Virologic and Immunologic Monitoring

Medical Care Criteria Committee, updated November 2019

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Monitori

ng Intervals

RECOMMENDATIONS

- Regular monitoring of HIV RNA levels remains the most accurate and meaningful measure of effective ART (see Table 1: Virologic and Immunologic Monitoring for Non-Pregnant Patients, below, for recommended intervals). (A1)
- Clinicians should monitor HIV RNA levels and CD4 counts according to the recommended intervals in Table 1. Follow-up visits should be scheduled more frequently as clinically necessary to address non-HIV-related conditions, secondary prevention, and issues that may affect adherence to ART or retention in care, such as substance use, mental health disorders, unstable housing, or need for supportive services. (A2)
- Clinicians should assess response to ART using viral load assays. (A1)
- CD4 cell counts should not be used for diagnosis of HIV infection. (A1)
- Quarterly CD4 count monitoring is no longer recommended for non-pregnant patients receiving ART who have consistently undetectable HIV RNA levels and CD4 counts >200 cells/mm³ (see Table 1 for recommended intervals). (A2)

Periodic laboratory tests are necessary to evaluate the response to ART and its potential related side effects. In the setting of ART failure, viral resistance assays should be used.

Regular monitoring of CD4 counts in patients with consistently undetectable HIV viral loads and CD4 counts >200 cells/mm³ offers little utility in clinical practice today. Clinicians rarely use this information to guide decision-making for clinically stable, virologically suppressed patients. Monitoring of HIV RNA levels to confirm appropriate response to treatment and durable viral suppression is the most accurate and meaningful measure of the effectiveness of ART [Gale, et al. 2013].

Very few studies address the appropriate frequency of viral load monitoring. A recent retrospective study noted that the strongest predictor of virologic failure at 12 months was a missed or canceled appointment rather than the interval of follow-up [Buscher, et al. 2013]. However, this and other similar studies [Reekie, et al. 2008; Romih, et al. 2010] have significant limitations, including their retrospective nature and short follow-up periods. Data indicate that the linked sexual transmission of HIV in serodiscordant couples in which the HIV infected partner maintains sustained viral suppression is negligible [Rodger, et al. 2016]. Based on this information, persons with HIV may rely on their antiretroviral therapy as a strategy to prevent viral transmission to an uninfected partner. Studies do not indicate the appropriate interval for viral suppression monitoring for the purposes of ongoing transmission prevention. Until more definitive data are available, the decision to lengthen monitoring intervals for HIV RNA level should be individualized. Patients who are monitored at longer intervals should be carefully selected based on length of viral suppression, CD4 count, use of antiretroviral therapy for transmission prevention, and adherence to medical care, including visit attendance and retention in care.

KEY POINT

- Quarterly HIV RNA monitoring remains appropriate for patients with a recent history of non-adherence, mental health disorders, substance use, homelessness, poor social support system, or other major medical conditions. Semiannual monitoring may be appropriate for patients with persistently undetectable HIV RNA and none of the above characteristics.
Table 1 provides a guide for monitoring HIV RNA levels and CD4 counts.

### Table 1: Virologic and Immunologic Monitoring for Non-Pregnant Patients [a]

<table>
<thead>
<tr>
<th>At Baseline</th>
<th>HIV RNA Levels (copies/mL)</th>
<th>CD4 Lymphocyte Count (cells/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>• Yes (A1)</td>
<td>• Yes (A1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment Monitoring</th>
<th>HIV RNA Levels (copies/mL)</th>
<th>CD4 Lymphocyte Count (cells/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Following (1) initiation of ART or (2) a change in ART regimen after virologic failure [b] with new resistance to prior ART</td>
<td>• Within 4 weeks of initiation of ART or change in regimen (A3) • At least every 8 weeks until complete suppression [c] is documented (A3)</td>
<td>• Repeat at 12 weeks and then every 4 months until CD4 &gt;200 cells/mm³ on two measurements obtained at least 4 months apart (A2); then monitor as below once suppressed</td>
</tr>
</tbody>
</table>

| Following a change in ART to simplify treatment regimen or reduce toxicity for patients with suppressed virus | • Within 4 weeks after change in regimen to ensure continued suppression (A3); then monitor as below for suppressed | Monitor as below for suppressed |

| Patients on ART who achieve complete suppression [c] | • At least every 4 months after complete suppression (A3) • May extend intervals to every 6 months in selected stable patients with CD4 counts >200 cells/mm³ after 1 year of complete suppression [Buscher, et al. 2013] (B2) | If CD4 count is ≤350 cells/mm³: At least every 6 months (B2) If CD4 count is >350 cells/mm³: Further monitoring is optional (B2) |

| Patients on previously suppressive ART with new HIV RNA [d] above the lower limit of detection using a highly sensitive assay [c] | All patients: • Assess adherence (A3) • Assess for drug-drug interactions (A3) Viral load ≥500 copies/mL: • Have patient return within 2 weeks and: - Repeat viral load (A2) and obtain resistance testing (A1) - Obtain CD4 count if not done within previous 6 months (B3) Viral load <500 copies/mL: • Repeat viral load test within 4 weeks to differentiate low-level transient viremia ("blip") from virologic failure [b,e]. (A2) • If viral load remains detectable on repeat test: - Obtain CD4 count if not done within previous 6 months (B3) - Consider resistance testing [f] (B3) | |

| Patients not on ART: According to NYSDOH recommendations, ART is recommended for all patients with HIV [g] | • If CD4 ≤500 cells/mm³: At least every 4 months (A2) • If CD4 >500 cells/mm³: At least every 6 months (A2) • Continue to discuss ART initiation (A1) | • If CD4 ≤500 cells/mm³: At least every 4 months (A3) • If CD4 >500 cells/mm³: At least every 6 months (A3) • Continue to discuss ART initiation (A1) |

### Notes:

- **a.** For monitoring HIV RNA levels and CD4 counts in pregnant women with HIV, see DHHS/AIDInfo > Recommendations for the Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States.
- **b.** Virologic failure is defined as the inability to achieve or maintain suppression of viral replication to an HIV RNA level <200 copies/mL [AIDInfo 2019].
- **c.** Complete suppression is generally considered below the lower limit of detection of a highly sensitive assay (<20 to <50 copies/mL).
- **d.** Patients with repeated intermittent low-level viremia ≤200 copies/mL over a period of years without demonstrated failure may continue routine testing intervals.
- **e.** ART should not be changed based on a single viral load elevation. The risk of virologic rebound (breakthrough) increases when values are >500 copies/mL [Grennan, et al. 2012].
Viral Load

Plasma HIV-1 RNA Level (Viral Load)

Plasma levels of viral RNA have been shown to correlate with clinical outcome, including overall mortality, and measurement of HIV RNA levels provides the most precise means of establishing whether a response to ART has occurred [Mellors, et al. 1997; Marschner, et al. 1998; Murray, et al. 1999; HIV Surrogate Marker Collaborative Group 2000; Thiebaut, et al. 2000]. HIV RNA levels should be obtained from all patients at baseline [Gulick, et al. 2003; Wu, et al. 2003; Tarwater, et al. 2004; Molina, et al. 2013; Behrens, et al. 2014; Porter, et al. 2015].

For patients beginning ART, or those changing therapy as a result of virologic failure, HIV RNA should be measured at 4 weeks after initiation of therapy and should decrease by at least 1 log (10-fold) in the presence of effective therapy [Haubrich, et al. 2011] (see Table 2: Interpretation of Viral Load, below). For patients who do not have background antiretroviral resistance, an undetectable viral load (<50 copies/mL) is usually achieved within 3 months. Patients with a baseline HIV viral load >100,000 copies/mL can be expected to achieve an undetectable viral load within 6 months of effective treatment.

<table>
<thead>
<tr>
<th>HIV-1 RNA Copy Number</th>
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<tbody>
<tr>
<td>Log 10</td>
</tr>
<tr>
<td>1,000,000</td>
</tr>
<tr>
<td>100,000</td>
</tr>
<tr>
<td>10,000</td>
</tr>
<tr>
<td>1,000</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

Reduction with ART if Patient has 100,000 copies/mm³

<table>
<thead>
<tr>
<th>Log Change</th>
<th>Percent Decrease</th>
<th>Fold Reduction</th>
<th>Resultant Copy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>66.00</td>
<td>3</td>
<td>33,000</td>
</tr>
<tr>
<td>1.0</td>
<td>90.00</td>
<td>10</td>
<td>10,000</td>
</tr>
<tr>
<td>2.0</td>
<td>99.00</td>
<td>100</td>
<td>1,000</td>
</tr>
<tr>
<td>3.0</td>
<td>99.99</td>
<td>1,000</td>
<td>100</td>
</tr>
</tbody>
</table>

An absent or incomplete response of viral load to ART should raise concerns about poor adherence to therapy and/or viral resistance [Baxter, et al. 2000; Townsend, et al. 2009].

Patients on previously suppressive ART with newly detectable HIV RNA levels of 50 to 500 copies/mL may be experiencing low-level transient viremia (“blip”) and not virologic failure. A blip by definition means that the viral load is again below the level of quantification on repeat testing performed promptly after a detectable result in someone previously suppressed. Persistent elevation, even at low levels, warrants further investigation. Acute concurrent illness and/or recent vaccination may cause this transient rise; however, studies have suggested that low-level transient viremia represents random biologic and statistical variation or false elevations of viral load resulting from laboratory processing [Nettles, et al. 2005; Lee, et al. 2006]. Blips are not known to be associated with the development of resistance mutations or virologic failure and do not require a change in ART [Lee, et al. 2006]. Retesting should be performed within 4 weeks to differentiate low-level transient viremia (a blip) from sustained viremia and possible virologic failure. The risk of virologic rebound (breakthrough) increases when values are >500 copies/mL [Grennan, et al. 2012]. However, ART should not be changed based on a single viral load elevation.
Advances in molecular detection technology have led to the development of HIV nucleic acid tests (NATs) that are highly sensitive and more reliable than earlier versions. Real-time polymerase chain reaction (PCR) technology has been widely adopted for HIV-1 RNA quantification, but new technologies are continually emerging and being adapted to viral detection and quantification. The currently available HIV-1 viral load tests that use real-time PCR technology offer larger dynamic range of quantification than early-version viral load tests. The lower and upper limits of quantification of the currently available FDA-approved HIV-1 viral load tests are shown in Table 3. Several different HIV viral load tests have been developed, and four are currently approved for use in the United States.

**Table 3: FDA-Approved Quantitative HIV-1 RNA Assays for Viral Load Monitoring**

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Method</th>
<th>Lower and Upper Limits of Quantification (LOQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 (Abbott Laboratories)</td>
<td>Real-time PCR</td>
<td>• 40* copies/mL&lt;br&gt;• 10,000,000 copies/mL</td>
</tr>
<tr>
<td>Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, version 2.0 (Roche Diagnostics)</td>
<td>Real-time PCR</td>
<td>• 20 copies/mL&lt;br&gt;• 10,000,000 copies/mL</td>
</tr>
<tr>
<td>Cobas HIV-1 quantitative NAT for use on Cobas 6800/8800 systems (Roche Diagnostics)</td>
<td>Real-time PCR</td>
<td>• 20 copies/mL&lt;br&gt;• 10,000,000 copies/mL</td>
</tr>
<tr>
<td>Cobas TaqMan HIV-1 Test, v2.0 for use with the high pure system (Roche Diagnostics)</td>
<td>Real-time PCR</td>
<td>• 34 copies/mL&lt;br&gt;• 10,000,000 copies/mL</td>
</tr>
</tbody>
</table>

*This lower LOQ applies when 1.0 mL of plasma is used. When 0.5 mL and 0.2 mL of plasma are used, the lower LOQ is 75 copies/mL and 150 copies/mL, respectively.

All of the current FDA-approved viral load assays quantify the level of cell-free virus in an individual’s plasma and are approved for monitoring response to ART, tracking viral suppression, and detecting treatment failure. Successful ART should decrease viral load 1.5 to 2 logs (30- to 100-fold) within 6 weeks, with the viral load decreasing below the limit of detection within 6 months [AIDSinfo 2019]. Cohort studies strongly suggest that patients with viral loads <50 copies/mL have more sustained viral suppression than patients with viral loads between 50 and 400 copies/mL. Assays that can detect <50 copies/mL are recommended for determining prolonged viral suppression and for monitoring patients who are on ART.

**→ KEY POINT**

- Achieving and maintaining an undetectable viral load is always the goal of ART.

**CD4 Cell Count**

**Lymphocyte Subsets (CD4 Cell Count)**

CD4 lymphocyte count is used to evaluate immunologic staging, predict the risk of clinical progression, and make decisions regarding prophylaxis of opportunistic infections [El-Sadr, et al. 2000; Lopez Bernaldo de Quiros, et al. 2001]. Low CD4 cell counts can be seen in other disease processes and should therefore not be used for diagnosis of HIV. Although, historically, CD4 cell count was used to establish a threshold for initiating ART, current guidelines in New York State recommend ART for all patients with HIV regardless of CD4 cell count. For patients who may not be ready to initiate ART, CD4 cell count can be used to guide discussions between patient and provider regarding the urgency of initiating ART.

Although CD4 counts should be obtained from patients at baseline [Fischl, et al. 1988; Schneider, et al. 1992; Havlir, et al. 1996; Oldfield, et al. 1998; Moore and Keruly 2007], clinicians are unlikely to use CD4 counts to guide clinical decision-making in practice for virologically suppressed patients once their CD4 count remains above 200 cells/mm³. However, for
persons infected with HIV-2 or HIV-1 variants that cannot be accurately quantified using viral load assays, CD4 count remains the most effective monitoring tool for progression of disease (see the NYSDOH AI guideline HIV-2 Infection).

Although a significant CD4 count increase often occurs among patients treated with effective ART, the absence of such an increase should not be interpreted as treatment failure if the viral load declines appropriately. ART regimens are generally not changed in patients with undetectable viral loads who experience immunologic failure, although patients should remain on appropriate prophylaxis for opportunistic infections based on CD4 count. One study of a cohort of more than 62,000 individuals in New York City over 1.9 years of observation reported that in those who entered the cohort with a CD4 count ≥350 cells/mm$^3$, there was a >90% likelihood of sustaining a CD4 count >200 cells/mm$^3$ during that time period [Myers, et al. 2016]. Reassuringly, other data suggest that in patients with sustained viral suppression and CD4 counts between 100 cells/mm$^3$ and 200 cells/mm$^3$, risk of pneumocystis pneumonia is very low even in the absence of prophylaxis [D'Egidio, et al. 2007; Mocroft, et al. 2010; Chaiwarith, et al. 2013].


Absolute CD4 cell counts are calculated values that may fluctuate widely. The calculation is made by multiplying the total white blood cell count (in thousands) by the percentage of total lymphocytes and then by the percentage of CD4 lymphocytes. Therefore, any change in one of these three parameters will cause the absolute CD4 count to vary. CD4 percentage is a direct measurement and more reliable than the calculated absolute CD4 value, especially over time. A stable CD4 percentage, even in the setting of fluctuations in the absolute CD4 cell count, can reassure both the patient and the clinician that immunologic stability is present.

Some factors that can cause these fluctuations include sex, age, race, drugs (zidovudine, cephalosporins, cancer chemotherapy, nicotine, interferon, and corticosteroids), anti-lymphocyte antibodies, and splenectomy. Differences in reagents and equipment both within a laboratory and between laboratories may further contribute to variations in CD4 cell counts. There is also interlaboratory variation of normal range.

All Recommendations

**ALL RECOMMENDATIONS: VIROLOGIC AND IMMUNOLOGIC MONITORING**

- Regular monitoring of HIV RNA levels remains the most accurate and meaningful measure of effective ART (see Table 1: Virologic and Immunologic Monitoring for Non-Pregnant Patients, below, for recommended intervals). (A1)
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- Clinicians should assess response to ART using viral load assays. (A1)
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**References**


Gras L, Kesselring AM, Griffin JT, et al. CD4 cell counts of 800 cells/mm3 or greater after 7 years of highly active antiretroviral therapy are feasible in most patients starting with 350 cells/mm3 or greater. *J Acquir Immune Defic Syndr* 2007;45(2):183-192. [PMID: 17414934]


