

HIV Laboratory Monitoring

Allison L. Agwu, MD

Allison Agwu: Hello. I'm Dr. Allison Agwu, a Combined Adult and Pediatric Infectious Diseases Fellow at the Johns Hopkins School of Medicine. As part of our Center for Global Health's Pediatric HIV lecture series, I will be discussing with you today HIV laboratory monitoring, including surrogate markers for settings with limited access to regular CD4 and viral load measurements.

Our lecture objectives today are threefold.

Number one: to understand the gold standard immunologic and biologic measures for laboratory monitoring HIV in treated and untreated HIV infections.

Number two: to understand the alternatives available for HIV monitoring when CD4 and viral load measures are not available.

And third and importantly: to recognize the limitations of standard and alternative measurements.

In this schematic is a standard representation of HIV infection. On the left-hand side of the panel in blue is CD4 cell. On the right, HIV RNA. And what you see is the interplay of CD4 and viral RNA over time, of HIV infection. Being in the beginning of a primary infection, there's a high number of CD4 cells and maybe a high number of viral load -- viral RNA -- available.

Over time, the viral load drops as HIV infection goes into sort of a latent phase. Over time, as the viral load rises, the CD4 continues to plummet, until finally resulting in death, usually from opportunistic infections.

So, first, what are CD4 cells? In this schematic on the right, what you see in red is the HIV virus. In green below is a T-cell, or CD4 cell. And you're seeing the interaction or the connection or the attachment of the HIV virus to the T-cell. So, CD4 cells -- or T-cells, or T-helper cells, as you will find them called -- are the primary targets of the HIV virus.

Typically, as stated in the prior slide, they decrease in number over time as HIV infection progresses. Third CD4 and/or CD4 percent is the best test for evaluating HIV stage and prognosis and for monitoring progression to AIDS, as well as the risk of opportunistic infections.

CD4 testing guides differential diagnosis in asymptomatic patients, guides decisions about initiating anti-retroviral therapy -- or ARTs -- and, thirdly, guides decisions about prophylaxis for opportunistic infections.

CD4 laboratory monitoring. The CD4 count is reported as the number of CD4 cells per microliter of blood. Most laboratories will report the CD4 count as part of a list of several types of lymphocytes, as both an absolute count and a relative

percentage.

In this example below, what you see is an absolute CD4 count of 240 cells per microliter, which is correlating with a CD4 percentage of 17%.

Some more about the CD4. There can be significant fluctuations in absolute CD4 count, therefore, providers should never draw definitive conclusions based on one value, particularly if that value is different from prior trends or values. So, usually the recommendation is for decisions to be made on greater than two or more values.

Factors that may transiently affect the CD4 count include illness, vaccinations, diurnal variations in the CD4 count, and fluctuating lymphocyte counts, which can occur with illness itself or just in general. And laboratory error, as well as differences between laboratories and how they measure the CD4.

CD4/CD4 percentage in children. In children less than five years old, CD4 percentage is considered the best measurement to use. Particularly in the very young, less than one year of age, the CD4 count may be markedly higher and fluctuate significantly versus the older individuals. Therefore, CD4 count in the very young is less predictive of mortality than CD4 percentage. In those greater than five years of age, CD4 count can be used more reliably, sort of similar to what happens in adult patients.

This is a slide from the WHO, the World Health Organization's management of HIV in infants and children. And what you see across the top of the graph are the age-related CD4 values and the classification of HIV-related immunodeficiency.

So, first we will look at the less than 11-month-old individual, where CD4 percentage is the rule to use in classifying disease. Insignificant and not significant immunosuppression is considered a CD4 count greater than 35%, while mild suppression is considered 30%-35%, advanced is 25%-39%, and severe immunosuppression is less than 25%.

In contrast to that, the greater than five year old patient, where a CD4 percentage of less than 15% correlating with a CD4 count of 200 is considered severe disease. So, more importantly again, noting the CD4 count, the percentage and the age of the patient is important for the provider actually to actually classify that patient.

In this schematic, what you see on the X-axis is CD4 percentage. On the Y-axis is the probability of death. And what you see is that over time, regardless of age-range, the lower the CD4 percentage, the higher the probability of death within a 12-month period.

CD4 in untreated or treated patients. As untreated HIV infection progresses, CD4 count decreases by about approximately 4% every year. With successful ART -- or anti-retroviral therapy -- the CD4 count may increase by greater than 50 cells per

microliter within weeks after viral suppression. This is variable. And then it may increase by 50-100 cells per microliter per year thereafter, until a threshold is reached. Note: in some patients, a CD4 count may not increase this quickly or steadily, even with durable viral suppression.

Recommendations for CD4 monitoring. CD4 count is one of many factors including clinical status, viral load status, and medication adherence or the likelihood of medication adherence, that should be assessed before starting or changing anti-retroviral therapy in a patient.

Ideally, the CD4 count should be repeated approximately every 2-4 months in patients on stable anti-retroviral therapy and every six months in untreated individuals or patients. In some situations, the CD4 count may need to be checked more frequently, for example in infants, where significant fluctuations can occur.

The disadvantages of CD4 testing include: one, it's expensive; two -- particularly in research-limited settings -- there may not be availability of CD4 testing; and three, laboratory availability in CD4 reporting.

Next we will discuss the HIV viral load. The viral load is the best indicator of how active HIV is in the patient's body. Higher amounts of viral activity has been correlated with more rapid disease progression, as there is simply more HIV available to attack the CD4 cells. When HIV antibody tests may be negative misleading, for example in acute HIV infection or in infants with neo-natal infection, the HIV viral load may be used to help diagnose HIV infection.

Antiretrovirals are used in combination to reduce the amount of virus in the body in someone who has HIV infection. The goal of therapy is always to lower the activity of HIV in the body and to, therefore, lower the viral load. With less HIV present, the body is able to produce more CD4 cells and therefore to improve the immune system.

In this schematic, you see on the X-axis, HIV RNA represented as a log₁₀ HIV RNA, and on the Y-axis, the probability of death. Regardless of age, the higher the viral load level, the higher the probability of death within a 12-month period.

So, more about viral load testing: The goal of therapy is to have an undetectable viral load. Having an undetectable viral load does mean that the virus cannot be detected in the body by the measures that you were using, although it may exist in other parts of the body.

Lack of detection does not mean a cure, and this is an important fact as patients may misinterpret this information when you tell them their viral load is undetectable. When therapy is stopped, reliably, the HIV activity increases again in the patient's body. As viral load declines, the patient's CD4 count usually increases, and he or she may be protected from infections and other illnesses

related to HIV.

Viral load testing: Where available, the viral load is reported as copies per mil, or milliliter, or log₁₀ HIV RNA.

The standard tool used to monitor viral activity and ARV response: There are many different types of viral load assays, and I have listed a few here, including the HIV polymerase reaction, the Amplicor assay, as well as other testing such as the bDNA, or branched DNA assay. So, knowing what your laboratory is using is very important, as interpretation of testing would be different based on that.

The lowest level of detection of the virus varies for each test, but most commonly, a viral load level less than 400 copies per mL, or milliliter, is utilized. There are ultra-sensitive assays that are available, which are preferred in circumstances when patients are in therapy, and that may measure viral loads to as low as 50 to 80 copies per milliliter, depending, again, on the specific test that your institution or laboratory is using.

Now, interpreting viral load results is important. First, when a viral load is below the level of detection, it is listed as "undetectable." It means that the level of testing is at the limit of detection of that specific assay and the virus was not detected. The virus may still be detected with more sensitive assays. Again, this does not mean that there is no virus in the body.

Suppressing HIV RNA to an undetectable level, less than 50 to 75 or 80 copies per mil, as measured by the ultra-sensitive assay, as stated before, is truly an important goal of therapy. The different assays also have different values for the highest level of detection, usually between 550,000 to 750,000 copies per mL. Higher viral loads can be reported, as high as, or greater than 500,000, for example.

Again, more on interpreting viral load results: Viral loads can, as CD4 cells can, vary considerably. Laboratory variations, assay fluctuations, acute illness, or recent vaccinations can lead to elevations of viral loads, or variability in viral loads. Variations of less than approximately .5 log₁₀ copies per mL, or less than three full, usually may not be clinically significant, and should be taken as part of a trend in viral load levels.

As with CD4, viral load results that are inconsistent with previous trends should be repeated, and treatment decisions should never be made based on one viral load level, but as with CD4, based on two or more similar values in the same direction.

When patients have had recent illnesses or vaccinations, viral load measurement should be deferred for as much as four weeks, if possible, and viral load testing should be checked at least twice at baseline before starting antiretroviral therapy.

The disadvantages of viral load testing are: First, they're expensive; they may not be widely available, particularly in resource-limited settings; they are labor-intensive, and there is laboratory variability.

The recommendations for viral load monitoring: Where available, viral load measurements should be performed at regular intervals, and usually that interval is from three to four months. Closer intervals may be necessary if there is a new therapy, a change in therapy, or there's been a significant change viral load or CD4 count, or there's a declining clinical status in a patient.

So, putting CD4 and viral load together: Absolute CD4 count is more predictive of clinical disease progression than is the baseline viral load, and this has been shown in cohort studies over time. Studies have shown that patients with high plasma viral load levels have an increased risk of progression to symptomatic HIV disease and AIDS, compared with patients who have low or undetectable viral load levels, and this is a rationale for the Department of Health and Human Services, the HHS, guidelines.

In acute seroconversion and in advanced disease, viral load may be markedly elevated, greater than 500,000, or above the limit of the assay that you are using, while in chronic infection, the viral load levels are usually lower. There are some concerns of utilizing CD4 monitoring alone, as it does not give an indication of what the viral load is given, and there is a possibility that a resistance may be developing and the practitioner not aware of it, as they're not aware of what is happening to the viral load.

So, how can we monitor HIV disease progression where CD4 and viral load levels are not available? There are other proposed laboratory markers, including total lymphocyte count, hemoglobin, serum albumin, and p24 antigen. When analyzed, low total lymphocyte count and albumin have been shown to be independent predictive markers for mortality, and this has been shown in children.

The WHO recommends the total lymphocyte count can be used as an alternative for CD4 disease progression, but not for an assessment of response to treatment. I will say that there are some studies that show that there may be some ability to do that, but has not been consistent, and therefore the WHO recommends not to use it as a response for treatment.

Now, utilizing total lymphocyte count: I want to show you here, in this figure which is from the WHO management of HIV infection in infants and children, of some of the age related total lymphocyte count, and how it compares to CD4 counts. For example, again, in a less than 11-month-old, a total lymphocyte count of less than 4,000 correlates with a CD4 count of less than 1,500, while in a greater than five-year-old, a less than 2,000 total lymphocyte count correlates to a CD4 count of less than 200, all in this panel showing severe immunosuppression.

Also in this panel is shown how total lymphocyte count is calculated, simply as a percentage of lymphocytes multiplied by the total white blood cell count.

In this schematic here, also from the WHO laboratory monitoring guidelines, simply correlates CD4, CD4 percent, and total lymphocyte count in age, showing in the youngest individuals the higher probability of death, but at lower levels of immune suppression, marked over here with the red circle.

So, monitoring children without laboratory parameters at all: There are clinical parameters that are useful: improvement in growth in children with prior growth failure; improvement or resolution of neurologic symptoms and improvement in development in children with encephalopathy or developmental delay; attainment of developmental milestones in those previously with developmental delay; and a decrease in infection or the frequency of infections, for example, of bacterial infections, oral thrush and other opportunistic infections all can be markers of improvement in clinical parameters, can be used to stage children, or monitor children's progress.

Thank you very much for paying attention to my Lab Monitoring lecture, and I look forward to discussing with you more in further lecture series.