.32	1.18	1.76–1.82	0.46	0.97	0.62–1.53	0.90
Region (global $P=0.05$)												
South	1.00	–	–	1.00	–		1.00	–	–	1.00	–	–
Central	1.92	1.43–2.59	<0.0001	1.65	1.18–2.32	0.003	1.35	0.99–1.85	0.06	1.16	0.83–1.63	0.38
North	1.25	0.94–1.66	0.13	1.26	0.92–1.73	0.14	0.95	0.70–1.28	0.72	0.88	0.64–1.21	0.42
East	1.74	1.27–2.39	0.0005	0.99	0.67–1.45	0.94	1.98	1.45–2.70	<0.0001	1.34	0.92–1.96	0.12
Argentina	0.78	0.25–2.43	0.67	1.36	0.42–4.43	0.60	1.28	0.47–3.48	0.62	2.01	0.70–5.72	0.19
Date of sample (month/year) (global $P<0.0001$)												
≤7/98	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
1/99–1/01	1.12	0.83–1.51	0.47	1.13	0.81–1.56	0.47	1.34	0.99–1.82	0.05	1.31	0.96–1.80	0.09
2/01/4/04	1.91	1.43–2.56	<0.0001	1.92	1.35–2.75	0.0003	1.45	1.07–1.97	0.01	1.26	0.87–1.82	0.21
≥5/04	3.03	2.22–4.16	<.0001	2.98	2.03–4.36	<0.0001	2.80	2.20–3.86	<0.0001	2.39	1.63–3.52	<0.0001
Season[a] (global $P<0.0001$)												
Spring (3–5)	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
Summer (6–8)	0.33	0.24–0.45	<0.0001	0.29	0.21–0.40	<0.0001	0.46	0.34–0.63	<0.0001	0.46	0.33–0.63	<0.0001
Autumn (9–11)	0.40	0.28–0.57	<0.0001	0.38	0.26–0.56	<0.0001	0.55	0.39–0.78	0.0009	0.56	0.39–0.81	0.002
Winter (12, 1–2)	1.26	0.93–1.71	0.14	1.35	0.98–1.86	0.06	0.85	0.61–1.18	0.33	0.91	0.65–1.27	0.56
Current treatment (global $P<.0001$)												
None	1.00	–	–	1.00	–	–	1.00	–	–	1.00	–	–
NNRTI based cART	0.99	0.70–1.41	0.97	0.85	0.58–1.26	0.42	0.80	0.70–1.16	0.23	0.73	0.49–1.09	0.12
PI based cART	0.55	0.42–0.73	<0.0001	0.62	0.45–0.83	0.001	0.63	0.47–0.83	0.001	0.70	0.51–0.95	0.02
Other	0.83	0.55–1.24	0.35	0.87	0.55–1.36	0.53	0.72	0.47–1.11	0.14	0.84	0.53–1.33	0.46
Age (global $P=0.06$)												
Per 10 year older	1.08	0.97–1.20	0.17	1.16	1.02–1.31	0.01	0.98	0.87–1.10	0.68	1.06	0.94–1.20	0.37

Multinomial logistic regression. CI, confidence interval; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor.

[a]Except for Argentina, where seasons were defined as spring (months 9–11), summer (months 12, 1–2), autumn (months 3–5) and winter (months 6–8).

of the small number of persons of non-white ethnic origin. Furthermore, there was no interaction between 25(OH)D level and HIV RNA viral load or use of cART for the prediction of AIDS, non-AIDS events or death. Further, adjusting for current RNA or CD4 value (i.e. taking account of measurements made during follow-up) did not significantly alter our findings. For example, for death, after additionally adjusting for CD4 and viral load as time-updated covariates, there was a significantly reduced incidence of death in both the middle (IRR 0.65, 95% CI 0.44–0.95, $P=0.025$) and higher 25(OH)D tertiles (IRR 0.67, 95% CI 0.45–1.00, $P=0.048$). Adjusting for cumulative time on treatment in the 1650 persons on cART at baseline did not significantly impact the analysis (data not shown).

Additional analyses

Hemoglobin was measured within 6 months of baseline in 1262 persons. There was very little correlation between 25(OH)D value and hemoglobin (correlation coefficient 0.087, $P=0.0020$), across 25(OH)D tertiles. A multivariate model adjusting additionally for baseline or current anemia status showed consistent results to those in Fig. 2 (data not shown). In addition, serum creatinine values within 6 months of baseline were available for 355 persons. There was no correlation between estimated glomerular filtration rate (eGFR) and 25(OH)D (correlation coefficient -0.091, $P=0.086$), and no patient had chronic kidney disease (two consecutive eGFR <60 ml/min/1.73 m^2) at or before 25(OH)D evaluation.

Body mass index (BMI) was available for 1518 persons within 6 months of baseline, and there was no correlation with 25(OH)D levels (correlation coefficient 0.016, $P=0.53$). After adjustment, compared to those with medium BMI, persons with a low BMI (<18) had marginally increased odds of having a 25(OH)D level in the lowest tertile (aOR 1.71; 95% CI 0.92–3.17, $P=0.091$), and persons with a high BMI (>25) had similar odds of having a 25(OH)D level in the lowest tertile (aOR 1.02; 95% CI 0.79–1.32, $P=0.88$).

Table 3. Incidence rates of events, expressed per 100 person-years of follow-up.

	Total	Lowest 25(OH)D tertile (<12 ng/ml)	Middle 25(OH)D tertile (12.1–20 ng/ml)	Higher 25(OH)D tertile (>20 ng/ml)
AIDS				
Events	159	73	39	47
PYFU	11 720	3737	3654	4329
Incidence (95% CI)	1.36 (1.15–1.87)	1.95 (1.51–2.40)	1.07 (0.73–1.40)	1.09 (0.78–1.40)
Death				
Events	188	87	47	54
PYFU	12 225	3963	3780	4482
Incidence (95% CI)	1.54 (1.32–1.76)	2.20 (1.73–2.66)	1.24 (0.89–1.60)	1.20 (0.88–1.53)
Non-AIDS[a]				
Events	149	58	38	53
PYFU	9714	3188	3062	3464
Incidence (95% CI)	1.53 (1.29–1.78)	1.82 (1.35–2.29)	1.24 (0.85–1.64)	1.53 (1.1–1.94)
Cardiovascular disease				
Events	71	31	19	21
PYFU	9828	3226	3086	3515
Incidence (95% CI)	0.72 (0.55–0.89)	0.96 (0.62–1.30)	0.62 (0.37–0.96)	0.60 (0.34–0.85)
Non-AIDS malignancies				
Events	62	21	15	26
PYFU	9909	3290	3106	3513
Incidence (95% CI)	0.63 (0.47–0.78)	0.64 (0.37–0.91)	0.48 (0.27–0.80)	0.74 (0.46–1.02)

CI, confidence interval; PYFU, person-years of follow-up.
[a]Ninety-four patients were excluded as they had no prospective follow-up after 1 January 2001 (see Methods section).

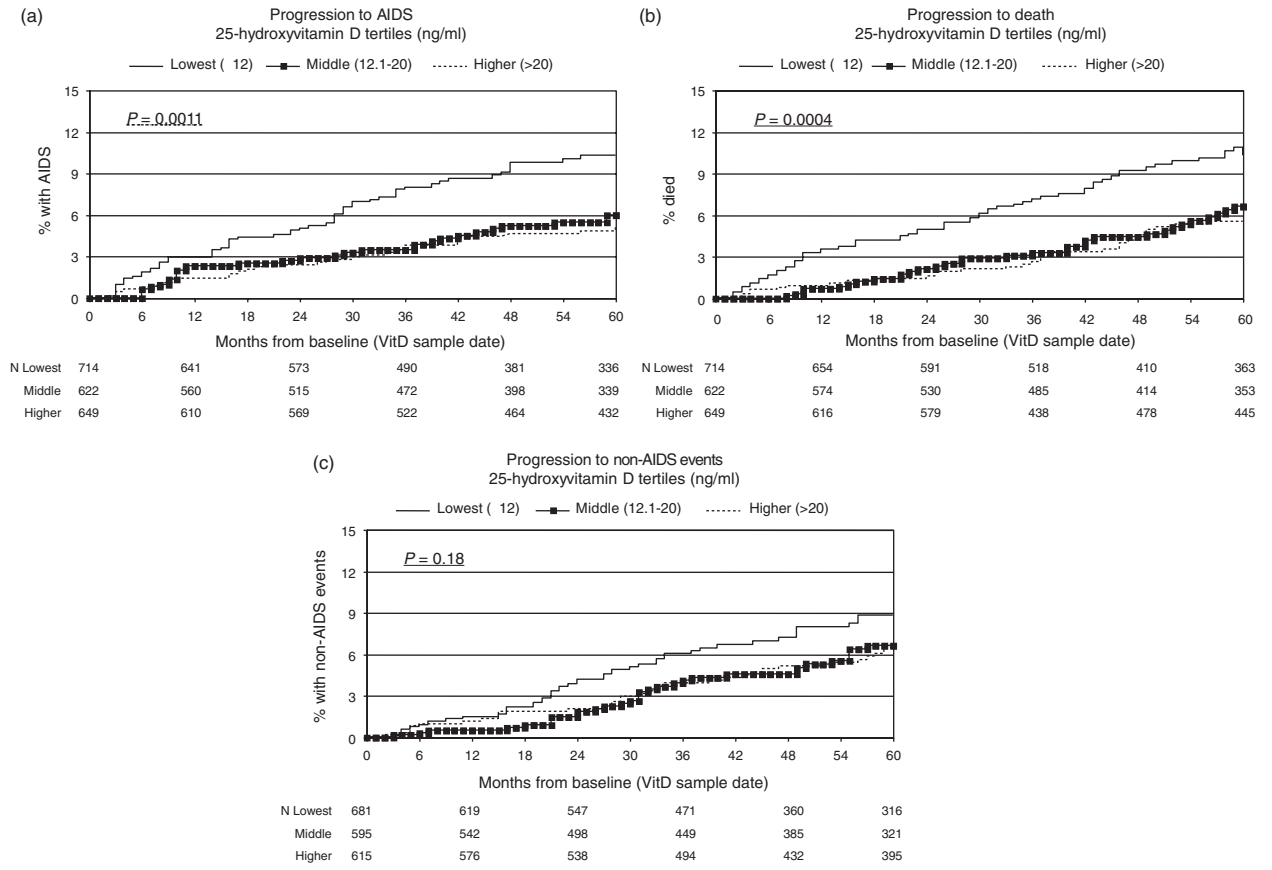

Fig. 1. Kaplan–Meier estimation of progression. Kaplan–Meier estimation of progression to (a) AIDS-defining events, (b) all-cause mortality, and (c) non-AIDS-defining events according to 25(OH)D concentration tertile at baseline.

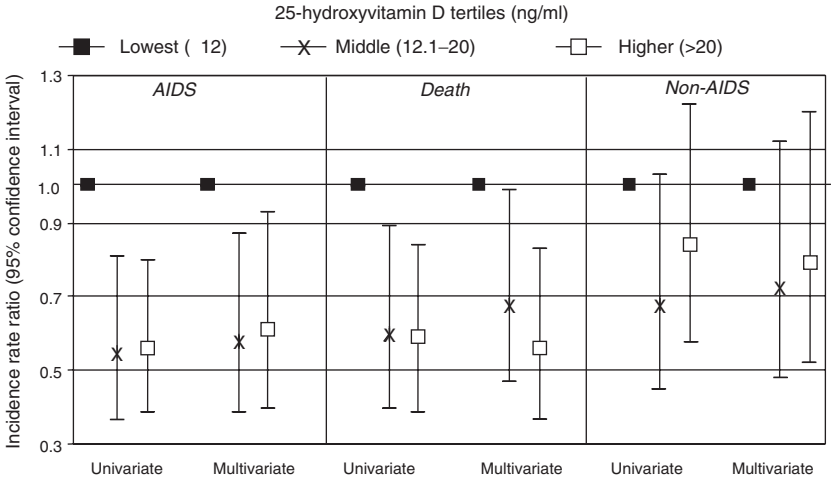

Fig. 2. Univariate and multivariate incidence rate ratios of AIDS-defining events, all-cause mortality and non AIDS-defining events. The lowest 25(OH)D concentration tertile is the reference category for all analyses. Multivariate analysis was adjusted for all factors listed in Table 1.

Discussion

The study examined 25(OH)D levels in a large population of well characterized HIV-infected persons. The objectives were to describe the prevalence and correlates of vitamin D deficiency, and to determine whether a 25(OH)D level was associated with subsequent all-cause death, AIDS-defining or non-AIDS events.

The present results confirm [15–19] that vitamin D insufficiency or deficiency is frequent in HIV-infected persons. Studies across Europe have found highly variable 25(OH)D levels in the general population, with levels below 10 ng/ml in 2–30% of adults [26]. In the US, 25(OH)D levels below 30 ng/ml and below 20 ng/ml were similarly prevalent in the SUN cohort of HIV-infected persons vs. in the general population (NHANES study), after adjustment for age, sex and race: 70.3 vs. 79.1% and 29.7 vs. 38.8%, respectively [27]. Therefore, vitamin D deficiency might not be more frequent in people living with HIV than in the general population. Unsurprisingly, the season of sampling was associated with 25(OH)D levels, and persons with less, or benefiting less from, sun exposure (living outside Southern Europe and Argentina, or of black ethnic origin) were more likely to have very low 25(OH)D levels. Nonhomosexual route of HIV-1 transmission was also associated with lower 25(OH)D levels. This is in agreement with an earlier study suggesting that intravenous drug use is a risk factor for low 25(OH)D [18]. Of note, the association with heterosexual transmission remained after adjustment for ethnicity. These differences across transmission groups may reflect other factors we were not able to adjust for, such as nutrition and sun exposure. As in the general population, increasing age was also associated with lower 25(OH)D levels. Exposure to cART [19] and the use of efavirenz [17,18,28,29] have been associated with lower 25(OH)D levels, but we were unable to reproduce this finding in the present study. Neither the use of NRTIs or protease inhibitors was associated with lower levels of 25(OH)D. The finding that protease inhibitor use was associated with higher 25(OH)D levels, as also shown in the SUN cohort [27], is of unclear biological relevance.

Having a 25(OH)D level in the lowest tertile (<12 ng/ml) was strongly associated with the occurrence of AIDS events and all-cause mortality over a median follow-up of 5 years, after adjusting for a large number of variables, including season, ethnic and geographic origin, CD4 cell count and viral load (at baseline and time-adjusted), and there was no interaction between these variables and 25(OH)D levels for the association with events. Thus, a very low 25(OH)D level was associated with events, even in the case of virologically controlled HIV infection and immune restoration. The prognostic value of vitamin D deficiency was also independent of anemia, another prognostic factor of HIV infection [30], that has been associated with low 25(OH)D levels in untreated HIV-infected African women [20]. In addition, although we only had information on a subgroup of persons, low 25(OH)D levels were not correlated with low eGFR, an indicator of cardiovascular risk in the general and HIV-infected populations [31], associated with all-cause and cardiovascular mortality [32].

We thus found a strong association between the lowest 25(OH)D tertile (<12 ng/ml) and the two main endpoints of all-cause death and AIDS events, but no differences between the medium and high 25(OH)D tertiles. Vitamin D deficiency therefore represents a new, independent, unfavorable prognostic marker in HIV infection, but without further research this cannot translate into clinical recommendations.

The association of low 25(OH)D with clinical progression in HIV infection echoes the multiple functions

now attributed to vitamin D, which, like other steroid hormones, can regulate gene activity in numerous cell types [2,5,6]. Vitamin D deficiency has been associated with cancer risk [1,2,5], with a large study suggesting that vitamin D supplementation could lower the risk of malignancies [33]. Studies have also associated vitamin D insufficiency with hypertension, insulin resistance and high BMI, thus establishing a link with cardiovascular risk [2], clinical cardiovascular disease [34,35], and cardiovascular and all-cause death [10,14]. Vitamin D regulates endothelial function [36], down-regulates the renin gene activity [37], and a placebo-controlled trial has shown that vitamin D administration lowers blood pressure in hypertensive persons [38]. Vitamin D has regulatory effects on immune functions [39]: the vitamin D receptor is expressed on adaptive and innate immune cells; vitamin D plays a role in innate immunity, particularly in the defence against mycobacteria, and in T-cell activation, with vitamin D insufficiency leading to altered T-cell proliferation [40]. Vitamin D deficiency has also been associated with inflammation [41]. In a study of patients referred for a coronary angiography, vitamin D deficiency was associated with all-cause and cardiovascular mortality, and correlated with high levels of markers of cell adhesion, oxidative stress and inflammation [C-reactive protein (CRP) and interleukin 6 (IL-6)] [10]. In a placebo-controlled study, vitamin D supplementation lowered the level of the inflammatory cytokine TNF-α and increased the level of the anti-inflammatory cytokine IL-10, in patients with congestive heart failure [42]. In a large cohort, low vitamin D levels have also been associated with increased levels of the coagulation activation markers tissue plasminogen activator and D-dimer [43].

Taken together, these results are of particular relevance to our findings, because of the well described increased vascular risk in HIV-infected persons, and because markers of inflammation (CRP and IL-6) and coagulation activation (D-dimers) have been associated with mortality and opportunistic diseases in HIV-infected persons [44,45]. In the present study, whereas non-AIDS events were not significantly associated with low 25(OH)D (possibly due to a lack of power), non-AIDS deaths were: this could reflect an aggravating effect of vitamin D deficiency, possibly linked with immune dysfunction or increased inflammation, on different conditions.

The study has several limitations. The study population may not be representative of the whole cohort, with excluded persons being older and having been sampled during spring less often: however, this would only attenuate the effect of 25(OH)D level on events. There were also a relatively low number of non-AIDS events: the impact of vitamin D on these events should therefore be addressed in larger studies. Information on vitamin D supplementation is not collected in EuroSIDA, but it is unlikely that HIV-infected persons sampled around 2001 were widely receiving vitamin D supplementation, and persons who were included earlier in the cohort had higher 25(OH)D levels, as observed in the general population [46]. This study was based on a single 25(OH)D measurement, at entry into the cohort, which leaves open the questions whether the length of exposure to low 25(OH)D, or its level at a given time point, is the key factor, and whether 25(OD) level has a short or mid-term, rather than long-term, prognostic value. Lastly, this is an observational study, from which causal relations cannot be drawn. However, the association between vitamin D deficiency and immune dysfunction, inflammation and coagulation activation may suggest a coherent, testable, pathogenic link between low 25(OH)D levels and events in HIV-infected persons.

These results provide strong evidence that vitamin D deficiency is an important cofactor in HIV disease progression, even in the setting of widespread, efficient cART. Whether the relationship between vitamin D deficiency and events is causal must now be addressed, because of potential public health consequences.

Acknowledgements

Primary support for EuroSIDA is provided by the European Commission BIOMED 1 (CT94-1637), BIOMED 2 (CT97-2713), the 5th Framework (QLK2-2000-00773) and the 6th Framework (LSHP-CT-2006-018632) programs. Current support also includes unrestricted grants by Gilead, Pfizer, and Merck and Co. The participation of centers from Switzerland was supported by The Swiss National Science Foundation (Grant 108787). 25-hydroxyitamin D measurements were funded by SIDACTION.

Authors acknowledge the technical expertise of Mélanie Ducarteron who performed all 25-hydroxyvitamin D determinations.

J.-P.V., J.-C.S., O.K., J.D.L., and A.M. designed the study, planned the analyses and wrote the first draft of the manuscript. They had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. J.-C.S. supervised vitamin D measurements. A.M. and J.R. performed the statistical analyses. B.K., M.L., J.G., C.P., J.R.B. contributed major suggestions for analysis and participated in the writing of the manuscript. All clinicians enrolled persons in the study and collected data.

Funding sources were not involved at any step of the present work (design and conduct of the study, collection, management, analysis, or interpretation of data; preparation, review or approval of the manuscript).

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References

1. Holick MF. **Vitamin D deficiency.** *N Engl J Med* 2007; **357**:266–281.
2. Norman AW, Bouillon R. **Vitamin D nutritional policy needs a vision for the future.** *Exp Biol Med* 2010; **235**:1034–1045.
3. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. **Estimates of optimal vitamin D status.** *Ostoporos Int* 2005; **16**:713–716.
4. Vieth R, Bischoff-Ferrari H, Boucher BJ, Dawson-Hughes B, Garland CF, Heaney RP, *et al*. **The urgent need to recommend an intake of vitamin D that is effective.** *Am J Clin Nutr* 2007; **85**:649–650.
5. Souberbielle JC, Body JJ, Lappe JM, Plebani M, Shoenfeld Y, Wang TJ, *et al*. **Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice.** *Autoimmunity Rev* 2010; **9**:709–715.
6. Adams JS, Hewison M. **Update in vitamin D.** *J Clin Endocrinol Metab* 2010; **95**:471–478.
7. Bischoff-Ferrari HA, Willett WC, Wong JB, Stuck AE, Staehelin HB, Orav EJ, *et al*. **Prevention of nonvertebral fractures with oral vitamin D and dose dependency.** *Arch Intern Med* 2009; **169**:551–561.
8. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, *et al*. **The 2011 report on dietary reference intakes for calcium and vitamin D form the Institute of Medicine: what clinicians need to know.** *J Clin Endocrinol Metab* 2011; **96**:53–58.
9. Pilz S, Dobnig H, Nijpels G, Heine RJ, Stehouwer CD, Snijder MB, *et al*. **Vitamin D and mortality in older men and women.** *Clin Endocrinol* 2009; **71**:666–672.
10. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, *et al*. **Independent association of low serum 25-hydroxy-vitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality.** *Arch Intern Med* 2008; **168**:1340–1349.
11. Kovesdy CP, Ahmadzadeh S, Anderson JE, Kalantar-Zadeh K. **Association of activated vitamin D treatment and mortality in chronic kidney disease.** *Arch Intern Med* 2008; **168**:397–403.
12. Zittermann A, Schleithoff SS, Götting C, Fuchs U, Kuhn J, Kleesiek K, *et al*. **Calcitriol deficiency and 1-year mortality in cardiac transplant recipients.** *Transplantation* 2009; **87**:118–124.
13. Ng K, Meyerhardt JA, Wu K, Feskanich D, Hollis BW, Giovanucci EL, *et al*. **Circulating 25-hydroxyvitamin D levels and survival in patients with colorectal cancer.** *J Clin Oncol* 2008; **26**:2984–2991.
14. Joergensen C, Gall MA, Schmedes A, Tarnow L, Parving HH, Rossing P. **Vitamin D levels and mortality in type 2 diabetes.** *Diabetes Care* 2010; **33**:2238–2243.
15. Kuehn EW, Anders HJ, Bogner JR, Obermaier J, Goebel FD, Schlöndorff D. **Hypocalcaemia in HIV infection and AIDS.** *J Intern Med* 1999; **245**:69–73.
16. Rodriguez M, Daniels B, Gunawardene S, Robbins GK. **High frequency of vitamin D deficiency in ambulatory HIV-positive patients.** *AIDS Res Human Retroviruses* 2009; **25**:9–14.
17. Van Den Bout-Van Den Beukel CJP, Fievez L, Michels M, Sweep FC, Hermus AR, Bosch ME, *et al*. **Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy.** *AIDS Res Hum Retroviruses* 2008; **24**:1375–1382.
18. Mueller NJ, Fux CA, Ledergerber B, Elzi L, Schmid P, Dang T, *et al*. **High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients.** *AIDS* 2010; **24**:1127–1134.
19. Welz T, Childs K, Ibrahim F, Poulton M, Taylor CB, Moniz CF, Post FA. **Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase.** *AIDS* 2010; **24**:1923–1928.
20. Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS. **HIV-protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D.** *AIDS* 2003; **17**:513–520.
21. Mehta S, Giovanucci E, Mugusi FM, Spiegelman D, Aboud S, Hertzmark E, *et al*. **Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality.** *PLoS One* 2010; **5**:e8770; doi: 10.1371/journal.pone.0008770.
22. Mehta S, Hunter DJ, Mugusi FM, Spiegelman D, Manji KP, Giovanucci EL, *et al*. **Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania.** *J Infect Dis* 2009; **200**:1022–1030.
23. Mocroft A, Reiss P, Gasiorowski J, Ledergerber B, Kowalska J, Chiesi A, *et al*. **Serious fatal and non fatal non-AIDS defining illnesses (non-ADI) in Europe.** *J Acquir Immune Defic Syndr* 2010; **55**:262–270.
24. Hollis BW. **Comparison of commercially available (125)I-based RIA methods for the determination of circulating 25-hydroxyvitamin D.** *Clin Chem* 2000; **46**:1657–1661.
25. Kowalska J, Mocroft A, Ledergerber B, Colebunders R, Ristola M, Begovac J, *et al*. **Estimating AIDS and non-AIDS related deaths for patients with missing death data**. *12th European AIDS Clinical Society, November 11–14 2009*, Cologne, Germany. Abstract PS6/6.
26. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, *et al*. **Global vitamin D status and determinants of hypovitaminosis D.** *Osteoporos Int* 2009; **20**:1807–1820.
27. Dao CN, Patel P, Overton ET, Rhame F, Pals SL, Johnson C, *et al*. **Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population.** *Clin Infect Dis* 2011; **52**:396–405.
28. Brown TT, McComsey GA. **Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D.** *Antivir Ther* 2010; **15**:425–429.
29. Fox J, Peters B, Prakash M, Arribas J, Hill A, Moecklinghoff C. **Improvement in vitamin D deficiency following antiretroviral regime change: results form the MONET trial**. *AIDS Res Human Retroviruses* 2011; **27**:29–34.
30. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, *et al*. **Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group.** *AIDS* 1999; **13**:943–950.
31. Choi AI, Li Y, Deeks SG, Grunfeld C, Volberding PA, Shlipak MG. **Association between kidney function and albuminuria with cardiovascular events in HIV-infected persons.** *Circulation* 2010; **121**:651–658.
32. Chronic Kidney Disease Prognosis Consortium. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, *et al*. **Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis.** *Lancet* 2010; **375**:2073–2081.
33. Lappe JM, Travers-Gustafson D, Davies KM, Recker M, Heaney R. **Vitamin D and calcium supplementation reduces cancer risk : results of a randomized trial.** *Am J Clin Nutr* 2007; **85**:1586–1591.

34. Giovanucci E, Liu Y, Hollis BW, Rimm EB. **25-hydroxyvitamin D and risk of myocardial infarction in men.** *Arch Intern Med* 2008; **168**:1174–1180.
35. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, *et al*. **Vitamin D deficiency and risk of cardiovascular disease.** *Circulation* 2008; **117**:503–511.
36. Suqden JA, Davies JI, Witham MD, Morris AD, Struthers AD. **Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels.** *Diabet Med* 2008; **25**:320–325.
37. Li YC, Kong J, Wei M, Chen ZF, Lui SQ, Cao LP. **1,25-dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system.** *J Clin Invest* 2002; **110**:229–238.
38. Judd SE, Raiser SN, Kumari M, Tangpricha V. **1,25-dihydroxyvitamin D(3) reduces systolic blood pressure in hypertensive adults. A pilot feasibility study.** *J Steroid Biochem Mol Biol* 2010; **121**:445–447.
39. Mora JR, Iwata M, von Andrian UH. **Vitamin effects on the immune system; vitamins A and D take centre stage.** *Nature Rev Immunol* 2008; **8**:685–698.
40. Von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C. **Vitamin D controls T cell antigen receptor signaling and activation of human T cells.** *Nature Immunol* 2010; **11**:344–349.
41. Boxer RS, Dauser DA, Walsh SJ, Hager WD, Kenny AM. **The association between vitamin D and inflammation with the 6-min walk and frailty in patients with heart failure.** *J Am Geriatr Soc* 2008; **56**:454–461.
42. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. **Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled study.** *Am J Clin Nutr* 2006; **83**:754–759.
43. Hyppönen E, Berry D, Cortina-Borja M, Power C. **25-Hydroxyvitamin D and preclinical alterations in inflammatory and hemostatic markers: a cross sectional analysis in the 1958 British birth cohort.** *PLoS ONE* 2010; **5**:e10801; doi/10.1371/journal.pone.0010801.
44. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, *et al*. **Inflammatory and coagulation biomarkers and mortality in patients with HIV infection.** *PLoS Med* 2008; **5**:e203; doi/10.1371/journal.pmed.0050203.
45. Rodger AJ, Fox Z, Lundgren JD, Kuller LH, Boesecke C, Gey D, *et al*. **Activation and coagulation markers are independent predictors of the development of opportunistic disease in patients with HIV infection.** *J Infect Dis* 2009; **200**:973–983.
46. Ginde AA, Liu MC, Camargo CA. **Demographic differences and trends of vitamin D insufficiency in the US population.** *Arch Intern Med* 2009; **169**:626–632.