Impact of antiretroviral therapy on HIV-1 transmission dynamics

Bezemer, D.O.

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IV

Combination antiretroviral therapy failure and HIV super-infection

Daniela Bezemer\textsuperscript{a}, Ard van Sighem\textsuperscript{b}, Frank de Wolf\textsuperscript{b,}\textsuperscript{c}, Marion Cornelissen\textsuperscript{c}, Antoinette C. van der Kuyl\textsuperscript{c}, Suzanne Jurriaans\textsuperscript{c}, Lia van der Hoek\textsuperscript{c}, Maria Prins\textsuperscript{d,}\textsuperscript{e}, Roel A. Coutinho\textsuperscript{d,}\textsuperscript{f} and Vladimir V. Lukashov\textsuperscript{c}

In addition to development or selection of resistance, failure to continuously suppress HIV-1 production while still using initially effective combination antiretroviral therapy (cART) may result from super-infection with a drug-resistant strain. Both transmission of drug resistant HIV and super-infection have been demonstrated. We analysed HIV \textit{pol} genes obtained before start of initially successful cART and during failure while still on cART in 101 patients. Difference in precART and cART failure sequences were explained by evolution and not by super-infection.

A considerable proportion of new HIV-1 infections are caused by viruses carrying antiretroviral drug-resistant mutations [1–3]. In The Netherlands, 6\% of recent infections were found to contain mutations associated with resistance [4]. Although rare, super-infection with another and even drug resistant HIV-1 strain has been demonstrated [5–13]. Given the popularity of seroconversion amongst HIV-1-infected males having sex with males (MSM) [14–20], we therefore investigated to what extent super-infection of initially successfully cART-treated individuals could explain treatment failure.

We analyzed HIV-1 \textit{pol} sequences obtained from patients before starting cART and during failure, while still on cART. The clinical, virological and immunological data of patients were collected within the framework of the ATHENA national observational cohort [21]. Failure was defined by a detectable HIV-1 RNA load while on cART, after at least one successfully suppressed plasma sample taken following start of cART. In total, 9390 patients started cART and experienced initial success. There were 22,395 person-years of follow-up from the first successful load measurement until the earliest of cART failure and the last available load measurement, with a rate of failure of 0.25 per person-year. Three thousand seven hundred and twelve patients with a median follow-up of 2.5 years since the first suppressed measurement [interquartile range (IQR) = 0.8–5.3 years] did not fail cART. Five thousand six hundred and seventy-eight (60\%) failed within a median of 0.9 years (IQR = 0.3–2.2 years). Of these patients, 32\% were pretreated before start of cART, which was significantly larger compared to the group of patients that did not fail (13\%, \textit{P} < 0.001). HIV-1 polymerase gene (\textit{pol}) sequences, both before starting cART and during virological failure while still on cART, were available for 101 patients older than 16 years. Population-based nucleotide sequencing of the HIV-1 \textit{pol} gene was performed as described previously [4], and sample contamination was checked for at the respective sequencing sites. Multiple sequence alignment was performed using the default parameters of ClustalX, release 1.83. From 55 patients, more than two sequences were available, which allowed for analysis of 338 sequences in total. The median percentage of ambiguous sites among the total 338 sequences was 0.6\% (IQR = 0.2–1.0\%). Pairwise sequence distances were calculated taking into account ambiguous sites by the mixed weighted distance method, as previously described by Gonzales \textit{et al.} [12]. Phylogenetic analysis was performed using the MEGA program, Neighbour-joining model with
Kimura two-parameter distances and bootstrap analysis (1000 replications), ignoring the ambiguous sites. Both the protease (PR) and reverse transcriptase (RT) region could be included in the analysis for 98 patients, and, either PR or RT was available for three patients. Therefore, two phylogenetic analyses were performed: one for the sequence set sharing at least RT, the other for sequences sharing at least PR. Bootstrap values higher than 80 were considered to be significant. Sequences were screened for resistance-conferring mutations at the amino acid positions described by the International AIDS Society (USA) [22], these sites were not ignored in the analysis.

The study group included 85 men and 16 women. Transmission risk groups were MSM (n = 68), heterosexual transmission (n = 21), injecting drug use (IDUs, n = 6), and blood transfusion (n = 2). For four patients the route of transmission was unknown. Median CD4 cell count at cART initiation was 200 × 10⁶ cells/µL (IQR = 70–310 cells/µL). Median time between the start of cART and viral suppression was 4.1 months (IQR = 1.5–7.1 months). Initial viral suppression lasted 4.9 months (IQR = 2.5–9.6 months). Median time between the start of failure while still on cART and the first blood sample used for HIV isolation and sequencing was 3.3 months (IQR = 0.2–20.9 months). Half of the patients (n = 51) were antiretroviral treatment naïve at the start of cART, of whom 23% (n = 11) presented with resistance-conferring mutations before cART. The other half of the patients (n = 50) had experienced antiretroviral treatment before, and 72% (n = 36) presented with drug-resistant mutations before start of cART. Sequences obtained after initial cART failure showed in 83 (81%) of 101 patients drug-resistant mutations and in all sequences obtained thereafter.

Phylogenetic analysis showed 85 patients being infected with HIV-1 subtype B viruses. For 101 patients, the sequences obtained before start and during failure of cART clustered together with bootstrap values above 90. Two pairs of patients had sequences that clustered together with bootstrap values of 99 but, within those clusters, the sequence clusters from the respective patients did not intermix.

Table 1 shows median pairwise nucleotide sequence distances, which were significantly smaller at intra- than at inter-patient level (P < 0.001). The highest absolute distances between the last sample taken before cART and the first sample taken during failure become smaller after correction for time between sequences, whereas the distance corresponding to the shortest time interval (0.4 years) became highest when extrapolated to a yearly rate. Positive selection between the last sample taken before cART and the first sample taken during failure at PR (nonsynonymous mutations per nonsynonymous site/synonymous mutations per synonymous site > 1) was found in 14 patients, 12 at PR and two at RT. Those intra-patient sequence pairs with a nucleotide distance > 4.5% in PR (seven patients) or > 3.0% in RT (15 patients) revealed no signs of recombination with a different strain at the amino acid level because the distance was only due to single (several ambiguous) substitutions, many at known resistance conferring sites.

In conclusion, in this selected subgroup of patients who experienced virological failure while still on initially successful cART, no evidence for super-infection with resistant HIV-1 was observed. Transmission risk behaviour around cART failure was reported in this small study group. Three IDUs reported risk behaviour between the cART start date and the date of cART failure: injecting drugs in two, one including needle sharing, and unprotected sex with a steady HIV-1 positive partner in the third. Four MSM reported unprotected anal sex between the date of starting cART and that of virological failure. When HIV is transmitted from a donor in a tight and limited transmission network (e.g. the originally infecting or infected partner),
detecting super-infection is almost impossible. Different treatment regimens for sero-concordant couples might be protective, but substantial cross-resistance between drugs should be considered [22].

4 HIV Monitoring Foundation, Academic Medical Centre, University of Amsterdam, the Netherlands; 5 Department of Infectious Disease Epidemiology, Imperial College London, UK; 6 Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands; 7 Department of Internal Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands; 8 Department of Infectious Diseases, Health Service Amsterdam, the Netherlands; and 9 Center for Infectious Disease Control, National Institute of Public Health and the Environment, Bilthoven, the Netherlands.

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