



Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection

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Laboratory Monitoring of Pediatric HIV Infection Before Initiation of Therapy (Updated August 11, 2011)

Panel's Recommendations

- The age of the child must be considered when interpreting the risk of disease progression based on CD4 percentage or count and plasma HIV RNA level **(AII)**. For any given CD4 percentage or count, younger children, especially those in the first year of life, face higher risk of progression than do older children.
- In children younger than 5 years of age, CD4 percentage is preferred for monitoring immune status because of age-related changes in absolute CD4 count in this age group **(AII)**.
- CD4 percentage or count should be measured at the time of diagnosis of HIV infection and at least every 3-4 months thereafter **(AIII)**.
- Plasma HIV RNA should be measured to assess viral load at the time of diagnosis of HIV infection and at least every 3-4 months thereafter **(AIII)**.
- More frequent CD4 cell and plasma HIV RNA monitoring should be considered in children with suspected clinical, immunologic, or virologic deterioration or to confirm an abnormal value **(AIII)**.

Immunologic Monitoring in Children

Clinicians interpreting CD4 counts in children must consider age as a variable. CD4 count and percentage values in healthy infants who are not infected with HIV are considerably higher than values observed in uninfected adults and slowly decline to adult values by age 5 years¹⁻². In children younger than age 5 years, the absolute CD4 count tends to vary more with age than does CD4 percentage. Therefore, in HIV-infected children younger than age 5 years, CD4 percentage is preferred for monitoring immune status, whereas absolute CD4 count can be used in older children³⁻⁵.

In HIV-infected children, as in infected adults, the CD4 count and percentage decline as HIV infection progresses, and patients with lower CD4 values have a poorer prognosis than patients with higher values ([Tables 3–5](#)). Consequently, CD4 values should be obtained as soon as possible after a child has a positive test for HIV and every 3 to 4 months thereafter. More frequent evaluation may be needed for children with suspected clinical, immunologic, or virologic deterioration; to confirm an abnormal value; or when initiating or changing therapy. Because young infants with HIV infection may have rapid disease progression⁶⁻⁷, some experts monitor CD4 percentage more frequently (e.g., every 1-2 months) in untreated infants younger than 6-12 months of age. Because of the risk of rapid progression, initiation of antiretroviral therapy (ART) is now recommended for all HIV-infected infants younger than age 12 months (see [When to Initiate Therapy in Antiretroviral-Naive Children](#)).

The prognostic value of CD4 percentage and HIV RNA copy number was assessed in a large individual patient meta-analysis (the HIV Paediatric Prognostic Markers Collaborative Study [HPPMCS]), which incorporated clinical and laboratory data from 17 pediatric studies and included 3,941 HIV-infected children receiving either no therapy or only zidovudine monotherapy⁴. The analysis looked at the short-term (12-month) risk of developing AIDS or death based on the child's age and selected values of CD4 percentage and HIV RNA copy number at baseline. [Figures 1 and 2](#) and [Table 3](#) depict age-associated 1-year risk of developing AIDS or death as a function of CD4 percentage. In a separate analysis of this data set, predictive value of absolute CD4 cell count for risk of death or AIDS/death in HIV-infected

children age 5 years or older was similar to that observed in young adults, with an increase in the risk of mortality when CD4 cell count fell below 350 cells/mm³ ([Table 4](#) and [Figure 3](#))^{3, 8}.

The risk of disease progression associated with a specific CD4 percentage or count varies with the age of the child. Infants in the first year of life experience higher risks of progression or death than older children for any given CD4 stratum. For example, comparing a 1-year-old child with a CD4 percentage of 25% to a 5-year-old child with the same CD4 percentage, there is an approximately fourfold increase in the risk of AIDS and sixfold increase in the risk of death in the 1-year-old child ([Figures 1 and 2](#)). Children age 5 years or older have a lower risk of progression than younger children, with the increase in risk of AIDS or death corresponding to absolute CD4 levels more similar to those in young adults ([Figure 3](#)). In the HPPMCS, there were no deaths among children age 5 years of age or older with CD4 counts greater than 350 cells/mm³, although in younger children there continued to be a significant risk of death even with CD4 cell counts greater than 500 cells/mm³ ([Table 4](#)).

These risk profiles form the rationale for recommendations on when to initiate therapy in a treatment-naive HIV-infected child (see [When to Initiate Therapy in Antiretroviral-Naive Children](#)). A Web site using the meta-analysis from the HPPMCS is available to estimate the short-term risk of progression to AIDS or death in the absence of effective ART according to age and the most recent CD4 percentage or HIV-1 RNA viral load measurement (<http://hppmcs.org>)⁴.

Measurement of CD4 values can be associated with considerable inpatient variation⁵. Even mild inter-current illness or the receipt of vaccinations can produce a transient decrease in CD4 count and percentage; thus, CD4 values are best measured when patients are clinically stable. No **decision about** therapy

Table 3. Likelihood of Developing AIDS or Death Within 12 Months, by Age and CD4+ T-Cell Percentage or Log₁₀ HIV-1 RNA Copy Number in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy

Age	CD4 Percentage					Log ₁₀ HIV RNA Copy Number		
	10%	20%	25%	30%		6.0	5.0	4.0
Percent Mortality (95% Confidence Interval)								
6 Months	28.7	12.4	8.5	6.4		9.7	4.1	2.7
1 Year	19.5	6.8	4.5	3.3		8.8	3.1	1.7
2 Years	11.7	3.1	2.0	1.5		8.2	2.5	1.1
5 Years	4.9	0.9	0.6	0.5		7.8	2.1	0.7
10 Years	2.1	0.3	0.2	0.2		7.7	2.0	0.6
Percent Developing AIDS (95% Confidence Interval)								
6 Months	51.4	31.2	24.9	20.5		23.7	13.6	10.9
1 Year	40.5	20.9	15.9	12.8		20.9	10.5	7.8
2 Years	28.6	12.0	8.8	7.2		18.8	8.1	5.3
5 Years	14.7	4.7	3.7	3.1		17.0	6.0	3.2
10 Years	7.4	2.2	1.9	1.8		16.2	5.1	2.2

Table modified from: HIV Paediatric Prognostic Markers Collaborative Study Group. *Lancet* 2003;362:1605-1611.

Table 4. Death and AIDS/Death Rate per 100 Person-Years by Current Absolute CD4 Count and Age in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy (HIV Paediatric Prognostic Markers Collaborative Study) and Adult Seroconverters (CASCADE Study)*

Age (Years)	Absolute CD4 cell count (cells/mm ³)					
	<50	50-99	100-199	200-349	350-499	500+
Rate of Death Per 100 Patient-Years						
0-4	59.3	39.6	25.4	11.1	10.0	3.5
5-14	28.9	11.8	4.3	0.89	0.00	0.00
15-24	34.7	6.1	1.1	0.71	0.58	0.65
25-34	47.7	10.8	3.7	1.1	0.38	0.22
35-44	58.8	15.6	4.5	0.92	0.74	0.85
45-54	66.0	18.8	7.7	1.8	1.3	0.86
55+	91.3	21.4	17.6	3.8	2.5	0.91
Rate of AIDS or Death per 100 Patient-Years						
0-4	82.4	83.2	57.3	21.4	20.7	14.5
5-14	64.3	19.6	16.0	6.1	4.4	3.5
15-24	61.7	30.2	5.9	2.6	1.8	1.2
25-34	93.2	57.6	19.3	6.1	2.3	1.1
35-44	88.1	58.7	25.5	6.6	4.0	1.9
45-54	129.1	56.2	24.7	7.7	3.1	2.7
55+	157.9	42.5	30.0	10.0	5.1	1.8

* Modified from HIV Paediatric Prognostic Markers Collaborative Study and the CASCADE Collaboration. *J Infect Dis* 2008;197:398-404.

should be made in response to a change in CD4 values until the change has been substantiated by at least a second determination, with a minimum of 1 week between measurements.

HIV RNA Monitoring in Children

Viral burden in peripheral blood can be determined by using quantitative HIV RNA assays. During the period of primary infection in adults, HIV RNA copy number initially rises to high peak levels and then declines by as much as 2 to 3 log₁₀ copies to reach a stable lower level (the virologic set point) approximately 6 to 12 months following acute infection⁹⁻¹⁰. In infected adults, the viral set point correlates with the subsequent risk of disease progression or death¹¹⁻¹².

The HIV RNA pattern in perinatally infected infants differs from that in infected adults and adolescents. High HIV RNA copy numbers persist in infected children for prolonged periods¹³⁻¹⁴. In one prospective study, HIV RNA levels generally were low at birth (i.e., <10,000 copies/mL), increased to high values by age 2 months (most infants had values >100,000 copies/mL, ranging from undetectable to nearly 10 million copies/mL), and then decreased slowly; the mean HIV RNA level during the first year of life was 185,000 copies/mL¹⁵. In addition, in contrast to the adult pattern, after the first year of life, HIV

Table 5. Association of Baseline Human Immunodeficiency Virus (HIV) RNA Copy Number and CD4+ T-Cell Percentage with Long-Term Risk of Death in HIV-Infected Children*

Baseline HIV RNA [§] (copies/mL)/Baseline CD4+ T-cell percentage	No. patients [¶]	Deaths [†]	
		No.	(%)
≤ 100,000			
≥ 15%	103	15	(15%)
< 15%	24	15	(63%)
> 100,000			
≥ 15%	89	32	(36%)
< 15%	36	29	(81%)

* Data from the National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial.

† Mean follow-up: 5.1 years.

§ Tested by NASBA[®] assay (manufactured by Organon Teknika, Durham, North Carolina) on frozen stored serum.

¶ Mean age: 3.4 years.

Source: Mofenson LM, Korelitz J, Meyer WA, et al. The relationship between serum human immunodeficiency virus type 1 (HIV-1) RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1-infected children. *J Infect Dis.* 1997;175(5):1029–1038.

Figure 1. Estimated Probability of AIDS Within 12 Months by Age and CD4 Percentage in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy [Modified from *Lancet* 2003;362:1605-1611]

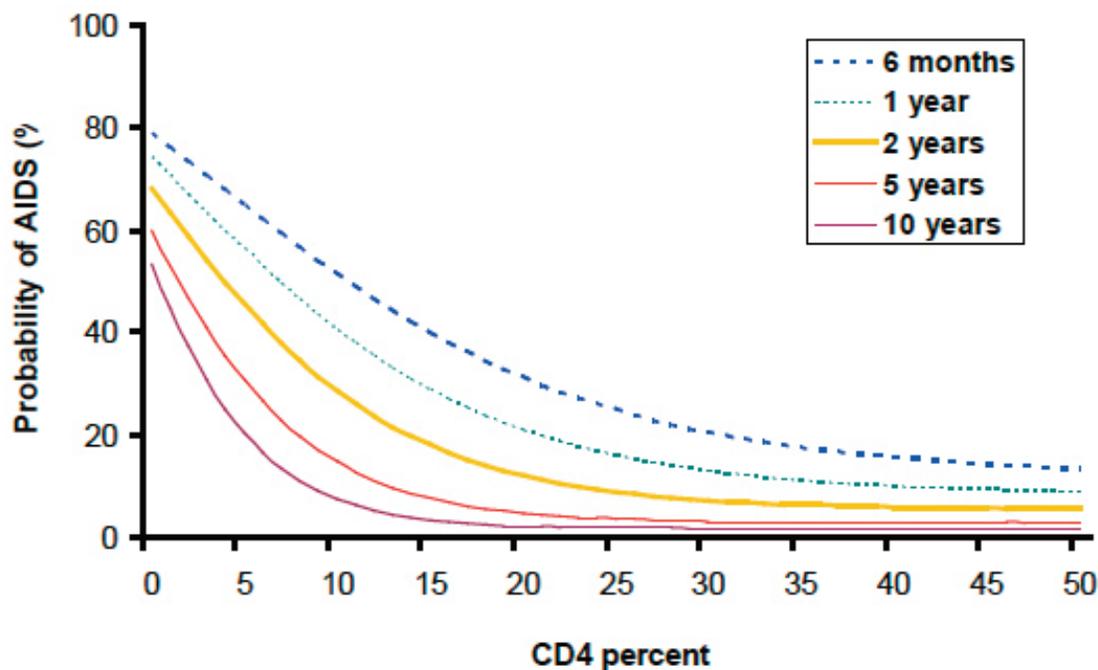


Figure 2. Estimated Probability of Death Within 12 Months by Age and CD4 Percentage in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy [Modified from *Lancet* 2003;362:1605-1611]

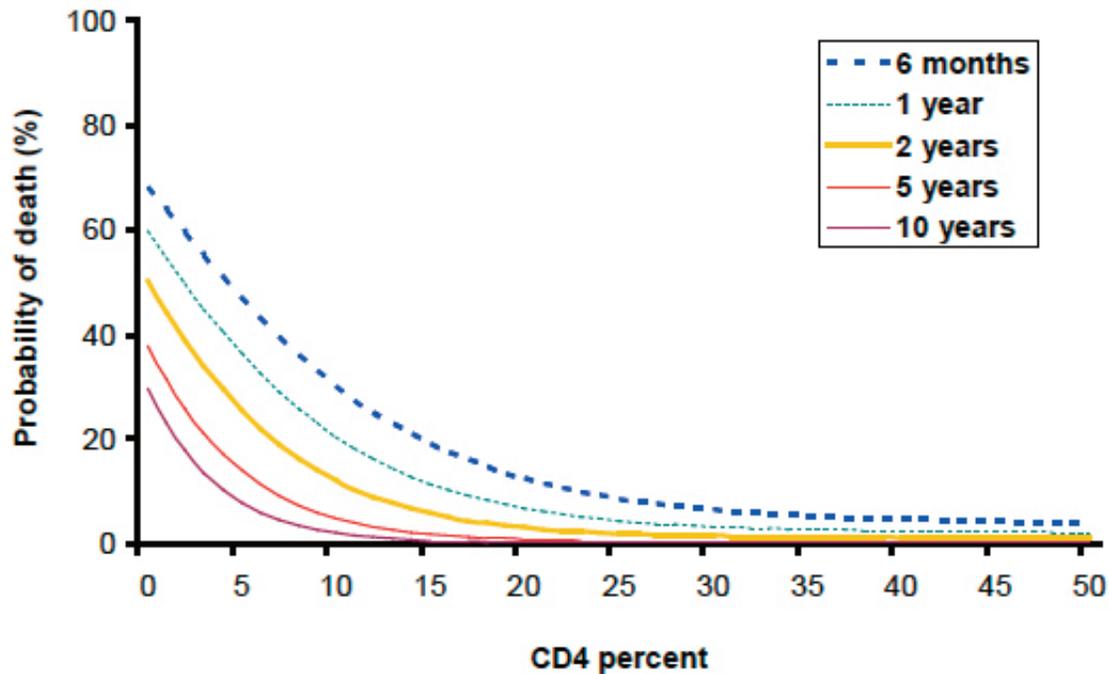


Figure 3. Death Rate per 100 Person-Years in HIV-Infected Children Age 5 Years or Older in the HIV Paediatric Prognostic Marker Collaborative Study and HIV-Infected Seroconverting Adults in the CASCADE Study [Modified from *HIV Paediatric Prognostic Markers Collaborative Study and the CASCADE Collaboration. J Infect Dis.* 2008;197:398-404.]

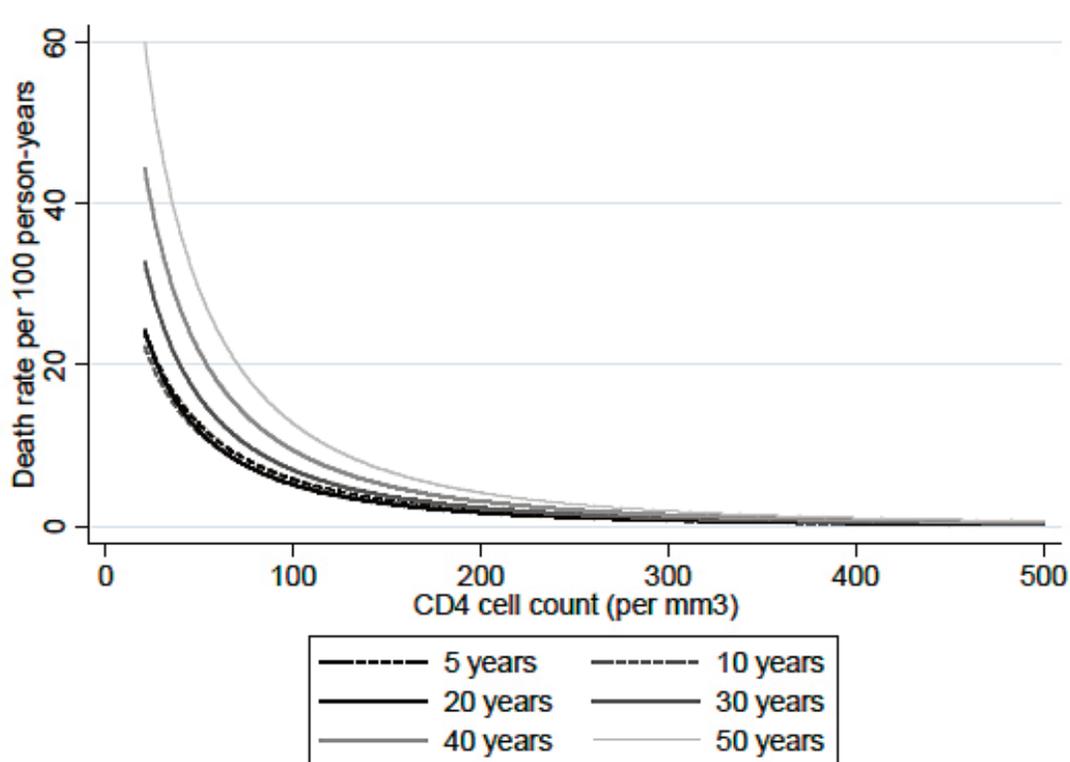


Figure 4. Estimated Probability of AIDS Within 12 Months by Age and HIV RNA Copy Number in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy [Modified from *Lancet* 2003;362:1605-1611.]

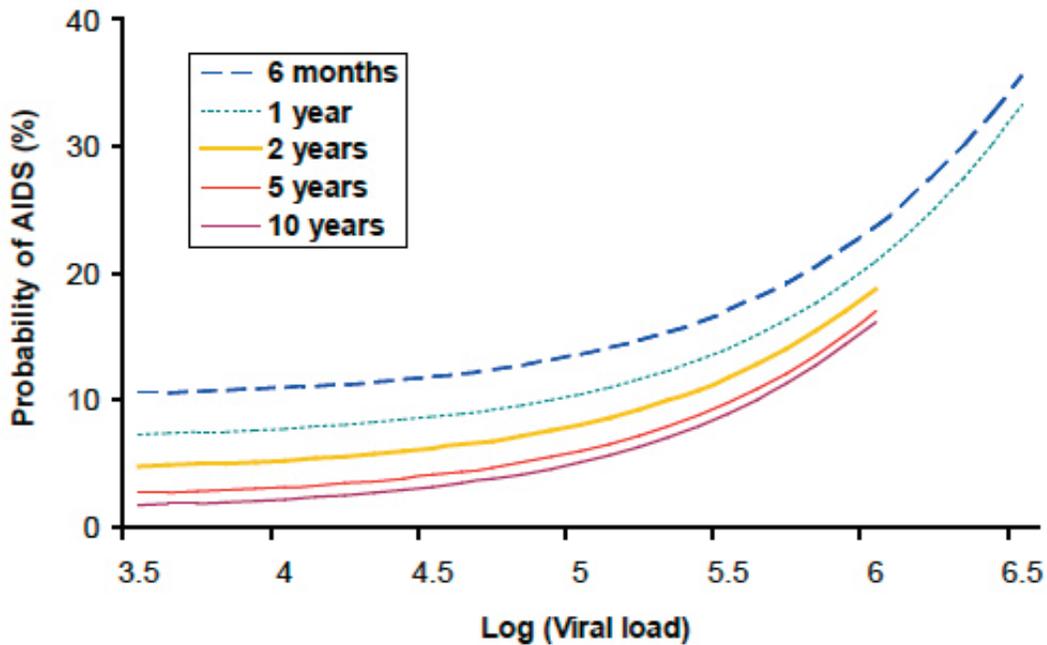
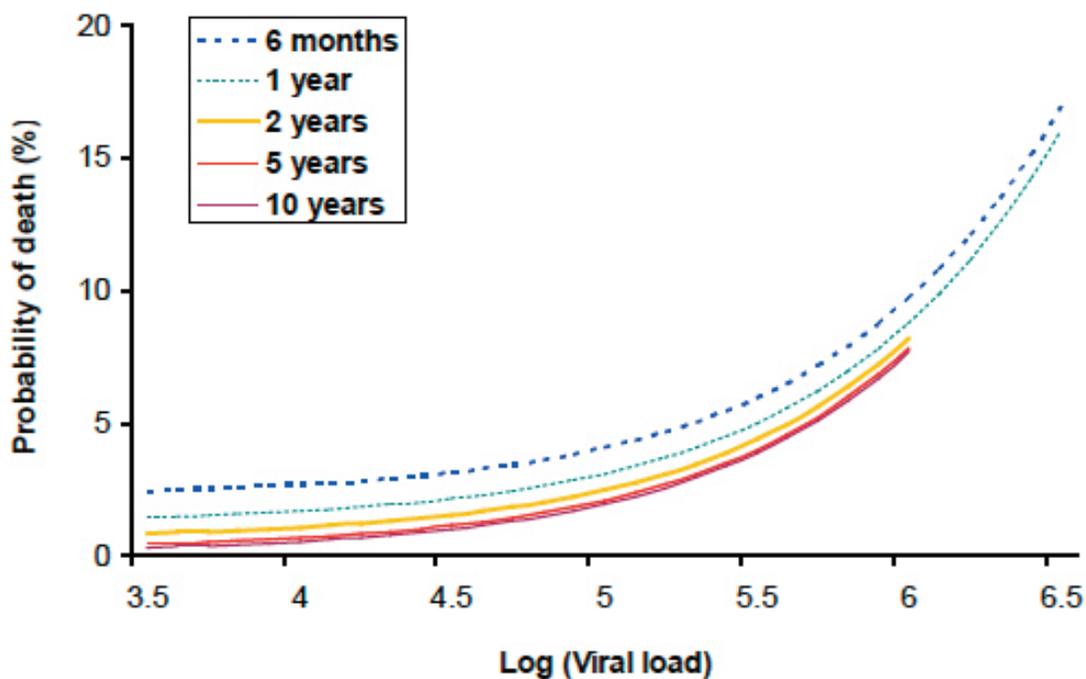


Figure 5. Estimated Probability of Death Within 12 Months by Age and HIV RNA Copy Number in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy [Modified from *Lancet* 2003;362:1605-1611.]



RNA copy number slowly declines over the next few years¹⁵⁻¹⁸. This pattern probably reflects the lower efficiency of an immature but developing immune system in containing viral replication and possibly the rapid expansion of HIV-susceptible cells that occurs with somatic growth¹⁹.

High HIV RNA levels (i.e., >299,000 copies/mL) in infants younger than age 12 months have been correlated with disease progression and death, but RNA levels overlap considerably in young infants who have rapid disease progression and those who do not¹³⁻¹⁵. High RNA levels (i.e., levels of >100,000 copies/mL) in older children have also been associated with high risk of disease progression and mortality, particularly if CD4 percentage is less than 15% ([Table 5](#))¹⁷⁻¹⁸. The most robust data set available to elucidate the predictive value of plasma RNA for disease progression in children was assembled in the HPPMCS (see Immunologic Monitoring in Children)⁴. As for CD4 percentage, analyses were performed for age-associated risk in the context of plasma RNA levels in a cohort of children receiving either no therapy or only zidovudine monotherapy. Similar to data from previous studies¹⁷⁻¹⁸, the risk of clinical progression to AIDS or death dramatically increases when HIV RNA exceeds 100,000 copies (5.0 log₁₀ copies)/mL; at lower values, only older children show much variation in risk ([Figures 4 and 5](#) and [Table 3](#)). At any given level of HIV RNA, infants younger than 1 year of age were at higher risk of progression than older children, although these differences were less striking than those observed for the CD4 percentage data.

Despite data indicating that high plasma HIV RNA concentrations are associated with disease progression, the predictive value of specific HIV RNA concentrations for disease progression and death for an individual child is moderate¹⁷. HIV RNA concentration may be difficult to interpret during the first year of life because values are high and are less predictive of disease progression risk than in older children¹⁴. In both HIV-infected children and adults, CD4 percentage or count and HIV RNA copy number are independent predictors of disease progression and mortality risk, and use of the two markers together more accurately defines prognosis^{17-18, 20-22}.

HIV RNA copy number should be assessed as soon as possible after a child has a positive virologic test for HIV and every 3 to 4 months thereafter; more frequent evaluation may be necessary for children experiencing virologic, immunologic, or clinical deterioration or to confirm an abnormal value (see [Anti-retroviral Treatment Failure in Infants, Children, and Adolescents](#)).

Methodological Considerations in Interpretation and Comparability of HIV RNA Assays

The use of HIV RNA assays for clinical purposes requires specific considerations²³, which are discussed more completely elsewhere²⁴. Several different methods can be used for quantitating HIV RNA, each of which has a different level of sensitivity. Although the results of the assays are correlated, the absolute HIV RNA copy number obtained from a single specimen tested by two different assays can differ by twofold (0.3 log₁₀ copies/mL) or more²⁵⁻²⁸.

Five Food and Drug Administration (FDA)-approved viral load assays using one of three different methodologies currently exist:

- HIV-1 reverse transcriptase (RT) quantitative polymerase chain reaction (PCR) assays: the Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostics), for which the lower limit of detection differs between the “ultrasensitive” assay (<50 copies/mL) and the “regular sensitivity” assay (<400 copies/mL); the AmpliPrep/TaqMan HIV-1 Test (Roche Diagnostics); and the Real Time HIV-1 Assay (Abbott Molecular Incorporated);
- HIV-1 nucleic acid sequence-based amplification test (NucliSens HIV-1 QT, bioMerieux); and

- HIV-1 *in vitro* signal amplification, branched chain nucleic acid probe assay (VERSANT HIV-1 RNA 3.0 Assay, Bayer).

The lower limits of detection of the assays differ (<40 copies/mL for the Abbott Real Time HIV-1 test, <48 copies/mL for the AmpliPrep/TaqMan HIV-1 Test, <50 copies/mL for the Amplicor HIV-1 Monitor Test, <80 copies/mL for the NucliSens HIV-1 QT assay, and <75 copies/mL for the VERSANT assay). Use of ultrasensitive viral load assays is recommended to confirm that ART is producing maximal suppression of viremia. Because of the variability among assays in techniques and quantitative HIV RNA measurements, if possible, a single HIV RNA assay method should be used consistently to monitor an individual patient.

The predominant virus subtype in the United States is B, which is the subtype for which all initial assays were targeted. Current kit configurations for all companies have been designed to detect and quantitate essentially all viral subtypes, with the exception of the uncommon O subtypes²⁹⁻³⁰. This is important for many regions of the world where non-B subtypes are predominant as well as for the United States, where a small subset of individuals are infected with non-B viral subtypes³¹⁻³³. It is particularly relevant for children who **are born outside the United States or to foreign-born parents**. Choice of HIV RNA assay, particularly for young children, may be influenced by the amount of blood required for the assay. The NucliSens assay requires the least amount of blood (100 µL of plasma), followed by the RT-PCR assays such as Amplicor HIV-1 Monitor (200 µL of plasma) and the VERSANT assays (1 mL of plasma).

Biologic variation in HIV RNA levels within one person is well documented. In adults, repeated measurement of HIV RNA levels using the same assay can vary by as much as threefold (0.5 log₁₀ copies/mL) in either direction over the course of a day or on different days^{20, 24, 27}. This biologic variation may be greater in infected infants and young children. In children with perinatally acquired HIV infection, RNA copy number slowly declines even without therapy during the first several years after birth, although it persists at higher levels than those observed in most infected adults¹⁵⁻¹⁷. This decline is most rapid during the first 12-24 months after birth, with an average decline of approximately 0.6 log₁₀ copies/mL per year; a slower decline continues until approximately 4-5 years of age (average decline of 0.3 log₁₀ copies/mL per year).

This inherent biologic variability must be considered when interpreting changes in RNA copy number in children. Thus, on repeated testing, only differences greater than fivefold (0.7 log₁₀ copies/mL) in infants younger than age 2 years and greater than threefold (0.5 log₁₀ copies/mL) in children ages 2 years and older should be considered reflective of changes that are biologically and clinically substantial.

No alteration in therapy should be made as a result of a change in HIV copy number unless the change is confirmed by a second measurement. Because of the complexities of HIV RNA testing and the age-related changes in HIV RNA in children, interpretation of HIV RNA levels for clinical decision making should be done by or in consultation with an expert in pediatric HIV infection.

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