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5 **The effects of HIV-1 subtype and ethnicity on CD4 decline in antiretroviral naïve patients:**
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7 **a Canadian-European collaborative cohort study**
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11 **Manuscript Type:** Cohort (Retrospective)
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Competing interests

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Abstract

Objectives: We compared CD4 decline in the most prevalent HIV-1 subtypes during chronic infection focusing on distinguishing between effects of viral subtype and ethnicity.

Methods: We merged data from four European and six Canadian cohorts selecting adults in the stable chronic phase of untreated HIV infection. We estimated the change in square root CD4 cell count over time for subtypes and ethnicities using mixed models adjusting for covariates selected because of their potential effect on initial CD4 cell count or its decline.

Results: Data from 9,772 patients were analysed, contributing 79,175 CD4 measurements and 24,157 person-years of follow-up. Overall, there were no appreciable differences in CD4 decline relative to subtype B for subtypes A, CRF01_AE, CRF02_AG, C or G; whereas CD4 decline in patients of black ethnicity was considerably slower than in patients of other ethnicities. When ethnic groups were studied separately, there was evidence for slower CD4 decline in subtypes C, and possibly A and G, relative to B in patients of black ethnicity but not among those of other ethnicities suggesting an interaction between subtype and ethnicity. Only subtype CRF01_AG appeared to have a similar effect in both ethnic groups.

Interpretation: Ethnicity is a major determinant of CD4 decline; subtype differences may also exist but were small relative to the effect of ethnicity and were most apparent in patients of black ethnicity. In developing countries, slower CD4 decline among individuals of African descent may translate to a longer asymptomatic phase and increase the opportunity for HIV transmission.

Keywords: HIV infection, subtypes, Ethnicity, CD4 cell count, Immunologic Progression, cohort

Introduction

HIV-1 has acquired extensive genetic diversity with nine recognized subtypes (A-D, F-H, J, K) and many circulating recombinant forms (CRFs).^{1,2} Subtype B is most widely studied being the predominant virus in North America and Europe. The majority (89%) of HIV infections worldwide, however, are of “non-subtype B” virus with subtypes C, A, and recombinants CRF01_AE and CRF02_AG accounting for 73% of all HIV-1 infections.³ Due to patterns of immigration, a growing number of HIV infections in developed countries are among migrant ethnic communities infected with non-B subtypes,^{4,5} with increasing transmission of subtypes between ethnicities.⁶ In Canada, non-B subtypes now account for about 15% of newly reported HIV infections.⁷

CD4 cell counts are both the primary prognostic marker for HIV-related disease and the main indicator for the initiation of antiretroviral treatment (ART). As ART is expanded worldwide, it is important to understand whether differences in immunologic progression exist that might necessitate subtype-specific monitoring and treatment guidelines. Subtype specific estimates of CD4 cell count trajectories are also essential for public health models used to predict the course of the HIV epidemic and are helpful for estimating what proportion of persons born abroad acquire HIV infection in their adopted country.⁸

HIV-1 viral diversity might affect immunologic progression through differential interactions with the human host.⁹⁻¹³ Determining subtype specific effects on immunologic progression is challenging:¹⁴ non-B subtypes predominantly affect individuals of African descent and several studies have suggested that rates of CD4 decline differ according to ethnicity. Ethnic differences

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4 have the potential to confound any association between viral subtype and immunologic
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6 progression.¹⁵⁻¹⁷ We conducted a Canadian and European collaborative study to examine CD4
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8 cell decline in untreated patients during the stable chronic phase of HIV infection according to
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10 the most prevalent viral subtypes and focused on distinguishing between the effects of viral
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12 subtype and ethnicity.
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Confidential

Methods

Data collection

Ten cohorts, or cohort collaborations, contributed to this study; four from Europe and six from Canada (Table 1). This project was approved by the relevant Scientific Boards or Steering Committees. EuroSIDA omitted patients also enrolled in other contributing cohorts. For each patient, we requested all available CD4 cell counts from cohort enrolment until the date of ART initiation and the dates of any newly acquired AIDS defining illness or death.

Seven cohorts provided information on ethnicity using a variety of classifications (Table 1); hence ethnicity was coded as black (e.g. of African descent), other, or unknown. Two Canadian cohorts provided information on country of origin; we assumed black ethnicity for patients from African or Caribbean countries. The ANRS CO3 Aquitaine cohort cannot by French law collect information on ethnicity or country of origin. Viral subtype was determined via the REGA HIV 1&2 Automated Subtyping Tool (version 2.0) based on HIV-1 pol sequences during genotypic resistance testing or, in some earlier cases, with the Subtype Analyzer tool.¹⁸

Our analysis was restricted to adults (age ≥ 16 years) enrolled between January 1998 and June 2010 with known subtype and with at least two CD4 measurements within one year while ART naïve. We selected patients and CD4 measurements within a stable chronic phase of untreated HIV infection. We omitted any patient with a first AIDS event either before or within three months after their first CD4 measurement – such patients were likely to be late presenters. In each patient's series of CD4 measurements, we removed early measurements if within six months of seroconversion (known for only 16%). We removed measurements both at the beginning of the series if below 100 cells per μl and at the end of the series starting with the first

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4 of any two consecutive measurements below 100 cells per μl – such CD4 cell counts may reflect
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6 advanced infection. We also removed measurements until consecutive measurements both at the
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8 beginning and end of the series were at least 2 and no more than 12 months apart to avoid re-
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10 measurement or measurement prompted by clinical deterioration.
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14 **Statistical analysis**

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17 Patients were followed from the time of the first CD4 measurement in their series until they
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19 started ART, the last CD4 measurement in their series, or death. In the main analysis, we
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21 compared CD4 cell count (square root transformed to stabilise the variance) over time for the
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23 most common HIV-1 subtypes (at least 100 patients available: A, CRF01_AE, CRF02_AG, B,
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25 C, or G) using a mixed linear regression model. This mixed model included both patient-specific
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27 random intercepts and random slopes over time to allow for heterogeneity in patients' initial
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29 CD4 cell count and its decline. In the main analysis, models were adjusted for covariates selected
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31 because of their potential effect on initial CD4 cell count (age, sex, ethnicity, initial HIV RNA,
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33 IDU and cohort) or on its decline (age, sex, ethnicity, initial HIV RNA, IDU). In a descriptive
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35 analysis of ethnic and subtype effects, we plotted random effects representing each patient's
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37 estimated CD4 decline from a mixed model without ethnicity or subtype slope parameters. We
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39 also report CD4 intercept and slope estimates for each subtype from mixed models fit to
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41 untransformed data, because rates of CD4 decline are easier to interpret on the original scale than
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43 on a square-root scale.
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51 We explored the results from our main analysis in a variety of sensitivity analyses. First,
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53 the main analysis model was re-fit to all CD4 cell counts since January 1998 without further
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55 selection (so as to include measurements made in both the acute and advanced stages of
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4 infection). Second, separate mixed models were fit for patients of black and other ethnicities.

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7 Third, the main analysis model was fit as part of a joint model, together with an exponential
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9 model for the time to ART initiation.¹⁹ This joint model allows for informative censoring if CD4
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11 cell count series are shorter for some subtypes because patients with these subtypes start therapy
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13 at higher CD4 cell counts. Finally, we varied the covariates and cohorts in the analysis because
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15 not all cohorts were able to provide all covariates of interest.
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20 Clinical events such as time to a first AIDS defining illness and death prior to ART
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22 initiation were summarised according to viral subtype and ethnicity. No formal analyses were
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24 conducted because of the small numbers of events and the potential for bias due to unobserved
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26 events prior to cohort enrolment.
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30 We used SAS 9.2 (SAS Institute, Cary, NC, USA) for all analyses.
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Results

A total of 14,590 patients met inclusion criteria: 13,682 had one of the subtypes A, CRF01_AE, CRF02_AG, B, C, or G. After applying CD4 selection criteria, 9,772 patients remained contributing 79,175 CD4 measurements and 24,157 person-years of follow-up with a median of 6 CD4 measurements per patient (IQR 3, 11). For most patients, CD4 measurements were censored either by ART initiation (37%) or by irregular measurement at the end of the series (33%) but 144 patients (1.5%) reached a CD4 cell count below 100 without starting ART, and 22 patients (0.2%) died.

Subtype B accounted for the majority of infections (81%, Table 2) followed by subtype C (8%), A (4%), CRF02_AG (4%), CRF01_AE (2%), and G (1%). The demographic and clinical characteristics of the non-B subtypes were similar, with the exception of CRF01_AE. Of note, subtypes B and CRF01_AE had a very low percentage of patients of black ethnicity whereas other subtypes were more ethnically diverse. Initial CD4 cell counts were higher among patients infected with subtypes CRF01_AE and B, and initial HIV RNA was higher in subtypes A and B.

For the main analysis, we omitted 1162 patients because either their ethnicity or initial HIV RNA was unknown. Note that patients of unknown ethnicity had characteristics similar to those of other ethnicities (Table 3). In the main analysis, patients of black ethnicity had an appreciably slower rate of CD4 decline relative to patients of other ethnicities (Table 4). Differences between subtypes, however, were minor relative to the difference between the two ethnic categories. Subtype differences became apparent when the main analysis model was fit to unselected CD4 cell counts, with appreciably slower declines in patients with subtypes A, C and CRF02_AG relative to subtype B. If ethnicity was ignored in an analysis of selected CD4 cell

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4 counts, then again these three subtypes appeared to have slower declines than patients of subtype
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7 B. In our descriptive analysis of ethnic and subtype effects, within each subtype patients of black
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9 ethnicity had a slower decline relative to patients of other ethnicities (Figure 1a), including
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11 within subtype B (although no conclusion can be drawn about subtype CRF01_AE). Within
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13 subtype B, patients of black ethnicity from Caribbean countries had a CD4 decline intermediate
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15 between other patients of black ethnicity and patients of other ethnicities (Figure 1b).
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20 In sensitivity analyses, we fit separate models for patients of black and other ethnicities
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22 (Table 4). The results from these analyses suggest little difference between subtypes in patients
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24 of other ethnicities but in patients of black ethnicity, slower declines in subtypes C and possibly
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26 A and G relative to subtype B. Only subtype CRF01_AG appeared to have a similar effect in
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28 both ethnic groups. This amounts to an interaction between the effects of subtype and ethnicity;
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30 however we did not have sufficient data to estimate this interaction in a single model. Our data
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32 suggest that in patients of other ethnicities, there is little variation between subtypes in both
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34 initial CD4 cell count and its decline (Table 5). Figure 2 shows the estimated average CD4
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36 decline over 5 years for patients of black ethnicity with subtype C and for patients of other
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38 ethnicities with subtype B.
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45 Estimates of CD4 decline did not change appreciably in a joint model (Table 6). In the
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47 longitudinal component of the joint model, lower initial CD4 cell count and more rapid decline
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49 were associated with older age, female sex, IDU, and higher initial HIV RNA. In the time to
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51 event component of the joint model, earlier ART initiation was associated with older age, female
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53 sex, higher initial HIV RNA, more recent calendar time and black ethnicity. There was no
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4 evidence from this model that subtype and ethnicity slope estimates were biased by informative
5 censoring due to early ART.
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9 In other sensitivity analyses, we added time dependent CD8 cell count (not available
10 from EuroSIDA); we replaced IDU with covariates for hepatitis co-infection (not available from
11 HOMER). Subtype and ethnicity slope estimates were not materially different in these analyses.
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17 We report clinical outcomes using unselected CD4 cell count series (Table 7) because
18 selected series were often censored by irregular measurement. There were no appreciable
19 differences between subtypes and ethnicities in either the proportion of patients starting ART or
20 the median CD4 cell count when starting. However, the median time to ART initiation was
21 shorter among patients of black ethnicity (0.51 vs. 1.27 years – Table 7), consistent with their
22 lower initial CD4 cell count (400 vs. 470 cells/ μ l – Table 3). Within each subtype, no more than
23 14% of patients developed an AIDS illness and 1% of patients died during a median follow up of
24 around two years. There was no obvious relationship between the number of these clinical events
25 and the rate of CD4 decline by either subtype or ethnicity.
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Interpretation

Main findings

This is the first study to explore the effects of both viral subtype and ethnicity on CD4 decline in untreated patients during the chronic phase of HIV infection.¹⁴ We focused on the chronic phase because it constitutes the longest period of untreated HIV infection. We were able to compare all of the predominant subtypes directly to one another. Indeed, the proportions of subtypes studied (other than B) mirrored their proportions in the worldwide HIV epidemic.³ We found that ethnicity is the major determinant of CD4 decline; subtype differences may also exist but were small relative to the effect of ethnicity and were most apparent in patients of black ethnicity. Patients of black ethnicity had considerably slower rates of CD4 decline overall. Although the non-B subtypes, with the exception of CRF01_AE, appeared to be associated with slower CD4 decline in models that did not include a slope for black ethnicity, these effects were greatly attenuated once this slope was included. Furthermore, in plots of CD4 decline by subtype and ethnicity, patients of black ethnicity had slower rates of CD4 decline within each subtype, including within subtype B. Together these results suggest that differences between subtypes are small relative to a broader effect of ethnicity on immunologic progression. Failure to account for ethnicity in the evaluation of subtype effects could therefore potentially lead to erroneous conclusions.

Strong associations between viral subtype and ethnicity exist. Only 5% of patients infected with subtype B and 3% of those infected with CRF01_AE were of black ethnicity compared with more than 50% of those infected with other subtypes. We therefore examined the effects of subtype in each ethnic group separately. Among patients of other ethnicities, there was

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4 little evidence for an effect of subtype on immunologic progression. In contrast, patients of black
5 ethnicity may have slower rates of decline when infected with subtype C (and possibly A,
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7 CRF02_AG and G) relative to subtype B. This suggests viral subtype may have different effects
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9 depending on ethnicity.
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13 14 15 **Comparisons with other studies**

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17 Indeed, biological differences between subtypes do exist that might affect immunologic
18 progression. For example, coreceptor use varies between subtypes, with A and C predominantly
19 using the coreceptor (R5) that is associated with slower disease progression and subtype C has
20 lower replicative fitness when compared with other viral subtypes in vitro.¹³ Observational
21 studies also support differences in disease progression among patients of black ethnicity infected
22 with different viral subtypes. For example, faster immunologic progression has been reported in
23 Ugandan and Kenyan patients infected with subtype D compared to subtype A.²⁰⁻²² Co-evolution
24 of HIV-1 subtypes and African populations may reduce the virulence of more dominant
25 subtypes, such as subtype C, possibly leading to an interaction between subtype and ethnicity.²
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27 In the absence of ART, which is still the reality for the majority of HIV-infected persons in
28 Africa, our results suggest that a patient of black ethnicity infected with a non-B subtype could
29 take considerably longer to reach a CD4 count of 200 cells/ul (9.3 vs. 6.2 years; Table 5), the
30 level at which the risk of AIDS increases, thus prolonging the asymptomatic period and
31 increasing the opportunity for HIV transmission.
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51 Several previous studies both in incident^{23, 24} and prevalent^{4, 16, 17} cohorts have shown that
52 that black ethnicity is associated with slower rates of CD4 decline but with one exception,²³ have
53 not included subtype and ethnicity in the same analysis.¹⁴ Yet it remains unclear how ethnicity
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4 could affect the underlying pathogenesis of HIV infection.²⁵ Those classified as being of black
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6 ethnicity in our study came from a large number of African and Caribbean countries, suggesting
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8 no single host characteristic is likely to be responsible for differences in disease progression.
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10 Differential patterns of migration and racial admixture in Caribbean black populations should
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12 dilute ethnic differences as in an analysis where Haitians and Canadians (both infected with
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14 subtype B) had similar rates of CD4 decline, both faster than that observed among recent African
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16 immigrants.²⁶ In our study, within subtype B, Caribbeans appeared to have rates of CD4 decline
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18 intermediate to those from African and other countries.
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25 Individuals of similar ethnicity do share genetic characteristics that have been associated
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27 with HIV disease progression. Examples include protective human leukocyte antigen (HLA)
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29 haplotypes, chemokine and chemokine receptor polymorphisms and mutational variants in genes
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31 involved in immune regulation.^{27, 28} It is also possible that ethnicity simply serves as a marker for
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33 socioeconomic, cultural and environmental factors that may influence immunologic
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35 progression.²⁵ Black patients in our study were more likely to be female and of childbearing age,
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37 have lower initial CD4 cell counts and initiated ART sooner. We were not able to adjust for
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39 socio-demographic factors beyond age and sex. However, our joint model showed no evidence
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41 of informative censoring due to early ART initiation. The healthy migrant effect is an alternate
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43 explanation for differences between black and other ethnicities receiving healthcare in developed
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45 countries,²⁹ but this does not explain reported differences in patients native to Africa or within
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47 the United States.^{15, 16, 24, 30} In our study, adjustment for time updated CD8 cell count – a crude
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49 marker of immune activation – or for hepatitis co-infection did not affect estimates of differences
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4 in CD4 decline. A healthy migrant effect implies that ethnic differences should be attenuated
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6 after adjusting for comorbidities.
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9 10 **Limitations**

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12 Our classification of patients as either of black or other ethnicity was rudimentary based on
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14 different classifications in each cohort or derived from country of origin. We also had
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16 insufficient Asian patients to study these separately. Patients included in this study were being
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18 treated in countries with publicly-funded healthcare, minimising the potential effects of
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20 healthcare access and quality on immunologic progression, but our results might not be
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22 generalizable to resource-limited settings. We did not have enough patients with other subtypes
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24 linked to faster CD4 decline, such as subtype D.^{20-22, 30} Also, limited numbers of both subtype G
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26 and subtype CRF01_AE in black patients meant our estimates for these subtypes were imprecise.
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31 32 **Conclusions**

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34 This large collaborative analysis with a broad representation of the most common circulating
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36 HIV-1 subtypes worldwide suggests that ethnicity is more prognostic of immunologic
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38 progression than viral subtype during untreated chronic HIV infection. While we have some
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40 evidence of differences in CD4 decline between subtypes, particularly in patients of black
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42 ethnicity, research to uncover the underlying biologic or sociologic reasons for slower
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44 immunologic progression among black ethnicities is warranted. Further study of subtype specific
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46 effects on immunologic progression will require comparisons of different subtypes in ethnically
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48 homogeneous populations.
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Table 1. Cohorts contributing patients to this study.

Cohort	Reference	Co-author responsible for data	Number of patients contributing CD4 cells counts		Information available with which to assign ethnicity and region
			Unselected	Selected	
Canadian	[1, 2]				
- Calgary		JG	79	62	Ethnicity (black, white, aboriginal, asian, other, missing)
- Hamilton		FS	30	7	Ethnicity (black, first nations, white) Country (no Caribbean countries for patients of black ethnicity)
- HOMER		RSH	1831	1112	Ethnicity (black, white, asian, first nation, hispanic, missing)
- Montreal		MBK	346	196	Country (black assumed if Burundi, Cameroon, Congo, Democratic Republic of the Congo, Ethiopia, Gabon, Guinea, Ivory Coast, Morocco, Mali, Rwanda, Senegal, South Africa, Tchad, Zimbabwe, Barbados, Dominican Republic, Haiti, Jamaica, Martinique, Saint Lucia – last six are Caribbean)
- Ottawa		CC	65	50	Country (black assumed if Angola, Burundi, Congo, Ethiopia, Haiti, Rwanda, Somalia, Zambia)
- Toronto		SW	187	125	Ethnicity (black, white, asian, south asian, other, missing) Country (Barbados, Cuba, Dominica, Grenada, Haiti, Jamaica, Trinidad & Tobago listed)
European					
- Aquitaine	[3]	FD	442	348	Not available
- Eurosidea	[4]	AC	349	267	Ethnicity (black, white, asian, missing) Country (no Caribbean countries for patients of black ethnicity)

- SHCS	[5]	BL	2802	2087	Ethnicity (black, white, hispanic, asian, other, missing) Region (Caribbean)
- UK CHIC	[6]	CS	7551	5518	Ethnicity (black, white, indian, other, missing) Country (Barbados, Bermuda, Cuba, Jamaica, Saint Kitts and Nevis listed)

SHCS, Swiss HIV Cohort Study; UK CHIC, UK Collaborative HIV Cohort Study; HOMER, HAART Observational Medical Evaluation and Research Study

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Table 2. Initial characteristics according to HIV-1 viral subtype – median or proportion (n missing).

	A n=356	AE n=193	AG n=352	B n=7937	C n=822	G n=112	Total n=9772
Age (years)	34	38	34	35	33	33	35
Sex (female)	0.53	0.24	0.57	0.11	0.59	0.48	0.19
Mode of transmission							
- Heterosexual	0.70	0.58	0.74	0.10	0.71	0.71	0.21
- Homosexual	0.14	0.32	0.12	0.67	0.09	0.15	0.57
- Injection drug use	0.06	0.03	0.00	0.12	0.02	0.08	0.11
- Other or unknown	0.10	0.08	0.14	0.11	0.18	0.06	0.11
Black ethnicity	0.49 (20)	0.03 (13)	0.60 (52)	0.05 (973)	0.72 (30)	0.55 (8)	0.15 (1096)
Hepatitis C	0.10 (43)	0.06 (18)	0.03 (22)	0.18 (1094)	0.04 (172)	0.13 (15)	0.16 (1364)
Hepatitis B	0.05 (42)	0.03 (19)	0.06 (31)	0.04 (1550)	0.04 (163)	0.02 (14)	0.04 (1819)
Calendar year of cohort enrolment							
<2000	0.15	0.09	0.06	0.22	0.07	0.07	0.20
2000 - <2005	0.44	0.39	0.38	0.38	0.39	0.28	0.39
2005 - <2010	0.41	0.51	0.56	0.39	0.54	0.65	0.42
Number of CD4 measures	6	6	5	6	5	5	6
CD4 (cells/ul)	420	450	440	470	400	420	460
CD4%	24 (73)	27 (32)	24 (42)	26 (901)	23 (143)	23 (17)	26 (1208)
CD8 (cells/ul)	910 (82)	900 (39)	860 (50)	930 (1125)	900 (177)	850 (18)	920 (1491)
HIV RNA (log copies/ml)	4.1 (13)	4.2 (1)	4.1 (2)	4.4 (44)	4.0 (9)	4.1	4.3 (69)
Follow up time (years)	1.59	1.78	1.40	1.75	1.39	1.51	1.69

Table 3. Initial characteristics by ethnicity – median or proportion (n unknown).

	Black ethnicity n=1303	Other ethnicities n=7373	Unknown ethnicity n=1096	Total n=9772
Age (years)	33	35	36	35
Sex (female)	0.55	0.12	0.19	0.19
Mode of transmission				
- Heterosexual	0.68	0.14	0.13	0.21
- Homosexual	0.15	0.68	0.35	0.57
- Injection drug use	0.01	0.11	0.17	0.11
- Other or unknown	0.15	0.07	0.36	0.11
Hepatitis C	0.03 (201)	0.18 (810)	0.19 (353)	0.16 (1364)
Hepatitis B	0.06 (192)	0.04 (1078)	0.02 (549)	0.04 (1819)
Calendar year <2000	0.09	0.21	0.22	0.20
2000 - <2005	0.40	0.38	0.43	0.39
2005 - <2010	0.51	0.41	0.35	0.42
Number of CD4 measures	5	6	5	6
CD4 (cells/ul)	400	470	470	460
CD4%	23 (225)	26 (932)	26 (51)	26 (1208)
CD8 (cells/ul)	880 (275)	940 (1163)	900 (53)	920 (1491)
HIV RNA (log copies/ml)	4.0 (10)	4.4 (56)	4.5 (3)	4.3 (69)
Follow up time (years)	1.42	1.76	1.56	1.69

Table 4. Change over time in square root CD4 cell count according to HIV-1 viral subtype and ethnicity.

Slope estimate [95% CI] Analysis of: Mixed model ³ :	Selected CD4 cell counts ¹										Unselected CD4 cell counts ²	
	All patients				Black ethnicity only				Other ethnicities only		All patients	
	Ethnicity included		Ethnicity ignored						Ethnicity included			
Change per year: patients of other ethnicities with subtype B												
	-1.23	[-1.27, -1.19]	-1.21	[-1.25, -1.17]	-0.88	[-1.03, -0.73]	-1.23	[-1.27, -1.19]	-1.35	[-1.40, -1.31]		
Difference in change per year: relative to the change in patients of other ethnicities with subtype B												
Subtype A	0.06	[-0.13, 0.25]	0.21	[0.02, 0.39]	0.22	[-0.04, 0.49]	0.03	[-0.22, 0.28]	0.30	[0.10, 0.49]		
AE	-0.03	[-0.26, 0.21]	-0.05	[-0.28, 0.19]	-3.2 ⁴	[-8.6, 2.2]	-0.04	[-0.28, 0.20]	0.03	[-0.23, 0.28]		
AG	0.17	[-0.04, 0.39]	0.34	[0.13, 0.55]	0.26	[-0.02, 0.54]	0.24	[-0.08, 0.55]	0.23	[0.00, 0.46]		
C	0.11	[-0.04, 0.27]	0.32	[0.19, 0.46]	0.32	[0.12, 0.52]	-0.02	[-0.24, 0.20]	0.31	[0.15, 0.47]		
G	0.02	[-0.32, 0.36]	0.19	[-0.14, 0.52]	0.46	[0.06, 0.86]	-0.42	[-0.92, 0.09]	0.00	[-0.37, 0.36]		
Black ethnicity	0.37	[0.25, 0.50]	-		-		-		0.44	[0.31, 0.57]		

¹ Patients aged 16 or over after January 1998 with CD4 cell counts likely to be within the stable phase of chronic untreated HIV infection.

² All CD4 cell counts for patients aged 16 and over after January 1998.

³ Mixed model with a random intercept and random slope for each patient; all cohorts excluding Aquitaine; with intercepts for cohort and with both intercepts and slopes for age, sex, ethnicity, initial HIV RNA, and likely transmission by injection drug use.

⁴ Few patients of black ethnicity had subtype AE – hence this point estimate is unreliable.

Table 5. Initial CD4 cell count and change over time according to HIV-1 viral subtype – results from separate mixed models for black and other ethnicities fit to the original CD4 cell counts with a random intercept and random slope for each patient.

	CD4 cell count			
	Initial ¹ [95% CI]		Change per year ² [95% CI]	
Model for patients of black ethnicity:				
Subtype B	477	[447, 507]	-35	[-42, -28]
Subtype A	392	[353, 430]	-23	[-35, -12]
Subtype AE	348 ³	[173, 523]	-146 ³	[-386, 95]
Subtype AG	398	[361, 435]	-23	[-35, -11]
Subtype C	395	[366, 425]	-19	[-27, -11]
Subtype G	413	[361, 465]	-13	[-31, 5]
Model for patients of other ethnicities:				
Subtype B	492	[469, 516]	-49	[-51, -47]
Subtype A	480	[441, 519]	-49	[-59, -38]
Subtype AE	484	[447, 522]	-50	[-60, -40]
Subtype AG	505	[462, 548]	-41	[-54, -27]
Subtype C	489	[454, 524]	-52	[-62, -43]
Subtype G	468	[406, 530]	-60	[-82, -38]

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5 ¹ Covariate adjustment implies a reference patient treated in a Canadian cohort, male, 35 years old,
6 not infected through injection drug use and with an initial HIV RNA of 4 log 10 copies.
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8 ² For example, these estimates imply that a black reference patient with subtype C and a CD4 cell
9 count of 395 cells/ul would take on average 2.4 years to reach a CD4 cell count below 350 cells/ul
10 $([395-350])/19$, whereas a reference patient of another ethnicity with subtype B and a CD4 cell
11 count of 492 cells/ul would take on average 2.9 years to reach this threshold $([492-350])/49$. Note
12 that according to mixed models for square root CD4 cell count, the first of these two patients would
13 take 1.2 years to reach a threshold of 350 cells/ul while the second patient would take 2.5 years.
14 However, to reach a threshold of 200 cells/ul, the first patient would take 9.3 years while the second
15 patient would take 6.2 years.
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21 ³ Few patients of black ethnicity had subtype AE – hence these point estimates are unreliable.
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Table 6. Full results from the main mixed model for square root CD4 cell decline and from an exponential accelerated failure time (AFT) model for time to initiation of antiretroviral therapy, as separate models and as a joint model (all models without patients from the Aquitaine cohort).

Mixed model	Separate models		Joint model	
	Estimate	95% CI	Estimate	95% CI
Starting CD4 cell count ([cells/ul] ^{1/2})				
Reference patient ¹	21.88	[21.47, 22.29]	22.02	[21.60, 22.43]
Female	-0.20	[-0.49, 0.08]	-0.24	[-0.51, 0.04]
IDU ²	-1.16	[-1.50, -0.82]	-1.12	[-1.49, -0.78]
Age (per 10 years)	-0.19	[-0.29, -0.08]	-0.18	[-0.29, -0.07]
Log ₁₀ HIV RNA (per log copy)	-1.51	[-1.62, -1.40]	-1.51	[-1.62, -1.40]
Cohort (reference ROC ³)				
- EuroSIDA	1.55	[0.88, 2.21]	1.36	[0.69, 2.03]
- HOMER	-0.77	[-1.32, -0.23]	-0.98	[-1.53, -0.43]
- Swiss HIV Cohort Study	1.10	[0.66, 1.55]	0.95	[0.50, 1.39]
- UK CHIC	0.82	[0.41, 1.24]	0.71	[0.29, 1.13]
Subtype (reference B)				
- A	-0.69	[-1.20, -0.18]	-0.63	[-1.12, -0.14]
- AE	-0.24	[-0.88, 0.40]	-0.20	[-0.81, 0.40]
- AG	-0.43	[-0.98, 0.11]	-0.39	[-0.91, 0.13]
- C	-0.78	[-1.18, -0.38]	-0.71	[-1.10, -0.33]
- G ⁴	-0.58	[-1.43, 0.27]	-	-
Black ethnicity	-1.57	[-1.91, -1.23]	-1.60	[-1.94, -1.25]
Rate of CD4 cell count decline ([cells/ul] ^{1/2} /year)				
Reference patient ¹	-1.23	[-1.27, -1.19]	-1.29	[-1.34, -1.25]
Female	-0.08	[-0.19, 0.02]	-0.13	[-0.23, -0.02]
IDU ²	-0.10	[-0.22, 0.02]	-0.16	[-0.28, -0.04]
Age (per 10 years)	-0.09	[-0.13, -0.05]	-0.11	[-0.15, -0.07]
Log ₁₀ HIV RNA (per log copy)	-0.28	[-0.32, -0.23]	-0.30	[-0.34, -0.26]
Subtype (reference B)				
- A	0.06	[-0.13, 0.25]	0.08	[-0.10, 0.26]
- AE	-0.03	[-0.26, 0.21]	-0.01	[-0.24, 0.21]
- AG	0.17	[-0.04, 0.39]	0.17	[-0.04, 0.38]

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- C	0.11	[-0.04, 0.27]	0.14	[0.00, 0.29]
- G ⁴	0.02	[-0.32, 0.36]	-	-
Black ethnicity	0.37	[0.25, 0.50]	0.29	[0.17, 0.42]
<hr/>				
AFT model	Estimate	95% CI	Estimate	95% CI
Reference patient ¹	1.60	[1.44, 1.76]	1.81	[1.62, 2.00]
Female	-0.21	[-0.31, -0.11]	-0.26	[-0.38, -0.15]
IDU ²	0.04	[-0.08, 0.17]	-0.05	[-0.19, 0.10]
Age (per 10 years)	-0.07	[-0.11, -0.03]	-0.11	[-0.15, -0.06]
Log ₁₀ HIV RNA	-0.37	[-0.41, -0.32]	-0.54	[-0.60, -0.48]
Cohort (reference ROC ³)				
- EuroSIDA	0.01	[-0.24, 0.26]	0.05	[-0.23, 0.33]
- HOMER	-0.14	[-0.35, 0.07]	-0.10	[-0.34, 0.14]
- Swiss HIV Cohort Study	-0.10	[-0.28, 0.07]	-0.02	[-0.21, 0.18]
- UK CHIC	0.54	[0.37, 0.71]	0.62	[0.43, 0.81]
Calendar time (per 10 years)	-0.66	[-0.78, -0.53]	-0.34	[-0.47, -0.21]
Black ethnicity	-0.10	[-0.21, 0.02]	-0.33	[-0.47, -0.19]
CD4 (square root)	0.12	[0.11, 0.13]	0.02	[0.00, 0.04]

¹ Covariate adjustment implies a reference patient treated in a Canadian cohort,, male, 35 years old, not infected through injection drug use, with a starting HIV RNA of 4 log 10 copies, infected with subtype B and not of black ethnicity.

² Transmission most likely by injection drug use.

³ Rest of Canada – Calgary, Hamilton, Montreal, Ottawa and Toronto cohorts.

⁴ A joint model including patients with subtype G gave implausible parameter estimates.

Table 7. Clinical outcomes in unselected CD4 cell counts by subtype and ethnicity - median or proportion (n).

		Outcome							CD4 cell count prior to ART initiation (cells/ul)
		Follow up time (years)	AIDS event before starting ART	Died before starting ART	Started ART during follow up	Time to ART initiation (years)			
Subtype A	(566)	2.1	0.13 (75)	0.01 (5)	0.74 (419)	0.77	240		
Subtype AE	(274)	2.0	0.14 (38)	0.00 (0)	0.74 (203)	0.90	220		
Subtype AG	(527)	1.7	0.09 (50)	0.00 (2)	0.70 (368)	0.49	240		
Subtype B	(10787)	2.3	0.11 (1206)	0.00 (53)	0.73 (7870)	1.28	230		
Subtype C	(1351)	1.7	0.14 (187)	0.00 (4)	0.69 (933)	0.46	210		
Subtype G	(177)	1.6	0.08 (14)	0.01 (1)	0.68 (121)	0.44	220		
Black ethnicity	(2086)	1.8	0.13 (275)	0.00 (6)	0.71 (1476)	0.51	220		
Other ethnicities	(10006)	2.3	0.12 (1159)	0.01 (54)	0.71 (7062)	1.27	230		
Missing ethnicity	(1590)	2.1	0.09 (136)	0.00 (5)	0.87 (1376)	1.16	230		
Total	(13682)	2.2	0.11 (1570)	0.00 (65)	0.72 (9914)	1.10	230		

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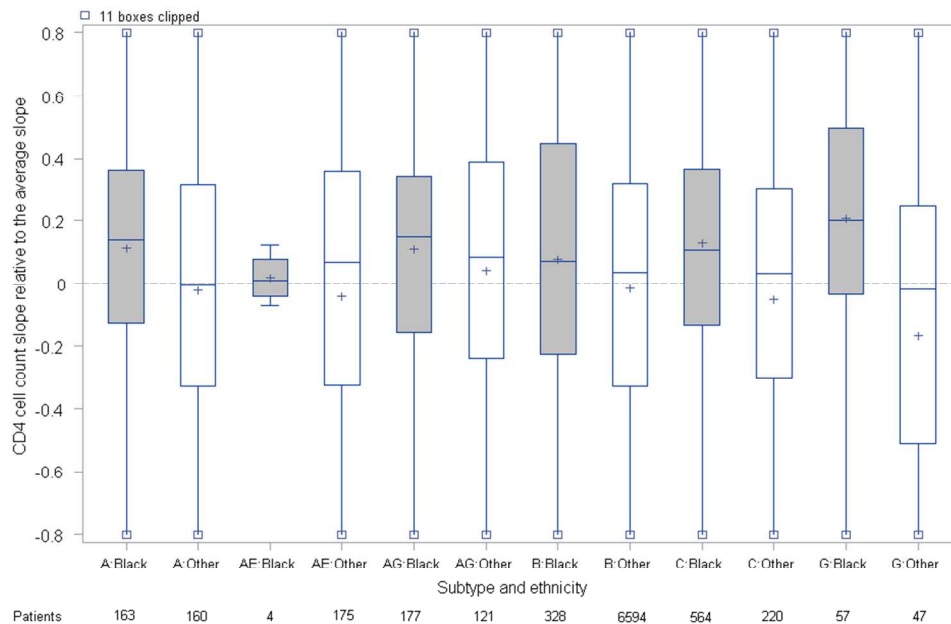


Figure 1a. Estimated CD4 slope decline for each patient. Random effects representing each patient's CD4 cell decline (relative to the average slope) estimated in a mixed model without ethnicity or subtype slope parameters, but adjusted for covariates selected because of their potential effect on initial CD4 cell count (age, sex, initial HIV RNA, transmission by injection drug use (IDU), cohort, ethnicity and subtype) or on its decline (age, sex, initial HIV RNA, IDU). All cohorts included except Aquitaine. The '+' symbol denotes the mean.

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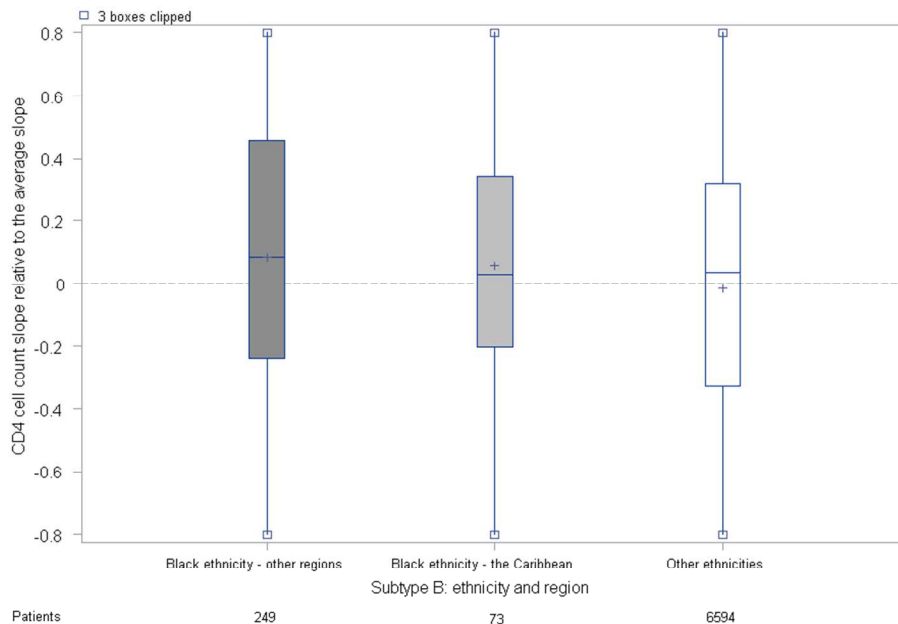


Figure 1b. Estimated CD4 slope decline for each patient with subtype B.

Random effects representing each patient's CD4 cell decline (relative to the average slope) estimated in a mixed model without ethnicity or subtype slope parameters, but adjusted for covariates selected because of their potential effect on initial CD4 cell count (age, sex, initial HIV RNA, transmission by injection drug use (IDU), cohort, ethnicity and subtype) or on its decline (age, sex, initial HIV RNA, IDU). All cohorts included except Aquitaine. The '+' symbol denotes the mean.

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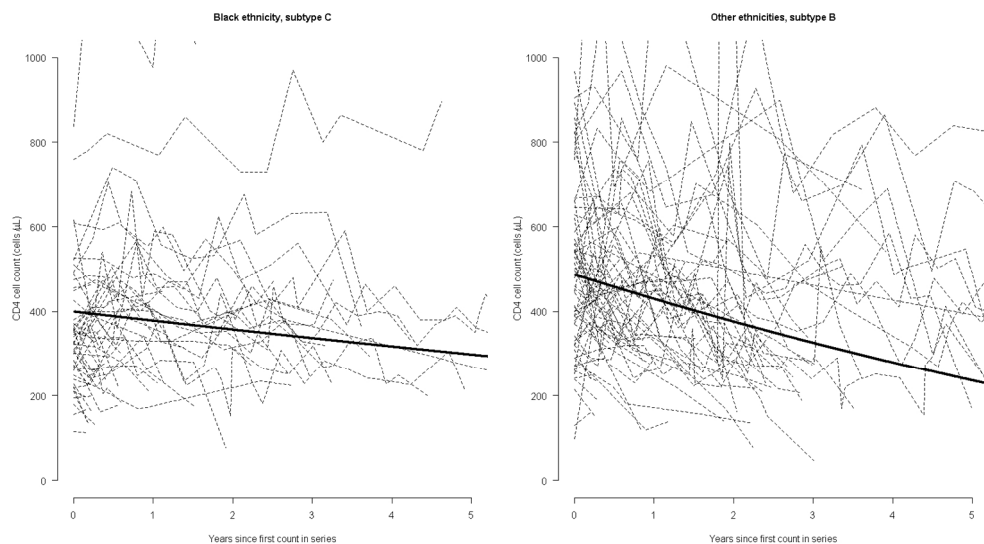


Figure 2. Estimated average CD4 cell count over 5 years.

The average CD4 cell count estimated for patients of black ethnicity with subtype C and for patients of other ethnicities with subtype B. Estimates were made using separate mixed models, for black or other ethnicities respectively, and represent CD4 cell counts for a male patient, not infected by injection drug use (IDU) and with age and initial HIV RNA set at the median for that ethnicity and subtype. Each mixed model was adjusted for covariates selected because of their potential effect on initial CD4 cell count (age, sex, initial HIV RNA, IDU, cohort and subtype) or on its decline (age, sex, initial HIV RNA, IDU and subtype). Patient CD4 cell trajectories are shown for a 10% sample of those of black ethnicity with subtype C and for a 1% sample of those of other ethnicities with subtype B to illustrate the variability of CD4 cell counts both between patients and over time.

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3 **Appendix. Membership of contributing cohorts.**
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21 Julia Zhu, Susan Shurgold; Statisticians: Viviane Lima, Zishan Cui, Jeong Min, Wendy Zhang,
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23 Keith Chan.
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28 **Southern Alberta Clinic Cohort**, Calgary Alberta. John Gill
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31 **Toronto General Hospital Cohort**, Toronto Ontario. Sharon Walmsley and Darrell Tan.
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34 **Ottawa General Hospital**, Ottawa, Ontario. Curtis Cooper
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27 Leen, A Wilson); North Bristol NHS Trust (M Gompels, S Allan); University of Leicester NHS
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obtaining subtype data.

STROBE Statement: The effects of HIV-1 subtype and ethnicity on CD4 decline in antiretroviral naïve patients: a Canadian-European collaborative cohort study

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract In title
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found Done
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Introduction - second paragraph
Objectives	3	State specific objectives, including any prespecified hypotheses Introduction - final paragraph
Methods		
Study design	4	Present key elements of study design early in the paper Methods - first paragraph; Table 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Methods - second and third paragraphs
Participants	6	<i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Table 1 - references given for each contributing cohort
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Methods - first paragraph; Statistical Analysis - first paragraph
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Methods - second paragraph; Table 1
Bias	9	Describe any efforts to address potential sources of bias Methods - third paragraph; Statistical Analysis - second paragraph
Study size	10	Explain how the study size was arrived at Results - first paragraph
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Not relevant
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding Statistical Analysis - first and second paragraphs
		(b) Describe any methods used to examine subgroups and interactions Statistical Analysis - second paragraph
		(c) Explain how missing data were addressed Results - third paragraph; Table 3
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed Not applicable

(e) Describe any sensitivity analyses

[Statistical analysis - second paragraph](#)

Results

Participants 13* (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed

[Results - first and third paragraphs](#)

(b) Give reasons for non-participation at each stage

[Results - first and third paragraphs](#)

(c) Consider use of a flow diagram

Descriptive data 14* (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders

[Table 2](#)

(b) Indicate number of participants with missing data for each variable of interest

[Tables 2 and 3](#)

(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)

[Results - first paragraph; Table 2](#)

Outcome data 15* *Cohort study*—Report numbers of outcome events or summary measures over time

[Table 7](#)

Main results 16 (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

[Statistical Analysis - first paragraph; Table 4](#)

(b) Report category boundaries when continuous variables were categorized

[Not applicable](#)

(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

[Not relevant - however untransformed results presented in Table 5 for ease of interpretation](#)

Other analyses 17 Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

[Results - paragraphs four, five and six](#)

Discussion

Key results 18 Summarise key results with reference to study objectives

[Discussion - first paragraph](#)

Limitations 19 Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias

[Limitations](#)

Interpretation 20 Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

[Conclusions](#)

Generalisability 21 Discuss the generalisability (external validity) of the study results

[Limitations](#)

Other information

Funding 22 Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

[Financial Disclosure statement](#)