



# Prevalence of hypovitaminosis D among HIV+ patients enrolled in a large Italian cohort

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## BACKGROUND

Hypovitaminosis D is a frequent condition in industrialised countries associated with several pathological states. A high prevalence of hypovitaminosis D in HIV-positive patients has been reported, but the reasons are unclear. Moreover, there is little in the way of estimating the prevalence of hypovitaminosis D in large observational studies, on the prevalence of hypovitaminosis according to geographical areas, on the role of demographic variables as well as HIV related variables. Finally, the possible toxicity role of antiretroviral combination therapy (cART) and of specific regimens still needs to be tested in this context.

## OBJECTIVES

The aims of this study were:

-to estimate the prevalence of hypovitaminosis D in a cohort of HIV-positive patients living in Italy, before and after start of cART

-to evaluate its association with anthropometric, environmental and clinical data

-to evaluate the changes of plasma levels of vitamin D after the initiation of cART and according to specific cART regimens

As a secondary objective we evaluated the possible correlation between vitamin D levels and risk of developing diabetes, cardio-vascular events and renal disease, all known to be associated with hypovitaminosis D in the HIV negative population.

## PATIENTS AND METHODS

Patients of Icona Foundation cohort for whom a stored plasma sample was available before or after starting cART were used for dosing 25(OH)-vitamin D concentration; patients' characteristics recorded at the time of sample collection were considered for the analysis. The original sample extraction was designed to obtain 2 samples per patient (one before and one after starting a PI or a NNRTI-containing regimen) and powered to test for a difference in the change over time according to drug class initiated; however several of the extracted samples were not available or usable for dosing vitD, resulting in an incomplete data set.

Vitamin D was tested by quantitative enzyme-immunoassay (IDS Octeia 25OH-vitamin D- IDS, Bolton, USA)

**Vitamin D insufficiency** was defined as 25(OH)-vitD <75 nmol/l, while values <30 nmol/l were considered as **vitamin D deficiency**.

## Statistical Analysis

Differences in the proportion of patients with VitD-insufficiency and VitD-deficiency were tested by chi-square test. A binary outcome was constructed to compare patients with VitD deficiency with those with normal VitD levels. Independent predictors of VitD deficiency were identified using a logistic regression analysis. Patients could contribute more than one test in this analysis and therefore standard errors were corrected accordingly.

The change in absolute levels of vitD pre/post cART was modelled by linear regression controlling for baseline vitD levels, potential confounders (shown in Table 1) and seasonality (by including only pairs of tests measured in the same season).

Finally, we investigated the prognostic value of vitD to predict diabetes, cardiovascular diseases (myocardial infarction, stroke and hypertension) and renal insufficiency using a composite event and a time to event analysis with time zero the date of the first measurement per patient. The multivariable analysis was performed by means of a proportional hazards Cox regression model.

## RESULTS

We studied 852 pts contributing 1,498 vitD measures: 262 obtained before and 1,236 after the initiation of ART.

VitD insufficiency and deficiency were found in 804 (54%) and 98 (7%) of the tests, respectively.

Demographic and clinical characteristics of the cohort tested according to vitD plasma levels are reported in Table 1. In univariable analysis, the prevalence of VitD insufficiency and deficiency varied according to: nationality (higher in patients of African origin compared to Italians), calendar season (higher in spring and winter compared to autumn and summer), HIV viral load (higher with higher values), CD4 count (higher with lower count) and duration of ART usage prior to the test (lower in untreated patients, higher in those treated for some time). There was no univariable association with the prevalence of severe disease such as diabetes (p=0.26), cardiovascular (MI, stroke and hypertension, p=0.19) and chronic renal disease (p=0.45).

Table 1 Prevalence of VitD insufficiency and deficiency according to patients characteristics

Characteristic	vitD deficiency (<=30 nmol/l) N=98	VitD insufficiency (31-75 nmol/l) N=706	vitD normal values (>75 nmol/l) N=694	P-value
Female, n (%)	33 (34%)	212 (30%)	196 (28%)	0.49
Age, years, n (%)				0.006
18-35	41 (42%)	296 (42%)	355 (51%)	
36-50	49 (50%)	339 (48%)	292 (42%)	
>50	8 (8%)	71 (10%)	47 (7%)	
Mode of HIV transmission, n (%)				0.15
IDU				
Heterosexual contacts	31 (32%)	196 (28%)	212 (30%)	
Homosexual contacts	48 (49%)	310 (44%)	282 (30%)	
Other/unknown	17 (17%)	147 (21%)	160 (23%)	
	2 (2%)	53 (8%)	40 (6%)	
Nationality, n (%)				0.0001
Africa	9 (9%)	27 (4%)	5 (1%)	
Asia	0 (0%)	5 (1%)	2 (0.2%)	
Centre/South America	3 (3%)	17 (2%)	15 (2%)	
Rest of Europe	0 (0%)	12 (2%)	5 (1%)	
Italy	86 (88%)	645 (91%)	667 (96%)	
Season, n (%)				0.0001
Autumn	16 (16%)	123 (17%)	249 (36%)	
Spring	42 (43%)	253 (36%)	125 (18%)	
Summer	9 (9%)	103 (15%)	182 (26%)	
Winter	31 (32%)	227 (32%)	138 (20%)	
BMI (n=1435), n (%)				0.13
<18.5	54 (57%)	440 (66%)	446 (67%)	
18.5-25	30 (32%)	193 (29%)	195 (29%)	
>25	10 (11%)	35 (5%)	26 (4%)	
HIV-RNA (=1490) (copies/ml),n(%)				0.04
<500				
501-10,000	53 (54%)	423 (61%)	461 (67%)	
10,001-100,000	14 (14%)	74 (11%)	73 (11%)	
>100,000	15 (15%)	110 (16%)	88 (13%)	
	16 (16%)	92 (13%)	64 (9%)	
CD4, cells/mm3, n (%)				0.0001
<200	30 (31%)	176 (25%)	108 (16%)	
201-350	20 (20%)	132 (19%)	143 (21%)	
350-500	20 (20%)	125 (18%)	140 (20%)	
>500	28 (29%)	268 (38%)	301 (44%)	
Drug class, n (%)				0.13
ART-naive	21 (21%)	134 (19%)	107 (15%)	
NNRTI	28 (29%)	163 (23%)	175 (25%)	
PI	49 (49%)	409 (58%)	412 (59%)	
Duration of ART (months),n (%)				0.005
0				
1-12	21 (21%)	134 (19%)	107 (15%)	
13-24	28 (29%)	209 (30%)	258 (37%)	
>24	36 (37%)	283 (40%)	280 (40%)	
	13 (13%)	80 (11%)	49 (7%)	
Clinical events, n (%)				0.16
Diabetes	2 (2.0%)	14 (2%)	6 (0.9%)	
Cardio-vascular	6 (6.0%)	18 (3%)	18 (2.6%)	
Renal	1 (1.0%)	7 (1%)	3 (0.4%)	
Free from clinical events	89 (91%)	667 (94%)	667 (96.1%)	

Table 2 shows the independent predictors of VitD deficiency when compared to the patients with normal values. After mutual adjustment, factors that remained significantly associated with a higher risk of VitD deficiency were older age, non Caucasian origin, a lower BMI, a lower CD4 count and prior exposure to NNRTI (Efavirenz=187, Nevirapine=179) compared to PI (n=870, of which 142 boosted-PI). There was a high variability associated with the season in which the sample was collected, those collected in spring and winter showing much lower levels than those stored in autumn or in summer.

In the subset of 664 measures stored when patients were ART-experienced (77 with vitD≤30 and 587 with vitD>75) there was little evidence for an association with the duration of previous exposure to ART (adjusted OR=1.19, per year longer on ART, 95% CI:0.92-1.54, p=0.19).

Out of 240 patients with both a sample pre- and post-ART initiation 116 have samples stored in the same season. In these we studied the factors associated with the change of vitD over time. The first sample was collected close to ART initiation (median=20 days, range:1-309) and the median time between first and second sample was 13 months (range:1-30). Overall, vitD decreased from pre- to post-ART initiation levels on average by 7.57 nmol/l per year (SD=7.34, p-value for a difference from zero =0.11). 87 patients (75%) started a PI-containing regimen vs. 29 (25%) who started a NNRTI-containing regimen. There was no evidence from the multivariable linear regression model that the change in vitD levels from pre-ART to post-ART varied according to whether patients had started PI vs. NNRTI, although power of the analysis was low (mean difference/year=+13 nmol/l, 95% CI:-8; +34, p=0.22, Table 3).

67 patients developed severe events potentially related to hypovitaminosis D over a median follow-up of 6.5 years (3 renal insufficiency, 31 diabetes and 33 cardiovascular events such as myocardial infarction, stroke or hypertension) in an analysis including all 852 patients followed-up from the date of their first vitD measure. In a Cox regression analysis adjusted for all factors shown in Table 2 and compared to patients with a vitD value >75, the RH associated with a vitD of 30-75 was 1.83 (95% CI:1.05-3.17, p=0.03) and the RH associated with a vitD of ≤30 was 1.51 (95% CI:0.56-4.07, p=0.41).

## LIMITATIONS

Limitations typical of any cross-sectional analysis apply here for the results of the logistic regression analysis in that causality between studied exposure factors and prevalence of vitD deficiency cannot be established. Also we cannot exclude that selection bias may have been introduced by including only patients of the cohort with a usable blood sample. The longitudinal analyses have limited power.

## CONCLUSIONS

This is the first large observational study confirming a very high prevalence of hypovitaminosis D in HIV positive patients living in western countries. Traditional risk factors for vit D deficiency are confirmed also in this setting: age, BMI, nationality, seasonality. Moreover, both immunodepression and high HIV viral load are associated with hypovitaminosis D.

Regarding the association with exposure to cART, we found that previous use of NNRTI-including regimens were associated with higher risk of vitD deficiency than previous exposure to PI. The analysis of the longitudinal changes in vitD according to the type of regimen started was largely unpowered and therefore the results are inconclusive. Our data also seems to confirm that, in HIV positive individuals, vitamin D insufficiency is predictive of the risk of subsequent development of a number of severe events such as cardiovascular, renal disease and diabetes.

Overall, these data should be carefully considered in the management of HIV positive patients. Due to both the high prevalence of hypovitaminosis D and the safety of cholecalciferol administration, vitamin D supplements might be taken into account in our therapeutic choices.

## Icona Foundation Study

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Table 2 Odds ratio of VitD deficiency vs. normal values from fitting a logistic regression model

Characteristic	OR (95% CI) p-value	
	Unadjusted	Adjusted
<b>Age</b>		
Per 10 years older	1.21 (0.95-1.56) p=0.13	1.53 (1.11-2.09) p=0.009
<b>Mode of transmission</b>		
Heterosexual	1.00	1.00
Homosexual	0.62 (0.35-1.12) p=0.12	0.46 (0.22-0.96) p=0.04
IDU	0.86 (0.53-1.40) p=0.54	1.10 (0.62-1.95) p=0.73
Other/unknown	0.29 (0.07-1.26) p=0.10	0.21 (0.04-0.99) p=0.05
<b>Origin</b>		
Non Caucasian	1.00	1.00
Caucasian	0.24 (0.11-0.49) p=0.0001	0.17 (0.07-0.42) p=0.0001
<b>CD4 count</b>		
X 100 higher	0.87 (0.81-0.95) p=0.001	0.90 (0.82-0.99) p=0.04
<b>BMI</b>		
Per unit higher	0.96 (0.90-1.03) p=0.24	0.90 (0.83-0.98) p=0.01
<b>Exposure to ART</b>		
NNRTI	1.00	1.00
PI	0.73 (0.44-1.20) p=0.22	0.47 (0.27-0.84) p=0.01
<b>Season</b>		
Summer	1.00	1.00
Spring	6.80 (3.19-14.46) p=0.0001	8.30 (3.61-19.09) p=0.0001
Autumn	1.30 (0.57-3.00) p=0.54	1.24 (0.51-3.05) p=0.64
Winter	4.54 (2.09-9.85) p=0.0001	4.84 (2.07-11.33) p=0.0003
<b>Cardiovascular disease</b>		
No	1.00	1.00
Yes	2.32 (0.90-5.95) p=0.08	3.17 (0.97-10.35) p=0.06

Table 3 Mean change in vitD levels from pre-ART to post-ART from fitting a linear regression model

Characteristic	Unadjusted	Adjusted*
<b>Drug class in first ART</b>		
NNRTI	Ref	Ref
PI	+8.7 (-16.1; +33.4) p=0.49	+13.0 (-8.4; +34.3) p=0.22
<b>Age</b>		
Per 10 years	-1.1 (-10.5; +8.4) p=0.82	-0.8 (-12.4; +10.8) p=0.89
<b>Origin</b>		
Non Caucasian	Ref	Ref
Caucasian	+22.4 (-10.9; +55.7) p=0.19	+16.2 (-25.7; +58.2) p=0.44
<b>CD4 count</b>		
X 100 cells higher	-0.7 (-4.3; +2.9) p=0.72	-1.5 (-8.4; +34.4) p=0.48

Model restricted to n=116 pre and post cART samples measured in the same season

\*Adjusted for all factors shown in Table 2