Worldwide HIV Virulence Evolution in Response to Changes in Prevalence and Treatment Coverage

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Abstract

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Introduction

Whether worldwide HIV virulence has been increasing, decreasing, or remaining constant through time is still debated. Modeling work has suggested that prevalence and treatment coverage within countries may impact HIV virulence evolution at the population level, but these factors have not yet been considered in data analyses of HIV virulence changes. Additionally, disparities in HIV burden, including in prevalence and treatment coverage, exist between black and white men who have sex with men (MSM) in the US and worldwide. If differences in prevalence and treatment coverage impact mean population HIV virulence, this impact could be seen in disparities in virulence levels between these groups.

Methods

I utilized the NESCENT-CASCADE dataset, which combines data from 32 HIV cohorts representing individuals from 184 countries around the world. This contained individual-level HIV virulence marker data as well as other individual-level covariates. Prevalence data came from the Institute of Health Metrics and Evaluation and treatment coverage data from the World Bank. Because different treatment initiation guidelines would likely affect virulence evolution

differently, these analyses only consider the time period in which treatment was based on CD4 guidelines. I utilized a multilevel modelling approach to allow for random effects at the country level and also utilized univariate and multivariable linear regression analyses to examine differences in HIV virulence with prevalence and treatment coverage differences in heterosexuals. As prevalence and treatment coverage were assessed on the country level, they are more likely to be representative of rates in the heterosexual population than the MSM population, given the much larger size of the former. Therefore, we only included heterosexuals in these analyses. I utilized linear regression and a Welch two-sample t-test to examine differences in virulence between black and white MSM in the US and Europe.

Results

The proportion of variance attributable to the country of origin was so small that it indicated that this effect was not meaningful. Higher prevalence was significantly associated with higher virulence in both univariate and multivariable analyses ($p<0.001$). Higher CD4-based treatment coverage was also significantly associated with lower virulence level in the multivariable analysis ($p=0.001$). No differences in virulence marker level were found between black and white MSM ($p=0.556$) but slight differences in virulence change though time were seen.

Conclusion

Consistent with previous modeling findings, a comparative analysis of 32 HIV cohorts finds that prevalence and treatment coverage both impact HIV virulence at the population level in heterosexuals. These factors should be considered when examining virulence levels through time. More data should be analyzed to determine if this effect extends to other groups, such as MSM.

INTRODUCTION

Research has yielded conflicting reports of whether worldwide HIV virulence has been increasing [1, 2], decreasing [3, 4], or remaining stable [5, 6] through time. These conflicts may be in part due to the heterogeneous environments in which the virus is evolving. Modeling work has shown that factors such as anti-retroviral therapy (ART) coverage [4, 7] and prevalence [8] may influence HIV virulence evolution. However, to my knowledge at the time of this writing no empirical analysis has included these predictors in comparing virulence evolution through time across populations.

HIV virulence is likely to evolve in response to increasing ART coverage in distinct ways depending on the treatment scheme and the environment in which transmissions take place. The current WHO recommendation, made in 2015, is to initiate ART as soon as HIV is diagnosed (test-and-treat [9]). My recent modeling work has suggested that, with test-and-treat, HIV virulence may decrease with higher ART coverage [10], although this depends on model assumptions and the population of interest, and is in contrast to previous literature on the impact of test-and-treat scale up on HIV virulence [7, 11, 12].

Prior to test-and-treat, the WHO recommendation was to treat individuals based on their CD4 counts [13]. Modeling work has suggested that with CD4-based treatment initiation schemes, HIV virulence may remain stable [7, 14] or decrease [4] with higher ART coverage. Evolutionary arguments can be made in favor of either virulence decrease or stability in the presence of CD4-based treatment coverage. Because more virulent viruses lead to lower CD4 levels in their hosts faster than less virulent viruses [15], Payne et al's [4] model suggests that, in a CD4-based treatment scheme, individuals with highly virulent strains of HIV would be treated sooner than individuals with less virulent strains. When an individual becomes virally suppressed, they cease to be able to transmit the virus onward. Therefore, highly virulent strains would be unable to transmit more often than less virulent strains, leading to decreasing virulence in the overall population. In contrast, Herbeck et al. [7] found in their modeling study that this effect was balanced by the highly virulent viruses' greater chance of transmission before treatment initiation.

HIV virulence is also likely to be affected by the overall prevalence levels within its environment. Goodreau et al.'s modeling study [8] found that simulations with higher prevalence values also had higher virulence levels, although this effect was non-linear. One likely explanation for this pattern is that with high prevalence there are fewer susceptible individuals in a population. This shifts the evolutionary landscape so that a highly virulent virus' increased transmission probability is advantageous enough to offset the decreased host lifespan, and overall mean population virulence levels will increase over time.

HIV may also evolve differently in men who have sex with men (MSM) and heterosexual populations. Anal intercourse has a higher transmission probability than vaginal [16]. Relationship patterns are also different between MSM and heterosexuals [17]. Changes in relationship patterns and probability of transmission may change the evolutionary environment and shift the optimal virulence of HIV depending on the groups in which the majority of infections in a country occur.

Large disparities in HIV burden exist between black and white MSM worldwide [18]. In the US, black gay and bisexual men made up 37% of HIV diagnoses among all gay and bisexual men in 2017 [19]. This disparity is further illustrated in Atlanta, GA, where prevalence is 43% in black MSM and 13% in white MSM [20]. Treatment coverage disparities are also present between these groups [21, 22]. In the HIV Outpatient Study, fewer black men than white men had achieved viral suppression (72% and 91% respectively) [22]. If prevalence and treatment coverage affect virulence evolution, virulence differences may be apparent when examining patterns of virulence through time between these groups.

Combined together, these various lines of modeling work suggest that there may be multiple different reasons why empirical populations appear to be undergoing a range of changes in virulence with time. Comparisons of multiple populations in terms of these various predictive factors and the observed changes in virulence over time within them may help to determine the role each seems to be playing in practice. In this analysis, I address three questions about HIV virulence, prevalence, and treatment coverage: (1) does HIV prevalence impact the virulence of the viruses that individuals acquire? (2) does treatment coverage impact the virulence of viruses that individuals acquire? and (3) is the large disparity in HIV burden between black and white MSM reflected in the virulence of viruses men acquire?

METHODS

HIV Virulence Measure

The outcome in each analysis is HIV virulence. The measure of virulence used here will be set point viral load (SPVL): the viral load (VL) that occurs at the beginning of the chronic phase of HIV, after the high peak in VL during the acute phase has stabilized. SPVL is a good proxy for HIV virulence, as high SPVLs are associated with fast disease progression, as well as high risk of transmission [23]. SPVL is generally measured on the $\log_{10}$ scale, and differences in SPVL as small as 0.3 $\log_{10}$ copies/mL are associated with faster progression to AIDS [23].

Study Population

The NESCent-CASCADE dataset (Table 1) combines 32 HIV cohorts from around the world. It consists of patients from 184 different countries who are over the age of 15, with ART-naïve CD4 or VL data, and with a known date of seroconversion based on either an HIV-positive antibody test taken within three years of a documented negative antibody test or laboratory evidence for recent seroconversion (real-time PCR positivity or incomplete Western blot). Seroconversion dates range from 1981-2014. Country of origin information is also known, and here refers to patients' original country of origin, not the location of the cohort study that they were enrolled in. Risk group information is available for MSM, heterosexuals, and persons who inject drugs (PWID). Derived variables include the date of seroconversion (based on laboratory evidence, reported seroconversion illness, or the midpoint of last negative and first positive tests) and SPVL (calculated as the mean of $\log_{10}$ VL data points between one and three years after seroconversion, excluding points after treatment initiation).

Table 1: Derivation of sample

Variable (nested)	**n**		**%**
NESCENT-CASCADE total	32874		-
With SPVL	17177	/ 32874	52.3
SPVL & Prevalence Analysis and SPVL & Treatment Coverage Analysis			
With seroconversion after 1990	16289	/17177	94.8
With seroconversion before 2011	15394	/16289	94.5
With country of origin	10234	/15394	66.5
SPVL & Prevalence Analysis			
With country-specific prevalence data	9898	/10234	96.7
In heterosexual risk group	2896	/9898	29.3
SPVL & Treatment Coverage Analysis			
With country-specific treatment coverage data	7336	/10234	71.7
In heterosexual risk group	2107	/7336	28.7
SPVL in Black & White MSM Analysis			
With seroconversion before 2011	16282	/17177	94.8
In Europe or the United States	11219	/16282	68.9
In MSM risk group	7869	/11219	70.1
With black or white race*	2226	/7869	28.3

***** The low proportion here reflects that many cohorts did not report participant race.

In order to facilitate comparison with prevalence and treatment coverage data (described below), only those patients with seroconversion date after 1990 will be included in those analyses. All three analyses will examine only those seroconversions occurring before 2011, prior to the HPTN 052 trial's announcement of the role of ART in the reduction in transmission of HIV between partners in serodiscordant relationships [24]. Treatment based on CD4 guidelines would no longer have been universal after that time, as PEPFAR, the US Department of Health

and Human Services, and the World Health Organization (WHO) then amended their treatment recommendations regarding those in serodiscordant relationships [25]. As different treatment initiation guidelines would likely affect SPVL evolution differently, these analyses only consider the time period in which treatment was based on CD4 guidelines.

Prevalence and Treatment Coverage Data

Predictors in the first two analyses included country- and year-specific prevalence and treatment coverage data. Annual prevalence estimates for the period 1990-2017 were obtained from the Global Burden of Disease Study [26], via the Institute of Health Metrics and Evaluation (IHME) online data center. Prevalence was measured as cases/100,000 total population. There were 85 countries represented in this dataset that were also found in the NESCENT-CASCADE dataset.

Annual ART coverage estimates (% of persons living with HIV (PLWH) on ART) between 1990-2017 were obtained from the World Development Indicators dataset [27], through the World Bank data center. This dataset covers many countries, especially those in the developing world, but does not include information on all countries globally. Notably, data on ART coverage in the United States were not present in the World Bank dataset, nor in similar large datasets, including WHO, AVERT, AIDSvu, or UNAIDS. Data from the CDC on linkage to care, receipt of HIV medical care, and viral suppression in the United States were available between 2010-2015 [28]; however, as there were only three cases in the study population that occurred in individuals of United States origin with seroconversion during or after 2010, the United States was not included in the analysis of ART coverage and SPVL. There were 69 countries represented in the World Bank dataset also found in the NESCENT-CASCADE dataset.

Risk-group specific prevalence or ART coverage data were not available in sufficient detail (country- and year- specific rates for countries in this analysis) in order to analyze MSM data separately from heterosexuals. Country-level prevalence and ART coverage data was more likely to be representative of rates in heterosexuals than in MSM given the much larger size of the former population. Although heterosexuals made up a smaller proportion of infections in our dataset than MSM, they comprise a large majority of the denominator in country-wide assessments of prevalence and treatment. This makes these assessments better reflections of the environments in which heterosexuals become infected than they are of MSM environments. Therefore, only heterosexuals were included in the analysis of the effects of prevalence and treatment coverage.

Statistical Analysis

SPVL Differences with Prevalence and Treatment Coverage

In order to estimate differences in SPVL with prevalence or treatment coverage changes, I used multilevel models (specifically linear mixed effects models) of the association between individual-level SPVLs (dependent) and country-level estimated prevalence or ART coverage (independent). Covariates included individual-level sex and age at seroconversion, as these have both been shown to impact SPVL [29, 30]. This model allowed for homogeneity within countries and heterogeneity across countries: I hypothesized that individuals within a country would be more alike in unmeasured factors that impact SPVL, such as relationship duration or the likelihood of concurrent relationships [31]. I used a random intercepts model, which allows for country-level differences in the starting virulence level. This approach contrasts with a random slopes model, which implies that there would be country-level effects on the relationship between SPVL and prevalence or SPVL and treatment coverage. The random intercepts model took this form:

$$Y_{ij} = \gamma_{0j} + X\beta + \varepsilon_{ij}$$

where Y_{ij} is the value of the outcome variable for a particular case with individual *i* and country *j*. γ_{0j} represents the random intercept with:

$$\gamma_{0j} = \beta_0 + \alpha_{0j}, \alpha_{0j} \sim N(0, \sigma_\alpha^2)$$

where α_{0j} represents the country-level impact on the intercept. X represents the fixed covariates (X_1 = sex, X_2 = age, X_3 = country-specific prevalence or treatment coverage) that impact the slope of the equation (β), and

$$\varepsilon_{ij} \sim N(0, \sigma^2)$$

gives the individual-level randomness. This model was implemented in the lme4 R package. I performed an additional ANOVA analysis to determine p-values for the fixed covariates.

The proportion of random effect variance attributable to country in either analysis was so small (see results) that it indicated that this effect was not meaningful and random effects were not present here, so the linear mixed modeling approach suggested by my hypothesis was not appropriate [32]. As this was the case, I removed the random effects from the model and utilized a linear regression analysis instead.

I performed univariate and multivariable linear regression of the association between individual-level SPVLs (dependent) and country-level estimated prevalence or ART coverage

(independent) and the same covariates of individual-level sex and age at seroconversion as in the previous model.

SPVL Differences between Black and White MSM

I assessed differences in mean population SPVL (MPSPVL) between black and white MSM from US and Europe through time. Only the US and Europe were included in this analysis due to the paucity of data from black MSM of other regions of origin in this dataset. I performed a linear regression analysis of the association between MPSPVLs (dependent) and year of seroconversion, race, and their interaction (independent) to assess differences through time, and a Welch two-sample t-test between SPVLs (dependent) and race (independent) to assess overall MPSPVL differences.

In all analyses, statistical associations were performed using R 3.6.0. Statistical significance was determined at the p=0.05 level. Confounding was determined by comparing estimated measures of association before and after adjusting for potential confounding factors; a factor was considered a confounder if the difference between the measures was 10% or greater.

RESULTS

The demographic characteristics of the study population are presented in Table 2. The first two analyses examined heterosexuals with countries of origin found in the different datasets relevant to each analysis, while the third analysis examined MSM in the US and Europe. Therefore, all samples are presented separately.

Table 2: Summary of subject characteristics by analysis

	SPVL & Prevalence Analysis		SPVL & Treatment Coverage Analysis		SPVL in Black & White MSM Analysis	
N	2897		2107		2226	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
SPVL	4.137	0.856	4.173	0.860	4.354	0.714
Age at Seroconversion	**n**	**%**	**n**	**%**	**n**	**%**
>20	83	2.9	61	2.9	25	1.1
20-29	1097	37.9	813	38.6	719	32.3
30-39	983	33.9	696	33.0	930	41.8
40-49	466	16.1	334	15.9	407	18.3
50+	268	9.3	203	9.6	145	6.5
Ethnic Group	**n**	**%**	**n**	**%**	**n**	**%**

	Asian	14	0.5	9	0.4	-	-
	Black	643	22.2	630	29.9	51	2.3
	Hispanic	1	<00.1	0	0.0	-	-
	White	486	16.9	250	11.9	2175	97.7
	Unknown	1753	60.5	1218	57.8	-	-
Year of Seroconversion		**n**	**%**	**n**	**%**	**n**	**%**
	1984-1989	-	-	-	-	453	20.4
	1990-1994	101	3.5	0	0.0	149	6.7
	1995-1999	536	18.5	0	0.0	219	9.8
	2000-2004	1035	35.7	974	46.2	397	17.8
	2005-2011	1225	42.3	1133	53.8	1008	45.3
Sex		**n**	**%**	**n**	**%**	**n**	**%**
	Male	1124	38.8	805	38.2	2226	100
	Female	1773	61.2	1302	61.8	-	-

SPVL Differences with Prevalence

In the SPVL and prevalence analysis, the multilevel model found that the proportion of random effect variance attributable to country was 1.18%. This indicated that no meaningful country-level effects on SPVL were present. Male sex, higher age at seroconversion, and higher prevalence were significantly associated with higher SPVL (Table 3a). The positive change in SPVL with increasing prevalence can be seen in Figure 1.

Table 3: Linear mixed model and linear regression analyses of the relationship between prevalence and SPVL. 3a: Linear mixed model. 3b: Univariate linear regression model. 3c: Multivariable linear regression model.

a			
Random Effects			
	Variance	SD	
Country	0.008	0.091	
Residual	0.691	0.831	
Fixed Effects			
	Estimate	Std Error	Significance
Intercept	3.665	0.062	-
Sex (ref. = female)	0.278	0.034	<0.001
Age at Seroconversion	0.009	0.002	<0.001
Prevalence	3.177	0.783	<0.001
b			
	Estimate	Std Error	Significance
Intercept	4.099	0.018	<0.001
Prevalence	2.039	0.490	<0.001
c			
	Estimate	Std Error	Significance

Intercept	3.678	0.056	<0.001
Sex (ref. = female)	0.287	0.034	<0.001
Age at Seroconversion	0.008	0.002	<0.001
Prevalence	3.273	0.491	<0.001

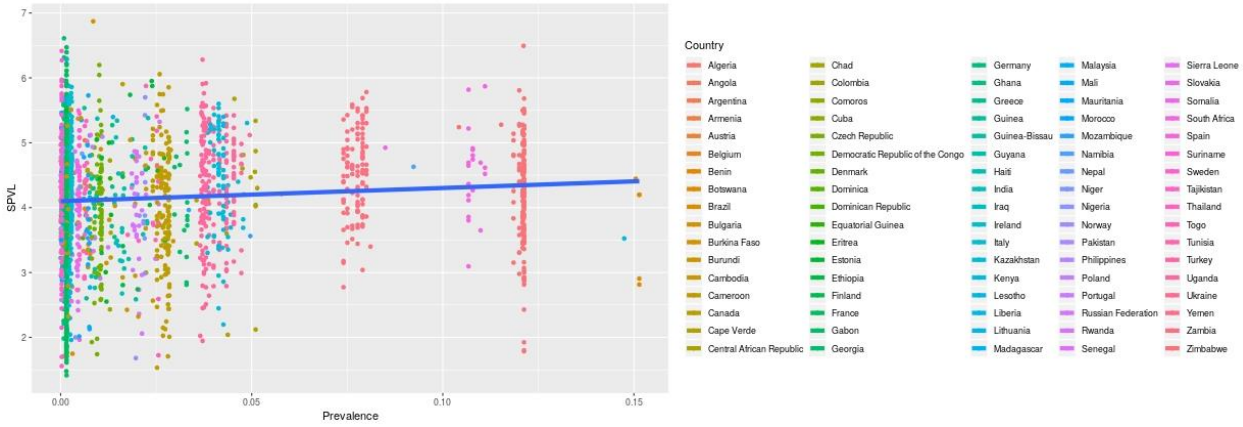

Figure 1: Relationship between SPVL and prevalence by country. The blue line shows the regression line for the relationship.

In the univariate linear regression analysis, we found a significant association between higher prevalence and higher SPVL (Table 3b). We also found a significant positive association in the multivariable linear regression analysis (Table 3c). Both male sex and higher age at seroconversion were also significantly associated with higher SPVL.

SPVL Differences with Treatment Coverage

In the SPVL and treatment coverage analysis, the multilevel model found that the proportion of random effect variance attributable to country was 3.46%. Again, this indicated that meaningful effects on SPVL were not found at the country level. While a significant association between treatment coverage and SPVL was not found here, SPVL was significantly positively associated with male sex and higher age at seroconversion (Table 4a). Changes in SPVL with increasing treatment coverage are shown in Figure 2.

Table 4: Linear mixed model and linear regression analyses of the relationship between prevalence and SPVL. 4a: Linear mixed model. 4b: Univariable linear regression model. 4c: Multivariable linear regression model.

a

Random Effects		
	Variance	SD
Country	0.025	0.157
Residual	0.691	0.831

Fixed Effects			
	Estimate	Std Error	Significance
Intercept	3.762	0.075	-
Sex (ref. = female)	0.300	0.041	<0.001
Age at Seroconversion	0.009	0.002	<0.001
Treatment Coverage	-0.104	0.154	0.499
b			
	Estimate	Std Error	Significance
Intercept	4.185	0.031	<0.001
Treatment Coverage	-0.033	0.068	0.631
c			
	Estimate	Std Error	Significance
Intercept	3.868	0.062	<0.001
Sex (ref. = female)	0.307	0.040	<0.001
Age at Seroconversion	0.008	0.002	<0.001
Treatment Coverage	-0.226	0.069	0.001

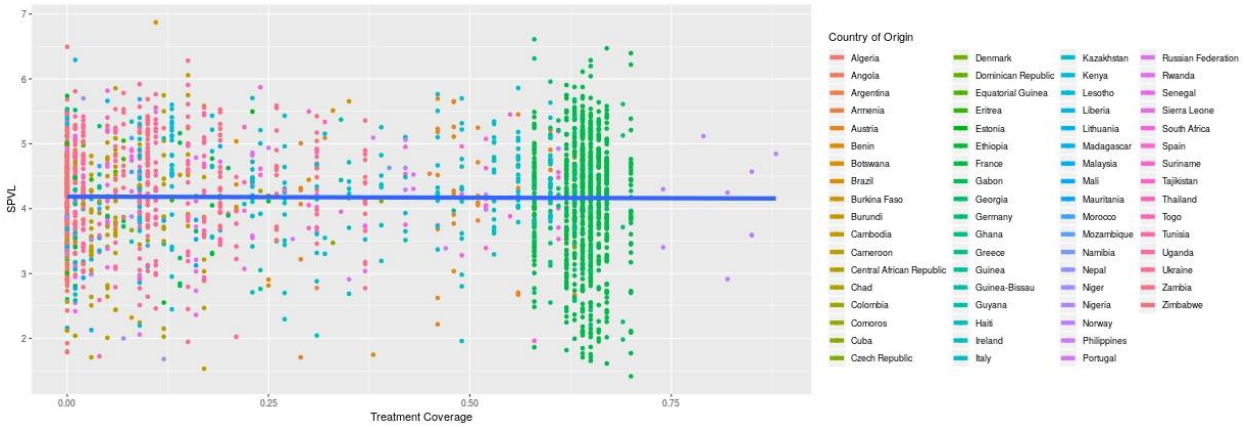

Figure 2: Relationship between SPVL and treatment coverage by country. The blue line shows the regression line for the relationship.

In the univariate linear regression analysis, we did not find a significant association between treatment coverage and SPVL (Table 4b). However, in the multivariable linear regression analysis, higher treatment coverage was significantly associated with lower SPVL (Table 4c). This suggests that the relationship between treatment coverage and SPVL is confounded by sex and age at seroconversion. SPVL was also significantly positively associated with both male sex and higher age at seroconversion.

SPVL Differences between Black and White MSM

We found a suggestion of a difference in trends in SPVL through time between black and white MSM (Table 5), although this was not significant. The change in SPVL in black and white MSM over time is shown in Figure 3a. In the regression analysis, when accounting for year of seroconversion and the interaction of seroconversion year and race, white MSM had lower SPVLs, although this was not significant. No significant differences in overall SPVL between black and white MSM were found, with mean SPVLs of 4.31 (95% CI 4.15, 4.47) and 4.36 (95% CI 4.33, 4.39) in black and white men respectively (p=0.556). Overall SPVL differences between black and white MSM are shown in Figure 3b.

Table 5: Linear regression analysis of the differences in SPVL between black and white MSM through time

	Estimate	Std Error	Significance
Intercept	4.516	0.185	<0.001
Year of Seroconversion*	-0.014	0.011	0.181
Race (ref. = black)	-0.250	0.189	0.186
Interaction between Year of Seroconversion and Race	0.019	0.011	0.072

***** Year of seroconversion was entered into the model with 1984 (the earliest seroconversion year) as 1, 1985 = 2, etc. to aid in parameter interpretability

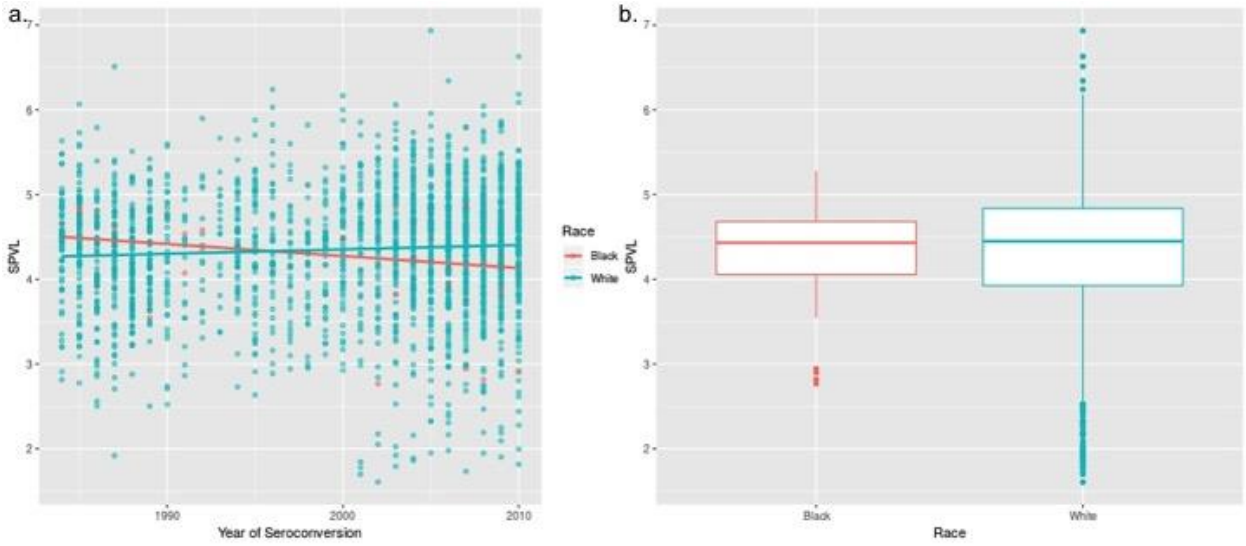

Figure 3: SPVL and race. 3A: The relationship between SPVL and year of seroconversion. Lines are the regression lines by race. This suggests there may be a difference in trends through by race, although this difference is not statistically significant. 3B: Boxplot of SPVL by race. Mean SPVL in black men = 4.31 (95% CI 4.15, 4.47) and mean SPVL in white men = 4.36 (95% CI 4.33, 4.39).

DISCUSSION

In this analysis, I explored potential hypotheses about the associations between SPVL and prevalence or treatment coverage in order to determine if these factors may partially explain the large amount of variability between estimates of HIV virulence through time in different populations, as previous modeling work has predicted. These results show that prevalence within a country is indeed predictive of incident SPVL so that individuals who become infected in higher prevalence countries will be more likely to acquire viruses with higher SPVLs. This relationship is complicated by the fact that prevalence level was highly, though not entirely, determined by country. However, this still supports modeling work that showed differences in SPVL evolution with prevalence level [31, 33]. This also suggests that future work examining HIV virulence evolution should consider changing prevalence levels as a predictor of virulence.

The results further show that treatment coverage under CD4-based treatment initiation programs may also affect SPVL, as the multivariable analysis showed a small decrease in SPVL with higher treatment coverage. This effect was not seen in the univariate analysis, suggesting that not accounting for sex or age at seroconversion suppressed this relationship. These results are in agreement with data from the Rakai Community Cohort Study. Here, treatment was administered using CD4-based initiation guidelines and SPVL decreased with date of seroconversion (−0.022 log10 copies/mL per year after adjusting for other covariates, CI −0.04; −0.002) [14]. This relationship contrasts slightly with previous modeling work that showed no changes in SPVL evolution with most CD4-based treatment initiation schemes, although some small decreases in virulence were seen with higher treatment coverage in other scenarios in the same analysis [7].

These results showed that there were no discernable country-level effects on SPVL. This conflicted with the hypothesized impacts of unmeasured factors, such as relationship duration or concurrency level, that are more likely to be similar for individuals within the same country than in different countries. However, the small amounts of variance attributable to the country showed that either this was not the case here or that the assumption that countries were a good proxy for behavioral homogeneity was incomplete.

I also examined potential differences SPVL between black and white MSM in the US and Europe both in absolute terms and through time. While there were not significant differences in SPVL by race, there was a suggestion that SPVL may be changing through time differently in black and white MSM. This may reflect how disparities in prevalence and treatment coverage between these groups further change the evolutionary environment for HIV, causing it to evolve differently over time in each group. However, this analysis was constrained by the small number of black MSM in this dataset.

This project has several limitations. The prevalence and ART uptake data only extend to 1990 and likely predominantly reflect the environments in which heterosexual infections were acquired. While extensive, the NESCent-CASCADE dataset is missing SPVL, country-specific origin data, and race for many individuals, limiting the numbers available for this analysis. Restricting the first two analyses to heterosexuals further limited the available data. Country of origin data may also not capture where an individual was at the time of their seroconversion. VL measurements in the NESCent-CASCADE dataset span decades and countries, making complete comparability of VL measurement methods unlikely. A fairly wide window of time was allowed in estimating seroconversion date (up to 3 years between positive and negative HIV tests). This makes seroconversion dates and ages less precise and more open to misclassification.

Using country-specific prevalence and ART coverage data represent county level averages and may not capture the actual environment in which individual infections were acquired. However, heterogeneity in transmission environments that is not captured in the country-wide data should, if anything, make our results conservative. In addition, data on prevalence and ART coverage are limited by the availability of health data in developing countries and may not be complete. ART coverage data were not available for all countries represented in the NESCent-CASCADE dataset, and were not available mainly for high-income countries instead of being missing randomly. By missing more high-income countries, where the majority of infections occur in MSM, we may be mainly assessing trends of treatment coverage and SPVL in heterosexual epidemics. This will limit the generalizability of our results to countries with heterosexual epidemics.

In conclusion, this analysis suggests that prevalence and treatment coverage within a country may impact HIV virulence levels in that country. Viral evolution may work to amplify ongoing prevalence changes: in an expanding epidemic, prevalence increases could coincide with parallel virulence increases, increasing the transmission rate and fueling higher prevalence. In contrast, once prevalence begins to decline, corresponding virulence declines will magnify the effect. In the past, increasing CD4-based treatment coverage may have had a similar amplifying effect where increasing treatment coverage both decreased new infections by preventing transmission directly, as well as by reducing viral virulence and thus transmission rate. Future work continuing to examine this question with more complete data, in MSM, and with modern test-and-treat approaches is necessary to determine if these effects extend further and to all communities.

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