Protease Inhibitors and Cardiac Autonomic Function in HIV-infected Patients: A cross sectional analysis from the Strategies for Management of Antiretroviral Therapy (SMART) Trial

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Protease Inhibitors and Cardiac Autonomic Function in HIV-infected Patients: A cross sectional analysis from the Strategies for Management of Antiretroviral Therapy (SMART) Trial

Short title:

Protease inhibitors and heart rate variability

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ABSTRACT

Objective: To compare cardiac autonomic function as measured by heart rate variability for HIV-infected participants taking protease inhibitors (PIs) with those taking a non-nucleoside reverse transcriptase inhibitor without a PI (NNRTI- no PI) regimen.

Design: Cross sectional analysis

Setting: Multicenter study

Participants: 2998 participants (average age 44 years, 28% females) enrolled in the SMART trial

Primary outcome measures: Heart rate and two heart rate variability measures [standard deviation of all normal RR intervals (SDNN) and root mean square of successive differences in normal RR intervals (rMSSD)]

Results: At study entry, 869 participants were taking a boosted PI (PI/r), 579 a non-boosted PI and 1550 an NNRTI-no PI. Median values (IQR) of heart rate, SDNN and rMSSD were: 68 (60-75) beats per minute (bpm), 21 (13-33) millisecond (ms), 22 (13-35) ms in the PI/r group, 68 (60-75) bpm, 21 (13-33) ms, and 21 (14-33) ms in the non-boosted PI group and 69 (62-77) bpm, 20 (13-31) ms, and 21(13-33) ms in the NNRTI-no PI group. After adjustment for baseline factors, for those given PI/r and non-boosted PI, heart rate was 2.2 and 2.8 bpm, respectively, lower than the NNRTI-no PI group ($p<0.001$ for both). On the other hand, compared with the NNRTI-no PI group, log SDNN and log rMSSD were significantly greater for those in the non-boosted PI (p-values for baseline adjusted differences in log- transformed SDNN and rMSSD were 0.004 and 0.001) but not for those in PI/r group at 0.01 alpha level.

Conclusions: Compared to an NNRTI-no PI regimen, heart rate was lower for those taking a PI/r or non-boosted PI and heart rate variability was greater, reflecting better cardiac autonomic function, for those taking a non-boosted PI regimen but not PI/r.

Key words: HIV/AIDS, Protease inhibitors, Electrocardiogram, Heart rate variability

Article Summary

Article focus

- Cardiac autonomic dysfunction manifested as reduced heart rate variability has been reported in HIV infection▪
- The impact of protease inhibitors on cardiac autonomic function considering their favorable effect on HIV suppression and the unfavorable diabetogenic and atherogenic effects is unclear

Key messages

- Different protease inhibitors have different impact on cardiac autonomic function as measured by heart rate variability.
- Compared to a regimen that does not include protease inhibitors, a non-boosted protease inhibitor regimen was associated with better heart rate variability
- Compared to a regimen that does not include protease inhibitors, a boosted protease inhibitor regimen was not associated with better heart rate variability

Strengths and limitations of this study

- This is the largest study to extensively examine cardiac autonomic function as measured by heart rate variability in HIV-infected participants taking protease inhibitors
- Limitations of this study include lack of examining the prognostic significance of the differences in heart rate variability among protease inhibitors.

INTRODUCTION

The introduction and wide use of combination antiretroviral therapy (ART) have made it possible to obtain long-term HIV viral suppression and increased CD4+ T-cell counts. This has resulted in improved mortality rates in HIV-infected patients, but has also led to long-term concern about possible adverse effects of treatment including greater risk of cardiovascular disease (1). Adverse effects could be both due to the drugs themselves, as well as caused indirectly through development of dyslipidemia, insulin resistance and metabolic syndrome, well known to be associated with ART (2, 3). Protease inhibitors (PIs) in particular have been linked to both hypercholesterolemia and development of insulin resistance [2-5], and subsequently can negatively influence the cardiovascular system, including cardiac autonomic function. Nevertheless, the overall impact of PIs on cardiac autonomic function considering their favorable effect on HIV suppression and the unfavorable diabetogenic and atherogenic effects is unclear.

Heart rate variability is a noninvasive and easy to obtain electrocardiographic measure of cardiac autonomic nervous system function [6, 7]. Cardiac autonomic dysfunction manifested as reduced heart rate variability and increased resting heart rate has been reported in HIV infection (8-10), and has been demonstrated to severely debilitate HIV-infected patients, namely by postural hypotension and syncope as well as possible life-threatening cardiac arrest [11-13].

The purpose of this cross-sectional analysis was to compare heart rate and cardiac autonomic function as measured by heart rate variability for HIV-infected participants taking PI-based regimens (boosted and non-boosted) with those taking a non-nucleoside reverse transcriptase inhibitors without a PI (NNRTI-no PI) regimen in the Strategies for Management of Antiretroviral Therapy (SMART) trial.

METHODS

Study population

SMART is an open-label randomized trial comparing two antiretroviral treatment (ART) strategies. Detailed descriptions of the design and aims of the study have been published elsewhere [14, 15]. Briefly, individuals infected with HIV who were older than 13 years and were not pregnant or breast-feeding were eligible for inclusion in the SMART study if their CD4+ T-cell count exceeded 350 cells per cubic millimeter and they were willing to participate. Before randomization (baseline), an ART and medical history were obtained, CD4+ T-cell count and plasma HIV RNA levels were measured, and a 12-lead electrocardiogram (ECG) was obtained.

All SMART participants (N=5472) were considered eligible for the present analysis, except those who were off ART, on an ART regimen not containing a PI and/or an NNRTI at baseline, on a regimen containing both a PI and an NNRTI, or on an ART regimen not containing a nucleoside reverse transcriptase inhibitor (NRTI), or those who were missing their baseline ECG or with ECG conditions that interfere with appropriate measurement of heart rate variability. After these exclusions, 2998 participants remained and were included in this analysis **(Figure 1)**.

Electrocardiography and heart rate variability measures

Detailed description of ECG recording in SMART has been published elsewhere [16]. Briefly, identical electrocardiographs (GE MAC 1200 models, GE Milwaukee, WI) were used in all of the study clinical sites, and standard 12-lead ECGs were recorded in all participants using strictly standardized procedures. The digital ECG tracings stored in the electrocardiographs were transmitted regularly over analog phone lines to the SMART ECG Reading Center, EPICARE, located at Wake Forest School of Medicine, Winston-Salem, NC for analysis. ECGs were evaluated blinded to treatment group and ART use. After being visually checked for quality, the study ECGs were automatically processed using the 2001 version of the GE Marquette 12-SL

program (GE, Milwaukee, WI). Heart rate variability indices were automatically calculated after excluding any ECG with non-sinus-originated beats (supraventricular and ventricular ectopic beats, AV blocks, atrial fibrillation) and artifacts. Two time-domain heart rate variability indices were calculated: The standard deviation of all filtered RR intervals over the length of the recording (SDNN) and the root mean square of the difference of successive RRs (RMSSD) [6].

Statistical analysis

Participants were categorized into one of three groups based on the ART regimen they were receiving at the time of randomization as follows: 1) boosted PI (PI/r); 2) non-boosted PI; or 3) an NNRTI-no PI. The distribution of heart rate variability measures across these three groups was tabulated.

Linear regression analysis was used to examine the association between heart rate and each of heart rate variability measures, separately, with different ART regimens [PI/r, and non-boosted PI regimens, separately, versus an NNRTI-no PI regimen]. SDNN and rMSSD were log-transformed due to their skewed distributions. Four different models were considered: Model 1: unadjusted; Model 2: adjusted for age, sex, race (Black, Asian, white and others) and NRTI backbone regimen; Model 3: adjusted as in Model 2 plus smoking status, total /HDL cholesterol ratio, body-mass index (BMI), prior cardiovascular disease , diabetes mellitus, use of blood pressure-lowering drugs and use of lipid-lowering drugs; and Model 4: adjusted as in model 3 plus baseline time since first prescribed ART, baseline CD4+ T-cell count and plasma HIV RNA levels.

Two-sided p-values are cited. A more stringent p-value of <0.01 was considered significant to minimize type-I error due to multiple comparisons. Analyses were performed using SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina) and *R* version 2.9.

RESULTS

This analysis included 2998 participants. Average age was 44 years, 28% were women, 54% were white, 27% were blacks and 6% were Asian. As shown in **Table 1** and **Supplemental Table 1**, 869 (29%) of the participants were receiving a PI/r [187 on saquinavir boosted with ritonavir (SQV/r), 410 on lopinavir boosted with ritonavir (LPV/r), 139 on atazanavir boosted with ritonavir (ATV/r) and 133 on other PI/r], 579 (19%) were receiving a non-boosted PI [345 on nelfinavir (NFV), 109 on indinavir (IDV), 84 on atazanavir (ATV) and 41 on other PIs] and 1550 (52%) were receiving an NNRTI- no PI.

A number of baseline factors varied by type of ART regimen used. Notably, most Asians (129 out of 167) were receiving a PI/r while most blacks (426 out of 816) and whites (886 out of 1632) were receiving an NNRTI-no PI (unadjusted p <0.001). The highest levels of total cholesterol and the longer time since first prescribed ART as well as the highest prevalence of the use of lipid lowering drugs and lowest levels of baseline CD4+ T-cell count were observed in the PI/r group compared to the non-boosted PI and NNRTI-no PI groups (unadjusted p<0.001 for all comparisons). The highest prevalence of diabetes was observed in the non-boosted PI compared to the boosted PI and NNRTI-no PI. Participants on an NNRTI-no PI regimen were more likely to have HIV RNA <400 copies/mL and higher levels of HDL-cholesterol compared to both PI-based regimens (unadjusted p<0.001) **(Table 1).**

Figure 2 shows the distribution of heart rate variability measures in all study population and across different types of ART regimens. The median (IQR) values of the resting heart rate, SDNN and rMSSD in all study population were 68 (61-77) beats per minute (bpm), 20 (13-32) millisecond (ms), and 21 (13-34) ms, respectively. There was a positive correlation between SDNN and rMSSD (Spearman rank correlation (r) = 0.88; p<0.001) but negative correlation between heart rate and SDNN (r = -0.39) and rMSSD (r = -0.55) with p-value <0.001 for all.

There was no significant difference in heart rate, SDNN, or rMSSD among individual boosted PIs and non-boosted PIs at alpha level of 0.01 as shown in **Supplemental Table 1**, and therefore, we combined all boosted PIs together and the same for non-boosted PIs in the linear regression analysis.

Table 2 shows the results of different regression models examining the association between PI based regimens, compared to an NNRTI-no PI regimen, with heart rate, log-SDNN and log-rMSSD, separately. After adjustment for baseline factors (full model; model 4), amongst those given boosted PIs and non-boosted PIs, heart rate was 2.2 and 2.8 bpm, respectively, lower than the NNRTI-no PI group ($p<0.001$ for both). On the other hand, compared with the NNRTI-no PI group, log SDNN and log rMSSD were significantly greater for those in the non-boosted PI (p-values for baseline adjusted differences in log- transformed SDNN and rMSSD were 0.004 and 0.001), but not those in PI/r group at 0.01 alpha level **(Table 2).**

In the full model (Model 4), older age, higher total/HDL cholesterol ratio, higher body mass index, and diabetes were significantly associated with lower SDNN and rMSSD. There were no significant associations between baseline CD4+ T-cell count, plasma HIV RNA levels, and type of NRTI backbone regimen with any of the heart rate variability measures **(supplemental Table 2)**

DISCUSSION

The key findings of our study were: 1) use of protease inhibitors, whether boosted or non-boosted, was associated with slower (favorable) resting heart rate compared to NNRTI-no PI use; 2) non-boosted PI use was associated with higher levels of heart rate variability measures (i.e. better cardiac autonomic function) compared to NNRTI-no PI use; 3) no significant difference in heart rate variability measures between PI/r and NNRTI- no PI groups, and 4) no significant differences in heart rate and heart rate variability measures among individual drugs in the PI/r and non-boosted PI groups, which suggest that the observed associations are class

associations. The clinical relevance of these observed differences in cardiac autonomic function among ART regimens and how they may influence cardiovascular outcomes in HIV-infected individuals needs to be investigated.

Former studies that have been carried out in participants with and without cardiovascular disease showed that higher resting heart rate and lower heart rate variability are associated with poor prognosis in both the general population (16-28) and HIV-infected individuals (8-13). These measures of cardiac autonomic function are dynamic rather than static; affected by disease processes as well as cardio-active medications. Given the reported higher prevalence of cardiac autonomic dysfunction in HIV-infected individuals and the reported atherogenic and diabetogenic effects of PIs, examining the association between this class of ART and autonomic function carries special importance. Since today's most relevant group of HIV-infected individuals is those receiving ART, examining the association of protease inhibitors with cardiac autonomic function in comparison with other ART regimens, rather than no treatment, provides more practical information. Hence, we examined the association between resting heart rate and heart rate variability (SDNN and rMSSD) with the use of PI-based regimens (boosted and non-boosted) compared to an NNRTI-no PI regimen.

In theory, an increase in resting heart rate could be either due to lower parasympathetic and/or higher sympathetic tone (6, 8). On the other hand, the SDNN component of heart rate variability is a measure of overall combined parasympathetic and sympathetic modulation of heart rate, while rMSSD reflects the degree of parasympathetic modulation. Thus, hypothetically, the slower resting heart rate accompanied by higher values of SDNN and rMSSD in the non-boosted PI group might reflect a favorable influence on both sympathetic and parasympathetic modulation of the cardiac autonomic function. On the other hand, the lower values of resting heart rate accompanied by non-significant associations with SDNN and rMSSD in the boosted-PI group might reflect a favorable influence on sympathetic but not the parasympathetic

modulation of the cardiac autonomic function. Determining the exact mechanism by which non-boosted PIs can improve heart rate variability and autonomic cardiac regulation and why they differ from boosted PIs will require additional research. Nevertheless, a number of possible explanations could be hypothesized.

Autonomic dysfunction in untreated patients with advanced disease was generally believed to be caused by HIV-1 virus itself, which is well known to be neurotropic (29, 30). So, it is possible that suppression of HIV virus by protease inhibitors might have reduced the chances of developing cardiac autonomic dysfunction. Nevertheless, we did not find a significant difference in heart rate variability for those with plasma HIV RNA <=400 vs > 400 copies/mL. Also, in a recent case-control study in which 97 HIV-infected individuals receiving ART for at least 12 months were compared to an age-matched control group of 52 healthy volunteers, autonomic dysfunction was present in the HIV-infected group even with suppressed plasma HIV load by ART (4). These results accord with another small study of 16 treated HIV individuals where reduced heart rate variability was found as well (8). This suggests that viral suppression cannot fully explain the favorable association between non-boosted PI (compared to NNRTI/no PI) in heart rate variability.

Differences between NNRTI and non-boosted PI regimens in heart rate variability could be the result of differences in the balance between their favorable viral suppression (even if not the major driving force) and their harmful atherogenic impacts. This could be partially supported by what we observed as differences in the lipid profile among different ART regimens. Similarly, several ART drugs have been associated with development of toxic neuropathy (31, 32). Hence, another possibility is that differences in the neurotoxicity might have resulted in differences in the associations of ART drugs with markers of cardiac autonomic function.

Our study has some limitations. Similar to any cross-sectional analysis, residual confounding by factors we did not consider or measure is a possibility. While we adjusted for many potentially

confounding factors, information on antiarrhythmic drug use, which could affect resting heart rate and heart rate variability, was not collected in SMART. Nevertheless, by adjusting for blood pressure lowering drugs which include beta-blockers and calcium channel blockers, we have adjusted for class II (beta-blockers) and class IV (calcium channel blockers) antiarrhythmic drugs - unless these agents were used specifically for arrhythmia not for blood pressure lowering. Information on the exact time of HIV infection was not available, and practically difficult to obtain. However, we adjusted for the time since prescribed first ART which is likely correlated with the time of infection. Another limitation inherent to all cross-sectional analysis is the inability to confirm the temporal relationship between ART use and changes in resting heart rate and heart rate variability. Despite these limitations, our study has many strengths. This is the first study to examine the association between various PI-based regimens and cardiac autonomic function in a large unselected cohort from a well-defined diverse population of HIV-infected individuals. Detailed medical history including ART use as well as clinical and laboratory data were available in the majority of our study population. Also, the ECG acquisition was performed in a consistent manner by trained research staff, and resting heart rate and heart rate variability were measured automatically (0% variability) in a central ECG core laboratory.

Conclusions: Compared to an NNRTI-no PI regimen, both boosted and non-boosted PI regimens were associated with better (i.e. slower) resting heart rate, but only non-boosted PI use was associated with better cardiac autonomic function manifested as higher levels of heart rate variability.

Conflicts of interest: None declared

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Contributions: EZS and JDN conceived the idea of the study (JDN Guarantors). All authors provided input into the data interpretation. MPR conducted the statistical analysis. EZS drafted the manuscript. DAD, HK, RE and JDN critically revised the manuscript. All authors gave final approval for submission of the manuscript.

Trial registration: The trial was registered with ClinicalTrials.gov (trial no. NCT00027352).

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Table 1 Baseline characteristics stratified by baseline antiretroviral use

Characteristic*	RTV boosted PI N = 869	Non-boosted PI N = 579	NNRTI -no PI N = 1550	*p-value***
Age (in years)	44.2 ± 9.0	44.9 ± 9.5	44.0 ± 9.6	0.20
Gender (% female)	252 (29.0%)	178 (30.7%)	423 (27.3%)	0.27
Race				<0.001
Black	199 (22.9%)	191 (33.0%)	426 (27.5%)	
Asian	129 (14.8%)	7 (1.2%)	31 (2.0%)	
White	458 (52.7%)	288 (49.7%)	886 (57.2%)	
Other	83 (9.6%)	93 (16.1%)	207 (13.4%)	
Smoking Status				0.47
Current Smoker	316 (36.4%)	213 (36.8%)	613 (39.5%)	
Past Smoker	223 (25.7%)	157 (27.1%)	390 (25.2%)	
Never Smoker	330 (38.0%)	209 (36.1%)	547 (35.3%)	
Total Cholesterol (mg/dl)	202.5 ± 47.0	199.4 ± 44.8	200.5 ± 47.7	<0.001
LDL Cholesterol (mg/dl)	115.0 ± 34.6	120.0 ± 35.9	116.8 ± 35.6	0.44
HDL Cholesterol (mg/dl)	42.8 ± 14.0	41.2 ± 14.4	46.2 ± 14.9	<0.001
Triglycerides (mg/dl)	259.8 ± 237.1	226.2 ± 189.6	216.2 ± 229.2	0.03
Total/HDL Cholesterol	5.2 ± 2.1	5.4 ± 2.5	4.8 ± 2.4	<0.001
Body mass index (kg/m^2)	25.5 ± 5.3	26.6 ± 5.4	25.8 ± 5.3	<0.001
Heart Rate (bpm)	68.7 ± 11.2	68.4 ± 11.2	70.2 ± 11.5	<0.001
Prior CVD	32 (3.7%)	24 (4.1%)	61 (3.9%)	0.90
Diabetes	46 (5.3%)	52 (9.0%)	121 (7.8%)	0.02
BP lowering drugs	137 (15.8%)	118 (20.4%)	314 (20.3%)	0.02
Lipid lowering drugs	173 (19.9%)	92 (15.9%)	262 (16.9%)	0.09
Baseline CD4 (cells/mm^3)	640.4 ± 239.0	711.6 ± 265.6	690.8 ± 262.2	<0.001
HIV RNA (% ≤ 400 copies/mL)	723 (83.4%)	434 (75.0%)	1357 (87.8%)	<0.001
Time since first prescribed ART (in years)	6.7 ± 3.9	6.4 ± 3.1	5.8 ± 3.4	<0.001
Baseline NRTI regimen				<0.001
AZT+3TC (without ABC)	302 (34.8%)	280 (48.4%)	639 (41.2%)	
TNF (without ABC)	223 (25.7%)	30 (5.2%)	268 (17.3%)	
ABC (without TNF)	130 (15.0%)	65 (11.2%)	236 (15.2%)	
3TC+D4T	81 (9.3%)	132 (22.8%)	239 (15.4%)	
Other NRTI regimens	133 (15.3%)	72 (12.4%)	168 (10.8%)	

*Values expressed as mean ± SD or N (%)
** p-value <0.01 is considered significant
Abbreviations 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; CVD, cardiovascular disease; D4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TNF, tenofovir

Table 2 Differences in heart rate and heart rate variability between protease inhibitor based regimens and NNRTI based regimens

	Model 1: unadjusted		**Model 2**: adjusted for Model 1 plus age, gender, race, and NRTI Backbone		**Model 3**: adjusted for Model 2 plus smoking status, total cholesterol/HDL ratio, BMI, history of CVD events* at baseline, diabetes, blood pressure lowering drugs and lipid lowering drugs		**Model 4**: adjusted for Model 3 plus baseline time since first prescribed ART, baseline CD4 and HIV-RNA	
	Coef. (SE)	p-value	Coef. (SE)	p-value	Coef. (SE)	p-value	Coef. (SE)	p-value*
Heart Rate (bpm)								
Boosted PI	-1.52 (0.48)	0.002	-1.67 (0.50)	<0.001	-1.93 (0.50)	<0.001	-2.15 (0.50)	<0.001
Non-boosted PI	-1.84 (0.55)	<0.001	-2.08 (0.56)	<0.001	-2.62 (0.55)	<0.001	-2.81 (0.56)	<0.001
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--
SDNN (log_{10} ms)								
Boosted PI	0.01 (0.01)	0.35	0.01 (0.01)	0.38	0.02 (0.01)	0.19	0.02 (0.01)	0.12
Non-boosted PI	0.02 (0.01)	0.22	0.03 (0.01)	0.03	0.04 (0.01)	0.006	0.04 (0.01)	0.004
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--
rMSSD (log_{10} ms)								
Boosted PI	0.02 (0.01)	0.22	0.01 (0.01)	0.27	0.02 (0.01)	0.09	0.03 (0.01)	0.04
Non-boosted PI	0.02 (0.02)	0.14	0.03 (0.02)	0.04	0.04 (0.01)	0.003	0.05 (0.02)	0.001
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--

* p-value <0.01 is considered significant

Abbreviations: bpm, beats per minute; ms, millisecond; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; rMMSD, the root mean square of the difference of successive RRs; SD, standard deviation; SDNN, the standard deviation of all filtered RR intervals over the length of the recording

Figure 1 Study flow and inclusion and exclusion criteria

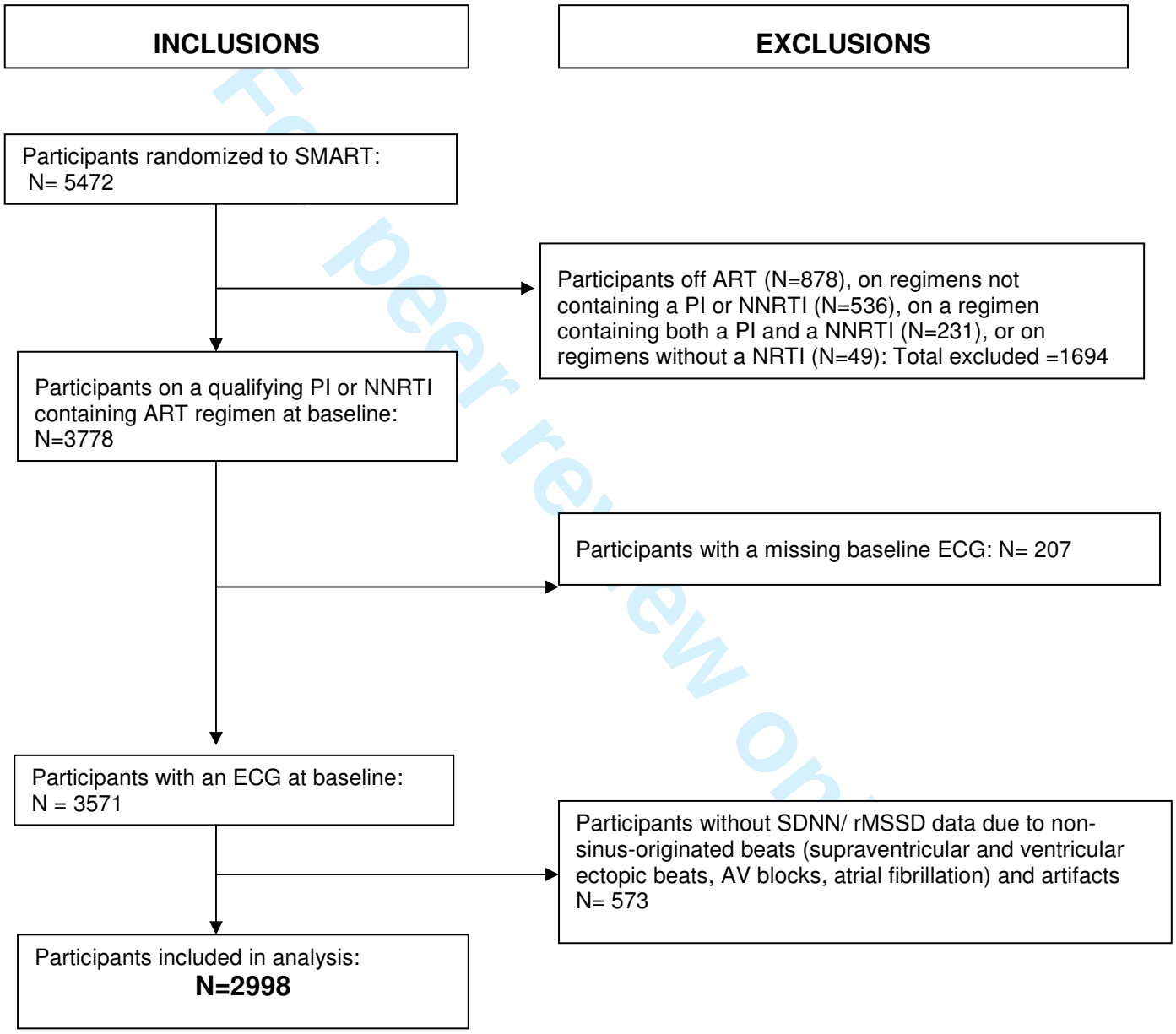
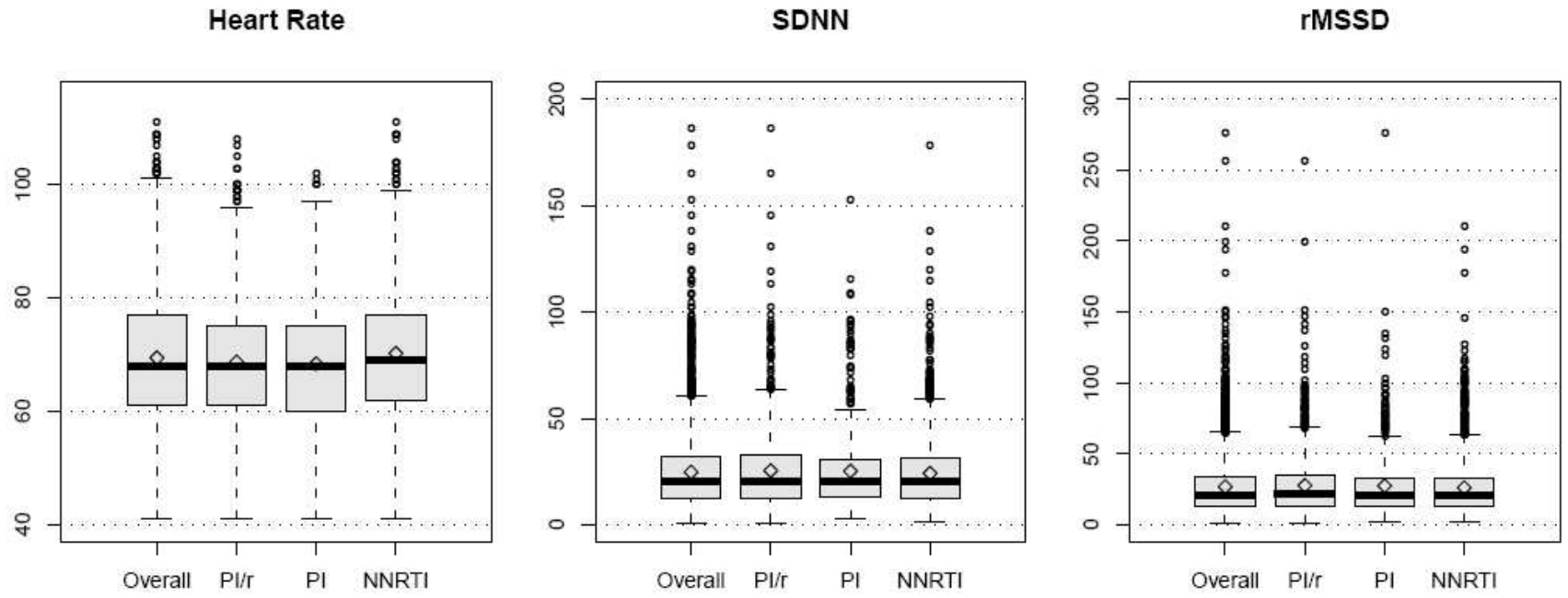


Figure 2 Distribution of resting heart rate variability measures across different types of antiretroviral treatment



	Heart Rate				SDNN				rMSSD			
N	2998	869	579	1550	2998	869	579	1550	2998	869	579	1550
Minimum	41	41	41	41	1.0	1.0	2.6	1.3	1.3	1.3	2.5	2.0
Q1	61	61	60	62	12.7	12.5	13.4	12.6	12.8	12.6	13.5	12.6
Median	68	68	68	69	20.4	20.5	20.6	20.2	21.1	21.5	21.1	20.9
Q3	77	75	75	77	31.8	33.1	30.9	31.4	33.9	35.1	33.1	33.2
Maximum	111	108	102	111	186.7	186.7	153.3	178.5	276.6	256.9	276.6	210.7
Mean	69.4	68.7	68.4	70.2	24.8	25.5	25.3	24.3	26.9	27.8	27.5	26.2
Std. Dev.	11.4	11.2	11.2	11.5	18.2	19.8	18.8	17.0	22.2	23.6	23.3	20.9

Abbreviations: PI, protease inhibitors; PI/r, boosted PI; NNRTI, non-nucleoside reverse transcriptase inhibitor; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; RMSSD, the root mean square of the difference of successive RRs

Supplemental Table 1 Distribution of heart rate variability measures across individual boosted and non-boosted protease inhibitors.

		Ritonavir Boosted PIs (N= 869)					Non-boosted PI (N= 579)				
		SQV/r N = 187	LPV/r N = 410	AZV/r N = 139	Other PI/r N = 133	p-value*	NFV N = 345	IDV N = 109	AZV N = 84	Other PI N = 41	p-value*
Heart Rate											
	Mean	69.8	68.1	68.8	69.1	0.35	67.8	70.0	69.3	67.4	0.24
	SD	10.1	11.2	11.7	12.0		11.7	10.2	10.7	10.1	
	Median	69.0	66.0	68.0	69.0		67.0	69.0	68.0	64.0	
	IQR	63.0, 77.0	60.0, 75.0	59.0, 76.0	60.0, 77.0		59.0, 75.0	63.0, 76.0	62.0, 74.0	59.0, 75.0	
	Min - Max	49.0, 103.0	41.0, 105.0	43.0, 108.0	42.0, 100.0		41.0, 101.0	47.0, 95.0	50.0, 102.0	52.0, 93.0	
SDNN											
	Mean	24.1	26.8	26.0	22.9	0.16	26.5	21.8	25.2	23.7	0.03
	SD	15.7	21.4	20.3	18.7		19.6	15.6	19.6	17.3	
	Median	20.7	21.2	20.8	18.7		22.3	17.3	20.1	20.6	
	IQR	14.8, 28.7	11.9, 35.5	13.7, 35.3	10.0, 29.4		14.1, 32.0	11.1, 26.8	12.5, 29.2	12.3, 25.5	
	Min - Max	3.1, 95.8	1.0, 186.7	2.2, 165.8	2.5, 119.3		3.6, 153.3	2.6, 91.4	3.8, 115.6	5.6, 85.9	
rMSSD											
	Mean	28.0	29.0	27.6	23.9	0.03	29.0	23.3	28.4	24.2	0.05
	SD	19.1	25.3	27.7	18.5		25.3	18.9	22.6	15.5	
	Median	23.6	22.1	20.0	17.5		23.1	18.2	20.5	22.6	
	IQR	15.4, 34.9	12.9, 35.9	13.3, 34.1	10.4, 32.6		14.4, 34.8	12.9, 27.6	13.3, 38.2	13.1, 30.0	
	Min - Max	3.0, 119.1	1.3, 200.0	1.9, 256.9	2.7, 83.1		2.5, 276.6	2.5, 120.3	4.3, 124.4	7.0, 83.5	

*unadjusted p-value for omnibus F-test after lost transformation of SDNN and rMSSD, p-value <0.01 is considered significant;
Abbreviations: SD, standard deviation; IQR,inter quartile range; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; rMSSD, the root mean square of the difference of successive RRs; SQV/r, LPV/r, ATV/r, and PI/r, saquinavir, lopinavir, atazanavir and other protease Inhibitors boosted with ritonavir; NFV, Nelfinavir ; IDV, Indinavir ; ATV, Atazanavir

Supplemental Table 2 Multivariable adjusted associations between participant characteristics and heart rate variability

	Heart Rate (bpm)		SDNN (log_{10} ms)		rMSSD (log_{10} ms)	
Factor*	**Coef. (SE)**	**P****	**Coef. (SE)**	**P****	**Coef. (SE)**	**P****
Age (per year)	-0.054 (0.024)	0.02	-0.008 (0.001)	<0.001	-0.008 (0.001)	<0.001
Gender (*F vs. M)*	1.565 (0.489)	0.001	0.018 (0.013)	0.15	0.054 (0.013)	<0.001
Race						
Black (vs. White)	0.668 (0.522)	0.20	-0.005 (0.014)	0.74	0.032 (0.014)	0.03
Asian (vs. White)	3.839 (1.002)	<0.001	-0.042 (0.026)	0.10	-0.022 (0.027)	0.43
Other Races (vs. White)	0.777 (0.649)	0.23	-0.037 (0.017)	0.03	-0.022 (0.018)	0.20
Smoking Status						
Current (vs. Never)	0.736 (0.495)	0.14	-0.012 (0.013)	0.35	-0.013 (0.013)	0.34
Past (vs. Never)	-0.776 (0.540)	0.15	0.011 (0.014)	0.43	0.013 (0.015)	0.38
Total/HDL Cholesterol Ratio	0.560 (0.093)	<0.001	-0.010 (0.002)	<0.001	-0.014 (0.003)	<0.001
Body mass index (kg/m^2)	0.206 (0.042)	<0.001	-0.003 (0.001)	0.005	-0.004 (0.001)	<0.001
Diabetes *(Y vs. N)*	4.706 (0.826)	<0.001	-0.082 (0.021)	<0.001	-0.102 (0.022)	<0.001
Prior CVD *(Y vs. N)*	-3.445 (1.101)	0.002	0.002 (0.029)	0.95	0.040 (0.030)	0.18
Use of BP-lowering drugs *(Y vs. N)*	1.111 (0.580)	0.06	-0.035 (0.015)	0.02	-0.041 (0.016)	0.01
Use of lipid lowering drugs *(Y vs. N)*	0.607 (0.586)	0.30	-0.018 (0.015)	0.22	-0.027 (0.016)	0.09
Baseline CD4 (per 100)	-0.012 (0.082)	0.88	0.003 (0.002)	0.16	0.003 (0.002)	0.22
Baseline HIV-RNA ≤ 400 *(Y vs. N)*	-0.897 (0.579)	0.12	-0.007 (0.015)	0.63	0.001 (0.016)	0.97
Time since first prescribed ART (per year)	0.157 (0.063)	0.01	-0.003 (0.002)	0.03	-0.005 (0.002)	0.002
NRTI backbone regimen						
AZT+3TC (without ABC)	Ref.	--	Ref.	--	Ref.	--
TNF (without ABC)	-0.867 (0.611)	0.16	0.028 (0.016)	0.08	0.014 (0.017)	0.39
ABC (without TNF)	-0.052 (0.648)	0.94	0.004 (0.017)	0.81	0.006 (0.018)	0.75
3TC+d4T	0.506 (0.625)	0.42	-0.012 (0.016)	0.46	-0.018 (0.017)	0.29
Other NRTI regimens	0.295 (0.678)	0.66	0.001 (0.018)	0.96	-0.006 (0.018)	0.76

*All variables are included in the model in addition to ART use. Multivariable association of ART use with resting heart rate, SDNN and rMSSD are listed in Table 2 (Model 4);

**p-value <0.01 is considered significant

Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; bpm, beats per minute; CVD, cardiovascular disease; D4T, stavudine; ms, millisecond; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; rMMSD, the root mean square of the difference of successive RRs; SD, standard deviation; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; TNF, tenofovir

Protease Inhibitors and Cardiac Autonomic Function in HIV-infected Patients: A cross sectional analysis from the Strategies for Management of Antiretroviral Therapy (SMART) Trial

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ABSTRACT

Objective: To compare cardiac autonomic function as measured by heart rate variability for HIV-infected participants taking protease inhibitors (PIs) with those taking a non-nucleoside reverse transcriptase inhibitor without a PI (NNRTI- no PI) regimen.

Design: Cross sectional analysis

Setting: Multicenter study

Participants: 2998 participants (average age 44 years, 28% females) enrolled in the SMART trial

Primary outcome measures: Heart rate and two heart rate variability measures [standard deviation of all normal RR intervals (SDNN) and root mean square of successive differences in normal RR intervals (rMSSD)]

Results: At study entry, 869 participants were taking a boosted PI (PI/r), 579 a non-boosted PI and 1550 an NNRTI-no PI. Median values (IQR) of heart rate, SDNN and rMSSD were: 68 (60-75) beats per minute (bpm), 21 (13-33) millisecond (ms), 22 (13-35) ms in the PI/r group, 68 (60-75) bpm, 21 (13-33) ms, and 21 (14-33) ms in the non-boosted PI group and 69 (62-77) bpm, 20 (13-31) ms, and 21(13-33) ms in the NNRTI-no PI group. After adjustment for baseline factors, for those given PI/r and non-boosted PI, heart rate was 2.2 and 2.8 bpm, respectively, lower than the NNRTI-no PI group ($p<0.001$ for both). On the other hand, compared with the NNRTI-no PI group, log SDNN and log rMSSD were significantly greater for those in the non-boosted PI (p-values for baseline adjusted differences in log- transformed SDNN and rMSSD were 0.004 and 0.001) but not for those in PI/r group at 0.01 alpha level.

Conclusions: Compared to an NNRTI-no PI regimen, heart rate was lower for those taking a PI/r or non-boosted PI and heart rate variability was greater, reflecting better cardiac autonomic function, for those taking a non-boosted PI regimen but not PI/r.

Key words: HIV/AIDS, Protease inhibitors, Electrocardiogram, Heart rate variability

Article Summary

Article focus

- Cardiac autonomic dysfunction manifested as reduced heart rate variability has been reported in HIV infection▪
- The impact of protease inhibitors on cardiac autonomic function considering their favorable effect on HIV suppression and the unfavorable diabetogenic and atherogenic effects is unclear

Key messages

- Different protease inhibitors have different impact on cardiac autonomic function as measured by heart rate variability.
- Compared to a regimen that does not include protease inhibitors, a non-boosted protease inhibitor regimen was associated with better heart rate variability
- Compared to a regimen that does not include protease inhibitors, a boosted protease inhibitor regimen was not associated with better heart rate variability

Strengths and limitations of this study

- This is the largest study to extensively examine cardiac autonomic function as measured by heart rate variability in HIV-infected participants taking protease inhibitors
- Limitations of this study include lack of examining the prognostic significance of the differences in heart rate variability among protease inhibitors.

INTRODUCTION

The introduction and wide use of combination antiretroviral therapy (ART) have made it possible to obtain long-term HIV viral suppression and increased CD4+ T-cell counts. This has resulted in improved mortality rates in HIV-infected patients, but has also led to long-term concern about possible adverse effects of treatment including greater risk of cardiovascular disease (1). Adverse effects could be both due to the drugs themselves, as well as caused indirectly through development of dyslipidemia, insulin resistance and metabolic syndrome, well known to be associated with ART (2, 3). Protease inhibitors (PIs) in particular have been linked to both hypercholesterolemia and development of insulin resistance (2-5), and subsequently can negatively influence the cardiovascular system, including cardiac autonomic function. Nevertheless, the overall impact of PIs on cardiac autonomic function considering their favorable effect on HIV suppression and the unfavorable diabetogenic and atherogenic effects is unclear.

Heart rate variability is a noninvasive and easy to obtain electrocardiographic measure of cardiac autonomic nervous system function (6, 7). Cardiac autonomic dysfunction manifested as reduced heart rate variability and increased resting heart rate has been reported in HIV infection (8-10), and has been demonstrated to severely debilitate HIV-infected patients, namely by postural hypotension and syncope as well as possible life-threatening cardiac arrest (11-13). Nevertheless, several of these studies were conducted before the wide use of highly active ART (HAART).

The purpose of this cross-sectional analysis was to compare heart rate and cardiac autonomic function as measured by heart rate variability for HIV-infected participants taking PI-based regimens (boosted and non-boosted) with those taking a non-nucleoside reverse transcriptase inhibitors without a PI (NNRTI-no PI) regimen in the Strategies for Management of Antiretroviral Therapy (SMART) trial.

METHODS

Study population

SMART is an open-label randomized trial comparing two antiretroviral treatment (ART) strategies. The study was approved by the institutional review board of all participants sites. Detailed descriptions of the design and aims of the study have been published elsewhere (14, 15). Briefly, individuals infected with HIV who were older than 13 years and were not pregnant or breast-feeding were eligible for inclusion in the SMART study if their CD4+ T-cell count exceeded 350 cells per cubic millimeter and they were willing to participate. At baseline, an ART and medical history were obtained, CD4+ T-cell count and plasma HIV RNA levels were measured, and a 12-lead electrocardiogram (ECG) was obtained. This analysis only utilized data from the baseline visit. All SMART participants (N=5472) were considered eligible for the present analysis, except those who were off ART, on an ART regimen not containing a PI and/or an NNRTI at baseline, on a regimen containing both a PI and an NNRTI, or on an ART regimen not containing a nucleoside reverse transcriptase inhibitor (NRTI), or those who were missing their baseline ECG or with ECG conditions that interfere with appropriate measurement of heart rate variability. After these exclusions, 2998 participants remained and were included in this analysis **(Figure 1)**.Baseline characteristics comparing participants with SDNN/rMSSD data to those without are detailed in Supplemental Table 1.

Electrocardiography and heart rate variability measures

Detailed description of ECG recording in SMART has been published elsewhere (16). Briefly, identical electrocardiographs (GE MAC 1200 models, GE Milwaukee, WI) were used in all of the study clinical sites, and standard 12-lead ECGs were recorded in all participants using strictly standardized procedures. The digital ECG tracings stored in the electrocardiographs were transmitted regularly over analog phone lines to the SMART ECG Reading Center, EPICARE,

located at Wake Forest School of Medicine, Winston-Salem, NC for analysis. ECGs were evaluated blinded to treatment group and ART use. After being visually checked for quality, the study ECGs were automatically processed using the 2001 version of the GE Marquette 12-SL program (GE, Milwaukee, WI). Heart rate variability indices were automatically calculated after excluding any ECG with non-sinus-originated beats (supraventricular and ventricular ectopic beats, AV blocks, atrial fibrillation) and artifacts. Two time-domain heart rate variability indices were calculated: The standard deviation of all filtered RR intervals over the length of the recording (SDNN) and the root mean square of the difference of successive RRs (RMSSD) [6].

Statistical analysis

Participants were categorized into one of three groups based on the ART regimen they were receiving at the time of randomization as follows: 1) boosted PI (PI/r); 2) non-boosted PI; or 3) an NNRTI-no PI. The distribution of heart rate variability measures across these three groups was tabulated. Baseline characteristics were also summarized by these three ART groups. F-tests were used to compare means, X^2 tests to compare percentages.

Linear regression analysis was used to examine the association between heart rate and each of heart rate variability measures, separately, with different ART regimens [PI/r, and non-boosted PI regimens, separately, versus an NNRTI-no PI regimen]. SDNN and rMSSD were log-transformed due to their skewed distributions. Four different models were considered: Model 1: unadjusted; Model 2: adjusted for age, sex, race (Black, Asian, white and others) and NRTI backbone regimen; Model 3: adjusted as in Model 2 plus smoking status, total /HDL cholesterol ratio, body-mass index (BMI), prior cardiovascular disease , diabetes mellitus, use of blood pressure-lowering drugs and use of lipid-lowering drugs; and Model 4: adjusted as in model 3 plus baseline time since first prescribed ART, baseline CD4+ T-cell count and plasma HIV RNA levels.

Two-sided p-values are cited. A more stringent p-value of <0.01 was considered significant to minimize type-I error due to multiple comparisons. Analyses were performed using SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina) and *R* version 2.9.

RESULTS

This analysis included 2998 participants. Average age was 44 years, 28% were women, 54% were white, 27% were blacks and 6% were Asian. As shown in **Table 1** and **Supplemental Table 2**, 869 (29%) of the participants were receiving a PI/r [187 on saquinavir boosted with ritonavir (SQV/r), 410 on lopinavir boosted with ritonavir (LPV/r), 139 on atazanavir boosted with ritonavir (ATV/r) and 133 on other PI/r], 579 (19%) were receiving a non-boosted PI [345 on nelfinavir (NFV), 109 on indinavir (IDV), 84 on atazanavir (ATV) and 41 on other PIs] and 1550 (52%) were receiving an NNRTI- no PI.

A number of baseline factors varied by type of ART regimen used. Notably, most Asians (129 out of 167) were receiving a PI/r while most blacks (426 out of 816) and whites (886 out of 1632) were receiving an NNRTI-no PI (unadjusted p <0.001). The highest levels of total cholesterol and the longer time since first prescribed ART as well as the highest prevalence of the use of lipid lowering drugs and lowest levels of baseline CD4+ T-cell count were observed in the PI/r group compared to the non-boosted PI and NNRTI-no PI groups (unadjusted p<0.001 for all comparisons). The highest prevalence of diabetes was observed in the non-boosted PI compared to the boosted PI and NNRTI-no PI. Participants on an NNRTI-no PI regimen were more likely to have HIV RNA <400 copies/mL and higher levels of HDL-cholesterol compared to both PI-based regimens (unadjusted p<0.001) **(Table 1).**

Figure 2 shows the distribution of heart rate variability measures in all study population and across different types of ART regimens. The median (IQR) values of the resting heart rate, SDNN and rMSSD in all study population were 68 (61-77) beats per minute (bpm), 20 (13-32) millisecond (ms), and 21 (13-34) ms, respectively. There was a positive correlation between

SDNN and rMSSD (Spearman rank correlation (r) = 0.88; p<0.001) but negative correlation between heart rate and SDNN (r = -0.39) and rMSSD (r = -0.55) with p-value <0.001 for all.

There was no significant difference in heart rate, SDNN, or rMSSD among individual boosted PIs and non-boosted PIs at alpha level of 0.01 as shown in **Supplemental Table 2**, and therefore, we combined all boosted PIs together and the same for non-boosted PIs in the linear regression analysis.

Table 2 shows the results of different regression models examining the association between PI based regimens, compared to an NNRTI-no PI regimen, with heart rate, log-SDNN and log-rMSSD, separately. After adjustment for baseline factors (full model; model 4), amongst those given boosted PIs and non-boosted PIs, heart rate was 2.2 and 2.8 bpm, respectively, lower than the NNRTI-no PI group (p<0.001 for both). On the other hand, compared with the NNRTI-no PI group, log SDNN and log rMSSD were significantly greater for those in the non-boosted PI (p-values for baseline adjusted differences in log- transformed SDNN and rMSSD were 0.004 and 0.001), but not those in PI/r group at 0.01 alpha level **(Table 2).**

In the full model (Model 4), older age, higher total/HDL cholesterol ratio, higher body mass index, and diabetes were significantly associated with lower SDNN and rMSSD. There were no significant associations between baseline CD4+ T-cell count, plasma HIV RNA levels, and type of NRTI backbone regimen with any of the heart rate variability measures **(Supplemental Table 3)**

DISCUSSION

The key findings of our study were: 1) use of protease inhibitors, whether boosted or non-boosted, was associated with slower (favorable) resting heart rate compared to NNRTI-no PI use; 2) non-boosted PI use was associated with higher levels of heart rate variability measures (i.e. better cardiac autonomic function) compared to NNRTI-no PI use; 3) no significant

difference in heart rate variability measures between PI/r and NNRTI- no PI groups, and 4) no significant differences in heart rate and heart rate variability measures among individual drugs in the PI/r and non-boosted PI groups, which suggest that the observed associations are class associations. The clinical relevance of these observed differences in cardiac autonomic function among ART regimens and how they may influence cardiovascular outcomes in HIV-infected individuals needs to be investigated.

Former studies that have been carried out in participants with and without cardiovascular disease showed that higher resting heart rate and lower heart rate variability are associated with poor prognosis in both the general population (17-28) and HIV-infected individuals (8-13). These measures of cardiac autonomic function are dynamic rather than static; affected by disease processes as well as cardio-active medications. Given the reported higher prevalence of cardiac autonomic dysfunction in HIV-infected individuals and the reported atherogenic and diabetogenic effects of PIs, examining the association between this class of ART and autonomic function carries special importance. Since today's most relevant group of HIV-infected individuals is those receiving ART, examining the association of protease inhibitors with cardiac autonomic function in comparison with other ART regimens, rather than no treatment, provides more practical information. Hence, we examined the association between resting heart rate and heart rate variability (SDNN and rMSSD) with the use of PI-based regimens (boosted and non-boosted) compared to an NNRTI-no PI regimen.

In theory, an increase in resting heart rate could be either due to lower parasympathetic and/or higher sympathetic tone (6, 8). On the other hand, the SDNN component of heart rate variability is a measure of overall combined parasympathetic and sympathetic modulation of heart rate, while rMSSD reflects the degree of parasympathetic modulation. Thus, hypothetically, the slower resting heart rate accompanied by higher values of SDNN and rMSSD in the non-boosted PI group might reflect a favorable influence on both sympathetic and parasympathetic

modulation of the cardiac autonomic function. On the other hand, the lower values of resting heart rate accompanied by non-significant associations with SDNN and rMSSD in the boosted-PI group might reflect a favorable influence on sympathetic but not the parasympathetic modulation of the cardiac autonomic function. Determining the exact mechanism by which non-boosted PIs can improve heart rate variability and autonomic cardiac regulation and why they differ from boosted PIs will require additional research. Nevertheless, a number of possible explanations could be hypothesized.

Autonomic dysfunction in untreated patients with advanced disease was generally believed to be caused by HIV-1 virus itself, which is well known to be neurotropic (29, 30). So, it is possible that suppression of HIV virus by protease inhibitors might have reduced the chances of developing cardiac autonomic dysfunction. Nevertheless, we did not find a significant difference in heart rate variability for those with plasma HIV RNA <=400 vs > 400 copies/mL. Also, in a recent case-control study in which 97 HIV-infected individuals receiving ART for at least 12 months were compared to an age-matched control group of 52 healthy volunteers, autonomic dysfunction was present in the HIV-infected group even with suppressed plasma HIV load by ART (4). These results accord with another small study of 16 treated HIV individuals where reduced heart rate variability was found as well (8). This suggests that viral suppression cannot fully explain the favorable association between non-boosted PI (compared to NNRTI/no PI) in heart rate variability.

Differences between NNRTI and non-boosted PI regimens in heart rate variability could be the result of differences in the balance between their favorable viral suppression (even if not the major driving force) and their harmful atherogenic impacts. This could be partially supported by what we observed as differences in the lipid profile among different ART regimens. Similarly, several ART drugs have been associated with development of toxic neuropathy (31, 32). Hence,

another possibility is that differences in the neurotoxicity might have resulted in differences in the associations of ART drugs with markers of cardiac autonomic function.

Our study has some limitations. Similar to any cross-sectional analysis, residual confounding by factors we did not consider or measure is a possibility. While we adjusted for many potentially confounding factors, information on antiarrhythmic drug use, which could affect resting heart rate and heart rate variability, was not collected in SMART. Nevertheless, by adjusting for blood pressure lowering drugs which include beta-blockers and calcium channel blockers, we have adjusted for class II (beta-blockers) and class IV (calcium channel blockers) antiarrhythmic drugs - unless these agents were used specifically for arrhythmia not for blood pressure lowering. Information on the exact time of HIV infection was not available, and practically difficult to obtain. However, we adjusted for the time since prescribed first ART which is likely correlated with the time of infection. Another limitation inherent to all cross-sectional analysis is the inability to confirm the temporal relationship between ART use and changes in resting heart rate and heart rate variability. Despite these limitations, our study has many strengths. This is the first study to examine the association between various PI-based regimens and cardiac autonomic function in a large unselected cohort from a well-defined diverse population of HIV-infected individuals. Detailed medical history including ART use as well as clinical and laboratory data were available in the majority of our study population. Also, the ECG acquisition was performed in a consistent manner by trained research staff, and resting heart rate and heart rate variability were measured automatically (0% variability) in a central ECG core laboratory.

Conclusions: Compared to an NNRTI-no PI regimen, both boosted and non-boosted PI regimens were associated with better (i.e. slower) resting heart rate, but only non-boosted PI use was associated with better cardiac autonomic function manifested as higher levels of heart rate variability.

Conflicts of interest: None declared

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Contributions: EZS and JDN conceived the idea of the study (JDN Guarantors). All authors provided input into the data interpretation. MPR conducted the statistical analysis. EZS drafted the manuscript. DAD, HK, RE and JDN critically revised the manuscript. All authors gave final approval for submission of the manuscript.

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Table 1: Baseline characteristics stratified by baseline antiretroviral use

Characteristic*	RTV boosted PI N = 869	Non-boosted PI N = 579	NNRTI -no PI N = 1550	*p-value***
Age (in years)	44.2 ± 9.0	44.9 ± 9.5	44.0 ± 9.6	0.20
Gender (% female)	252 (29.0%)	178 (30.7%)	423 (27.3%)	0.27
Race				<0.001
Black	199 (22.9%)	191 (33.0%)	426 (27.5%)	
Asian	129 (14.8%)	7 (1.2%)	31 (2.0%)	
White	458 (52.7%)	288 (49.7%)	886 (57.2%)	
Other	83 (9.6%)	93 (16.1%)	207 (13.4%)	
Smoking Status				0.47
Current Smoker	316 (36.4%)	213 (36.8%)	613 (39.5%)	
Past Smoker	223 (25.7%)	157 (27.1%)	390 (25.2%)	
Never Smoker	330 (38.0%)	209 (36.1%)	547 (35.3%)	
Total Cholesterol (mg/dl)	202.5 ± 47.0	199.4 ± 44.8	200.5 ± 47.7	<0.001
LDL Cholesterol (mg/dl)	115.0 ± 34.6	120.0 ± 35.9	116.8 ± 35.6	0.44
HDL Cholesterol (mg/dl)	42.8 ± 14.0	41.2 ± 14.4	46.2 ± 14.9	<0.001
Triglycerides (mg/dl)	259.8 ± 237.1	226.2 ± 189.6	216.2 ± 229.2	0.03
Total/HDL Cholesterol	5.2 ± 2.1	5.4 ± 2.5	4.8 ± 2.4	<0.001
Body mass index (kg/m^2)	25.5 ± 5.3	26.6 ± 5.4	25.8 ± 5.3	<0.001
Heart Rate (bpm)	68.7 ± 11.2	68.4 ± 11.2	70.2 ± 11.5	<0.001
Prior CVD	32 (3.7%)	24 (4.1%)	61 (3.9%)	0.90
Diabetes	46 (5.3%)	52 (9.0%)	121 (7.8%)	0.02
BP lowering drugs	137 (15.8%)	118 (20.4%)	314 (20.3%)	0.02
Lipid lowering drugs	173 (19.9%)	92 (15.9%)	262 (16.9%)	0.09
Baseline CD4 (cells/mm^3)	640.4 ± 239.0	711.6 ± 265.6	690.8 ± 262.2	<0.001
HIV RNA (% ≤ 400 copies/mL)	723 (83.4%)	434 (75.0%)	1357 (87.8%)	<0.001
Time since first prescribed ART (in years)	6.7 ± 3.9	6.4 ± 3.1	5.8 ± 3.4	<0.001
Baseline NRTI regimen				<0.001
AZT+3TC (without ABC)	302 (34.8%)	280 (48.4%)	639 (41.2%)	
TNF (without ABC)	223 (25.7%)	30 (5.2%)	268 (17.3%)	
ABC (without TNF)	130 (15.0%)	65 (11.2%)	236 (15.2%)	
3TC+D4T	81 (9.3%)	132 (22.8%)	239 (15.4%)	
Other NRTI regimens	133 (15.3%)	72 (12.4%)	168 (10.8%)	

*Values expressed as mean ± SD or N (%)
** Means were compared with F-tests, X^2 tests for percentages; p-value <0.01 is considered significant
Abbreviations 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; CVD, cardiovascular disease; D4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TNF, tenofovir

Table 2: Differences in heart rate and heart rate variability between protease inhibitor based regimens and NNRTI based regimens

Measure	**Model 1:** *unadjusted*		**Model 2:** *adjusted for Model 1 plus age, gender, race, and NRTI Backbone*		**Model 3:** *adjusted for Model 2 plus smoking status, total cholesterol/HDL ratio, BMI, history of CVD events* at baseline, diabetes, blood pressure lowering drugs and lipid lowering drugs*		**Model 4**: *adjusted for Model 3 plus baseline time since first prescribed ART, baseline CD4 and HIV-RNA*	
	Difference (95% CI)	p-value*	Difference (95% CI)	p-value*	Difference (95% CI)	p-value*	Difference. . (95% CI)	p-value*
Heart Rate (bpm)								
Boosted PI	-1.52 (-2.46, -0.57)	0.002	-1.67 (-2.65, -0.69)	<0.001	-1.93 (-2.91, -0.96)	<0.001	-2.15 (-3.14, -1.16)	<0.001
Non-boosted PI	-1.84 (-2.92, -0.75)	<0.001	-2.08 (-3.18, -0.98)	<0.001	-2.62 (-3.70, -1.53)	<0.001	-2.81 (-3.90, -1.71)	<0.001
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--
SDNN (log_{10} ms)								
Boosted PI	0.01 (-0.01, 0.04)	0.35	0.01 (-0.01, 0.04)	0.38	0.02 (-0.01, 0.04)	0.19	0.02 (-0.01, 0.05)	0.12
Non-boosted PI	0.02 (-0.01, 0.05)	0.22	0.03 (0.00, 0.06)	0.03	0.04 (0.01, 0.07)	0.006	0.04 (0.01, 0.07)	0.004
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--
rMSSD (log_{10} ms)								
Boosted PI	0.02 (-0.01, 0.04)	0.22	0.01 (-0.01, 0.04)	0.27	0.02 (-0.00, 0.05)	0.09	0.03 (0.00, 0.05)	0.04
Non-boosted PI	0.02 (-0.01, 0.05)	0.14	0.03 (0.00, 0.06)	0.04	0.04 (0.01, 0.07)	0.003	0.05 (0.02, 0.08)	0.001
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--

*p-value*ue<0.01 is considered significant.

Abbreviations bpm, beats per minute; ms, millisecond; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; rMMSD, the root mean square of the difference of successive RRs; SD, standard deviation; SDNN, the standard deviation of all filtered RR intervals over the length of the recording

FIGURE LEGENDS

Figure 1 Study flow and inclusion and exclusion criteria

Figure 2 Distribution of resting heart rate variability measures across different types of antiretroviral treatment

Full title:

Protease Inhibitors and Cardiac Autonomic Function in HIV-infected Patients: A cross sectional analysis from the Strategies for Management of Antiretroviral Therapy (SMART) Trial

Short title:

Protease inhibitors and heart rate variability

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ABSTRACT

Objective: To compare cardiac autonomic function as measured by heart rate variability for HIV-infected participants taking protease inhibitors (PIs) with those taking a non-nucleoside reverse transcriptase inhibitor without a PI (NNRTI- no PI) regimen.

Design: Cross sectional analysis

Setting: Multicenter study

Participants: 2998 participants (average age 44 years, 28% females) enrolled in the SMART trial

Primary outcome measures: Heart rate and two heart rate variability measures [standard deviation of all normal RR intervals (SDNN) and root mean square of successive differences in normal RR intervals (rMSSD)]

Results: At study entry, 869 participants were taking a boosted PI (PI/r), 579 a non-boosted PI and 1550 an NNRTI-no PI. Median values (IQR) of heart rate, SDNN and rMSSD were: 68 (60-75) beats per minute (bpm), 21 (13-33) millisecond (ms), 22 (13-35) ms in the PI/r group, 68 (60-75) bpm, 21 (13-33) ms, and 21 (14-33) ms in the non-boosted PI group and 69 (62-77) bpm, 20 (13-31) ms, and 21(13-33) ms in the NNRTI-no PI group. After adjustment for baseline factors, for those given PI/r and non-boosted PI, heart rate was 2.2 and 2.8 bpm, respectively, lower than the NNRTI-no PI group ($p<0.001$ for both). On the other hand, compared with the NNRTI-no PI group, log SDNN and log rMSSD were significantly greater for those in the non-boosted PI (p-values for baseline adjusted differences in log- transformed SDNN and rMSSD were 0.004 and 0.001) but not for those in PI/r group at 0.01 alpha level.

Conclusions: Compared to an NNRTI-no PI regimen, heart rate was lower for those taking a PI/r or non-boosted PI and heart rate variability was greater, reflecting better cardiac autonomic function, for those taking a non-boosted PI regimen but not PI/r.

Key words: HIV/AIDS, Protease inhibitors, Electrocardiogram, Heart rate variability

Article Summary

Article focus

- Cardiac autonomic dysfunction manifested as reduced heart rate variability has been reported in HIV infection▪
- The impact of protease inhibitors on cardiac autonomic function considering their favorable effect on HIV suppression and the unfavorable diabetogenic and atherogenic effects is unclear

Key messages

- Different protease inhibitors have different impact on cardiac autonomic function as measured by heart rate variability.
- Compared to a regimen that does not include protease inhibitors, a non-boosted protease inhibitor regimen was associated with better heart rate variability
- Compared to a regimen that does not include protease inhibitors, a boosted protease inhibitor regimen was not associated with better heart rate variability

Strengths and limitations of this study

- This is the largest study to extensively examine cardiac autonomic function as measured by heart rate variability in HIV-infected participants taking protease inhibitors
- Limitations of this study include lack of examining the prognostic significance of the differences in heart rate variability among protease inhibitors.

INTRODUCTION

The introduction and wide use of combination antiretroviral therapy (ART) have made it possible to obtain long-term HIV viral suppression and increased CD4+ T-cell counts. This has resulted in improved mortality rates in HIV-infected patients, but has also led to long-term concern about possible adverse effects of treatment including greater risk of cardiovascular disease (1). Adverse effects could be both due to the drugs themselves, as well as caused indirectly through development of dyslipidemia, insulin resistance and metabolic syndrome, well known to be associated with ART (2, 3). Protease inhibitors (PIs) in particular have been linked to both hypercholesterolemia and development of insulin resistance [2-5], and subsequently can negatively influence the cardiovascular system, including cardiac autonomic function. Nevertheless, the overall impact of PIs on cardiac autonomic function considering their favorable effect on HIV suppression and the unfavorable diabetogenic and atherogenic effects is unclear.

Heart rate variability is a noninvasive and easy to obtain electrocardiographic measure of cardiac autonomic nervous system function [6, 7]. Cardiac autonomic dysfunction manifested as reduced heart rate variability and increased resting heart rate has been reported in HIV infection (8-10), and has been demonstrated to severely debilitate HIV-infected patients, namely by postural hypotension and syncope as well as possible life-threatening cardiac arrest [11-13]. Nevertheless, several of these studies were conducted before the wide use of highly active ART (HAART).

The purpose of this cross-sectional analysis was to compare heart rate and cardiac autonomic function as measured by heart rate variability for HIV-infected participants taking PI-based regimens (boosted and non-boosted) with those taking a non-nucleoside reverse transcriptase inhibitors without a PI (NNRTI-no PI) regimen in the Strategies for Management of Antiretroviral Therapy (SMART) trial.

METHODS

Study population

SMART is an open-label randomized trial comparing two antiretroviral treatment (ART) strategies. The study was approved by the institutional review board of all participants sites. Detailed descriptions of the design and aims of the study have been published elsewhere [14, 15]. Briefly, individuals infected with HIV who were older than 13 years and were not pregnant or breast-feeding were eligible for inclusion in the SMART study if their CD4+ T-cell count exceeded 350 cells per cubic millimeter and they were willing to participate. ~~Before randomization (baseline)~~At baseline, an ART and medical history were obtained, CD4+ T-cell count and plasma HIV RNA levels were measured, and a 12-lead electrocardiogram (ECG) was obtained. This analysis only utilized data from the baseline visit ~~which was collected within 45 days of randomization~~. All SMART participants (N=5472) were considered eligible for the present analysis, except those who were off ART, on an ART regimen not containing a PI and/or an NNRTI at baseline, on a regimen containing both a PI and an NNRTI, or on an ART regimen not containing a nucleoside reverse transcriptase inhibitor (NRTI), or those who were missing their baseline ECG or with ECG conditions that interfere with appropriate measurement of heart rate variability. After these exclusions, 2998 participants remained and were included in this analysis **(Figure 1)**. Baseline characteristics comparing participants with SDNN/rMSSD data to those without are detailed in Supplemental Table 1.

Electrocardiography and heart rate variability measures

Detailed description of ECG recording in SMART has been published elsewhere [16]. Briefly, identical electrocardiographs (GE MAC 1200 models, GE Milwaukee, WI) were used in all of the study clinical sites, and standard 12-lead ECGs were recorded in all participants using strictly

standardized procedures. The digital ECG tracings stored in the electrocardiographs were transmitted regularly over analog phone lines to the SMART ECG Reading Center, EPICARE, located at Wake Forest School of Medicine, Winston-Salem, NC for analysis. ECGs were evaluated blinded to treatment group and ART use. After being visually checked for quality, the study ECGs were automatically processed using the 2001 version of the GE Marquette 12-SL program (GE, Milwaukee, WI). Heart rate variability indices were automatically calculated after excluding any ECG with non-sinus-originated beats (supraventricular and ventricular ectopic beats, AV blocks, atrial fibrillation) and artifacts. Two time-domain heart rate variability indices were calculated: The standard deviation of all filtered RR intervals over the length of the recording (SDNN) and the root mean square of the difference of successive RRs (RMSSD) [6].

Statistical analysis

Participants were categorized into one of three groups based on the ART regimen they were receiving at the time of randomization as follows: 1) boosted PI (PI/r); 2) non-boosted PI; or 3) an NNRTI-no PI. The distribution of heart rate variability measures across these three groups was tabulated. Baseline characteristics were also summarized by these three ART groups. F-tests were used to compare means, X^2 tests to compare percentages.

Linear regression analysis was used to examine the association between heart rate and each of heart rate variability measures, separately, with different ART regimens [PI/r, and non-boosted PI regimens, separately, versus an NNRTI-no PI regimen]. SDNN and rMSSD were log-transformed due to their skewed distributions. Four different models were considered: Model 1: unadjusted; Model 2: adjusted for age, sex, race (Black, Asian, white and others) and NRTI backbone regimen; Model 3: adjusted as in Model 2 plus smoking status, total /HDL cholesterol ratio, body-mass index (BMI), prior cardiovascular disease , diabetes mellitus, use of blood pressure-lowering drugs and use of lipid-lowering drugs; and Model 4: adjusted as in

model 3 plus baseline time since first prescribed ART, baseline CD4+ T-cell count and plasma HIV RNA levels.

Two-sided p-values are cited. A more stringent p-value of <0.01 was considered significant to minimize type-I error due to multiple comparisons. Analyses were performed using SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina) and *R* version 2.9.

RESULTS

This analysis included 2998 participants. Average age was 44 years, 28% were women, 54% were white, 27% were blacks and 6% were Asian. As shown in **Table 1** and **Supplemental Table 1~~2~~**, 869 (29%) of the participants were receiving a PI/r [187 on saquinavir boosted with ritonavir (SQV/r), 410 on lopinavir boosted with ritonavir (LPV/r), 139 on atazanavir boosted with ritonavir (ATV/r) and 133 on other PI/r], 579 (19%) were receiving a non-boosted PI [345 on nelfinavir (NFV), 109 on indinavir (IDV), 84 on atazanavir (ATV) and 41 on other PIs] and 1550 (52%) were receiving an NNRTI- no PI.

A number of baseline factors varied by type of ART regimen used. Notably, most Asians (129 out of 167) were receiving a PI/r while most blacks (426 out of 816) and whites (886 out of 1632) were receiving an NNRTI-no PI (unadjusted p <0.001). The highest levels of total cholesterol and the longer time since first prescribed ART as well as the highest prevalence of the use of lipid lowering drugs and lowest levels of baseline CD4+ T-cell count were observed in the PI/r group compared to the non-boosted PI and NNRTI-no PI groups (unadjusted p<0.001 for all comparisons). The highest prevalence of diabetes was observed in the non-boosted PI compared to the boosted PI and NNRTI-no PI. Participants on an NNRTI-no PI regimen were more likely to have HIV RNA <400 copies/mL and higher levels of HDL-cholesterol compared to both PI-based regimens (unadjusted p<0.001) **(Table 1).**

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Figure 2 shows the distribution of heart rate variability measures in all study population and across different types of ART regimens. The median (IQR) values of the resting heart rate, SDNN and rMSSD in all study population were 68 (61-77) beats per minute (bpm), 20 (13-32) millisecond (ms), and 21 (13-34) ms, respectively. There was a positive correlation between SDNN and rMSSD (Spearman rank correlation (r) = 0.88; p<0.001) but negative correlation between heart rate and SDNN (r = -0.39) and rMSSD (r = -0.55) with p-value <0.001 for all.

There was no significant difference in heart rate, SDNN, or rMSSD among individual boosted PIs and non-boosted PIs at alpha level of 0.01 as shown in **Supplemental Table ~~1~~2**, and therefore, we combined all boosted PIs together and the same for non-boosted PIs in the linear regression analysis.

Table 2 shows the results of different regression models examining the association between PI based regimens, compared to an NNRTI-no PI regimen, with heart rate, log-SDNN and log-rMSSD, separately. After adjustment for baseline factors (full model; model 4), amongst those given boosted PIs and non-boosted PIs, heart rate was 2.2 and 2.8 bpm, respectively, lower than the NNRTI-no PI group (p<0.001 for both). On the other hand, compared with the NNRTI-no PI group, log SDNN and log rMSSD were significantly greater for those in the non-boosted PI (p-values for baseline adjusted differences in log- transformed SDNN and rMSSD were 0.004 and 0.001), but not those in PI/r group at 0.01 alpha level **(Table 2).**

In the full model (Model 4), older age, higher total/HDL cholesterol ratio, higher body mass index, and diabetes were significantly associated with lower SDNN and rMSSD. There were no significant associations between baseline CD4+ T-cell count, plasma HIV RNA levels, and type of NRTI backbone regimen with any of the heart rate variability measures **(supplemental Table ~~2~~3)**

DISCUSSION

The key findings of our study were: 1) use of protease inhibitors, whether boosted or non-boosted, was associated with slower (favorable) resting heart rate compared to NNRTI-no PI use; 2) non-boosted PI use was associated with higher levels of heart rate variability measures (i.e. better cardiac autonomic function) compared to NNRTI-no PI use; 3) no significant difference in heart rate variability measures between PI/r and NNRTI- no PI groups, and 4) no significant differences in heart rate and heart rate variability measures among individual drugs in the PI/r and non-boosted PI groups, which suggest that the observed associations are class associations. The clinical relevance of these observed differences in cardiac autonomic function among ART regimens and how they may influence cardiovascular outcomes in HIV-infected individuals needs to be investigated.

Former studies that have been carried out in participants with and without cardiovascular disease showed that higher resting heart rate and lower heart rate variability are associated with poor prognosis in both the general population (~~16~~17-28) and HIV-infected individuals (8-13). These measures of cardiac autonomic function are dynamic rather than static; affected by disease processes as well as cardio-active medications. Given the reported higher prevalence of cardiac autonomic dysfunction in HIV-infected individuals and the reported atherogenic and diabetogenic effects of PIs, examining the association between this class of ART and autonomic function carries special importance. Since today's most relevant group of HIV-infected individuals is those receiving ART, examining the association of protease inhibitors with cardiac autonomic function in comparison with other ART regimens, rather than no treatment, provides more practical information. Hence, we examined the association between resting heart rate and heart rate variability (SDNN and rMSSD) with the use of PI-based regimens (boosted and non-boosted) compared to an NNRTI-no PI regimen.

In theory, an increase in resting heart rate could be either due to lower parasympathetic and/or higher sympathetic tone (6, 8). On the other hand, the SDNN component of heart rate variability is a measure of overall combined parasympathetic and sympathetic modulation of heart rate, while rMSSD reflects the degree of parasympathetic modulation. Thus, hypothetically, the slower resting heart rate accompanied by higher values of SDNN and rMSSD in the non-boosted PI group might reflect a favorable influence on both sympathetic and parasympathetic modulation of the cardiac autonomic function. On the other hand, the lower values of resting heart rate accompanied by non-significant associations with SDNN and rMSSD in the boosted-PI group might reflect a favorable influence on sympathetic but not the parasympathetic modulation of the cardiac autonomic function. Determining the exact mechanism by which non-boosted PIs can improve heart rate variability and autonomic cardiac regulation and why they differ from boosted PIs will require additional research. Nevertheless, a number of possible explanations could be hypothesized.

Autonomic dysfunction in untreated patients with advanced disease was generally believed to be caused by HIV-1 virus itself, which is well known to be neurotropic (29, 30). So, it is possible that suppression of HIV virus by protease inhibitors might have reduced the chances of developing cardiac autonomic dysfunction. Nevertheless, we did not find a significant difference in heart rate variability for those with plasma HIV RNA <=400 vs > 400 copies/mL. Also, in a recent case-control study in which 97 HIV-infected individuals receiving ART for at least 12 months were compared to an age-matched control group of 52 healthy volunteers, autonomic dysfunction was present in the HIV-infected group even with suppressed plasma HIV load by ART (4). These results accord with another small study of 16 treated HIV individuals where reduced heart rate variability was found as well (8). This suggests that viral suppression cannot fully explain the favorable association between non-boosted PI (compared to NNRTI/no PI) in heart rate variability.

Differences between NNRTI and non-boosted PI regimens in heart rate variability could be the result of differences in the balance between their favorable viral suppression (even if not the major driving force) and their harmful atherogenic impacts. This could be partially supported by what we observed as differences in the lipid profile among different ART regimens. Similarly, several ART drugs have been associated with development of toxic neuropathy (31, 32). Hence, another possibility is that differences in the neurotoxicity might have resulted in differences in the associations of ART drugs with markers of cardiac autonomic function.

Our study has some limitations. Similar to any cross-sectional analysis, residual confounding by factors we did not consider or measure is a possibility. While we adjusted for many potentially confounding factors, information on antiarrhythmic drug use, which could affect resting heart rate and heart rate variability, was not collected in SMART. Nevertheless, by adjusting for blood pressure lowering drugs which include beta-blockers and calcium channel blockers, we have adjusted for class II (beta-blockers) and class IV (calcium channel blockers) antiarrhythmic drugs - unless these agents were used specifically for arrhythmia not for blood pressure lowering. Information on the exact time of HIV infection was not available, and practically difficult to obtain. However, we adjusted for the time since prescribed first ART which is likely correlated with the time of infection. Another limitation inherent to all cross-sectional analysis is the inability to confirm the temporal relationship between ART use and changes in resting heart rate and heart rate variability. Despite these limitations, our study has many strengths. This is the first study to examine the association between various PI-based regimens and cardiac autonomic function in a large unselected cohort from a well-defined diverse population of HIV-infected individuals. Detailed medical history including ART use as well as clinical and laboratory data were available in the majority of our study population. Also, the ECG acquisition was performed in a consistent manner by trained research staff, and resting heart rate and heart rate variability were measured automatically (0% variability) in a central ECG core laboratory.

Conclusions: Compared to an NNRTI-no PI regimen, both boosted and non-boosted PI regimens were associated with better (i.e. slower) resting heart rate, but only non-boosted PI use was associated with better cardiac autonomic function manifested as higher levels of heart rate variability.

Conflicts of interest: None declared

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Contributions: EZS and JDN conceived the idea of the study (JDN Guarantors). All authors provided input into the data interpretation. MPR conducted the statistical analysis. EZS drafted the manuscript. DAD, HK, RE and JDN critically revised the manuscript. All authors gave final approval for submission of the manuscript.

Trial registration: The trial was registered with ClinicalTrials.gov (trial no. NCT00027352).

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Table 1: Baseline characteristics stratified by baseline antiretroviral use

Characteristic*	RTV boosted PI N = 869	Non-boosted PI N = 579	NNRTI -no PI N = 1550	*p-value***
Age (in years)	44.2 ± 9.0	44.9 ± 9.5	44.0 ± 9.6	0.20
Gender (% female)	252 (29.0%)	178 (30.7%)	423 (27.3%)	0.27
Race				<0.001
Black	199 (22.9%)	191 (33.0%)	426 (27.5%)	
Asian	129 (14.8%)	7 (1.2%)	31 (2.0%)	
White	458 (52.7%)	288 (49.7%)	886 (57.2%)	
Other	83 (9.6%)	93 (16.1%)	207 (13.4%)	
Smoking Status				0.47
Current Smoker	316 (36.4%)	213 (36.8%)	613 (39.5%)	
Past Smoker	223 (25.7%)	157 (27.1%)	390 (25.2%)	
Never Smoker	330 (38.0%)	209 (36.1%)	547 (35.3%)	
Total Cholesterol (mg/dl)	202.5 ± 47.0	199.4 ± 44.8	200.5 ± 47.7	<0.001
LDL Cholesterol (mg/dl)	115.0 ± 34.6	120.0 ± 35.9	116.8 ± 35.6	0.44
HDL Cholesterol (mg/dl)	42.8 ± 14.0	41.2 ± 14.4	46.2 ± 14.9	<0.001
Triglycerides (mg/dl)	259.8 ± 237.1	226.2 ± 189.6	216.2 ± 229.2	0.03
Total/HDL Cholesterol	5.2 ± 2.1	5.4 ± 2.5	4.8 ± 2.4	<0.001
Body mass index (kg/m^2)	25.5 ± 5.3	26.6 ± 5.4	25.8 ± 5.3	<0.001
Heart Rate (bpm)	68.7 ± 11.2	68.4 ± 11.2	70.2 ± 11.5	<0.001
Prior CVD	32 (3.7%)	24 (4.1%)	61 (3.9%)	0.90
Diabetes	46 (5.3%)	52 (9.0%)	121 (7.8%)	0.02
BP lowering drugs	137 (15.8%)	118 (20.4%)	314 (20.3%)	0.02
Lipid lowering drugs	173 (19.9%)	92 (15.9%)	262 (16.9%)	0.09
Baseline CD4 (cells/mm^3)	640.4 ± 239.0	711.6 ± 265.6	690.8 ± 262.2	<0.001
HIV RNA (% ≤ 400 copies/mL)	723 (83.4%)	434 (75.0%)	1357 (87.8%)	<0.001
Time since first prescribed ART (in years)	6.7 ± 3.9	6.4 ± 3.1	5.8 ± 3.4	<0.001
Baseline NRTI regimen				<0.001
AZT+3TC (without ABC)	302 (34.8%)	280 (48.4%)	639 (41.2%)	
TNF (without ABC)	223 (25.7%)	30 (5.2%)	268 (17.3%)	
ABC (without TNF)	130 (15.0%)	65 (11.2%)	236 (15.2%)	
3TC+D4T	81 (9.3%)	132 (22.8%)	239 (15.4%)	
Other NRTI regimens	133 (15.3%)	72 (12.4%)	168 (10.8%)	

*Values expressed as mean ± SD or N (%)

** Means were compared with F-tests, X^2 tests for percentages; p-value <0.01 is considered significant

Abbreviations 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; CVD, cardiovascular disease; D4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TNF, tenofovir

Table 2: Differences in heart rate and heart rate variability between protease inhibitor based regimens and NNRTI based regimens

Measure	**Model 1:** *unadjusted*		**Model 2:** *adjusted for Model 1 plus age, gender, race, and NRTI Backbone*		**Model 3:** *adjusted for Model 2 plus smoking status, total cholesterol/HDL ratio, BMI, history of CVD events* at baseline, diabetes, blood pressure lowering drugs and lipid lowering drugs*		**Model 4**: *adjusted for Model 3 plus baseline time since first prescribed ART, baseline CD4 and HIV-RNA*	
	Difference (95% CI)	p-value*	Difference (95% CI)	p-value*	Difference (95% CI)	p-value*	Difference. . (95% CI)	p-value*
Heart Rate (bpm)								
Boosted PI	-1.52 (-2.46, -0.57)	0.002	-1.67 (-2.65, -0.69)	<0.001	-1.93 (-2.91, -0.96)	<0.001	-2.15 (-3.14, -1.16)	<0.001
Non-boosted PI	-1.84 (-2.92, -0.75)	<0.001	-2.08 (-3.18, -0.98)	<0.001	-2.62 (-3.70, -1.53)	<0.001	-2.81 (-3.90, -1.71)	<0.001
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--
SDNN (log_{10} ms)								
Boosted PI	0.01 (-0.01, 0.04)	0.35	0.01 (-0.01, 0.04)	0.38	0.02 (-0.01, 0.04)	0.19	0.02 (-0.01, 0.05)	0.12
Non-boosted PI	0.02 (-0.01, 0.05)	0.22	0.03 (0.00, 0.06)	0.03	0.04 (0.01, 0.07)	0.006	0.04 (0.01, 0.07)	0.004
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--
rMSSD (log_{10} ms)								
Boosted PI	0.02 (-0.01, 0.04)	0.22	0.01 (-0.01, 0.04)	0.27	0.02 (-0.00, 0.05)	0.09	0.03 (0.00, 0.05)	0.04
Non-boosted PI	0.02 (-0.01, 0.05)	0.14	0.03 (0.00, 0.06)	0.04	0.04 (0.01, 0.07)	0.003	0.05 (0.02, 0.08)	0.001
NNRTI - no PI	**Ref.**	--	**Ref.**	--	**Ref.**	--	**Ref.**	--

*p-value*ue<0.01 is considered significant.

Abbreviations bpm, beats per minute; ms, millisecond; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; rMMSD, the root mean square of the difference of successive RRs; SD, standard deviation; SDNN, the standard deviation of all filtered RR intervals over the length of the recording

Figure 1 Study flow and inclusion and exclusion criteria

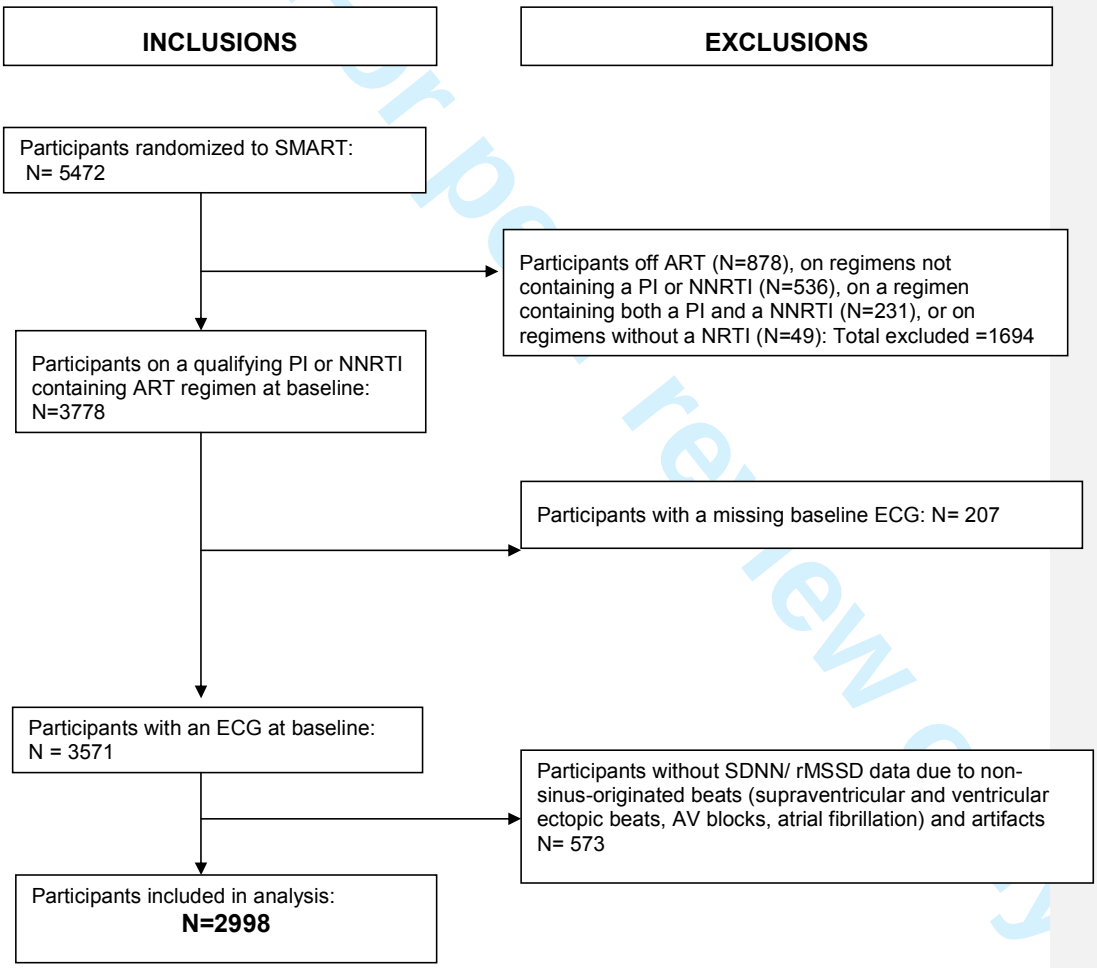
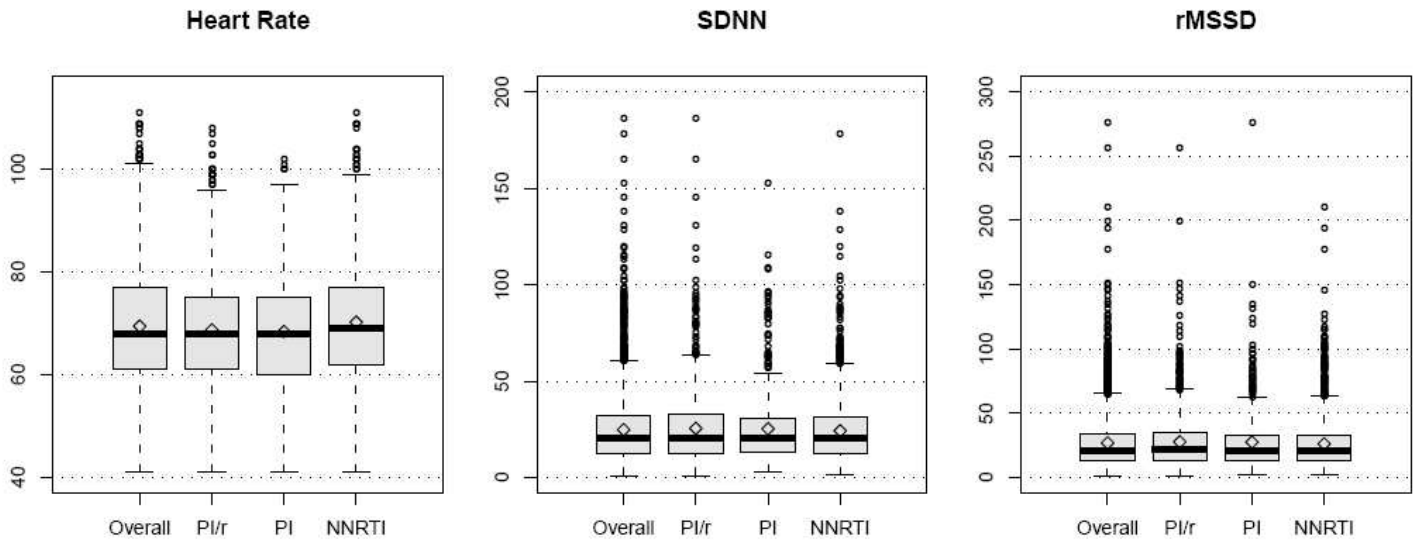


Figure 2 Distribution of resting heart rate variability measures across different types of antiretroviral treatment



	Heart Rate				SDNN				rMSSD			
N	2998	869	579	1550	2998	869	579	1550	2998	869	579	1550
Minimum	41	41	41	41	1.0	1.0	2.6	1.3	1.3	1.3	2.5	2.0
Q1	61	61	60	62	12.7	12.5	13.4	12.6	12.8	12.6	13.5	12.6
Median	68	68	68	69	20.4	20.5	20.6	20.2	21.1	21.5	21.1	20.9
Q3	77	75	75	77	31.8	33.1	30.9	31.4	33.9	35.1	33.1	33.2
Maximum	111	108	102	111	186.7	186.7	153.3	178.5	276.6	256.9	276.6	210.7
Mean	69.4	68.7	68.4	70.2	24.8	25.5	25.3	24.3	26.9	27.8	27.5	26.2
Std. Dev.	11.4	11.2	11.2	11.5	18.2	19.8	18.8	17.0	22.2	23.6	23.3	20.9

Abbreviations: PI, protease inhibitors; PI/r, boosted PI; NNRTI, non-nucleoside reverse transcriptase inhibitor; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; RMSSD, the root mean square of the difference of successive RRs

Supplemental Table 1: Baseline characteristics comparing participants with SDNN/rMSSD data to those without

	Included N = 2998	Excluded N = 573
Age (in years)	44.3 (9.4)	45.0 (9.9)
Gender (% female)	853 (28.5%)	133 (23.2%)
Race		
Black	816 (27.2%)	167 (29.1%)
Asian	167 (5.6%)	26 (4.5%)
White	1632 (54.4%)	335 (58.5%)
Other	383 (12.8%)	45 (7.9%)
Smoking Status		
Current Smoker	1142 (38.1%)	228 (39.8%)
Past Smoker	770 (25.7%)	142 (24.8%)
Never Smoker	1086 (36.2%)	203 (35.4%)
Total Cholesterol (mg/dl)	200.9 (47.0)	201.2 (46.1)
LDL Cholesterol (mg/dl)	116.9 (35.4)	118.0 (37.5)
HDL Cholesterol (mg/dl)	44.2 (14.7)	46.3 (15.8)
Triglycerides (mg/dl)	230.8 (225.2)	209.3 (216.6)
Total/HDL Cholesterol	5.0 (2.3)	4.8 (2.2)
Body mass index (kg/m^2)	25.9 (5.3)	25.3 (5.1)
Heart Rate (bpm)	69.4 (11.4)	69.9 (11.6)
Prior CVD	117 (3.9%)	18 (3.1%)
Diabetes	219 (7.3%)	44 (7.7%)
BP lowering drugs	569 (19.0%)	110 (19.2%)
Lipid lowering drugs	527 (17.6%)	98 (17.1%)
Hepatitis B or C	480 (16.2%)	75 (13.4%)
Baseline CD4 (cells/mm^3)	680.2 (257.7)	665.4 (239.9)
HIV RNA (% ≤ 400 copies/mL)	2514 (84.0%)	487 (85.3%)
Time since first prescribed ART (in years)	6.2 (3.5)	6.4 (3.6)
Baseline NRTI regimen		
AZT+3TC (without ABC)	1221 (40.7%)	218 (38.0%)
TNF (without ABC)	521 (17.4%)	120 (20.9%)
ABC (without TNF)	431 (14.4%)	86 (15.0%)
3TC+D4T	452 (15.1%)	71 (12.4%)
Other NRTI containing regimens	373 (12.4%)	78 (13.6%)

Values expressed as mean (SD) or N (%)

Abbreviations 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; CVD, cardiovascular disease; D4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TNF, tenofovir

Supplemental Table 2: Distribution of heart rate variability measures across individual boosted and non-boosted protease inhibitors.

		Ritonavir Boosted PIs (N= 869)					Non-boosted PI (N= 579)				
		SQV/r N = 187	LPV/r N = 410	AZV/r N = 139	Other PI/r N = 133	p-value*	NFV N = 345	IDV N = 109	AZV N = 84	Other PI N = 41	p-value*
Heart Rate											
	Mean	69.8	68.1	68.8	69.1	0.35	67.8	70.0	69.3	67.4	0.24
	SD	10.1	11.2	11.7	12.0		11.7	10.2	10.7	10.1	
	Median	69.0	66.0	68.0	69.0		67.0	69.0	68.0	64.0	
	IQR	63.0, 77.0	60.0, 75.0	59.0, 76.0	60.0, 77.0		59.0, 75.0	63.0, 76.0	62.0, 74.0	59.0, 75.0	
	Min - Max	49.0, 103.0	41.0, 105.0	43.0, 108.0	42.0, 100.0		41.0, 101.0	47.0, 95.0	50.0, 102.0	52.0, 93.0	
SDNN											
	Mean	24.1	26.8	26.0	22.9	0.16	26.5	21.8	25.2	23.7	0.03
	SD	15.7	21.4	20.3	18.7		19.6	15.6	19.6	17.3	
	Median	20.7	21.2	20.8	18.7		22.3	17.3	20.1	20.6	
	IQR	14.8, 28.7	11.9, 35.5	13.7, 35.3	10.0, 29.4		14.1, 32.0	11.1, 26.8	12.5, 29.2	12.3, 25.5	
	Min - Max	3.1, 95.8	1.0, 186.7	2.2, 165.8	2.5, 119.3		3.6, 153.3	2.6, 91.4	3.8, 115.6	5.6, 85.9	
rMSSD											
	Mean	28.0	29.0	27.6	23.9	0.03	29.0	23.3	28.4	24.2	0.05
	SD	19.1	25.3	27.7	18.5		25.3	18.9	22.6	15.5	
	Median	23.6	22.1	20.0	17.5		23.1	18.2	20.5	22.6	
	IQR	15.4, 34.9	12.9, 35.9	13.3, 34.1	10.4, 32.6		14.4, 34.8	12.9, 27.6	13.3, 38.2	13.1, 30.0	
	Min - Max	3.0, 119.1	1.3, 200.0	1.9, 256.9	2.7, 83.1		2.5, 276.6	2.5, 120.3	4.3, 124.4	7.0, 83.5	

*unadjusted p-value for omnibus F-test after log transformation of SDNN and rMSSD, p-value <0.01 is considered significant;
Abbreviations: SD, standard deviation; IQR,inter quartile range; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; rMSSD, the root mean square of the difference of successive RRs; SQV/r, LPV/r, ATV/r, and PI/r, saquinavir, lopinavir, atazanavir and other protease Inhibitors boosted with ritonavir; NFV, Nelfinavir ; IDV, Indinavir ; ATV, Atazanavir

Supplemental Table 3: Multivariable adjusted associations between participant characteristics and resting heart rate ~~variablity~~variability

	Heart Rate (bpm)		SDNN (log_{10} ms)		rMSSD (log_{10} ms)	
Factor*	**Difference (95% CI)**	**p-value****	**Difference (95% CI)**	**p-value****	**Difference (95% CI)**	**p-value****
ART regimen at Baseline						
Boosted PI	-2.149 (-3.14, -1.16)	<0.001	0.020 (-0.01, 0.05)	0.12	0.028 (0.00, 0.05)	0.04
Non-boosted PI	-2.808 (-3.90, -1.71)	<0.001	0.042 (0.01, 0.07)	0.004	0.048 (0.02, 0.08)	0.001
NNRTI - no PI	Ref.	--	Ref.	--	Ref.	--
Age (per year)	-0.054 (-0.10, -0.01)	0.02	-0.008 (-0.01, -0.01)	<0.001	-0.008 (-0.01, -0.01)	<0.001
Gender (*F vs. M*)	1.565 (0.61, 2.52)	0.001	0.018 (-0.01, 0.04)	0.15	0.054 (0.03, 0.08)	<0.001
Race						
Black (vs. White)	0.668 (-0.36, 1.69)	0.20	-0.005 (-0.03, 0.02)	0.74	0.032 (0.00, 0.06)	0.03
Asian (vs. White)	3.839 (1.87, 5.80)	<0.001	-0.042 (-0.09, 0.01)	0.10	-0.022 (-0.07, 0.03)	0.43
Other Races (vs. White)	0.777 (-0.49, 2.05)	0.23	-0.037 (-0.07, -0.00)	0.03	-0.022 (-0.06, 0.01)	0.20
Smoking Status						
Current (vs. Never)	0.736 (-0.23, 1.71)	0.14	-0.012 (-0.04, 0.01)	0.35	-0.013 (-0.04, 0.01)	0.34
Past (vs. Never)	-0.776 (-1.83, 0.28)	0.15	0.011 (-0.02, 0.04)	0.43	0.013 (-0.02, 0.04)	0.38
Total/HDL Cholesterol Ratio	0.560 (0.38, 0.74)	<0.001	-0.010 (-0.01, -0.00)	<0.001	-0.014 (-0.02, -0.01)	<0.001
Body mass index (kg/m^2)	0.206 (0.12, 0.29)	<0.001	-0.003 (-0.01, -0.00)	0.005	-0.004 (-0.01, -0.00)	<0.001
Diabetes *(Y vs. N)*	4.706 (3.09, 6.33)	<0.001	-0.082 (-0.12, -0.04)	<0.001	-0.102 (-0.15, -0.06)	<0.001
Prior CVD *(Y vs. N)*	-3.445 (-5.60, -1.29)	0.002	0.002 (-0.05, 0.06)	0.95	0.040 (-0.02, 0.10)	0.18
Use of BP-lowering drugs *(Y vs. N)*	1.111 (-0.03, 2.25)	0.06	-0.035 (-0.06, -0.01)	0.02	-0.041 (-0.07, -0.01)	0.01
Use of lipid lowering drugs *(Y vs. N)*	0.607 (-0.54, 1.76)	0.30	-0.018 (-0.05, 0.01)	0.22	-0.027 (-0.06, 0.00)	0.09
Baseline CD4 (per 100)	-0.012 (-0.17, 0.15)	0.88	0.003 (-0.00, 0.01)	0.16	0.003 (-0.00, 0.01)	0.22
Baseline HIV-RNA ≤ 400 *(Y vs. N)*	-0.897 (-2.03, 0.24)	0.12	-0.007 (-0.04, 0.02)	0.63	0.001 (-0.03, 0.03)	0.97
Time since first prescribed ART (per year)	0.157 (0.03, 0.28)	0.01	-0.003 (-0.01, -0.00)	0.03	-0.005 (-0.01, -0.00)	0.002
NRTI backbone regimen						
AZT+3TC (without ABC)	Ref.	--	Ref.	--	Ref.	--
TNF (without ABC)	-0.867 (-2.07, 0.33)	0.16	0.028 (-0.00, 0.06)	0.08	0.014 (-0.02, 0.05)	0.39
ABC (without TNF)	-0.052 (-1.32, 1.22)	0.94	0.004 (-0.03, 0.04)	0.81	0.006 (-0.03, 0.04)	0.75
3TC+d4T	0.506 (-0.72, 1.73)	0.42	-0.012 (-0.04, 0.02)	0.46	-0.018 (-0.05, 0.02)	0.29
Other NRTI regimens	0.295 (-1.04, 1.63)	0.66	0.001 (-0.03, 0.04)	0.96	-0.006 (-0.04, 0.03)	0.76

*All variables are included in the model in addition to ART use. Multivariable association of ART use with resting heart rate, SDNN and rMSSD are listed in Table 2 (Model 4).

**p-value <0.01 is considered significant.

Abbreviations 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; bpm, beats per minute; CVD, cardiovascular disease; D4T, stavudine; ms, millisecond; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor;
rMMSD, the root mean square of the difference of successive RRs; SD, standard deviation; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; TNF, tenofovir

Supplemental Table 1: Baseline characteristics comparing participants with SDNN/rMSSD data to those without

	Included N = 2998	Excluded N = 573
Age (in years)	44.3 (9.4)	45.0 (9.9)
Gender (% female)	853 (28.5%)	133 (23.2%)
Race		
Black	816 (27.2%)	167 (29.1%)
Asian	167 (5.6%)	26 (4.5%)
White	1632 (54.4%)	335 (58.5%)
Other	383 (12.8%)	45 (7.9%)
Smoking Status		
Current Smoker	1142 (38.1%)	228 (39.8%)
Past Smoker	770 (25.7%)	142 (24.8%)
Never Smoker	1086 (36.2%)	203 (35.4%)
Total Cholesterol (mg/dl)	200.9 (47.0)	201.2 (46.1)
LDL Cholesterol (mg/dl)	116.9 (35.4)	118.0 (37.5)
HDL Cholesterol (mg/dl)	44.2 (14.7)	46.3 (15.8)
Triglycerides (mg/dl)	230.8 (225.2)	209.3 (216.6)
Total/HDL Cholesterol	5.0 (2.3)	4.8 (2.2)
Body mass index (kg/m^2)	25.9 (5.3)	25.3 (5.1)
Heart Rate (bpm)	69.4 (11.4)	69.9 (11.6)
Prior CVD	117 (3.9%)	18 (3.1%)
Diabetes	219 (7.3%)	44 (7.7%)
BP lowering drugs	569 (19.0%)	110 (19.2%)
Lipid lowering drugs	527 (17.6%)	98 (17.1%)
Hepatitis B or C	480 (16.2%)	75 (13.4%)
Baseline CD4 (cells/mm^3)	680.2 (257.7)	665.4 (239.9)
HIV RNA (% ≤ 400 copies/mL)	2514 (84.0%)	487 (85.3%)
Time since first prescribed ART (in years)	6.2 (3.5)	6.4 (3.6)
Baseline NRTI regimen		
AZT+3TC (without ABC)	1221 (40.7%)	218 (38.0%)
TNF (without ABC)	521 (17.4%)	120 (20.9%)
ABC (without TNF)	431 (14.4%)	86 (15.0%)
3TC+D4T	452 (15.1%)	71 (12.4%)
Other NRTI containing regimens	373 (12.4%)	78 (13.6%)

Values expressed as mean (SD) or N (%)

Abbreviations 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BP, blood pressure; CVD, cardiovascular disease; D4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TNF, tenofovir

Supplemental Table 2: Distribution of heart rate variability measures across individual boosted and non-boosted protease inhibitors.

		Ritonavir Boosted PIs (N= 869)					Non-boosted PI (N= 579)				
		SQV/r N = 187	LPV/r N = 410	AZV/r N = 139	Other PI/r N = 133	p-value*	NFV N = 345	IDV N = 109	AZV N = 84	Other PI N = 41	p-value*
Heart Rate											
	Mean	69.8	68.1	68.8	69.1	0.35	67.8	70.0	69.3	67.4	0.24
	SD	10.1	11.2	11.7	12.0		11.7	10.2	10.7	10.1	
	Median	69.0	66.0	68.0	69.0		67.0	69.0	68.0	64.0	
	IQR	63.0, 77.0	60.0, 75.0	59.0, 76.0	60.0, 77.0		59.0, 75.0	63.0, 76.0	62.0, 74.0	59.0, 75.0	
	Min - Max	49.0, 103.0	41.0, 105.0	43.0, 108.0	42.0, 100.0		41.0, 101.0	47.0, 95.0	50.0, 102.0	52.0, 93.0	
SDNN											
	Mean	24.1	26.8	26.0	22.9	0.16	26.5	21.8	25.2	23.7	0.03
	SD	15.7	21.4	20.3	18.7		19.6	15.6	19.6	17.3	
	Median	20.7	21.2	20.8	18.7		22.3	17.3	20.1	20.6	
	IQR	14.8, 28.7	11.9, 35.5	13.7, 35.3	10.0, 29.4		14.1, 32.0	11.1, 26.8	12.5, 29.2	12.3, 25.5	
	Min - Max	3.1, 95.8	1.0, 186.7	2.2, 165.8	2.5, 119.3		3.6, 153.3	2.6, 91.4	3.8, 115.6	5.6, 85.9	
rMSSD											
	Mean	28.0	29.0	27.6	23.9	0.03	29.0	23.3	28.4	24.2	0.05
	SD	19.1	25.3	27.7	18.5		25.3	18.9	22.6	15.5	
	Median	23.6	22.1	20.0	17.5		23.1	18.2	20.5	22.6	
	IQR	15.4, 34.9	12.9, 35.9	13.3, 34.1	10.4, 32.6		14.4, 34.8	12.9, 27.6	13.3, 38.2	13.1, 30.0	
	Min - Max	3.0, 119.1	1.3, 200.0	1.9, 256.9	2.7, 83.1		2.5, 276.6	2.5, 120.3	4.3, 124.4	7.0, 83.5	

*unadjusted p-value for omnibus F-test after log transformation of SDNN and rMSSD, p-value <0.01 is considered significant;
Abbreviations: SD, standard deviation; IQR,inter quartile range; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; rMSSD, the root mean square of the difference of successive RRs; SQV/r, LPV/r, ATV/r, and PI/r, saquinavir, lopinavir, atazanavir and other protease Inhibitors boosted with ritonavir; NFV, Nelfinavir ; IDV, Indinavir ; ATV, Atazanavir

Supplemental Table 3: Multivariable adjusted associations between participant characteristics and resting heart rate variability

	Heart Rate (bpm)		SDNN (log_{10} ms)		rMSSD (log_{10} ms)	
Factor*	**Difference (95% CI)**	**p-value****	**Difference (95% CI)**	**p-value****	**Difference (95% CI)**	**p-value****
ART regimen at Baseline						
Boosted PI	-2.149 (-3.14, -1.16)	<0.001	0.020 (-0.01, 0.05)	0.12	0.028 (0.00, 0.05)	0.04
Non-boosted PI	-2.808 (-3.90, -1.71)	<0.001	0.042 (0.01, 0.07)	0.004	0.048 (0.02, 0.08)	0.001
NNRTI - no PI	Ref.	--	Ref.	--	Ref.	--
Age (per year)	-0.054 (-0.10, -0.01)	0.02	-0.008 (-0.01, -0.01)	<0.001	-0.008 (-0.01, -0.01)	<0.001
Gender (*F vs. M)*	1.565 (0.61, 2.52)	0.001	0.018 (-0.01, 0.04)	0.15	0.054 (0.03, 0.08)	<0.001
Race						
Black (vs. White)	0.668 (-0.36, 1.69)	0.20	-0.005 (-0.03, 0.02)	0.74	0.032 (0.00, 0.06)	0.03
Asian (vs. White)	3.839 (1.87, 5.80)	<0.001	-0.042 (-0.09, 0.01)	0.10	-0.022 (-0.07, 0.03)	0.43
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Smoking Status						
Current (vs. Never)	0.736 (-0.23, 1.71)	0.14	-0.012 (-0.04, 0.01)	0.35	-0.013 (-0.04, 0.01)	0.34
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Total/HDL Cholesterol Ratio	0.560 (0.38, 0.74)	<0.001	-0.010 (-0.01, -0.00)	<0.001	-0.014 (-0.02, -0.01)	<0.001
Body mass index (kg/m^2)	0.206 (0.12, 0.29)	<0.001	-0.003 (-0.01, -0.00)	0.005	-0.004 (-0.01, -0.00)	<0.001
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Prior CVD *(Y vs. N)*	-3.445 (-5.60, -1.29)	0.002	0.002 (-0.05, 0.06)	0.95	0.040 (-0.02, 0.10)	0.18
Use of BP-lowering drugs *(Y vs. N)*	1.111 (-0.03, 2.25)	0.06	-0.035 (-0.06, -0.01)	0.02	-0.041 (-0.07, -0.01)	0.01
Use of lipid lowering drugs *(Y vs. N)*	0.607 (-0.54, 1.76)	0.30	-0.018 (-0.05, 0.01)	0.22	-0.027 (-0.06, 0.00)	0.09
Baseline CD4 (per 100)	-0.012 (-0.17, 0.15)	0.88	0.003 (-0.00, 0.01)	0.16	0.003 (-0.00, 0.01)	0.22
Baseline HIV-RNA ≤ 400 *(Y vs. N)*	-0.897 (-2.03, 0.24)	0.12	-0.007 (-0.04, 0.02)	0.63	0.001 (-0.03, 0.03)	0.97
Time since first prescribed ART (per year)	0.157 (0.03, 0.28)	0.01	-0.003 (-0.01, -0.00)	0.03	-0.005 (-0.01, -0.00)	0.002
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ABC (without TNF)	-0.052 (-1.32, 1.22)	0.94	0.004 (-0.03, 0.04)	0.81	0.006 (-0.03, 0.04)	0.75
3TC+d4T	0.506 (-0.72, 1.73)	0.42	-0.012 (-0.04, 0.02)	0.46	-0.018 (-0.05, 0.02)	0.29
Other NRTI regimens	0.295 (-1.04, 1.63)	0.66	0.001 (-0.03, 0.04)	0.96	-0.006 (-0.04, 0.03)	0.76

*All variables are included in the model in addition to ART use. Multivariable association of ART use with resting heart rate, SDNN and rMSSD are listed in Table 2 (Model 4).

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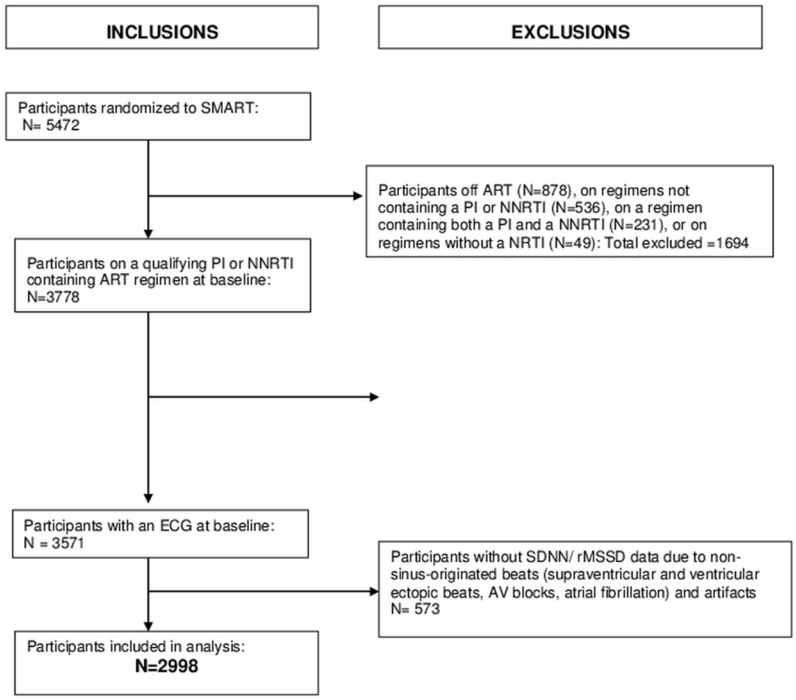
rMMSD, the root mean square of the difference of successive RRs; SD, standard deviation; SDNN, the standard deviation of all filtered RR intervals over the length of the recording; TNF, tenofovir

Figure 1 Study flow and inclusion and exclusion criteria



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