Markers associated with persisting low-level viraemia under antiretroviral therapy in HIV-1 infection

Leen Vancoillie1, Els Demecheleer1, Steven Callens2, Dirk Vogelaers2, Linos Vandekerckhove2, Virginie Mortier1 and Chris Verhofstede1*

1AIDS Reference Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, De Pintelaan 185-Blok A, B-9000 Ghent, Belgium; 2AIDS Reference Centre, Department of General Internal Medicine and Infectious Diseases, Ghent University Hospital, De Pintelaan 185-1P2, B-9000 Ghent, Belgium

*Corresponding author. Tel: +32-9-332-51-61; Fax: +32-9-332-38-41; E-mail: chris.verhofstede@ugent.be

Received 12 September 2013; returned 2 October 2013; revised 28 October 2013; accepted 14 November 2013

Objectives: To identify host and viral characteristics associated with long-term persisting low-level viraemia (PLLV) under antiretroviral therapy (ART).

Patients and methods: Seventy-one ART-treated patients with long-term PLLV (20–250 copies/mL) and 102 control patients with systematically undetectable viral load (VL) were selected retrospectively from ART-treated patients followed at the Ghent HIV reference centre. Host and viral characteristics were compared using univariate and multivariate analyses.

Results: Higher plasma VL at therapy initiation (OR 3.52; 95% CI 1.86–6.65; P = 0.001), therapy re-initiation after an interruption (OR 3.94; 95% CI 1.70–9.16; P = 0.001), male gender (OR 4.28; 95% CI 1.40–13.00; P = 0.011), a protease inhibitor-based regimen (OR 2.90; 95% CI 1.20–6.97; P = 0.017) and predicted CCR5 co-receptor tropism (OR 2.53; 95% CI 1.05–6.11; P = 0.039) were independently associated with PLLV.

Conclusions: VL at ART initiation, therapy history, gender, ART regimen and co-receptor tropism were independently associated with PLLV. Gender, therapy history, co-receptor tropism and VL at ART initiation could be valuable predictive markers to identify patients at risk for PLLV.

Keywords: HIV, viral load, therapy response

Introduction

In clinical studies, a plasma HIV RNA load below 50 copies/mL is generally considered as reflecting optimal virological suppression.1 This endpoint was originally based on the detection limit of the Roche Cobas Amplicor HIV-1 test, a commercial viral load (VL) assay that has been widely used for over 10 years. During the last 6 years, new VL assays using real-time PCR technology have been developed with detection limits as low as 20 copies/mL.2 The increase in assay sensitivity has resulted in an increasing number of patients with detectable, but low, VL and/or persisting low-level viraemia (PLLV).3–7

The clinical implications of PLLV under antiretroviral therapy (ART) remain controversial.8 A recent study by Laprise et al.9 showed a significant higher risk of subsequent virological failure (VL >1000 copies/mL) in patients with VL above 50 copies/mL compared with patients with VL consistently <50 copies/mL. Others have reported similar findings,10–13 but there are also several studies that could not find an association between PLLV and virological failure.14–17 Likewise there is no agreement in the literature on the potential association of occasional or persistent low viraemia and the emergence of drug resistance.12–15,18–20 An association between low-level viraemia and persistent immune activation was also hypothesized. This hypothesis was supported by the findings of Karlsson et al.21 and Reus et al.22 but not by the results of Taiwo et al.23 There are some plausible explanations for the many discordant findings on the subject of PLLV; several reports have relied on limited sample numbers, many of the studies were designed as cross-sectional and were therefore based on one or a few VL results per patient and, most importantly, different studies have used different definitions of PLLV and different cut-offs. These shortcomings are the main reasons for the current failure to generate a consensus about the clinical implications of PLLV and the lack of guidelines on how to manage patients with PLLV. Such guidelines cannot be composed as long as the consequences of and the driving mechanisms behind PLLV under ART remain elusive. Meanwhile PLLV remains a cause of concern for both patients and clinicians.
The purpose of this study was to assess whether specific host or viral characteristics correlate with PLLV in patients under ART. The major plus of this study in comparison to other studies performed on the subject was the use of longitudinal VL data for patient selection.

Patients and methods

Patients were retrospectively selected from the cohort of HIV-1-infected individuals followed at Ghent University Hospital. All patients initiating (or re-initiating) treatment from January 1997 to December 2012 were considered, but the selection of PLLV or control group members was based exclusively on VL measurements carried out between March 2009 and December 2012, after the introduction of the Cobas AmpliPrep/Cobas TaqMan HIV-1 test v2. For inclusion, a regular follow-up from the initiation (or re-initiation) of ART until the time of inclusion of the patient in the study (January 2013) was required. Only patients with at least six VL measurements, from 6 months after the initiation of therapy onwards, were considered eligible. For inclusion in the PLLV group, at least half of all VL measurements had to be higher than 20 copies/mL, but lower than 250 copies/mL. For inclusion in the control group of optimal responders, VL measurements needed to be systematically undetectable, although one isolated measurement above the detection limit of the assay was accepted.

The study protocol was approved by the Ethics Committee of the Ghent University Hospital (reference number: 2009/093). Patients provided written informed consent.

Host and viral characteristics were compared between the two patient groups based on information collected anonymously from the patients’ records. The parameters compared were age, gender, origin, plasma VL at ART initiation, VL zenith (highest ever VL), VL testing frequency, CD4 count at ART initiation, CD4 nadir, virus subtype, therapy regimen, therapy adjustments, duration of follow-up after therapy initiation, therapy history (first-line ART versus ART re-initiation after interruption), host CCR5 phenotype and virus co-receptor tropism.

All VL determinations were performed with assays from Roche (Basel, Switzerland). The Cobas AmpliPrep/Cobas Amplicor assay, with a detection limit of 50 copies/mL, was used until April 2007 and then replaced by the Cobas AmpliPrep/Cobas TaqMan HIV-1 test v1 (detection limit of 40 copies/mL). In March 2009, the Cobas AmpliPrep/Cobas TaqMan HIV-1 test v2 (detection limit of 20 copies/mL) was introduced. For patients to be included in the PLLV or control group, results of a minimum of six VL measurements with the Cobas AmpliPrep/Cobas TaqMan HIV-1 test v2 needed to be available.

Additional testing was performed on available leftover blood or plasma samples to determine the CCR5 phenotype, the HIV-1 co-receptor tropism and the HIV-1 DNA concentration.

The presence or absence of a defective CCR5 gene in the host was determined after PCR amplification of a region flanking the 32 bp deletion. Primers and amplification conditions were as described by de Roda Husman et al.,25 but the reverse primer was fluorescently labelled with FAM to facilitate length analysis on the ABI Prism 3130XL Genetic Analyzer (Applied Biosystems). The CCR5 fragments detected had a length of 239 bp (wild-type allele, wt) or 207 bp (mutant allele, 32).

The HIV-1 co-receptor tropism was genotypically determined after env V3 sequencing as previously described.16 Sequences were submitted to geno2pheno[co-receptor] 2.5 for prediction of the co-receptor tropism.26,27 Classification as a CCR5-using or CXCR4-using virus was based on a false-negative rate cut-off of 10%.

Total HIV DNA was quantified with qPCR using LTR-specific primers and a TaqMan probe as described by Gaut et al.29 The amplification reaction and analysis were performed using the Roche LightCycler 480 system and software v1.5.

All statistical analyses were done using SPSS v.20 (IBM, NY, USA). In the univariate analysis, the $\chi^2$ test was used for categorical variables and the Mann–Whitney U non-parametric test for continuous variables. All characteristics with a $P$ value $\leq 0.25$ were included in the multivariate logistic regression analysis. The significance level was set at $P<0.05$.

Results

A total of 173 patients met the criteria for inclusion in one of the two study groups. The overall median age was 44 years (IQR 38–51 years); 135 (78%) patients were male and 113 (65%) were infected with a subtype B virus. The median pre-ART VL zenith was 5.05 log_{10} copies/mL (IQR 4.71–5.54 log_{10} copies/mL) and the median CD4 nadir was 225 cells/mm³ (IQR 139–270 cells/mm³). At the time of ART initiation or re-initiation, the median plasma VL was 4.95 log_{10} copies/mL (IQR 4.45–5.28 log_{10} copies/mL) and the median CD4 + T cell count was 260 cells/mm³ (IQR 172–338 cells/mm³). The initiated or re-initiated ART regimen was non-nucleoside reverse transcriptase inhibitor (NNRTI) based in 69 (28.3%), protease inhibitor (PI) based in 122 (70.5%) or an alternative (in 1.2%). For 66.5% of the patients, the therapy regimen was adjusted during the study, with adjustments not necessarily involving a switch to another drug class. Patients were followed for a median of 5 years (IQR 3.7–7.4 years) and a minimum of 1.7 years after ART initiation or re-initiation.

Of the 173 selected patients, 71 (41%) met the criteria for inclusion in the PLLV group and 102 (59%) for inclusion in the control group. The viral and host characteristics of both groups are summarized in Table 1. For the 71 patients in the PLLV group, 1344 VL measurements were registered, of which 707 were recorded as ‘detectable’, with a median VL of 52 copies/mL (IQR 35–84 copies/mL). Measurements were done over time using one of three Roche assays and the results were >50 copies/mL in 19, >40 copies/mL in 72 and >20 copies/mL in 616 for Cobas Amplicor, Cobas TaqMan v1 and Cobas TaqMan v2, respectively. For the 102 control patients, a total of 1800 VL measurements were registered; the results were <20 copies/mL in 1097, <40 copies/mL in 322 and <50 copies/mL in 352. For only 29 of the 1800 measurements in the control group was an isolated ‘detectable’ VL reported, with a median of 31 copies/mL (IQR 23.5–76.5 copies/mL).

Male gender, treatment re-initiation, a shorter duration of follow-up, a PI-based ART regimen, a higher VL zenith and a higher VL at ART initiation were identified as being significantly associated with PLLV after univariate analysis (Table 1). For multivariate analysis, the VL zenith and the origin of the host were excluded because of collinearity (a correlation with VL at ART initiation and virus subtype, respectively), and two variables that showed a tendency for correlation with PLLV (P<0.25) were additionally included: the subtype and co-receptor tropism. Multivariate analysis identified six factors as independent predictors of PLLV: higher VL at therapy initiation (OR 3.52; 95% CI 1.86–6.65; P<0.001), therapy re-initiation (OR 3.94; 95% CI 1.70–9.16; P=0.001), male gender (OR 4.28; 95% CI 1.40–13.00; P=0.011), a PI-based regimen (OR 2.90; 95% CI 1.20–6.97; P=0.017), a shorter duration of follow-up (OR 0.98; 95% CI 0.97–0.99; P=0.032) and CCR5 co-receptor tropism (OR 2.53; 95% CI 1.05–6.11; P=0.039) (Table 1). Correcting for VL testing frequency, however, resulted in a loss of the association between the duration of follow-up and PLLV (OR 1.00; 95% CI 0.98–1.01; P=0.524).
Table 1. Viral and host characteristics associated with PLLV by univariate and multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 102)</th>
<th>PLLV group (n = 71)</th>
<th>P (univariate)</th>
<th>OR (95 CI) (multivariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44 (37–50)</td>
<td>45 (38–52)</td>
<td>0.440</td>
<td></td>
</tr>
<tr>
<td>European origin</td>
<td>74 (72.5)</td>
<td>58 (81.7)</td>
<td>0.164</td>
<td>NA</td>
</tr>
<tr>
<td>Male gender</td>
<td>72 (70.6)</td>
<td>63 (88.7)</td>
<td>0.005</td>
<td>4.28 (1.40–13.00)</td>
</tr>
<tr>
<td>Duration of follow-up (months)</td>
<td>64 (47–98)</td>
<td>53 (43–71)</td>
<td>0.003</td>
<td>0.98 (0.97–0.99)</td>
</tr>
<tr>
<td>ART re-initiation</td>
<td>15 (14.7)</td>
<td>29 (40.8)</td>
<td>&lt;0.001</td>
<td>3.94 (1.70–9.16)</td>
</tr>
<tr>
<td>Therapy adjustments</td>
<td>67 (65.7)</td>
<td>48 (67.6)</td>
<td>0.793</td>
<td></td>
</tr>
<tr>
<td>PI-based ART regimen</td>
<td>63 (61.8)</td>
<td>59 (83.1)</td>
<td>0.002</td>
<td>2.90 (1.20–6.97)</td>
</tr>
<tr>
<td>Virus subtype B</td>
<td>61 (59.8)</td>
<td>52 (73.2)</td>
<td>0.068</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma VL (log_{10} copies/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at ART initiation</td>
<td>4.7 (4.1–5.1)</td>
<td>5.1 (5.0–5.4)</td>
<td>&lt;0.001</td>
<td>3.52 (1.86–6.65)</td>
</tr>
<tr>
<td>zenith</td>
<td>4.9 (4.4–5.4)</td>
<td>5.3 (5.0–5.7)</td>
<td>&lt;0.001</td>
<td>NA</td>
</tr>
<tr>
<td>CD4 count (cells/mm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at ART initiation</td>
<td>260 (165–337)</td>
<td>260 (178–341)</td>
<td>0.913</td>
<td></td>
</tr>
<tr>
<td>nadir</td>
<td>228 (142–275)</td>
<td>204 (132–265)</td>
<td>0.601</td>
<td></td>
</tr>
<tr>
<td>wt/wt CCR5 phenotype</td>
<td>94 (92.2)</td>
<td>62 (87.3)</td>
<td>0.294</td>
<td></td>
</tr>
<tr>
<td>Virus CCR5 co-receptor tropism</td>
<td>73 (71.6)</td>
<td>57 (80.3)</td>
<td>0.229</td>
<td>2.53 (1.05–6.11)</td>
</tr>
</tbody>
</table>

NA, not applicable; NS, not significant; wt/wt, homozygous wild-type.

Only the OR and 95% CI of variables with independent predictive value are shown.

Discussion

Since the introduction of highly sensitive VL assays, the number of HIV-infected individuals on ART with low, but detectable, VL, either episodically or persistently, has increased. The clinical relevance of this persistent or recurrent low-level viraemia remains a subject of debate. The aim of this study was to identify viral and host characteristics associated with PLLV and to define potential predictive markers for PLLV. For that purpose, 71 patients experiencing PLLV and 102 control patients with an optimal VL response were compared. The results revealed an independent association between PLLV and high VL at therapy initiation or re-initiation, a shorter time on ART, a PI-based ART regimen, therapy re-initiation, male gender and the presence of a virus population predicted as CCR5-using.

Other studies have tried to identify characteristics associated with PLLV under ART.17,30–32 Most of them were cross-sectional or used a limited number of VL measurements to classify patients as PLLV or optimal responders. Additionally, differences in cut-offs used to define low-level viraemia (ranging between 1 and 50 copies/mL) hamper comparison of the results. An important strength of the study described here is that long-term follow-up data are taken into account, with patient selection being based on a minimal follow-up period of 1.7 years and at least six independent VL measurements.

The association found between the plasma VL at the initiation of ART and the risk of developing PLLV is in line with the hypothesis that ART induces a drop in VL to a set point that is partly determined by the VL before treatment initiation,33 and it confirms the observation of others.30,32 Charpentier et al.17 however, could not find an association between a higher pre-therapy VL and low-level viraemia. Although the total population in the study by Charpentier et al.17 consisted of 656 individuals, only 38 were identified as PLLV patients. This low sample number may have influenced the outcome of the comparison.

Despite significant differences in the pre-ART VL, the pre-ART CD4 counts were comparable between the PLLV group and the control group. This suggests that the mean duration of infection in both groups was comparable and argues against the assumption that evolution towards PLLV is more likely in patients initiating ART in a more advanced stage of infection. Although based on limited data because of the scarce availability of pre-ART
Markers associated with persisting low-level viraemia

blood cell samples, a clear association was observed between the HIV-1 DNA load at treatment initiation and PLLV, indicating the potential importance of the burden of infected cells. Additional studies are needed to further explore the value of the HIV DNA load as an independent predictor of suboptimal treatment response. The recent results of Chun et al. suggested an association between the size of the cellular reservoir at ART initiation and the magnitude of the residual viraemia. It can be hypothesized that a higher burden of infected cells will result in a higher level of virus release after activation and a longer ‘wear out’ period of residual virus production. That viral blips and PLLV mainly reflect viral production from activated latently infected cells was already suggested a long time ago.35

Patients included in the PLLV group had overall a shorter duration of follow-up than the control patients. An inverse relationship between the time on therapy and PLLV was also reported by Pascual-Pareja et al., but others have failed to confirm this.17,32 Although our observations seemed to support the findings of Pascual-Pareja et al., we have to acknowledge a bias induced by the criteria for patient selection. A requirement for inclusion was the availability of at least six VL measurements. It was noticed, however, that the VL testing frequency was slightly higher in the PLLV group (four measurements per year) than in the control group (three measurements per year), leading to a shorter time to reach at least six VL determinations in the PLLV group compared with the control group. After correcting for the VL testing frequency, the association between duration of follow-up and PLLV was indeed lost.

Several studies have shown an association between NNRTI use and a more profound control of residual viraemia.31,32,36,37 Our observations are in line with these findings, but a potential bias, introduced by the fact that patients were selected retrospectively from a cohort of individuals initiating ART according to routine clinical practices, should be considered. Some clinicians may preferentially prescribe a PI-based regimen in patients re-initiating ART after treatment interruption or in patients with a high baseline VL. No difference in the backbone of the regimen taken up to the point when PLLV first manifested was observed between patients initiating and patients re-initiating therapy (P = 0.255). The therapy regimen and baseline VL, however, showed a trend towards association (P = 0.054) so a bias in the prescribing behaviour of the clinician cannot be fully excluded.

Male patients showed a significantly higher probability of developing PLLV. Previous studies did not report an association between gender and low-level viraemia.17,30,32 The reason for these discordances, apart from possible differences in the study population or power, are not immediately clear. Furthermore, Saison et al. reported an association between non-B subtypes and low-level viraemia, but these results could not be confirmed by our data. Differences between the study of Saison et al. and our own were the use of a different VL assay, the Abbott RT HIV-1 assay m2000rt v4.0 and the Roche CAP/ICTM v2 assay, respectively.

To our knowledge, this study is the first to show that the probability of developing PLLV is lower in patients initiating a first-line regimen than in patients re-initiating ART after a treatment interruption. A study comparing treatment outcome in children receiving continuous ART versus children undergoing planned treatment interruptions was unable to show a difference in the number of patients reaching virological suppression (<50 copies/mL) 1 or 2 years after the final treatment resumption.33 Correspondingly, in a study of short non-structured treatment interruptions, all of the patients achieved viral suppression (<50 copies/mL) after resumption of therapy.40 None of these studies, however, specifically addressed PLLV or viral blips during follow-up.

An association between predicted CCR5 co-receptor tropism and PLLV has also not previously been reported. A possible explanation for this observation is that ART preferentially suppresses CXCR4-using viruses, as has been suggested.61 Available data in the literature do not, however, support this assumption. Waters et al. showed no effect of virus co-receptor tropism on virological suppression under ART and Séclen et al. reported lower rates of virological response in patients with predicted CCRX4 use. Differences in the methodology used and in the definition of virological suppression again impede a direct comparison between these studies. The relationship between co-receptor tropism and therapy outcome definitely needs to be addressed further in the future.

A slightly, but statistically non-significant, higher number of patients with a wt/Δ32 CCR5 phenotype was found in the PLLV group. This was surprising as the wt/Δ32 phenotype in general is associated with a lower viral replicative capacity, lower VL and slower disease progression and thus a presumed faster suppression by ART.44 Further investigation showed that, in our study population, the VL in the patients with a wt/Δ32 CCR5 phenotype was even slightly higher than in the patients with a wt/wt CCR5 phenotype [median 5.5 (IQR 4.5–5.3) copies/mL versus 4.9 (4.4–5.3) copies/mL; P = 0.225], which might explain the counterintuitive observation.

An overall limitation of the current study was its observational nature, making it impossible to assess the causality of the reported associations. PLLV under ART remains a challenge to face and a cause of uneasiness for both the clinician and the patient because there are currently no clear guidelines on how to handle patients with long-term PLLV.

In conclusion, the results of this study show that high VL at therapy initiation or re-initiation, a PI-based ART regimen, therapy re-initiation, male gender and predicted CCR5 co-receptor tropism are independently associated with PLLV. Four of these parameters—baseline VL, gender, therapy history and co-receptor tropism—can be assessed at the time of ART initiation or re-initiation and as such have the potential for use as predictive markers for PLLV. The possibility of identifying patients at risk of progression towards PLLV will facilitate the evaluation of strategies to avoid or overcome PLLV and help the design of prospective trials aimed at studying the clinical impact of PLLV, both in patients whose baseline characteristics suggest a higher risk for PLLV and in patients whose baseline characteristics favour an optimal response.

Acknowledgements
Presented in part at the Eleventh European Meeting on HIV and Hepatitis, Rome, Italy, 2013 (Abstract O.03).

We would like to thank Jolanda Pelgrom, Erica Sermijn, Bea Van Der Gucht and Filip Van Wanzeele from the AIDS Reference Centre of the Ghent University Hospital and Sylvie Dynakais, Marlies Schauvliege and Delfien Staels for technical assistance.

Funding
This study was carried out as part of the routine laboratory monitoring of HIV-1-infected individuals. The AIDS Reference Laboratory of Ghent is
supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System. L. Vandekerckhove is supported by the Research Foundation—Flanders (FWO) (grant no. 1.8.020.09.N.00).

Transparency declarations
None to declare.

References
30. Pascual-Pareja JF, Martinez-Prats L, Luczkiowiak J et al. Detection of HIV-1 at between 20 and 49 copies per milliliter by the Cobas Amplicor HIV-1 v2.0 assay is associated with higher pretherapy viral load and less time on antiretroviral therapy. *J Clin Microbial* 2010; 48: 1911–2.
Markers associated with persisting low-level viraemia


