Higher Risk of Incident Hepatitis C Virus Coinfection Among Men Who Have Sex With Men, in Whom the HIV Genetic Bottleneck at Transmission Was Wide

Roger D. Kouyos,[1] Andri Rauch,[3] Dominique L. Braun,[1] Wan-Lin Yang,[1] Jürg Böni,[2] Sabine Yerly,[4] Thomas Klimkait,[6] Vincent Aubert,[8] Cyril Shah,[2] Helen Kovari,[1] Alexandra Calmy,[5] Matthias Cavassini,[9] Manuel Battegay,[7] Pietro L. Vernazza,[10] Enos Bernasconi,[11] Bruno Ledergerber,[1] Huldrych F. Günthard,[1] and the Swiss HIV Cohort Study (SHCS)

[1]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, [2]Institute of Medical Virology, University of Zurich, [3]Department of Infectious Diseases, Bern University Hospital and University of Bern, [4]University Hospital Geneva, [5]Division of Infectious Diseases, Geneva University Hospital, [6]University of Basel, [7]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, [8]University of Lausanne, [9]Division of Immunology, University Hospital Lausanne, [10]Division of Infectious Diseases, Cantonal Hospital St. Gallen, and [11]Division of Infectious Diseases, Regional Hospital Lugano, Switzerland

Background. High-risk sexual behaviors have been suggested as drivers of the recent dramatic increase of sexually transmitted hepatitis C virus (HCV) among human immunodeficiency virus (HIV)–infected men who have sex with men (MSM).

Methods. We assessed the association between the genetic bottleneck of HIV at transmission and the prevalence and incidence of HCV coinfection in HIV-infected MSM from the Swiss HIV Cohort Study (SHCS). As a proxy for the width of the transmission bottleneck, we used the fraction of ambiguous nucleotides detected by genotypic resistance tests sampled during early HIV infection. We defined a broad bottleneck as a fraction of ambiguous nucleotides exceeding a previously established threshold (0.5%).

Results. From the SHCS, we identified 671 MSM with available results of HCV serologic tests and with an HIV genotypic resistance test performed during early HIV infection. Of those, 161 (24.0%) exhibited a broad HIV transmission bottleneck, 38 (5.7%) had at least 1 positive HCV test result, and 26 (3.9%) had an incident HCV infection. Individuals with broad HIV transmission bottlenecks exhibited a 2-fold higher odds of having ever experienced an HCV coinfection (odds ratio, 2.2 [95% confidence interval {CI}, 1.1–4.3]) and a 3-fold higher hazard of having an incident HCV infection (hazard ratio, 3.0 [95% CI, 1.4–6.6]) than individuals with narrow HIV transmission bottlenecks.

Conclusions. Our results indicate that the currently occurring sexual spread of HCV is focused on MSM who are prone to exhibit broad HIV transmission bottlenecks. This is consistent with an important role of high-risk behavior and mucosal barrier impairment in the transmission of HCV among MSM.

Keywords. HIV; hepatitis C virus; coinfection; transmission bottleneck; sexual transmission.

Hepatitis C virus (HCV) is a major cause of morbidity and mortality, with 130–150 million infections worldwide causing approximately 350 000 deaths/year [1]. Until recently, sexual transmission of HCV was considered an unlikely route of acquisition or was assumed to play only a marginal role [2]. However, recent outbreaks of HCV infection among human immunodeficiency virus (HIV)–infected men who have sex with men (MSM) in several industrialized countries have called these views into question [3–7]. The driving mechanisms behind the recent change in the epidemiology of HCV infection are unclear. The extent to which sexual transmission occurs only or predominantly as a

consequence of high-risk practices is especially unclear. Several lines of evidence support this view, such as the absence of an increased incidence of HCV infection in HIV-infected heterosexuals [7] and the increased frequency of high-risk practices in HCV/HIV-coinfected MSM [6].

The majority of sexually transmitted HIV infections are founded by a single virus, such that the entire virus population observed in an HIV-infected patient consists of the descendants of a single transmitted virion [8]. This narrow genetic bottleneck at transmission (hereafter, the "transmission bottleneck") is reflected by the very low diversity observed in most recent HIV infections. However, in a minority (10%–20%) of sexually transmitted HIV infections, the transmission bottleneck is wide [8–12]; that is, the infections are founded by ≥2 viruses and accordingly exhibit a high genetic diversity even during the early phase of infection. Intuitively, one would expect that the chance of such a wide transmission bottleneck is larger for high-risk sexual contacts, given that practices that increase the infection risk (ie, the transmission of at least 1 virion) should also increase the chance that >1 virus is transmitted simultaneously upon an infection event. To what extent this is the case is, however, still an open question, as some studies confirmed it [10–12], while others failed to find evidence for a wider transmission bottleneck in HIV transmitted by high-risk sexual contact [9].

Here, we used HIV/HCV-coinfection data from the Swiss HIV Cohort Study (SHCS) and diversity measures derived from the SHCS Drug Resistance Database (SHCS-DRDB) to assess the association between the HIV bottleneck and the prevalence and incidence of HCV infections and (as an additional indicator of high-risk behavior) of other sexually transmitted infections. The SHCS is the ideal setting for this analysis because it is highly representative for the HIV-infected population in Switzerland and because HCV-seronegative individuals enrolled in the SHCS have, since 1998, undergone testing at least every 2 years for incident HCV infections.

METHODS

We considered the clinical and demographic data from the SHCS and ambiguous-nucleotide scores derived from genotypic resistance tests in the SHCS-DRDB. The SHCS is an open cohort with continuous enrollment of HIV-infected individuals and semiannual follow-up visits [13]. In total, 18 200 individuals have been enrolled into the SHCS (as of December 2013). Of those, 11 291 have at least 1 genotypic resistance test (GRT) result in the SHCS-DRDB [13–15]. HCV status was ascertained by enzyme-linked immunosorbent assays or by use of quantitative polymerase chain reaction to detect HCV RNA (see the article by Wandeler et al [7] for more details). The SHCS was approved by the individual local institutional review boards of all participating centers (a list of the centers is available

at: http://www.shcs.ch/31-health-care-providers). Written informed consent was obtained for each SHCS participant.

The size of the transmission bottleneck can be estimated from the diversity observed in sequences sampled during early infection [8]: if only 1 virus founds the new infection, diversity is low during early HIV infection; otherwise, diversity is high. Elsewhere [16], we have shown that the fraction of ambiguous nucleotides ($f_{ambiguous}$) is a good marker for viral diversity, with an excellent correlation with clonal diversity in early infection. Therefore, we used this quantity with the threshold of 0.5% (established previously [16]) as a measure for the size of the transmission bottleneck. Specifically, the transmission bottleneck in a given patient was classified as wide if the earliest GRT for that patient was performed on a sample obtained within the first year after HIV seroconversion and exhibited a high diversity ($f_{ambiguous} \geq 0.5\%$). Conversely, the transmission bottleneck was classified as narrow if the earliest GRT for that patient was performed on a sample obtained within the first year after HIV seroconversion and exhibited a low diversity ($f_{ambiguous} < 0.5\%$).

Since only diversity observed in early HIV-1 infection is indicative of the width of the HIV transmission bottleneck, we focus our analysis on individuals with an accurate estimate of the infection date and a GRT performed on samples obtained during early infection. The group of patients with an accurate HIV infection/seroconversion date consisted of patients fulfilling at least 1 of the following conditions: (1) negative and positive HIV serologic test results <1 year apart, with the seroconversion date estimated as the midpoint between the last negative and the first positive HIV test result; (2) presence of symptoms indicating primary HIV infection (antiretroviral syndrome, as defined by the SHCS [17]); and (3) participation in the Zurich Primary HIV Infection Study [9].

Statistical analyses were performed in Stata, version 12.0. Associations were assessed by univariable and multivariable logistic regression and Cox proportional hazards model for HCV infection incidence. In the logistic regression model, cases were defined as individuals who had at least 1 HCV-positive test result recorded in the SHCS, and controls were defined as individuals who never had a positive result of a test for HCV. In the Cox proportional hazards model for HCV infection incidence, we focused on individuals whose first HCV test result was negative and for whom at least 1 subsequent test result was available. Time at risk started at the time of this first negative HCV test result and ended at the date of the last HCV test. The multivariable models were adjusted for the potential confounders: laboratory that determined the sequences, year of HIV seroconversion, and age at HIV seroconversion. The variables "laboratory" and "width of the transmission bottleneck" were included as categorical variables, and the variables "year of HIV seroconversion" and "age at HIV seroconversion" were included as continuous variables. The validity of the

proportional hazards assumptions were tested by Schoenfeld residuals [18] and could not be rejected at a *P* value of .05.

RESULTS

Here, we assessed the relation between the size of the HIV transmission bottleneck and the risk of acquiring an HCV coinfection. The size of the transmission bottleneck was derived from the diversity detected by GRTs. A high HIV diversity indicates a broad HIV transmission bottleneck only if the sample stems from a recently infected patient. Therefore, we restricted our analysis to patients for whom the SHCS and the SHCS-DRDB contained both an accurate estimate of their date of HIV seroconversion and the result of a GRT that was performed within the first year after HIV seroconversion. A total of 1194 patients fulfilled these 2 criteria. Because the HCV serostatus of individuals enrolled in the SHCS has been systematically tested since 1998 (tests are performed on individuals at least every 2 years), we further restricted our analysis to patients with a date of HIV seroconversion during 1998 or later (1104 patients). Accordingly, information on the HCV status was available for most of those individuals (1084 of 1104 patients). Since sexually transmitted HCV has been observed almost exclusively among MSM [3–7], the main analysis presented here focuses on this transmission group. The MSM transmission group was defined as males in the SHCS who stated that their likely route of acquiring HIV was homosexual sex and who did not indicate any injection drug use at their follow-up visits. These corresponded to 671 of 1084 patients with a GRT result from early infection period and information on their HCV status (Figure 1). Overall, we included all MSM in the SHCS with an accurate estimation of their date of HIV seroconversion after 1998 who had a GRT performed within 1 year of their estimated HIV seroconversion and for whom at least 1 HCV test result was available (Figure 1). According to these criteria, 671 patients were included in the analysis (Table 1).

We found that HIV-infected MSM with a wide HIV transmission bottleneck had a substantially higher risk of experiencing an HCV coinfection: among HIV-infected MSM with a narrow HIV transmission bottleneck, 4.5% (23 of 510) had ever been coinfected with HCV, compared with 9.3% of HIV-infected MSM (15 of 161) with a wide HIV bottleneck. This corresponds to an odds ratio (OR) of 2.2 (95% confidence interval [CI], 1.1–4.3). To assess the impact of the HIV bottleneck on the HCV infection incidence, we focused on the 558 HIV-infected MSM whose first HCV test had a negative result and for whom results of subsequent tests were available. If the HIV transmission bottleneck was narrow, we found 14 HCV infections in 2300 person-years, resulting in an HCV infection incidence of 6 cases/1000 person-years. If the HIV transmission bottleneck was broad, we found 12 infections in 656 person-years, resulting in an HCV infection incidence of 18 cases/
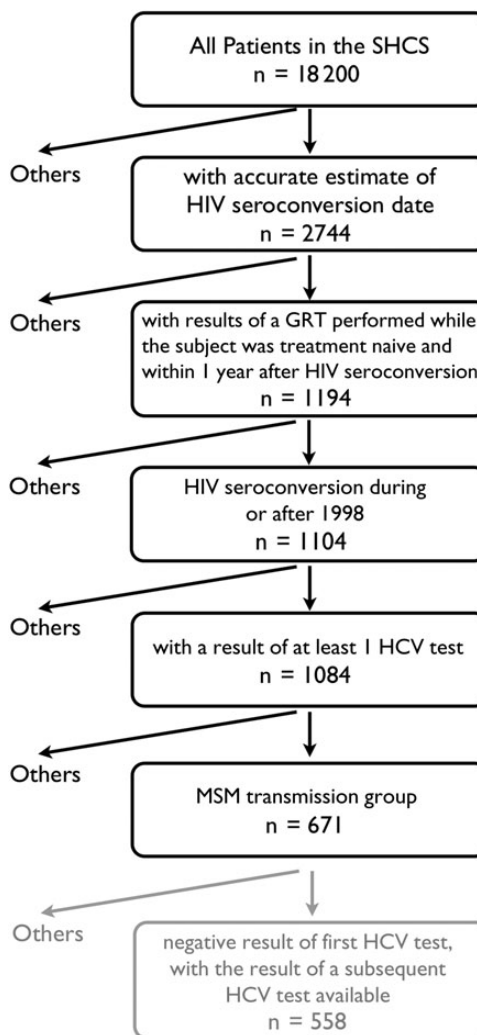
Figure 1. Flow diagram for the study population. The last selection (in grey) corresponds to the subpopulation used for the analysis of incident hepatitis C virus (HCV) infections. Abbreviations: GRT, genotypic resistance test; HIV, human immunodeficiency virus; MSM, men who have sex with men; SHCS, Swiss HIV Cohort Study.

1000 person-years. In a Cox proportional hazards model, we found that the hazard ratio (HR) of HCV acquisition was 3.0 (95% CI, 1.4–6.6) if the HIV transmission bottleneck was wide, compared with a narrow bottleneck (Figure 2). Thus, both the prevalence and incidence of HCV coinfection were >2-fold higher if the bottleneck at HIV transmission was wide.

The associations between HCV infection incidence and prevalence on the one hand and the size of the HIV transmission bottleneck on the other hand were not affected by age, year of HIV seroconversion, or laboratory performing the GRT. The analysis that controlled for these variables yielded an OR for experiencing an HCV infection of 2.2 (95% CI, 1.1–4.4) and the corresponding HR in the Cox proportional hazards model for

**Table 1.   Characteristics of the Study Population**

Characteristic	Without HCV	With HCV	Total	P Value[a]
HIV transmission bottleneck				.03
Narrow	487 (95.5)	23 (4.5)	510 (100)	
Broad	146 (90.7)	15 (9.3)	161 (100)	
Age at HIV seroconversion, y	35 (28–41)	33 (27–41)	34 (28–41)	.56
Year of HIV seroconversion	2007 (2004–2009)	2008 (2005–2009)	2007 (2004–2009)	.39
Ethnicity				.35
White	582 (94.0)	37 (6.0)	619 (100)	
Not white	51 (98.1)	1 (1.9)	52 (100)	
Laboratory				.63
Zurich	294 (95.2)	15 (4.9)	309 (100)	
Geneva	193 (93.2)	14 (6.8)	207 (100)	
Lausanne	107 (93.0)	8 (7.0)	115 (100)	
Basel	39 (97.5)	1 (2.5)	40 (100)	
First available CD4$^+$ T-cell count, cells/μL	470 (327–631)	464 (320–634)	470 (327–631)	.8
First available HIV RNA value, $\log_{10}$ copies/mL	5.0 (4.4–5.9)	5.3 (4.5–6.7)	5.0 (4.4–5.9)	.27

Data are no. (%) of study participants or median (interquartile range). The study population was defined as men who have sex with men, who had results of a genotypic resistance test performed during early human immunodeficiency virus (HIV) infection, and who had results of at least 1 test for detection of hepatitis C virus (HCV; Figure 1).

[a] By the Fisher exact test, for count data, and by the Wilcoxon rank sum test, for continuous variables.

HCV infection incidence was 3.2 (95% CI, 1.5–6.9; Tables 2 and 3). Among the demographic confounders, 2 should be noted in particular: the time of the GRT and the laboratory performing the GRT. These variables are potential confounders because in one of the laboratories the frequency of patients reporting a GRT with an $f_{ambiguous}$ value of ≥ 0.5% has increased over time (because of a higher propensity of calling ambiguous

nucleotides in the laboratory; logistic regression OR, 1.1 per year [95% CI, 1.0–1.2 per year]), while the incidence of HCV infection has also increased over time in Switzerland [7]. However, because the incidence and HR remained almost unchanged by controlling for laboratory and time of HIV seroconversion in the multivariable models, this potential confounding has only a weak effect and cannot explain the observed
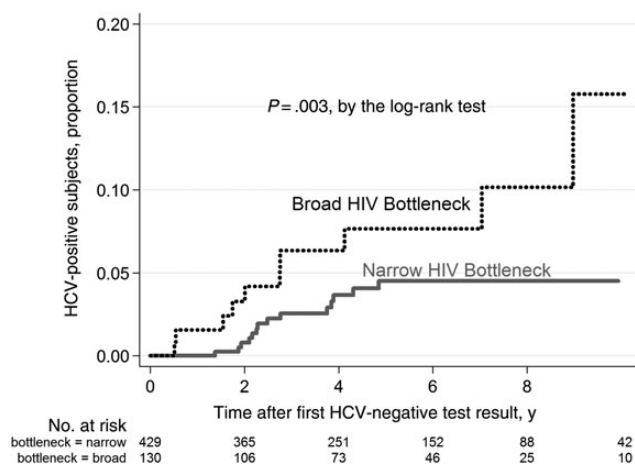
Figure 2.   Kaplan–Meyer estimates for hepatitis C virus (HCV) infection incidence among patients with narrow (solid gray line) and broad (dashed black line) transmission bottlenecks. The graph has been truncated when the number of patients at risk in the smaller stratum was <10 (see Supplementary Figure 1 for a version of the graph without truncation). The log-rank test was performed on the nontruncated data.

Table 2.   Results of Multivariable Logistic Regression Analysis for Prevalent Cases of Hepatitis C Virus Infection

Factor	Univariable Analysis OR (95% CI)	P Value	Multivariable Analysis OR (95% CI)	P Value
HIV transmission bottleneck		.02		.02
Narrow	1 (Reference)		1 (Reference)	
Broad	2.17 (1.11–4.28)		2.21 (1.12–4.39)	
Age at HIV seroconversion (per y)	0.99 (.96–1.03)	.72	0.99 (.96–1.03)	.66
Year of HIV seroconversion (per y)	1.04 (.94–1.14)	.43	1.07 (.96–1.18)	.23
Laboratory		.55		.31
Zurich	1 (Reference)		1 (reference)	
Geneva	1.42 (.67–3.01)		1.91 (.84–4.35)	
Lausanne	1.42 (.67–3.01)		1.61 (.66–3.95)	
Basel	0.50 (.06–3.91)		0.56 (.07–4.41)	

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio.

Table 3. Results of Multivariable Cox Proportional Hazards Modeling for Incident Hepatitis C Virus Infection

Factor	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	P Value	HR (95% CI)	P Value
HIV transmission bottleneck		.005		.004
Narrow	1 (Reference)		1 (Reference)	
Broad	3.02 (1.40–6.55)		3.17 (1.45–6.91)	
Age at HIV seroconversion (per y)	0.96 (.91–1.01)	.12	0.96 (.91–1.01)	.11
Year of HIV seroconversion (per y)	1.60 (1.28–2.00)	<.001	1.62 (1.29–2.02)	<.001
Laboratory		.2		.08
Zurich	1 (Reference)		1 (Reference)	
Geneva	0.85 (.33–2.19)		1.61 (.62–4.13)	
Lausanne	1.76 (.66–4.74)		1.87 (.69–5.11)	
Basel	0		0	

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio.

association between diversity and HCV coinfection risk. Because of the small numbers of HCV infections in our study population (38 patients who ever had an HCV infection, and 26 with incident HCV infection), we did not include additional covariables in the multivariable models shown in Tables 2 and 3. However, we found similarly strong effects of the transmission bottleneck (OR > 2 and HR > 3) when additionally adjusting for the other variables listed in Table 1.

DISCUSSION

Here, we have shown that HIV-infected MSM from the SHCS who experienced a broad HIV transmission bottleneck had a >3-fold higher risk of acquiring HCV. This result is robust against correction for potential demographic confounders such as age, calendar year, or the laboratory performing the GRT. Our finding suggests that the HCV epidemic among MSM is currently focused on individuals who were also prone to experience a broad transmission bottleneck.

In this study, we focused on MSM because the recent increase in HCV infection incidence has so far been found only in this transmission group [3–7]. We extended our analysis to other transmission groups but did not find significant associations between the size of the transmission bottleneck and the risk of an HCV infection for individual transmission groups other than MSM (data not shown). It should be noted that the number of individuals with GRTs available for early infection was much smaller for these transmission groups (Figure 1). Moreover, a test for homogeneity could not reject the null-hypothesis that the ORs are the same across groups (P = .33). Thus, because

of the limited data available, it is currently unclear whether this association between the transmission bottleneck and the risk of an HCV infection also extends to other transmission groups.

Previous work has shown an association between high-risk sexual practices and both the risk of HCV infection [3–7] and a broader HIV transmission bottleneck, although the latter association has not been found in all studies [8–12]. Similarly, injection drug use has also been associated with a broad HIV transmission bottleneck [19]. In the light of these previous studies on both high-risk sex and injection drug use, our results can be explained as follows: MSM engaging in high-risk behavioral practices have a larger chance of experiencing both a wide HIV transmission bottleneck and infection with HCV. Hence, a wide transmission bottleneck is an indicator for high-risk behavior and therefore for an increased risk of an HCV transmission.

Even though the association between broad HIV transmission bottlenecks and HCV infection might have been caused by undisclosed injection drug use among the MSM population, we believe that high-risk sexual behavior is a more likely cause for the association. It should be noted that the SHCS collects very detailed information on injection drug use at the semiannual follow-up visits and that even a single report of injection drug use in a follow-up visit led to exclusion from our study population, thereby minimizing the potential problem of undisclosed injection drug use. Moreover, similar to Wandeler et al [7], we found that the incidence of HCV infection was positively associated with reporting unprotected sex and with a history of concomitant syphilis, highlighting the role of sexual transmission (data not shown). Including those factors into the multivariate model for HCV infection incidence (summarized in Table 3) caused only minor changes in the effect of a broad bottleneck (adjusted HR, 2.8). In addition, in a large molecular epidemiology study, we have clearly shown that HIV sequences from MSM hardly ever cluster together with those of injection drug users (≤2%), thus rendering injection drug use highly unlikely as a confounder [20]. Finally, in recent work [21] we found that the prevalence and incidence of HCV coinfection in HIV-infected MSM is clearly associated with the HIV phylogeny, which again provides evidence that at least some cases of transmission occur via sex.

One may speculate that a wide HIV transmission bottleneck and an increased risk for HCV coinfection are linked because both are facilitated by high-risk, traumatic sex causing breaches/impairment of the mucosal barrier during sexual transmission. This interpretation of the results (but not the results as such) assumes that the risk behavior at the time point of HIV acquisition is indicative of the behavior during later time points. This assumption still allows for changes of risk behavior over time because it only involves the much weaker requirement that some residual correlation persists over time. An alternative mechanism that may cause the observed association is that syphilitic ulcers increase the probability both for broad HIV transmission bottlenecks and for HCV coinfection. Individuals

with a broad HIV transmission bottleneck exhibited a slight but nonsignificant increase in the risk of a syphilis coinfection (OR, 1.23 [95% CI, .86–1.8]), lending partial support for this alternate mechanism. Finally, we found that 24 of 26 incident HCV infections occurred after antiretroviral therapy initiation and that the 2 individuals who acquired HCV while therapy naive exhibited both a narrow HIV transmission bottleneck. However, this association is not significant ($P = .48$, by the Fisher exact test), and it is not clear which mechanism could have generated the association. Independently of these possible interpretations, our results indicate that HCV coinfections are currently focused on a subgroup of MSM: namely those who—for behavioral or biological reasons—are more prone to experience a broad bottleneck at HIV transmission.

This study has several limitations. First, the propensity of calling ambiguous nucleotides might vary between laboratories and over time. In fact, we found such an effect of time for one laboratory. However, our results remained robust after controlling for laboratory and year of HIV seroconversion in multivariable models, indicating that this effect did not have a major impact on our results. Second, our interpretation that high-risk sex is causing the association between HCV infections and a broad HIV transmission bottleneck hinges on the assumption that undisclosed injection drug use is negligible. Finally, our study has been conducted in the particular epidemiological setting of the SHCS, and it remains to be shown whether similar effects occur also in other settings. This problem is mitigated by the fact that the rise of sexually transmitted HCV has occurred in a similar way (eg, with similar time scales and populations at risk) in many different industrialized countries [3–7]. This suggests that high-risk sexual behavior might also play a similar role across epidemiological settings.

Overall, our results show that HCV transmission among MSM occurs preferentially among individuals who also exhibit a broad HIV transmission bottleneck. From a general perspective, this provides a proof of principle of how molecular tools can be used to identify core groups for disease transmission. For HCV, it indicates that high-risk behavioral transmission and possibly high-risk sexual behavior leading to mucosal breaches may have played an important role in the recent transition of HCV from an almost uniquely parenterally transmitted pathogen to one that is transmitted, in at least some cases, via sex.

STUDY GROUP MEMBERS

The members of the Swiss HIV Cohort Study are as follows: V. Aubert, J. Barth, M. Battegay, E. Bernasconi, J. Böni, H. C. Bucher, C. Burton-Jeangros, A. Calmy, M. Cavassini, M. Egger, L. Elzi, J. Fehr, J. Fellay, P. Francioli, H. Furrer (chairman of the Clinical and Laboratory Committee), C. A. Fux, M. Gorgievski, H. Günthard (president of the SHCS), D. Haerry (deputy of the Positive Council), B. Hasse, H. H. Hirsch, B. Hirschel, I. Hösli, C. Kahlert, L. Kaiser, O. Keiser, C. Kind, T. Klimkait, H. Kovari, B. Ledergerber, G. Martinetti, B. Martinez de Tejada, K. Metzner, N. Müller, D. Nadal, G. Pantaleo, A. Rauch (chairman of the Scientific Board), S. Regenass, M. Rickenbach (head of the data center), C. Rudin (chairman of the Mother and Child Substudy), P. Schmid, D. Schultze, F. Schöni-Affolter, J. Schüpbach, R. Speck, P. Taffe, P. Tarr, A. Telenti, A. Trkola, P. Vernazza, R. Weber, and S. Yerly.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.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

References

1. Who Factsheet HCV. http://www.who.int/mediacentre/factsheets/fs164/en/. Accessed 23 June 2014.
2. Bresters D, Reesink HW, van der Poel CL, et al. Sexual transmission of hepatitis C virus. Lancet **1993**; 342:210–1.

3. Danta M, Rodger AJ. Transmission of HCV in HIV-positive populations. Curr Opin HIV and AIDS 2011; 6:451–8.

4. van de Laar TJW, van der Bij AK, Prins M, et al. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. J Infect Dis 2007; 196:230–8.

5. Götz HM, van Doornum G, Niesters HG, Hollander den JG, Thio HB, de Zwart O. A cluster of acute hepatitis C virus infection among men who have sex with men–results from contact tracing and public health implications. AIDS 2005; 19:969–74.

6. Vogel M, Boesecke C, Rockstroh JK. Acute hepatitis C infection in HIV-positive patients. Curr Opin Infect Dis 2011; 24:1–6.

7. Wandeler G, Gsponer T, Bregenzer A, et al. Hepatitis C virus infections in the swiss HIV cohort study: a rapidly evolving epidemic. Clin Infect Dis 2012; 55:1408–16.

8. Keele BF, Giorgi EE, Salazar-Gonzalez JF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proc Natl Acad Sci 2008; 105:7552–7.

9. Rieder P, Joos B, Scherrer AU, et al. Characterization of human immunodeficiency virus type 1 (HIV-1) diversity and tropism in 145 patients with primary HIV-1 infection. Clin Infect Dis 2011; 53:1271–9.

10. Li H, Bar KJ, Wang S, et al. High multiplicity infection by HIV-1 in men who have sex with men. PLoS Pathog 2010; 6:e1000890.

11. Sagar M, Lavreys L, Baeten JM, et al. Identification of modifiable factors that affect the genetic diversity of the transmitted HIV-1 population. AIDS 2004; 18:615–9.

12. Long EM, Martin HL, Kreiss JK, et al. Gender differences in HIV-1 diversity at time of infection. Nat Med 2000; 6:71–5.

13. Swiss HIV Cohort StudySchoeni-Affolter F, Ledergerber B, et al. Cohort profile: the Swiss HIV Cohort study. Int J Epidemiol 2010; 39:1179–89.

14. Wyl von V, Kouyos RD, Yerly S, et al. The role of migration and domestic transmission in the spread of HIV-1 non-B subtypes in Switzerland. J Infect Dis 2011; 204:1095–103.

15. Wyl von V, Yerly S, Böni J, et al. Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral treatment: a comparison of different regimen types. Arch Intern Med 2007; 167:1782–90.

16. Kouyos RD, Wyl von V, Yerly S, et al. Ambiguous nucleotide calls from population-based sequencing of HIV-1 are a marker for viral diversity and the age of infection. Clin Infect Dis 2011; 52:532–9.

17. Swiss HIV Cohort Study. Definitions: primary HIV infection (database code PRI). http://www.shcs.ch/56-definitions#2.1. Accessed 23 June 2014.

18. Schoenfeld D. Partial residuals for the proportional hazards regression-model. Biometrika 1982; 69:239–41.

19. Bar KJ, Li H, Chamberland A, et al. Wide variation in the multiplicity of HIV-1 infection among injection drug users. J Virol 2010; 84:6241–7.

20. Kouyos RD, Wyl von V, Yerly S, et al. Molecular epidemiology reveals long-term changes in HIV type 1 subtype B transmission in Switzerland. J Infect Dis 2010; 201:1488–97.

21. Kouyos RD, Rauch A, Böni J, et al. Clustering of HCV coinfections on HIV phylogeny indicates domestic and sexual transmission of HCV. Int J Epidemiol 2014; 43:887–96.