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HIV-1 Transmission During Recent Infection and During Treatment Interruptions as Major Drivers of New Infections in the Swiss HIV Cohort Study

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Background. Reducing the fraction of transmissions during recent human immunodeficiency virus (HIV) infection is essential for the population-level success of "treatment as prevention".

Methods. A phylogenetic tree was constructed with 19 604 Swiss sequences and 90 994 non-Swiss background sequences. Swiss transmission pairs were identified using 104 combinations of genetic distance (1%–2.5%) and bootstrap (50%–100%) thresholds, to examine the effect of those criteria. Monophyletic pairs were classified as recent or chronic transmission based on the time interval between estimated seroconversion dates. Logistic regression with adjustment for clinical and demographic characteristics was used to identify risk factors associated with transmission during recent or chronic infection.

Findings. Seroconversion dates were estimated for 4079 patients on the phylogeny, and comprised between 71 (distance, 1%; bootstrap, 100%) to 378 transmission pairs (distance, 2.5%; bootstrap, 50%). We found that 43.7% (range, 41%–56%) of the transmissions occurred during the first year of infection. Stricter phylogenetic definition of transmission pairs was associated with higher recent-phase transmission fraction. Chronic-phase viral load area under the curve (adjusted odds ratio, 3; 95% confidence interval, 1.64–5.48) and time to antiretroviral therapy (ART) start (adjusted odds ratio 1.4/y; 1.11–1.77) were associated with chronic-phase transmission as opposed to recent transmission. Importantly, at least 14% of the chronic-phase transmission events occurred after the transmitter had interrupted ART.

Conclusions. We demonstrate a high fraction of transmission during recent HIV infection but also chronic transmissions after interruption of ART in Switzerland. Both represent key issues for treatment as prevention and underline the importance of early diagnosis and of early and continuous treatment.

Keywords. HIV recent (early) infection; treatment as prevention; treatment interruptions; HIV transmission; endgame.

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Human immunodeficiency virus (HIV) remains an immense public health threat, with a global prevalence of 35.3 million infected individuals in 2013 [1]. Whereas in most high-income countries the incidence of male-female transmission has been stable or decreasing, the incidence of male-male transmission is rising or remains high [2]. In this context, one pivotal question is

the relative contribution of the early and chronic disease phases to HIV transmission. Previous studies have shown discrepant estimations of the fraction of transmissions attributable to recent infection ranging from <10% [3] to 70%–80% [4].

Knowing the burden of early-phase transmission is important for public health policy, especially in the context of the latest efforts to introduce immediate and early antiretroviral therapy (ART)—that is, "treatment as prevention" (TasP)—as one of the main global containment strategies of the HIV pandemic [5]. A growing body of evidence suggests that once an HIV-positive individual is diagnosed and successfully treated with ART, the hazard of onward transmission drops dramatically [6,7].

A high proportion of recent-phase HIV transmissions will compromise the effectiveness of TasP for several reasons. First, a substantial fraction of recently infected patients are still unaware of their HIV-positive status and thus remain untreated and infectious [8]. Secondly, infectiousness during primary HIV-infection has been estimated to be up to 26 times higher than during later (pre-AIDS) stages of the infection [9], which is further supported by a higher HIV-1 concentration in semen [10]. Finally, ongoing transmission of drug-resistant viral variants by patients unaware of their infection may compromise the effectiveness of ART [11].

In this work, we addressed this question by retrospectively analyzing transmission pairs from the unique data from the Swiss HIV Cohort Study (SHCS), the associated drug resistance database, and the Zurich Primary HIV Infection Study (ZPHI). The aims of this study were to determine the fraction of HIV transmissions that occurs during recent infection and to evaluate HIV transmission in relation to the timing of ART initiation.

METHODS

Study Population: SHCS Drug Resistance Database and ZPHI

The SHCS is a large prospective multicentered, study established in 1988 [12]. During the biannual outpatient follow-up visits, extensive clinical and demographic data are collected for each participant. The drug resistance database contains HIV sequences for approximately 60% of the patients in the SHCS. The SHCS is highly representative of the HIV epidemic in Switzerland, with an estimated coverage of ≥45% of all HIV cases, 69% of all patients with AIDS in Switzerland and 72% of all ART-treated individuals [12]. The ZPHI [13, 14], specifically enrolls patients with documented acute or recent primary HIV-1 infection.

Phylogenetic Tree Construction

A total of 19 604 partial *pol* sequences from 10 970 SHCS cohort participants (40% of patients had ≥2 sequences) were pooled with 90 994 background sequences from the Los Alamos database. The phylogenetic tree was generated (see Supplementary Text 2 for details) with FastTree software (version 2.1.7, SSE3, OpenMP) [15] using a generalized time-reversible model. Support values for internal nodes were derived based on 100 bootstrapped trees. With use of the R package APE (version 3.1) [16] and custom scripts, potential transmission pairs were identified as monophyletic pairs if their genetic distance and bootstrap support values met the predefined thresholds of 104 combinations of genetic distance (1%, 1.5%, 2%, and 2.5%) and bootstrap (50%–100% in 2% increments) support. This was done to estimate the effect of various transmission cluster definitions on the dependent variable, because there is no consensus on optimal thresholds [17].

Estimation of Infection Dates

Seroconversion dates were estimated based on a hierarchical algorithm (Figure 1; see Supplementary Text 1 for detailed description), which relied on participation in the ZPHI, immunological markers, dates of HIV-positive/negative tests, clinical symptoms and ambiguous nucleotides [18, 19].

Classification of Transmission Pairs as Recent or Chronic

After construction of the phylogenetic tree and the estimations of the patients' infection dates, a time interval between the infection dates of the members of each phylogenetically established transmission pair was calculated (Supplementary Figure 1). Clusters with an interval of ≤6 or ≤12 months within a pair were classified as recent transmission (to account for variable definitions of the duration of recent infection [20], 2 analyses were performed, 1 for each threshold), and those with an interval of >6 or >12 months as chronic transmission.

Determining the Potential Transmitter and the Infection Window of the Recipient Within Each Pair

By default, a "transmitter" was defined as the member of the pair with the earliest seroconversion date. For the analysis of transmission in relation to time of ART initiation, we also defined for each potential recipient the most plausible infection window. Its upper bound is given by the first positive HIV test. Its lower bound is given by the latest of 3 dates: (1) 90 days before the last HIV negative test; (2) for individuals with a diagnosis of primary HIV infection (categories I and II in Supplementary Text 1), 365 days before the first HIV positive test; and (3) 730 days before the first positive test, if the patients' seroconversion date was estimated based on a resistance test with <0.5% of ambiguous nucleotides within 3 years after diagnosis and a first CD4 cell count >250/μL [18].

Estimation of Infectiousness During the Chronic Phase

Among the phylogenetically inferred transmitters, we identified risk factors associated with the relative odds of being a chronic- or recent-phase transmitter using logistic regression. To quantify the transmission potential [21] during the chronic phase,

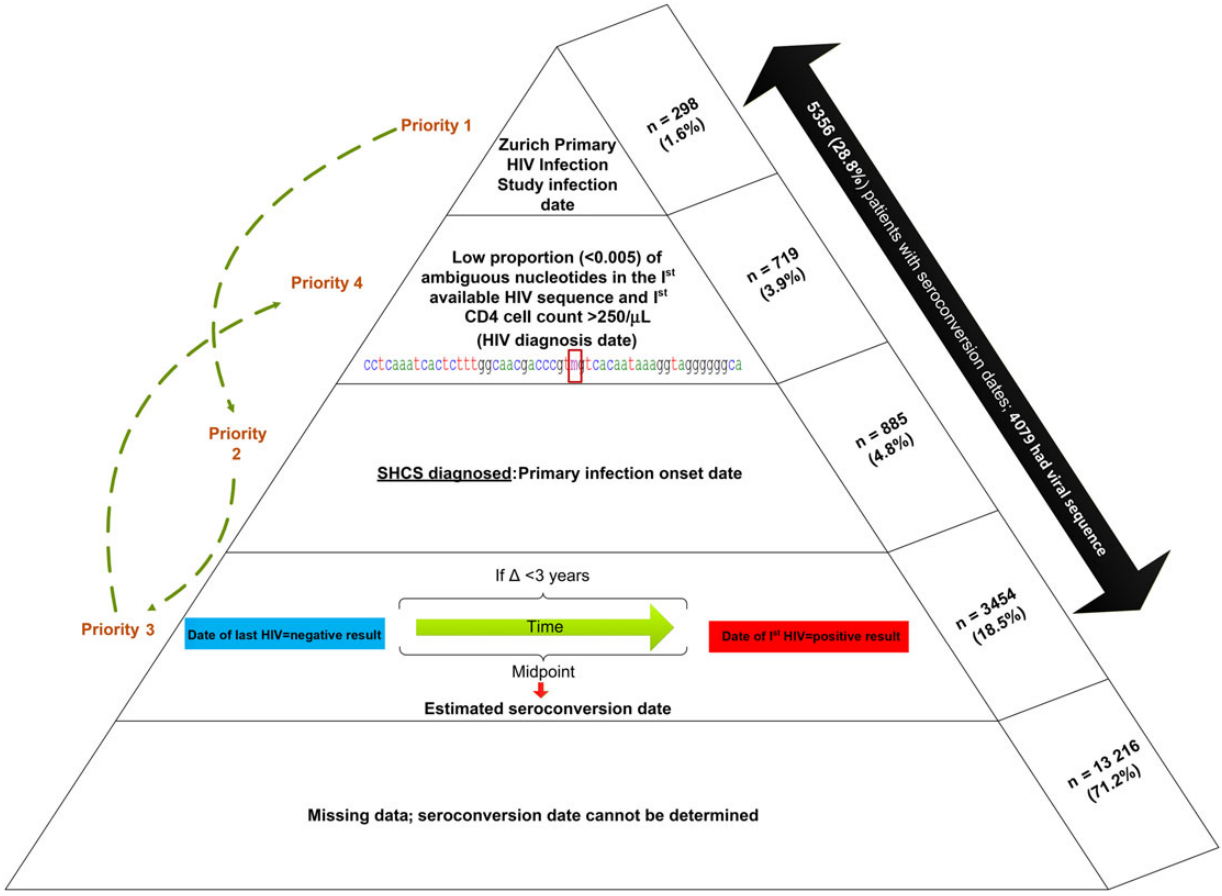

Figure 1. Hierarchical algorithm for the determination of the infection dates for patients enrolled in the Swiss HIV Cohort Study (SHCS) (n = 18 572). Abbreviation: HIV, human immunodeficiency virus.

which depends both on viral load (VL) magnitude and the duration with detectable VL, we calculated for each patient with ≥2 chronic-phase RNA measurements, the area under the curve (AUC) of the $\log_{10}$-transformed RNA VL from the end of the recent infection (1 year after the seroconversion date) to the last laboratory result of the chronic phase. To facilitate the regression interpretation, this variable was standardized (such that its mean is zero and one unit is one standard deviation). For the comparison of chronic-phase post-ART and pre-ART transmitters, VL AUC was calculated from the time of ART initiation to the last RNA measurement.

Statistical Analysis

Statistical analysis was performed with R software (version 3.0.3; http://cran.r-project.org).

Ethics

Ethical approval of the SHCS and the ZPHI and written informed consent for all participants were obtained.

RESULTS

Data Description

Of 18 572 SHCS participants, 10 970 (59%) had ≥1 sequence in the SHCS drug resistance database and were hence considered for further analysis (Table 1). Their year of HIV diagnosis ranged from 1984 to 2014. Of these patients, 7799 (71%), were men, 8314 (75%) were infected with subtype B, 4205 (38%) were men who have sex with men (MSM), and 8495 (77%) were white. Depending on the phylogenetic threshold, 3%–20.6% of the patients represented on the phylogenetic tree were members of a putative transmission pair (Figure 2). Seroconversion dates could be estimated for 4079 patients represented on the phylogeny, 82% with diagnosis during the first year after seroconversion. As expected, stricter bootstrap thresholds were associated with fewer transmission pairs (Figure 2) (Spearman $\rho = -1$; $P < .001$ for all 4 distances). For all 104 phylogenetic thresholds the predominant risk group among transmission pairs was MSM, ranging between 62% and 66%.

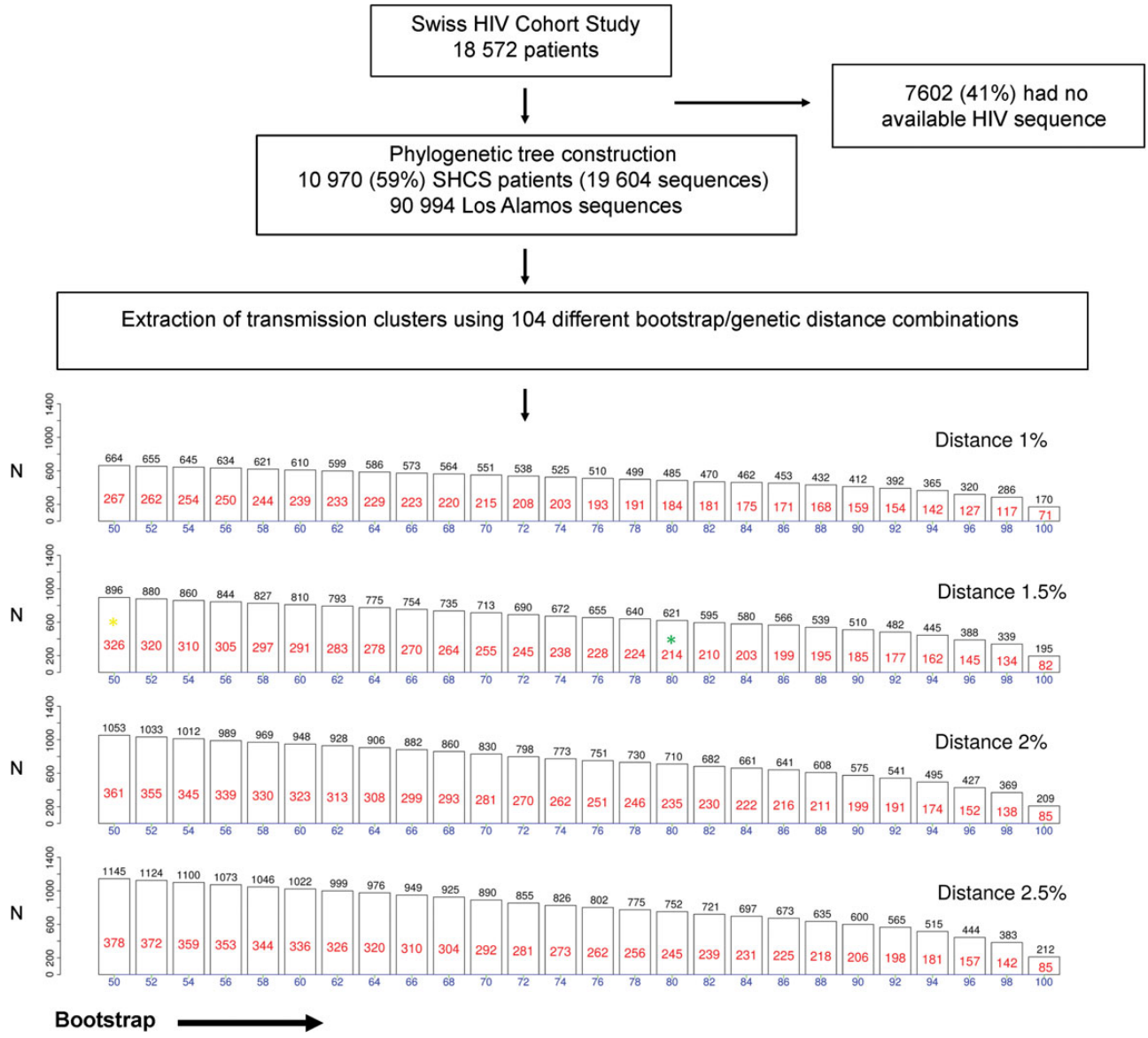

Figure 2. Outline of this study. Each bar represents a different combination of bootstrap and genetic distance thresholds. Black numbers above the bars represent the number of transmission pairs that correspond to the specific combination; red numbers, the number with available seroconversion dates for both members; blue numbers at the x-axis, ascending bootstrap thresholds; green and yellow asterisks, data sets used for the main logistic regression and sensitivity analyses, respectively. Abbreviations: HIV, human immunodeficiency virus; N, number of pairs; SHCS, Swiss HIV Cohort Study.

Estimation of HIV Transmission During Recent Infection

To estimate the fraction of transmissions attributable to recent infections, we selected potential transmission pairs using 104 different combinations of bootstrap and genetic distance. For each combination, we calculated the fraction of the recent-phase transmission (see "Methods" section). Overall, we found a high fraction of transmission during recent infection. This fraction was higher, but not proportionally higher, when recent infection was defined as first year of infection (vs the first 6 months) and increased with the strictness of the criterion used to define transmission pairs. When recent HIV infection was defined as the first year since seroconversion, the median fraction of transmission during recent infection was 43.7% and ranged from a minimum of 41% (95% confidence interval [CI], 36%–46%) for a bootstrap of 50% and a distance of 2.5% to a maximum of 56.5% (95% CI, 45%–67%) for a bootstrap of 100% and distances of 2% and 2.5%. When recent HIV infection was defined as 6 months since seroconversion, the median fraction of transmission during recent infection was lower (31.6%) and ranged from a minimum of 28% (95% CI, 23%–

Table 1. Clinical and Demographic Characteristics of the Study Population

Characteristic	All SHCS	Phylogenetic Tree	Distance: 1%; Bootstrap: 100%[a]	Distance 1.5%; Bootstrap: 80%[a]	Distance 2.5%; Bootstrap: 50%[a]
Patients, No.	18 572	10 970	142	428	744
Age at diagnosis, mean (IQR), y	34 (26.4–40)	34.5 (26.5–40)	36.4 (29–42)	36 (28.6–42)	35.3 (28–41)
Sex, No. (%)					
Male	13 369 (71.98)	7799 (71.09)	122 (85.92)	363 (84.81)	632 (84.95)
Female	5203 (28.02)	3171 (28.91)	20 (14.08)	65 (15.19)	112 (15.05)
Risk group, No. (%)					
MSM	6929 (37.3)	4205 (38.3)	93 (65.5)	271 (63.3)	463 (62.2)
Heterosexuals	6118 (32.9)	3919 (35.7)	40 (28.2)	102 (23.8)	158 (21.2)
Injection drug users	3281 (17.7)	1627 (14.8)	6 (4.2)	35 (8.2)	76 (10.2)
Other	2244 (12.1)	1219 (11.1)	3 (2.1)	20 (4.7)	47 (6.3)
Subtype, No. (%)					
B	8314 (75.8)	8314 (75.8)	96 (67.6)	335 (78.3)	616 (82.8)
Non-B	2656 (24.2)	2656 (24.2)	46 (32.4)	93 (21.7)	128 (17.2)
Ethnicity					
White	12 528 (67.5)	8495 (77.5)	116 (81.7)	371 (86.9)	660 (88.9)
Other	6039 (32.5)	2472 (22.5)	26 (18.3)	56 (13.1)	82 (11.1)
RNA viral load, median (IQR),[b] $\log_{10}$ copies/mL	4.65 (3.96–5.2)	4.65 (4–5.2)	4.81 (4–5.41)	4.82 (4.18–5.45)	4.76 (4.11–5.36)
CD4 cell counts, median (IQR), cells/μL[b]	342 (167–546)	370 (200–562)	420 (291–622)	440 (302–636.5)	471 (319.5–655.5)
ART start year, median (range)	1999 (1986–2014)	2000 (1986–2014)	2009 (1990–2013)	2008 (1990–2013)	2008 (1990–2014)
Time to ART, median (IQR), mo	20.8 (6.03–50.7)	21.5 (6–51.55)	14.5 (2.68–32.93)	14.7 (3.1–36.6)	16.6 (4.27–40.58)
Cohort recruitment year, median (range)	1997 (1981–2014)	2000 (1984–2014)	2008 (1989–2013)	2007 (1989–2013)	2006 (1987–2013)
Progressed to AIDS, No. (%)	6657 (35.8)	3032 (27.6)	11 (7.7)	40 (9.3)	73 (9.8)

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; SHCS, Swiss HIV Cohort Study.

[a] Based on pairs with available seroconversion dates.

[b] Earliest treatment-naive measurement after enrollment.

33%) with a bootstrap of 50% and distance of 2.5% to a maximum of 42.3% (95% CI, 32%–54%) with a bootstrap of 100% and distances of 2% and 2.5%.

For all 4 distance thresholds, a positive correlation was observed between the bootstrap thresholds and the recent-phase (12-month) transmission fractions (Figure 3) (Spearman $\rho > 0.70$; $P < .001$). Thus, a higher bootstrap threshold resulted in a higher fraction of recent-phase transmission. Importantly, the fraction of recent transmission increased sharply for higher bootstrap values (>92%), indicating that high bootstrap thresholds may bias the selection toward recently infected transmission pairs. For the 6-month definition of recent HIV infection the correlation between bootstrap and the fraction of attributable recent-phase transmissions was even stronger, and was significant for all 4 genetic distances tested (Spearman $\rho > 0.93$; $P < .001$). Thus, our phylogenetic analysis indicates that a large share of infections can be attributed to recent-phase transmission but that the exact proportion varies depending on the definition of a transmission pair (bootstrap and distance thresholds) and the duration of recent infection (12 vs 6 months).

Risk Factors for Chronic Transmission

HIV-1 transmission in the chronic phase, as opposed to the recent phase, was strongly associated with higher AUC of chronic-phase VL and delayed initiation of ART. Logistic regression was applied to the data set that corresponded to a genetic distance of 1.5% and bootstrap of 80% (Table 2). These thresholds were chosen as a compromise between 3 criteria: (1) avoiding the above-mentioned selection bias toward recent infection, which occurs for very strict criteria; (2) providing a fair statistical power (170 complete cases); and (3) minimizing the probability of false-positive clustering. In a bivariate analysis, transmitting HIV during chronic infection was positively correlated with time until the initiation of ART (odds ratio, 1.5/y;

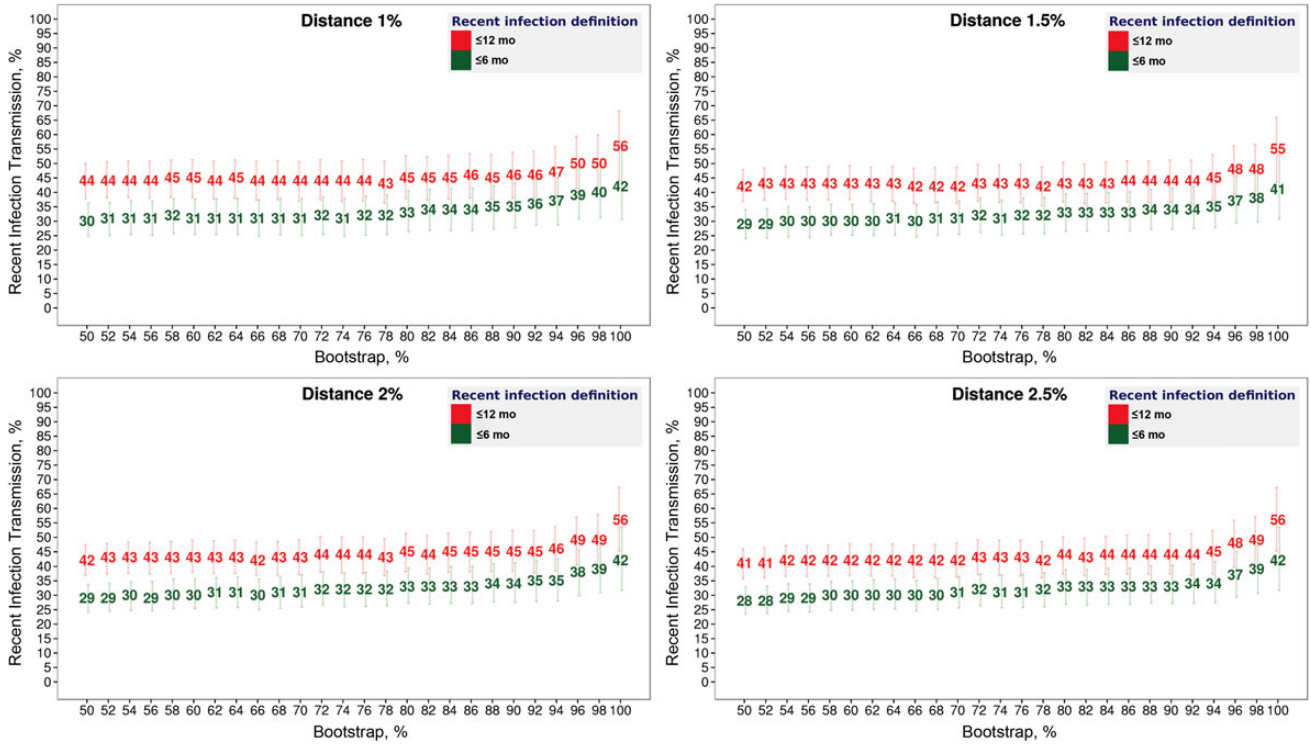

Figure 3. Swiss HIV Cohort Study–based estimation of transmission during recent human immunodeficiency virus infection. Red numbers represent the fraction of transmissions during recent infection, according to a definition of recent infection as 12 months since seroconversion; green numbers, recent transmission fraction for a definition of 6 months since seroconversion. In all, 104 combinations of genetic distance (1%, 1.5%, 2%, or 2.5%) and bootstrap (50%–100% in 2% increments) support values are shown; vertical lines represent 95% confidence intervals for proportion. Abbreviation: HIV, human immunodeficiency virus.

Table 2. Logistic Regression Analysis for Chronic Versus Recent Phylogenetically Linked Human Immunodeficiency Virus Transmitters[a]

Variable	Bivariate OR (95% CI)	*P* Value	Multivariable OR (95% CI)	*P* Value
Age at infection	0.96 (.93–.99)	.01	0.97 (.93–1.01)	.11
Sex				
Male	Reference	. . .	. . .	. . .
Female	1.04 (.49–2.23)	.92	2.43 (.6–9.94)	.22
Risk group				
MSM	Reference	. . .	. . .	. . .
Heterosexuals	1.08 (.56–2.1)	.81	0.61 (.15–2.52)	.49
Injection drug users	0.92 (.33–2.51)	.86	0.54 (.09–3.38)	.51
Subtype				
Non-B	Reference	. . .	. . .	. . .
B	1.4 (.72–2.73)	.32	1.28 (.45–3.67)	.65
$\sqrt{}$CD4 cell counts[b]	1.02 (.98–1.07)	.34	0.93 (.87–1)	.04
Transmission year	0.99 (.93–1.06)	.82	1.13 (1.02–1.26)	.02
Time to ART (years)	1.5 (1.26–1.8)	<.001	1.4 (1.11–1.77)	.005
Chronic RNA VL AUC	2.62 (1.74–3.97)	<.001	3 (1.64–5.48)	<.001

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; MSM, men who have sex with men; OR, odds ratio; VL AUC, viral load area under the curve.

[a] Of 170 transmitters (complete cases), 94 were chronic and 76 recent. Chronic transmission was defined as >1 year since seroconversion (coded as 1); recent transmission, as ≤1 year (coded as 0); phylogenetic linkage thresholds: bootstrap, 80%, genetic distance 1.5%.

[b] Earliest treatment-naive measurement after enrollment.

95% CI, 1.26–1.8) and with higher chronic-phase VL AUC (2.62; 95% CI, 1.74–3.97) (Table 2). In the multivariable model, both time to ART and the AUC of HIV plasma RNA during the chronic phase remained significantly associated with higher odds of chronic as opposed to recent transmission (adjusted odds ratio, 1.4/y [95% CI, 1.11–1.77] and 3 [1.64–5.48], respectively). Thus 1 standard-unit change in chronic VL AUC was associated with 3-fold increased odds of chronic-phase HIV transmission compared with recent-phase transmission, after adjustment for potential confounders and time to initiation of ART. Moreover, we found—only in the multivariable analysis—that later transmission years were associated with chronic-phase transmission, and higher baseline CD4 cell counts with recent transmission. In a sensitivity analysis, we found similar results with the more lenient criteria of 1.5% distance and 50% bootstrap (Supplementary Table 1). In summary, we showed that increased delay to initiation of ART shifts the relative odds of transmission toward the chronic phase. Moreover, our data indicate that the total RNA VL in the chronic phase increases the relative odds of transmitting HIV during this phase, even after adjustment for treatment initiation.

Transmission in Relation to ART Initiation

To explain the above-mentioned, ART-adjusted association of total chronic-phase VL with chronic-phase transmission, we further examined the chronic-phase transmitters (n = 121) in relation to ART initiation. Our data show that a substantial fraction of chronic-phase transmission occurred after ART was started by the transmitter.

For 54 of 121 chronic-phase transmitters (45%), the seroconversion date of the recipient was after the ART initiation date of the transmitter. In line with post-ART transmission, the mean post-ART VL AUC of post-ART transmitters was higher than that of pre-ART transmitters (0.17 vs −0.38; $P = .002$, Wilcoxon rank sum test) (Figure 4). Restricting the transmitters' VL measurements only to those obtained during the recipients' infection window (see "Methods" section) further corroborated transmission after ART: 44 of 54 transmitters had ≥1 VL measurement in the relevant period, and 35 of these 44 transmitters had ≥1 VL value >400 copies/mL [22] with a median nonzero maximal VL of 70 800 copies/mL (range, 2340 to 4.99×10^6 copies/mL). The remaining 9 transmitters might represent a nondirect transmission pair (eg, with a missing intermediate transmitter) or a false-positive cluster; alternatively, the intermittent VL rebounds might have been missed by the 3–4 monthly VL measurements.

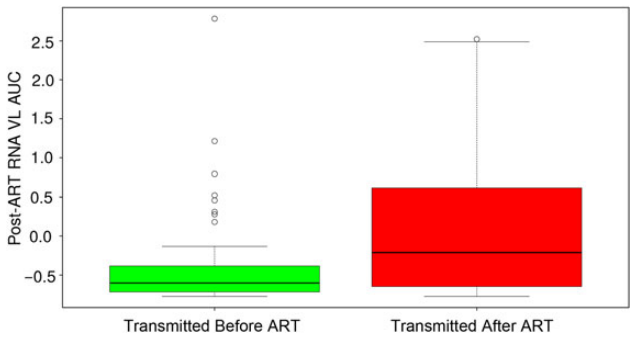

Figure 4. Total post–antiretroviral therapy (ART) viral load area under the curve (VL AUC) of pre-ART (*green*) and post-ART (*red*) transmitters. The median post-ART VL AUC of post-ART transmitters was higher than that of pre-ART transmitters (based on 121 chronic transmitters selected using a bootstrap of 80% and a genetic distance of 1.5%).

Finally, we determined in more detail the treatment status of the 35 VL-confirmed post-ART transmitters. For 18 transmitters, the date of ART initiation for the transmitter lay within the transmission window for the recipient. Hence, even though the estimated seroconversion date suggests post-ART transmission, we cannot exclude for those patients the possibility that the transmission occurred shortly before ART (Supplementary Figure 2*A*). Thus, these individuals transmitted either briefly before or briefly after ART initiation. For the remaining 17 transmitters, the transmitter's date of ART initiation lay completely before the recipients' infection window (Supplementary Figure 2*B*). Importantly, 16 of 17 had a documented period of treatment interruption during the recipient's infection window. These therapy interruptions lasted between 42 to 859 days within the infection window of the recipient. The remaining transmitter had no documented treatment interruption but carried high-level resistance mutations (M184V and K103N) and did not achieve viral suppression in the 6 years after treatment initiation, including the recipient's transmission window.

Overall, these results indicate that a substantial fraction of chronic-phase transmission events—at least 17 of 121 (14%) and up to 54 of 121 (45%)—occurred after ART initiation by the transmitter. This observation underlines the important contribution of treatment interruptions and the periods close to ART initiation for onward HIV transmission.

DISCUSSION

In Switzerland, despite increasing treatment coverage and decreased time to ART initiation, the number of newly diagnosed HIV infections remains stable [23]. Our study revealed 2 key challenges for achieving a population level effect of TasP: recent infections and HIV transmission during treatment interruptions in patients with chronic infection.

We demonstrated that a substantial fraction of HIV transmissions in the SHCS can be attributed to recently infected patients, for whom the preventive effect of treatment is weaker, due to underdiagnosis and lack of patient's awareness of his

seropositive status. In addition, immediate treatment of acute or recent infection was recommended only recently [24]. Moreover, our data show a strong effect of total VL on transmission in the chronic phase, even after adjustment for time to initiation of ART. This effect is partly due to transmission after ART initiation, notably during treatment interruptions. This observation implies that rapid administration of treatment, while the patient is still in the early phase of infection, is necessary but not sufficient to prevent transmission (because transmission may also occur after ART interruption in the chronic phase).

Our findings imply that TasP needs to be accompanied by interventions to tackle treatment continuity, adherence, retention in care, and, importantly, early diagnosis [25, 26]. A systematic review has shown that the median proportion of patients interrupting treatment was 23% for a median duration of 150 days [27]. Furthermore, 54% of HIV-diagnosed patients in Europe were late presenters—individuals who had a CD4 cell count <350/μL or an AIDS-defining illness within 6 months of HIV diagnosis [28]. Cumulatively, our data imply that treatment interruptions, whether structured or due to toxic effects, patient's wishes, or lack of adherence, are not only unfavorable for the individual [29] but also bear public health consequences [26].

Our work further underlines the need for validated and consensual thresholds for phylogeny-based detection of HIV transmission. The observed positive correlation between the strictness of the transmission pair selection criteria (higher bootstrap and lower genetic distance) and the fraction of transmissions attributed to recent infections implies that too-strict selection criteria overestimate the fraction of recent-phase transmission. Several other studies that implemented strict genetic distance and bootstrap thresholds (eg, 1.5% distance and 98% bootstrap) have found recent infection as a predictor of membership in HIV transmission clusters (reviewed in [17]). Our data suggest that some of these results might have been affected by the strictness of the chosen thresholds, which inadvertently favored selection of recent transmission clusters over the chronic clusters.

This study has several limitations. One intrinsic challenge is that neither the timing nor the order of transmission events are strictly reflected in the pathogen phylogeny, which is also highly dependent on the sampling density of the target population [30]. However, the SHCS coverage of the Swiss HIV epidemic was estimated to be high [12], with >10 000 genotypic resistance tests done retrospectively using the SHCS biobank [11]. Moreover, 90 994 Los Alamos HIV-1 sequences were included to reduce the chances of random clustering.

Another limitation is that we were able to estimate the seroconversion date for only 29% of the cohort participants. This resulted in selection toward the MSM risk group, possibly because of the high rate of HIV testing, a key criterion in our estimation of seroconversion dates. We speculate that this selection toward MSM, combined with the high fraction of patients that were diagnosed while still at the recent phase, led to an overestimation of recent-phase transmission in our sample compared with the general Swiss HIV-positive population.

Finally, in contrast to the chronic-phase total VL, an accurate estimate of the total (AUC) recent-phase VL was not possible and was not incorporated into our statistical models, because most patients were enrolled in the cohort at variable times in relation to the acute-phase viremic peak. Despite these limitations, our work highlights the high fraction of recent-phase transmission and transmission during therapy interruptions, two key challenges for curbing HIV incidence with TasP.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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