Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome

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Objectives: The aim of our study was to identify factors associated with persistent low-level viraemia (LLV) in HIV-infected patients under suppressive antiretroviral therapy and to assess the virological outcome of these patients.

Methods: LLV was defined as at least two HIV-1 RNA values between 20 and 50 copies/mL during 1 year of follow-up. We compared patients with all values <20 copies/mL (LLV–) and patients with LLV (LLV+). The ‘blip ratio’ was defined as (number of HIV-1 RNA values >50 copies/mL)/(number of HIV-1 RNA determinations) before study inclusion.

Results: Among the 656 patients included, 5.8% were in group LLV+. CDC stage B/C at study inclusion and a higher blip ratio before the study period were the only factors independently associated with LLV. During the 1 year follow-up, the proportion of patients experiencing virological failure was not different between the LLV– and LLV+ groups, and 40% of patients shifted from LLV+ to LLV– status.

Conclusions: LLV was infrequent in our series and the follow-up did not evidence a higher rate of virological failure than in fully suppressed patients. LLV seems to be a transient phenomenon that might be driven by residual ongoing viral replication and/or viral release and/or accuracy of viral load assay at lower values.

Keywords: HIV, viral load assay, low-level viraemia

Introduction

The objective of combined antiretroviral therapy (cART) is to reach and maintain plasma virological suppression <50 copies/mL.1 Several studies using ultra-sensitive techniques evidenced residual low-level viraemia (LLV) in a high proportion of virologically suppressed patients under cART.2–6

In clinical trials and international recommendations, the level of 50 copies/mL is the current accepted threshold for virological suppression. Upgraded commercial assays are now able to detect viral loads <50 copies/mL, such as the COBAS Ampliprep/COBAS TaqMan HIV-1 v2.0 assay with a limit of detection of 20 copies/mL. Thus, it is expected that some patients display HIV-1 RNA values between 20 and 50 copies/mL. However, the relevance in the clinical setting of LLV between 20 and 50 copies/mL is still uncertain.

The aim of our study was to identify factors associated with the detection of persistent LLV, defined as several HIV-1 RNA values between 20 and 50 copies/mL, in patients receiving stable cART and to assess the virological outcome of these patients.

Patients and methods

Study patients

This was a longitudinal study among the 4820 patients followed in the clinical unit of infectious diseases of Hôpital Bichat-Claude Bernard,
Patients with LLV; and (iii) proportion of patients with virological failure. Patients were eligible for inclusion in Period 1 if they met the following inclusion criteria: (i) stable cART for at least 6 months before study inclusion; (ii) all HIV-1 RNA values <50 copies/mL during Period 1; and (iii) at least three HIV-1 RNA determinations during Period 1. Two groups of patients were defined: group LLV− included patients with all HIV-1 RNA values <20 copies/mL during Period 1; and group LLV+ included patients with at least two HIV-1 RNA values between 20 and 50 copies/mL, consecutive or not, during Period 1. Patients with an isolated HIV-1 RNA value between 20 and 50 copies/mL during Period 1 were excluded from the study.

The study protocol was approved by an institutional ethics review board (Saint-Germain en Laye, France) and all patients provided their written informed consent.

**Measurement of plasma HIV-1 RNA level**

HIV-1 RNA quantification assay was performed using the new COBAS AmpliPrep COBAS TaqMan HIV-1 test, version 2.0 (CAP/CTM v2.0) (Roche Molecular Systems, Branchburg, NJ, USA) with the lower quantification limit of 20 copies/mL. No repeat of the HIV-1 RNA quantification assay was performed.

In order to assess the occurrence of transient detectable viraemia >50 copies/mL and <1000 copies/mL a ‘blip ratio’ was defined as (number of HIV-1 RNA values >50 copies/mL)/(number of HIV-1 RNA determinations during period of interest). The blip ratio was calculated: (i) before study inclusion during all the time the patient received ongoing cART; and (ii) during Period 2. By definition, no detectable viraemia >50 copies/mL was allowed during Period 1.

**Genotypic resistance tests**

Bulk sequencing of protease and reverse transcriptase was performed according to the complete sequencing procedures and primer sequences described at www.hivfrenchresistance.org. Resistance mutations were identified according to the IAS-USA list.

**Virological outcome**

To assess the impact of LLV on virological outcome, all patients included in Period 1 were further followed up during Period 2. All HIV-1 RNA values available during Period 2 were collected. As in Period 1, only patients with at least three HIV-1 RNA determinations during this period were included in Period 2. Virological failure was defined as two consecutive HIV-1 RNA values >50 copies/mL.

Several outcomes were assessed during Period 2: (i) proportion of patients with all HIV-1 RNA values <20 copies/mL; (ii) proportion of patients with LLV; and (iii) proportion of patients with virological failure.

**Statistical analysis**

Continuous variables were expressed as medians and IQRs, and categorical variables were expressed as numbers and percentages. To compare clinical and virological characteristics, at study inclusion or before, of patients in group LLV− or LLV+ of Period 1, the Wilcoxon test was used for continuous variables and Fisher’s exact test was used for categorical variables in the univariate analysis. Variables with P<0.2 in univariate analysis were included in a multivariate logistic regression model using a backward elimination procedure and a significance level of P=0.05. Two-way interactions were studied between significant variables in multivariate analysis; when interactions were significant at the level of P=0.05 they were retained in the final multivariate model.

For Period 2, the occurrence of virological failure was compared between group LLV− and group LLV+ using Fisher’s exact test and the blip ratio using the Wilcoxon test.

All tests were two-sided at the 0.05 significance level. Analyses were performed with SAS statistical software (version 9.2; SAS Institute, Cary, NC, USA).

**Results**

**Factors associated with persistent LLV**

During Period 1, 656 patients fulfilled the inclusion criteria. Among them, 618 patients (94.2%) displayed all HIV-1 RNA values <20 copies/mL and were included in group LLV−. The 38 remaining patients (5.8%) had at least two HIV-1 RNA values between 20 and 50 copies/mL and were included in group LLV+. The median number of HIV-1 RNA determinations was similar in both groups (n=4).

The characteristics at study inclusion of the patients in both groups and the results of univariate analysis are reported in Table 1. No differences between the LLV− and LLV+ groups could be observed in the therapeutic history of the patients (Table 1). Neither the duration of virological suppression nor the nature of the ongoing cART regimen differed significantly between the two groups. A protease inhibitor (PI)-based regimen was received by 49% and 50% of patients in groups LLV− and LLV+, respectively. In the univariate analysis, the following factors were associated with being in group LLV+: age (P=0.04); CDC clinical stage at study inclusion (P=0.004); CDC clinical stage at time of initiation of the first cART regimen (P=0.006); HIV-1 RNA value at initiation of the ongoing cART (P=0.02); CD4 cell count at initiation of the ongoing cART (P=0.02); duration of ongoing cART (P=0.047); and a higher blip ratio during ongoing cART before study inclusion (P=0.007).

In the multivariate analysis, only CDC stage B/C at study inclusion (OR 2.9; 95% CI 1.4−5.9; P=0.003) and a higher blip ratio (OR 0.9; 95% CI 0.9−1.0; P=0.001) were independently associated with persistent LLV. No significant difference in CD4 cell count variations during Period 1 was observed between LLV− and LLV+ patients (median change in CD4 during Period 1: 11 versus 53 cells/mm³ in groups LLV− and LLV+, respectively).

**Virological characteristics in study groups**

Virological analysis was performed in order to evidence possible differences in viral characteristics between viruses from patients of groups LLV− and LLV+.

Genotypic resistance tests were available before study inclusion in 135 patients in group LLV− and 24 patients in group LLV+. No significant difference was evidenced in the proportion of HIV-1 ‘non-B’ subtypes between the two groups: 66% in group LLV− versus 67% in group LLV+ (P=1.0). The median number of resistance-associated mutations was similar in both groups (two versus one in groups LLV− and LLV+, respectively; P=0.7), with no differences among the antiretroviral drug classes (data not shown).
Table 1. Characteristics of study patients included in group LLV− (all HIV-1 RNA values <50 copies/mL) and in group LLV+ (at least two HIV-1 RNA values between 20 and 50 copies/mL)

<table>
<thead>
<tr>
<th>At inclusion</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group LLV− (n=618)</td>
<td>Group LLV+ (n=38)</td>
</tr>
<tr>
<td>age (years)</td>
<td>44 (38–50)</td>
<td>47 (43–53)</td>
</tr>
<tr>
<td>male (%)</td>
<td>61</td>
<td>74</td>
</tr>
<tr>
<td>CD4 cell count (/mm³)</td>
<td>552 (405–738)</td>
<td>612 (403–734)</td>
</tr>
<tr>
<td>CD4 cell count nadir (/mm³)</td>
<td>168 (69–255)</td>
<td>124 (26–252)</td>
</tr>
<tr>
<td>time since HIV diagnosis (years)</td>
<td>11 (7–17)</td>
<td>14 (6–18)</td>
</tr>
<tr>
<td>duration of any ARV therapy (months)</td>
<td>92 (44–144)</td>
<td>127 (31–156)</td>
</tr>
<tr>
<td>duration of ongoing ARV therapy (months)</td>
<td>28 (16–41)</td>
<td>21 (10–36)</td>
</tr>
<tr>
<td>total number of ARV drugs received</td>
<td>7 (4–9)</td>
<td>7 (4–11)</td>
</tr>
<tr>
<td>number of virological failuresa</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>ongoing ARV-based treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 NRTIs + 1 PI/ritonavir</td>
<td>302 (49)</td>
<td>19 (50)</td>
</tr>
<tr>
<td>2 NRTIs + 1 NNRTI</td>
<td>211 (34)</td>
<td>9 (24)</td>
</tr>
<tr>
<td>ARV-based regimen without INI</td>
<td>29 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>ARV-based regimen with INI</td>
<td>37 (6)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>other triple combination ARV regimen</td>
<td>23 (4)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>monotherapy or bitherapy ARV regimen</td>
<td>16 (2)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>duration of HIV-1 RNA values &lt;50 copies/mL before inclusion (months)</td>
<td>9 (3–23)</td>
<td>7 (2–17)</td>
</tr>
<tr>
<td>CDC stage (%)</td>
<td>56</td>
<td>32</td>
</tr>
<tr>
<td>HIV-1 RNA level (log_{10} copies/mL)</td>
<td>1.7 (1.7–3.4)</td>
<td>2.0 (1.7–4.5)</td>
</tr>
<tr>
<td>CD4 cell count (/mm³)</td>
<td>402 (262–587)</td>
<td>254 (142–583)</td>
</tr>
<tr>
<td>blip ratio (%)</td>
<td>0 (0–8)</td>
<td>6 (0–13)</td>
</tr>
<tr>
<td>At initiation of ongoing ARV therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA level (log_{10} copies/mL)</td>
<td>4.6 (3.7–5.3)</td>
<td>4.8 (4.5–5.1)</td>
</tr>
<tr>
<td>CDC stage (%)</td>
<td>70</td>
<td>47</td>
</tr>
<tr>
<td>HIV-1 RNA level (log_{10} copies/mL)</td>
<td>30</td>
<td>53</td>
</tr>
<tr>
<td>CD4 cell count (/mm³)</td>
<td>228 (122–346)</td>
<td>204 (48–310)</td>
</tr>
</tbody>
</table>

ARV, antiretroviral; INI, integrase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor. Continuous variables are expressed as medians and IQRs, and categorical variables are expressed as numbers and percentages (unless otherwise stated).

Blip ratio [(number of HIV-1 RNA values >50 copies/mL)/(number of HIV-1 RNA determinations before study inclusion)].

aA virological failure was defined as two consecutive HIV-1 RNA values >200 copies/mL.

**Virological outcome of patients with persistent LLV**

During Period 2, 413 (66.8%) patients from group LLV− and 25 (65.8%) from group LLV+ were assessed.

The proportion of patients experiencing virological failure was not significantly different between groups LLV− and LLV+ (4% versus 8%, respectively; P=0.32) (Table 2). The median HIV-1 RNA value at the time of failure was 250 copies/mL (IQR 94–1342) in group LLV− and 88 copies/mL in group LLV+ (IQR 84–123). During Period 2, the blip ratio was not different between the two groups. The follow-up showed that 267 (65%) and 11 (44%) patients displayed all HIV-1 RNA values <20 copies/mL in groups LLV− and LLV+, respectively (Table 2). The proportion of patients experiencing LLV was higher in group LLV+ (16%, n=4) when compared with group LLV− (2%, n=7) (P=0.002).

**Discussion**

The new commercial HIV-1 RNA quantification assays led to the identification of patients with HIV-1 RNA values between 20 and
probably reflecting a distinct physiopathological mechanism different stratum of viral replication, between 20 and 50 copies/mL, nature of cART and the detection of LLV. However, we assessed a study, we did not demonstrate an association between the residual viraemia when compared with the use of PIs, 4 – 6 and reverse transcriptase inhibitors was associated with lower levels of regimen. Thus, two studies showed that the use of non-nucleoside 20 and 50 copies/mL were HIV-1 RNA level before initial cART factors associated with the detection of HIV-1 RNA between 20 and 40 copies/mL (n=69 patients), similarly showed no significant association with the development of virological failure when compared with patients with undetectable HIV-1 RNA values (i.e. <12 copies/mL).12 However, patients with detectable LLV in this latter study were significantly less likely to achieve subsequent virological suppression. Conversely, a recent study reported the association between a very low level of viral replication (>3 copies/mL) and the risk of virological failure.13 One limitation of our study could be the duration of follow-up, which was 1 year. We can hypothesize that a longer period of follow-up might have led evidence of a higher number of virological failures in the LLV+ group.

In conclusion, persistent LLV was infrequent in our series and the 1 year follow-up did not evidence a higher rate of virological failure than in patients with all HIV-1 RNA values <20 copies/mL. LLV seems to be a transient and dynamic phenomenon, with ~40% of patients shifting from LLV status to complete viral load suppression. For the moment, no selection of resistance mutations has been described in the literature at LLV >50 copies/mL. Indeed, selection of resistance mutations has been described only in case of persistent viraemia >50 copies/mL, which might lead to a lack of statistical power; however, we showed no difference in the frequency of virological failure during the year of follow-up in patients with LLV compared with patients with sustained HIV-1 RNA values <20 copies/mL. In addition, 44% of the patients with LLV during the inclusion period displayed all HIV-1 RNA values <20 copies/mL during the follow-up period and 16% still displayed LLV. A previous study, assessing the significance of LLV between 12 and 40 copies/mL, showed no significant association with the development of virological failure when compared with patients with undetectable HIV-1 RNA values (i.e. <12 copies/mL).12

We assessed the virological outcome of the 38 patients displaying persistent LLV during the year following the detection of LLV between 20 and 50 copies/mL. This small sample size might lead to a lack of statistical power; however, we showed no difference in the frequency of virological failure during the year of follow-up in patients with LLV compared with patients with sustained HIV-1 RNA values <20 copies/mL. In addition, 44% of the patients with LLV during the inclusion period displayed all HIV-1 RNA values <20 copies/mL during the follow-up period and 16% still displayed LLV. A previous study, assessing the significance of LLV between 12 and 40 copies/mL, showed no significant association with the development of virological failure when compared with patients with undetectable HIV-1 RNA values (i.e. <12 copies/mL).12 However, patients with detectable LLV in this latter study were significantly less likely to achieve subsequent virological suppression. Conversely, a recent study reported the association between a very low level of viral replication (>3 copies/mL) and the risk of virological failure.13 One limitation of our study could be the duration of follow-up, which was 1 year. We can hypothesize that a longer period of follow-up might have led evidence of a higher number of virological failures in the LLV+ group.

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Transparency declarations

None to declare.

References

8. Pascual-Pareja JF, Martinez-Prats L, Luczkowiak J et al. Detection of HIV-1 at between 20 and 49 copies per milliliter by the Cobas TaqMan HIV-1 v2.0 assay is associated with higher pretherapy viral load and less time on antiretroviral therapy. J Clin Microbiol 2010; 48: 1911–2.