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Original Article

Cardiovascular risks in Asian HIV-infected patients receiving boosted-protease inhibitor-based antiretroviral treatment

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Abstract

Introduction: Increased risk of cardiovascular disease in HIV-infected patients was tought to be the cause of multiple mechanistic factors, which changing the HIV care landscape. Antiretroviral therapy (ART), especially protease inhibitors (PI), is one of common HIV treatments that may have some association with this. The mechanism of PI in comparison to other regimens, however, are not clearly understood.

Methodology: Age-and gender-match HIV-infected patients treated with either boosted-PI-based regimen (boosted-PI group, N=30) or NNRTI-based ART (non-PI group, N = 30) were recruited for this cross-sectional study. Parameters determined cardiovascular risks, inflammation, endothelial function, and bone metabolic function were evaluated.

Results: Compared with non-PI, patients in the boosted-PI group had more evidence of dyslipidemia. No statistical difference in the prevalence of subclinical atherosclerosis was found between the two groups. Circulating levels of inflammatory markers, C-reactive protein (CRP) (5.4±9.1 *vs.* 14.9 ± 19.4 mg/L, $p = 0.019$) and lectin-liked oxidized lipoprotein receptor-1 (LOX-1) (387 ± 299 *vs.* 554 ± 324 pg/mL, $p = 0.042$) were lower in boosted-PI group. Contrastingly, Vascular adhesion molecules-1 (VCAM-1) (160.2 ± 80.0 *vs.* 147.8 ± 66.3 ng/mL, $p = 0.010$), and osteoprotegerin (OPG) (153.7 ± 57.1 *vs.* 126.4 ± 35.8, $p = 0.031$) were higher. After adjustment in the multivariate analysis, PI treatment is the only independent parameter associated with the changes of CRP, LOX-1, VCAM-1, and OPG. Subgroup analysis showed that ARV treatment effects differed among participant having dyslipidemia.

Conclusions: The major mechanism in which PI-mediated was triggering atherogenesis could be through alteration of lipid metabolism and endothelial function, but no evidence of accelerated pro-inflammatory response was attested.

Key words: Cardiovascular disease; HIV; antiretroviral therapy; protease inhibitors; inflammation; endothelial function.

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Introduction

Advances in Human Immunodeficiency Virus (HIV) treatment with antiretroviral therapy (ART) has led to a decrease in AIDS-related morbidity and mortality among people living with HIV (PLWH) [1]. However, non-AIDS-related co-morbidities have emerged, despite the controlled viremia; this becomes a challenging problem in the ART era. Studies revealed that PLWH is at higher risk of developing chronic non-communicable diseases, including cardiovascular diseases (CVD) comparing to HIV-negative patients [2-4]. CVD, especially coronary artery diseases (CAD), has become one of the leading causes of death among PLWH [5]. Thus, an increased understanding of ART mechanism on CVD association may develop optimal guidelines and HIV-specific clinical management for PLWH.

In general, endothelial dysfunction, lipid deposition, and inflammation were commonly described in atherogenesis [6]. For PLWH, the mechanism of atherogenesis is more complicated and not clearly understood. In addition to HIV infection itself, microbial translocation and cytomegalovirus co-infection, potentially promote atherogenesis through ongoing inflammation. Thus, it facilitates vascular disease progression [7]. Increased prevalence of traditional risk factors among PLWH, such as smoking, hypertension, diabetes, and dyslipidemia, were

consistently reported. The higher prevalence of these risk factors is associated with ART with a varying degree depends on the regimen. Protease inhibitors (PI), especially ritonavir-boosted lopinavir (LPV/r), was described as a risk factor of CAD among PLWH in the large D:A:D cohort [8] even after adjustment for CVD risk factors and lipid concentration.

It has been proposed that PI promote premature atherogenesis by several mechanisms. Since PI have a high affinity for HIV protease, it can also bind to lipoprotein-receptor-related proteins involved in lipid metabolism, i.e., chylomicron uptake and triglyceride clearance at the liver, which subsequently directly lead to hyperlipidemia [3,4,9]. Besides, PI modulated CD36, major fatty acid transporter, and gene expression. Changes of CD36 function in various tissues result in impaired fatty acid utilization, decrease insulin sensitivity, and thus induced hyperlipidemia [10].

There was also evidence of PI inducing inflammation [11]. Two short-term follow-up studies suggested the positive association between carotid intimal medial thickness (CIMT) and increase the risk of cardiovascular events in an HIV-positive person [12,13]. However, the data remains controversial as several other studies revealed no such correlation between HIV infection and PI drug using the surrogate markers of atherosclerosis [12,14,15].

Several serum markers have been proposed as an early detector for subclinical atherosclerosis. Inflammation and endothelial cell activation are crucial elements in the initiation, progression, and thrombotic complications of atherosclerotic coronary artery disease [16]. Increased levels of pro-inflammatory cytokines, namely C-reactive protein (CRP) and Interleukin-6 (IL-6), were the independent prognostic factors in patients with CVD and acute coronary syndromes [17]. Elevated CRP and IL-6 in association with serious non-AIDS defining events, including CVD, was noted [18,19].

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) plays a vital role in cellular uptake of internalized oxidized-LDL (ox-LDL) in the arterial wall. The interplay of LOX-1 in the induction of signaling pathways involving activation of oxidative stress and inflammation supported its essential role in various steps of atherogenesis [20]. Once activated by ox-LDL, LOX-1 stimulates expression of cellular adhesion molecules and triggering pro-inflammatory signalling pathway in the endothelial cells. Worsening endothelial dysfunction in response of LOX-1 was also occured by accelerated production of vasoconstrictors, decreased endothelial nitric oxide and increased reactive oxygen species (ROS) [21] .

Vascular cellular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) have been widely used as markers of endothelial dysfunction and have found a significant association with CVD risk and mortality in the general population.

Osteoprotegerin (OPG) is a soluble glycoprotein of the TNF- receptor superfamily. As a critical element of the RANKL-OPG axis, OPG plays a crucial role in the control of bone metabolism, endocrine function, and immune activation. Recent data demonstrated that OPG levels increased in response to the vascular insult and ongoing process of inflammation within an atherosclerotic plaque lesion [22]. Hence, it has been proposed as a prognostic factor for vascular pathology, including atherosclerotic vascular disease.

Furthermore, ankle-brachial index (ABI), carotid intimal medial thickness (CIMT), cardio-ankle vascular index (CAVI), and pulse wave velocity (PWV), which reflex vascular endothelial function have been used as testing techniques to identify subclinical vascular atherosclerotic changes [23].

The incidence of CAD among PLWH may vary by race [24]. Despite several studies that were conducted to determine CAD pathogenesis in PLWH, none was done among Asian PI users. Our research aims to explore the importance of PI in CAD pathogenesis among Asian populations.

Methodology

Study design and patient enrollment

This cross-sectional, comparative study was conducted during November 2015 and October 2016 at the Out-Patient Department (OPD), Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai, Thailand. Sixty HIV-infected patients were enrolled in the study voluntarily, including 30 boosted-PI and 30 age-and-sex-matched non-PI users. Inclusion criteria were 1) Age 18 years or older, and 2) being on consistent ART regimens within 12 months before enrollment. Patients starting PI therapy during the observation period could only be included in the boosted-PI group. Patients with a previous history of cardiovascular events (e.g., stroke, coronary artery diseases, myocardial infarction, or large aneurysm that required significant surgery) were excluded from the study. Informed consent was obtained from all study participants. All clinical research protocol was approved by the ethics committee of the Faculty of Medicine, Chiang Mai University (SUR-2558-03117).

Data collection and laboratory method

A questionnaire was administered to each patient to obtain demographic data (gender, age, medical right, occupation, education), anthropometric data (height, weight, waist circumstance), clinical history (family history of CVD, hypertension, chronic kidney disease, diabetes, dyslipidemia), and behavior (smoking, alcohol drinking). Clinical examination and laboratory findings were also recorded in the case report form. Current status of diagnosed diabetes (defined as currently treated with anti-diabetic medication or fasting blood glucose ≥ 126 mg/dL), hypertension (defined as currently treated with antihypertensive agents or systolic/diastolic BP ≥ 140/90 mmHg), dyslipidemia (defined as currently treated with lipid modifying agent or any of the following criteria: fasting total triglyceride ≥ 150 mg/dL, total cholesterol ≥ 200 mg/dL, or LDL-cholesterol ≥ 140 mg/dL) and chronic kidney disease (defined as proteinuria > 1+ or calculated glomerular filtration rate < 60 ml/min) were obtained from medical records as well as most recent absolute CD4 lymphocyte count and HIV-RNA level. Subclinical atherosclerosis was evaluated by ankle-brachial index (ABI), cardio-ankle vascular index (CAVI), carotid intima-media thickness (CIMT), and pulse wave velocity (PWV). Standardized venipuncture protocol was used to collect blood samples in all patients on enrollment. Serums were separated by centrifugation at 3,000 rpm, 4 °C for 10 minutes within 15 minutes of collection. Aliquots were stored at -70 °C and were thawed only once for analysis. Serum levels of selected biomarkers were quantified by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturer's instructions. Those included, 1) systemic inflammatory markers; CRP (Merck, CYT298, USA), IL-6 (Merck, EZHIL6, USA), LOX-1 (Abcam, ab212161, UK), 2) endothelial dysfunction markers; ICAM-1 (Abcam, ab100640, UK), VCAM-1 (Abcam, ab46118, UK) and 3) bone metabolic marker; OPG (Abcam, ab100617, UK).

Statistical analysis

Statistical analysis was performed with program STATA for Windows version 14.0 (StataCorp LP, USA). Descriptive for continuous variables were presented as mean ± standard deviation [Inter quartile range]. In the case of normally distributed data, Student's *t*-test was selected for statistical analysis of the difference. Otherwise, the Mann-Whitney U test was used. The significant difference between variables in the two groups was defined as a *p*-value of less than 0.05.

Pearson's correlation coefficients were determined for multiple variables. Potential confounding variable including gender, age, BMI, waist circumstance, CD4 level, viral load, lipid and glucose levels, blood pressure, heart rate, ABI, CAVI, CIMT, PWV, smoke,

Table 1. Demographic and clinical characteristics of the patients enrolled in the study.

Characteristics	Boosted-PI (*N* = 30)	Non-PI (*N* = 30)	*p-value*
Gender, Male, *n* (%)	17 (56.7)	17 (56.7)	1.000
Age (year)	43.9 ± 6.9 [38 – 47]	43.6 ± 7.3 [41 – 48]	0.885
Body mass index (kg/m^2)	22.3 ± 3.4 [19.9-23.9]	24.2 ± 5.3 [21.4 – 26.3]	0.112
Absolute CD4+ (cells/μL)	593 ± 303 [370-760]	564 ± 244 [376-698]	0.701
Viral load, > 50 copies/mL, *n* (%)	2 (8.3)	1 (3.8)	0.260
Duration of ART (month)	51.0 ± 28.1 [27 – 76]	40.6 ± 27.6 [17 – 63]	0.153
Diabetes Mellitus, *n* (%)	3 (10.0)	2 (6.7)	0.640
Fasting blood sugar (mg/dL)	90.4 ± 12.5 [83.5 – 93.5]	109. 5 ± 35.4 [90 - 116]	0.110
Dyslipidemia, *n* (%)	11 (36.7)	4 (13.3)	0.037*
Hypertension, *n* (%)	8 (26.7)	8 (26.7)	1.000
Systolic BP (mmHg)	124.1 ± 10.47 [114 - 132]	132.53 ± 16.64 [121 - 144]	0.023*
Diastolic BP (mmHg)	78.87 ± 7.85 [78 - 90]	84.97 ± 10.28 [76 - 86]	0.012*
Heart rate (bpm)	67.87 ± 9.68 [60 - 74]	74.6 ± 14.25 [65 - 83]	0.037*
Family history of premature atherosclerosis, *n* (%)	1 (3.3)	0	0.313
Former or current smoker, *n* (%)	15 (50.0)	11 (36.7)	0.297
Former or current alcohol drinker, *n* (%)	19 (63.3)	20 (66.7)	0.336
ABI < 0.9, *n* (%)	0	0	-
CAVI > 8, *n* (%)	5 (16.7)	8 (26.7)	0.347
CIMT > 0.8 mm, *n* (%)	6 (20.0)	5 (17.9)	0.150
PWV > 12 m/s, *n* (%)	5 (17.9)	5 (22.7)	0.669

N: number of tested; *n*: number of tested within indicated criteria; ART: antiretroviral treatment; BP: blood pressure; ABI: ankle-brachial index; CAVI: cardio-ankle vascular index; CIMT: carotid intimal medial thickness; PWV: pulse wave velocity. Quantitative data presented as mean ± SD [Inter quartile range]. * *p*-values are statistically significant.

history of dyslipidemia, diabetes, hypertension, type of ART use and duration on ART were evaluated. Factors with $p \leq 0.2$ in univariate analysis were considered in the multivariate model.

Multivariate analysis was stratified by PI treatment (yes or no) as indicator variable. Logarithmic transformation was used for those variables which showed a non-normal distribution. The model followed the stepwise backward strategy, adjusted by gender and age, and the criterion for the exclusion of the variables was the highest *p*-value. Variables with statistical significance ($p < 0.05$) were kept in final model. The diagnostic test on model assumptions and fit was verified.

Results

Patient and control characteristics

The demographic and baseline characteristics of patients are shown in Table 1. There was no statistical difference in sex, mean age, body mass index (BMI), waist circumference, occupation, education, duration of ART, absolute or percentage of CD4 lymphocyte count, and HIV RNA levels between boosted-PI and non-PI groups.

All except one (29/30, 96.7%) in the boosted-PI group received ritonavir-boosted lopinavir (LPV/r) based regimen. Only one patient (1/30, 3.3%) received ritonavir-boosted darunavir (DRV/r) based regimen. For the non-PI group, the majority (13/30, 43.3%) received a nevirapine (NVP) based regimen. Others received efavirenz (EFV; 11/30, 36.7%) or rilpivirine (RPV; 6/30, 20.0%). The most common NRTI backbone used was tenofovir disoproxil fumarate/lamivudin (TDF/3TC) or tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) (56/60, 93.3%). Meanwhile, abacavir/lamivudin (ABC/3TC) was the least common NRTI backbone in this cohort (1/30, 3.3% in the PI and 2/30, 6.7% in non-PI groups). Only one patient received a single NRTI (lamivudin) as a backbone.

Conventional atherosclerotic risk factors**,** including the prevalence of hypertension, chronic kidney disease, dyslipidemia, diabetes, smoking, alcohol consumption, and family history of premature atherosclerosis, were demonstrated in Table 1. Nearly half (26/60, 43.0%) of study participants were former or current smokers. No statistical difference was found in most CAD risk factors (including gender, age, occurrences of diabetes, hypertension or kidney disease, smoking, and alcohol drinking status) between the two study groups. The prevalence of dyslipidemia was higher in the boosted-PI group (36.7% vs. 13.3%, p=0.037) compared to non-PI.

Laboratory findings and clinical characteristics

Physical examination revealed significantly lower mean systolic and diastolic blood pressure in the boosted-PI group, but no statistical difference in the proportion of patients with increased blood pressure (defined as BP ≥ 140/90 mmHg).

Imaging for subclinical atherosclerosis detection found no statistical difference in the subclinical atherosclerotic event, as indicated by ABI (< 0.9), CAVI (> 8.0), CIMT (> 0.8 mm) or PWV (>12 m/sec) was observed between the two groups.

To evaluate the level of systemic inflammation, serum level of inflammatory markers, CRP, IL-6, and LOX-1 were quantified. The non-PI group of patients demonstrated a statistically significant higher mean level of CRP and LOX-1, but no difference of IL-6 level between the two groups was observed (Table 2).

Elevated levels of CRP were markedly more prevalent in the non-PI group of patients (19/30 *vs.* 14/30, respectively; $p = 0.033$). Nevertheless, the possible coexisting infection should be noted for these patients. Of patients in the non-PI group; one was recorded to have the positive result of Anti-HCV, and

Table 2. Laboratory parameters of inflammation and endothelial dysfunction.

Parameters	Boosted-PI (*N* = 30)	Non-PI (*N* = 30)	*p-value*
Systemic inflammatory markers:			
CRP (µg/mL)	5.4 ± 9.1 [1.9 – 5.3]	14.9 ± 19.4 [2.6 – 22.3]	0.019*
IL-6 (pg/mL)	2.1 ± 1.7 [0.8 – 3.1]	1.7 ± 1.8 [0.7 – 2.1]	0.346
LOX-1 (pg/mL)	387.0 ± 299.0 [172.1 – 444.8]	554.4 ± 324.0 [306.5 – 808.8]	0.042*
Endothelial dysfunction markers:			
ICAM-1 (pg/mL)	160.2 ± 80.0 [103.4 – 192.6]	147.8 ± 66.3 [117 – 165.1]	0.516
VCAM-1 (ng/mL)	1135.3 ± 224.3 [1012.7 – 1281.0]	985.1 ± 214.6 [816.1 – 1087.6]	0.010*
Bone metabolic marker:			
OPG (pg/mL)	153.7 ± 57.1 [118 – 180.9]	126.4 ± 35.8 [103.3 - 150]	0.031*

CRP: C-reactive protein; IL-6: interleukin-6; LOX-1: lectin-like oxidized low-density lipoprotein receptor-1; ICAM-1: intercellular adhesion Molecule-1; VCAM-1: vascular cell adhesion molecule-1; OPG: osteoprotegerin. Quantitative data presented as mean ± SD [Inter quartile range]. * *p*-values are statistically significant.

Table 3. Linear regression and multiple regression analyses of log(CRP) value.

Parameters	Correlation	*p-value*	Multiple regression		
			Regression coefficient	95 % CI	*p-value*
PI use	- 0.3081	0.017*	- 2.606	- 4.519, - 0.693	0.009*
Female Gender	- 0.0248	0.851	0.022	- 0.690, 0.733	0.951
Age (years)	- 0.1875	0.151	- 0.034	- 0.077, 0.009	0.118
Body mass index ≥ 25 kg/m^2	0.1701	0.194			
Waist circumference (cm)	0.2157	0.098			
Duration of ARV treatment (months)	- 0.1720	0.193			
Dyslipidemia	- 0.2143	0.100	- 1.916	- 3.503, - 0.329	0.019*
Pulse wave velocity >12 m/sec	- 0.3160	0.025*	- 1.368	- 2.406, - 0.330	0.011*
PI use and DLP			1.998	0.005, 3.992	0.049*
PI use and PWV			1.213	- 0.319, 2.745	0.118

CRP: C-Reactive protein; PI: protease inhibitor; ARV: antiretroviral drug; DLP: dyslipidemia; PWV: pulse wave velocity. * *p*-values are statistically significant.

one was recorded for reactive VDRL of 1:32. In those of boosted-PI group, two patients were recorded to have the positive effect of Anti-HCV, one was urinary tract infected with *E.coli*, one was positive for sputum squamous epithelial, and one with high blood lactate (3.91 mmol/L).

For markers of endothelial function, the level of ICAM-1 tends to be higher in boosted-PI group but not statistically significant. However, a significantly higher VCAM-1 level ($p < 0.01$) was observed in the boosted-PI group. The moderate positive correlation between serum level of VCAM-1 and CRP was found among patients in the non-PI group ($r = 0.396$, $p = 0.030$) but not in the boosted-PI group ($r = 0.186$, $p = 0.324$). The mean level of OPG, selected bone metabolic marker, was also significantly higher in the boosted-PI group.

Multivariate linear regression analysis was undertaken to assess the most comprehensive confounders of the increase in the value of CRP, LOX-1, VCAM-1, and OPG. A model incorporating known cardiovascular risk factors (gender, age, BMI, waist circumference, diabetes, hypertension, dyslipidemia, smoking status) and vascular parameters (ABI, CAVI, CIMT, PWV) are shown in Tables 3-6. In the case of CRP, LOX-1 and OPG, log transformation was used to adjust constant variance to the standard plot. Univariate and multivariate analysis model indicated that PI treatment was an independent predictor of decrease in log CRP (Table 3**)** and log LOX-1 (Table 4**)** value, whereas contributed to increasing in VCAM-1 (Table 5) and log OPG value (Table 6). Subgroup analysis from multiple linear regression model demonstrated that, beside PI treatment, living with dyslipidemia (DLP) or arterial stiffness (PWV>12 m/s) relatively affect lower circulating CRP level (Table 3). Levels of CRP in patients with DLP (predictive mean=4.871 μg/mL) will be 64.5% lower than patients without DLP. Interaction of DLP effects more potent in the non-PI group of patients. In non-PI group, having DLP (predictive mean = 5.637 μg/mL) will be 85.2% lower CRP level than no DLP (p=0.019). In boosted-PI group, having DLP (predictive mean = 3.912 μg/mL), may resulted in 8.5% higher CRP level than no DLP

Table 4. Linear regression and multiple regression analyses of log(LOX-1) value.

Parameters	Correlation	*p-value*	Multiple regression		
			Regression coefficient	95 % CI	*p-value*
PI use	- 0.2658	0.042*	- 0.420	- 0.984, 0.144	0.139
Female Gender	0.1422	0.278	- 0.016	- 0.603, 0.570	0.956
Age (year)	0.0556	0.673	- 0.007	- 0.037, 0.023	0.623
Viral load > 50 copies/mL	0.2719	0.059	0.345	- 0.531, 1.220	0.428
Smoking	- 0.2991	0.020*	- 0.802	- 1.496, - 0.107	0.025*
Dyslipidemia and Pulse wave velocity					
DLP and PWV ≤ 12			- 1.010	- 1.9690, - 0.051	0.040*
DLP and PWV > 12			- 0.884	- 1.9192, 0.1518	0.092
Smoke and Duration of ART					
Never smoke and Duration of ART			- 0.0050	- 0.016, 0.006	0.343
Smoke and Duration of ART			0.0125	- 0.002, 0.026	0.079

LOX-1: lectin-like oxidized low-density lipoprotein receptor-1; PI: protease inhibitor; ART: antiretroviral treatment; DLP: dyslipidemia; PWV: pulse wave velocity. * *p*-values are statistically significant.

Table 5. Linear regression and multiple regression analyses of VCAM-1 value.

Parameters	Correlation	*p-value*	Multiple regression		
			Regression coefficient	95 % CI	*p-value*
PI use	0.3285	0.010*	189.507	48.736, 330.278	0.010*
Female Gender	- 0.0758	0.565	- 96.802	- 241.010, 47.496	0.183
Age (year)	0.1137	0.387	5.526	- 3.364, 14.417	0.216
Waist circumference (cm)	0.2021	0.122			
Viral load > 50 copies/mL	0.2866	0.046*	121.363	- 140.160, 382.886	0.354
Dyslipidemia	- 0.4065	0.001*	-299.550	- 573.671, -25.428	0.033*
CIMT and Duration of ART					
CIMT ≤ 0.8 mm. and Duration of ART			- 1.378	- 3.961, 1.205	0.287
CIMT > 0.8 mm. and Duration of ART			- 3.770	- 7.883, 0.343	0.071

VCAM-1: vascular cell adhesion molecule-1; PI: protease inhibitor; DLP: dyslipidemia; CIMT: carotid intima-media thickness; ART: antiretroviral treatment. * *p*-values are statistically significant.

(*p*=0.902). Likely trends in the changes of CRP level, in according to PWV status, were observed.

Living with dyslipidemia or currently treated with lipid-modifying agents also significantly affect decreased in VCAM-1 and LOX-1. Regression analysis indicated that patients living with DLP will be 22.6% lower in VCAM-1 level compared to those without DLP. For the participant without abnormal PWV: having DLP (geometric mean= 369.767) will be 63.6% lower LOX-1 than no DLP. Patient's serum lipid profile (level of triglyceride, cholesterol, LDL-cholesterol, HDL-cholesterol), yet, were not statistically associated with the circulating level of VCAM-1 or LOX-1. Other potential confounding factor, however, not found in this study.

Beside PI-treatment, our study found that increasing of age, absolute CD4 lymphocyte count and viral load were positively correlated with the increase of serum OPG levels. Those factors, however, not show statistical relative effect to each other.

Discussion

In this study, we explored several markers that have evidence of association with CVD and also imaging for subclinical atherosclerosis. As expected, the results were divergent but quite interesting. Because ART has been shown to improve inflammation markers in HIV-infected individuals, but not normalized, the effects of various regimens could be different and complicated.

Our data suggested that a PI-based regimen could potentially reduce systemic inflammation from HIV infection. This was certainly consistent with previous reports [25,26]. In the meantime, VCAM-1 and OPG levels, which both are markers of vascular injury, were significantly higher in those of PI-group. This revealed other potential vital CVD mechanism of PI, also, to increase the incidence of insulin resistance and dyslipidemia, through endothelial dysfunction and vascular injury. Conditions like dyslipidemia and arterial stiffness also show relative effect on the changes of parameters investigated in this study. The alternate explanation of the lower inflammatory markers, but raised in the OPG level in the boosted-PI group, could be the regulation of the RANKL-OPG axis. The certain pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α that are under the regulation of the RANKL-OPG axis in mediated bone metabolism also play remarkable roles in atherogenesis [22]. Increase OPG level, on the other hand, may lead to decrease RANKL-OPG ratio and, therefore, reduction of pro-inflammatory response. Higher levels of OPG and VCAM-1, along with lower levels of CRP and LOX-1, were found in the boosted-PI group. The findings may suggest the two-face modulating effect of PI in attenuating inflammation while accelerating

Table 6. Linear regression and multiple regression analyses of log(OPG) value.

Parameters	Correlation	*p-value*	Multiple regression		
			Regression coefficient	95 % CI	*p-value*
PI use	0.2874	0.026*	0.168	0.023, 0.312	0.024*
Female Gender	- 0.0090	0.945	- 0.011	- 0.163, 0.141	0.884
Age (year)	0.3114	0.015*	0.011	0.001, 0.022	0.037*
Absolute CD4+ (cells/uL)	0.3529	0.008*	0.001	0.000, 0.001	0.001*
Viral load > 50 copies/mL	0.2257	0.119	0.403	0.089, 0.718	0.013*

OPG: osteoprotegerin; PI: protease inhibitor. * *p*-values are statistically significant.

endothelial dysfunction in HIV-infected patients. In addition, studies report associations by OPG with PI exposure are consistent with greater bone loss and increased fracture risk with this ART class [27-29]. Further study to investigate how levels of OPG, in response with PI treatment, modulate other inflammatory markers and its impact on cardiovascular risk is warranted. Non-PI based regimen seemed to have a more substantial impact on inflammation, which was also supported by the positive correlation of CRP, LOX-1, and abnormal CIMT. However, when possible co-infection was taken into account, it may affect individual CVD risk. Recent studies stated increased CVD risk among people with HIV/HCV co-infection [30]. The mechanism underlies this association; however, not yet clearly explained. Despite the lower OPG level in the non-PI group, the mechanism of vascular injury cannot be excluded in this group as there was a positive correlation with abnormal CIMT. Of note, the number of patients with unusual imaging for subclinical atherosclerosis was relatively low in this study. Asian race might have certain protective factors leading to the lower incidence of CVD when compared to the western countries. Further study is required to identify the protective factors so that they could be strengthened or improved. Our research has limitations related to the cross-sectional study design and the small size of the study population. Since most subjects received combined ART regimens, the influence of drug users cannot be seen as mono-therapy. Moreover, with a lack of baseline markers before ART use or PI exposure, we could not make a strong conclusion whether any of the study findings were directly affected by PI regimen. However, a study in the population free of severe comorbidity allowed us to imply that the effect of any concurrent morbidities on systemic inflammation and immune activation by other causes can be ruled out. Nonetheless, our study was conducted in a single-center, and all study population was Asian. Perhaps it may not be generalizable to other people. This present study suggested that the increased risk of atherosclerotic cardiovascular disease among HIV-infected patients receiving a PI-based regimen may not be directly due to the acceleration of pro-inflammatory response. This was previously described in a previous report [31] with a different race. However, the process could mainly trigger by impaired lipid metabolism and endothelial dysfunction. It is also possible that other typical cardiovascular risk factors such as gender, age, cigarette smoking, alcohol drinking, and family history of atherosclerosis, all of which were matched in this study, are more potent mediators of atherogenesis than the impact of exposure to PI therapy *per se* [32]. Further clinical research with larger population size and with controlled baseline quantification is needed to warrant evidence found in this preliminary study.

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