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Clinical, behavioral and biomarker predictors of PCSK9 levels in HIV-infected patients naïve of statin therapy: A cross-sectional analysis from the Swiss HIV cohort

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HIGHLIGHTS

- Better characterization of Proprotein Convertase Subtilisin Kexin 9 (PCSK9) profile is needed in HIV patients.
- In HIV-infected individuals naïve of statin treatment, marijuana consumption and low CD4 values are associated with higher PCSK9 levels.
- A future randomized controlled study assessing PCSK9 inhibitors in HIV patients should be considered in order to improve control of dyslipidaemia.

ABSTRACT

Background and aims: Better characterization of Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) profile is currently needed to tailor appropriate lipid-lowering strategies in HIV patients.
Methods: HIV-infected individuals aged ≥ 40 years and naive of statin therapy included in the Swiss HIV cohort study were screened for PCSK9 levels with a routine blood sample collection in 2014 at the Geneva University Hospitals. An exploratory linear regression model was built including clinical (age, sex, ethnicity, cardiovascular risk factors, body mass index, low CD4 defined as ≤200 cells/μl, leucocytes, lymphocytes, platelet, antiretroviral therapy), behavioral (tobacco and marijuana smoking, alcohol use and physical activity) and biomarker (CRP, TNF-α, IL-8, Il-10 and MCP-1) to investigate association with continuous PCSK9 levels.
Results: We studied 239 HIV-infected individuals who met inclusion criteria and available PCSK9 levels with a mean age of 49 years. 35 subjects (14.6%) reported marijuana consumption, of whom 20 (57.1%) reported daily consumption and 15 (6.3%) occasional use. PCSK9 levels were correlated with low-density lipoprotein-cholesterol (LDL-C). Our exploratory model identified marijuana consumption ($p = 0.023$) and low CD4 values ($p = 0.020$) as significantly associated factors with higher PCSK9 levels. No association was found with Framingham risk score. Patients with marijuana consumption had significantly higher levels of PCSK9 with a dose-response effect ($p < 0.001$); the association persisted after adjustment for the calculated Framingham risk score ($p = 0.003$) and additional adjustment for clinical variables ($p = 0.027$).
Conclusions: In HIV-infected individuals naïve of statin treatment, marijuana consumption and low CD4 values are associated with higher PCSK9 levels independently of clinically relevant confounding factors.

1. Introduction

Proprotein convertase subtilisin/kexin 9 (PCSK9) has gained attention in the last decade as an emergent target for the treatment of hypercholesterolemia [1]. PCSK9 inhibitors have been shown to reduce low-density lipoprotein cholesterol (LDL-C) by 50% in subjects with hypercholesterolemia [2]. In addition, PCSK9 inhibitors have been shown to stabilize atherosclerosis process as demonstrated with coronary imaging studies and to reduce the major adverse cardiovascular events in high-risk patients [3,4]. Defining the characteristics of high-

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risk population is the cornerstone of individualizing cardiovascular prevention strategies. Among the subgroups at risk, HIV-infected patients are particularly vulnerable, as they might present with an altered lipid profile related to the use of certain antiretroviral drugs, or because they may be exposed to abicavir [5], as well as pro-atherosclerotic activity related to viral replication, both of which may contribute to higher cardiovascular (CV) risk compared to non-HIV subjects [6,7].

Reports on PCSK9 levels in HIV-infected patients are scarce [8], and HIV-infected patients have been typically excluded from published clinical trials assessing efficacy of monoclonal antibodies against PCSK9, although bococizumab was being evaluated in this group of patients (NCT02524106 trial, Bococizumab HIV Evaluation (B-HIVE) Study), as well as evolocumab (NCT02833844). Pilot studies indicated that HIV-positive patients have PCSK9 levels on the opposite direction than low-density lipoprotein cholesterol (LDL-C) levels, called an apparent "PCSK9-Lipid Paradox" [8]. Coinfection with hepatitis C virus (HCV) was, furthermore, associated with an additional increase in circulating PCSK9 levels, in parallel with an elevation of the inflammatory and proatherogenic cytokine interleukin-6 [8].

A better characterization of the PCSK9 profile is currently needed to tailor appropriate lipid-lowering strategies in HIV patients, taking also into account that HIV patients often lead unhealthy lifestyles, with a high prevalence of smokers or consumers of recreational drugs, such as marijuana or alcohol. In this cross-sectional study design including patients from the Swiss HIV Cohort Study (SHCS), we aimed to assess exploratory factors associated with high PCSK9 levels.

2. Materials and methods

2.1. Study population

All HIV-infected individuals included in the SHSC (www.shcs.ch) were screened for PCSK9 levels based on a routine blood sample collected in 2014 during a clinical visit at Geneva University Hospitals [9]. Exclusion criteria comprised preexisting CV disease or use of lipid-lowering therapy, as those patients are already considered to be at very high-risk, independently of the HIV status. In addition, we did not measure PCSK9 levels in subjects younger than 40 years, as the European Society of Cardiology/European Atherosclerosis Society guidelines for cardiovascular prevention do not recommend systematic cardiovascular risk assessment in men < 40 and women < 50 of age with no known CV risk factors [10].

2.2. Biomarker measurement

Whole blood and EDTA plasma were collected and centrifuged at 800 g for 10 min within 6 h of collection for fasting total cholesterol (TC), HDL, triglycerides, glucose, CD4 lymphocyte count and HIV RNA. Low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedwald formula. The SHCS scheduled regular follow-up visits every 6 months at which CD4 and CD8 lymphocyte cell counts and plasma HIV-1 viral load were measured [11]. The lymphocyte cell counts per μL blood were measured by flow cytometry [12]. Since the year 2000 all assays used for HIV-1 RNA detection had a detection limit of 50 copies per mL or lower. We classified then HIV related data as low CD4 if less than 200 cells/μl and HIV viral load as detectable if more or equal to 20 copies *vs.* undetectable if less than 20 copies.

All biomarker analyses were performed on EDTA plasma samples. PCSK9 was determined by colorimetric enzyme-linked immunosorbent assay [ELISA] (R&D Systems, Minneapolis, Minnesota), as previously described [13]. Interleukine (IL-8), interleukine (IL-10), Tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP) were measured using the Meso Scale Discovery (MSD) platform (Rockville, MD, USA) [9]. Analyte concentrations were determined with Discovery Workbench ® software 4.0, using a 4 parameter logistic fit model. The lower limits of detection in pg/ml were: IL-8, 0.04; IL-10, 0.03; TNF-α 0.04; CRP, 1.33; and MCP-1, 0.09. Intra-run CVs were below 7% and inter-run CVs below 15%.

2.3. Clinical and lifestyle measurements

Marijuana consumption was investigated based on a physician-administered questionnaire and information was gathered regarding frequency (daily, less frequently and never). Self-reported marijuana has been used as a validated measurement in large cohorts assessing its association with clinical outcomes [14]. Physical activity (classified as sedentary for ≤2 days monthly, moderately active for > 2 days monthly but < 5 days weekly, or highly active for ≥5 days weekly), alcohol consumption (never, ≤2 days weekly or > 2 days weekly) and smoking connsuption defined as at least 1 cigarette a day were also evaluated [15]. Given the reported impact of anti-retroviral therapy (ART) on humoral autoimmune response, lipid profile and CV diseases, we reported data on the current use of protease-inhibitors and abacavir. For complications related to fat redistribution disorders [16], we collected body mass index (BMI), waist-to-hip ratio and physician-reported lipodystrophy, defined as fat accumulation in any of the following regions (face, arms, legs, buttocks, abdomen, breasts, neck). Ethnicity was coded as white or others.

2.4. Statistical analyses

Baseline characteristics were presented by higher versus lower PCSK9 levels. The data were expressed as medians ± interquartile range for continuous variables and as numbers and percentages for categorical variables. Two-tailed Fisher's exact tests for dichotomous variables, larger Chi-squared tests for independence, and Mann-Whitney-U tests for continuous variables were utilized as appropriate. The correlation between total PCSK9 levels and other biomarkers was evaluated by a nonparametric test (Spearman rank correlation). Using scatterplot figures, we assessed the relationship between PCSK9 levels and LDL-C and CD4 levels, and calculated the Framingham risk score (age, sex, diabetes, current smoking, total and high-density lipoprotein cholesterol, systolic blood pressure and treatment for hypertension) [17]. The use of the 1-year Framingham risk score has been previously performed in the SHCS and validated against HIV-uninfected individuals [18]. Using a linear regression model, we explored the association between previously described clinical, behavorial and biomarker variables with continuous PCSK9 levels in univariate and multivariate variables. For variables presenting a distribution that did not follow a non-normal distribution, we performed a log transformation. Post-doc analyses were performed for marijuana consumption and PCSK9 levels. Continuous PCSK9 levels were transformed into a logarithm scale as the dependent outcome for linear regression and three models were built, based on the following independent variables: (1) unadjusted for marijuana consumption, (2) adjusted for the calculated Framingham risk score (age, sex, diabetes, current smoking, total and high-density lipoprotein cholesterol, systolic blood pressure and treatment for hypertension) [17], and (3) additional adjustments for available clinical variables relevant to HIV-individuals, such as body mass index, total leucocyte count, CD4 count (< 200 cells/μl), HIV viral load (detectable ≥ 20 copies vs undetectable < 20 copies), current use of protease-inhibitor regimens, current use of abacavir, physical activity) [19,20], alcohol consumption and transaminases as done in previous publications. We checked for multicollinearity using variance inflation factor (VIF). As a rule of thumb, a variable whose VIF values were greater than 10 have been omitted from the multivariate model. All hypothesis tests were two-sided and the significance level was set at 5%. Statistical analyses were performed using STATA software® (Version 15, STATA Corp, College Station, TX, USA).

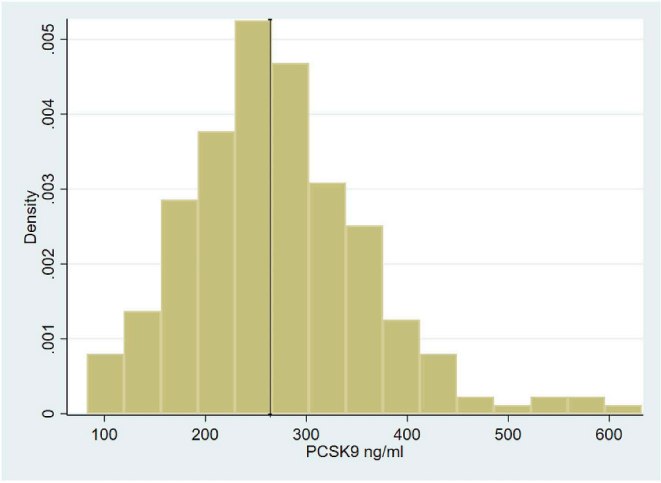

Fig. 1. Distribution of PCSK9 levels in 239 HIV-infected patients naive of statin therapy.

3. Results

Among 239 HIV-infected individuals who met the inclusion criteria with available PCSK9 levels, the distribution of PCSK9 levels is shown on Fig. 1. The mean value was 271.93 ng/ml, ranging from 82.67 ng/ml to 632.13 ng/ml. Baseline characteristics of patients classified as being in the higher median levels compared to those in the lower median levels are presented in Table 1. Mean age was 49.8 years, the median Framingham risk score over 10 years was 7.9% in the lowest group versus 8.6% in the highest group ($p = 0.663$). Patients with higher PCSK9 levels were more likely to be women (37.8% *vs.* 29.2%, $p = 0.157$), to consume frequently marijuana (10.9% *vs.* 5.8%, $p = 0.155$) or tobacco (43.4% *vs.* 30.5%, $p = 0.043$), but less alcohol (65.8% *vs.* 78.3%, $p = 0.032$). In term of HIV characteristics, those with higher PCSK9 levels had higher leucocytes (6050 *vs.* 5550 cells/μl, $p = 0.059$), lymphocytes (2028 *vs.* 1697 cells/μl, $p = 0.030$) and platelet (234 *vs.* 218 cells 10^9/l, $p = 0.010$) counts with higher proportion of low CD4 levels (11.8% *vs.* 5.0%, $p = 0.059$) and detectable HIV-RNA (26.0% *vs.* 18.3%, $p = 0.151$). Higher PCSK9 levels correlated with higher total cholesterol levels, LDL-C, MCP-1 and platelet count (Table 2). Borderline significance was observed for TNF-α levels, and for leucocyte and lymphocyte counts. No correlation was found with higher Framingham risk scores (Fig. 2).

In the multivariate models containing potential predictors of PCSK9 levels, two predictors reached the statistical significance: daily marijuana consumption ($p = 0.027$) and low CD4 levels ($p = 0.031$, Table 3). Borderline significance was observed for IL-8, gender and ethnicity (all P values ≤ 0.10). Patients with marijuana consumption had significantly higher levels of PCSK9 levels ($p < 0.001$, Fig. 3). Among 35 participants (14.6%) reporting marijuana consumption, 20 (57.1%) reported daily consumption

Table 1
Baseline characteristics of patients with PCSK9 values and naïve of statin therapy, selected from the Swiss HIV Cohort Study.

	Lower PCSK9 (82.7–264.4 ng/ml)	Higher PCKS9 (264.4–632.1 ng/ml)	*p*-value
Patients, n	120	119	
Age (years), mean ± SD	49.8 ± 7.4	49.9 ± 7.2	0.845
Women, n (%)	35 (29.2)	45 (37.8)	0.157
CVRF			
BMI (kg/m^2), mean ± SD	26.3 ± 5.1	25.4 ± 4.7	0.172
SBP (mm Hg), mean ± SD	126.1 (12.6)	125.1 (16.0)	0.591
Diabetes, n (%)	6 (5.0)	6 (5.0)	0.988
Hypertension, n (%)	10 (8.3)	12 (10.2)	0.547
FRS (% over 10 years), median (p25-p75)	7.9 (4.0–14.6)	8.6 (5.1–15.5)	0.663
Waist-to-hip ratio	0.93 ± 0.08	0.92 ± 0.08	0.910
Lifestyle			
Daily marijuana consumption, n (%)	7 (5.8)	13 (10.9)	0.155
Current tobacco consumption, n (%)	**36 (30.5)**	**49 (43.4)**	**0.043**
Weekly alcohol consumption	42 (35.0)	46 (39.3	0.492
Highly physical activity	22 (18.3)	15 (12.6)	0.221
Lipids			
Total cholesterol (mmol/l), mean ± SD	**4.9 ± 1.1**	**5.2 ± 1.1**	**0.017**
LDL-C (mmol/l), mean ± SD	2.9 ± 0.9	3.2 ± 1.1	0.068
HDL-C (mmol/l), mean ± SD	1.3 ± 0.3	1.2 ± 0.4	0.788
Triglycerides (mmol/l), median (p25-p75)	1.3 (0.9–1.9)	1.3 (1.0–2.1)	0.226
Non HDL-C (mmol/l), mean ± SD	3.6 ± 1.0	4.0 ± 1.1	0.010
PCSK9 (ng/ml), median (p25-p75)	214 (178–238)	320 (295–369)	< 0.001
HIV characteristics			
Disease duration in years, median (p25-p75)	11.5 (5.1–17.9)	12.0 (5.0–20.6)	0.702
Leucocytes (cells/μl), median (p25-p75)	5550 (4450–6700)	6050 (4700–7350)	0.059
Lymphocytes (cells/μl), median (p25-p75)	1697 (1475–2277)	2028 (1513–2563)	0.030
Platelet [10^9/l], median (p25-p75)	218 (183–252)	234 (195–287)	0.010
CD4 (cells/μl), median (p25-p75)	521 (357–698)	551 (335–847)	0.168
Low CD4 (< 200 cells/μl), n (%)	6 (5.0)	14 (11.8)	0.059
Undetectable HIV-RNA (≤20 copies)	98 (81.7)	88 (74.0)	0.151
HAART-current regimen, n (%)	114 (95.0)	108 (90.8)	0.202
PI-current regimen, n (%)	34 (29.6)	35 (30.7)	0.906
Abacavir-current regimen, n (%)	27 (22.5)	26 (21.9)	0.904
Co-infection status			
HBV	0 (0)	1 (0.8)	0.314
HCV	0 (0)	1 (0.8)	0.314
Alanine transaminase (U/l), median (p25-p75)	24 (17–35)	22.5 (16–34)	0.683
Aspartate transaminase (U/l), median (p25-p75)	20 (15–29)	22 (16–27)	0.626

BMI, body mass index; CVRF, cardiovascular risk factors; FRS, Framingham risk score; HAART, highly active antiretroviral therapy; HBV, hepatitis B; HCV, hepatitis C; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; LDL-C, low density lipoprotein-cholesterol; PCSK9, proprotein convertase subtilisin kexin 9; PI, protease-inhibitor; RNA, ribonucleic acid; SD, standard deviation; SBP, systolic blood pressure.

Table 2
Correlation between PCSK9 levels and other biomarkers.

Biomarkers	Rho coefficient[a]	*p*-value
Total cholesterol (mg/dl)	0.213	0.001
LDL-C (mmol/l)	0.200	0.003
HDL-C (mmol/l	0.052	0.441
Triglycerides (mmol/l)	0.007	0.919
Non HDL-C (mmol)	0.200	0.003
TNF-α (pg/ml)	0.121	0.064
CRP (mg/l)	0.062	0.343
Il-10 (pg/ml)	0.050	0.445
Il-8 (pg/ml)	−0.023	0.727
MCP-1 (pg/ml)	0.180	0.006
CD4 (cells/μl)	0.051	0.440
Leucocytes (cells/μl)	0.127	0.052
Lymphocytes (cells/μl)	0.120	0.066
Platelets (cells/μl)	0.167	0.010
Alanine transaminase (U/l)	−0.043	0.520
Aspartate transaminase (U/l)	0.015	0.823

HDL-C, high-density lipoprotein cholesterol; IL-8, interleukin-8; IL-10, interleukin-10; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; PCSK9, proprotein convertase subtilisin kexin 9; TNF-α, tumor necrosis factor alpha.

[a] Spearman's rank correlation rho for continuous variables; rho = 1 denotes perfect positive correlation; rho = −1 denotes perfect negative correlation, and rho = 0 absent correlation.

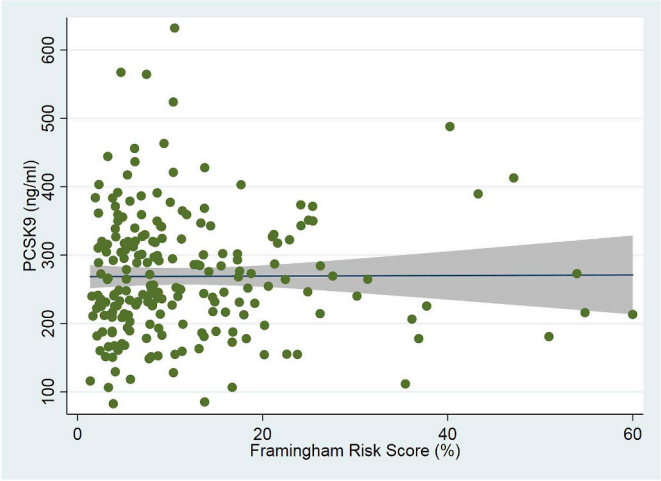

Fig. 2. Scatter plot to show the absence of relationship between PCSK9 levels and the Framingham Risk Score (%) in 239 HIV-infected patients naive of statin therapy.

and 15 (6.3%) occasional use. A dose-response effect was observed between marijuana consumption and PCSK9 levels ($p < 0.001$); the association persisted after adjustment for the calculated Framingham risk score ($p = 0.003$) and additional adjustment for relevant clinical variables ($p = 0.027$; l Table 4). No significant linear correlation was found between PCSK9 levels and CD4 count (Fig. 4A), higher PCSK9 levels were observed in those with low CD4 (< 200 cells/μl) without a specific trend in those with normal CD4 values (Fig. 4B).

4. Discussion

In HIV-infected individuals naïve of statin treatment, we found several novel findings potentially associated with PCSK9 levels. First, we did not find a paradoxical association between PCSK9 levels and LDL-C, but a positive correlation as observed in non-HIV subjects. No association was found with high cardiovascular risk, as expressed by the Framingham risk score. In exploratory analyses, marijuana consumption and lower CD4 cell count values were associated with higher PCSK9 levels independently of CV risk

Table 3
Association between clinical, behavioral and biomarker variables with PCSK9 levels in 239 HIV-infected patients naive of statin therapy.

Variables	Univariate		Multivariate	
	Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value
Clinical characteristics				
Age (per 10 years)	6.291	0.426	2.099	0.848
Women	14.566	0.233	37.460	0.063
White	22.053	0.062	35.654	0.084
Diabete	−1.429	0.957	10.841	0.724
Hypertension	−3.203	0.872	28.168	0.372
BMI (per 5 kg/m^2)	−6.174	0.341	−0.273	0.888
Waip-to-hip ratio (per 0.1 ratio)	−2.620	0.734	0.395	0.974
Fat accumulation	3.506	0.783	13.692	0.484
Lifestyle characteristics				
Daily Marijuana consumption	88.487	< 0.001	62.599	0.027
Daily smoking consumption	28.832	0.018	16.331	0.389
Weekly alcohol consumption	0.786	0.948	−8.632	0.591
Highly physical activity	−19.317	0.225	−24.232	0.261
Lipids				
Total cholesterol (mmol/l)	19.825	< 0.001	omitted	0.271
LDL-C (mmol/l)	17.864	0.002	11.723	0.154
HDL-C (mmol/l)	22.662	0.150	−4.904	0.854
Log Triglycerides (mmol/)	4.415	0.655	1.579	0.921
Non HDL-C (mmol/l)	18.650	0.001	omitted	
HIV related data				
Low CD4 (≤200 cells/μl)	30.451	0.143	68.682	0.031
Detectable RNA (< 20 copies)	3.694	0.790	−10.334	0.604
Leucocytes (per 1000 cells/μl)	6.454	0.023	1.882	0.744
Lymphocytes (per 1000 cells/μl)	16.334	0.041	4.464	0.764
Platelet (per 10^9/l)	0.225	0.007	0.068	0.588
PI-current regimen	11.229	0.382	0.753	0.967
Abacavir-current regimen	−10.506	0.449	−18.664	0.290
Alanine transaminase (U/l)	−5.754	0.511	4.498	0.844
Aspartate transaminase (U/l)	−4.289	0.671	−3.534	0.884
Inflammatory markers				
Log TNF-alpha (pg/mL)	24.469	0.050	8.356	0.685
Log MCP-1 (pg/ml)	38.967	0.002	21.004	0.352
Log CRP (mg/l)	1.094	0.760	−0.685	0.892
Log Il-8 (pg/ml)	−2.945	0.581	−13.080	0.104
Log Il-10 (pg/ml)	−0.466	0.943	3.29	0.787

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; IL-8, interleukin-8: IL-10, interleukin-10; LDL-C, low density lipoprotein-cholesterol; MCP-1, monocyte chemoattractant protein-1; PCSK9, proprotein convertase subtilisin kexin 9; PI, protease-inhibitor; RNA, ribonucleic acid; SD, standard deviation; TNF-α, tumor necrosis factor alpha.

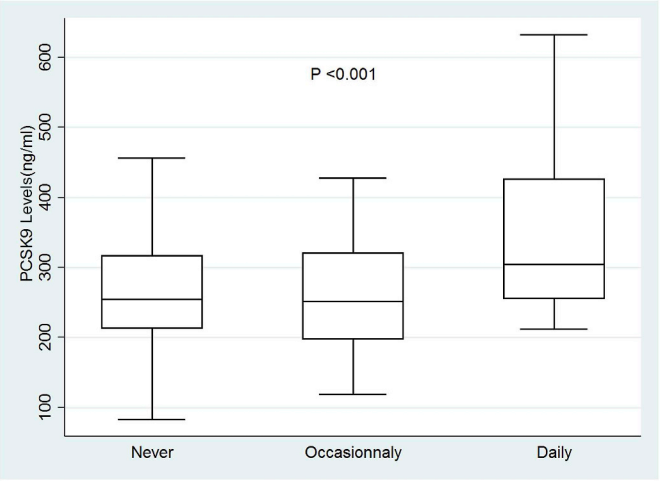

Fig. 3. Median PCSK9 levels in 239 HIV-infected patients according to marijuana consumption. *p* value was assed using a non-parametric Wilcoxon test.

Table 4
Association between marijuana consumption and PCSK9 levels in 239 HIV-infected patients naive of statin therapy.

Variables	Univariate		Adjusted for Framingham Score[a]		Adjusted for clinical variables[b]	
	Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value
Marijuana consumption						
Never	Reference		Reference		Reference	
Occasionally	0.031 (−0.142 to 0.204)	0.727	0.038 (−0.139 to 0.215)	0.670	0.046 (−0.138 to 0.229)	0.625
Daily	0.289 (0.137–0.444)	< 0.001	0.273 (0.096–0.450)	0.003	0.216 (0.025–0.407)	0.027

[a] Framingham score was a calculated risk score of fatal and nonfatal coronary heart disease events over 10 years and included age, sex, current smoking status, low-density lipoprotein cholesterol levels, diabetes, treatment status for hypertension and systolic blood pressure.

[b] Further adjustment for clinical variables was for high body mass index (> 25 kg/m^2), low CD 4 (≤ 200 cells/μl), undetectable ribonucleic acid (< 20 copies), log leucocytes count (cells/μl), physical activity (sedentary. moderately active and highly active), alcohol consumption (never, ≤ 2x weekly, > 2 x weekly), use of abacavir regimen, use of protease-inhibitor regimen, log transaminases.

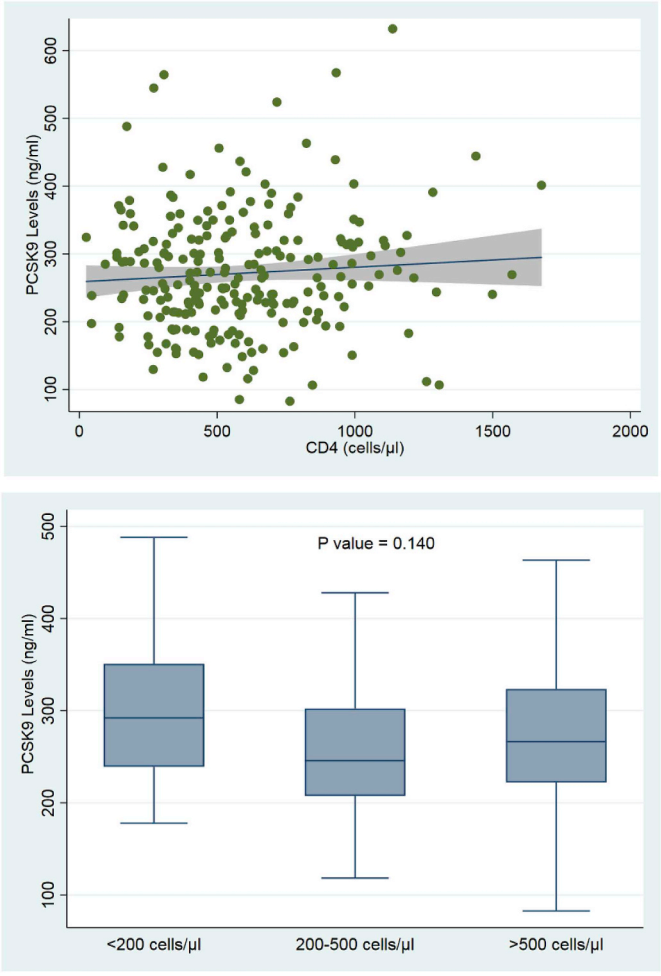

Fig. 4. Scatter and box plots.
(A) Scatter plot to show the absence of a strong correlation between PCSK9 levels and the CD4 lymphocyte cell count in 239 HIV-infected patient. (B) The box plot suggests higher PCSK9 levels in those with low CD4 (< 200 cells/μl) without a specific trend in those with normal CD4 values.

factors and clinically relevant confounding factors. Our findings are the first to suggest an association between marijuana consumption and PCSK9 levels. Of note, we did not find significant association between PCSK9 levels and the use of abacavir.

To our knowledge, only one study has reported PCSK9 levels in HIV-infected patients [8]. Despite low LDL-C levels in this study, a significant increase of PCSK9 levels was found in subjects who had HIV and hepatitis C co-infection, as well with higher levels of IL-6. In addition, 21% of participants were on statin, which could increase PCSK9 levels [8]. Our study population has different characteristics, as none were on statin and co-infection with HCV was extremely low. Furthermore, the strength of our study is that it integrates behavioral aspects. Compared with non-HIV studies using the same ELISA method, our study had a similar range of PCSK9 levels. For example, in the Brisighella Heart Study, young men had mean PCSK9 levels of 260 ± 80.4 ng/mL [21]. On the other hand, in a study including patients with familial hypercholesterolemia PCSK9 mean values were almost 2-fold higher (464 ng/ml, range 364–553 ng/ml) [22].

The key challenges that remain for cardiologists and HIV specialists are the mechanisms underlying HIV-associated dyslipidaemia and atherosclerosis on the one hand, and the ability to predict precisely which individuals are at risk on the other hand, with the aim to provide adequate interventions to reduce this risk [23]. Cardiovascular disease is an increasing cause of death in HIV-infected subjects as other causes are being increasingly well managed [24]. Typically, HIV + persons have more extensive myocardial scarring than uninfected persons in the post-myocardial infarction setting [25]. Inflammation plays a key role in the atherosclerosis process of HIV-infected subjects [26]. This mechanism can potentially explain the association between low CD4 values and high PCSK9 levels found in the present study. With the same population, we previously found that patient with low CD4 had higher inflammatory markers making this subgroup population at higher risk of CVD events [9,27]. The impact of ART on arterial wall inflammation is still not established [28], while other interventions focusing on CV risk factor control are lacking [29]. There is still controversial data regarding the safety and associated risk of CVD events in HIV-patients treated with abacavir: a recent meta-analysis suggested no increased risk of myocardial infarction in clinical trials [30], while some observational data reported a 2.2 fold increased risk of myocardial infarction with the use of abacavir [5]. The potential mechanisms of abicavir effects on CVD events are probably unrelated to lipid abnormalities, but explained by changes in platelet function [31]. Gathering data suggest that HIV should be considered as an independent cardiovascular risk factor [23]. Pharmacological PCSK9 inhibition was suggested as treatment strategy to abrogate the residual cardiovascular risk unaffected by statins [11]. However, HIV-infected patients have been typically excluded from published clinical trials assessing efficacy of monoclonal antibodies against PCSK9, except for bococizumab (NCT02524106 trial, Bococizumab HIV Evaluation (B-HIVE) Study). The use of PCSK9 inhibitors to lower LDL-C in the HIV population has not yet been proven in terms of safety and efficacy. Such trial is currently ongoing with evolocumab (NCT02833844, as this would add novel, interesting, relevant and ethical evidence for the prevention of CV disease in HIV-infected patients. In addition, an alternate non statin lipid-lowering drug is a need for the HIV-population which is characterized by drug-interaction [10].

With regard to the prognostic value of PCSK9 to predict cardiovascular events, current data in non-HIV populations is controversial with conflicting results [32], although it could be relevant in selected high-risk populations [33]. The pharmacological inhibition of PCSK9 has led to indisputable benefits in terms of LDL-C and CV risk lowering, but the validity of clinical measurements of plasma PCSK9 for CV risk prediction is not fully

supported. This discrepancy may lay on the following observations: (i) plasma PCSK9 levels differ across study design and clinical outcomes, (ii) assay methods for PCSK9 do not discriminate between active and inactive truncated forms. In a cohort of 500 obese subjects, higher PCSK9 levels were associated with the Framingham Risk Score and with daminag effects of long-term exposure of environment air pollution, but not with inflammation [34]. But, other studies suggest that PCSK9 have pro-inflammatory action on macrophages mainly by LDL-receptor with a positive correlation with TNF-α plasma levels [35].

In a high-risk population, such as HIV-infected patients, we showed that marijuana was associated with higher levels of PCSK9 independently of other clinical predictors. Although marijuana is widely consumed in the United States it remains illicit as a recreational and medicinal drug in many States, and controversy persists regarding its association with CV risk factors [36]. Our findings demonstrate that, conversely to mice where cannabinoids were associated with a reduction in atherogenesis [37], exogenous cannabinoidsappear to increase the level of circulating PCSK9, and thereby also the gap to achieve therapeutic targets in CV diseases. Our results are in line with previous evidence showing that high levels of endogenous cannabinoids were associated with dysfunctional coronary circulation in obese subjects [38]. Data from observational surveys reported that marijuana users had a higher risk of mortality from hypertension and subclinical atherosclerosis [39,40]. The relationship between marijuana use and CV death in 1213 individuals who responded to questions regarding marijuana use in the National Health and Nutrition Examination Survey (NHANES) [39]. Marijuana users had a greater than three-fold increase in risk of death from hypertension, Of interest, risk of death attributed to either heart disease or cerebrovascular disease was not increased in marijuana users [41]. In order to explain the underlying molecular mechanisms, we might speculate on cannabinoid system pathophysiology that is characterized by a redundant triggering of both protective and deleterious pathways via receptors, such as the cannabinoid receptor type 1 (CB1), CB2 or a variety of orphan receptors (GPRs) [38]. Marijuana smoking implies concomitant exposure to thousands of compounds that do not guarantee a selective activation of the protective cannabinoid receptors. Conversely, smoking might activate a complex signaling cascade, resulting a in a final pro-atherosclerotic effect.

We have previously shown an association between (1) high levels of endogenous cannabinoids and dysfunctional coronary circulation in obese subjects, and (2) between lower PCSK9 levels and healthy lifestyle habits, such as physical activity [38,42]. Furthermore, we also recently demonstrated an association between high initial PCSK9 plasma levels and inflammation in the acute phase following acute coronary syndromes (ACS) [13]. Growing evidence suggests an association between PCSK9 levels and higher platelet reactivity in the ACS setting [43]. Higher PCSK9 levels were shown to be predictive of increased platelet reactivity and aggregation on the coronary plaque thrombus, while mice with loss of mutation in PCSK9 −/− were protected of occlusive carotid thrombi [44]. In addition, mechanistic studies demonstrated pleiotrophic effects of PCSK9, including both via LDL-C receptor and cholesterol-independent mechanisms, and with a direct pro-atherogenic effect on the arterial wall [45,46]. In our model, we did measure platelet reactivity. The platelet count was associated with higher PCSK9 levels in the univariate model, but lost significance in the multivariate model. Larger primary prevention studies are warranted to clarify the potential association between marijuana consumption and cardiovascular risk. The present study also identified a novel pathway (related to PCSK9) by which marijuana could increase the cardiovascular risk. Large cohort studies are needed to clarify a possible association between marijuana consumption and emerging cardiac biomarkers, such as demonstrated here for PCSK9.

4.1. Limitations

The study has limitations that warrant consideration. First, as with any observational analysis, we cannot exclude the possibility of residual confounding factors. Our study had the strength to adjust for clinical, behavorial and biomarkers, not systematically reported by previous studies. Second, although marijuana consumption is a marker of higher risk behavior, the causality with PCSK9 levels is not proven and the value of quitting marijuana as therapeutic target can only be confirmed with appropriately powered clinical trials. Data in individuals without an HIV infection are lacking to evaluate the possible association between PCSK9 levels and marijuana consumption. Our findings should be confirmed in the general population, although marijuana use has been scarcely recorded in previous available cohort studies. Third, the sample size was not sufficiently powered to demonstrate a true association for other potential predictors. However, to our knowledge our study is the second largest available on this topic in the HIV setting. Fourth, we did not integrate longitudinal data to observe changes in PCSK9 levels with modifications of other predictors. Our study is a cross-sectional assessment of the HIV cohort. Fifth, alipoprotein B (ApoB) was not measured in this subsample of subjects, it is well known that altered ApoB metabolism has been reported in HIV-infected patients under antiretroviral therapy with a preferential shift to increased number of pro-atherogenic small dense lipoprotein particles. As a matter of fact, previous data in the SHSC showed an association between elevated small dense LDL lipoproteins and ApoB with coronary events [47]. Sixth, previous studies suggested that hepatic steatosis could also be associated with higher PCSK9 levels [48], our data reported clinically relevant fat accumulation without measuring formally hepatic steatosis. To address this potential unmeasured confounding factor, we reported data for hepatic enyzmes and did not find association with PCSK9 levels. Finally, although we did not measure cannabis in the blood, previous studies in large cohorts using self-reported methods have already demonstrated an association with clinical outcomes.

4.2. Conclusions

In this large population of subjects infected with HIV, higher PCSK9 levels were associated with daily marijuana consumption and a low CD4 count. Given that this specific combination of high-risk factors is found more frequently in HIV-infected patients, a future randomized controlled assessing the use of PCSK9 inhibitors to improve control of dyslipidaemia in HIV patients should be considered.

Conflicts of interest

F.M. has received speaker and consultant fees from Amgen, AstraZeneca, Eli Lilly, MSD, Novartis, Sanofi, and Pfizer. The other authors have nothing to disclose.

Author contributions

Writing of the manuscript (BG), critical review of the manuscript (all), statistical analysis (BG and CD), conseption of design (BG and AC). acquisition of data (SP, NS, AC).

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