Low-Level Viremia in HIV

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Last Updated: May 12, 2016

Many slides courtesy of Dr. David Spach

This presentation is intended for educational use only, and does not in any way constitute medical consultation or advice related to any specific patient.
Case

- A 45-year-old man with history of cryptococcal meningitis presents for follow-up. His baseline HIV RNA level was 368,000 copies/ml, which decreased to undetectable by 6 months on TDF-FTC-EFV (Atripla). However, now his last 3 HIV RNA checks have been: 52 copies, 41 copies, and 86 copies. He reports 100% adherence to ART and pharmacy confirms on-time monthly med fills.

Low-Level Viremia in HIV: Outline

- **Part 1: Low-level viremia (LLV, <200 copies/mL)**
  - Definitions
  - Clinical significance
  - Considerations for evaluating cause and for management

- **Part 2: Very low-level viremia (VLLV, detected not quantified)**
  - Definitions
  - Clinical significance
  - Considerations for evaluating cause and for management
DEFINITIONS

What is Low-Level Viremia (LLV)?
A confirmed HIV RNA level below the limit of assay detection, usually by 3-6 months.

Virologic Responses on Antiretroviral Therapy
Virologic Rebound/Failure

Confirmed detectable HIV RNA (to >200 copies/mL) after virologic suppression

**Virologic failure**: In contrast to patients with HIV RNA persistently below 200 copies/ml, those with HIV RNA >200 copies/ml often develop resistance, especially if >500 copies/ml. Persistent HIV RNA >200 copies/ml should be considered virologic failure and resistance testing should be attempted, especially if >500 copies/ml.

After virologic suppression, an isolated detectable HIV RNA level followed by return to virologic suppression.
Virologic Responses on Antiretroviral Therapy
Low-Level Viremia

HIV RNA (copies/ml)

Weeks

Persistent HIV RNA in range between lower limit of detection of assay and 200 copies/mL

Assessing Viral Load Response to ART

- **Adequate virologic response**: HIV RNA reduction to below limit of detection of the assay within 3-6 months

- **Low-level viremia (LLV)**: persistent HIV RNA between lower limit of detection of the assay and 200 copies/ml

- **Very low-level viremia (VLLV)**: persistent detected HIV RNA below limit of quantification of assay (ie. <40 or <20 copies/ml)

- **Virological failure**: HIV RNA increase to above 200 copies/ml

Is Low-Level Viremia Clinically Significant? 
*Risk of Virological Failure*
### Study Design

- **N = 1860** HIV-infected patients enrolled in study
- Observational cohort study in Montreal Clinic started in 1997
- Received at least 12 months of antiretroviral therapy
- Duration of persistent viremia was ≥ 6 months
- Virologic failure defined as HIV RNA >1,000 copies/ml
- Subjects followed until virologic failure occurred

Virologic Failure Following Persistent Low-Level Viremia

Is Low-Level Viremia Clinically Significant?  
*Risk of Clinical Events*
Clinical Events Associated with Low-Level Viremia

- Data conflicting regarding inflammation, AIDS events, non-AIDS events
  - Italian cohort (n=4,400): VL 50-400 does not predict AIDS, CV events, or death
  - Icona cohort (n=7,277): VL 51-500 raises risk of AIDS & death, not non-AIDS events
  - Dutch ATHENA cohort (n=6,440): VL <400 does not predict non-AIDS events
  - ART-CC cohort (N=17,902): VL 51-500 does not predict AIDS or death

- Very scant data related to transmission risk

Possible Causes of Low-Level Viremia and Considerations for Management
Potential Causes of Low-Level Viremia

- **Patient factors**: adherence, absorption, food requirements
- **Medication factors**: drug-drug interactions
- **Virological factors**: intermittent activation of latently infected cells, ongoing replication at sanctuary sites, viral mutations, non-B subtype virus
- **Collection/assay factors**: use of plasma preparation tubes, spillage of DNA from PBMC’s
Persistent Low Level HIV Replication
Overestimation of Human Immunodeficiency Virus Type 1 Load Caused by the Presence of Cells in Plasma from Plasma Preparation Tubes

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Received 13 March 2009/Returned for modification 17 April 2009/Accepted 29 April 2009

The human immunodeficiency virus type 1 (HIV-1) load is an important marker of disease progression and treatment efficacy in patients with HIV-1 infection. In recent years, an increase in the number of samples with detectable HIV-1 RNA has been reported among patients with previously suppressed viral loads, affecting clinical patient care and leading to repeated measurements of viral load and drug resistance. This rise seems to have coincided with the increased use of plasma preparation tubes (PPTs) for sample collection, and we have aimed to explain why PPTs might yield elevated HIV-1 RNA levels. The impacts of different sample-processing procedures on HIV-1 RNA levels were compared retrospectively. Prospective, the presence of different cells and cell-associated HIV-1 nucleic acids in paired plasma samples from PPTs centrifuged before (PPT1) and after (PPT2) transportation to the laboratory was compared. A retrospective analysis of 4,049 patient samples with <1,000 HIV-1 RNA copies/ml showed elevated HIV-1 RNA levels in plasma from PPT1 compared with the levels from PPT2 and standard EDTA-containing tubes. Prospective data revealed cell-associated HIV-1 nucleic acids and abundant blood cells in plasma from PPT1 but not from the corresponding PPT2. The levels of HIV-1 RNA correlated with the lymphocyte counts in plasma in PPT1. Cells could be removed by the centrifugation of PPT2 before analysis. In conclusion, the transportation of PPTs after centrifugation may render cells in the plasma fraction containing cell-associated HIV-1 nucleic acids that contribute significantly to the HIV-1 RNA copy numbers in patients with low viral loads.

Successful use of Plasma Preparation Tubes™ (PPTs) in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

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Using plasma preparation tubes for the collection and storage of plasma resulted in factitious, low-level human immunodeficiency virus type 1 (HIV-1) viremia among patients receiving highly active antiretroviral therapy who incurred unnecessary additional clinic visits, laboratory testing, and medication changes. We caution clinicians against the routine

N=63 HIV-infected adult subjects (Anticipated viral loads <5000 copies/ml)

EDTA
- 20 min spin (1,100 x g)
- Pour off
  - Frozen at -20°C
  - Thawed on day of testing

PPT2
- 20 min spin (1,100 x g)
- Transported by courier from clinic site to molecular laboratory
  - Pour off
    - Frozen at -20°C

PPT3
- 10 min spin (1,100 x g)
- Pour off
  - 10 min spin (2,000 x g)
  - Frozen at -20°C

PPT1
- 10 min spin (1,100 x g)
- Refrigerated at 4°C
- Pour off
  - 10 min spin (2,000 x g)
  - Tested

January 2016 HHS Antiretroviral Therapy Guidelines
Management of Low-Level Viremia

- No consensus regarding how to manage patients with HIV RNA levels between lower limit of detection of assay and 200 copies
- Assess adherence, drug-drug interactions (including with OTC’s and supplements), and drug-food requirements
- Follow HIV RNA levels at least every 3 months to assess the need for an ART change (AIII)

Additional Considerations for Evaluation and Management of Persistent Low-Level Viremia

1) Assess adherence and exactly how patient takes medications
2) Assess drug interactions, food requirements, absorption
3) Consider DNA genotype resistance assay
4) If regimen with low resistance barrier, consider change
5) Review collection process/use of plasma preparation tubes (PPT’s)
6) If access, consider lab testing for spillage of proviral DNA
Summary
(Includes Some Personal Opinion)

• LLV can be the result of multiple factors, including behavioral, virologic, or collection/assay factors. It is often difficult to determine the exact cause or clinical significance. It is possible, though not confirmed, that persistent LLV portends higher risk of virologic failure and clinical events.

• For persistent LLV, assess adherence, drug-drug interactions, and behavioral factors, and monitor HIV RNA closely.

• If feasible, review lab collection process, consider checking DNA genotype, and consider changing regimen with low resistance barrier to regimen with high resistance barrier.
Significance and Clinical Management of Persistent Low-Level Viremia and Very-Low-Level Viremia in HIV-1-Infected Patients

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A goal of HIV therapy is to sustain suppression of the plasma viral load below the detection limits of clinical assays. However, widely followed treatment guidelines diverge in their interpretation and recommended management of persistent viremia of low magnitude, reflecting the limited evidence base for this common clinical finding. Here, we review the incidence, risk factors, and potential consequences of low-level HIV viremia (LLV; defined in this review as a viremia level of 50 to 500 copies/ml) and very-low-level viremia (VLLV; defined as a viremia level of <50 copies/ml) detected by clinical assays that have quantification cutoffs of <50 copies/ml. Using this framework, we discuss practical issues related to the diagnosis and management of patients experiencing persistent LLV and VLLV. Compared to viral suppression at <50 or 40 copies/ml, persistent LLV is associated with increased risk of antiretroviral drug resistance and overt virologic failure. Higher immune activation and HIV transmission may be additional undesirable consequences in this population. It is uncertain whether LLV of <200 copies/ml confers independent risks, as this level of viremia may reflect assay-dependent artifacts or biologically meaningful events during suppression. Resistance genotyping should be considered in patients with persistent LLV when feasible, and treatment should be modified if resistance is detected. There is a dearth of clinical evidence to guide management when genotyping is not feasible. Increased availability of genotypic assays for samples with viral loads of <400 copies/ml is needed.