Predictors of CD4 cell count response to first-line combination antiretroviral treatment among HIV-positive adult patients from the Asia-Pacific region.

Workplace Project Portfolio

Masters in Biostatistics

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January 2016

Preface

Student's role

This project was completed between August 2015 and January 2016 with the Biostatistics and Databases Program at the Kirby Institute, UNSW Australia.

This project utilized observational data on HIV-positive patients that was previously collected for the TREAT Asia HIV Observational Databases Low Intensity Transfer (TAHOD-LITE). The study objective was to evaluate the predictors associated with CD4 cell count changes from combination antiretroviral treatment initiation for those who had sought care in 2010-13. The intention was to determine whether certain treatment related and demographic factors could contribute to improved CD4 cell count restoration. This was a useful preliminary analysis for a subsequent research studies.

I completed this project independently and sought statistical advice from my supervisor, Matthew Law, when required. My tasks included concept development, data management and extraction, creating STATA and SAS programs for statistical analyses and writing of the report.

Reflections on Learning

Communication Skills

This project was helpful in developing my communication skills between internal and external staff as well as concisely and clearly communicating the clinical significance and relevance of the project findings. Prior to the analysis, I discussed the project objectives, use of the data and whether the findings would be published with staff within my department as well as with staff from the clinical sites in the Asia-Pacific region that provided the data.

This project challenged me to clearly communicate statistical results that were reflective of the accuracy and reliability of the models produced. Additionally, I had to ensure that the findings could be easily translated into a clinically meaningful conclusion.

Work Planning

Prior to this project, I had completed two studies that utilized TAHOD-LITE data and, as such, much of the data collection and literature review was complete. As I was involved with most of the data transfer for the patient database, I was aware of the strengths and limitations of the data which allowed me to develop a feasible concept proposal. Once complete, the

concept proposal was approved by Dr Patrick Kelly, the unit coordinator, and the principal investigators from each of the clinical sites. After approval, I began to retrieve the patients that satisfied the eligibility criteria for the study and begin the analysis. The initial results were discussed with my statistics supervisor and approved to allow me to subsequently write the report.

Statistical Principles, Methods & Computing

This project examined the predictors of the mean CD4 cell count at 12 months from combination antiretroviral treatment initiation with the use of a multivariate linear regression model. Further, it required me to check the model assumptions were met and that the model provided a reasonable fit of the data.

Several units in the BCA program assisted me in completing this project, mainly being linear models and regression (LMR) and, data management and statistical computing (DMC). All of the analysis was completed in STATA v14 and SAS v9.4 programs. This experience taught me the importance of keeping organised and consistent file records to allow me to easily move between steps in the analysis.

Teamwork

My role as a student with the Biostatistics and Databases Program requires me to collaborate with several staff within the program but also with a collaborative network of clinical sites and a coordinating center site in the Asia-Pacific region. In the initial stages of this project, I communicated with the network to inform and agree on the concept for this project. However, during the statistical analysis, I worked independently on my project and consulted with my statistics supervisor, Matthew Law, when required.

Ethical Considerations

All ethical approvals for the data used from TAHOD-LITE had previously been obtained from Institutional Review Boards (IRBs) at each participating clinical site, the University of New South Wales, and the coordinating center at TREAT Asia/amfAR. Written informed consent for the collection of patient data was not obtained unless required by the site-specific IRBs.

Preface

Location and dates:	The Kirby Institute, UNSW Australia, Sydney, NSW August 2015 – January 2016
Context:	This project used observational data on HIV-positive patients that was previously collected and stored at the Biostatistics and Databases Program at the Kirby Institute. The intention was to determine whether certain factors could contribute to improved CD4 cell count restoration for HIV-positive patients undertaking care in the Asia-Pacific region.
Student Contribution:	Nicole completed all the data collection and management, literature review, study concept proposal, statistical methods and analyses, writing of the report and presentation of results.
Statistical issues involved:	• Multivariate linear regression

Declaration:	I declare this project is evidence of my own work, with direction and assistance provided by my project supervisor and has not been submitted for previous academic credit.
Student Name:	Nicole L. De La Mata
Student Signature:	

Supervisor Statement:	Nicole has completed all the work presented in this project. She has selected and applied the appropriate statistical methods involved in this analysis. Nicole has shown ample ability in completing a complex analysis and addressing any challenges that arose from using a large observational database. She has been a dedicated student who has worked independently to produce this substantial amount of work.
Supervisor Name:	Professor Matthew G. Law
Supervisor Signature:	

Introduction

The introduction of combination antiretroviral treatment (ART) has had substantial impact on the human immunodeficiency virus (HIV) epidemic [1, 2]. Patients responding to ART have reduced HIV viral replication and lower rates of HIV-associated morbidity, mortality and AIDS-related events [3-5]. Recommendations on when to initiate ART is largely based upon the CD4 T lymphocyte cell count [6-10]. The CD4 T lymphocyte cell is generally regarded as an indicator of immune deficiency and used as a prognostic marker of HIV disease progression [11-13].

Early World Health Organisation (WHO) guidelines recommended ART initiation when patients were at an advanced stage of HIV or in asymptomatic stages of HIV with a CD4 cell count below 200cells/μL [6]. However, recent research has suggested that initiating ART at higher CD4 cell count levels has greater benefits in preventing further disease progression, further HIV transmission and reduced occurrence of opportunistic infections [14-17]. Although ideal, earlier ART initiation has been difficult to implement, particularly in low-income or resource-limited countries, as it requires patients to access health care facilities to receive HIV testing and diagnosis at early stages of disease progression [18, 19].

The Asia Pacific region retains a heavy burden of the HIV epidemic, with close to 5 million people living with HIV in 2013 [20, 21]. Despite an increasing push towards earlier initiation of ART, barriers to receiving care have contributed to the inability of many HIV-positive patients, within the Asia-Pacific region, to be diagnosed and initiate ART at higher CD4 cell count levels [22, 23]. In 2012, UNAIDS estimates for the Asia-Pacific region indicated that the number of people accessing ART has increased yet, the treatment coverage rate remains lower than the global average at 51% and a majority of people living with HIV are not diagnosed [21]. Patients presenting with CD4 cell counts <200 cells/μL require longer periods of time to fully restore CD4 cell count levels which increases there susceptibility to treatment failure and death [23]. Other factors, such as previous exposure to mono/duo therapy and older age, are also known to hinder CD4 restoration [24, 25].

The study objective is to examine the CD4 cell count response to first-line ART among HIV-positive patients from the TREAT Asia HIV Observational database low intensity transfer (TAHOD-LITE). Factors associated with mean CD4 cell count at 12 months will also be evaluated. Study findings will be useful for guiding decision making in future programs addressing the HIV-epidemic in the Asia-Pacific region.

Methods

Data collection and Participants

The TREAT Asia HIV Observational database (TAHOD) is a collaboration of 21 HIV treatment clinics across the Asia-Pacific region. TAHOD collects detailed data on a subset of HIV-positive patients that present at the clinics. The TREAT Asia HIV Observational database Low Intensity Transfer (TAHOD-LITE) is a sub-study of TAHOD and currently involves eight clinical sites from the Asia-Pacific region, including Cambodia, Hong Kong, India, Indonesia, Singapore and Vietnam. TAHOD-LITE collects data on all patients that are seen at the clinical site from a certain time point and, to date, includes over 30 000 adult HIV-positive patients. TAHOD-LITE began collecting retrospective data in 2014 with most sites contributing patient data from 2003 through to May 2014. Some sites could only contribute data from the following years: 2006 (Singapore); 2004 (Cambodia); 2010 (Vietnam).

Patient data includes: demographics (most recent clinic visit, gender, date of birth, mode of HIV exposure, HIV diagnosis date, date of death and death reason); hepatitis serology (HBV surface antigen test result and date, and HCV antibody test result and date); HIV-related biomarkers (CD4 and CD8 T lymphocyte counts, HIV viral load counts and date of results); ART history (antiretroviral drugs undertaken, and dates when initiated and ceased).

Patient data is routinely collected in clinical care, and then anonymized before being electronically transferred to the Kirby Institute. Ethical approvals for TAHOD-LITE were obtained from Institutional Review Boards (IRB) at each of the clinical sites, the University of New South Wales and the coordinating center at TREAT Asia/amfAR. Written informed consent for collection of study data is not obtained unless required by the site-specific IRBs.

First-line ART initiation is considered as the first combination regimen consisting of three or more ARV drugs. This analysis included all patients who had initiated a first-line ART regimen from 01 January 2010 to 31 December 2013, aged ≥18 years at first clinic visit and had at least one subsequent clinic visit after ART initiation. The primary endpoint was CD4 cell count at 12 months from ART initiation. The secondary endpoints include the patient demographics at ART initiation and the CD4 cell count every 6 months from ART initiation up to 24 months.

Statistical methods

There were three main objectives. The first objective was to summarize the patient demographics by country and overall. The second objective was to describe the median CD4 cell count up to 24 months from ART initiation for each country and overall. The third objective was to identify the predictors that were associated with CD4 cell count at 12 months from ART initiation.

First, descriptive statistics were used to summarize the patient demographics by country and overall, including age at ART initiation, sex, mode of HIV exposure, pre-ART CD4 cell count and pre-ART HIV viral load, first-line ART regimen, previous mono/dual therapy, HBV and HCV co-infection ever. The pre-ART CD4 cell count and pre-ART HIV viral load was defined as the CD4 cell count or HIV viral load closest to, and within 6 months prior to, first-line ART initiation.

Second, the median CD4 cell count, with interquartile range, was determined every 6 months, up to 24 months, from first-line ART initiation for each country and overall. The CD4 cell count at a given time point was defined as the CD4 cell count closest to, and within 3 +/- months of, the given time point.

Third, linear regression models were used to examine the predictors associated with CD4 cell count at 12 months from ART initiation, adjusted for clinical site. In this approach, the regression model is fitted as:

$$\hat{Y}_i = \beta_0 + \beta_1 X_i + \beta_2 Z_i + \sum_{j=3}^{p} \beta_j W_i + \varepsilon_i$$

Where $\hat{Y}_i$ is the predicted CD4 cell count at 12 months from ART initiation, X_i is the pre-ART CD4 cell count, Z_i is the dummy variable that indexes the eight clinical sites and β_j is the subsequent coefficient estimates for the remaining p covariates included in the final regression model. The random error, ε_i, are assumed to be independently and identically distributed with a normal distribution of mean zero and variance σ^2 [26]. Under this model, the slope intercept is allowed to vary for each of the clinical sites, while sharing the same slope.

Several assumptions were required to be met to use linear regression model appropriately. These included:

1. Linear relationship between the baseline and follow-up CD4 cell counts.
2. Linear relationship between follow-up CD4 cell counts and continuous predictors.
3. No interaction between the baseline variable and grouping variable (that is, clinical site).
4. Normality of error distribution and constant, linear error variance.
5. Limited collinearity.

The predictors, chosen *a priori*, included all the patient demographic variables previously mentioned. Prior to model selection, the association between the predictors and the CD4 cell count at 12 months was explored. Scatterplots with lowess curves superimposed were used to examine the linear association between the outcome, CD4 cell count at 12 months, and the continuous predictors. Categorical predictors were explored with the use of dot plots.

Initially, linear regression models were performed for each of the predictors, adjusting for clinical site and pre-ART CD4 cell count. Only those covariates that were significantly associated with the outcome in these models were subsequently selected for the first multivariate model.

Assessing collinearity

Added variable plots and the variance inflation factor (VIF) were used to evaluate collinearity between the covariates in the first-order multivariate model. Added variable plots are graphical displays that allow us to investigate the partial correlation between the outcome and a given covariate, while adjusting for other covariates [27]. The VIF is a measure of how much of the variance of the *i*th independent variable is not related to the other independent variables. The VIF is given by: $1/(1-R_i^2)$ where R_i^2 represents the squared multiple correlation for the regression of the *i*th independent variable on the other independent variables. In general, larger VIF values indicate the presence of collinearity issues [28].

Assessing highly influential data points

Influential points were evaluated to ensure that the model provided a good fit of the data. We used leverage and delta-beta statistic (DFBETA) values to assess the presence of influential points that would impact upon the regression model fit. The leverage h_{ii}, is the diagonal elements of the hat matrix, H. H is given by:

$$\mathbf{H} = \mathbf{X}(\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'$$

Where **X** is the covariate matrix containing X_{ik} elements with *i* indicating the case and *j* indicating the covariate. The leverage is a measure of the distance between the X value for the *i*th observation and the mean X value for all *n* observations [29]. A high leverage does not necessarily mean that the data point will be influential to the coefficient estimates. By plotting the leverage against the squared normalized residuals we are able to evaluate outliers that have the potential to affect the fit of the model. The *i*th squared normalized residual is given by:

$$a_i^2 = \frac{e_i}{\sqrt{\mathbf{e}^{\mathrm{T}}\mathbf{e}}}$$

Where e_i the ith residual ($e_i = Y_i - \hat{Y}_i$), and **e** is the residual matrix with $\mathbf{e} = (\mathbf{I} - \mathbf{H})\mathbf{Y}$ [29, 30].

The DFBETA is given by:

$$DFBETA_i = \frac{b_1 - b_{1(-i)}}{s(b_1)}$$

Where b_1 is the estimated slope coefficient for all observations, $b_{1(-i)}$ is the estimated slope coefficient when observation *i* is omitted and $s(b_1)$ is the estimated standard error of the estimated slope coefficient for all observations [31]. The DFBETA value indicates the degree to which the estimated slope would change if observation *i* were omitted.

Final model selection

The final multivariate model was selected using a backwards stepwise approach to reduce the model to a set of statistically significant ($2p < 0.05$) covariates. The backwards stepwise selection process begins with all the potential predictors included in the model and obtaining the p-value results for each of the predictors. If any predictor has a p-value above the pre-specified threshold, then they are removed from the model until a reduced model is produced. Previously removed predictors are then re-added to the reduced model, one step at a time, to verify that the predictor still produces a p-value below the given threshold [29, 32].

A normal probability plot was used to evaluate the normality of the residuals in the final multivariate model. The residuals were also plotted by the predicted values to assess the constancy and linearity of the residual variance as well as to identify any potential outlying points.

All statistical analyses were performed using Stata version 14 (Stata Corporation, College Station, Texas, USA) and SAS/STAT software (Version 9.4 of the SAS system for Windows).

Results

Patient characteristics

A total of 7976 patients had initiated an ART regimen between 1st January 2010 and 31st December 2013. Subsequently, 136 patients were excluded as they were aged <18 years. The remaining 7840 patients had at least one subsequent clinic visit after ART initiation and were eligible to be included in the analysis.

Overall, the majority of patients were male (66%) and had heterosexual contact as the mode of HIV exposure (73%). The median age at ART initiation was 36 years (Interquartile Range, IQR: 30-43). A majority of patients had not received a HIV viral load test prior to ART initiation (72%), while most had received a CD4 cell count prior to ART initiation (86%). Approximately 94% of patients had initiated a first-line ART regimen consisting of nucleoside reverse transcriptase inhibitors (NRTIs) and a nonnucleoside reverse transcriptase inhibitor (NNRTI) and about 2% had previous mono/dual therapy (Table 1). By country analysis showed some differences in patient demographics (Appendix 1).

Table 1. Summary of patient characteristics at ART initiation.

	n (%)
Total	7840
Age at ART initiation (years)	
≤30	2,087 (27)
31-40	3,343 (43)
41-50	1,555 (20)
51+	855 (11)
Median [IQR]	36 [30, 43]
Sex	
Male	5,183 (66)
Female	2,657 (34)
Mode of HIV Exposure	
Heterosexual contact	5748 (73)
Homosexual contact	784 (10)
Injecting drug use	571 (7)
Other/Unknown	737 (9)
Pre-ART HIV viral load (copies/mL)	
≤100000	1073 (14)
>100000	1154 (15)
Not tested	5613 (72)
Median [IQR]	110 564 [30 563, 402 000]

Pre-ART CD4 cell count (cells/µL)	
≤50	1,620 (21)
51-100	833 (11)
101-200	1,307 (17)
>200	2,983 (38)
Not tested	1,097 (14)
Median [IQR]	172 [53, 286]
First ART regimen	
NRTI+NNRTI	7,381 (94)
NRTI+PI	381 (5)
Other	78 (1)
Previous mono/dual therapy	
No	7,680 (98)
Yes	160 (2)
HBV Co-infection	
Negative	4,702 (60)
Positive	473 (6)
Not tested	2,665 (34)
HCV Co-infection	
Negative	4,093 (52)
Positive	776 (10)
Not tested	2,971 (38)

CD4 cell count response 24 months from ART initiation

Of the 7840 patients, 1090 (14%) did not have a CD4 cell count within 6 months prior to ART initiation. Across all countries, there was an increasing trend in the median CD4 cell count from ART initiation, from 172 cells/µL (IQR: 53-286 cells/µL) at ART initiation to 399 cells/µL (IQR: 252-546 cells/µL) at 24 months from ART initiation (Figure 1). This increasing trend was also present when stratified by country (Figure 2). Additionally, the number of patients receiving a CD4 cell count test every 6 months decreased as time from ART initiation increased.

Figure 1. Median CD4 cell count (cells/µL) from ART initiation, with interquartile range shown and total patients with CD4 tests at each time point.

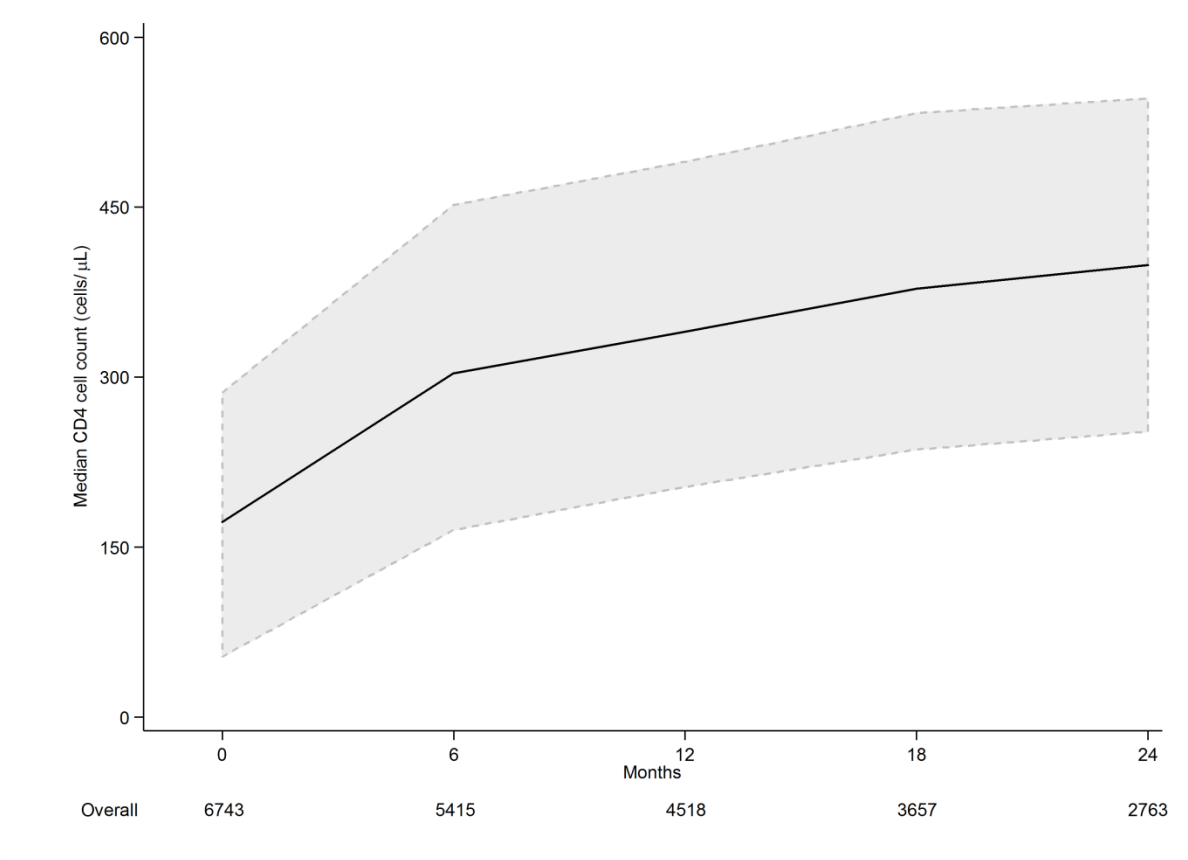
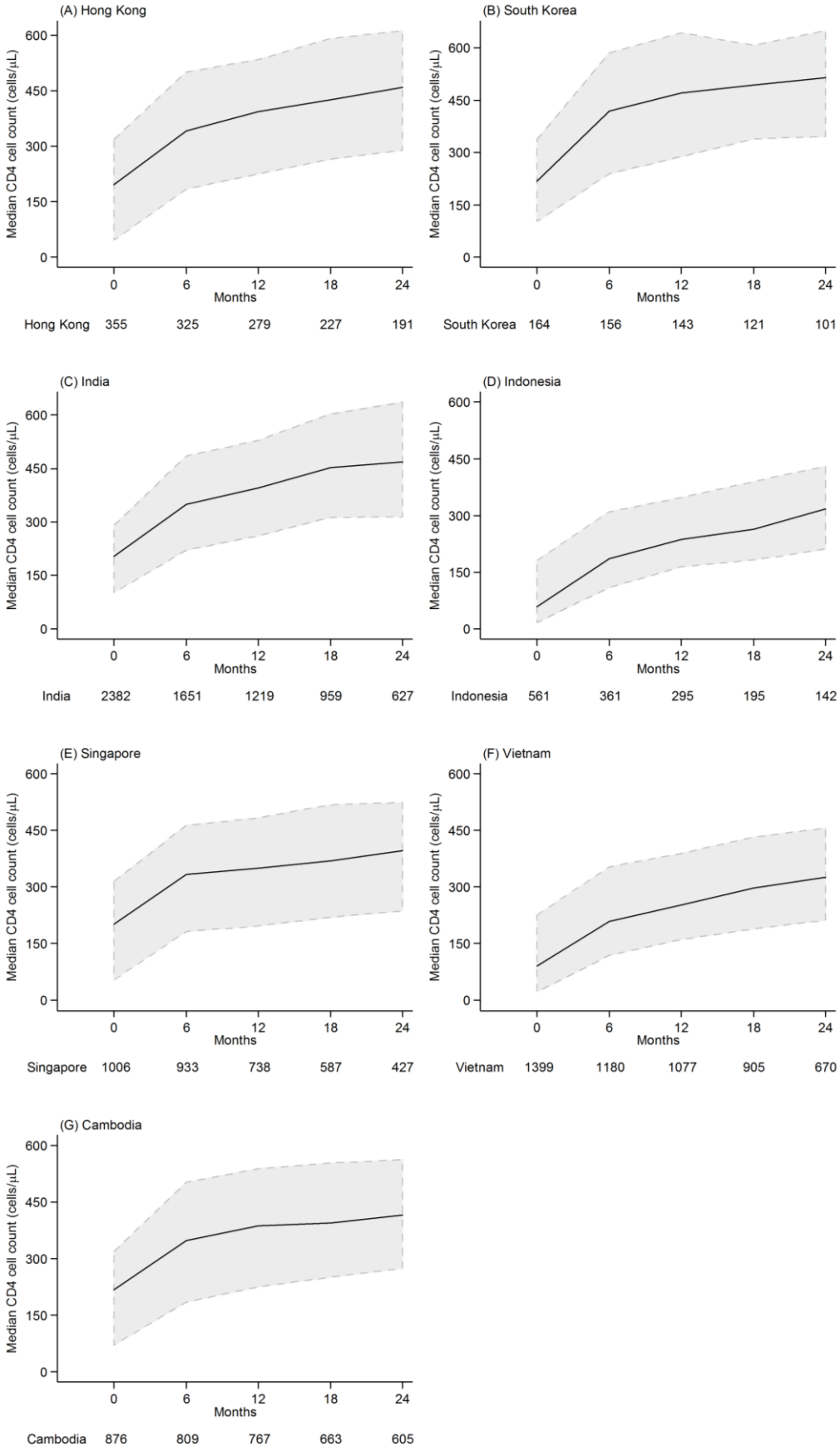


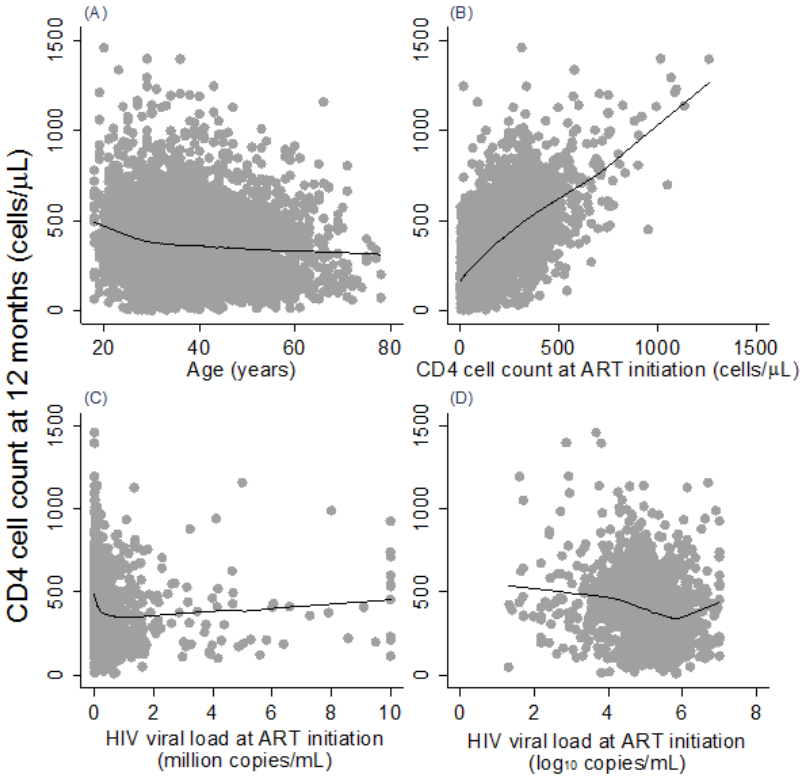
Figure 2. Median CD4 cell count (cells/μL) from ART initiation by country, with interquartile range shown and total patients with CD4 tests at each time point.



Exploring linear association of predictors

There were four continuous predictors selected to be potentially included in the linear regression model being age at ART initiation, CD4 cell count (cells/µL) at ART initiation and HIV viral load (copies/mL) at ART initiation. Initial scatterplots showed a clear linear association between the age (years) at ART initiation and the outcome, CD4 cell count at 12 months as well as the CD4 cell count at ART initiation and the outcome (Figure 3A- Figure 3B). The association between the outcome and HIV viral load at ART initiation was not monotonic or linear (Figure 3C). Log transformation of the HIV viral load did not rectify to show a linear association with the outcome (Figure 3D). As a linear, monotonic association to the outcome could not be shown, HIV viral load at ART initiation was not selected for subsequent linear regression models.

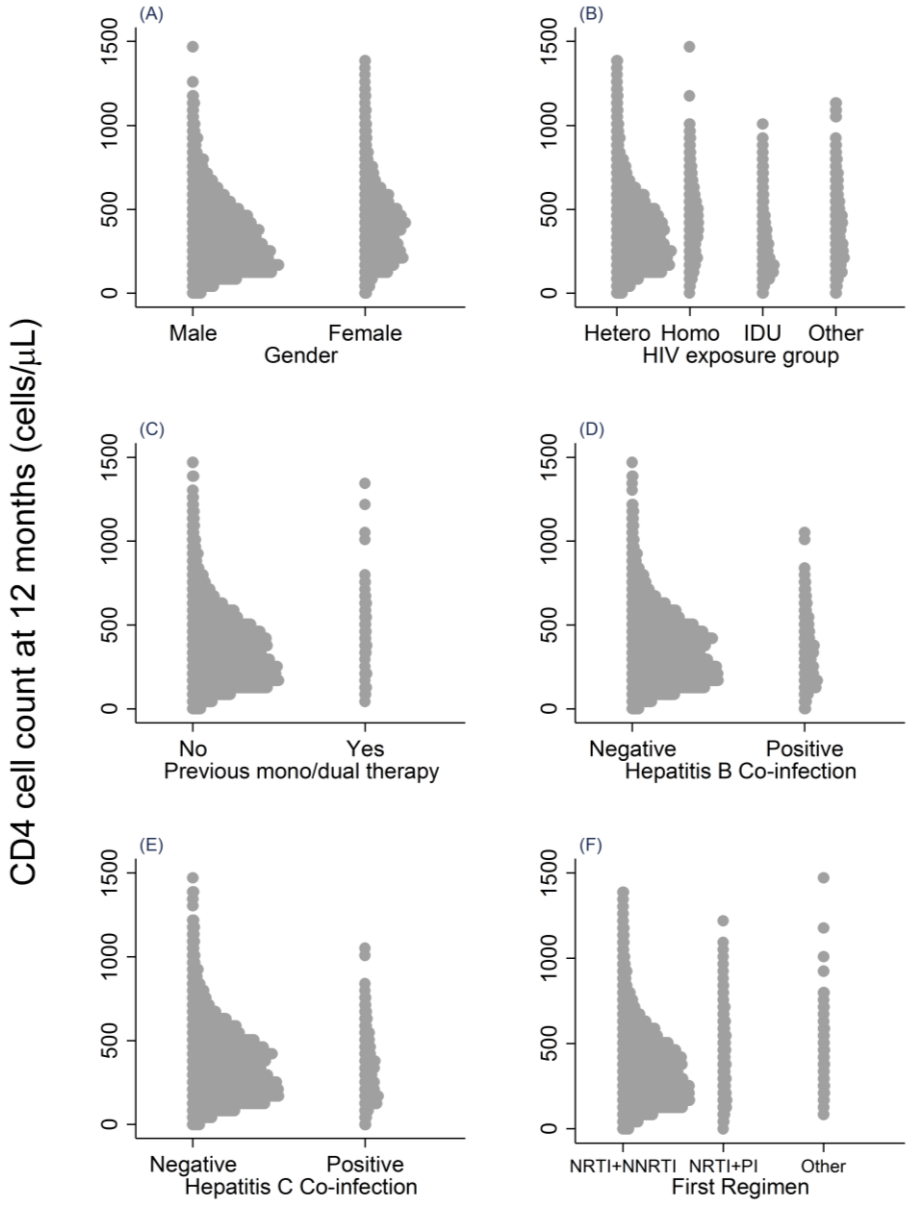
Figure 3. Scatterplot of the continuous covariates against the CD4 cell count at 12 months from ART initiation. *(A) Age (years) at ART initiation. (B) CD4 cell count (cells/µL) at ART initiation. (C) HIV viral load (million copies/mL) at ART initiation. (D) HIV viral load (log_{10}) copies/mL) at ART initiation.*



There were six categorical predictors selected to be potentially included in the linear regression model which were gender, HIV exposure group, previous mono/dual therapy, HBV co-infection, HCV co-infection and first ART regimen. The distributions for all the categorical variables showed a relatively normal distribution with most observations having a

CD4 cell count at 12 months ranging less than 1000 cells/µL (Figure 4A-Figure 4F). There was some skewing as there were some observations with a CD4 cell count greater than 1000 cells/µL. However, the sizes of the groups are large enough to reasonably assume the normality assumption holds, even though the distributions appear to somewhat deviate from a normal distribution [33].

Figure 4. Dot plots of the CD4 cell count at 12 months from ART initiation for each of the categorical variables. *(A) Gender. (B) HIV exposure group: hetero = heterosexual contact; homo = homosexual contact; IDU = injecting drug use. (C) Previous mono/dual therapy. (D) Hepatitis B co-infection (ever). (E) Hepatitis C co-infection (ever). (F) First ART regimen: NRTI= nucleoside reverse transcriptase inhibitor; NNRTI=nonnucleoside reverse transcriptase inhibitor; PI=protease inhibitor.*



Univariate linear regression models

All univariate models were adjusted for clinical site and CD4 cell count at ART initiation (per 100 cells/μL) and included all the predictors previously mentioned, except for HIV viral load at ART initiation. All univariate models were significantly associated with CD4 cell count at 12 months, except for HBV co-infection (p=0.220), first ART regimen (p=0.190) and previous mono/dual therapy (p=0.331) (Table 2). Therefore, first multivariate linear regression model (Model 1) only included the significant predictors: age at ART initiation, gender, HIV exposure group, HCV co-infection and CD4 cell count at ART initiation.

Clinical site was included in all univariate models to account for the patient differences that arise between the clinical sites. In the univariate model, adjusting for CD4 cell count at ART initiation, all coefficient estimates for each clinical site were significant, except for India (p value = 0.398). The mean change in CD4 cell count at 12 months from ART initiation for each site, with Hong Kong as the reference level, is given in Table 2.

Table 2. Univariate models for outcome, CD4 cell count at 12 months from ART initiation, and respective covariates, adjusting for CD4 cell count at ART initiation and clinical site.

	Mean Diff.	(95% CI)	p value
CD4 cell count at ART initiation (per 100 cells/μL)[a]	89.1	(86.0, 92.2)	<0.001
Age (per 10 years)[b]	-11.5	(-16.1, -6.8)	<0.001
Female[b]	27.3	(16.9, 37.7)	<0.001
HIV exposure[b]			<0.001[c]
Heterosexual	ref		
Homosexual	23.5	(6.3, 40.8)	<0.001
IDU	-40.9	(-60.1, -21.8)	<0.001
Other	-11.2	(-27.5, 5.1)	0.177
HBV Co-infection (ever)[b]			0.220[c]
Negative	ref		
Positive	-10.5	(-28.3, 7.4)	0.249
Not tested	-13.2	(-26.2, -0.1)	0.048
HCV Co-infection (ever)[b]			<0.001[c]
Negative	ref		
Positive	-33.6	(-50.1, -17.2)	<0.001
Not tested	-12.2	(-25.5, 1.0)	0.070
First regimen[b]			0.190[c]
NRTI+NNRTI	ref		
NRTI+PI	-7.4	(-30.1, 15.3)	0.524
Other	37.7	(-9.3, 84.8)	0.116

Previous mono/dual therapy[b]	16.3	(-16.6, 49.3)	0.331
Clinical site[b]			<0.001[c]
Hong Kong	ref		
South Korea	78.1	(47.5, 108.7)	<0.001
India	8.5	(-11.3, 28.3)	0.398
Singapore	-23.6	(-44.4, -2.8)	0.026
Indonesia	-28.3	(-54.3, -2.2)	0.033
Cambodia	-23.6	(-44.3, -2.9)	0.025
Vietnam 1	-45.4	(-66.8, -24.0)	<0.001
Vietnam 2	-53.9	(-76.9, -30.8)	<0.001

[a]Adjusting for clinical site.
[b]Adjusting for CD4 cell count at ART initiation and clinical site.
[c] Global p value from the Wald test for heterogeneity.

Assessing collinearity in Model 1

As expected, there was a very strong positive partial correlation between CD4 cell count at ART initiation and at 12 months from ART initiation (Figure 5A). There was also a good partial correlation between the outcome CD4 cell count and age (Figure 5B), and gender (Figure 5C). The partial correlation between the outcome CD4 cell count and the HIV exposure groups, and HCV co-infection was less prominent (Figure 5D-Figure 5G). The added variable plot clearly illustrates that once accounting for the other significant predictors, HIV exposure of IDU or other, and HCV co-infection have very little predictive association with the outcome CD4 cell count. Additionally, the VIF values also remained quite low for all the predictors suggesting that collinearity is not be an issue (Table 3).

Table 3. VIF values for each of the predictors included in Model 1.

Predictor	**VIF**	**1/VIF**	**Predictor**	**VIF**	**1/VIF**
CD4 cell count at ART initiation (cells/μL)	1.15	0.87	Clinical site		
Age at ART initiation (years)	1.22	0.82	Hong Kong	ref	
Female gender	1.40	0.71	South Korea	1.46	0.68
HIV exposure group			India	2.60	0.39
Heterosexual	ref		Singapore	2.88	0.35
Homosexual Contact	1.81	0.55	Indonesia	1.42	0.71
IDU	1.88	0.53	Cambodia	3.43	0.29
Other	1.16	0.86	Vietnam 1	3.70	0.27
HCV co-infection	1.87	0.54	Vietnam 2	2.98	0.34
Mean VIF			2.07		

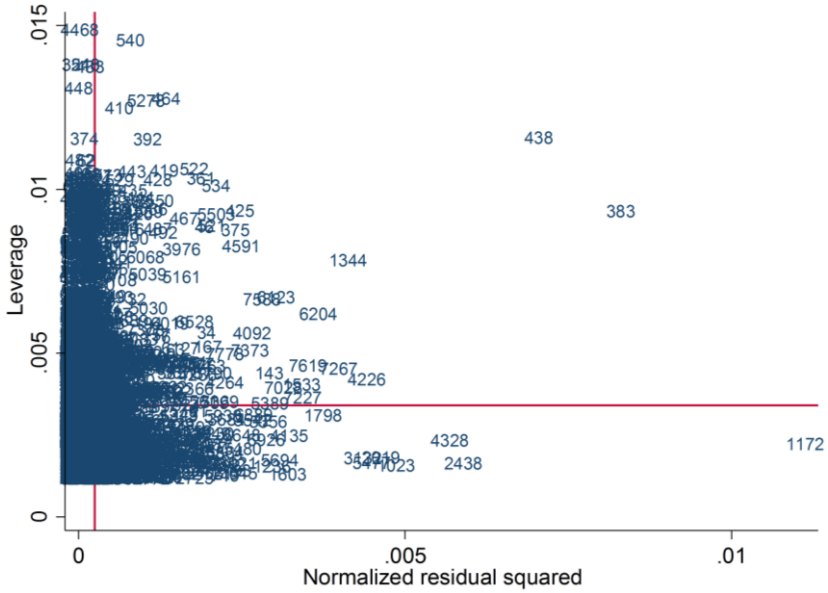
Figure 5. Added variable plots for the model 1 covariates, where the line represents the partial correlation of the CD4 cell count at 12 months (cells/μL) and the respective covariate. *(A) CD4 cell count at ART initiation (cells/μL). (B) Age at ART initiation. (C) Female gender. (D) Homosexual contact HIV exposure. (E) Injecting Drug Use (IDU) HIV exposure. (F) Other HIV exposure. (G) Hepatitis C co-infection (ever).*

Model selection

As HCV co-infection was not significant in model 1, model 2 removed HCV co-infection as a predictor. In model 2, the coefficient estimate for HIV exposure of IDU changed from -22.1 (95% CI: -45.2, 0.9) to -32.1 (95% CI: -51.8, -12.5) and, as such, became a significant predictor of the outcome (p value < 0.001). In model 3, HIV exposure was removed while HCV co-infection was included. The HCV positive co-infection coefficient estimates changed from -16.3 (95% CI: -35.9, 3.3) in model 1 to -27.2 (95% CI: -43.9, -10.6) in model 3 which was significant (p value < 0.001) (Table 4).

The significant changes in the coefficient estimates and, hence, the switching of significance suggests that there is collinearity between HCV co-infection and HIV exposure of IDU. Therefore, only one of the two can be selected for the final model. As HIV exposure also has heterosexual contact as a significant predictor of the outcome, it would be preferred as it contributes more to the final model than HCV co-infection would. As such, model 2 was selected and, subsequently, highly influential data points were evaluated.

Figure 6. Leverage values for model 2 against the squared residuals, with lines indicating the mean leverage and mean squared residual. Patient IDs are also shown.



Assessing highly influential data points in Model 2

The mean leverage was 0.003 (standard deviation: 0.002), with a minimum value of 0.001 and a maximum value of 0.015. Although the range of leverage values was quite large, the leverage plot clearly indicates that a majority of data points either had a higher than average leverage or a higher than average normalized residual squared value or some minor

combination of both (Figure 6). There were only two observations which were of concern as they had a combination of both extremely higher than average leverage and normalized residual squared (ID= 438 & 383). However, removal of these two observations had little impact on the coefficient estimates (Appendix 2).

Most DFBETA values for each of the covariates in model 2 are within 0.2 and -0.2 (Figure 7). Although there are a few observations outside of these limits, these are very few and not extremely large. The most extreme DFBETA value was -0.33 (DFBETA for age, ID = 1344) which indicates that the regression slope would increase by approximately 0.33 standard errors if this point was omitted. Overall, it is clear that this would have little impact on the coefficient estimates and clinical interpretation of the results. Hence, overall there is little evidence to suggest that there are any highly influential points that would produce a linear regression model with a poor fit of the data.

Final model selection

Model 1 displayed a collinearity issue between injecting drug user as mode of HIV exposure and HCV co-infection. Model 2 removed HCV co-infection as it had a weaker partial correlation to the outcome compared to mode of HIV exposure. Assessing the highly influential points in model 2 also showed little evidence to suggest the model was poorly fit.

Interaction terms between the clinical sites and all other predictors were also evaluated in Model 2. The Wald test for heterogeneity indicated that all interaction terms were not significant between the clinical site and CD4 cell count at ART initiation (p value = 0.335), age at ART initiation (p value = 0.460), gender (p value = 0.241) and HIV exposure group (p value = 0.236).

Table 4. Results from the first, second and third models for the selection of the final multivariate model.

	Model 1			Model 2			Model 3		
	Mean Diff.	**(95% CI)**	**p value**	**Mean Diff.**	**(95% CI)**	**p value**	**Mean Diff.**	**(95% CI)**	**p value**
CD4 cell count at ART initiation (per 100 cells/μL)	86.7	(83.6, 89.9)	<0.001	86.8	(83.7, 90.0)	<0.001	87.0	(83.9, 90.2)	<0.001
Age (per 10 years)	-8.9	(-13.7, -4.1)	<0.001	-8.9	(-13.7, -4.1)	<0.001	-10.1	(-14.8, -5.5)	<0.001
Female	21.5	(10.6, 32.5)	<0.001	22.3	(11.4, 33.2)	<0.001	20.8	(10.1, 31.5)	<0.001
HIV exposure			0.005			<0.001			
Heterosexual	ref			ref					
Homosexual	20.8	(3.0, 38.7)	0.022	21.2	(3.4, 39.0)	0.020			
IDU	-22.1	(-45.2, 0.9)	0.060	-32.1	(-51.8, -12.5)	<0.001			
Other	-11.3	(-27.5, 5.0)	0.175	-11.6	(-27.8, 4.7)	0.163			
HCV Co-infection (ever)			0.102						0.002
Negative	ref						ref		
Positive	-16.3	(-35.9, 3.3)	0.103				-27.2	(-43.9, -10.6)	<0.001
Not tested	-10.0	(-23.1, 3.2)	0.138				-10.8	(-24.0, 2.4)	0.107
Clinical site									
Hong Kong	ref			ref			ref		
South Korea	83.8	(53.2, 114.5)	<0.001	84.1	(53.4, 114.7)	<0.001	78.2	(47.7, 108.6)	<0.001
India	16.4	(-7.1, 39.9)	0.170	10.6	(-11.6, 32.8)	0.348	6.3	(-15.1, 27.7)	0.562
Singapore	-18.6	(-39.4, 2.2)	0.079	-18.4	(-39.2, 2.3)	0.082	-20.8	(-41.4, -0.1)	0.049
Indonesia	-26.0	(-54.8, 2.8)	0.077	-31.4	(-59.2, -3.7)	0.026	-35.5	(-62.9, -8.1)	0.011
Cambodia	-24.7	(-48.0, -1.4)	0.038	-27.2	(-50.4, -4.0)	0.022	-34.3	(-55.5, -13.1)	0.002
Vietnam 1	-38.2	(-62.6, -13.8)	0.002	-41.1	(-65.2, -17.1)	0.001	-50.0	(-72.5, -27.6)	<0.001
Vietnam 2	-43.6	(-69.7, -17.5)	0.001	-45.9	(-71.7, -20.0)	0.001	-56.6	(-80.6, -32.5)	<0.001
Constant	247.6	(216.2, 279.0)	<0.001	246.4	(215.1, 277.8)	<0.001	261.7	(234.1, 289.4)	<0.001

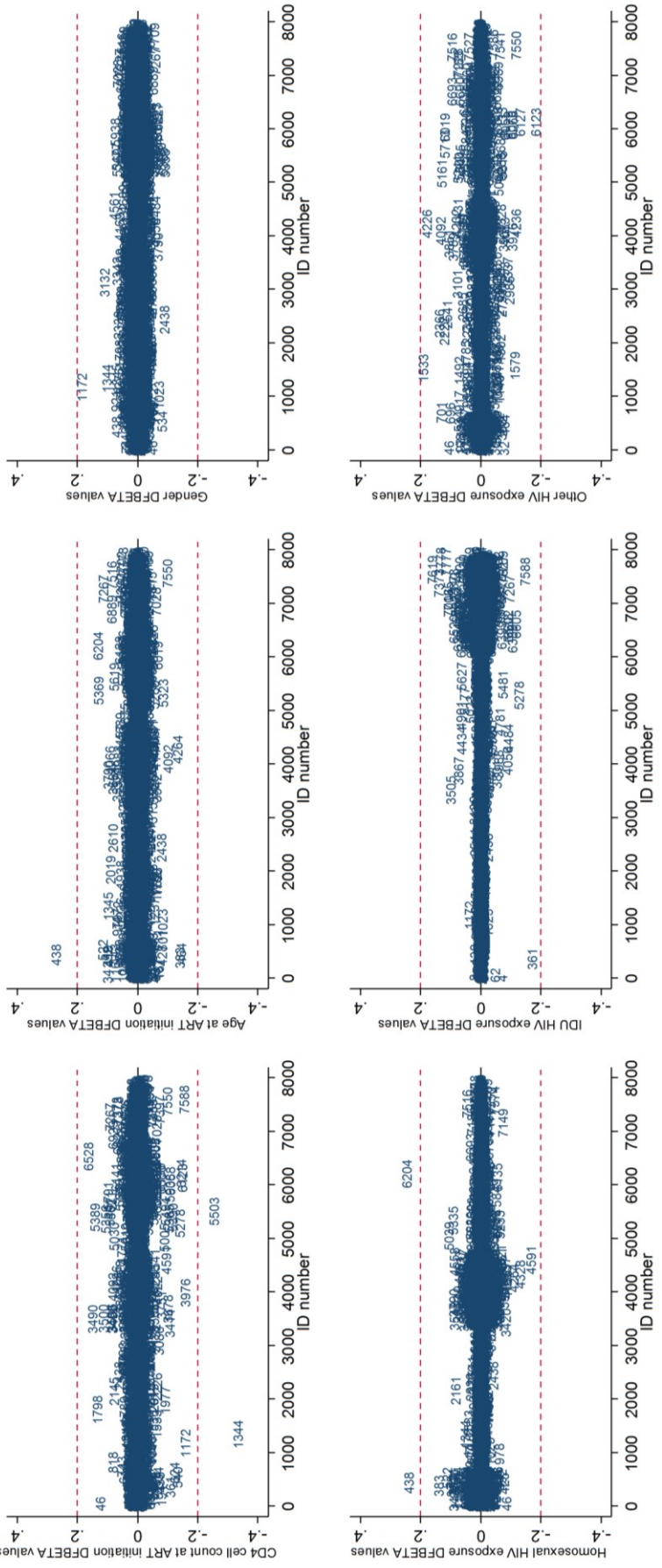


Figure 7. The DFBETA values for each of the covariates in model 2. *(A) CD4 cell count at ART initiation DFBETA values. (B) Age at ART initiation DFBETA values. (C) Gender DFBETA values. (D) Homosexual contact HIV exposure DFBETA values. (E) Injecting drug use HIV exposure DFBETA values. (F) Other HIV exposure DFBETA values.*

Last, we evaluate the residuals in Model 2 to determine if they have constant variance and normally distributed. The normal probability residual plots for each of the countries indicate only minor deviations around the line of equality (Figure 8). The only indication of non-normality is the heavy tails, shown quite prominently in Vietnam, Singapore, India and South Korea. However, this is only of slight concern and likely a result of the few extreme CD4 cell count values, those greater than 1000 cells/μL, that are generally uncommon. The residuals against the predicted values indicated a fairly constant and linear residual variance for all the countries (Figure 8). Hence, Model 2 satisfied the requirements of normally distributed, constant and linear residuals.

The final multivariate model had the general structure for the mean CD4 cell count at 12 months from ART initiation given by:

$$\widehat{Y}_i = 246.4 + 86.8 \times \frac{\textbf{Baseline CD4}}{100}_i - 8.9 \times \frac{\textbf{Age}}{10}_i + \begin{Bmatrix} 0 \text{ if } \textbf{MALE} \\ 22.3 \text{ if } \textbf{FEMALE} \end{Bmatrix} + \begin{Bmatrix} 0 \text{ if } \textbf{EXPOSURE}=1 \\ 21.2 \text{ if } \textbf{EXPOSURE}=2 \\ -32.1 \text{ if } \textbf{EXPOSURE}=3 \\ -11.6 \text{ if } \textbf{EXPOSURE}=4 \end{Bmatrix} + \begin{Bmatrix} 0 \text{ if } \textbf{SITE}=1 & -31.4 \text{ if } \textbf{SITE}=5 \\ 84.1 \text{ if } \textbf{SITE}=2 & -27.2 \text{ if } \textbf{SITE}=6 \\ 10.6 \text{ if } \textbf{SITE}=3 & -41.1 \text{ if } \textbf{SITE}=7 \\ -18.4 \text{ if } \textbf{SITE}=4 & -45.9 \text{ if } \textbf{SITE}=8 \end{Bmatrix}$$

Where:

$\widehat{Y}_i$ Mean CD4 cell count (cells/μL) predicted at 12 months from ART initiation

Age: Age at ART initiation (years)

Baseline CD4: CD4 cell count at ART initiation (cells/μL)

Exposure: HIV exposure
- 1 = Heterosexual contact
- 2 = Homosexual contact
- 3 = Injecting Drug User
- 4 = Other

Site: Clinical site
- 1 = Hong Kong
- 2 = South Korea
- 3 = India
- 4 = Singapore
- 5 = Indonesia
- 6 = Cambodia
- 7 = Vietnam 1
- 8 = Vietnam 2

Figure 8. The normal probability residual plots with the line of equality (left panel) and the residuals against the predicted values of the CD4 cell count at 12 months from ART initiation (right panel) in Model 2 for the patients in: *(A) Hong Kong. (B) South Korea. (C) India. (D) Indonesia. (E) Singapore. (F) Vietnam. (G) Cambodia.*

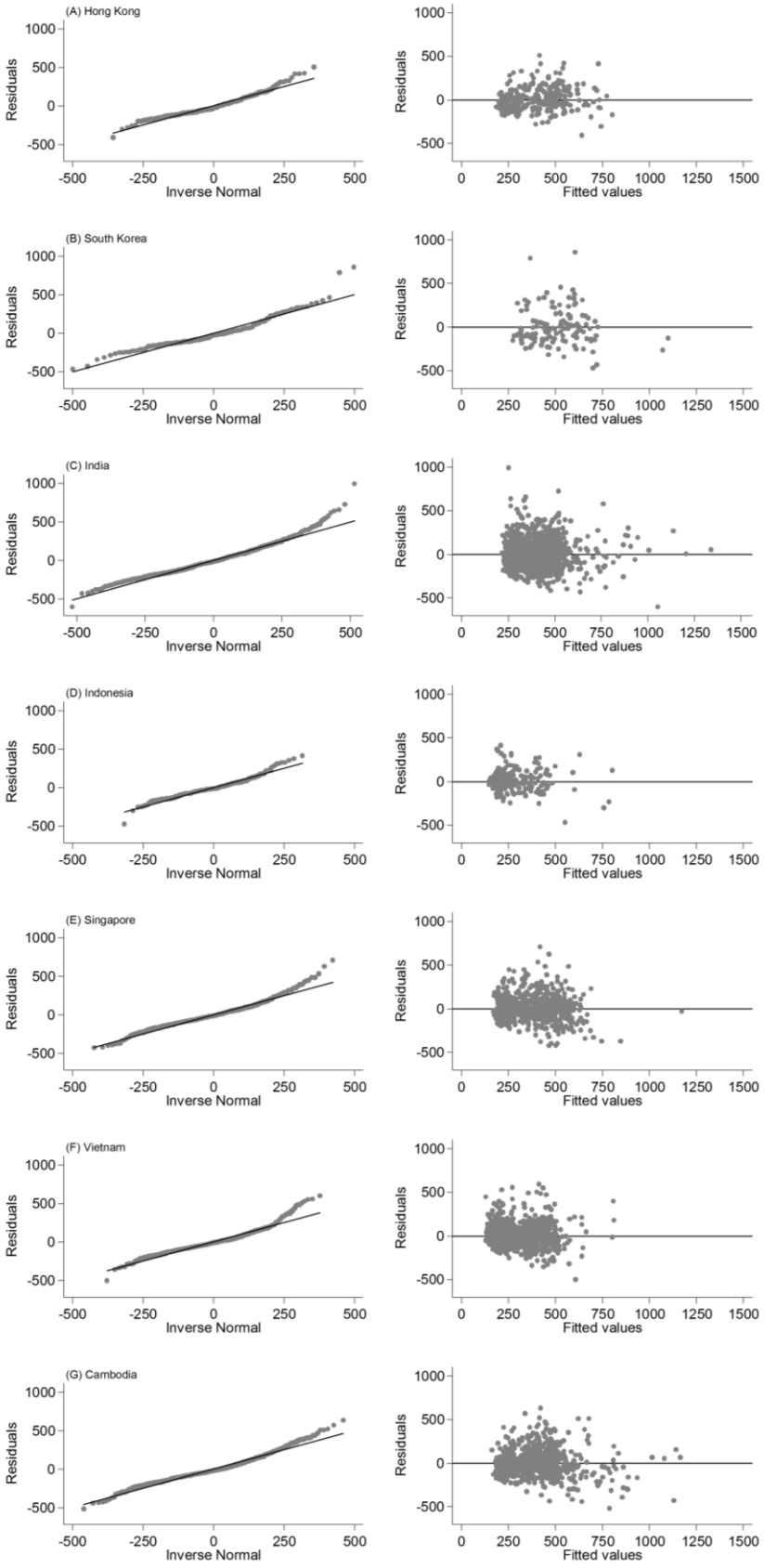
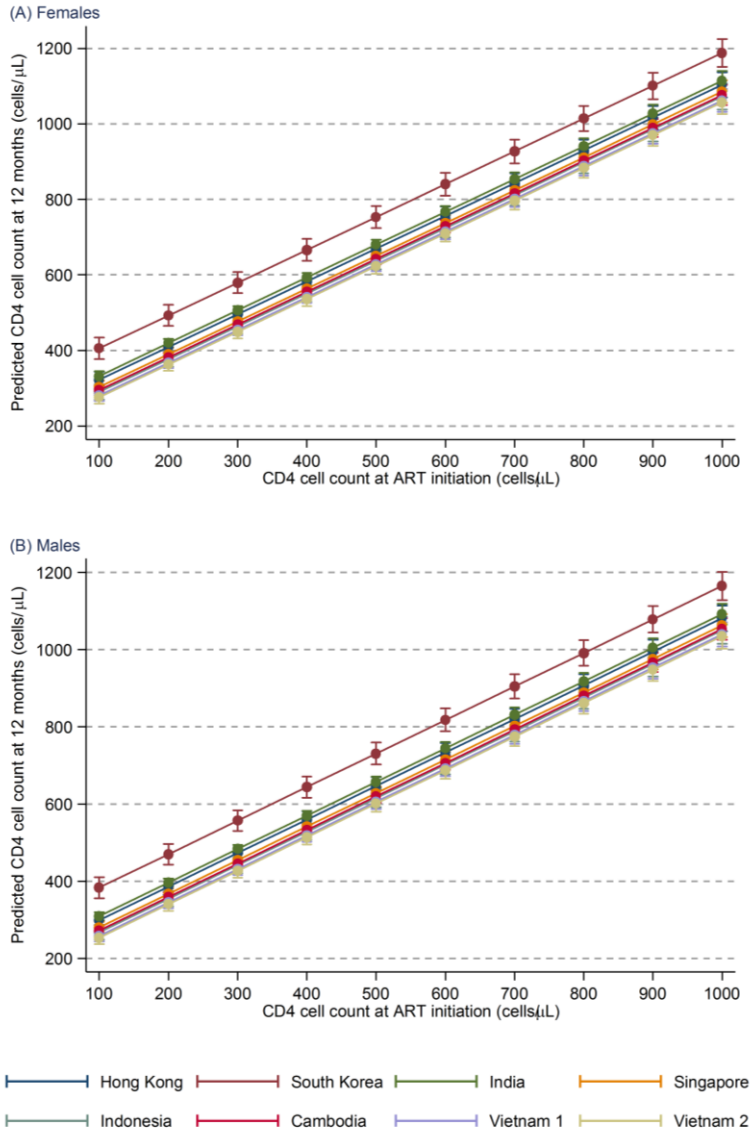


Figure 9 demonstrates the predicted CD4 cell count (cells/µL) at 12 months from ART initiation, with 95% confidence interval, from the final multivariate model for each of the clinical sites and by gender and CD4 cell count at ART initiation (cells/µL), assuming heterosexual contact as the HIV exposure and age at ART initiation of 37.9 years. Therefore, a female patient aged 37.9 years at ART initiation with heterosexual contact as HIV exposure attending the clinical site in Indonesia and initiating ART with a CD4 cell count of 350cells/µL is predicted to have a CD4 cell count of 507cells/µL at 12 months from ART initiation.

Figure 9. The adjusted predicted CD4 cell count at 12 months from Model 2, with 95% confidence intervals, assuming HIV exposure of heterosexual contact and mean age at ART initiation of 37.9 years for: *(A) Females and (B) Males, by clinical site and CD4 cell count at ART initiation.*



Discussion

In this cohort of 7840 patients receiving care at 8 clinical sites in the Asia-Pacific region, predictors associated with the mean CD4 cell count at 12 months from ART initiation included age at ART initiation, gender and HIV mode of exposure, after controlling for CD4 cell count at ART initiation and clinical site. The mean CD4 cell count at 12 months increased by 22.3 cells/μL (95% CI: 11.4 to 33.2 cells/μL) for females compared to males, by 21.2 cells/μL (95% CI: 3.4 to 39.0 cells/μL) for those who had homosexual contact compared to heterosexual contact as mode of HIV exposure and by 86.8 cells/μL (95% CI: 83.7 to 90.0 cells/μL) for every 100 cells/μL of CD4 cell count at ART initiation, adjusting for other significant predictors. While the mean CD4 cell count at 12 months decreased by 8.9 cells/μL (95% CI: -13.7 to -4.1 cells/μL) for every 10 years of age and by 32.1 cells/μL (95% CI: -51.8 to -12.5 cells/μL) for those with injecting drug use compared to heterosexual contact as mode of HIV exposure, adjusting for other significant predictors. Also, the mean CD4 cell count at 12 months was influenced by the clinical site, after adjusting for other significant predictors, where, compared to Hong Kong, those who had attended South Korea had an increase of 84.1 cells/μL (95% CI: 53.4 to 114.7 cells/μL) while those who attended Indonesia, Cambodia and Vietnam (site 1 and 2) had a decrease of 31.4 cells/μL (95% CI: -59.2 to -3.7 cells/μL), 27.2 (95% CI: -50.4 to -4.0 cells/μL), 41.1 cells/μL (95% CI: -65.2 to -17.1 cells/μL) and 45.9 (95% CI: -71.7 to -20.0 cells/μL), respectively.

The predictors associated with the CD4 cell count at 12 months are consistent with other studies for HIV-positive patients receiving ART. Older age in HIV-positive patients is associated with poorer outcomes to ART, including slower CD4 cell count restoration and higher risk of AIDS-related events [34]. It is postulated that the impaired CD4 cell count restoration is due to a decrease in thymic function [35]. We noted that the decrease in mean CD4 cell count at 12 months from ART was relatively quite small. For example, the expected CD4 cell count at 12 months would be reduced by 44.5 cells/μL (95% CI: -68.5 to -20.5) for a patient aged 50 years, controlling for other significant factors. However, during the first year on ART, CD4 cell count restoration is usually rapid and comparable between age groups [36]. Beyond that is the second phase of CD4 restoration, where older individuals are more likely to have reduced thymic output of naïve CD4 cells and reduced ability to produce mature CD4 cells, leading to poorer long-term CD4 restoration [37]. Therefore, the effect of age on expected CD4 cell count may have been greater if we had examined beyond 12 months from ART initiation.

Previous studies have also reported injecting drug users as having a reduced CD4 cell count response to ART [25, 38]. Several factors may be contributing to their poorer response including decreased access to HIV care facilities and treatment [39, 40], more drug-related comorbidities [41, 42], poorer adherence to ART [43, 44] and more adverse drug-related events [45, 46]. Last, we found that females were associated with an increased mean CD4 cell count at 12 months from ART initiation, which is consistent with some other previous studies [47, 48]. Some possible explanations include females have better adherence [49, 50] or better overall health with fewer comorbidities [51]. However, there has been much recent research to suggest that females have a more optimal biological response to ART [52, 53].

There are limitations to our study. Adherence to ART is critical to the effectiveness of ART in restoring CD4 and reducing HIV viral load [54]. We were unable to include adherence in our model as the patient database does not collect this information. HIV viral load and CD4 cell count are both considered as strong prognostic markers of HIV disease progression [13, 55]. The depletion of CD4 cell count is typically associated with an increase in HIV viral load [56]. We believe that the large proportion of patients without a HIV viral load test significantly contributed to the lack of linear association to CD4 cell count and hence, exclusion from the model. If these influencing factors had been included the model it may have accounted for more variability in the CD4 cell count at 12 months and allowed for more precise predictions.

Our model assumes a constant mean change in the CD4 cell count at 12 months from ART initiation as the CD4 cell count at ART initiation varies. However, in practice, as the CD4 cell count at ART initiation increases than the expected mean change in CD4 cell count at 12 months from ART initiation decreases. That is, those initiating with a lower CD4 cell count have a greater mean change in CD4 cell count than those initiating with a higher CD4 cell count [57, 58]. Therefore, our model would not be suitable for accurately predicting the expected CD4 cell count at 12 months from ART initiation for those initiating ART with a CD4 cell count >500 cells/μL. Alternate models that would be more appropriate for predicting CD4 cell count change for those initiating at higher baseline CD4 cell count could include an additional quadratic term for baseline CD4 cell count or baseline CD4 cell count could be fitted as a categorical variable to allow for different estimates of the mean CD4 cell count change.

In addition, our study utilized data from several clinical sites in the Asia-Pacific region, mainly from different countries with distinct differences in economies and cultures. As most countries had only one contributing clinical site, there were many site-based differences including when the patients present for ART, the type of ART and the level of care that can be provided. Therefore, the model did include clinical site to account for the heterogeneity between the sites. The results were also heavily weighted by the Indian site which contributed 38% of the patients. Despite this, the results were reasonably consistent among the clinical sites, showing a clear linear increasing trend in the CD4 cell count as the time from ART initiation increases.

In conclusion, this analysis describes the CD4 cell count response from ART initiation for HIV-positive patients receiving care in the Asia-Pacific region. Our findings suggest that age at ART initiation, gender and HIV mode of exposure are all significant predictors of CD4 cell count at 12 months from ART initiation, adjusting for CD4 cell count at ART initiation and clinical site. Further advancements in more tolerable and efficient ART regimens, and patient management, including greater access to care and earlier initiation of ART, are likely to lead to faster CD4 cell count restoration. In addition, targeting vulnerable populations, such as injecting drug users, by improving access HIV care and retaining in care would improve treatment outcomes as well as reduce mortality and morbidity rates in this population [59].

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Appendix 1. Summary of patient characteristics at ART initiation, by country.

	Hong Kong		South Korea		India		Indonesia		Singapore		Vietnam		Cambodia	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Total	368		177		2965		725		1061		1611		933	
Age at ART initiation														
≤30	65	(18)	60	(34)	622	(21)	298	(41)	223	(21)	535	(33)	284	(31)
31-40	127	(34)	52	(29)	1,386	(47)	290	(40)	305	(29)	798	(50)	385	(41)
41-50	106	(29)	28	(16)	701	(24)	101	(14)	261	(24)	177	(11)	181	(19)
51+	70	(19)	37	(21)	256	(8)	36	(5)	272	(26)	101	(6)	83	(9)
Median [IQR]	40	[33, 47]	37	[28, 47]	37	[32, 43]	32	[28, 38]	41	[32, 51]	33	[29, 38]	34	[30, 41]
Sex														
Male	306	(83)	164	(93)	1,864	(63)	452	(62)	986	(93)	1,009	(63)	402	(43)
Female	62	(17)	13	(7)	1,101	(37)	273	(38)	75	(7)	602	(37)	531	(57)
Mode of HIV Exposure														
Heterosexual contact	116	(32)	44	(25)	2685	(91)	588	(81)	450	(42)	1014	(63)	851	(91)
Homosexual contact	189	(51)	61	(34)	23	(1)	53	(7)	436	(41)	16	(1)	6	(1)
Injecting drug use	8	(2)	1	(1)	9	(0)	48	(7)	30	(3)	471	(29)	4	(0)
Other/Unknown	55	(15)	71	(40)	248	(8)	36	(5)	145	(14)	110	(7)	72	(8)
Pre-ART HIV viral load (copies/mL)														
≤100000	155	(42)	89	(50)	247	(8)	25	(3)	321	(30)	231	(15)	5	(1)
>100000	196	(53)	75	(43)	254	(9)	34	(5)	348	(33)	247	(15)	0	(0)
Not tested	17	(5)	13	(7)	2464	(83)	666	(92)	392	(37)	1133	(70)	928	(99)
Median [IQR]	131165	[37225, 394145]	83850	[25800, 526500]	103377	[19800, 411914]	157480	[39500, 518705]	112641	[32631, 406000]	111000	[30500, 370000]	6392	[269, 18910]
Pre-ART CD4 cell count (cells/µL)														
≤50	93	(25)	25	(14)	275	(9)	259	(36)	238	(22)	557	(34)	173	(19)
51-100	41	(11)	15	(9)	320	(11)	86	(12)	116	(11)	171	(11)	84	(9)
101-200	47	(13)	38	(21)	584	(20)	88	(12)	146	(14)	256	(16)	148	(16)
>200	174	(47)	86	(49)	1,203	(41)	128	(18)	506	(48)	415	(26)	471	(50)
Not tested	13	(4)	13	(7)	583	(19)	164	(22)	55	(5)	212	(13)	57	(6)
Median [IQR]	196	[46, 319]	218	[103, 338]	203	[101, 290]	59	[17, 181]	202	[54, 315]	91	[22, 226]	218	[71, 319]

First ART regimen							
NRTI+NNRTI	268 (73)	59 (33)	2,845 (96)	720 (99)	966 (91)	1,606 (99)	917 (98)
NRTI+PI	96 (26)	76 (43)	113 (4)	5 (1)	76 (7)	3 (<0.2)	12 (1)
Other	4 (1)	42 (24)	7 (<0.3)	0 (0)	19 (2)	2 (<0.2)	4 (<0.5)
Previous mono/dual therapy							
No	366 (99)	176 (99)	2,879 (97)	719 (99)	1,045 (98)	1,578 (98)	917 (98)
Yes	2 (1)	1 (1)	86 (3)	6 (1)	16 (2)	33 (2)	16 (2)
HBV Co-infection							
Negative	314 (85)	155 (88)	1,017 (34)	272 (37)	903 (86)	1,371 (85)	670 (72)
Positive	36 (10)	7 (4)	55 (2)	21 (3)	79 (7)	216 (13)	59 (6)
Not tested	18 (5)	15 (8)	1,893 (64)	432 (60)	79 (7)	24 (2)	204 (22)
HCV Co-infection							
Negative	330 (90)	156 (88)	792 (26)	248 (34)	953 (90)	957 (59)	657 (71)
Positive	17 (4)	1 (1)	15 (1)	19 (3)	45 (4)	628 (39)	51 (5)
Not tested	21 (6)	20 (11)	2,158 (73)	458 (63)	63 (6)	26 (2)	225 (24)

Appendix 2. Results from the second model when two potential highly influential points are removed.

	Model 2 All observations			Model 2 Remove ID 438			Model 2 Remove ID 383			Model 2 Remove ID 438 & 383		
	Mean Diff.	**(95% CI)**	**p value**	**Mean Diff.**	**(95% CI)**	**p value**	**Mean Diff.**	**(95% CI)**	**p value**	**Mean Diff.**	**(95% CI)**	**p value**
CD4 cell count at ART initiation (per 100 cells/μL)	86.8	(83.7, 90.0)	<0.001	87.0	(83.8, 90.1)	<0.001	86.8	(83.7, 89.9)	<0.001	86.9	(83.8, 90.1)	<0.001
Age (per 10 years)	-8.9	(-13.7, -4.1)	<0.001	-9.6	(-14.4, -4.8)	<0.001	-8.6	(-13.4, -3.8)	<0.001	-9.2	(-14.0, -4.5)	<0.001
Female	22.3	(11.4, 33.2)	<0.001	21.9	(11.1, 32.8)	<0.001	22.4	(11.6, 33.3)	<0.001	22.0	(11.2, 32.9)	<0.001
HIV exposure			<0.001									
Heterosexual	ref			ref			ref			ref		
Homosexual	21.2	(3.3, 39.0)	0.020	19.0	(1.2, 36.8)	0.036	19.9	(2.1, 37.7)	0.028	17.7	(0.0, 35.5)	0.050
IDU	-32.1	(-51.8, -12.5)	0.001	-32.3	(-51.9, -12.8)	<0.001	-32.1	(-51.6, -12.5)	<0.001	-32.3	(-51.8, -12.8)	0.001
Other	-11.6	(-27.8, 4.7)	0.163	-11.2	(-27.4, 5.0)	0.175	-11.0	(-27.2, 5.2)	0.184	-10.6	(-26.8, 5.5)	0.197
Clinical site												
Hong Kong	ref			ref			ref			ref		
South Korea	84.1	(53.4, 114.7)	<0.001	77.6	(46.9, 108.2)	<0.001	77.4	(46.8, 108.0)	<0.001	70.8	(40.2, 101.4)	<0.001
India	10.6	(-11.6, 32.8)	0.348	9.3	(-12.8, 31.4)	0.409	10.1	(-12.0, 32.1)	0.372	8.7	(-13.3, 30.8)	0.436
Singapore	-18.4	(-39.2, 2.3)	0.082	-18.6	(-39.3, 2.1)	0.078	-18.6	(-39.3, 2.1)	0.078	-18.8	(-39.5, 1.8)	0.073
Indonesia	-31.4	(-59.2, -3.7)	0.026	-32.7	(-60.3, -5.0)	0.021	-31.8	(-59.4, -4.2)	0.024	-33.1	(-60.6, -5.5)	0.019
Cambodia	-27.2	(-50.4, -4.0)	0.022	-28.5	(-51.6, -5.4)	0.016	-27.7	(-50.8, -4.6)	0.019	-29.0	(-52.0, -6.0)	0.014
Vietnam 1	-41.1	(-65.2, -17.1)	0.001	-42.5	(-66.4, -18.5)	0.001	-41.6	(-65.5, -17.7)	0.001	-42.9	(-66.8, -19.1)	<0.001
Vietnam 2	-45.9	(-71.7, -20.0)	0.001	-47.2	(-72.9, -21.4)	<0.001	-46.3	(-72.0, -20.6)	<0.001	-47.6	(-73.3, -22.0)	<0.001
Constant	246.4	(215.1, 277.8)	<0.001	250.1	(218.8, 281.4)	<0.001	245.7	(214.4, 276.9)	<0.001	249.3	(218.2, 280.5)	<0.001

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