

Original Article

Cumulative effects of hypertriglyceridemia in HIV-infected patients switching from NNRTIs to PI-based antiretroviral therapyYu Zhang^{1,2}, Jiang Xiao¹, Wen Zhang¹, Ning Han¹, Di Yang¹, Wei Liu¹, Hui Zeng^{3,4}, Junyan Han^{3,4}, Hongxin Zhao^{1,2}¹ *Clinical and Research Center of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China*² *Department of Infectious Disease, Peking University Ditan Teaching Hospital, Beijing, China*³ *Institute of Infectious diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China*⁴ *Beijing Key Laboratory of Emerging Infectious Diseases, Beijing, China***Abstract**

Introduction: The objective of this study was to investigate changes in serum lipids among HIV-infected patients switching from non-nucleoside-reverse transcriptase inhibitors (NNRTI) to protease inhibitor (PI)-based highly active antiretroviral therapy (HAART), and to determine if changes of lipid profiles impacted the monocyte subsets recovery.

Methodology: Fifty-seven subjects who switched from NNRTIs to PI-based HAART (NNRTIs to PI group) and fifty-five subjects who initially started with PI-based HAART (initial PI group) were recruited. According to their baseline triglyceride (TG) levels, the NNRTIs to PI and initial PI groups were further divided into non-hypertriglyceridemia and hypertriglyceridemia subgroups, respectively. The effects of PI-based HAART on lipid profiles and monocyte subsets were analyzed.

Results: At 48 weeks, the TG changes in the NNRTIs to PI group was higher than that of the initial PI group. The increases of serum TG levels in the initial PI non-hypertriglyceridemia group was greater than that of the NNRTIs to PI non-hypertriglyceridemia group. For the hypertriglyceridemia group at baseline, significant increment in TG levels were observed in the NNRTIs to PI hypertriglyceridemia group. The percentages of circulating CD14^{high}CD16⁺ and CD14^{low}CD16⁺ subsets were elevated in the two groups. At 48 weeks, the proportion of CD14^{high}CD16⁺ monocytes declined gradually, and the proportion of CD14^{low}CD16⁺ monocytes decreased independently of the TG level.

Conclusions: For non-hypertriglyceridemia individuals at baseline, PI-based regimens increased the TG level in the initial PI group. For the NNRTIs to PI hypertriglyceridemia group, PI-based regimens reinforced HAART-related hypertriglyceridemia.

Key words: Non-nucleoside reverse transcriptase inhibitors; protease inhibitors; highly active antiretroviral therapy; hypertriglyceridemia.

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Introduction

The wide use of highly active antiretroviral therapy (HAART) among HIV-infected patients has been proven to decrease plasma HIV RNA viral load, increase peripheral blood CD4⁺ lymphocyte counts, and decrease morbidity and mortality [1,2]. However, with increased life expectancy, the benefits of HAART have been tempered by a growing concern for HAART-related adverse events, including metabolic abnormalities such as HAART-related dyslipidemia, which occurs in 70%–80% of HIV-infected individuals receiving HAART [3]. Studies have indicated a high rate of dyslipidemia in HIV-infected patients receiving either non-nucleoside-reverse transcriptase inhibitors (NNRTIs)-based or protease inhibitor (PI)-based HAART [4-6]. The elevated serum lipids are associated

with increased cardiovascular disease (CVD) and lower cognitive score [7-9].

HIV-associated hyperlipidemia can increase the risk of atherosclerosis, coronary artery disease, and has long-term effects on immune recovery [7,10-11]. It has been reported that the incidence of myocardial infarction was elevated with increasing length of exposure to anti-HIV drugs, which was partly explained by dyslipidemia [12].

Exposure to all PIs (especially ritonavir) was associated with hypertriglyceridemia, elevated concentrations of low-density lipoprotein (LDL), and reduced high-density lipoprotein (HDL) levels [3]. Furthermore, even though NNRTIs have been associated with the occurrence of dyslipidemia, abnormalities of plasma lipid levels appear to be

prevalent among patients receiving PI-based regimens [13,14]. Second-line PI-based regimens are recommended for HIV-infected patients failing first-line NNRTI treatments [15]. We previously reported that the prevalence of first-line ART failure was 3.39% in our cohort [16]. Another study showed the prevalence was 4.1% [17]. However, lipid changes in patients who switched to PI-based HAART after NNRTI-based HAART failure were not determined.

The lipid droplets appear to be essential for the regulation and modulation of immune responses, and have a vital role in antigen cross-presentation, interferon responses, production of inflammatory mediators, and pathogen clearance [18]. Monocytes/macrophages, as important target cells and reservoirs of actively replicating HIV virus, have been found to contribute to the progression of HIV/AIDS and the increased risk of noninfectious chronic complications such as cardiovascular and thromboembolic disease [19,20]. Monocytes are divided into three subsets: classic CD14^{high}CD16⁻, intermediate CD14^{high}CD16⁺, and nonclassical CD14^{low}CD16⁺ monocytes [21, 22]. In our previous study, elevated percentages of both CD14^{high}CD16⁺ and CD14^{low}CD16⁺ monocyte subsets were observed in HIV-infected HAART-naïve patients, and CD14^{high}CD16⁺ subsets were correlated with increased viral loads [23]. NNRTIs-based HAART only successfully reduced the percentages of CD14^{high}CD16⁺ monocytes, but did not return the percentages of CD14^{low}CD16⁺ monocytes to normal levels [23]. In addition, a failure of CD14^{low}CD16⁺ subset recovery was observed in patients with NNRTIs-based HAART-related hypertriglyceridemia at 48 weeks [24]. Thus, HAART-related hypertriglyceridemia altered the homeostasis of monocyte subsets during antiviral therapy, which might further affect immune reconstitution. Whether PI-based HAART has similar effects on lipid profiles and monocyte subsets was not fully understood.

Thus, in the present study, in order to explore whether there were undesirable changes in serum lipids among HIV-infected patients who switched from NNRTIs- to PI-based HAART, and whether these changes of lipid profiles impacted the monocyte subset recovery, we examined changes in serum lipid profile concentrations, including total cholesterol (TC), triglyceride (TG), LDL, and HDL levels in a retrospective cohort of patients, and monocyte subsets in a longitudinal cohort of patients undergoing therapy with PI-based HAART following NNRTIs-based HAART.

Methodology

Study subjects and treatments

This retrospective study consisted of HIV-infected patients from Beijing Ditan Hospital, Capital Medical University, recruited between July 2009 and December 2013. Profiles of 223 individuals were collected, including 157 patients who switched from NNRTIs-based to PI-based HAART (NNRTIs to PI group) and 66 subjects initially started with PI-based HAART (initial PI group). Among the 157 patients, 56 patients switched to PI-based HAART unrelated to treatment failure, and 25 patients had missing baseline data. Out of 76 remaining patients, 19 patients were excluded because they received NNRTIs- or PI-based HAART for less than 24 weeks. Thus, 57 patients were used to analyze the lipid profiles, and 19 patients were included to analyze the effects of HAART on monocyte subsets. Ten patients had missing baseline data and one patient received HAART less than 24 weeks in the PI-based HAART group. Therefore, there were 55 individuals that met the inclusion criteria in the PI-based HAART group and 16 out of those 55 (29%) patients were used for monocyte subset analyses. The selection scheme is shown in Supplementary Figure 1. After analyzing the changes of lipid profiles between the NNRTIs to PI and initial PI groups, we further divided the two groups into two subgroups; the non-hypertriglyceridemia (serum TG \leq 1.72 mmol/L) and hypertriglyceridemia (serum TG $>$ 1.72 mmol/L) groups, according to baseline serum concentrations of TG. Fifty healthy donors were recruited in the present study and written informed consent was obtained.

Among the NNRTIs to PI group ($n = 57$), the regimens before switching therapy were as follows: 63.2% of the patients underwent the AZT (zidovudine)/D4T (stavudine) + 3TC (lamivudine) + NVP (nevirapine) regimen, 33.4% of the patients underwent the AZT/D4T/TDF (tenofovir) + 3TC + EVP (efavirenz) regimen, and 0.4% of the patients underwent the IDV (indinavir) + 3TC + EFV regimen. In the NNRTIs to PI group, 86% of patients were switched to the Lopinavir/r (LPV, lopinavir/ritonavir, Kaletra) + 3TC + TDF regimen and 14% were switched to the Lopinavir/r (LPV, Kaletra) + 3TC + AZT regimen. Among the initial PI group ($n = 55$), 78.2% patients received Lopinavir/r (LPV, Kaletra) + 3TC+TDF, and 21.8% received Lopinavir/r (LPV, Kaletra) + 3TC + AZT regimens (Supplementary Table 1). In the NNRTIs to PI group, eight (14%) patients were treated with hypolipidemic agents (seven taking fenofibrate, one taking acipimox), while six (10.9%) patients in the initial PI group had taken fenofibrate.

Furthermore, when the above two groups were divided into subgroups, eight (23.5%), and five (21.7%) received hypolipidemic agents in the NNRTIs to PI hypertriglyceridemia group and the initial PI hypertriglyceridemia group, respectively; and 3.2% in the NNRTIs to PI non-hypertriglyceridemia and initial PI non-hypertriglyceridemia groups received hypolipidemic agents.

HIV-1 viral load and CD4⁺ T cell count

Plasma HIV-1 RNA level (viral load, VL) was measured using the Standard Amplicor HIV Monitor assay, version 1.5 (Roche Diagnostics, Indianapolis, IN, USA) using RT-PCR. CD4⁺ T cell counts were determined using a standard flow cytometry technique with a Trucount™ tube assay (BD Biosciences, San Jose, CA, USA).

Measurement of serum lipid profiles and definitions of dyslipidemia

Plasma total cholesterol (TC), TG (triglyceride), HDL-c (high-density lipoprotein cholesterol), and LDL-c (low-density lipoprotein cholesterol) were measured at weeks 0 (baseline), 24, and 48 after HAART using the Hitachi 7600 Automatic Biochemical Analyzer (Hitachi, San Jose, CA, USA). We defined hyperlipidemia and divided the groups into two subgroups according to the ATP III Guidelines of National Cholesterol Education Program (NCEP) criteria: TC ≤ 5.18 mmol/L, TG > 1.72 mmol/L.

Immunophenotypic characterization of monocytes

Whole blood from HIV-negative and HIV-positive subjects was collected for the monocyte subsets analyses by flow cytometry according to a previous study [23]. The samples were performed within 4 hours. After red blood cell lysis, the following monoclonal antibodies were used for analyses: anti-human CD14, -CD16, and -CD45 (BD Biosciences). Matched isotype antibodies were used as negative controls. Samples were analyzed on a FACS Calibur (BD Biosciences) using CellQuest™ software (BD Biosciences).

Statistical analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA). Data were presented as the mean + standard deviation (SD) for continuous variables and for categorical variables such as numbers and percentages. The possible differences between two groups were analyzed using the independent *t*-test (parametric) or Mann-Whitney U test (nonparametric). For more than two-group analyses, one-way ANOVA (parametric) or Kruskal-Wallis (nonparametric) followed by post-hoc tests (S-N-K method) were performed. Cross-tabulations were conducted to compare the incidence rate of hypertriglyceridemia between the two groups, using Pearson's chi-squared (χ^2) tests. Statistical significance was set at a level of $p < 0.05$.

Results

Demographic characteristics

The NNRTIs to PI group (n = 57) and initial PI group (n = 55) patients were enrolled in the present study. The demographic characteristics of the study groups are included in Table 1. There was no difference between the groups in terms of age, sex, and absolute CD4⁺ T cell counts (Table 1). After 48 weeks of treatment, CD4⁺ T cell counts were increased and the virologic suppression was obtained in the NNRTIs to PI and initial PI groups (Supplementary Figure 2). There was no difference between the two groups in CD4⁺ T cell counts, virologic suppression rate, or the use of hypolipidemic agents.

The lipid profiles at baseline and the effects of PI-based HAART on lipid profiles

HAART, especially PI-based regimens related to dyslipidemia, were highly prevalent and were associated with advanced disease in HIV-infected patients. In our study, at baseline (0 weeks), the TG levels in the NNRTIs to PI group were higher than that of the initial PI group ($p = 0.027$). There were no differences in the TC, HDL-c, and LDL-c levels between the two groups (Supplementary Table 2). The

Table 1. Characterization of studied groups.

	Initial PI (n=55)	NNRTIs to PI (n=57)	<i>p</i> value
Age (years)	37.49 ± 8.53	38.45 ± 7.84	0.508
Sex, n (%)			
Male	48 (87.3)	53 (93)	0.055
Female	7 (12.7)	4 (7)	
Absolute CD4 ⁺ T count (cells/μL)	213 ± 163	259 ± 128	0.313
Viral load (log ₁₀ copies/mL)	4.45 ± 1.166	3.86 ± 0.9087	0.016

Baseline characteristics of HIV-positive patients before beginning therapy with a protease inhibitor (PI). Data are presented as n (%) for sex, and the mean ± SD for all others. Initial PI group: the patients were initially started with PI-based HAART; NNRTIs to PI group: the patients who switched from NNRTIs-based to PI-based HAART. HAART, highly active antiretroviral therapy; NNRTIs, non-nucleoside-reverse transcriptase inhibitors.

proportion of patients with higher TG levels (> 1.72 mmol/L) in the NNRTIs to PI and the initial PI group was 43.6% and 59.6%, respectively. Notably, the difference between the initial PI and NNRTIs to PI groups at baseline was only found in serum TG levels. In addition, the patients in the NNRTIs to PI group had higher TG levels before receiving PI-based regimens.

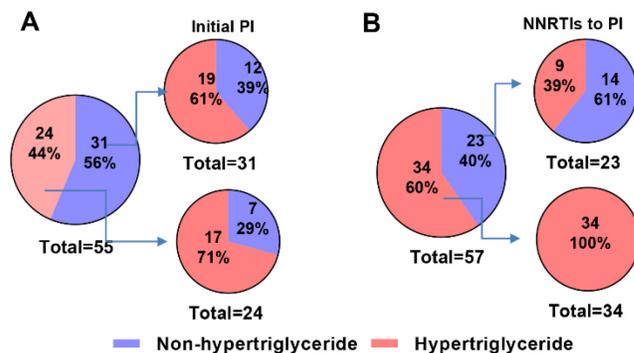
In order to explore the effects of switching the NNRTIs- to PI-based regimens on lipid profiles, we measured the serum lipids, including TC, TG, HDL-c, and LDL-c at 0, 24, and 48 weeks after PI-based HAART. There was no difference between or within groups at 24 or 48 weeks in terms of TC and LDL-c levels (Supplementary Table 2 and Figure 1). An increment ($p < 0.05$) in TG was observed in the initial PI group as well as in the NNRTIs to PI group at 48 weeks. Moreover, the TG level in the NNRTIs to PI group was significantly higher than that of the initial PI group (4.11 ± 4.30 vs. 2.51 ± 1.67 , $p = 0.029$) (Supplementary Table 2, Figure 1). Interestingly, HDL-c levels at 48 weeks were significantly higher than baseline in the initial PI group, while it was unchanged in the NNRTIs to PI group. Thus, PI-based HAART led to higher TG levels in both the initial PI and NNRTIs to PI groups, and PI-based HAART exacerbated NNRTIs-related hypertriglyceridemia.

The effects of PI-based regimens on TG levels in non-hypertriglyceridemia groups at baseline

In order to limit the influence of baseline TG levels, both the initial PI and NNRTIs to PI groups were further divided into two subgroups; the non-hypertriglyceridemia ($TG \leq 1.72$ mmol/L) and hypertriglyceridemia ($TG > 1.72$ mmol/L) subgroups according to the baseline TG levels. The demographic characteristics of these groups are displayed in

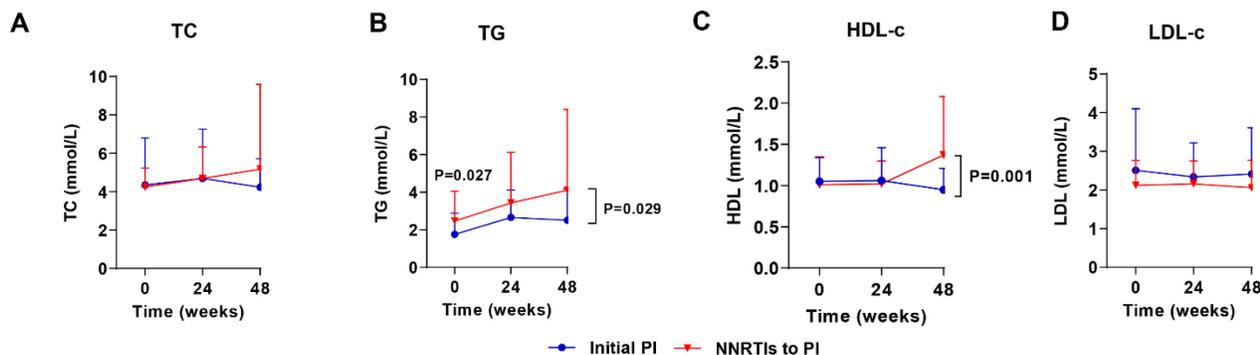
Supplementary Table 3. At 48 weeks, in the initial PI non-hypertriglyceridemia group, 19 of 31 patients (61.29%) were hypertriglyceridemia while 9 of 23 patients (39.13%) were hypertriglyceridemic in the NNRTIs to PI non-hypertriglyceridemia group. However, no significant differences in incidence rates of hypertriglyceridemia were observed (Supplementary Table 4, Figure 2). At 24 weeks, the TG levels were significantly elevated compared to baseline in both the non-hypertriglyceridemia groups. At 48 weeks, the TG levels remained high in the initial PI non-hypertriglyceridemia group, while TG levels returned to normal in the NNRTIs to PI non-hypertriglyceridemia group (Supplementary Table 4, Figure 3). Moreover, the amplification of serum TG

Figure 2. The incidence rates of hypertriglyceridemia at 48 weeks in the different groups.



Both initial PI and NNRTIs to PI groups were divided into two subgroups, non-hypertriglyceridemia ($TG \leq 1.72$ mmol/L) and hypertriglyceridemia ($TG > 1.72$ mmol/L) groups, respectively, according to the baseline TG levels. At 48 weeks, the incidence rates of hypertriglyceridemia ($TG > 1.72$ mmol/L) were calculated. PI, protease inhibitor; NNRTIs, non-nucleoside-reverse transcriptase inhibitors; TG, triglyceride.

Figure 1. The effect of PI-based HAART on lipid profiles among study groups.



(A) Plasma total cholesterol (TC), (B) triglyceride (TG), (C) high-density lipoprotein (HDL-c), and (D) low-density lipoprotein (LDL-c) of the two groups at 0, 24, and 48 weeks after initiation of PI-based HAART. Significant p values between groups are shown. Comparisons were performed using two-sample t-tests.

levels in the initial PI non-hypertriglyceridemia group (Δ TG48w: 1.53 ± 1.48 mmol/L) was greater than that of the NNRTIs to PI non-hypertriglyceridemia group (Δ TG48w: 0.51 ± 0.68 mmol/L) ($p = 0.005$) (Supplementary Table 5, Figure 3). Thus, for non-hypertriglyceridemia individuals at baseline, the PI-based regimens were more effective on TG levels in the initial PI group.

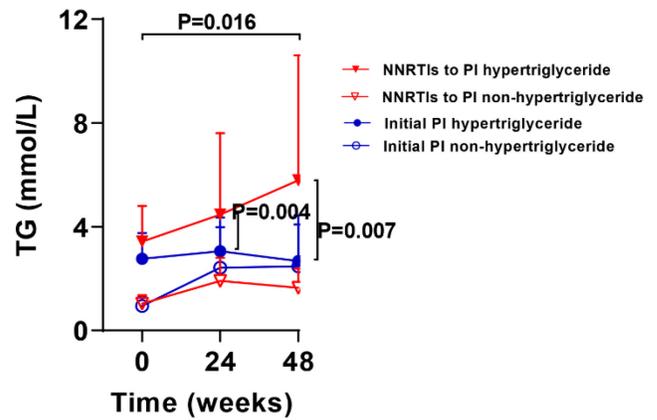
PI-based regimens reinforced NNRTIs-related hypertriglyceridemia

For the hypertriglyceridemia group at baseline, the levels of serum TGs were always high (≥ 2.27 mmol/L) in the initial PI hypertriglyceridemia group, and we did not find differences in TG levels at baseline, 24 weeks, and 48 weeks (Supplementary Table 5, Figure 3). Strikingly, significant increments ($p < 0.05$) in TG levels were observed in the NNRTIs to PI hypertriglyceridemia group (Supplementary Table 5, Figure 3). In this group, the concentration of TG at 48 weeks reached high levels (5.79 ± 4.82 mmol/L, TG ≥ 5.67 mmol/L), which were higher than that at baseline ($p = 0.016$). The TG levels at 48 weeks of NNRTIs to PI were significantly higher than that of the initial PI group ($p = 0.007$) (Figure 3). Thus, PI-based regimens further reinforced NNRTIs-related hypertriglyceridemia.

CD14^{high}CD16⁺ and CD14^{low}CD16⁺ monocyte subsets were elevated in the NNRTIs to PI and initial PI groups

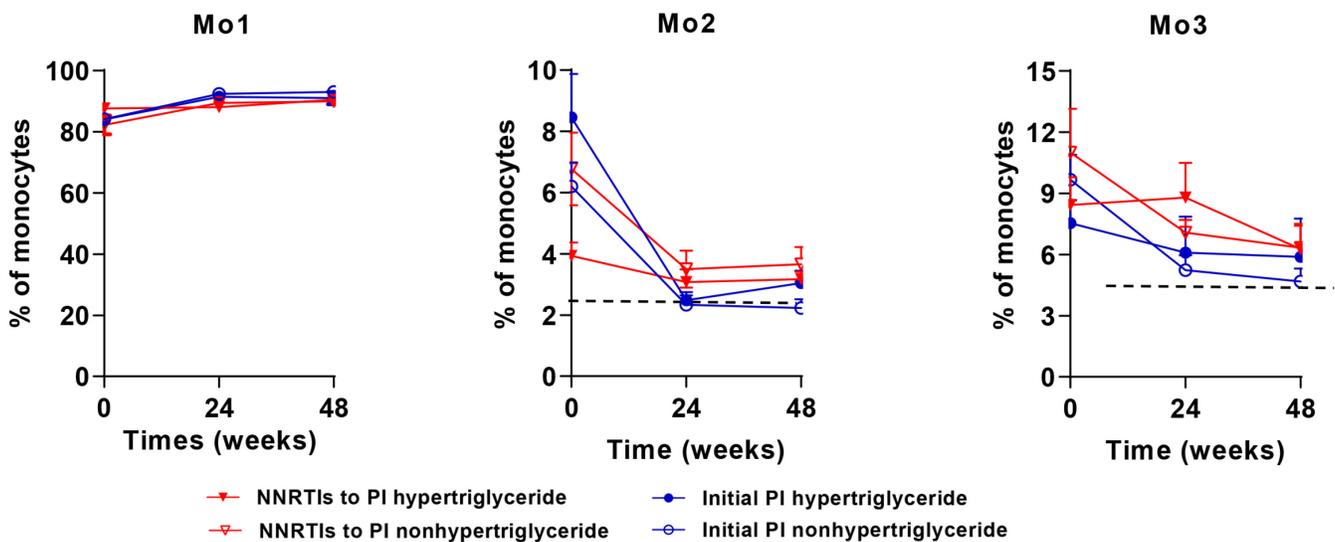
Monocyte subsets play important roles in HIV-infected patients and HAART-related dyslipidemia. Monocytes were divided into three monocyte subsets: CD14^{high}CD16⁻, CD14^{high}CD16⁺, and CD14^{low}CD16⁺ subsets as assessed by flow cytometry in our study

Figure 3. PI-based regimens reinforced NNRTIs HAART-related hypertriglyceridemia in the NNRTIs to PI hypertriglyceridemia groups.



The serum TG level of initial PI non-hypertriglyceridemia (n = 31), initial PI hypertriglyceridemia (n = 24), NNRTIs to PI non-hypertriglyceridemia (n = 23), and NNRTIs to PI hypertriglyceridemia group (n = 34), at 0, 24, 48 weeks. TG, triglyceride; PI, protease inhibitor; NNRTIs, non-nucleoside-reverse transcriptase inhibitors; HAART, highly active antiretroviral therapy.

Figure 4. The changes of monocyte subsets after 48 weeks of receiving PI-based HAART.



The changes of monocyte subsets (percentage) of the initial PI non-hypertriglyceridemia (n = 13), initial PI hypertriglyceridemia (n = 6), NNRTIs to PI non-hypertriglyceridemia (n = 9), and NNRTIs to PI hypertriglyceridemia groups (n = 7) were analyzed in a longitudinal study by flow cytometry during regular follow-ups at 0, 24, and 48 weeks. PI, protease inhibitor; NNRTIs, non-nucleoside-reverse transcriptase inhibitors.

(Supplementary Figure 3A). Consistent with the results of our previous studies [23], the percentages of CD14^{high}CD16⁺ and CD14^{low}CD16⁺ subsets were elevated in the initial PI group. The percentage of the CD14^{high}CD16⁺ subset in the NNRTIs to PI group remained high (Supplementary Figure 3B).

The PI-based regimens restored the monocyte subset independent of TG levels

NNRTIs-based HAART has been proven to restore the proportion of CD14^{high}CD16⁺ monocytes, whereas CD14^{low}CD16⁺ monocytes did not return to normal levels due to hypertriglyceridemia [23, 24]. To determine whether PI-based HAART recovered monocyte subsets and the effects of TG levels, the kinetic changes of monocyte subsets were analyzed in a longitudinal study. As shown in Figure 4, during regular follow-up at 0, 24, and 48 weeks, the proportion of circulating CD14^{high}CD16⁺ monocytes declined gradually and almost returned to the level observed in healthy controls at 48 weeks (Figure 4). Meanwhile, the proportion of CD14^{low}CD16⁺ monocytes decreased slightly despite the level of TG. These results showed that PI-based HAART totally restored the CD14^{high}CD16⁺ subset and partially restored the CD14^{low}CD16⁺ subset, and this effect was independent of the serum TG levels.

Discussion

HAART-related dyslipidemia is associated with increased CVD and cognitive decline [7-9]. Previous studies have proven that PI-based therapy had deleterious effects on lipid metabolism [13]. In the present study, we explored the changes of lipid profiles of the patients who switched from NNRTIs- to PI-based HAART after NNRTIs-based HAART failure. We found that the NNRTIs to PI group had higher TG levels than that of the initial PI group at baseline. More importantly, the patients with elevated TG levels before switching to PI-based HAART (NNRTIs to PI hypertriglyceridemia group) had even higher TG levels than that of the patients that initially started with PI-based treatment, even though eight patients had received hypolipidemic agents. These data suggested that patients with increased TG levels after NNRTIs-based HAART might be more prone to maintaining hypertriglyceridemia. In other words, PI-based HAART had a cumulative effect on hypertriglyceridemia and exacerbated the development of hypertriglyceridemia in patients switching from NNRTIs to PI-based HAART.

Regarding the lipid profile and the type of dyslipidemia, HAART had more effects on the TG levels and the incidence of hypertriglyceridemia in children and adults [25-28]. Consistent with the previous studies, hypertriglyceridemia was the main type of HAART- or HIV-related dyslipidemia, and the proportion of patients with hypertriglyceridemia in the NNRTIs to PI group at baseline was 59.6%. Although triglycerides do not accumulate in foam cells, triglyceride-rich lipoproteins can be directly taken up by macrophages without modification [29]. Triglycerides are related to remnant lipoproteins with more atherogenic potential [30]. High triglyceride levels lead to higher cholesteryl ester transfer protein activity, which in turn, gives rise to decreased levels of HDL particles and increased levels of LDL particles [31], and hypertriglyceridemia potentially accelerates the risk for CVD in the HIV-infected patients [32]. In addition, triglycerides were shown to generate a host of proinflammatory mediators and to cause inflammation [33], which was associated with the effects of HAART therapy.

The differences between the initial PI and NNRTIs to PI groups at baseline were only found in serum TG levels. In addition, during regular follow-up, there was no difference between or within groups at 24 or 48 weeks in terms of TC and LDL-c, and the increment of TG levels were significant. Thus, we explored the effects of PI-based HAART on TG levels and hypertriglyceridemia, in this study.

The kinetic change and increment of TG levels in the NNRTIs to PI group and the initial PI group were distinct. Similar to a previous report on a HIV-therapy naïve cohort [34], we found that, at baseline, initial PI patients already had abnormal TG concentrations compared to normal levels. The previous study showed that PI-based HAART increased TG concentrations, which may in turn increase the risk for CVD [35]. In our study, the TG levels of the initial PI group were significantly increased at 48 weeks of follow-up, and 61% of the patients in the initial PI non-hypertriglyceridemia groups at baseline developed hypertriglyceridemia after 48 weeks of PI-based HAART. However, for the patients in the initial PI hypertriglyceridemia groups at baseline, the TG level did not continue to increase. Thus, for HIV-infected initial PI patients, the prevention of hypertriglyceridemia was necessary for patients in the initial PI non-hypertriglyceridemia groups at baseline.

However, the effect of PI-based HAART on lipid profile in the NNRTIs to PI group was not clear. At baseline, the NNRTIs to PI group had higher TG but

normal HDL levels at baseline due to application of first-line treatments [5,36-37]. At 48 weeks of PI-based HAART, the TG level of the NNRTIs to PI non-hypertriglyceridemia group increased modestly. In contrast, the patients with elevated TG levels before switching to PI-based HAART (NNRTIs to PI hypertriglyceridemia group) had even higher TG concentrations than that of the initial PI group after 48 weeks of PI-based treatment. The changes of TG levels in the initial PI and NNRTIs to PI non-hypertriglyceridemia groups were significantly different, although there was no significant difference in incidence rate of hypertriglyceridemia, perhaps due to the small sample size. These data suggested that patients with high TG levels after NNRTIs-based HAART may be more prone to elevated TG levels. However, the post-NNRTIs-based HAART patients with normal TG levels tended to maintain normal TG levels after switching to PI-based HAART. Therefore, more consideration should be paid to the patients who have elevated TG levels before changing therapy regimens, and hypolipidemic agents should be used as early as possible. Considering the differences in the use of hypolipidemic agents, sex, age, or VL between the NNRTIs to PI and initial PI hypertriglyceridemia groups, and prolonged treatment times, the additional effects of different types of HAART [38, 39], and individual sensitivity might contribute to the cumulative effects of NNRTIs- to PI-based HAART regimens on hypertriglyceridemia.

For virological failure patients, PI-based HAART has been proven to significantly raise CD4⁺ T counts and suppress viral replication, in our study. The results agreed with previous data showing that PI-based HAART increased the CD4 cell count and achieved effective virologic suppression. In addition, no significant differences with respect to age, sex, CD4 count, and VL were observed for the groups studied, indicating that these factors did not significantly change the lipid profile, in the present study.

As target cells and reservoirs of HIV virus, activated monocytes may contribute to inflammation and cardiovascular disease. Monocyte heterogeneity provides the distinct functions of monocyte subsets. The classic CD14⁺CD16⁻ monocytes present antigen, the inflammatory CD14^{high}CD16⁺ monocytes produce high levels of inflammatory cytokines, and the patrolling CD14^{low}CD16⁺ monocytes home to the vascular endothelium and recognize viral products [21].

As we have described in the previous study [23], the elevated percentages of both CD14^{high}CD16⁺ and CD14^{low}CD16⁺ monocyte subsets were shown not only

in HIV-infected HAART-naïve patients (initial PI group), but also in the NNRTIs to PI group. NNRTIs-based HAART decreased the percentages of both CD14^{high}CD16⁺ and CD14^{low}CD16⁺ monocyte subsets in virological suppression patients. At baseline, the higher percentages of both CD14^{high}CD16⁺ and CD14^{low}CD16⁺ monocyte subsets were observed in the NNRTIs to PI group, although these patients had received NNRTIs-based HAART for more than 24 weeks. Thus, the higher percentage of CD14^{high}CD16⁺ and CD14^{low}CD16⁺ monocyte subsets might indicate treatment failure. During regular follow-up, PI-based HAART decreased the percentage of CD14^{high}CD16⁺ monocyte and CD14^{low}CD16⁺ monocyte subsets. Previously, NNRTIs-based HAART-related hypertriglyceridemia was associated with failure of recovery of CD14^{low}CD16⁺ monocyte subsets in AIDS patients [24]. Rosuvastatin, a lipid-lowering agent, was reported to decrease the proportions of tissue factor-positive patrolling CD14^{low}CD16⁺ monocytes and lowered markers of monocyte activation, although this effect was independent of the lipid-lowering effect [40]. The effect of PI-based HAART-related hypertriglyceridemia on CD14^{low}CD16⁺ monocyte recovery was different from that of NNRTIs-based HAART, and therefore a different mechanism could be involved. However, further study is needed to determine the mechanism.

It is challenging to collect an adequate number of samples to meet a criterion and acquire the comprehensive clinical profiles since the present study is a retrospective study. Our study was limited by the small sample size. However, according to previous studies, 112 patients in the present study are sufficient for the present study.

Conclusions

In conclusion, PI-based HAART exacerbated the development of hypertriglyceridemia in the patients switching from NNRTIs- to PI-based HAART with higher levels of TGs, and the recovery of monocyte subsets was independent of serum TG levels. The NNRTIs to PI patients with normal TG levels may be inclined to maintain normal levels of TGs. The results indicated that high TG levels among patients switching from NNRTIs- to PI-based HAART may be associated with individual sensitivity or individual hereditary factors. This study provided information regarding lipid levels and hypolipidemic agent use among HIV-infected patients during HAART in order to decrease cardiovascular risk factors and improve the quality of life among HIV-infected patients.

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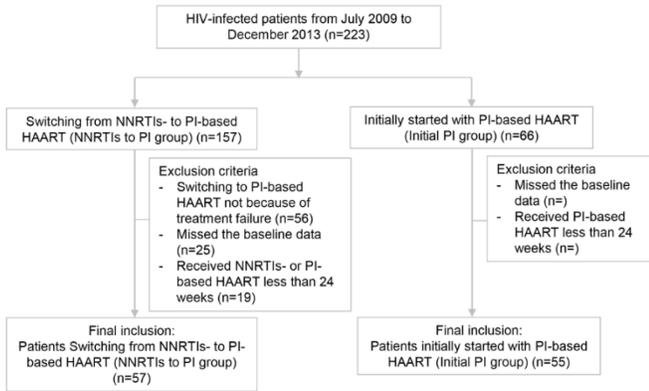
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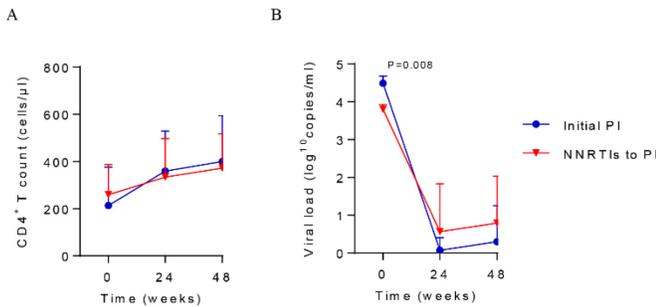
Annex – Supplementary Items

Supplementary Figure 1. Patient selection scheme used in our study.



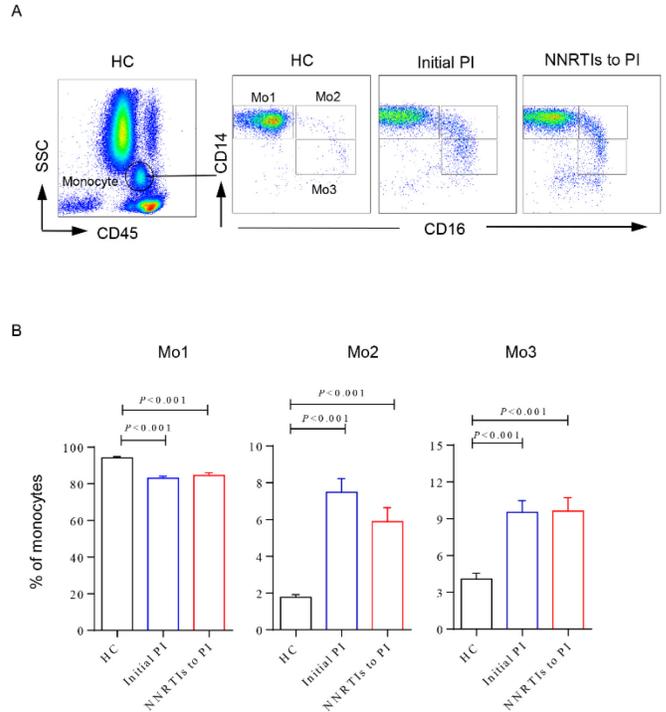
Subject enrollment and sequential application of inclusion and exclusion criteria used to define the study population. NNRTIs, non-nucleoside-reverse transcriptase inhibitors; PI, protease inhibitor; HAART, highly active antiretroviral therapy.

Supplementary Figure 2. Enrichment of CD4⁺ T cell count and successful suppression of virus replication after antiretroviral therapy.



Absolute CD4⁺ T-cell counts (A) and plasma HIV Viral loads (B) of NNRTIs to PI group and initial PI group at 0, 24 and 48 weeks after initiation of PI-based HAART. NNRTIs, non-nucleoside-reverse transcriptase inhibitors; PI, protease inhibitor; HAART, highly active antiretroviral therapy.

Supplementary Figure 3. The percentage of monocyte subsets in peripheral blood before PI-based HAART.



(A) Gating of monocyte subsets. Peripheral blood monocytes were divided into three subsets (CD14^{high}CD16⁻, CD14^{high}CD16⁺, and CD14^{low}CD16⁺); (B) The percentages of the three monocyte subsets (CD14^{high}CD16⁻, CD14^{high}CD16⁺, and CD14^{low}CD16⁺) among healthy donors (HC) (n = 50), NNRTIs to PI (n = 16), and initial PI groups (n = 19) were analyzed by flow cytometry within 4 hours. Data are shown as the mean ± SEM of indicated subjects. The data were analyzed using the one-way ANOVA test. NNRTIs, non-nucleoside-reverse transcriptase inhibitors; PI, protease inhibitor; HAART, highly active antiretroviral therapy.

Supplementary Table 1. Characterization of treatments.

HAART Agent	Abbreviation	Dose
NNRTI		
Efavirenz	EFV	600mg, once a day
Nevirapine	NVP	200mg, twice a day
PI		
Lopinavir/Ritonavir	LPV/r	LPV: 400mg, RTV: 100mg, twice a day
NRTI		
Zidovudine	AZT	300mg, twice a day
Stavudine	D4T	Weight ≥ 60kg, 40mg; Weight < 60kg, 30mg, twice a day
Lamivudine	3TC	150mg, twice a day
Tenofovir disoproxil	TDF	300mg, once a day
Indinavir	IDV	800mg, three times a day

Supplementary Table 2. Changes of lipids profiles in the study groups.

weeks	TC, mmol/L			TG, mmol/L			HDL-c, mmol/L			LDL-c, mmol/L		
	Initial PI (n = 55)	NNRTIs to PI (n = 57)	Value <i>p</i> ^b	Initial PI (n = 55)	NNRTIs to PI (n = 57) ^c	Value <i>p</i> ^b	Initial PI (n = 55) ^c	NNRTIs to PI (n = 57) ^c	Value <i>p</i> ^b	Initial PI (n = 55)	NNRTIs to PI (n = 57)	Value <i>p</i> ^b
0	4.35 ± 2.45	4.24 ± 0.99	0.743	1.75 ± 1.13	2.46 ± 1.59	0.027	1.01 ± 0.34	1.05 ± 0.29	0.469	2.51 ± 1.59	2.12 ± 0.64	0.094
24	4.96 ± 2.57	4.69 ± 1.64	0.509	2.65 ± 1.47**	3.43 ± 2.69*	0.153	1.02 ± 0.28	1.06 ± 0.40	0.715	2.34 ± 0.88	2.16 ± 0.59	0.232
48	4.23 ± 1.48	5.17 ± 4.42	0.167	2.51 ± 1.67**	4.11 ± 4.30**	0.029	1.37 ± 0.71***	0.95 ± 0.26	0.001	2.41 ± 1.20	2.06 ± 0.71	0.130
<i>p</i> ^a	0.201	0.212		0.005						0.801	0.705	

TC: total cholesterol; TG: triglycerides; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; NNRTIs: non-nucleoside-reverse transcriptase inhibitors; PI: protease inhibitor. ^a*p* is from ANOVA tests comparing the three time points within each cohort. ^b*p* is from two-sample t-tests comparing two cohorts at each time point. ^cANOVA not applicable. *Significant differences compare with baseline TG, **P* < 0.05; ***P* < 0.01; ****P* < 0.001

Supplementary Table 3. Characterization of studied subgroups.

	Initial PI non-hypertriglyceridemia (n = 31)	NNRTIs to PI non-hypertriglyceridemia (n = 23)	Initial PI hypertriglyceridemia (n = 24)	NNRTIs to PI hypertriglyceridemia (n = 34)	<i>p</i>
Age (years)	37.30 ± 8.171	38.43 ± 7.959	37.92 ± 9.500	37.65 ± 8.079	0.084
Sex, n (%)					
Male	27 (87.1)	21 (91.3)	20 (83.3)	32 (94.1)	0.5778
Female	4 (12.9)	2 (8.7)	4 (16.7)	2 (5.9)	
Absolute CD4 count (cells/μL)	177.4 ± 97.59	283.8 ± 180.7	251.3 ± 208.2	222.3 ± 102.1	0.2559
Viral load (log ₁₀ copies/mL)	4.451 ± 1.120	3.709 ± 0.9770	4.446 ± 1.265	3.992 ± 0.8343	0.0717

NNRTIs, non-nucleoside-reverse transcriptase inhibitors; PI, protease inhibitor. *p* values were obtained by one-way analysis of variance. (ANOVA; Kruskal–Wallis test) followed by post-hoc tests (S–N–K method).

Supplementary Table 4. Incidence rate of hypertriglyceridemia after 48 weeks PI-based HAART in two non-hypertriglyceridemia groups at baseline.

Group	Non-Hypertriglyceridemia	Hypertriglyceridemia	Total	IR (%)
Initial PI	12	19	31	61.29
NNRTIs to PI	14	9	23	39.13
Total	26	28	54	

IR: incidence rate; NNRTIs: non-nucleoside-reverse transcriptase inhibitors; PI: protease inhibitor; HAART: highly active antiretroviral therapy.

Supplementary Table 5. Changes of TG in the non-hypertriglyceridemia and hypertriglyceridemia group after PI-based treatment.

Weeks	non-hypertriglyceridemia			hypertriglyceridemia		
	Initial PI (n = 31)	NNRTIs to PI (n = 23)	<i>p</i> value	Initial PI (n = 24)	NNRTIs to PI (n = 34)	<i>p</i> value
0	0.94 ± 0.33	1.03 ± 0.34	0.339	2.71 ± 0.99	3.43 ± 1.36	0.111
24	2.42 ± 1.56***	1.90 ± 0.89***	0.193	2.96 ± 1.35	4.47 ± 3.13	0.040
48	2.47 ± 1.62***	1.64 ± 0.74**	0.042	2.58 ± 1.76	5.79 ± 4.82*	0.007

TG: triglyceride; NNRTIs: non-nucleoside-reverse transcriptase inhibitors; PI: protease inhibitor.