Episodes of low-level viral rebound in HIV-infected patients on antiretroviral therapy: frequency, predictors and outcome

Pilar García-Gascó, Ivana Maida, Francisco Blanco, Pablo Barreiro, Luz Martín-Carbonero, Eugenia Vispo, Juan González-Lahoz and Vincent Soriano*

Service of Infectious Diseases, Hospital Carlos III, Madrid, Spain

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Background: The rate, predictors and outcome following episodes of low-level viral rebound (LLVR) in HIV patients on highly active antiretroviral therapy (HAART) are unknown.

Methods: Retrospective assessment of all HIV patients who experienced LLVR episodes on HAART at one institution from January 1999 to December 2006. LLVR was defined as plasma HIV-RNA between 51 and 500 copies/mL after at least two consecutive undetectable plasma viral load measurements made during the last 6 months. Virological failure was defined as plasma HIV-RNA >500 copies/mL.

Results: Out of 2720 HIV patients on successful HAART during the 8 year study period, 779 (28.6%) developed at least one LLVR episode. Only 655 patients who kept unchanged their HAART regimen following LLVR episodes were further examined. After 12 weeks, undetectable viraemia was regained in 458 (71%), which were considered as blips. In contrast, 66 (9%) LLVR episodes were followed by virological failure, and drug resistance mutations developed in most cases, mainly rtM184V (66%) and rtK103N (29.5%). Plasma HIV-RNA remained between 51 and 500 copies/mL at 12 weeks in the remaining 131 (20%) patients with LLVR episodes. In the multivariate analysis, only plasma HIV-RNA level at the time of LLVR predicted subsequent virological failure.

Conclusions: Episodes of LLVR in HIV patients on successful HAART are relatively common and represent transient events (blips) in most cases (71%). Keeping the same treatment regimen, virological failure follows in <10% of the cases. Plasma HIV-RNA level at the time of LLVR is the best predictor of subsequent failure.

Keywords: blips, viral load, virological failure, drug resistance

Introduction

More than three-quarters of HIV-infected individuals on highly active antiretroviral therapy (HAART) currently show undetectable plasma HIV-RNA. Virological control in this population has translated into a dramatic clinical benefit.1–3 However, episodes of detectable viraemia have been described in up to 20% to 40% of patients followed longitudinally who have achieved complete viral suppression on HAART.4–8 Intermittent episodes of detectable low-level viral rebound (LLVR) preceded and followed by undetectable viraemia, without any change in therapy, are often called ‘blips’. However, LLVR episodes may occasionally anticipate subsequent virological failure and selection of drug-resistant viruses,9–11 a circumstance that forces switching antiretroviral therapy.

The clinical significance of episodes of LLVR in patients on successful HAART has been a matter of debate with controversial results. Poor drug compliance, prior antiretroviral drug resistance and intercurrent illnesses or vaccination have all been associated with a higher rate of virological failure following episodes of LLVR on HAART,4 while in other studies this adverse outcome has not been confirmed.5,8,12–14 Moreover, episodes of transient viraemia on HAART have occasionally been seen in patients carrying drug-resistant viruses in whom virological failure did not develop on subsequent follow-up.15,16 These LLVR episodes may be more frequent using antiretrovirals with poor penetration in some body compartments17 and/or having less potency.18,19 At this time, it is unclear whether the rate and subsequent outcome following LLVR episodes on HAART is influenced by CD4 counts, baseline viral load, duration of HIV

*Corresponding author. Tel: +34-91-4532500; Fax: +34-91-7336614; E-mail: vsoriano@dragonet.es

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infection or antiretroviral treatment modality.\textsuperscript{6,20–22} Herein, the incidence of episodes of LLVR is retrospectively examined in a relatively large group of HIV-infected individuals on HAART. Predictors of subsequent outcome, including regain of undetectable viraemia or virological failure, are further investigated.

Patients and methods

All HIV-infected individuals on at least triple antiretroviral drug therapy on regular follow-up from January 1999 to December 2006 at one HIV/AIDS reference hospital located in Madrid were examined. Patients currently under suboptimal therapy, including mono- or dual nucleoside reverse transcriptase inhibitor (NRTI) therapy, were excluded. Subjects with undetectable viraemia in the last two consecutive determinations covering at least the last 6 months who subsequently experienced LLVR episodes were identified and followed for another 12 weeks. LLVR was defined as plasma HIV-RNA between 51 and 500 copies/mL. Only the subset of patients who maintained the same treatment regimen during the follow-up were selected for the purpose of this study. Virological failure was defined as a plasma HIV-RNA value over 500 copies/mL after the LLVR episode.

Demographics as well as risk factors for HIV infection, time from HIV diagnosis and HAART regimen were all recorded in a single database. Information on CD4 counts and plasma HIV-RNA throughout the follow-up were also recorded. In patients experiencing virological failure following episodes of LLVR, a drug resistance test was performed.

Plasma HIV-RNA was determined in all cases using the Versant\textsuperscript{TM} bDNA assay (Bayer Diagnostics, Barcelona, Spain), which has a lower limit of detection of 50 HIV-RNA copies/mL. Genotypic resistance in the reverse transcriptase and protease genes was examined using the dRhodamine Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and the ABI 3100 sequencer (Applied Biosystems).

CD4 counts were determined using flow cytometry (Coulter, Barcelona, Spain). Adherence to therapy was evaluated using interview questionnaires made during the clinical visit and using monthly pharmacy records. Compliance was considered as good only when more than 95\% of prescribed pills had been taken during the last month.

Statistical analysis

All data were analysed using the SPSS software package version 13.0 (SPSS Inc., Chicago, IL, USA). The rate of LLVR episodes in patients on HAART with undetectable viraemia was estimated by taking into account the number of individuals who experienced a first episode of viral rebound between 51 and 500 HIV-RNA copies/mL.

Subjects with LLVR episodes were split into two groups according to their subsequent outcome, which was virological failure or not. Univariate analysis was performed to compare baseline characteristics of these two groups. Continuous data were analysed using Student’s \textit{t}-test for equal variances or the Wilcoxon rank-sum test for variables that were not normally distributed. Categorical data were compared using the Chi-square or the Fisher exact tests. A multivariate analysis was performed in order to find out which variables were associated with virological failure following LLVR episodes, using logistic regression.

Results

Out of 2720 distinct HIV-infected individuals on HAART who achieved undetectable plasma HIV-RNA during the 8-year study period, 779 (28.6\%) experienced at least one episode of LLVR. Of them, only patients who kept unchanged their antiretroviral medication following the first episode of LLVR were further examined, resulting in 655 (80.5\%) patients. Overall, 524 (80\%) of them were male, with a mean age of 44 ± 7.8 years (range, 18–80), and their distribution according to HIV transmission routes was as follows: injecting drug users 43.6\%, men who have sex with men 41.2\%, heterosexuals 13.7\% and infected by other routes 1.5\%. First HIV diagnosis had been made between 1982 and 2005. Mean time with undetectable plasma HIV-RNA until the moment of the first LLVR episode was 22.8 ± 16 months (range, 2.2–99), with no significant differences by calendar.

Of 779 first LLVR episodes, 38 (4.9\%) occurred in 1999, 71 (9.1\%) in 2000, 79 (10.1\%) in 2001, 178 (22.8\%) in 2002, 124 (15.9\%) in 2003, 110 (14.1\%) in 2004, 99 (12.7\%) in 2005 and 80 (10.3\%) in 2006. The rate of LLVR episodes was not associated with age, gender, risk group, treatment modality or time from HIV diagnosis. The proportion of patients on three NRTI, NNRTI and PI slightly changed during the study period. Briefly, the proportion of patients on 3 NRTIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs) was 5\%, 40\% and 55\% in year 1999; 20\%, 50\% and 30\% in year 2003 and 5\%, 60\% and 35\% in year 2006.

At the time of first LLVR, the mean CD4 count was 601 ± 308 cells/mm\(^3\) and the mean plasma HIV-RNA was 2.04 ± 0.3 log copies/mL. Of the 655 patients with LLVR episodes who did not modify their antiretroviral regimen, plasma HIV-RNA levels were between 51–100, 101–300 and 301–500 copies/mL in 345 (52.7\%), 246 (37.6\%) and 64 (9.8\%) cases, respectively. Subsequently, 66 (9.1\%) patients experienced virological failure, 458 (70.9\%) regained undetectable viraemia (and therefore were true \textit{blips}) and 131 (20\%) maintained low-level detectable viraemia between 51 and 500 HIV-RNA copies/mL (Figure 1).

The outcome following LLVR episodes did not differ significantly comparing different years (Figure 2), although it should be noted that the rate of virological failure has significantly diminished since 2001 (\textit{P} = 0.02). Overall, 20 (15\%) out of 131 cases who maintained low-level viraemia following a first LLVR episode subsequently experienced virological failure, 64 (49\%) regained undetectable plasma viraemia and 47 (36\%) persisted with detectable viraemia between 51 and 500 HIV-RNA copies/mL (Figure 1).

In patients who experienced virological failure after the first LLVR episode, the mean CD4 count did not differ significantly in comparison with patients who regained undetectable viraemia. Nevertheless, the CD4 count decreased after the LLVR episode in patients who failed while it remained stable in patients keeping virological suppression. Overall, there were no differences in the rate of virological failure following LLVR episodes after adjustment for CD4 counts (>200 or <200 CD4+ T cells/mm\(^3\)).

LLVR episodes occurred in 22.5\% of patients treated with triple NRTI regimens, in 44\% treated with NNRTI-based
regimens and in 33.5% on PI-based combinations. However, the rate of subsequent virological failure did not differ significantly when comparing these three groups of patients (Figure 3).

Among 101 subjects on Trizivir (zidovudine, lamivudine and abacavir), 8 (8%) developed virological failure. As reference, virological failure following LLVR episodes occurred in 45 (9%) out of 508 patients on NNRTI- or PI-based regimens (P = 0.2). Moreover, when all patients on distinct triple NRTI regimens were considered together, the rate of virological failure increased to 9.3% confirming that there were no differences between treatment modalities. Considering separately patients on NNRTI and PI, the rate of virological failure following LLVR episodes was 7.9% (23 out of 291) and 10.4% (21 out of 217) (P = 0.3).

The univariate analysis showed that only older age and higher viral load at the time of LLVR were significantly associated with subsequent virological failure [OR: 0.945 (95% CI: 0.908–0.989), P = 0.002; and OR: 1.25 (95% CI: 1.152–1.361), P < 0.001, respectively]. In the multivariate analysis (Table 1) including demographics, CD4 counts, time from HIV diagnosis, treatment modality and viral load at LLVR, only the degree of plasma viraemia at the time of LLVR predicted subsequent virological failure [OR: 1.281 (95% CI: 1.087–1.509); P = 0.003] (Figure 4). The best threshold to discriminate subsequent virological failure was a plasma HIV-RNA of 2.07 logs, equivalent to 120 copies/mL [r = 0.68 (95% CI: 0.62–0.74); P < 0.001].

Results from drug resistance testing could only be obtained from 44 patients with LLVR episodes who subsequently experienced virological failure. In the rest, low viral load values most likely precluded obtaining amplicons. The mean number of drug resistance mutations in patients experiencing virological failure was 7 ± 4 (range, 1–20), without significant differences when comparing subjects on distinct treatment modalities. However, as expected some drug-resistant genotypes were more prevalent according to the therapeutic regimen. For example, rtK103N was seen in 69% of patients failing on NNRTI while it was present in only 8% and 23% of subjects failing on triple NRTI- or PI-based regimens, respectively (P = 0.006). Overall, the changes more frequently found were rtM184V and rtK103N, and the L63P polymorphism in the protease (Table 2).

Discussion

The rate of virological failure following achievement of undetectable viraemia on HAART has been reported to vary during the subsequent year of follow-up between 20% and 53%.1–7 The rate of a first episode of LLVR in our study was 28.6%, which may be seen as relatively low. However, we restricted the definition of LLVR to patients experiencing viral rebound between 51 and 500 HIV-RNA copies/mL after at least two consecutive measurements with <50 copies/mL within the last 6 months of HAART. Thus, differences in definitions may explain at least in part our results.

While most studies have reported that episodes of intermittent plasma viraemia are not associated with subsequent virological failure,5,8,13,14,16 other reports have found a greater risk of virological failure.4,23,24 In our study, the rate of virological failure following a first episode of LLVR on HAART was below
10%, which is in agreement with the results from others.\textsuperscript{4,5,25} Furthermore, nearly three-quarters of patients experiencing LLVR episodes in our study subsequently regained undetectable viraemia without changing therapy. The recognition of these true ‘blips’ reinforces the notion that a conservative attitude in the clinical management of LLVR seems to be reasonable for most patients on HAART experiencing sporadic episodes of low-level viraemia, without an urgent need for switching therapy. However, it should be noted that the outcome following episodes of LLVR differed according to the level of plasma viral rebound, as already has been noticed by others.\textsuperscript{4,25} Our data strongly advise for a close follow-up in patients showing viral rebounds above 120 HIV-RNA copies/mL in whom the risk of subsequent virological failure is significantly increased.

Viral replication continues during successful treatment in most if not all patients.\textsuperscript{26,27} It is the recruitment of target uninfected cells by activation and the expression of proviral DNA integrated in latently infected cells which explain episodes of viral rebound in patients on HAART. The trigger is generally a transient activation of the immune system by intercurrent infections. Using mathematical modelling, Jones and Perelson\textsuperscript{28} have recently shown that the level of residual viraemia below 50 copies/mL in patients on successful HAART may predict the amplitude of blips. If the level of residual viraemia is 30 copies/mL, the blip will be of <300 copies/mL. If the level

\begin{table}[h]
\centering
\caption{Predictors of subsequent virological failure in patients on HAART experiencing LLVR}
\begin{tabular}{|l|c|c|c|c|}
\hline
 & Patients with LLVR and no & Patients with LLVR and & Bivariate & Multivariate \\
 & LLVR and no & subsequent VF & subsequent VF & analysis ($P$) & analysis ($P$) \\
 & ($n = 589$) & ($n = 66$) & & & \\
\hline
Gender & & & & & \\
male ($n = 524$) & 485 (92.6\%) & 39 (7.4\%) & NS & NS \\
female ($n = 131$) & 121 (92.6\%) & 10 (7.4\%) & & & \\
Age (years) & 44 $\pm$ 7 & 40 $\pm$ 9 & 0.002 & NS \\
Risk group & & & & & \\
IDU ($n = 286$) & 274 (96\%) & 12 (4\%) & NS & NS \\
MSM ($n = 270$) & 251 (93\%) & 19 (7\%) & NS & NS \\
HTS ($n = 90$) & 85 (95\%) & 5 (5\%) & & & \\
other ($n = 9$) & 9 (100\%) & 0 (0\%) & & & \\
Time from undetectable VL to LLVR (months) & 22.6 $\pm$ 16 & 24.6 $\pm$ 18 & NS & NS \\
CD4 count at LLVR (cells$/$mm$^3$) & 602 $\pm$ 311 & 588 $\pm$ 18 & NS & NS \\
Plasma HIV-RNA at LLVR (log copies/mL) & 2 $\pm$ 0.26 & 2.2 $\pm$ 0.28 & <0.001 OR: 1.25 & 0.003 OR: 1.281 \textendash (1.152$\textendash$1.361) & (1.087$\textendash$1.509) \\
Years from HIV diagnosis & 9.5 $\pm$ 5 & 11 $\pm$ 5 & NS & NS \\
Treatment group & & & & & \\
3 NRTIs ($n = 92$) & 90 (90.7\%) & 2 (9.3\%) & NS & NS \\
NNRTIs ($n = 319$) & 308 (92\%) & 11 (8\%) & & & \\
PIs ($n = 244$) & 236 (90\%) & 7 (10\%) & & & \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Drug resistance mutations in 44 patients on HAART experiencing virological failure following episodes of LLVR}
\begin{tabular}{|c|c|c|c|}
\hline
 & NRTIs & NNRTIs & PIs \\
\hline
\begin{tabular}{c}
\text{codon no.} \\
41 \\
67 \\
70 \\
184 \\
210 \\
215 \\
219
\end{tabular} & \begin{tabular}{c}
\text{no. (\%)} \\
17 (39\%) \\
22 (50\%) \\
15 (34\%) \\
29 (66\%) \\
12 (27\%) \\
24 (54.5\%) \\
18 (33\%)
\end{tabular} & \begin{tabular}{c}
\text{no. (\%)} \\
103 (29.5\%) \\
108 (23\%) \\
18 (16\%) \\
90 (18\%) \\
70 (27\%) \\
190 (9\%) \\
77 (34\%)
\end{tabular} & \begin{tabular}{c}
\text{no. (\%)} \\
36 (25\%) \\
63 (81\%) \\
77 (34\%) \\
15 (34\%) \\
77 (34\%) \\
77 (34\%)
\end{tabular} \\
\hline
\end{tabular}
\end{table}

VF, virological failure; VL, viral load; IDU, intravenous drug user; MSM, men who have sex with men; HTS, heterosexual; LLVR, low-level viral rebound; NS, not significant; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors.
of residual viraemia is 3 copies/mL, the blip amplitude will be of 100 copies/mL. The model predicts that using very potent drugs able to reduce residual viraemia to <7 copies/mL, no rebounds above 50 copies/mL will be seen. This linear log–log relationship between residual viraemia and blip amplitude may equally be seen between blip amplitude and subsequent viral outcome. According to our data, a critical threshold for selection of drug resistance might exist around 120 copies/mL, although differences in drug properties, and viral and host factors may account for the observed heterogeneity.

The different trends in CD4 counts following episodes of viral rebound in patients on HAART according to their subsequent outcome has already been reported by others.14,23 However, declines in CD4 counts in patients who subsequently experienced virological failure, as was seen in our study, have not been reported yet.8 Differences in the follow-up period, baseline CD4 counts and treatment modality may account for these discrepancies. Altogether, however, our data support that when possible earlier switch of therapy may be beneficial to avoid progression of immune deficiency.

Although poor drug adherence may make patients prone to a higher rate of episodes of viral rebound and/or virological failure, particularly when using antiretroviral drugs with short half-life and/or low genetic barrier for resistance, the results are still controversial.29–31 In our study, poor treatment compliance did not predict the outcome following LLVR episodes. However, most patients failing to adhere to their medication most likely experienced viral load rebounds above 500 HIV-RNA copies/mL from the beginning and therefore were not examined in this study.

Information about the influence of distinct antiretroviral regimens on the subsequent risk of virological failure following a first episode of viral rebound is scarce and controversial.18–20,22 Our results did not show any significant difference comparing NRTI-, NNRTI- and PI-based regimens. In contrast with the results derived from ACTG A5095,32 we did not find a higher rate of virological failure in patients on Trizivir33 than in patients under NNRTI- or PI-based regimens, at least in the short term. No significant differences in the rate of LLVR episodes and subsequent virological failure were found when comparing different years, in spite of the changes in drugs prescribed. However, a trend towards a lower incidence of LLVR episodes was noticed for the most recent period, which most likely was due to the availability of more convenient and potent antiretroviral drugs.

Resistance mutations found in patients who experienced virological failure following the episode of LLVR were those typically associated with drugs with low genetic barrier for resistance, such as rtM184V for lamivudine or emtricitabine and K103N for efavirenz or nevirapine. It remains unknown whether selection of these mutations preceded and was the cause of or alternatively followed and resulted from the episode of intermittent viraemia.15,16 Studies with a different design will be needed to clarify this issue.

In summary, episodes of low-level viraemia (51–500 HIV-RNA copies/mL) in patients on HAART and prior undetectable viral load are relatively common but transient (‘blips’) in most cases (71%). Only rarely (<10%) are they followed by virological failure despite keeping the same treatment regimen. Of note, plasma HIV-RNA levels at the time of LLVR predict the risk of subsequent virological failure. Therefore, close monitoring is warranted for subjects on HAART with intermittent viral load values above 120 HIV-RNA copies/mL.

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Transparency declarations
None to declare.

References


17. Phillips A, Staszewski S, Lampe F et al. HIV rebound after suppression to <400 copies/mL during initial highly active antiretroviral therapy regimens, according to prior nucleoside experience and duration of suppression. *J Infect Dis* 2002; 186: 1086–91.


