

# LPS and HIV gp120 modulate monocyte/macrophage CYP27B1 and CYP24A1 expression leading to vitamin D consumption and hypovitaminosis D in HIV-infected individuals

M.R. PINZONE<sup>1</sup>, M. DI ROSA<sup>1</sup>, B.M. CELESIA<sup>1</sup>, F. CONDORELLI<sup>2</sup>,  
M. MALAGUARNERA<sup>3</sup>, G. MADEDDU<sup>4</sup>, F. MARTELLOTTA<sup>5</sup>,  
D. CASTRONUOVO<sup>1</sup>, M. GUSSIO<sup>1</sup>, C. COCO<sup>1</sup>, F. PALERMO<sup>1</sup>,  
S. COSENTINO<sup>1</sup>, B. CACOPARDO<sup>1</sup>, G. NUNNARI<sup>1,6</sup>

<sup>1</sup>Department of Clinical and Molecular Biomedicine, Division of Infectious Diseases, University of Catania, Catania, Italy

<sup>2</sup>DiSCAFF & DFB Center, University of Piemonte Orientale A. Avogadro, Novara, Italy

<sup>3</sup>International PhD programme in Neuropharmacology, University of Catania, Catania, Italy

<sup>4</sup>Department of Clinical, Experimental and Oncological Medicine, Division of Infectious Diseases, University of Sassari, Sassari, Italy

<sup>5</sup>Department of Medical Oncology, National Cancer Institute, Aviano Italy

<sup>6</sup>Department of Microbiology and Immunology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, USA

**Abstract.** – **AIM:** Vitamin D deficiency is very common among HIV-infected subjects. We cross-sectionally evaluated the prevalence and risk factors for hypovitaminosis D in 91 HIV-infected Italian patients.

**PATIENTS AND METHODS:** We studied in a cohort of 91 HIV-infected Italian patients the metabolism of Vitamin D by evaluating the in vitro expression of CYP27B1, CYP24A1 and vitamin D receptor (VDR) by monocytes and macrophages stimulated with the viral envelope protein gp120 or lipopolysaccharide (LPS).

**RESULTS:** The prevalence of vitamin D deficiency (25OHD < 10 ng/ml) and vitamin D insufficiency (25OHD 10-30 ng/ml) was 31% and 57%, respectively. In univariate analysis, female sex ( $p = 0.01$ ), increasing age ( $p = 0.05$ ), higher highly sensitive-C reactive protein ( $p = 0.025$ ), higher parathyroid hormone (PTH) ( $p = 0.043$ ) and lower BMI ( $p = 0.04$ ) were associated with vitamin D deficiency. In multivariate analysis, the association was still significant only for PTH ( $p = 0.03$ ) and female sex ( $p = 0.03$ ).

Monocyte stimulation with LPS (100 ng/ml) or gp120 (1  $\mu$ g/ml) significantly upregulated CYP27B1 mRNA expression. Moreover, gp120 significantly increased VDR mRNA levels. On the contrary, neither LPS nor gp120 modified CYP24A1 levels. Macrophage stimulation with LPS (100 ng/ml) significantly upregulated CYP27B1 and CYP24A1 mRNA expression.

When monocytes were cultured in the presence of 25OHD (40 ng/ml) and stimulated with LPS we detected significantly lower levels of 25OHD in the supernatant.

**CONCLUSIONS:** Vitamin D deficiency was very common in our cohort of HIV-infected patients. Chronic inflammation, including residual viral replication, may contribute to hypovitaminosis D, by modulating vitamin D metabolism and catabolism. Systematic screening may help identifying subjects requiring supplementation.

*Key Words:*

Vitamin D deficiency, Vitamin D receptor, Parathormone, HIV-infected patients, gp 120, lipopolysaccharide.

## Introduction

Even though highly active antiretroviral therapy (HAART) has significantly changed the course of Human immunodeficiency virus (HIV) infection, it is not able to eradicate HIV infection<sup>1</sup>. HIV persistence in latent reservoirs represents a major barrier to curing HIV<sup>2-12</sup>. HIV infection is characterized by chronic inflammation and immune activation. These factors have been shown to significantly contribute to the in-

creased prevalence of metabolic and cardiovascular disease, malignancies, bone and renal disease observed in HIV-infected subjects<sup>13-28</sup>. In fact, direct or indirect markers of inflammation, such as highly sensitive (hs)-C reactive protein (CRP) and Interleukin (IL)-6, and coagulation, such as D-dimer, and circulating levels of bacterial lipopolysaccharide (LPS), have been associated with increased morbidity and mortality in the setting of HIV<sup>29,30</sup>.

Vitamin D is involved not only in bone homeostasis, but it also has non-skeletal functions, including immune regulation<sup>31,32</sup>. Vitamin D deficiency has been associated with increased levels of pro-inflammatory cytokines and increased systemic inflammation<sup>33-35</sup>.

In recent years a growing number of studies have reported high prevalence rates of vitamin D deficiency among HIV-infected patients<sup>36-38</sup>. Hypovitaminosis D has a multifactorial origin, including non-HIV-related risk factors, such as female sex, low dietary intake and low sun exposure, and HIV-related factors, i.e. immune activation and antiretroviral adverse effects<sup>39,40</sup>. Several reports have identified vitamin D deficiency as an independent risk factor not only for osteoporosis and fragility fractures<sup>41,42</sup>, but also for cardiovascular and metabolic disorders among HIV-positive subjects<sup>43-45</sup>.

In the present study, we cross-sectionally evaluated the prevalence of vitamin D deficiency in a cohort of HIV-infected subjects and the risk factors associated with hypovitaminosis D, including inflammation and coagulopathic markers. Then, we studied *in vitro* the expression of CYP27B1 and CYP24A1, the enzymes involved in vitamin D metabolism and catabolism, respectively, and vitamin D receptor (VDR), upon monocyte and macrophage stimulation with LPS or the HIV envelope protein gp120. We also measured vitamin D levels in the supernatant of monocytes stimulated with LPS, LPS plus 25-hydroxyvitamin D (25OHD) or 25OHD alone.

## Patients and Methods

### Study Population

From March to May 2011, 91 consecutive HIV-positive patients were enrolled for this study during their regularly scheduled ambulatory care visits at the Unit of Infectious Disease, Garibaldi Nesima Hospital, Catania, Italy. Written informed consent was obtained by all participants at enrolment.

The following parameters were extracted from medical records: patient demographics, HCV and/or HBV co-infection, body mass index (BMI) and HIV-related parameters, including time since HIV diagnosis and initiation of HAART, route of infection, CDC stage, HAART regimen. Information on smoking habit was obtained by subject interview. The following biochemical and viro-immunological parameters were measured: serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, insulin, creatinine, D-dimer, hs-CRP, parathyroid hormone (PTH), CD4+ and CD8+ T-cell count, HIV RNA (Cobas Amplicor HIV-1 monitor test, minimal detection threshold of 20 copies/ml). 25OHD levels were measured using the DiaSorin radioimmunoassay (Stillwater, MN, USA)<sup>46</sup>. The level of detection for 25OHD was < 4 ng/ml. The reference range is 32-100 ng/ml. According to EuroSida indications, 25OHD concentration was defined normal when > 30 ng/ml, insufficient when ranging between 10 and 30 ng/ml, deficient when < 10 ng/ml<sup>47</sup>.

### Cell Cultures

Human monocytes were isolated from fresh buffy coats of healthy volunteers as described in details elsewhere<sup>32</sup>. Peripheral blood mononuclear cells (PBMCs) were diluted with phosphate-buffered saline (PBS) supplemented with 2.5 mM EDTA and plated at a density of  $1 \times 10^6$  cells/well in 6-well plates (Becton Dickinson, Franklin Lakes, NJ, USA). Monocytes were allowed to attach to the wells for 2 hours (h) at 37°C in 5% CO<sub>2</sub>, after which non-adherent cells were removed by washing three times with PBS. Monocytes were cultured in Iscove's Modified Dulbecco's Medium supplemented with rHuman Macrophage-Colony Stimulating Factor (M-CSF) 5 ng/ml (PeproTech, BDA, Segrate, Milan, Italy), 10% fetal bovine serum (FBS), 2 mM glutamine and 1% of penicillin/streptomycin (Invitrogen, Milan, Italy). Mature macrophages were obtained from monocytes after 7 days of culture without changing the medium.

To determine the effects of glycoprotein 120 (gp120) on Vitamin D (1,25-dihydroxyvitamin D<sub>3</sub>) receptor (VDR), CYP24A1, CYP27B1 and IL-6 expression on freshly isolated monocytes, cells were treated immediately after adhesion with gp120 (1 µg/ml, ImmunoDiagnostics, Boston, MA, USA) or left untreated, samples were evaluated at 3h and 24h. Analogously, to evaluate the effects of LPS on VDR, CYP24A1,

CYP27B1 and IL-6 expression, monocytes were treated with LPS (100 ng/ml, Sigma-Aldrich, Milan, Italy) or left untreated and samples collected at different time points (2h, 4h, 8h and 24h). mRNA levels were measured by real-time RT-polymerase chain reaction (PCR). Similarly, macrophages were treated with LPS (100 ng/ml, Sigma-Aldrich, Milan, Italy) and samples evaluated at 2h, 4h and 6h. We also stimulated monocytes with LPS (100 ng/ml)  $\pm$  25OHD (40 ng/ml, Sigma-Aldrich, Milan, Italy) or with 25OHD alone for 24h and we measured 25OHD levels in the supernatants<sup>46</sup>.

In another series of experiments, we isolated monocytes from 5 vitamin D-sufficient healthy controls, 5 HIV-positive patients with 25OHD < 10 ng/ml and 5 HIV-positive patients with 25OHD > 10 ng/ml and we evaluated baseline expression of IL-6 mRNA as well as IL-6 levels after monocyte treatment with 1,25-dihydroxyvitamin D (1,25OH<sub>2</sub>D) (1  $\mu$ M, Sigma-Aldrich, Milan, Italy) for 24h.

#### Gene Expression Analysis by Real-Time PCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Milan, Italy). For reverse transcription-PCR (RT-PCR), 2  $\mu$ g of total RNA were reverse-transcribed with high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Monza, Italy) in a 20  $\mu$ l reaction volume. Real-time fluorescence PCR, based on SYBR Green, was carried out in a 30  $\mu$ l final volume containing 1  $\mu$ l SYBR Green PCR Master Mix (Applied Biosystems, Monza, Italy), 200 nM forward and 200 nM reverse primers (Table I) and 20 ng of cDNA. Thermal cycling was performed for each gene in triplicate on cDNA samples in MicroAmp Optical 96-well reaction plate (Applied Biosystems, Monza, Italy) with MicroAmp optical caps (Applied Biosystems, Monza, Italy), using the ABI PRISM 7700 sequence detection system (Applied Biosystems, Monza, Italy). Amplification was carried out with the fol-

lowing conditions: 50°C for 2 min, 95°C for 10 min and 40 cycles each of 95°C for 15 s and 60°C for 1 min.

#### Statistical Analysis

Statistical analysis was performed using SPSS statistical software package release 14.0.2 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 4 software (GraphPad, La Jolla, CA, USA).

Continuous variables are expressed as median (interquartile range, IQR) and compared by non-parametric Mann-Whitney and Kruskal-Wallis test, when appropriate. Categorical variables are presented as number of cases (percentage) and were compared using Fisher's exact and chi-square test, when appropriate. Correlations between different parameters were analyzed by Spearman's correlation coefficient. Univariate and multivariate regression analysis were used to identify significant factors associated with 25OHD deficiency. We included in multivariable analysis all variables with a *p*-value less than 0.2 in univariate analysis.

For *in vitro* experiments, data are expressed as mean  $\pm$  standard deviation (SD). Significance was assessed by one-way analysis of variance (ANOVA) and Student's *t*-test.

## Results

#### Study Population Characteristics and Vitamin D Levels

The characteristics of the study population are shown in Table II.

Median age was 45 (40-51) years and 74% were men. 20% of patients had hepatitis C coinfection, 54% were cigarette smokers. 11 patients (12%) were diabetic.

Median time from HIV diagnosis was 155 (62-202) months. Most patients (91.2%) were on HAART. 57% of them were receiving a protease inhibitor (PI)-based regimen, 26% an efavirenz (EFV)-containing regimen, 59% were receiving

**Table I.** Primer pairs used in real-time polymerase chain reaction (PCR) analysis.

Gene	Forward	Reverse	Size (bp)
GAPDH	CTGCACCAACTGCTTAG	AGGTCCACCACTGACACGTT	262
IL-6	GAAAGCAGCAAAGAGGCACT	TTTCACCAGGCAAGTCTCCT	108
VDR	TCCTCCTGCTCAGATCACTG	AGGGTCACAGAAGGGTCATC	107
CYP27B1	TTGCTATTGGCGGGAGTGG	TGCCGGGAGAGCTCATACAG	68
CYP24A1	ATGAGCACGTTTGGGAGGAT	TGCCAGACCTTGGTGTGAG	70

**Table II.** Characteristics of our study population.

Patients' characteristics	N = 91
Age (years), median (IQR)	45 (40-51)
Sex	
Male, n (%)	67 (73.6)
Female, n (%)	24 (26.4)
CDC stage A, n (%)	55 (60.4)
CDC stage B, n (%)	14 (15.4)
CDC stage C, n (%)	22 (24.2)
Risk	
Omosexual, n (%)	37 (40.6)
Eterosexual, n (%)	36 (39.6)
Intravenous drug use, n (%)	11 (12.1)
Other, n (%)	7 (7.7)
Hepatitis C coinfection, n (%)	18 (19.8)
Active smokers, n (%)	49 (53.8)
Diabetes mellitus, n (%)	11 (12)
Body mass index (kg/m <sup>2</sup> ), median (IQR)	23.9 (22-26.2)
Glomerular filtration rate (ml/min), median (IQR)	111 (90.7-134.6)
Current HAART, n (%)	83 (91.2)
Time since HIV diagnosis (months), median (IQR)	155 (62-202)
Time spent on HAART (months), median (IQR)	121 (25-171)
Current exposure to efavirenz, n (%)	24 (26.4)
Current exposure to protease inhibitors, n (%)	52 (57.1)
Current exposure to tenofovir, n (%)	54 (59.3)
Current CD4+ T-cell count (cells/ $\mu$ l), median (IQR)	538 (389-766)
HIV RNA < 20 copies/ml, n (%)	57 (62.6)
25OHD (ng/ml), median (IQR)	17 (9.15-24.1)
25OHD < 10 ng/ml, n (%)	28 (30.8)
25OHD 10-30 ng/ml, n (%)	52 (57.1)
25OHD > 30 ng/ml, n (%)	11 (12.1)
Parathyroid hormone ( $\mu$ g/ml), median (IQR)	49.6 (39.1-60.3)
Insulin (microU/ml), median (IQR)	8.8 (5.17-11.4)
D-Dimer (ng/ml), median (IQR)	164.5 (119.3-226.8)
Highly sensitive-C reactive protein (mg/dl), median (IQR)	0.17 (0.08-0.87)
Total cholesterol (mg/dl), median (IQR)	195 (160-220)
HDL cholesterol (mg/dl), median (IQR)	37 (31-43)

tenofovir (TDF). Median CD4+ T-cell count was 538 (389-766) cells/ $\mu$ l and 63% of patients had an undetectable viral load (< 20 copies/ml).

Median 25OHD concentration was 17 (9.15-24.1) ng/ml. In our population, only 12% of subjects had 25OHD levels above 30 ng/ml. Most of them (57%) were 25OHD insufficient, whereas the prevalence rate of 25OHD deficiency was 31%. Median hs-CRP level was 0.17 (0.08-0.87) mg/dl. Median PTH level was 49.6 (39.1-60.3)  $\mu$ g/ml, median insulin level was 8.8 (5.17-11.4) microu/ml. Median D-Dimer level was 164.5 (119.3-226.8) ng/ml.

**Factors Associated with Vitamin D Deficiency**

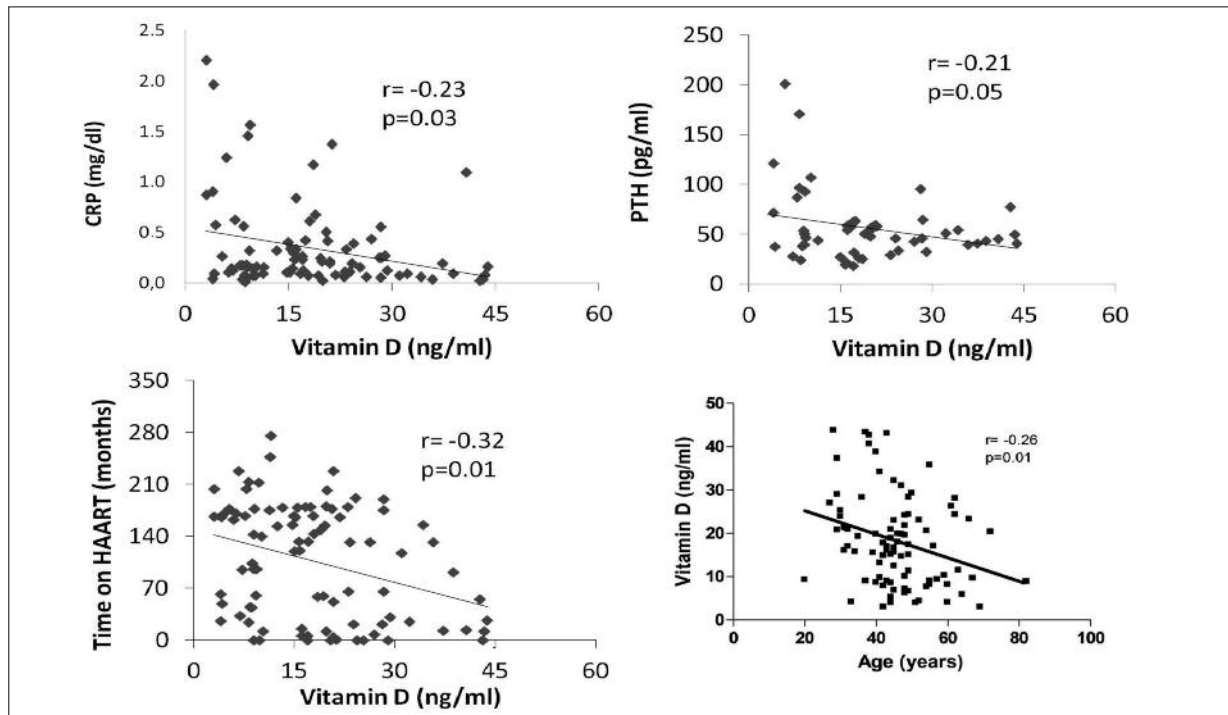
Table III summarizes factors associated with vitamin D deficiency in our cohort of patients.

In univariate analysis, vitamin D deficiency was found to correlate with age and female sex (Figure 1), as patients with 25OHD < 10 ng/ml were significantly older than those with 25OHD > 10 ng/ml (48 vs. 45 years,  $p = 0.05$ ;  $r = -0.26$ ,  $p = 0.01$ ). Similarly, women were more represented in the vitamin D-deficient group (42.8 vs. 17.4%,  $p = 0.01$ ). Vitamin D-deficient patients had lower BMI (22.2 vs. 24 kg/m<sup>2</sup>,  $p = 0.04$ ;  $r = 0.2$ ,  $p = 0.15$ ) and higher PTH (63 vs. 47  $\mu$ g/ml,  $p = 0.043$ ;  $r = -0.21$ ,  $p = 0.05$ ) in comparison with subjects having 25OHD > 10 ng/ml. In addition, hs-CRP levels were significantly higher among vitamin D-deficient patients (0.32 vs. 0.19 mg/dl,  $p = 0.025$ ;  $r = -0.23$ ,  $p = 0.033$ ) (Figure 1).

CD4+ and CD8+ T-cell count, viral load, time since HIV diagnosis, CDC stage and route of infection did not significantly differ between groups. HCV coinfection and diabetes did not affect vitamin D status. Analogously, we did not find any significant correlation between HAART and vitamin D levels, as well as between any antiretroviral class and 25OHD serum concentra-

**Table III.** Factors associated with vitamin D deficiency in our study population.

Patients' characteristics	25OHD < 10 ng/ml	25OHD > 10 ng/ml	Univariate analysis	Multivariate analysis
Age (years), median (IQR)	48 (42-57)	45 (38-49)	$p = 0.05$	$p = 0.4$
Sex: Female, n (%)	12 (42.8)	11 (17.4)	$p = 0.01$	$p = 0.03$
Body mass index (kg/m <sup>2</sup> ), median (IQR)	22.2 (19.2-25.5)	24 (22.2-27.2)	$p = 0.04$	$p = 0.24$
Glomerular filtration rate (ml/min), median (IQR)	104 (81-114)	116 (96-143)	$p = 0.13$	$p = 0.77$
Parathyroid hormone ( $\mu$ g/ml), median (IQR)	62.6 (39.1-109)	47.1 (33.4-57.8)	$p = 0.043$	$p = 0.03$
Highly sensitive-C reactive protein (mg/dl), median (IQR)	0.32 (0.08-0.88)	0.19 (0.09-0.33)	$p = 0.025$	$p = 0.07$
Time spent on HAART (months), median (IQR)	163 (66-177)	132 (26-176)	$p = 0.19$	$p = 0.65$



**Figure 1.** Factors associated with vitamin D levels in the univariate analysis by Spearman's correlation.

tion. Insulin, D-dimer and cholesterol levels were similar among patients who were vitamin D-deficient and patients who were not.

In a logistic regression model (Table III), only PTH ( $p = 0.03$ ) and female sex ( $p = 0.03$ ) were still associated with vitamin D deficiency.

### ***In vitro* Experiments**

#### ***Effects of HIV gp120 on VDR, CYP24A1, CYP27B1 and IL-6 Expression in Human Monocytes***

As shown in Figure 2, monocyte treatment with gp120 significantly increased CYP27B1 mRNA expression (356.7%,  $p < 0.05$ ) compared with the control group, as well as VDR (174.11%,  $p < 0.05$ ). As for CYP24A1, we noticed a slight, not significant increase in the expression at 3h.

#### ***Effects of LPS on VDR, CYP24A1, CYP27B1 and IL-6 Expression in Human Monocytes***

We examined the effect of LPS on VDR, CYP24A1, CYP27B1 and IL-6 expression on freshly isolated monocytes. As shown in Figure 3, monocyte treatment with LPS significantly increased CYP27B1 mRNA expression, with

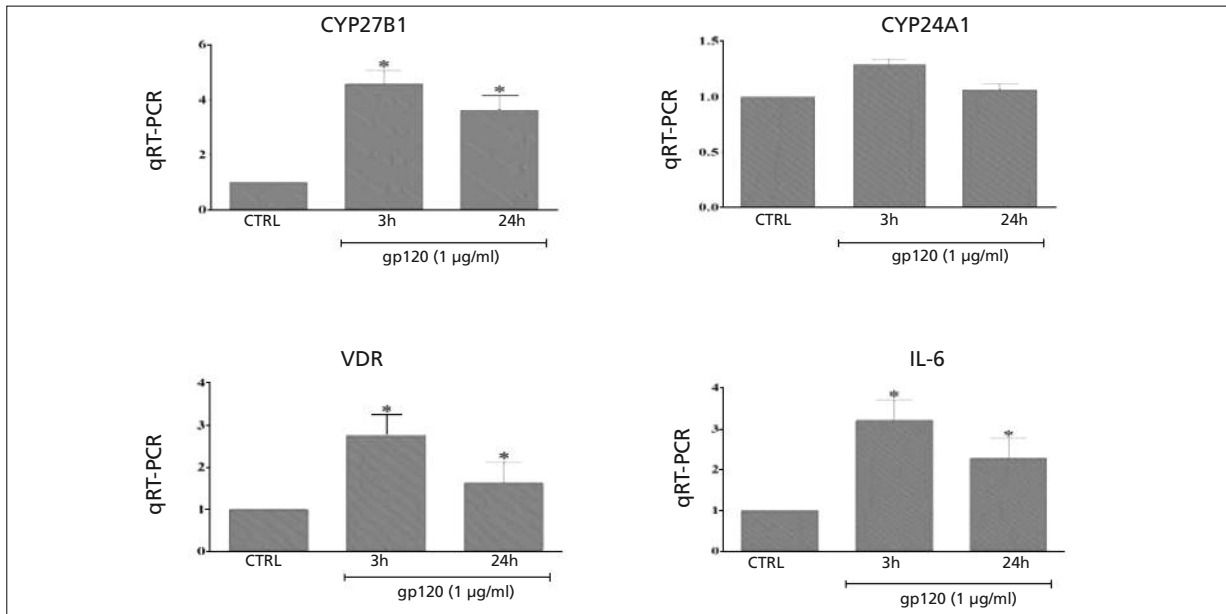
the highest stimulation at 24h (258%,  $p < 0.05$ ). CYP24A1 and VDR expression were not significantly affected by treatment with LPS. As for IL-6, its levels significantly increased at 4h (134%,  $p < 0.05$ ) and then decreased over time (75%,  $p < 0.05$ ).

#### ***Effects of LPS on VDR, CYP24A1, CYP27B1 and IL-6 Expression in Human Macrophages***

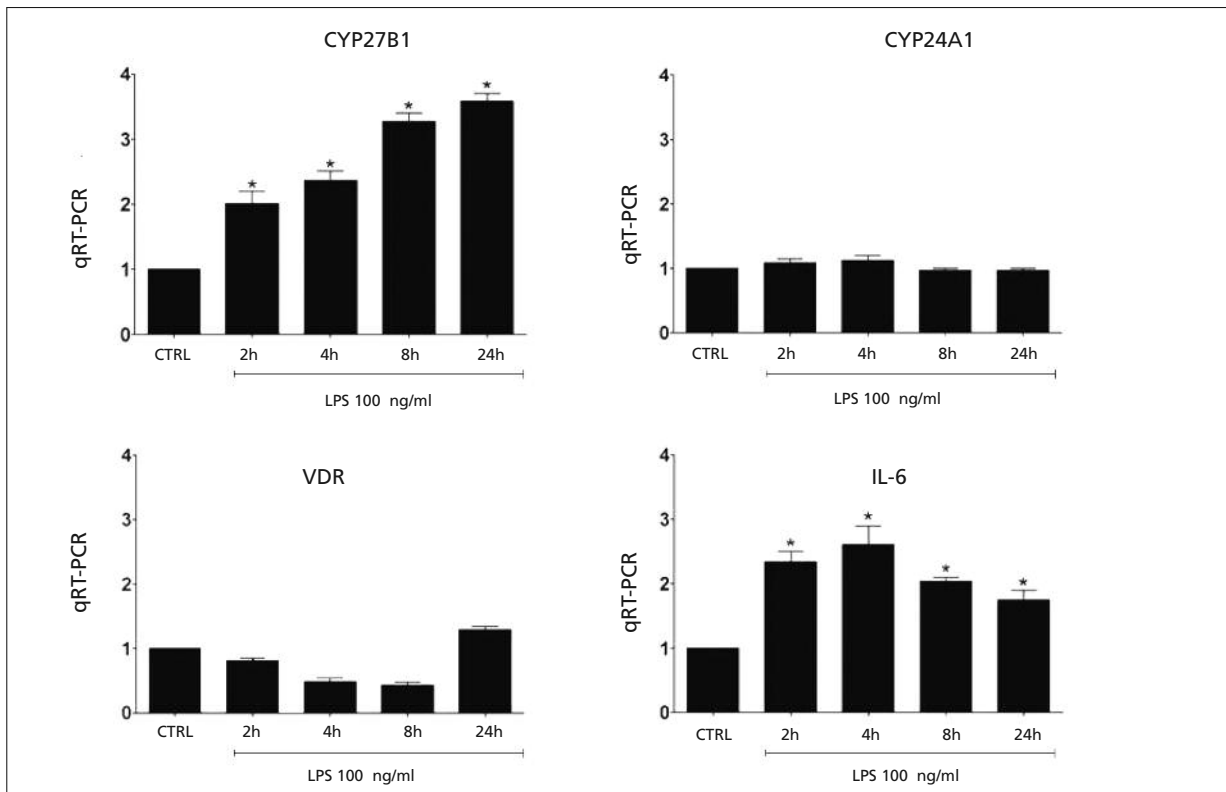
We examined the effect of LPS on VDR, CYP24A1, CYP27B1 and IL-6 expression on macrophages. As shown in Figure 4, macrophage treatment with LPS significantly increased CYP27B1 mRNA expression, with the highest stimulation at 6h (23-fold increase,  $p < 0.01$ ). CYP24A1 mRNA expression was significantly up-regulated at 2h (18-fold increase,  $p < 0.01$ ) and 4h (12-fold increase,  $p < 0.01$ ). VDR expression was not significantly affected by treatment with LPS. As for IL-6, its levels significantly increased upon LPS stimulation, with the highest expression at 6h (22-fold increase,  $p < 0.01$ ).

#### ***In vitro* Effects of LPS on Vitamin D Consumption**

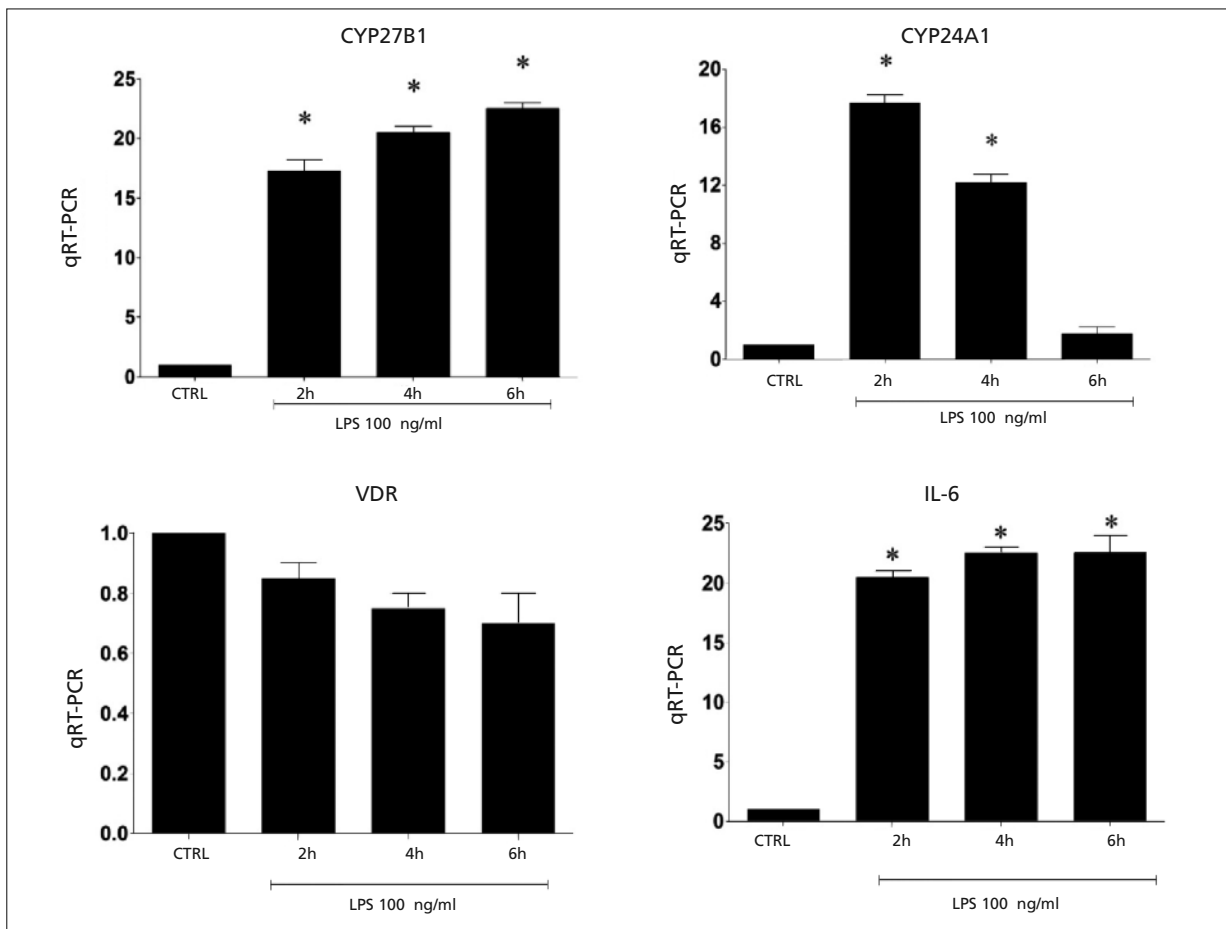
As shown in Figure 5, we found that vitamin D levels were significantly lower in the super-



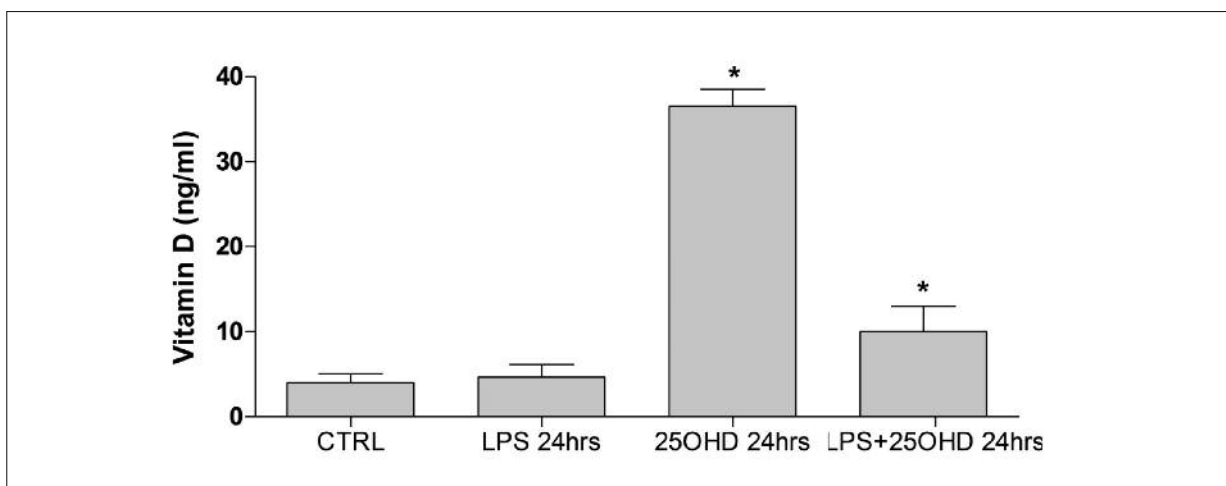
**Figure 2.** Quantification of CYP27B1, CYP24A1, vitamin D receptor (VDR) and Interleukin (IL)-6 mRNA expression by real-time polymerase chain reaction in human monocytes, at 3h and 24h, following treatment with gp120 (1 µg/ml). Expression levels are calculated as ΔCt value. Data are representative of three independent experiments. \**p* < 0.05 in comparison with control (CTRL).



**Figure 3.** Quantification of CYP27B1, CYP24A1, vitamin D receptor (VDR) and Interleukin (IL)-6 expression by real-time polymerase chain reaction in human monocytes, following treatment with lipopolysaccharide (LPS) (100 ng/ml) at different time points. Expression levels are calculated as ΔCt value. Data are representative of three independent experiments. \**p* < 0.05 in comparison with control (CTRL).



**Figure 4.** Quantification of CYP27B1, CYP24A1, vitamin D receptor (VDR) and Interleukin (IL)-6 expression by real-time polymerase chain reaction in human macrophages, following treatment with lipopolysaccharide (LPS) (100 ng/ml) at different time points. Expression levels are calculated as  $\Delta\Delta C_t$  value. Data are representative of three independent experiments. \* $p < 0.01$  in comparison with control (CTR).



**Figure 5.** Evaluation of 25-hydroxyvitamin D (25OHD) levels in the supernatant of monocytes stimulated for 24h with lipopolysaccharide (LPS) (100 ng/ml)  $\pm$  25OHD (40 ng/ml), 25OHD (40 ng/ml) and controls. Data are representative of three independent experiments. \* $p < 0.01$ .

nant of monocytes treated with LPS plus 25OHD in comparison with monocytes treated with 25OHD alone (75%,  $p < 0.01$ ). Vitamin D levels in the supernatant of controls and LPS-treated monocytes were unremarkable.

### **IL-6 mRNA Expression in Monocytes of Healthy Controls and HIV-positive Patients Stimulated with 1,25OH<sub>2</sub>D**

We found no baseline expression of IL-6 mRNA in monocytes of healthy controls. On the contrary, 3 out of 5 (60%) of monocytes isolated from vitamin D-deficient HIV-infected patients and 1 out of 5 (20%) of monocytes from patients with 25OHD > 10 ng/ml patients expressed IL-6 mRNA at baseline. IL-6 levels were higher in monocytes from vitamin D-deficient subjects ( $p < 0.05$ ). Treatment with 1,25OH<sub>2</sub>D for 24h significantly reduced IL-6 expression in both groups (74% and 48% respectively,  $p < 0.01$  in comparison with baseline values) (Figure 6).

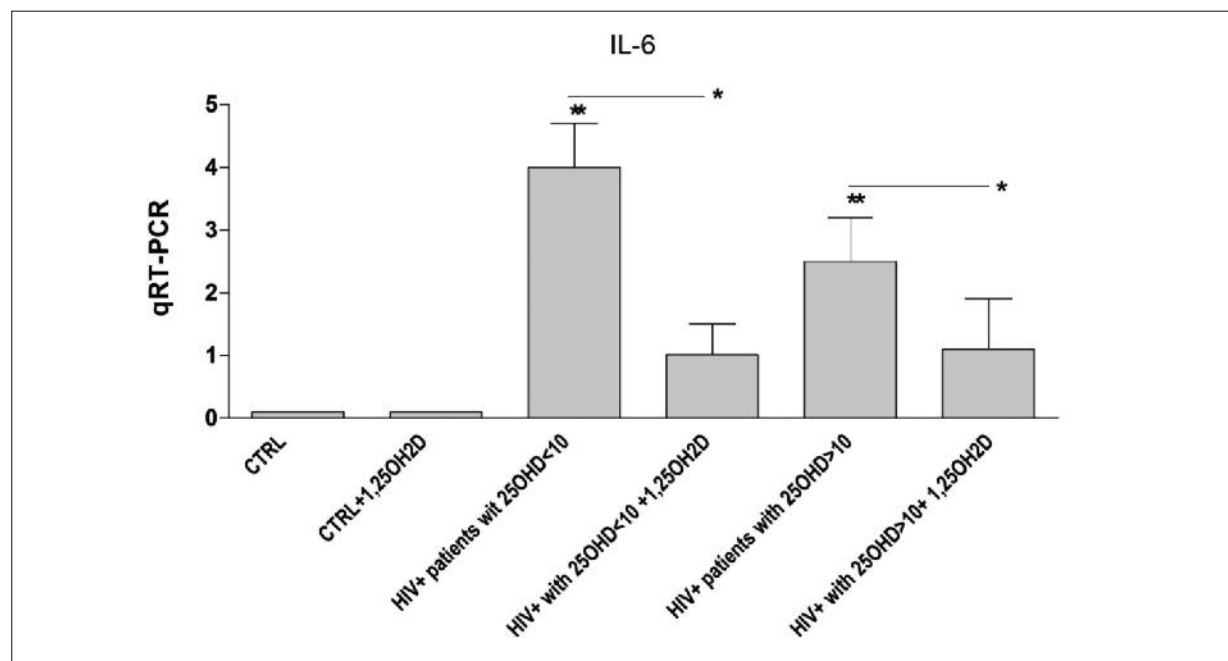
## **Discussion**

In our cross-sectional study, we evaluated the prevalence of vitamin D deficiency during spring months in a cohort of HIV-positive subjects. Al-

most 90% of patients had vitamin D levels below 30 ng/ml and about one-third had severe vitamin D deficiency. Similar prevalence rates have been described in several large HIV cohorts: in the SUN study<sup>36</sup>, 71% of HIV-positive subjects had 25OHD levels below 30 ng/ml, in the EuroSIDA study only 11% of patients had 25OHD levels above 30 ng/ml<sup>37</sup>. However, vitamin D deficiency seems to be very common even among HIV-negative subjects. In fact, in the SUN study no difference in the prevalence of hypovitaminosis D were found between HIV-positive and HIV-negative subjects<sup>36</sup>; analogously, Stein et al<sup>42</sup> described similar prevalence rates of vitamin D deficiency/insufficiency (about 75%) in both HIV-positive and HIV-negative postmenopausal women.

We observed a significant association between vitamin D deficiency and some established predictors of 25OHD levels, such as age<sup>37,38</sup> and female sex<sup>48</sup>. On the contrary, there was no association between exposure to HAART and 25OHD levels. In fact, even though vitamin D-deficient subjects were on HAART for longer time (163 vs. 132 months), the difference was not statistically significant, possibly because of the small sample size.

Several antiretroviral drugs are known to interfere, at least *in vitro*, with vitamin D metabo-



**Figure 6.** Quantification of Interleukin (IL)-6 expression by real-time polymerase chain reaction in human monocytes of healthy controls, HIV-infected patients with 25-hydroxyvitamin D (25OHD) < 10 ng/ml and patients with 25OHD > 10 ng/ml at baseline and after treatment with 1,25-dihydroxyvitamin D (1,25OH<sub>2</sub>D) (1  $\mu$ M) for 24h. Expression levels are calculated as  $\Delta$ DCt value. \*\* $p < 0.05$ ; \* $p < 0.01$ .



lism. PI have been shown to inhibit vitamin D  $1\alpha$ - and  $25\alpha$ -hydroxylation in hepatocyte and monocyte cultures<sup>49</sup>, EFV has been associated with increased 25OHD catabolism, through the induction of CYP24A1<sup>50</sup>. Some *in vivo* studies<sup>50-52</sup> have related the administration of EFV with an increased risk of developing vitamin D deficiency. In the present report, we failed to detect any association between 25OHD levels and specific antiretroviral drugs.

Even though some defect in PTH production/secretion in HIV-infected patients has been hypothesized<sup>53</sup>, we found that patients with 25OHD < 10 ng/ml had significantly higher PTH levels than those with 25OHD > 10 ng/ml. This observation is in keeping with the physiological regulation of PTH-vitamin D-calcium axis, as low vitamin D levels usually result in secondary hyperparathyroidism. Childs et al<sup>54</sup> have reported higher PTH levels among patients receiving TDF who were vitamin D-deficient; similar results have been reported by Labarga et al<sup>55</sup>.

In contrast with other studies, describing obesity as a risk factor for vitamin D deficiency<sup>36,56</sup>, we found that patients with 25OHD < 10 ng/ml had lower BMI in comparison with those with 25OHD > 10 ng/ml. In a work of Mehta et al<sup>57</sup>, the Authors reported low 25OHD to correlate with increased risk of wasting, defined as BMI < 18 kg/m<sup>2</sup>. Increased secretion of pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$ , IL-1 and IL-6 has been shown to contribute to wasting<sup>58-59</sup>. Considering the immunomodulatory properties of vitamin D, it can be supposed that vitamin D deficiency may be associated with higher levels of pro-inflammatory cytokines, hence, with an increased risk of wasting. However, even though this mechanism may partially explain the differences in BMI observed in our cohort, this association should be interpreted with caution as almost all patients in our study had BMI within the normal range.

There is a growing evidence relating vitamin D deficiency with inflammation. The present study suggests a positive correlation between hypovitaminosis D and hs-CRP levels. These findings are consistent with a recent report of Palmer et al<sup>60</sup>, in which participants with 25OHD levels < 20 ng/ml had a 3.2-fold higher odds of high CRP compared to those with a 25OHD serum level  $\geq$  20 ng/ml. Similarly, in a work of Piroth et al<sup>61</sup>, vitamin D deficiency correlated with IL-6 and hs-CRP levels, albeit the latter did not reach statistical significance. Given the cross-sectional design of both

studies, we could not establish if it is chronic inflammation to contribute to vitamin D deficiency or if it is vitamin D deficiency to increase production of pro-inflammatory cytokines.

It is well known that vitamin D has several immunomodulatory functions, related with both innate and adaptive immunity<sup>31,32</sup>. In our *in vitro* experiments, we found that monocytes from vitamin D-deficient subjects expressed IL-6 mRNA in a significantly higher number of cases in comparison with patients with 25OHD > 10 ng/ml, whereas healthy controls did not express IL-6 mRNA. Moreover, treatment with 1,25OH<sub>2</sub>D was able to reduce IL-6 levels when added to monocyte cultures in both groups. Monocyte stimulation with both gp120 and LPS upregulated the expression of CYP27B1, the enzyme catalyzing the conversion of 25OHD to 1,25OH<sub>2</sub>D, the biologically active metabolite of 25OHD. This mechanism, which can be amplified by residual chronic inflammation, may theoretically reduce circulating 25OHD levels by enhancing its conversion to 1,25OH<sub>2</sub>D. Moreover, we found increased CYP24A1 expression in macrophages but not monocytes after LPS stimulation, leading to an increased activation of the catabolic pathway. We also found lower 25OHD levels in the supernatant of monocytes treated with LPS plus 25OHD in comparison with monocytes treated with 25OHD alone. Our *in vitro* data are in keeping with the findings of Liu et al<sup>62</sup>, showing increased expression of CYP27B1 in activated macrophages, as well as with the study of Mathieu et al<sup>63</sup>, describing CYP27B1 upregulation in response to LPS-mediated TLR4/CD14 stimulation. In a recent work of Safdar et al<sup>64</sup>, higher 1,25OH<sub>2</sub>D levels correlated with higher HIV viral load. The Authors suggested immune activation as a possible explanation, as increasing viremia may lead to systemic chronic activation of the immune system, including monocytes/macrophages, which may in turn result in increased conversion of 25OHD to 1,25OH<sub>2</sub>D. Although we did not find any significant association between viral load and 25OHD levels, the observation that gp120 upregulates CYP27B1 expression is consistent with this hypothesis. It may be speculated that immune cells, such as monocyte/macrophages and T cells, may convert and utilize 25OHD and 1,25OH<sub>2</sub>D to regulate immune responses. This process could ultimately lead to vitamin D consumption and not to a release of 1,25OH<sub>2</sub>D in the circulation, which is, instead, what kidney cells do.

## Conclusions

Hypovitaminosis D was highly prevalent in our population of HIV-infected subjects. As suggested by current European AIDS Clinical Society guidelines<sup>47</sup>, HIV-positive patients should be routinely screened for vitamin D deficiency, in order to identify those requiring supplementation. There is a need for large prospective studies, in order to identify the causal relationship between risk factors and vitamin D deficiency and to extensively study the impact of vitamin D supplementation on immune activation and prevention of comorbidities, also in cancer patients with HIV infection<sup>65-72</sup>.

## Conflict of Interest

No conflict of interest to declare. The present study was supported by internal funds of the University of Catania, Italy. These data were presented in part at the SIMIT Conference, La Maddalena, Italy 2011 and at the International AIDS Conference, Washington D.C., USA 2012.

## References

- BERRETTA M, CINELLI R, MARTELOTTA F, SPINA M, VACCHER E, TIRELLI U. Therapeutic approaches to AIDS-related malignancies. *Oncogene* 2003; 22: 6646-6659.
- NUNNARI G, OTERO M, DORNADULA G, VANELLA M, ZHANG H, FRANK I, POMERANTZ RJ. Residual HIV-1 disease in seminal cells of HIV-1-infected men on suppressive HAART: latency without on-going cellular infections. *AIDS* 2002; 16: 39-45.
- NUNNARI G, GUSSIO M, PINZONE MR, MARTELOTTA F, COSENTINO S, CACOPARDO B, CELESIA BM. Cryptococcal meningitis in an HIV-1-infected person: relapses or IRIS? Case report and review of the literature. *Eur Rev Med Pharmacol Sci* 2013; 17: 1555-1559.
- NUNNARI G, SULLIVAN J, XU Y, NYIRJESY P, KULKOSKY J, CAVERT W, FRANK I, POMERANTZ RJ. HIV type 1 cervicovaginal reservoirs in the era of HAART. *AIDS Res Hum Retroviruses* 2005; 21: 714-718.
- KULKOSKY J, BOUHAM DAN M, GEIST A, NUNNARI G, PHINNEY DG, POMERANTZ RJ. Pathogenesis of HIV-1 infection within bone marrow cells. *Leuk Lymphoma* 2000; 37: 497-515.
- POMERANTZ RJ, NUNNARI G. HIV and GB virus C--can two viruses be better than one? *N Engl J Med* 2004; 350: 963-965.
- DORNADULA G, NUNNARI G, VANELLA M, ROMAN J, BABINCHAK T, DE SIMONE J, STERN J, BRAFFMAN M, ZHANG H, POMERANTZ RJ. Human immunodeficiency virus type 1-infected persons with residual disease and virus reservoirs on suppressive highly active antiretroviral therapy can be stratified into relevant virologic and immunologic subgroups. *J Infect Dis* 2001; 183: 1682-1687.
- OTERO M, NUNNARI G, LETO D, SULLIVAN J, WANG FX, FRANK I, XU Y, PATEL C, DORNADULA G, KULKOSKY J, POMERANTZ RJ. Peripheral blood Dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. *AIDS Res Hum Retroviruses* 2003; 19: 1097-1103.
- NUNNARI G, ARGYRIS E, FANG J, MEHLMAN KE, POMERANTZ RJ, DANIEL R. Inhibition of HIV-1 replication by caffeine and caffeine-related methylxanthines. *Virology* 2005; 335: 177-184.
- SMITH JA, NUNNARI G, PREUSS M, POMERANTZ RJ, DANIEL R. Pentoxifylline suppresses transduction by HIV-1-based vectors. *Intervirology* 2007; 50: 377-386.
- PINZONE MR, CACOPARDO B, CONDORELLI F, DI ROSA M, NUNNARI G. Sirtuin-1 and HIV-1: An overview. *Curr Drug Targets* 2013; 14: 648-652.
- WANG FX, XU Y, SULLIVAN J, SOUDER E, ARGYRIS EG, ACHEAMPONG EA, FISHER J, SIERRA M, THOMSON MM, NAJERA R, FRANK I, KULKOSKY J, POMERANTZ RJ, NUNNARI G. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J Clin Invest* 2005; 115: 128-137.
- ZANET E, BERRETTA M, DI BENEDETTO F, TALAMINI R, BALLARIN R, NUNNARI G, BERRETTA S, RIDOLFO A, LLESHI A, ZANGHI A, CAPPPELLANI A, TIRELLI U. Pancreatic cancer in HIV-positive patients: a clinical case-control study. *Pancreas* 2012; 41: 1331-1335.
- BERRETTA M, GARLASSI E, CACOPARDO B, CAPPPELLANI A, GUARALDI G, COCCHI S, DE PAOLI P, LLESHI A, IZZI I, TORRESIN A, DI GANGI P, PIETRANGELO A, FERRARI M, BEARZ A, BERRETTA S, NASTI G, DI BENEDETTO F, BALESTRERI L, TIRELLI U, VENTURA P. Hepatocellular carcinoma in HIV-infected patients: check early, treat hard. *Oncologist* 2011; 16: 1258-1269.
- BERRETTA M, LLESHI A, CAPPPELLANI A, BEARZ A, SPINA M, TALAMINI R, CACOPARDO B, NUNNARI G, MONTESAR-CHIO V, IZZI I, LANZAFAME M, NASTI G, BASILE F, BERRETTA S, FISICHELLA R, SCHIANTARELLI C, GARLASSI E, RIDOLFO A, GUELLA L, TIRELLI U. Oxaliplatin based chemotherapy and concomitant highly active antiretroviral therapy in the treatment of 24 patients with colorectal cancer and HIV infection. *Curr HIV Res* 2010; 8: 218-222.
- BERRETTA M, CAPPPELLANI A, DI BENEDETTO F, LLESHI A, TALAMINI R, CANZONIERI V, ZANET E, BEARZ A, NASTI G, LACCHIN T, BERRETTA S, FISICHELLA R, BALESTRERI L, TORRESIN A, IZZI I, ORTOLANI P, TIRELLI U. Clinical presentation and outcome of colorectal cancer in hiv-positive patients: a clinical case-control study. *Onkologie* 2009; 32: 319-324.
- BERRETTA M, ZANET E, BASILE F, RIDOLFO AL, DI BENEDETTO F, BEARZ A, BERRETTA S, NASTI G, TIRELLI U. HIV-positive patients with liver metastases from colorectal cancer deserve the same therapeutic approach as the general population. *Onkologie* 2010; 33: 203-204.
- ZANET E, BERRETTA M, MARTELOTTA F, CACOPARDO B, FISICHELLA R, TAVIO M, BERRETTA S, TIRELLI U. Anal cancer: focus on HIV-positive patients in the HAART era. *Curr HIV Res* 2011; 9: 70-81.

- 19) NUNNARI G, XU Y, ACHEAMPONG EA, FANG J, DANIEL R, ZHANG C, ZHANG H, MUKHTAR M, POMERANTZ RJ. Exogenous IL-7 induces Fas-mediated human neuronal apoptosis: potential effects during human immunodeficiency virus type 1 infection. *J Neurovirol* 2005; 11: 319-328.
- 20) NUNNARI G, POMERANTZ RJ. IL-7 as a potential therapy for HIV-1-infected individuals. *Expert Opin Biol Ther* 2005; 5: 1421-1426.
- 21) NUNNARI G, BERRETTA M, PINZONE MR, DI ROSA M, CAPPELLANI A, BERRETTA S, TIRELLI U, MALAGUARNERA M, SCHNELL JM, CACOPARDO B. Hepatocellular carcinoma in HIV positive patients. *Eur Rev Med Pharmacol Sci* 2012; 16: 1257-1270.
- 22) MARTELOTTA F, BERRETTA M, CACOPARDO B, FISICHELLA R, SCHIOPPA O, ZANGHI A, SPARTÀ D, CAPPELLANI A, TALAMINI R, IZZI I, RIDOLFO A, TORRESIN A, FIORICA F, TIRELLI U. Clinical presentation and outcome of squamous cell carcinoma of the anus in HIV-infected patients in the HAART-era: a GICAT experience. *Eur Rev Med Pharmacol Sci* 2012; 16: 1283-1291.
- 23) BERRETTA M, DI BENEDETTO F, DAL MASO L, CACOPARDO B, NASTI G, FACCHINI G, BEARZ A, SPINA M, GARLASSI E, DE RE V, FIORICA F, LLESHI A, TIRELLI U. Sorafenib for the treatment of unresectable hepatocellular carcinoma in HIV-positive patients. *Anticancer Drugs* 2013; 24: 212-218.
- 24) PINZONE MR, FIORICA F, DI ROSA M, MALAGUARNERA G, MALAGUARNERA L, CACOPARDO B, ZANGHI G, NUNNARI G. Non-AIDS-defining cancers among HIV-infected people. *Eur Rev Med Pharmacol Sci* 2012; 16: 1377-1388.
- 25) BEARZ A, VACCHER E, TALAMINI R, BERRETTA M, TIRELLI U. Comment on 'Lung cancer in the Swiss HIV Cohort Study: role of smoking, immunodeficiency and pulmonary infection'. *Br J Cancer* 2012; 106: 1899-1900.
- 26) NUNNARI G, SMITH JA, DANIEL R. HIV-1 Tat and AIDS-associated cancer: targeting the cellular anti-cancer barrier. *J Exp Clin Cancer Res* 2008; 27: 3.
- 27) CACOPARDO B, CAMMA C, PETTA S, PINZONE MR, CAPPELLANI A, ZANGHI A, NICOLOSI A, NUNNARI G. Diagnostic and therapeutic role of vitamin D in chronic hepatitis C virus infection. *Front Biosci (Elite Ed)*. 2012 Jan 1;4:1276-86. Review. PubMed mPMID: 22201953.
- 28) APPAY V, SAUCE D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J Pathol* 2008; 214: 231-241.
- 29) NIXON DE, LANDAY AL. Biomarkers of immune dysfunction in HIV. *Curr Opin HIV AIDS* 2010; 5: 498-503.
- 30) PINZONE MR, DI ROSA M, CACOPARDO B, NUNNARI G. HIV RNA suppression and immune restoration: can we do better? *Clin Dev Immunol* 2012; 2012: 515962.
- 31) DI ROSA M, MALAGUARNERA L, NICOLOSI A, SANFILIPPO C, MAZZARINO C, PAVONE P, BERRETTA M, COSENTINO S, CACOPARDO B, PINZONE MR, NUNNARI G. Vitamin D3: an ever green molecule. *Front Biosci (Schol Ed)* 2013; 5: 247-260.
- 32) DI ROSA M, MALAGUARNERA G, DE GREGORIO C, PALUMBO M, NUNNARI G, MALAGUARNERA L. Immuno-modulatory effects of vitamin D3 in human monocyte and macrophages. *Cell Immunol* 2012; 280: 36-43.
- 33) GUILLOT X, SEMERANO L, SAIDENBERG-KERMANACH N, FALGARONE G, BOISSIER MC. Vitamin D and inflammation. *Joint Bone Spine* 2010; 77: 552-557.
- 34) HELMING L, BOSE J, EHRCHEN J, SCHIEBE S, FRAHM T, GEFERS R, PROBST-KEPPER M, BALLING R, LENGELING A. Alpha,25-dihydroxyvitamin D3 is a potent suppressor of interferon gamma-mediated macrophage activation. *Blood* 2005; 106: 4351-4358.
- 35) AMER M, QAYYUM R. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous national health and nutrition examination survey 2001 to 2006). *Am J Cardiol* 2012; 109: 226-230.
- 36) DAO CN, PATEL P, OVERTON ET, RHAME F, PALS SL, JOHNSON C, BUSH T, BROOKS JT; STUDY TO UNDERSTAND THE NATURAL HISTORY OF HIV AND AIDS IN THE ERA OF EFFECTIVE THERAPY (SUN) INVESTIGATORS. 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.
- 37) VIARD JP, SOUBERBIELLE JC, KIRK O, REEKIE J, KNYSZ B, LOSSO M, GATELL J, PEDERSEN C, BOGNER JR, LUNDGREN JD, MOCROFT A; EUROSIDA STUDY GROUP. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. *AIDS* 2011; 25: 1305-1315.
- 38) VESCINI F, COZZI-LEPRI A, BORDERI M, RE MC, MAGGIOLLO F, DE LUCA A, CASSOLA G, VULLO V, CAROSI G, ANTINORI A, TOZZI V, MONFORTE AD; ICONA FOUNDATION STUDY GROUP. Prevalence of Hypovitaminosis D and Factors Associated With Vitamin D Deficiency and Morbidity Among HIV-Infected Patients Enrolled in a Large Italian Cohort. *J Acquir Immune Defic Syndr* 2011; 58: 163-172.
- 39) PINZONE MR, DI ROSA M, MALAGUARNERA M, MADEDU G, FOCÀ E, CECCARELLI G, D'ETTORRE G, VULLO V, FISICHELLA R, CACOPARDO B, NUNNARI G. Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic. *Eur Rev Med Pharmacol Sci* 2013; 17: 1218-1232.
- 40) NUNNARI G, COCO C, PINZONE MR, PAVONE P, BERRETTA M, DI ROSA M, SCHNELL M, CALABRESE G, CACOPARDO B. The role of micronutrients in the diet of HIV-1-infected individuals. *Front Biosci (Elite Ed)* 2012; 4: 2442-2456.
- 41) MADEDU G, SPANU A, SOLINAS P, CALIA GM, LOVIGU C, CHESSA F, MANNAZZU M, FALCHI A, MURA MS, MADEDU G. Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. *Q J Nucl Med Mol Imaging* 2004; 48: 39-48.
- 42) STEIN EM, YIN MT, McMAHON DJ, SHU A, ZHANG CA, FERRIS DC, COLON I, DOBKIN JF, HAMMER SM, SHANE E. Vitamin D deficiency in HIV-infected postmenopausal Hispanic and African-American women. *Osteoporos Int* 2010; 22: 477-487.

- 43) CHOI AI, LO JC, MULLIGAN K, SCHNELL A, KALAPUS SC, LI Y, HUNT PW, MARTIN JN, DEEKS SG, HSUE PY. Association of vitamin D insufficiency with carotid intima-media thickness in HIV-infected persons. *Clin Infect Dis* 2011; 52: 941-944.
- 44) SZEPEL Z, GUARALDI G, SHAH SS, LO RE V 3RD, RATCLIFFE SJ, ORLANDO G, CARLI F, ROSSI R, ROCHIRA V, TEBAS P. Vitamin D deficiency is associated with type 2 diabetes mellitus in HIV infection. *AIDS* 2011; 25: 525-529.
- 45) LAI H, GERSTENBLITH G, FISHMAN EK, BRINKER J, KICKLER T, TONG W, BHATIA S, HONG T, CHEN S, LI J, DETRICK B, LAI S. Vitamin D deficiency is associated with silent coronary artery disease in cardiovascularly asymptomatic African Americans with HIV infection. *Clin Infect Dis* 2012; 54: 1747-1755.
- 46) 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.
- 47) EUROPEAN AIDS CLINICAL SOCIETY. Guidelines: prevention and management of non-infectious comorbidities in HIV. Available at <http://www.europeanaidscinicalsociety.org/images/stories/EACS-Pdf/EacsGuidelines-v6.12edition.pdf>. Accessed January 2013.
- 48) VAN DEN BOUT-VAN DEN BEUKEL CJ, FIEVEZ L, MICHELS M, SWEEP FC, HERMUS AR, BOSCH ME, BURGER DM, BRAVENBOER B, KOOPMANS PP, VAN DER VEN AJ. Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy. *AIDS Res Hum Retroviruses* 2008; 24: 1375-1382.
- 49) 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.
- 50) BROWN T, MC COMSEY G. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. *Antivir Ther* 2010; 15: 425-429.
- 51) 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.
- 52) FUX CA, BAUMANN S, FURRER H, MUELLER NJ. Is lower serum 25-hydroxy vitamin D associated with efavirenz or the non-nucleoside reverse transcriptase inhibitor class? *AIDS* 2011; 25: 876-878.
- 53) HAUG CJ, AUKRUST P, HAUG E, MORKRID L, MULLER F, FROLAND SS. Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunologic hyperactivity and only minor changes in calcium homeostasis. *J Clin Endocrinol Metab* 1998; 83: 3832-3838.
- 54) CHILDS KE, FISHMAN SL, CONSTABLE C, GUTIERREZ JA, WYATT CM, DIETERICH DT, MULLEN MP, BRANCH AD. Short communication: inadequate vitamin D exacerbates parathyroid hormone elevations in tenofovir users. *AIDS Res Hum Retroviruses* 2010; 26: 855-899.
- 55) LABARGA P, BARREIRO P, MARTIN-CARBONERO L, ALVAREZ E, SOLERA C, PINILLA J. Prevalence and mechanisms of hyperparathyroidism in HIV+ patients treated with TDF. Abstr 18th Conference on Retroviruses and Opportunistic Infections Boston, MA, 2011.
- 56) MUELLER NJ, FUX CA, LEDERGERBER B, ELZI L, SCHMID P, DANG T, MAGENTA L, CALMY A, VERGOPOULOS A, BISCHOFF-FERRARI HA; SWISS HIV COHORT STUDY. High prevalence of severe vitamin D deficiency in combined antiretroviral therapy naive and successfully treated Swiss HIV patients. *AIDS* 2010; 24: 1127-1134.
- 57) MEHTA S, MUGUSI FM, SPIEGELMAN D, VILLAMOR E, FINKELSTEIN JL, HERTZMARK E, GIOVANNUCCI EL, MSAMANGA GI, FAWZI WW. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. *AIDS Patient Care STDS* 2011; 25: 579-585.
- 58) RIMANIOL AC, ZYLBERBERG H, ZAVALA F, VIARD JP. Inflammatory cytokines and inhibitors in HIV infection: Correlation between interleukin-1 receptor antagonist and weight loss. *AIDS* 1996; 10: 1349-1356.
- 59) GODFRIED MH, VAN DER POLL T, JANSEN J, ROMIJN JA, SCHATTEKERK JK, ENDERT E, VAN DEVENTER SJ, SAUERWEIN HP. Soluble receptors for tumour necrosis factor: A putative marker of disease progression in HIV infection. *AIDS* 1993; 7: 33-36.
- 60) POUDEL-TANDUKAR K, POUDEL KC, JIMBA M, KOBAYASHI J, JOHNSON CA, PALMER PH. Serum 25-hydroxyvitamin D levels and C-Reactive Protein in persons with human immunodeficiency virus infection. *AIDS Res Hum Retroviruses* 2013; 29: 528-534.
- 61) ANSEMANT T, MAHY S, PIROTH C, ORNETTI P, EWING S, GUILLAND JC, CROISIER D, DUVILLARD L, CHAVANET P, MAILLEFERT JF, PIROTH L. Severe hypovitaminosis D correlates with increased inflammatory markers in HIV infected patients. *BMC Infect Dis* 2013; 13: 7.
- 62) LIU PT, STENGER S, LI H, WENZEL L, TAN BH, KRUTZIK SR, OCHOA MT, SCHAUBER J, WU K, MEINKEN C, KAMEN DL, WAGNER M, BALS R, STEINMEYER A, ZÜGEL U, GALLO RL, EISENBERG D, HEWISON M, HOLLIS BW, ADAMS JS, BLOOM BR, MODLIN RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006; 311(57968): 1770-1773.
- 63) STOFFELS K, OVERBERGH L, GIULIETTI A, VERLINDEN L, BOUILLON R, MATHIEU C. Immune Regulation of 25-Hydroxyvitamin-D3-1alpha-Hydroxylase in Human Monocytes. *J Bone Miner Res* 2006; 21: 37-47.
- 64) BEARDEN A, ABAD C, GANGNON R, SOSMAN JM, BINKLEY N, SAFDAR N. Cross-sectional study of Vitamin D levels, immunologic and virologic outcomes in HIV-infected adults. *J Clin Endocrinol Metab* 2013; 98: 1726-1733.
- 65) DI BENEDETTO F, DE RUVO N, BERRETTA M, MASETTI M, MONTALTI R, DI SANDRO S, QUINTINI C, TIRELLI U,

- GERUNDA G. Don't deny Liver transplantation to HIV-positive patients with HCC in the HAART-era. *JCO* 2006; 24: e26-27.
- 66) DI BENEDETTO F, DI SANDRO S, DE RUVO N, BERRETTA M, MONTALTI R, GUERINI GP, BALLARIN R, DE BLASIS MG, SPAGGIARI M, SMERIERI N, IEMMOLO RM, GUARALDI G, Gerunda GE Human immunodeficiency virus and liver transplantation: our point of view: *Transplant Proc* 2008; 40: 1965-1971.
- 67) BERRETTA M, ZANET E, DI BENEDETTO F, SIMONELLI C, BEARZ A, MORRA A, BONANNO S, BERRETTA S, TIRELLI U. Unusual presentation of metastatic hepatocellular carcinoma in an HIV/HCV coinfecting patient: case report and review of the literature. *Tumori* 2008; 94: 589-591.
- 68) SIMONELLI C, TEDESCHI R, GLOGHINI A, TALAMINI R, BORTOLIN MT, BERRETTA M, SPINA M, MORASSUT A, VACCHER E, DE PAOLI P, CARBONE A, TIRELLI U. Plasma HHV-8 viral load in HHV-8 related lymphoproliferative disorders associated with HIV infection. *J Med Virol* 2009 24; 81: 888-896.
- 69) BERRETTA M, DI BENEDETTO F, BEARZ A, SIMONELLI C, BERRETTA S, SPINA M, TIRELLI U. Folfox-4 regimen with concomitant highly active antiretroviral therapy in metastatic colorectal cancer HIV-infected patients: a report of five cases and review of the literature. *Cancer Invest* 2008; 26: 610-614.
- 70) BERRETTA M, ZANET E, TAIBI R, MARTELLOTTA F, PAVONE P, BEARZ A, GOBITTI C, CIANCIA E.M, CANZONIERI V, TIRELLI U. Leiomyosarcoma of the parotid gland in a HIV-positive patient: therapeutic approach, clinical course and review of the literature. *J Chemother* 2009; 21: 215-218.
- 71) TAVIO M, GROSSI P, BACCARANI U, SCUDELLER L, PEA F, BERRETTA M, ADANI G, VIVARELLI M, RIVA A, TIRELLI U, BRESADOLA V, VIALE P. Risaliti A HIV-infected patients and liver transplantation: who, when and why. *Curr HIV Res* 2011; 9: 120-127.
- 72) MARTELLOTTA F, BERRETTA M, VACCHER E, SCHIOPPA O, ZANET E, TIRELLI U. AIDS-related Kaposi's sarcoma: state of art. *Curr HIV Res* 2009; 7: 634-638.