

Acute HIV infection: impact on the spread of HIV and transmission of drug resistance

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Objective: To assess the impact of primary HIV infection (PHI) on the spread of HIV and the temporal trends in transmission of HIV drug resistance between 1996 and 1999 in Switzerland.

Methods: Sequencing of the genes for reverse transcriptase (RT) and protease was performed for 197 individuals with documented PHI. Phylogenetic analyses were confronted with epidemiological data.

Results: Significant clustering was demonstrated for 29% of the RT sequences. All these cases occurred closely together in place and time; contact tracing demonstrated transmission at the time of PHI in 30% of them. Genotypic drug resistance was detected in 8.6% of PHI individuals in 1996, 14.6% in 1997, 8.8% in 1998 and 5.0% in 1999. Drug-resistant variants were identified in 11.3% of individuals infected by homosexual contacts, 6.1% by heterosexual contacts, 13% of intravenous drug users and more frequently in men (10.4%) than women (2.6%). Potential factors involved in the recent decrease of transmission of drug-resistant variants include increase of HIV non-B subtypes from 23% in 1996 to 35% in 1999 (only one non-B subtype had resistance mutations) and a steady increase of patients with undetectable viraemia as documented in Swiss HIV Cohort Study (10% in 1996 vs 53% in 1999).

Conclusions: Phylogenetic and epidemiological analyses underline the impact of PHI in the spread of HIV. Moreover, this study indicates that drug resistance transmission may have decreased recently in Switzerland through the increased frequency of infection with HIV non-B subtypes and the steady increase of patients with undetectable viraemia.

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Introduction

Molecular epidemiology for human pathogens has been successfully used as a tool to identify clusters in order to assess the modalities of the spread of infection and to monitor the impact of therapy on the transmission of

drug resistance [1–3]. These, in turn, may translate into prevention measures focused on populations at risk.

Transmission of HIV-1 infection is largely influenced by viraemic levels [4]. Individuals with primary HIV-1 infection (PHI) harbour high viral load in both blood

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and genital secretions [5–8] and it has been postulated that spread of HIV infection is driven in large part by these individuals [9,10]. However, because of the difficulty in identifying individuals with acute infection, there are only anecdotal reports on secondary transmission in this clinical setting [11,12].

Suboptimal therapy only partially contains viral replication and selects for drug-resistant variants. Non-nucleoside reverse transcriptase inhibitors and protease inhibitors (PI) are more potent than previously used drugs, which might have an impact on transmission of drug-resistant variants. Another factor that may influence the rate of transmission of drug-resistant variants is the prevalence of infection with non-B subtypes, since these non-B subtypes originate from countries where antiretroviral drugs are not readily available [13–15]. In Western Europe and in the United States, the prevalence of drug-resistant variants in patients with recent HIV infection has been approximately 10% in recent years [16–22]. This relatively high transmission rate of drug-resistant variants is a major concern for the efficacy of antiretroviral therapy in the future and has led to the recommendation of resistance testing in recently infected individuals [23,24].

In order to evaluate the impact of PHI in the spread of HIV and to assess the rate of transmission of drug-resistant variants, we have performed phylogenetic analyses based on *pol* sequences and collected epidemiological data in 197 individuals with documented PHI in Switzerland and neighbouring France over four years (1996–1999).

Methods

Study population

The study included all individuals with documented PHI identified in six AIDS centres of University Hospitals in Switzerland (Basel, Geneva, Lausanne, Lugano, St Gallen and Zurich) and two AIDS Centres of Hospitals close to Geneva (Annemasse and Annecy, France). For each patient, the clinician in charge completed an epidemiological questionnaire labelled with a code number. In the total of 197 individuals infected between January 1996 and January 2000, PHI was documented by evolving HIV antibody response and/or symptoms consistent with an acute retroviral syndrome [25] within 3 months in 70% of individuals and by seroconversion within 12 months of presentation in 30%. Sequence analyses were performed on plasma samples collected at the first visit, before initiation of antiretroviral treatment.

Sequence analysis

The procedure of viral isolation, reverse transcription,

amplification and sequencing were described earlier [16]. Briefly, viral RNA was isolated from 100 μ l plasma using Amplicor HCV Cobas reagents (Roche, Basel, Switzerland). Viral RNA was transcribed into complementary DNA (cDNA), which was subjected to nested polymerase chain reaction (PCR) for reverse transcriptase (RT) and protease genes. Direct double-stranded sequencing was performed on an automatic sequencer with Big Dye terminator kit (Applied Biosystems, Foster City, California, USA). Alignment of sequences was performed using CLUSTAL W version 1.7 [26]. Phylogenetic analyses were carried out using the PHYLIP package [27]. Neighbour-joining trees with 100 replicates were used in the bootstrap analysis [28], and values above 85 (85%) were considered as supporting the grouping. These clusters were confirmed using Fitch–Margoliash and least-squares analysis with 100 bootstrap replicates [29]. HIV-1 subtypes were identified on combined RT plus protease sequences using SimPlot software, which calculates and plots the percentage identity of the query sequence to a panel of reference sequences [30].

Viraemia

Quantification of HIV-1 RNA levels were performed using Amplicor HIV Monitor (Roche) according to the manufacturer's instruction. For the analysis of viraemia over time for each patient in the Swiss HIV Cohort Study, the first viraemia value for each year was used.

Immunological parameters

CD3, CD4 and CD8 lymphocyte cell counts were determined by flow cytometry (Coulter EPICS IV, Basel, Switzerland) using fluorescein-labelled DAKO-T3, DAKO-T8, and R-phycoerythrin DAKO-CD4 (Dako, Glostrup, Denmark).

Results

Study population

A total of 197 individuals with documented PHI were included. The mean age was 34 years (range, 18–71); 80% were male and 94% were Caucasians. The risk factors for HIV-1 transmission were homosexual contacts in 42%, heterosexual contacts in 42%, intravenous drug use (IDU) in 13% and unknown in 3%. Break-down of these data per year of HIV-1 infection is reported in Table 1. At the time of sequence analysis, the mean HIV-1 RNA was 5.08 \log_{10} copies/ml (range, 3.0–7.45) and the mean CD4 cell count was 593×10^6 cells/l (range, 100–1817).

HIV-1 subtypes

Sequences of the RT and protease genes were recovered for 191 and 176 individuals, respectively. All

Table 1. Characteristics of individuals according to year of HIV-1 infection.

Characteristics	1996	1997	1998	1999	Total
Number	35	41	60	61	197
Male (%)	68.6	85.4	83.3	78.7	79.7
Risk factors (%)					
Homosexual contacts	28.6	56.1	45.0	37.7	42.1
Heterosexual contacts	54.3	36.6	33.3	45.9	41.6
Intravenous drug use	11.4	4.9	16.7	14.8	12.7
Unknown	5.7	2.4	5.0	1.6	3.6
Non-B subtype (%)	22.9	22.0	21.1	35.0	25.9
Drug resistance (%)	8.6	14.6	8.8	5.0	8.8

sequences showed a full-length open reading frame. HIV-1 B subtypes were identified using SimPlot analysis in 143/193 (74%) individuals. Non-B subtypes were detected in 23% of individuals in 1996, 22% in 1997, 21% in 1998 and 35% in 1999 (Fig. 1). The percentages of non-B subtypes were 1.6% A, 3.6% C, 2% D, 1% F, 0.5% G, 5.8% J, 4.8% AE and 6.6% AG. Non-B subtypes were more frequently detected in female than in male patients (41% versus 21%). Among Caucasians, 22% were infected with non-B subtypes compared with 60% of non-Caucasians. Non-B subtypes were detected in 7.5% of individuals infected by homosexual contacts, 40% by heterosexual contacts and 26% by IDU. An increase in HIV-1 subtype diversity over time was observed in the heterosexual risk group. The J subtype, already observed in 1996 in IDU, was first detected in 1999 in the heterosexual contact risk group, suggesting transmission of this subtype from IDU to heterosexuals.

Phylogenetic analyses and epidemiological linkages

A phylogenetic tree constructed by using neighbour-joining method for the 193 available RT sequences

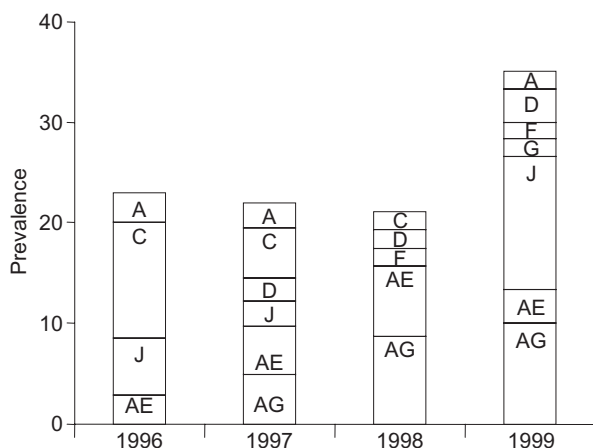


Fig. 1. Prevalence of HIV-1 subtypes in individuals with primary HIV infection. Subtypes were identified using SimPlot analysis [28] for reverse transcriptase and protease sequences.

revealed significant clustering (bootstrap value $\geq 85/100$) for 58 (30%) individuals (data not shown). Sequences from these individuals were re-analyzed using the Fitch–Margoliash method. The resulting phylogenetic tree demonstrates significant clustering (bootstrap value $\geq 90/100$) for 56 (29%) individuals (Fig. 2). Similar results were obtained using phylogenetic analyses of the protease gene (data not shown).

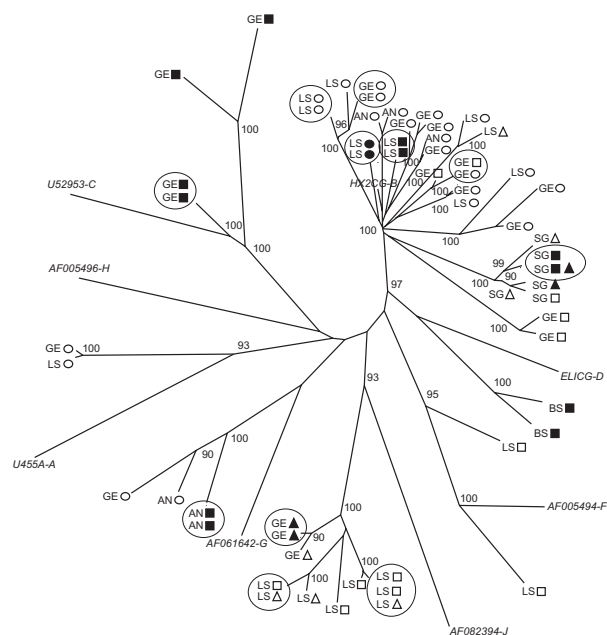


Fig. 2. Phylogenetic tree analysis of individuals with primary HIV infection in Switzerland. Data are based on sequences of the reverse transcriptase gene from 58 individuals using neighbour joining method with 100 replicates. Sequences from eight HIV-1 subtypes retrieved from the GeneBank are included (U455A-A, HXB2CG-B, U52953-C, ELICG-D, AF005494-F, AF06142-G, AF005496-H, AF08394-J). Bootstrap values over 90 (90% of 100 replicates) are shown. Large circles report sequences with 100% similarity. Geographical factors for HIV infection are reported: GE, Geneva; LS, Lausanne; AN, Annecy; SG, St Gallen; homosexual contact, ○; heterosexual contact, □; intravenous drug use, △; black symbols, linkage confirmed by epidemiological data.

Eighteen clusters of genetically related viruses, each including 2 to 11 PHI individuals were observed. Clusters were more frequently observed in IDU than in homosexuals and heterosexuals (48%, 30% and 26%, respectively).

Epidemiological linkages were formally documented for three individuals included in a cluster of six individuals infected in the St Gallen (SG) area. The index case was a female infected through rape. She transmitted the infection to her regular sexual partner and to at least one other individual through needle sharing at the time she presented symptoms of acute HIV infection. The six individuals shared drugs or had common friends. Another large cluster (HIV subtype J) of 11 individuals was observed in the western part of Switzerland. This cluster was subdivided into three subclusters (Fig. 2). Evidence of secondary infection through needle sharing was documented by contact tracing in two individuals.

The 16 other clusters included two to four individuals. These individuals lived in the same geographical areas (Annecy and Lausanne are about 60 km from Geneva), and had the same risk factor for HIV infection, except two (homosexual contact and IDU). The delay between the estimated dates of infection was less than 12 months for individuals included in the same cluster. Epidemiological linkages were documented for six couples (five heterosexual and one homosexual couple). Four couples (two non-Caucasian and two Caucasian couples) presented a similar history. In each case, the husband had a short stay (less than 2 months) in Africa, had sexual intercourse with commercial sex workers, developed acute retroviral syndrome shortly after returning in Switzerland and infected his wife within 10 days. All the female partners developed an acute retroviral syndrome within 3 to 6 weeks. In one case, the wife started highly active antiretroviral therapy (HAART) within 10 days after the return of her husband, before seroconversion or other evidence of HIV infection developed. Treatment was stopped after 1 month and acute HIV infection developed in the wife. Among the 56 PHI individuals with significant sequence clustering, contact tracing was documented in 17 individuals, demonstrating secondary HIV infection at the time of PHI. There were no epidemiological linkages between individuals with unrelated sequences.

Transmission of drug-resistant variants

Mutations previously described as associated with anti-retroviral drug resistance [31] were observed in 17/193 (8.8%) individuals. The prevalence of genotypic drug-resistant variants among patients with PHI was 8.6% (PI resistant, 3.0%) in 1996, 14.6% (PI resistant, 7.7%) in 1997, 8.8% (PI resistant, 2.0%) in 1998 and 5.0% (PI resistant, 1.9%) in 1999. A similar trend was observed when considering only the 143 PHI individuals in-

fectured with HIV-1 B subtype (8.0% in 1996, 15.6% in 1997, 11.6% in 1998 and 7.5% in 1999). Eleven (5.8%) individuals harboured variants with mutations associated with resistance to zidovudine and or stavudine (M41L, D67N, K70R, L210W and T215Y/F). The lamivudine-resistant mutation M184V was detected in three (1.6%) individuals and mutations associated with resistance to non-nucleoside reverse transcriptase inhibitors (G190A and/or Y181C) were detected in two (1.1%) individuals. Major mutations associated with PI resistance were detected in 6/176 (3.4%) individuals: the V82A/F mutation was detected in four of these and the L90M mutation in two. In these six individuals, two to five of the minor mutations associated with PI resistance (L10I, K20R, M36I, I54V, L63P, A71T/V and I84V) were also detected. Amino acid insertions in the protease (35ED, 37NN) were observed in three individuals; epidemiological linkage was documented for two of them. Drug-resistant variants were identified more frequently in male than female (10.4% versus 2.6%), and more frequently in IDU (13%) and homosexuals (11.3%) than in heterosexuals (6.1%).

There was a decrease in the prevalence of the transmission of drug-resistant variants at the later stages of the study. The proportion of patients with undetectable viraemia in the Swiss HIV Cohort Study increased from less than 10% to more than 50% between 1996 and 1999 (Fig. 3).

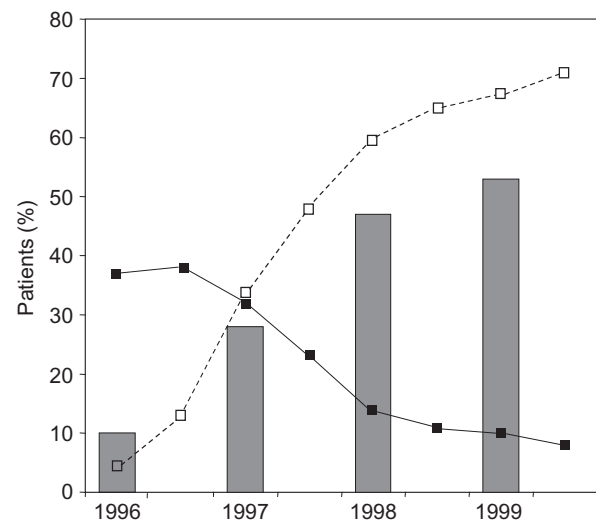


Fig. 3. Proportion of HIV-1 infected individuals with undetectable viraemia (HIV RNA < 400 copies/ml) in the Swiss HIV Cohort Study (n = 4500). Grey bars show the proportion of patients included in the Swiss HIV Cohort Study with viraemia < 400 copies/ml) in 1996–1999. Bold line shows the proportion of treated patients on mono or bitherapy and the dashed line show those on triple therapy including protease inhibitor(s) and/or non-nucleoside inhibitor of the reverse transcriptase.

Discussion

It has long been suspected that individuals with PHI are more likely to transmit infection, owing to their high viraemia and their unawareness of their infection status [9,10]. In this study, we found that approximately one-third of individuals with PHI harbour viral variants genotypically related to other individuals with recent HIV transmission and this was confirmed by contact tracing in one-third of them. This is likely a minimal estimate since during the 4 years of the study period there have been approximately 3000 newly reported HIV-1 infections in Switzerland [32] and we have identified only 197 individuals at the time of PHI.

In this large and systematic study, a peak in transmission of antiretroviral drug-resistant variants, reaching nearly 15%, was observed in 1997, followed by decreased prevalence in 1998 (8.8%) and 1999 (5.0%). As in previous studies, genotypic resistance to RT inhibitors was more frequent than to PI [16–22]. The prevalence of transmission of drug-resistant variants was higher in IDU (13%) and in the homosexual group (11%) than in the heterosexual group (6%). The high transmission rate of drug-resistant variants in the first two risk groups is likely because of the initial spreading of HIV-1 infection and, therefore, earlier antiretroviral treatment within these two risk groups. The lower transmission rate observed in the heterosexual risk group is also partially explained by the high percentage of women infected with HIV-1 non-B subtypes (41%).

The observed recent decrease in the prevalence of the transmission of drug-resistant variants is unexpected. Several factors might be contributive. Viral load is the most important factor determining the probability of HIV transmission among untreated serodiscordant individuals [4] as well as for vertical transmission [33,34]. It is, therefore, likely that any decrease in viraemia induced by HAART decreases infectiousness. Transmission of drug-resistant variants probably originates in a population of individuals who continue to be viraemic despite HAART. Conversely, transmission of drug-resistant variants is unlikely if HAART is effective, or if new cases of HIV infection derive from infected individuals who have not been exposed to antiretroviral drugs, such as African immigrants. We provide evidence that both factors might be involved in Switzerland. The proportion of patients with undetectable viraemia in the Swiss HIV Cohort Study increased from less than 10% to more than 50% between 1996 and 1999 (Fig. 3). Moreover in the period to 1997, a large proportion of patients were on mono- or bitherapy with nucleoside analogue inhibitors of RT. In this period, 563 (72%) of these patients had virological failure, with viraemia > 400 copies/ml 6 months after treatment initiation. In 67% of these patients, a switch of nucleoside analogue inhibitor and

addition of a PI and/or non-nucleoside inhibitor of RT was associated with a decrease in viraemia to < 400 copies/ml in 1997–1999. Overall, this resulted in a decrease of the population of infected patients susceptible to transmit drug-resistant variants. An additional factor contributing to the decrease in the transmission of drug-resistant variants is the steady increase of individuals infected with HIV-1 non-B subtypes. These non-B subtypes were observed at high prevalence, but not exclusively, in African immigrants and in women infected through heterosexual contacts.

New guidelines favour a less aggressive strategy for initiation of treatment, based more on CD4 cell counts than on viraemic levels, and treatment interruptions within or outside clinical trials are increasingly popular because of drug adverse events. This obviously leads to an increased number of infectious individuals. Measures to prevent HIV transmission should, therefore, be reinforced. Finally, if mutations conferring multiple drug resistance result in reduced viral fitness [35], the limitations in the replication capacity might also reduce the risk of transmission.

The data presented here underline two main points: first, it is not inevitability that transmission of antiretroviral drug resistance will increase, since preventive programmes and treatment availability may decrease it; second, individuals with acute HIV infection are frequently involved in the spread of HIV in a geographical area where newly diagnosed infections decreased over recent years. Hence, the identification of newly infected individuals and contact tracing should be considered as an important public health measure to reduce HIV transmission further.

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Appendix

The members of the Swiss HIV Cohort Study are R. Amiet, M. Battegay (Chairman of the Scientific Board), E. Bernasconi, H. Bucher, Ph. Bürgisser, M. Egger, P. Erb, W. Fierz, M. Flepp (Chairman of the Clinical and Laboratory Committee), P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, CH-1011-Lausanne), H. J. Furrer, M. Gorgievski, H. Günthard, P. Grob, B. Hirschel, C. Kind, Th. Klimkait, B. Ledergerber, U. Lauper, M. Opravil, F. Paccard, G. Pantaleo, L. Perrin, W. Pichler, J.-C. Piffaretti, M. Rickenbach (Head of Data Centre), C. Rudin (Chairman of the Mother & Child Substudy), P. Sudre, V. Schiffer, J. Schupbach, A. Telenti, P. Vernazza and R. Weber.