Executive Summary

I. Introduction

The goal of combination antiretroviral therapy (cART) is to suppress HIV replication so that viral evolution and resistance are prevented, immunologic function and clinical health are restored, and HIV transmission is curtailed. Plasma HIV concentration, commonly termed plasma viral load (pVL), has been shown to be a reliable marker of clinical disease progression and treatment response. “Suppression” of pVL has emerged as the primary determinant of treatment success.\(^1,2\) The target pVL during suppression has changed through the years, largely in parallel with the development of pVL assays with progressively lower limits of HIV RNA detection. For many years, the commonly used pVL assays had a lower limit of detection of 50 copies per milliliter (cpm). Suppression below 50 cpm was then validated in many studies as associated with cessation of new HIV resistance, immunologic restoration, durable virologic response, and a marked reduction in HIV transmission.\(^2,4\) Based on these findings, pVL suppression, below 50 cpm, was recommended as the goal of cART.\(^5,6\) More recently, real time HIV PCR assays with limits of HIV RNA detection below 50 cpm (i.e., 48, 40 and 20 cpm) have become widely adopted.

Two clinical dilemmas are commonly encountered when using available assays. First is how to interpret and manage very low-level viremia (VLLV) of 20 to 50 cpm detected with the new real time HIV PCR assays. Second is how to interpret and manage low-level viremia (LLV) of approximately 50 cpm to 400 or 500 cpm. The current understanding of viral dynamics, technical aspects of pVL assays, as well as the risk factors, clinical significance, and management of patients experiencing LLV and VLLV provide some insights that are useful in routine patient care.

II. What happens to HIV plasma viral load when a patient is started on cART?

Following cART initiation, HIV-1 decays in a predictable fashion through at least three phases, reflecting the different half-lives of HIV-1 infected cell types.\(^7,8\) Among adherent patients on long term cART, the majority will achieve viral suppression to less than 50 cpm by 12-24 weeks of therapy.\(^9\) Viral decay continues beyond this until, after months to years, most reach a plateau of 1-10 cpm.\(^9-13\) The source of this stable residual viremia residual viremia is a topic of debate. The prevailing opinions are that residual viremia reflects either stable, periodic release of HIV virus from latently infected cells, and/or ongoing viral replication.\(^14\) There is consensus that HIV cannot be cured by cART because all efforts to eradicate residual viremia by intensifying cART have failed.

III. How is HIV viral load measured?

The three nucleic acid-based molecular diagnostic methods for quantifying HIV RNA include reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid sequence-based amplification (NASBA), and branch copy DNA (b-DNA) (Table 1). Most contemporary assays utilize RT-PCR technology.
Enhancements to standard RT PCR technology led to ultrasensitive end point PCR assays with a lower limit of detection of approximately 50 cpm. These assays were widely adopted in research and clinical practice. More recently, real-time PCR pVL assays have begun to replace the existing end point PCR platforms, providing a fully automated, high throughput alternative to traditional assays, with enhanced assay performance. These assays detect RNA target with detection limits of 20-48 cpm and can qualitatively detect HIV RNA below these limits of quantification.

Table 1: Commercially available nucleic acid-based testing assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Roche COBAS Amplicor HIV-1 Monitor v1.5¹</th>
<th>bioMérieux NucliSENSE EasyQ HIV-1 v2.0</th>
<th>Siemens Versant HIV-1 v3.0</th>
<th>Abbott RealTime HIV-1 Assay</th>
<th>COBAS Ampliprep/COBAS TaqMan HIV-1 Test, v2.0.</th>
<th>Siemens VERSANT HIV-1 RNA 1.0 (kPCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year FDA Approved</td>
<td>1999</td>
<td>2001</td>
<td>2002</td>
<td>2007</td>
<td>2010</td>
<td>Not approved in U.S.²</td>
</tr>
<tr>
<td>Linear Range</td>
<td>Standard: 400-&gt;750⁵ cpm</td>
<td>10-10⁶</td>
<td>50-500,000 cpm</td>
<td>40-10⁶ cpm</td>
<td>20-10⁶ cpm</td>
<td>37-11⁶ cpm</td>
</tr>
<tr>
<td>Target Region</td>
<td>gag gene</td>
<td>gag gene</td>
<td>pol gene</td>
<td>pol gene (integrate region)</td>
<td>gag gene and LTR regions of the HIV-1 genome</td>
<td>pol gene (integrate region)</td>
</tr>
<tr>
<td>Time tResult</td>
<td>8 hours</td>
<td>3 hours</td>
<td>25 hours</td>
<td>4 hours</td>
<td>5.5 hours</td>
<td>6.5-7 hours</td>
</tr>
<tr>
<td>Nucleic acid testing approach</td>
<td>End point RT PCR</td>
<td>NASBA</td>
<td>bDNA</td>
<td>Realtime RT PCR</td>
<td>Realtime RT PCR</td>
<td>Realtime RT PCR</td>
</tr>
</tbody>
</table>
IV. What clinical issues have arisen in the era of real time HIV PCR testing?

The increased sensitivity of real time pVL assays has led to higher inter- and intra-assay variability around lower quantification limits. In particular, real time HIV PCR assays appear to report detectable LLV at a higher frequency than traditional end point HIV PCR assays. LLV used to be defined as a pVL of 50-1000 cpm but many recent studies are restricting the definition to 50-400 cpm or 50-500 cpm. LLV can be further stratified by its temporal pattern, such that transient LLV preceded and followed by a pVL < 50 cpm, is termed a viral blip, and ≥2 consecutive LLV episodes is termed persistent LLV. The clinical significance and management of LLV has gained increasing attention over the last decade, but this focus has intensified in the era of real time HIV PCR testing. Finally, the ability to routinely detect HIV RNA below 50 cpm now challenges clinicians and researchers to interpret the significance of these findings.

V. Management Considerations

A. Viral Blips

i. Incidence and Risk Factors

Viral blips can be expected in up to approximately one-third of cART recipients, depending on factors such as the cART regimen and length of observation. The magnitude of viremia during viral blips is often < 200 cpm but may be much higher. Risk factors (Table 2) may include advanced HIV disease stage prior to cART, poor adherence, and use of less potent ARV combinations (see “VLLV risk factors” below).
<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Frequency</th>
<th>Duration of follow up (months)</th>
<th>LLV and viremia &lt; 50 definition (cpm)</th>
<th>Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VIRAL BLIPS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Havlir 2001</td>
<td>101</td>
<td>29%</td>
<td>54</td>
<td>&gt;50</td>
<td>● Higher pre-cART HIV RNA</td>
</tr>
<tr>
<td>Sungkanuparph 2006</td>
<td>382</td>
<td>34%</td>
<td>24</td>
<td>50-1000</td>
<td>● Higher pre-cART CD4+ T cell count &lt; 200 cells/mm³</td>
</tr>
<tr>
<td>Mira 2002</td>
<td>330</td>
<td>11%</td>
<td>11</td>
<td>51-1000</td>
<td>None</td>
</tr>
<tr>
<td>Sklar 2002</td>
<td>448</td>
<td>27%</td>
<td>16</td>
<td>&gt;50</td>
<td>● Health payer status (non-private insurance)</td>
</tr>
<tr>
<td>Martinez 2005</td>
<td>43</td>
<td>19%</td>
<td>18</td>
<td>&gt;50</td>
<td>● Higher baseline level of residual viremia (7.5 vs 3 cpm)</td>
</tr>
<tr>
<td>Grennan 2012</td>
<td>3550</td>
<td>21%</td>
<td>32</td>
<td>50-999</td>
<td>● Boosted PI (vs.NNRTI) - based cART</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>● Higher pre-cART HIV RNA (&gt;10⁵ cpm)</td>
</tr>
<tr>
<td>Posadecki 2007</td>
<td>223</td>
<td>27%</td>
<td>22</td>
<td>50-1000</td>
<td>● cART adherence</td>
</tr>
<tr>
<td>Raboud 2002</td>
<td>165</td>
<td>21%</td>
<td>NA</td>
<td>&gt;50</td>
<td>● cART adherence</td>
</tr>
<tr>
<td>Geretti 2008</td>
<td>1386</td>
<td>19%</td>
<td>26</td>
<td>50-400</td>
<td>● Boosted PI (vs. NNRTI) - based cART</td>
</tr>
<tr>
<td>Easterbrook 2002</td>
<td>&gt;400</td>
<td>16%</td>
<td>28</td>
<td>&gt;400</td>
<td>● Younger age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>● Male</td>
</tr>
<tr>
<td>Greub 2002</td>
<td>2055</td>
<td>24%</td>
<td>22</td>
<td>51-500</td>
<td>● Mono- or dual- ARV therapy before cART</td>
</tr>
<tr>
<td>Masquelier 2005</td>
<td>219</td>
<td>9%</td>
<td>18</td>
<td>&gt;500</td>
<td>NA</td>
</tr>
<tr>
<td>Garcia-Gasco</td>
<td>2720</td>
<td>29%</td>
<td>96</td>
<td>51-500</td>
<td>None</td>
</tr>
<tr>
<td><strong>PERSISTENT LLV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raboud 2002</td>
<td></td>
<td>30%</td>
<td>NA</td>
<td>&gt;50</td>
<td>NA</td>
</tr>
<tr>
<td>Greub 2002</td>
<td>2055</td>
<td>8%</td>
<td>18</td>
<td>51-500</td>
<td>NA</td>
</tr>
<tr>
<td>Karlsson 2004</td>
<td>46</td>
<td>39%</td>
<td>27</td>
<td>50-1000</td>
<td>● Lower pre-cART CD4+ T cell count</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>%</td>
<td>Median CD4</td>
<td>Median HIV RNA</td>
<td>Key Features</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------</td>
<td>------</td>
<td>------------</td>
<td>----------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Geretti 2008</td>
<td>1386</td>
<td>6%</td>
<td>26</td>
<td>50-400</td>
<td>1. Boosted PI (vs. NNRTI) - based cART</td>
</tr>
<tr>
<td>Sungkanuparph 2006</td>
<td>362</td>
<td>28%</td>
<td>30</td>
<td>51-1000</td>
<td>None</td>
</tr>
</tbody>
</table>
| Taiwo 2011          | 1158   | 6%   | NA         | 51-1000        | 1. Boosted PI (vs. NNRTI) - based cART  
2. Higher pre-cART HIV RNA (>10^6)  
3. Lower pre-cART CD4+ T cell count |
| Doyle 2012          | 1247   | 60%  | 12         | <40 RNA detected or 40-49 | 1. Duration of viral suppression  
2. Higher pre-cART HIV RNA  
3. Lower pre-cART CD4+ T cell count  
4. Younger age  
5. Boosted PI (vs. NNRTI) - based cART  
6. cART adherence |
| Maggiolo 2012       | 1214   | 29%  | 12         | 3-50           | 1. Boosted PI (vs. NNRTI) - based cART  
2. Duration of viral suppression <50 cpm  
3. Higher pre-cART HIV RNA |
| Widdrington 2011    | 139    | 50%  | 36         | <40, RNA detected | 1. Non-NNRTI-based cART |
| Henrich 2012        | 778    | 23%  | 22         | <48, RNA detected | None |
| Alvarez 2013        | 290    | 54%  | 12         | 20-29 or 30-39 | 1. Duration of viral suppression <50 cpm |
| Havlir 2005         | 100    | 61%  | 17         | 2.5-50         | 1. Higher proviral HIV DNA in PBMC  
2. Higher pre-cART HIV RNA  
3. Stavudine (vs. tenofovir) therapy |
| Gianotti 2012       | 739    | 40%  | 11         | 1-49           | 1. Higher blip ratio prior to T_0  
2. Male gender  
3. Non-NNRTI-based cART |
| Charpentier 2012    | 656    | 6%   | NA         | 20-50 (≥ 2 measurements) | 1. Pre-cART CDC stage  
2. Higher blip ratio prior to T_0 |
Abbreviations: cpm: copies/mL; cART: combination antiretroviral therapy; PI: protease-inhibitor; ARV: antiretroviral; NNRTI: non-nucleoside reverse transcriptase inhibitor; PBMC: peripheral blood mononuclear cells

ii. Clinical significance

The significance of viral blips can be approached according to viremia magnitude (Table 3). Low level (<400 cpm) viral blips do not appear to increase risk for HIV resistance, virologic failure, or immunologic deterioration. High level (>400 cpm) viral blips, however, have been associated with increased likelihood of new drug resistance, virologic failure, and, possibly, decline in CD4 T cell trajectory in some patients, though it is not proven that these events are actually caused by the blip. The 400 cpm cut-off should be regarded as a guide as there are exceptions to this general rule. Limited studies suggest absence of a strong association between viral blips and chronic inflammation/immune activation.23,38,39 The effect of viral blips on morbidity/mortality and HIV transmission has not been studied.

Table 3: Summary of literature regarding clinical consequences of low level viremia and viremia < 50 cpm

<table>
<thead>
<tr>
<th>LLV Category</th>
<th>HIV Resistance (Baseline Available)</th>
<th>Virologic Failure/Rebound</th>
<th>Immunologic Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral Blips</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Magnitude (&lt;400 cpm)</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Contradicting studies: Macias</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Magnitude (≥400 cpm)</td>
<td>UNKNOWN</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Contradicting studies: Martinez No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Persistent</strong></td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Supporting studies: Taiwo 2011 Lo Re</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
iii. Management

Viremia >50 cpm should prompt an adherence assessment and repeat testing should be performed at an interval based on blip magnitude as well as patient risk factors, including time on current cART >12 months, a history of ARV resistance, and lower potency cART regimens (Figure 1). Low magnitude (<200 cpm) viremia is unlikely to be of clinical significance unless other risk factors are present (as above), whereas high magnitude viral blips should trigger a more urgent adherence assessment and prompt repeat pVL testing (such as within 3-6 weeks). Repeat pVL testing should always be performed using the same assay if possible. There is no clear benefit to empiric cART change and/or intensification following a viral blip. It is prudent to avoid routine pV testing within a few days of vaccination or during an acute illness since these have been associated with occurrence of blips in some, though not all, studies.
Persistent low level viremia

i. Incidence and risk factors

Approximately 4-8% of the HIV population receiving cART experience persistent LLV.\(^{24-27}\) The magnitude of viremia among those with persistent LLV is typically low (median 113-267 cpm in some studies).\(^{23,25,37,40}\) Potential risk factors largely overlap with those associated with viral blips (Table 2).

ii. Clinical significance

There are several well-documented adverse clinical outcomes associated with persistent LLV (Table 3). These include the development of antiretroviral resistance,\(^{23,37,40-46}\) increased risk of virologic failure,\(^{23-25,47,48}\) and increased markers of immune activation.\(^{23,49}\) The effect of persistent LLV on CD4 T cell trajectory, morbidity and mortality, and HIV transmission remain largely understudied. There is biologically plausibility for increased HIV transmission among those with persistent LLV compared to those with consistent pVL suppression.\(^{50}\)
iii. Management

Intensive adherence counseling is essential for all patients with persistent LLV. The management of persistent LLV of 400-1000 cpm should be genotype-guided, as at least two commercial laboratories in the U.S. will accept samples for genotypic analysis in this pVL range.\textsuperscript{51,52} If genotyping is successful and resistance is detected, a new regimen should be constructed with at least two, and ideally three, active ARVs. Patients with no evidence of resistance should continue their regimen and intensify adherence. Among those with persistent LLV of 50-400 cpm, an attempt should be made to obtain genotypic analysis, as many centers can perform in-house assays (not FDA approved). Reported success rates are as high as 75\% for pVL 50-249 cpm, and \textasciitilde 90\% for pVL 250-499 cpm.\textsuperscript{43} Although this has not been evaluated in prospective trials, some experts recommend empirically modifying the regimen if resistance information is not available and the pVL remains \textasciitilde 200 cpm despite intensified adherence.

Other factors that may further support empiric treatment modification include a relatively high pVL and an upward trajectory in LLV magnitude over time. When modifying the regimen without genotype results, the clinician should consider the patient’s treatment history and prior drug resistance. There are new concerns surrounding optimal management of patients with pVL of 50-200 cpm in light of evidence that even this range of viremia may be associated with increased risk of virologic failure later on. The DHHS currently defines virologic failure as persistent pVL > 200 cpm but this may change with time.

B. Very low-level viremia and residual viremia in patients with pVL < 50 cpm

i. Incidence and risk factors

The majority of patients will achieve pVL <50 cpm by the end of six months of effective cART. There are two categories of viremia that may be present in patients with pVL < 50 cpm. The first is VLLV of 20-48 cpm, which can be detected with commercially available assays that have detection limits lower than 50 cpm. Sometimes, the assays simply report VLLV as presence of HIV RNA using qualitative methods. The other category is residual viremia of approximately 1-10 cpm (average of around 2-3 cpm) that can only be detected (for now) using even more sensitive research assays. It takes longer than 6 months for most patients to reach this residual viremia plateau.\textsuperscript{9,53} The key feature of residual viremia is that it is present in most patients and cannot be eliminated by cART, no matter how intensive the cART is. Risk factors for detectable viremia after suppressing below 50 cpm may include correlates of HIV disease stage prior to cART (including pre-cART HIV RNA, CD4 count), duration of cART, cART adherence, and specific ARVs. In particular, NNRTI-, as compared to PI-based cART, has been associated with lower rates of this level of viremia, though the mechanism underlying this difference is not well-established.\textsuperscript{12,54-56}

ii. Clinical significance

Several studies have examined the risk of virologic rebound among those with evidence of viremia < 50 cpm. Many of these studies lumped together patients with VLLV and those with residual
viremia, reflecting the lack of consensus definitions (Table 3). Unfortunately, these studies differed in terms of patient population, duration of suppressive cART and follow up, and pVL assay platforms used. The largest of such studies found an increased risk of viral rebound (to levels >50 cpm) among those with evidence of viremia <50 cpm. Further, there may be a linear relationship between the magnitude of viremia < 50 cpm and risk of viral rebound, although three smaller studies found no association of presence of viremia <50 cpm with virologic rebound. Despite limitations due to discrepancies in study design, patient population and results in some cases, the accrued literature suggests an increased risk of virologic rebound to levels >50 cpm in patients with evidence of viremia < 50 cpm using newer assays compared with those who have no evidence of viremia whatsoever with these more sensitive assays. It is uncertain how much of this risk is driven by cART adherence and duration. Re-suppression sometimes occurs without a change in treatment. There has been no documented evolution of viral resistance when pVL is suppressed below 50 cpm and no consistent evidence of an increase in markers of immune activation and/or inflammation.

iii. Management

Given known patterns of viral decay following cART initiation, presence of VLLV should not cause concern in the first twelve months on cART. Such patients can continue to have routine pVL monitoring (e.g. every 12-24 weeks). Some experts advocate that patients with VLLV while on stable, long term (e.g. >12 months) should have an ARV adherence assessment performed and addressed, and perhaps be observed at closer intervals with repeat pVL testing using the same HIV PCR assay. This is not a consensus view as other experts consider it unnecessary to implement any changes in patients who have any degree of viremia under 50 cpm. At present there is insufficient evidence to support cART change or treatment intensification in patients with VLLV.
Viral dynamics following cART initiation

After cART initiation, HIV RNA levels decay in at least 3 distinct phases (Figure 1). The first phase of decay, which lasts several days, is believed to be due to death of short-lived, HIV-infected, activated T cells. The second phase has been attributed to the death of other HIV-infected cells that have longer half-lives such as macrophages, and produces a more modestly-sloped decay lasting several weeks to months. The third phase of viral decay is thought to be from the death of a reservoir of latently-infected T-cells. This phase possesses an even slower decay rate of months to years. Most patients on stable cART attain viral suppression to < 50 copies per mL (cpm) by six months and ultimately reach residual HIV RNA plateau of 1-10 cpm, with a mean of around 2-3 cpm. The source of this stable residual viremia has been the subject of much investigation and continues to be debated. One hypothesis is that RV is due to periodic release of HIV from latently-infected cells, possibly when triggered by antigens, such as reactivated herpes virus infections. This is supported by studies which demonstrated that genetic sequences of residual HIV in the peripheral blood were identical to those found in proviral DNA of resting CD4+ T cells. A competing hypothesis is that ongoing viral replication persists during suppressive cART, presumably from sites that antiretroviral drugs penetrate poorly (so called sanctuary sites) or via cell-to-cell spread. Supporters of this latter hypothesis point to potential signs of cryptic replication in gastrointestinal lymphoid tissue among those with peripheral pVL < 50 cpm. Arguing against ongoing replication is the fact that intensification of cART with a variety of antiretroviral drugs has failed to affect RV. It is conceivable that periodic release or ongoing viral replication coexist and/or are codependent.

HIV viral load testing

Quantified plasma HIV RNA (plasma viral load, or pVL) is an essential tool for HIV clinicians, yielding information regarding disease progression, therapeutic monitoring, and in some cases, HIV diagnosis. The three most common methods for quantifying HIV RNA are reverse transcription PCR (RT-PCR), nucleic acid sequence-based amplification (NASBA), and branched chain DNA (bDNA) (Table 1). The most widely available assays utilize RT-PCR technology, in which nucleic acid sequences are amplified and then detected through a colorimetric reaction. There are two major categories of RT-PCR assays, those which detect PCR product during (“real-time”) or at the end (“endpoint”) of the
Traditional pVL assays, epitomized by the Roche Amplicor HIV-1 Monitor (HIM) v1.5 platform, used endpoint RT-PCR technology, with lower limits of RNA detection of 50 cpm. The HIM assay was used in most registrational trials for new antiretroviral agents and became the gold standard for subsequent clinical trials as well as therapeutic monitoring. Over the last several years, newer generation real-time HIV PCR assays were introduced which were faster, fully-automated systems with less cross-contamination, broader linear dynamic ranges of detection, and improved overall test performance. Two commercial, FDA-approved real-time HIV PCR assays (the Roche COBAS Amplicor TaqMan assay and the Abbott RealTime RT-PCR assay) have now largely replaced endpoint RT PCR platforms. Important features of the Roche and Abbott real-time assays are lower limits of HIV RNA quantification (20 and 40 cpm, respectively) and ability to qualitatively detect HIV RNA below quantification limits. Finally, several research-based ultrasensitive pVL assays exist, many of which are able to quantify HIV RNA down to 1 cpm. Though not commercially available, these assays have provided important insight into the nature of residual HIV viremia.

The introduction of new real-time HIV PCR assays was followed by the observation of an apparent increase in rates of viral blips using these new platforms. Several studies confirmed that approximately 10-15% of patients with pVL <50 cpm using the HIM assay had viral blips using these real-time HIV PCR assays. Further, there was confusion regarding the clinical significance of detectable viremia below the traditional limits of detection (50 cpm). Interpreting these clinical dilemmas requires an understanding of the performance characteristics of these real-time HIV PCR platforms, including their accuracy, precision, sensitivity, specificity, and reproducibility across laboratories. Crucially, intra-assay variability, a measurement of assay precision, is high at lower ends of viremia (≤ 50 cpm). For example, in a study examining the variability of 3 commercial real-time HIV PCR assays, 80% of viral blips between 50-100 cpm were not reproducible when performed again on the same sample. Besides inherent assay characteristics, suboptimal specimen processing can affect assay results. Factors known to affect assay results include prolonged processing time, inadequate freezing and/or thawing, the use of EDTA as specimen anticoagulant, (which can decrease detection rates), and the use of plasma preparation tubes which may cause leakage and contamination from cell-associated HIV DNA, leading to false elevations in HIV plasma viral load. Finally, at lower levels of viremia, real-time HIV PCR assays show variable with traditional endpoint PCR assays much like the variable correlation between different real-time HIV PCR platforms. These differences may be more pronounced in non-B subtypes.
Specifically, at low level viral loads, real-time HIV PCR assays report higher pVL on the same sample compared to traditional end point PCR assays such as the Roche Amplicor HIV-1 Monitor test, version 1.5. These differences were more pronounced in the Roche AmpliPrep/COBAS Taqman HIV-1 Test, v1.0, but still exist with its next iteration (v2.0). Overall, these recognized inter- and intra-assay variability and peculiarities of different HIV PCR platforms has led to the recommendation to use consistent HIV PCR assays for individual patients whenever possible.

Definitions

Based in part on the performance characteristics of newer HIV PCR platforms, the DHHS now defines viral failure as confirmed pVL ≥200 cpm and viral suppression as <50 cpm. Many studies define low level viremia (LLV) as a pVL of 50-1000 cpm, but there is a trend towards restricting the definition of LLV to 50-400 or 500 cpm. LLV can be further defined by its temporal pattern. Transient LLV, preceded and followed by a pVL < 50 cpm, is termed a viral blip. When at least two consecutive LLV episodes occur, this is termed persistent LLV. Quantitative and/or or qualitative detection of HIV RNA below 50 cpm by commercially available assays (usually 20-48 cpm) is termed very low-level viremia (VLLV). Residual viremia refers to the stable viremia that is detected using research assays only and not affected by cART intensification. Though these classifications are in common use, other experts have used different definitions.

Incidence

LLV (of up to 1000 cpm) is common in clinical practice with up to approximately one-third of cART recipients having at least one episode during long-term follow up. It is well established that around 70% of all cases of LLV are viral blips. Most of the remaining cases lead to persistent LLV and only a minority (under 10%) progress to persistently higher pVL. When patients with pVL < 50 cpm were intensively sampled (every 2-3 days) in a study, 90% were found to have viral blips (median 79 cpm), suggesting that blips may be more common than described in large cohort studies. In several studies, the median magnitude of viral blip ranged from 82-162 cpm while the median pVL was 113-258 cpm among those with persistent LLV.
After six months on suppressive cART, the majority of patients will achieve pVL <50 cpm, and months to years later, most will reach a plateau, often 3-10 cpm.\textsuperscript{9,57} Several studies that utilized HIV RNA assays with different lower limits of detection to examine rates of viremia below 50 cpm during cART are summarized in Table 1.

**Risk factors for LLV and viremia below 50 cpm**

Risk factors for LLV and viremia below 50 cpm overlap as summarized in Table 2. While there is controversy about how much impact the pre-cART HIV stage has on viral blips, LLV and VLLV, a few points are noteworthy.\textsuperscript{23,25,28,29,40} In a small retrospective study, cART initiation during primary HIV infection led to a two-fold lower rate of viral blips compared to those initiating cART during chronic HIV infection.\textsuperscript{(91)} Other studies have suggested that those with very low CD4 T cell levels pre-ART may increase the risk of viral blips,\textsuperscript{47} persistent LLV,\textsuperscript{23,40} and VLLV.\textsuperscript{54,59} Having a very high pVL pre-cART also appears to increase the risk of viral blips,\textsuperscript{28,92} persistent LLV,\textsuperscript{40} and VLLV.\textsuperscript{13,54,93}

Higher magnitude of viremia below 50 cpm appears to increase risk for virologic rebound >50 cpm,\textsuperscript{54,57,58} including viral blips.\textsuperscript{13,94} Mathematic modeling has shown that the level of residual viremia is predictive of amplitude of subsequent viral blips.\textsuperscript{95} It appears that the longer a patient goes without evidence of any viremia using routine assays, the less likely it becomes for that individual to have viremia of any degree in the future,\textsuperscript{13,54,58} but it has not been rigorously examined for viral blips and/or persistent LLV. Maintenance of adherence is generally accepted as necessary for optimal suppression even though adherence is often difficult to quantitate in research studies and the clinic.\textsuperscript{31} The fact that some studies did not find a strong association between viral blips and adherence should not deter clinicians and patients from aiming for maximum adherence.\textsuperscript{30,96} Finally, a history of more extensive ARV exposure may increase risk for persistent LLV compared to viral blips or persistent viral suppression.\textsuperscript{34}

Lower potency cART regimens, such as triple NRTI therapy\textsuperscript{97} and PI-monotherapy,\textsuperscript{98} have been associated with higher rates of LLV which is one of the reasons why these regimens are not endorsed for routine use. Several studies found that NNRTI therapy, when compared to PI-based cART, is more likely to suppress HIV to lower levels under 50 cpm\textsuperscript{12,54,55,62} and have less frequent viral blips.\textsuperscript{28} Other studies, however, found no such association.\textsuperscript{24,26,29,34} If true, this difference may reflect a slower decay of plasma virus in PI-based regimens compared to NNRTI-based regimens, or selection bias by clinicians who may opt for PI-based therapy in less adherent patients.
Clinical significance of LLV

Before exploring the literature regarding clinical consequences of LLV and residual viremia, several caveats must be established. First, definitions of LLV, VLLV, residual viremia, and treatment failure have varied widely between studies. Second, patient populations have varied widely in terms of ARV treatment history, ARV resistance, and socio-demographics. Finally, nearly all LLV studies have analyzed results obtained from earlier-generation HIV PCR assays, primarily the HIM assay. These factors prevent broad generalization of study findings and highlight the need to validate results across patient populations using newer HIV PCR platforms.

HIV Resistance

Drug resistance mutations (DRM) are commonly found during LLV. According to an estimate derived by modeling clinical data, 65% of DRM among patients with extensive ART experience were predicted to occur while pVL was ≤ 500 cpm, though a recent study found lower rates (19%) of DRM among patient with LLV 250-1000 cpm.99 In a study where unboosted PI use was common, ≥1 DRM was present in 60% and 72% of samples with pVL <300 cpm and 300-999 cpm, respectively.99 Longer elapsed time with LLV appears to increase risk for DRM accrual.42 Since DRM seem to accumulate the longer a patients has LLV, it is not surprising that there is more evidence of ARV resistance emergence during persistent LLV compared to viral blips. In fact, there is no clear evidence of HIV resistance emergence during a viral blip, as only one study of heavily treatment-experienced patient demonstrated new DRM emergence during a viral blip.32 Interestingly, all seven patients in that study achieved viral suppression during the follow up period after empiric changes in their cART regimen. Unlike viral blips, persistent LLV clearly increases the risk of DRM accumulation (Table 2). The persistent VL does not have to be high for resistance to develop, ranging from 72 cpm to 374 cpm at the time of DRM emergence in some studies.33,37,42,43 Finally, it appears that resistance during LLV is more likely to arise against NNRTI/nucleos(t)ide reverse transcriptase inhibitors (NRTIs) and integrase strand transfer inhibitors (INSTIs) compared to ritonavir boosted PIs early during LLV, but over time all classes are at risk for DRM.37,42,91 Perhaps due to limitations of HIV resistance assays, there is no convincing evidence of DRM development during VLLV. One small study found sequence evolution, consisting of new DRM, in 1/20 patients with residual viremia of 6.5-50 cpm.101
Virologic failure/rebound

LLV

For viral blips, the risk of virologic failure appears to be low among patients with blips of low magnitude (<400-500 cpm).\(^\text{25,29,30,31,34,48,102}\) Several studies documented an increased risk of virologic failure with higher magnitude viral blips (>400-500 cpm),\(^\text{28,34,35,103}\) including a study of 3550 individuals in which viral blips of 500-999 cpm were associated with a nearly 3 fold higher risk of viral rebound (defined as \(\geq 2\) consecutive pVL >50 cpm or one pVL >1000 cpm).\(^\text{28}\) It is important to consider the apparent cut-off of 400-500 cpm as no more than a guide since not all studies found an association of virologic failure with blip magnitude.\(^\text{29}\)

Unlike viral blips, persistent LLV has been consistently shown to increase risk for virologic failure (Table 3). Higher persistent LLV magnitude may also be a risk factor. In a study of patients with initial LLV who were then followed for 69 weeks, median initial LLV magnitude was higher (426 cpm) among those who went on to develop high level persistent viral rebound > 400 cpm compared to those who developed isolated viral blips (96 cpm).\(^\text{34}\) Crucially, even low magnitude persistent LLV has been associated with virologic failure, as demonstrated in a recent study of 1860 patients which found that persistent LLV 50-199 cpm of at least 6 months duration doubled the risk of subsequent virologic failure.\(^\text{104}\) In theory, the risk of virologic failure should increase with duration of persistent LLV but this has not been well examined. Surprisingly, a study that found a three-fold higher risk of virologic failure among those with at least three months of persistent LLV > 400 cpm compared to those with viral suppression found no difference in virologic failure among subjects with persistent LLV > 6 months compared to those with persistent LLV of < 6 months.\(^\text{47}\) It is possible that the difference in duration of LLV between the > 6 months and < 6 months groups in the study may not have been significant.

Viremia under 50 cpm

In total, seven studies published over the last two years have examined the risk of virologic rebound (of varying magnitudes) among individuals with viremia under 50 cpm (Table 3). These studies categorized viremia variably, through a variety of research- or commercial-based quantitative assays with lower detection limits of 1, 3, 20, 40, or 50 cpm, and/or with assays reporting qualitative HIV RNA detection below limits of 40 or 48 cpm. Differences in patient populations and study methodologies again limit the ability to generalize these results. Nonetheless, a growing body of research indicates that
detection of viremia under 50 cpm, and particularly those of high magnitude viremia, may increase risk of virologic rebound.

The largest such study evaluated the risk of virologic rebound to >50 and >400 cpm among 1249 patients with pVL < 50 cpm. The patients were separated into 3 groups as follows: no detectable viremia (i.e pVL <40 cpm and RNA not detected); pVL <40 cpm but RNA detected; and pVL 40-49 cpm. Compared to individuals who had pVL <40 cpm and no RNA detected, there was almost 5-fold higher risk of virologic rebound >50 cpm among those with pVL 40-49 cpm while those with pVL <40 cpm but RNA detected had almost 2-fold increased risk. Further, this risk persisted for virologic rebound > 400 cpm, at almost 7-fold and 3-fold increases, respectively. A limitation of this study was that duration of cART was longer in the group with pVL <40 cpm and no detected RNA, indicating that some patients in the other groups may not yet have reached the steady state of viral decay. Another large study, using a research-based assay with lower detection limit of 3 cpm found over 7-fold higher risk of confirmed virologic rebound > 50 cpm (and over 4-fold >200 cpm). Importantly, they found a linear relationship between magnitude of viremia and risk of virologic rebound. To address concerns about potential survivor bias due to inadequate duration of cART in some of the participants, a sub-analysis was performed, restricted to only individuals with prolonged (>44 months) duration of pVL suppression. This again revealed an increased risk of rebound >50 cpm. Finally, two smaller studies, one utilizing an assay with qualitative RNA detection below 48 cpm, and another utilizing an assay with a lower limit of detection of 20 cpm, found increased risk of rebound >50 cpm, as well as, in one of the two studies, risk of rebound >200, and 400 cpm. The possibility of a survivor bias was not rigorously excluded in either of these studies.

By contrast, four studies found no risk of confirmed virologic rebound among individuals with evidence of viremia below 50 cpm. One of these studies did find an association between detectable residual viremia and transient viral rebound. Another study was limited by a smaller sample size and relatively low failure rate, which may have limited the statistical power to detect a difference. Regardless, lack of uniformity in results suggests that more robust study is required to definitely address the risk of virologic rebound in patients with evidence of viremia below 50 cpm. It will be important in future studies to carefully separate VLLV from residual viremia since their origins and consequences cannot be assumed to be the same.
**Immunologic function**

**CD4 T cell trends**

The immunologic sequelae of LLV, including viral blips and viremia below 50 cpm, have been evaluated in the modern era (Table 3). One study followed 101 patients on cART with pVL <200 cpm for 24 months, 68 of whom had intermittent or persistent LLV (median 81 cpm) and found that higher level of LLV was associated with reduced CD4 recovery.\(^{105}\) Two larger studies found a lower probability of an immunologic response only among those with intermittent or persistent LLV of higher magnitude (>200 and >400 cpm, respectively).\(^{106,107}\) LLV magnitude also has been examined in viral blip studies, which have generally found either no association with inferior CD4 trajectories,\(^{30,103}\) or suboptimal CD4 changes in the context of only high magnitude blips (>350 cpm).\(^{34,95}\) Very few published studies in the contemporary cART era have examined the effect of persistent LLV on CD4 trends; there is no evidence of altered T cell count trajectories in this context.\(^{40,90}\) In summary, CD4 T cell trajectories do not appear to be influenced by low magnitude viral blips.

Of the four studies that addressed T cell trends among patients with and without viremia below 50 cpm (variably defined), only one was able to demonstrate a modestly inferior CD4 recovery slope (+14.3 vs. +21.2 after 49 weeks of follow up).\(^{12}\) Though further study with consistent methodology is required, to date there is little evidence that presence of viremia below 50 cpm alters T cell trajectories in a clinically meaningful way.\(^{56,59,93}\)

**Immune activation and inflammation**

In addition to CD4 recovery, a goal of cART is to control immune activation and chronic inflammation.\(^{108}\) Markers of cellular immune activation assessed (including T cell co-expression of HLA-DR and CD38) have been found to be higher in patients with viral blips\(^{38}\) and persistent LLV\(^{40}\) compared to those with viral suppression, one two study found no such association with viral blips.\(^{39}\)

Several studies have examined whether markers of inflammation and/or endothelial activation are altered in individuals with LLV.\(^{109-112}\) All but one study found an association of LLV and soluble makers of inflammation (either C-reactive protein, interleukin-6, and/or von Willebrand factor).\(^{110,112}\) In addition, one of these studies found higher levels of molecular microbial translocation (46% vs. 18%) among patients with LLV (20-200 cpm) compared to those with undetectable (<20 cpm) pVL.\(^{109}\) Given...
the strong correlation between markers of immune activation and levels of HIV viremia, it is conceivable that this relationship would continue to exist in those with viremia below 50 cpm. Consistent with this, two small cross sectional studies did show an association between presence of viremia below 50 cpm and soluble markers of immune activation. An association has not been demonstrated with cellular markers of T cell activation or markers of inflammation, except among those with poor T cell response. Taken together, LLV appears to increase markers of immune activation and inflammation, perhaps through increased microbial translocation, whereas this association has not yet been definitively proven to exist during VLLV.

**Morbidity and mortality**

There is a well-established correlation between pVL level in untreated or failing patients and morbidity/mortality. It is unknown whether persistent LLV or viremia below 50 cpm during cART, lead to higher morbidity or mortality. It is likely that large studies with long follow up periods would be needed to study this issue comprehensively.

**HIV transmission**

Plasma viral load is a strong predictor of HIV transmission risk. However, despite the negligible expected transmission risk in patients with persistent viral suppression, residual free and cell-associated HIV RNA can be detected in genital secretions of patients with pVL suppression, likely due to imperfect ARV penetration into the genital compartment. Thus, there is a theoretical risk of increased viral shedding during episodes of LLV. It is unknown whether detection of viremia below 50 cpm increases HIV RNA levels in genital secretions compared to those without any evidence of viremia.

**Management:**

**Viral blips**

Viral blips are more common in the era of real-time HIV PCR testing. Given the high variability of real-time HIV PCR platforms, an isolated low magnitude (<200 cpm) LLV pVL should engender a patient-specific response. In patients within the first year of cART initiation, this finding likely represents random
variation around an expected mean and should not trigger an immediate pVL repeat or a change in ARV regimen. It is reasonable in such cases to repeat pVL testing within 6-12 weeks after an assessment of cART adherence. Repeat pVL testing should be performed using the same HIV PCR assay as the original one. Whenever possible, pVL testing should not be done close to vaccination or during an acute illness because some, but not all, studies have associated these events with transient increases in pVL.\textsuperscript{35,95,102,119}

For an isolated LLV episode ≥200 cpm or occurring in patients on cART > 12 months, or among those with extensive ARV resistance, or those on lower-potency regimens, many experts would recommend retesting pVL sooner (e.g. within 3-6 weeks) and completing a comprehensive ARV adherence assessment and optimization. If repeat pVL testing demonstrates pVL suppression <50 cpm, the LLV episode is termed a viral blip and is unlikely to be of clinical significance. One exception is viral blips of high magnitude (such as >400 cpm), which should trigger adherence education and close observation as some of these patients may be at higher risk for virologic failure.\textsuperscript{50}

**Persistent LLV**

If repeat pVL testing demonstrates LLV (50-1000 cpm), this is termed persistent LLV and management should be tailored based on LLV magnitude and patient-specific risk factors. Adherence assessment and counseling is an essential first step and identified barriers to adherence should be addressed. In general, the management of persistent LLV of 400-1000 cpm should be genotype-guided, as many commercial assays accept plasma samples with pVL as low as 400 cpm for genotyping, though none are FDA-approved for use below 1000 cpm. For persistent LLV <400 cpm, resistance testing should still be attempted, though the absence of a commercially available resistance assay for pVL 50-400 cpm necessitates the use of in-house assays (which are not widely available) but have success rates as high as 75% for pVL 50-249 cpm and 90% for pVL 250-499 cpm.\textsuperscript{43} Selecting a new cART regimen based on genotype results has been associated with improved viral suppression compared to empiric switching.\textsuperscript{31,120} When resistance testing is not possible, many experts would recommend considering empiric cART change, particularly if the pVL is > 200 cpm. The decision should be based primarily on patient-specific risk factors such as treatment and resistance history, as well as on the magnitude, trajectory, and duration of persistent LLV. When constructing an empiric regimen change, clinicians should aim for a regimen with at least 2, and ideally 3 fully active ARV drugs. The subset of patients with persistent LLV but pVL too low for genotyping is one of the most challenging to manage. There is a need for validated FDA approved genotypic assays for this population. There are new concerns surrounding
The optimal management of patients with pVL of 50-200 cpm in light of evidence that even this range of viremia may be associated with increased risk of virologic failure later on. The DHHS currently defines virologic failure as persistent pVL > 200 cpm but this may change with time.

**Viremia < 50 cpm**

The management of viremia < 50 cpm during routine clinical care is evolving. Patients with viremia < 50 cpm in the first 12 months following cART initiation may still be early in the third phase of viral decay, hence such viremia would be predictable in this setting. In this scenario, routine pVL monitoring is appropriate. Patients on stable, long term (e.g. >12 months) cART with viremia of 20-48 cpm or qualitative evidence of HIV RNA are now routinely encountered using routine assays. Given high assay variability near the limit of detection using real-time PCR platforms, an isolated viremia of this nature may be repeated according to usual monitoring routine, and using the same HIV PCR assay. If the viremia persists on repeat testing, some experts would recommend focused adherence assessment and optimization, although other experts still consider any viremia under 50 cpm as insignificant. Resistance testing is not an option in these patients in clinical settings and there is, at present, insufficient evidence to support treatment intensification or cART change.

**References**


60. Steel A, John L, Shamji MH, et al. CD38 expression on CD8 T cells has a weak association with CD4 T-cell recovery and is a poor marker of viral replication in HIV-1-infected patients on antiretroviral therapy. HIV Med. 2008;9:118-25.


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