HIV in the tropics: staging in the resource-limited setting

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Purpose of review
In 2010, the WHO updated HIV treatment guidelines for adults and children, expanding the eligibility of HIV-infected individuals for antiretroviral therapy (ART) on the basis of immunological staging. We discuss the barriers to HIV staging in under-resourced settings.

Recent findings
In industrialized countries, HIV-infected patients are immunologically staged using CD4 lymphocyte counts measured using flow cytometry, but reliable and timely CD4 testing is still not readily available for all patients in many poorly resourced countries. Often CD4 testing is only available in central hospitals and clinics and depends upon availability of reagents. This leaves clinical staging as the standard of care in many places. Significant discrepancies exist between clinical and immunologic staging. Lack of immunologic staging can lead to delayed or inappropriate initiation of ART, increased attrition before ART, and overall poorer outcomes as patients often initiate ART at lower CD4 cell count baselines. This has led to intensive efforts to develop cost-effective laboratory testing, particularly for accurate low-cost CD4 testing.

Summary
Simplified, low-cost alternatives for immunologic staging are vital to continued scale up of ART programs globally. Point-of-care CD4 testing in particular has shown promise in decreasing attrition rates before ART and improving overall mortality in resource-limited settings.

Keywords
CD4, clinical staging, HIV, resource limited

INTRODUCTION

Thirty years since the start of the HIV/AIDS epidemic, tremendous strides have been made from a political, social, and financial standpoint resulting in a remarkable expansion of HIV/AIDS prevention, treatment, and support [1,2▪]. Of the 34 million people infected with HIV worldwide, over 6.5 million people receive antiretroviral therapy (ART), which has led to improved survival of people living with HIV [1]. Mother-to-child transmission has also significantly decreased with an estimated 50% of pregnant women receiving antiretrovirals [1,2▪]. Access to HIV counseling and testing has increased, and the global incidence of newly acquired infections has decreased with an estimated 2.7 million new infections in 2010, 15% fewer than the 3.1 new infections estimated in 2001 [1]. Most of these improvements are due to expanded treatment programs in sub-Saharan Africa [1].

Despite these remarkable advances, significant barriers remain to the global provision of prevention, treatment, and support for people living with HIV/AIDS [3]. In particular, the success of pediatric HIV prevention and management witnessed in industrialized countries has not been realized in the developing world [4]. Of the 2.5 million deaths prevented with ART since 1995, only 4% were amongst children under the age of 15 [1]. Extraordinary discrepancies exist in treatment coverage between adults and children with less than 25% of eligible children receiving therapy [1]. In spite of improved access to testing, vast majorities of people in developing countries are unaware of their HIV status, and there has been a significant increase in incidence of new HIV infections and AIDS-related

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KEY POINTS

- Updated WHO adolescent and adult guidelines recommend initiation of ART for any patient with a CD4 cell count less than 350 cells/μl.
- Any child under the age of 12 months with a presumed or definitive diagnosis of HIV should initiate ART. There is also a conditional recommendation to initiate ART for any presumptively or definitively HIV-infected child aged 12–24 months, but this is left to the discretion of individual countries to implement.
- Lack of reliable and timely CD4 testing can lead to delayed or inappropriate ART initiation, increased attrition before ART, and overall poorer outcomes and mortality for patients initiating ART at lower CD4 baselines.
- POC CD4 testing has been shown to improve retention before ART and overall outcomes of HIV-infected patients, and further research is needed in this area.

mortality in areas of eastern Europe and central Asia [1].

The WHO and the joint United Nations program on HIV/AIDS continue to work diligently toward universal HIV testing and treatment resulting in increased local scale-up campaigns for expanded HIV testing and treatment [5]. In industrialized countries initiation of ART is based upon immunologic criteria, but in most developing countries access to CD4 testing is limited and WHO clinical staging is the standard of care [6,7]. Significant discrepancies exist between clinical and immunologic staging, leading to inferior outcomes in those patients initiating ART based solely on clinical staging [8–11].

Recent evidence also indicates that lack of reliable and timely CD4 testing may be associated with decreased retention before ART [12▪▪], resulting in increased loss to follow-up and mortality in patients, especially those initiating ART with lower baseline CD4 counts [1,11,13]. For successful scale up of HIV/AIDS programs, particularly for ART initiation, bolstering the connection between testing and follow-up services, especially access to CD4 testing, is critical.

CURRENT ADULT AND PEDIATRIC WHO GUIDELINES

Revised adult and pediatric WHO guidelines were published in 2010 and are expected to undergo reevaluation this year (Table 1). One of the most significant adolescent and adult guideline changes is the recommendation for earlier initiation of ART, at a CD4 cell count less than 350 cell/μl [6]. This change was made based on evidence showing improved morbidity and mortality outcomes in asymptomatic, ART-naive patients who initiated ART at a higher CD4 cell count of less than 350 cells/μl [14–18].

The landmark Comprehensive International Program of Research on AIDS (CIPRA) was a randomized, open-label trial of early initiation of ART versus standard initiation of therapy among two groups of HIV-infected, ART-naive Haitian adults with confirmed CD4 T-cell counts less than 200 cells/μl (standard treatment) or between 200 and 350 cells/μl (early treatment) at baseline with no history of an AIDS-defining illness. Patients were followed for 21 months with a primary study endpoint of survival. There were 23 deaths in the standard treatment group, as compared with six deaths in the early treatment group [hazard ratio (HR) 4, 95% confidence interval (CI) 1.6–9.8, $P = 0.001$]. There were also 36 incident cases of tuberculosis (TB) as compared with 18 cases in the early treatment group (HR 2.0, 95% CI 1.2–3.6, $P = 0.01$). Both study outcomes strongly favored the initiation of ART at a higher CD4 threshold of 350 cells/μl [16]. Although a follow-up evaluation of the CIPRA study by Koenig *et al.* [19▪] found cost-effectiveness evidence against early initiation of ART, the study authors believed that the long-term benefit was ultimately in favor of earlier ART as patients in the study were only followed over a 3-year period.

In the absence of CD4 testing, WHO clinical staging should be applied and ART begun for any patient with stage 3 or 4 disease. Updated guidelines

Table 1. WHO criteria for antiretroviral therapy initiation in children and adults

Age	Less than 24 months[a]	24–59 months	5 years and older (includes adults)
CD4%	All	25% or less	Not applicable
Absolute CD4	All	750 cells/μl or less	350 cells/μl or less

Table from WHO. Antiretroviral therapy for HIV infection in infants and children: toward universal access. Recommendations for a public health approach. 2010 (7). All children and adults with stage 3 or 4 clinical disease should be started on antiretrovirals irrespective of CD4 counts. In many resource-poor settings, pulmonary tuberculosis is the first clinical presentation of HIV infection (and is considered a stage 3 diagnosis).
[a]Implementation of guidelines in 12–24-month age group is left to the discretion of individual countries.

also promote the use of simplified and less toxic first-line and second-line ART regimens and recommend initiation of ART in all patients with HIV infection who are coinfected with TB after initiation of anti-TB therapy. Patients who are coinfected with HIV and hepatitis B who are to be started on hepatitis B therapy should also receive ART [6].

More deliberate monitoring of treatment efficacy and toxicity is also recommended, which may be an area in which costs can be significantly lowered. Although the DART (Clinically driven laboratory monitoring of HIV anti-retroviral therapy in Africa) trial showed that laboratory monitoring is most beneficial in the second year of ART to help guide switching to second-line therapy, routine laboratory monitoring for adverse effects may not be necessary. This information has considerable financial implications as funding can potentially be reallocated for diagnostics and drug delivery [20].

Compared with adults, absolute lymphocyte counts in children under the age of 5 years old exhibit considerable variation, making CD4 percentage the preferred immunologic parameter in determining disease stage and when to initiate ART in this age group [21]. Updated WHO pediatric guidelines published in 2010 focus on determining HIV exposure and infection status earlier – at birth or soon after [7]. There are significant difficulties with HIV diagnosis in children, particularly in resource-limited settings, which may hamper timely ART initiation. Because of the low sensitivity of the HIV DNA PCR test at birth (<50% versus up to 99% at 6 weeks), clinical practice in resource-poor setting is often to test only at 6 weeks. Maternal HIV antibodies can be present in infants up to 18 months of age, making CD4 and viral load testing preferred but not realistic in many developing countries. HIV serologic testing should be confirmed at 18 months of age in any patient with a presumptive diagnosis of HIV [7].

Epidemiologic studies indicate unequivocally that HIV-infected infants suffer extremely high morbidity and mortality rates [22–25]. This has led the WHO to recommend that any child under the age of 12 months with presumed or known HIV infection is started immediately on ART irrespective of immunologic or clinical status (Table 1). The WHO further makes a conditional recommendation for children age 12–24 months also to be initiated on ART regardless of clinical or immunologic status, but implementation of this recommendation has been left to the discretion of individual country governments [7].

Similar to adult guidelines, an additional objective for children is more simplified first-line and second-line ART regimens. There is also a suggestion for increased attention to nutritional status in the pediatric population receiving ART. For both adults and children, total lymphocyte count is no longer recommended to guide treatment initiation decisions [6,7].

Even in resource-limited areas that have the ability to implement current WHO guidelines, critical decisions must be made about which recommendations can be feasibly implemented. In a populated model using South African clinical and resource utilization data, Walensky *et al.* [26] found that for developing countries who cannot practically implement all of the new guidelines, the initiation of ART at a CD4 cell count less than 350 cells/μl afforded the greatest survival and cost benefit compared with all other guideline recommendations.

RELIABILITY OF CLINICAL HIV STAGING

The WHO advises that all patients should have access to immunologic testing in an effort to improve retention before ART, initiation of ART, and subsequent laboratory monitoring. In particular, patients classified as stage 1 or 2 should be tested for a CD4 count to determine whether treatment should be initiated based on immunologic criteria [6]. Adequate laboratory access is critical for improved classification of patients eligible for ART as well as for monitoring of treatment efficacy and toxicity [27,28]. Despite improved laboratory infrastructure in many regions, access to CD4 testing remains a significant barrier to the improvement of ART programs [29]. As a result, clinical staging remains the standard of care in most resource-limited settings. A number of studies, however, have shown significant disagreement between clinical and immunologic staging [30▪,31▪,32–36]. The discordance between clinical and immunologic staging has considerable medical, public health, and financial implications in the developing world.

Carter *et al.* [37] evaluated an African cohort of pregnant and recently postpartum HIV-positive women who were clinically and immunologically staged upon enrollment into a mother-to-child transmission prevention program. The study authors compared immunologic and clinical staging criteria according to 2009 WHO treatment guidelines in identifying ART-eligible women (stage 3 and 4 or $CD4^+$ cell count <350 cell/μl). Of the 6036 women enrolled, 2915 (48%) were ART eligible; although only 23% of those ART-eligible patients had WHO stage 3 or 4 disease, 94% of that group met CD4 cell count thresholds. Furthermore, WHO stage 1 or 2 disease was identified in 5356 women (84%), yet 2235 (42%) had $CD4^+$ cell count less than 350 cells/μl. On the basis of these results, the study

authors concluded that immunologic staging was superior to clinical staging in accurately determining ART eligibility in pregnant and postpartum HIV-infected women.

In a multicenter cross-sectional study done in Uganda by Baveewo *et al.* [30▪], the sensitivity and specificity of clinical staging and immunologic staging both at CD4 cell counts less than 250 and 350 cells/μl were compared in ART-naive adult patients. WHO clinical staging sensitivity at CD4 thresholds less than 250 and 350 cells/μl was 53.5 and 49.1% with specificities of 81.1 and 86.8%, respectively. In this study, specific clinical findings were also evaluated for a potential correlation with immunologic staging. Angular chelitis and papular eruptions were associated with a CD4 cell count less than 250 cells/μl. Angular chelitis along with recurrent upper respiratory tract infections was a more sensitive predictor of CD4 cell count less than 350 cells/μl.

Children pose additional challenges with regards to discrepancies between clinical and immunologic staging. Up to a third of children who are deemed ineligible for ART on the basis of clinical staging alone may actually have severely depressed $CD4^+$ T-cell counts [9]. Furthermore, in areas such as sub-Saharan Africa, children experience higher rates of TB, gastroenteritis, and pneumonia than their counterparts in industrialized countries. Such acute or chronic illnesses may contribute to underlying malnutrition resulting in misclassification when such children are clinically staged. In these instances, children may be starting ART too early as a consequence of symptoms that are not reflective of a suppressed immune system [9].

Despite limited resources, HIV/AIDS and ART initiation programs have significantly improved access to the testing and treatment of people living with HIV/AIDS. Populations with advanced HIV disease continue to decrease as ART becomes more available globally. This will continue to lead to clinical criteria being a less sensitive and less functional screening method [10].

EFFECTS OF LACK OF ACCESS TO IMMUNOLOGIC STAGING

The high costs associated with equipment purchase and maintenance as well as lack of trained personnel contribute to poor laboratory infrastructure in resource-limited settings. These factors also limit ART scale-up programs [38▪▪,39]. With clinical staging alone, stage 1 or 2 patients may not receive ART, although immune suppression may be more advanced than clinical symptoms suggest. Patients who initiate ART later when CD4 counts are lower have poorer success rates compared with those initiating therapy at a higher CD4 count [40▪]. An analysis done of patients in multiple resource-limited countries demonstrated that initiating ART at a CD4 cell count below 25 cells/μl posed a more than three-fold risk of death than starting ART at a CD4 cell count greater than 50 cells/μl. This same study also showed that patients with lower CD4 counts were less likely to return for further care [11].

Conversely, incorrectly classifying patients as clinical stage 3 or 4 can lead to the inappropriate early initiation of ART, which can increase risk for adverse effects as well as resistance to first-line medications. There are also important cost implications as placing patients on ART unnecessarily can place heavy financial burdens on resource-limited countries and local governments [35].

Lack of reliable and timely access to CD4 testing may also contribute to high attrition rates prior to initiation of ART [12▪▪]. Additional reasons for attrition before ART includes the lack of patient symptoms, long distances for travel to clinic, transfer to a different clinic, and death [41–44,45▪▪]. Over 40% of patients on ART are lost to follow-up in the first year [11,13,46]. Moreover, up to 80% of loss to follow-up occurs between HIV diagnosis and initiation of ART, with the majority of losses occurring between HIV diagnosis and CD4 staging [42,43,47]. Thus, timely CD4 testing upon HIV diagnosis is essential to alleviating late ART initiation, high attrition, and late presentation rates [45▪▪,48▪].

ALTERNATIVES TO CLINICAL STAGING

In resource-limited settings in which CD4 testing is not feasible, additional clinical and laboratory parameters have been evaluated. Markers such as degree of anemia or BMI have been studied with variable results [34,35]. Boniphace *et al.* [31▪] studied the correlation of region-specific clinical symptoms with immunologic and clinical staging and found individual symptoms such as headache and peripheral neuropathy to be more sensitive predictors of advanced immunosuppression. A number of investigators have studied the use of total lymphocyte count as an alternative to CD4 absolute count, however, total lymphocyte count is no longer recommended by the WHO for staging or determination of ART initiation in children or adults [6,7,49–51].

Flow cytometry can quickly evaluate cell surface markers and determine cell lines within a blood sample, making it the gold standard for immunologic staging in the developed world [52]. Flow cytometry, however, is expensive and requires trained personnel. Strategies for improving access

to CD4 testing throughout low-income countries include lowering equipment costs, simplifying flow cytometric procedures, and developing alternative CD4 counting techniques [33]. Recently, a number of studies evaluating simpler, more cost-effective alternatives to flow cytometric CD4 counting have produced results with comparable sensitivity to traditional flow cytometry methods [27–29,33,53–56]. Even in areas in which CD4 testing is available, medical providers are still faced with financial constraints, making additional research on lower cost methods a priority. One potential cost-saving measure is the use of capillary rather than venous blood samples for both traditional flow cytometry or alternative cytometry methods. Good correlation between capillary and venous blood samples has been shown and development of standard procedures could diminish the need for dedicated laboratory personnel to carry out testing [57▪].

Point-of-care (POC) CD4 testing is pivotal to the scale up of ART programs and decentralization of HIV care globally. The potential reduction in costs associated with POC testing can lead to improvement in access and quality of HIV care. Jani *et al.* [12▪▪] found that compared with standard CD4 testing, POC CD4 testing significantly improved patient retention and rates of initiation of ART. Following the implementation of POC CD4 testing, the percentage of patients lost to follow-up decreased from 57 to 21% (adjusted odds ratio 0.2, 95% CI 0.15–0.27). Time to complete CD4 staging decreased from 32 to 3 days ($P < 0.0001$), leading to a decrease in the median time from enrollment to ART initiation from 38 to 20 days ($P < 0.0001$). POC testing can be conducted by community healthcare workers, requires less preparation and fewer ancillary supplies, and provides results in real time [55,56].

CONCLUSION

The WHO 2015 strategic goals include achieving 'universal access to comprehensive HIV prevention, treatment, and care' [58▪]. With the expanded recommendation to initiate ART at CD4 cell counts less than 350 cells/μl or clinical stages 3 or 4, approximately 49% more people will start ART leading to a 20% reduction in HIV-related mortality by 2015. Early ART initiation is projected to increase costs by 57% [59]. Domestic and global HIV funding decreased to $15 billion in 2010, significantly lower than the predicted $22–24 billion needed in 2015 to promote comprehensive global scale up of HIV/AIDS care. Thus, governments and nongovernmental organizations face difficult decisions about how best to allocate funds for diagnostics, monitoring, and therapy [2▪]. Improved access to CD4 testing has potential cost benefits compared with clinical staging [8]. Reduction of costs associated with diagnosis and monitoring as well as decentralization of services is imperative in continued ART scale-up efforts [28]. Alternative CD4 testing methods, especially POC CD4 testing, will help lead to more timely ART initiation, decreased attrition before ART, and overall improved patient outcomes.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 596–597).

1. UNAIDS. Global HIV/AIDS Response. Epidemic update and healh sector progress towards Universal Access. 2011. http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/20111130_UA_Report_en.pdf. [Accessed 15 February 2012]
2. ▪ Schwartlander B, Stover J, Hallett T, *et al.* Towards an improved investment approach for an effective response to HIV/AIDS. Lancet 2011; 377:2031–2041.

This article summarizes the financial perspectives associated with HIV/AIDS care and program implementation, particularly in resource-limited settings, and provides a strategic investment tactic.

3. United Nations. Report of the Secretary-General: uniting for universal access: towards zero new HIV infections, zero discrimination and zero AIDS-related deaths. 2011. http://www.unaids.org/en/media/unaids/contentassets/documents/document/2011/20110331_SG_report_en.pdf. [Accessed 15 February 2012]
4. Kline MW. Perspectives on the pediatric HIV/AIDS pandemic: catalyzing access of children to care and treatment. Pediatrics 2006; 117:1388–1393.
5. Alcorn K. South Africa to launch mass HIV testing drive in April, to test 15 million in one year. 2010. http://www.aidsmap.com/South-Africa-to-launch-mass-HIV-testing-drive-in-April-to-test-15-million-in-one-year/page/1438260/. [Accessed 12 March 2012]
6. WHO. Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach. 2010. http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf. [Accessed 8 February 2012]
7. WHO. Antiretroviral therapy for hiv infection in infants and children: towards universal access. Recommendations for a public health approach. 2010. http://whqlibdoc.who.int/publications/2010/9789241599801_eng.pdf. [Accessed 8 February 2012]
8. Athan E, O'Brien DP, Legood R. Cost-effectiveness of routine and low-cost CD4 T-cell count compared with WHO clinical staging of HIV to guide initiation of antiretroviral therapy in resource-limited settings. AIDS 2010; 24:1887–1895.
9. Callens SF, Kitetele F, Lusiama J, *et al.* Computed CD4 percentage as a low-cost method for determining pediatric antiretroviral treatment eligibility. BMC Infect Dis 2008; 8:31.
10. Kagaayi J, Makumbi F, Nakigozi G, *et al.* WHO HIV clinical staging or CD4 cell counts for antiretroviral therapy eligibility assessment? An evaluation in rural Rakai district, Uganda. AIDS 2007; 21:1208–1210.

11. Brinkhof MW, Dabis F, Myer L, *et al.* Early loss of HIV-infected patients on potent antiretroviral therapy programmes in lower-income countries. Bull World Health Organ 2008; 86:559–567.
12. ■■ Jani IV, Sitoe NE, Alfai ER, *et al.* Effect of point-of-care CD4 cell count tests on retention of patients and rates of antiretroviral therapy initiation in primary health clinics: an observational cohort study. Lancet 2011; 378:1572–1579.

This is an observational cohort study evaluating POC CD4 testing that showed significant benefits in decreasing attrition before ART and improving time between diagnosis and initiation of ART.

13. Fox MP, Rosen S. Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007–2009: systematic review. Trop Med Int Health 2010; 15 (Suppl 1):1–15.
14. El-Sadr WM, Lundgren JD, Neaton JD, *et al.* CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006; 355:2283–2296.
15. Emery S, Neuhaus JA, Phillips AN, *et al.* Major clinical outcomes in antiretroviral therapy (ART)-naive participants and in those not receiving ART at baseline in the SMART study. J Infect Dis 2008; 197:1133–1144.
16. Severe P, Juste MA, Ambroise A, *et al.* Early versus standard antiretroviral therapy for HIV-infected adults in Haiti. N Engl J Med 2010; 363:257–265.
17. Siegfried N, Uthman OA, Rutherford GW. Optimal time for initiation of antiretroviral therapy in asymptomatic, HIV-infected, treatment-naive adults. Cochrane Database Syst Rev 2010:CD008272.
18. Sterne JA, May M, Costagliola D, *et al.* Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. Lancet 2009; 373:1352–1363.
19. ■ Koenig SP, Bang H, Severe P, *et al.* Cost-effectiveness of early versus standard antiretroviral therapy in HIV-infected adults in Haiti. PLoS Med 2011; 8:e1001095.

This study is a follow-up of the CIPRA study that focused on the cost effectiveness of ART initation at a CD4 level less than 350 cells/μl. Study authors found that a higher CD4 threshold for ART initiation was less cost effective; however, this finding was felt to be attributed to limited follow-up time.

20. Mugyenyi P, Walker AS, Hakim J, *et al.* Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised noninferiority trial. Lancet 2010; 375:123–131.
21. O'Gorman MR, Zijenah LS. CD4 T cell measurements in the management of antiretroviral therapy–A review with an emphasis on pediatric HIV-infected patients. Cytometry B ClinCytom 2008; 74 (Suppl 1):S19–S26.
22. Dunn D, Woodburn P, Duong T, *et al.* Current CD4 cell count and the short-term risk of AIDS and death before the availability of effective antiretroviral therapy in HIV-infected children and adults. J Infect Dis 2008; 197:398–404.
23. Prendergast A, Mphatswe W, Tudor-Williams G, *et al.* Early virological suppression with three-class antiretroviral therapy in HIV-infected African infants. AIDS 2008; 22:1333–1343.
24. Sutcliffe CG, Scott S, Mugala N, *et al.* Survival from 9 months of age among HIV-infected and uninfected Zambian children prior to the availability of antiretroviral therapy. Clin Infect Dis 2008; 47:837–844.
25. Violari A, Cotton MF, Gibb DM, *et al.* Early antiretroviral therapy and mortality among HIV-infected infants. N Engl J Med 2008; 359:2233–2244.
26. Walensky RP, Wood R, Ciaranello AL, *et al.* Scaling up the 2010 World Health Organization HIV Treatment Guidelines in resource-limited settings: a model-based analysis. PLoS Med 2010; 7:e1000382.
27. Dieye TN, Diaw PA, Daneau G, *et al.* Evaluation of a flow cytometry method for CD4 T cell enumeration based on volumetric primary CD4 gating using thermoresistant reagents. J Immunol Methods 2011; 372:7–13.
28. Mandy F, Janossy G, Bergeron M, *et al.* Affordable CD4 T-cell enumeration for resource-limited regions: a status report for 2008. Cytometry B Clin Cytom 2008; 74 (Suppl 1):S27–S39.
29. Mtapuri-Zinyowera S, Chideme M, Mangwanya D, *et al.* Evaluation of the PIMA point-of-care CD4 analyzer in VCT clinics in Zimbabwe. J Acquir Immune Defic Syndr 2010; 55:1–7.
30. ■ Baveewo S, Ssali F, Karamagi C, *et al.* Validation of World Health Organisation HIV/AIDS clinical staging in predicting initiation of antiretroviral therapy and clinical predictors of low CD4 cell count in Uganda. PLoS One 2011; 6:e19089.

This study compared WHO clinical staging versus CD4 testing in a resource-limited setting and found that WHO clinical staging had significantly limited sensitivity compared with immunologic staging leading to misclassification of HIV patients and inappropriate delay of ART.

31. ■ Boniphace I, Omari M, Susan Fred R, *et al.* HIV/AIDS clinical manifestations and their implication for patient clinical staging in resource limited settings in Tanzania. Open AIDS J 2011; 5:9–16.

This study evaluated the use of region-specific clinical symptoms that could be used as an aid to WHO clinical staging. Study investigators found that regional clinical symptoms were helpful in guiding clinical staging decisions, which could be a useful additional clinical resource in developing countries.

32. Edathodu J, Ali B, Alrajhi AA. CD4 validation for the World Health Organization classification and clinical staging of HIV/AIDS in a developing country. Int J Infect Dis 2009; 13:243–246.
33. MacLennan CA, Liu MK, White SA, *et al.* Diagnostic accuracy and clinical utility of a simplified low cost method of counting CD4 cells with flow cytometry in Malawi: diagnostic accuracy study. BMJ 2007; 335:190.
34. Miiro G, Nakubulwa S, Watera C, *et al.* Evaluation of affordable screening markers to detect CD4+ T-cell counts below 200 cells/mul among HIV-1-infected Ugandan adults. Trop Med Int Health 2010; 15:396–404.
35. Zachariah R, Teck R, Ascurra O, *et al.* Targeting CD4 testing to a clinical subgroup of patients could limit unnecessary CD4 measurements, premature antiretroviral treatment and costs in Thyolo District, Malawi. Trans R Soc Trop Med Hyg 2006; 100:24–31.
36. Araujo Cardoso CA, Pinto JA, Sanchez Candiani TM, *et al.* Assessment of the prognostic value of the World Health Organization clinical staging system for HIV/AIDS in HIV-infected children and adolescents in a cohort in Belo Horizonte, Brazil. J Trop Pediatr 2012. [Epub ahead of print]
37. Carter RJ, Dugan K, El-Sadr WM, *et al.* CD4+ cell count testing more effective than HIV disease clinical staging in identifying pregnant and postpartum women eligible for antiretroviral therapy in resource-limited settings. J Acquir Immune Defic Syndr 2010; 55:404–410.
38. ■■ Balakrishnan P, Iqbal HS, Shanmugham S, *et al.* Low-cost assays for monitoring HIV infected individuals in resource-limited settings. Indian J Med Res 2011; 134:823–834.

A number of HIV viral load and CD4 POC and flow cytometry alternatives are being evaluated and produced. This article provides a review of several of the alternative testing methods that are available or are currently being evaluated.

39. Katabira ET, Oelrichs RB. Scaling up antiretroviral treatment in resource-limited settings: successes and challenges. AIDS 2007; 21 (Suppl 4):S5–S10.
40. ■ Mossdorf E, Stoeckle M, Mwaigomole EG, *et al.* Improved antiretroviral treatment outcome in a rural African setting is associated with cART initiation at higher CD4 cell counts and better general health condition. BMC Infect Dis 2011; 11:98.

This prospective study evaluated patient outcomes with initiation of ART at CD4 cell count of less than 50 and 100 cells/μl and found evidence that patients who initiated ART at lower CD4 thresholds had higher mortality and loss to follow-up.

41. Amuron B, Namara G, Birungi J, *et al.* Mortality and loss-to-follow-up during the pretreatment period in an antiretroviral therapy programme under normal health service conditions in Uganda. BMC Public Health 2009; 9:290.
42. Bassett IV, Regan S, Chetty S, *et al.* Who starts antiretroviral therapy in Durban, South Africa?... not everyone who should. AIDS 2010; 24 (Suppl 1):S37–S44.
43. Bassett IV, Wang B, Chetty S, *et al.* Loss to care and death before antiretroviral therapy in Durban, South Africa. J Acquir Immune Defic Syndr 2009; 51:135–139.
44. Mulissa Z, Jerene D, Lindtjorn B. Patients present earlier and survival has improved, but pre-ART attrition is high in a six-year HIV cohort data from Ethiopia. PLoS One 2010; 5:e13268.
45. ■■ Rosen S, Fox MP. Retention in HIV care between testing and treatment in sub-Saharan Africa: a systematic review. PLoS Med 2011; 8:e1001056.

High attrition rates before ART contribute significantly to increased morbidity and mortality amongst patients with HIV/AIDS. This article provides a systematic literature review of studies evaluating attrition rates before ART in sub-Saharan Africa, associated causes, and subsequent patient outcomes.

46. Lawn SD, Myer L, Harling G, *et al.* Determinants of mortality and nondeath losses from an antiretroviral treatment service in South Africa: implications for program evaluation. Clin Infect Dis 2006; 43:770–776.
47. Losina E, Bassett IV, Giddy J, *et al.* The 'ART' of linkage: pretreatment loss to care after HIV diagnosis at two PEPFAR sites in Durban, South Africa. PLoS One 2010; 5:e9538.
48. ■ Nash D, Wu Y, Elul B, *et al.* Program-level and contextual-level determinants of low-median CD4+ cell count in cohorts of persons initiating ART in eight sub-Saharan African countries. AIDS 2011; 25:1523–1533.

This article provides a multilevel aggregate evaluation of program-level and contextual-level factors contributing to low CD4 counts upon ART initiation in a group of eight sub-Saharan countries.

49. Githinji N, Maleche-Obimbo E, Nderitu M, *et al.* Utility of total lymphocyte count as a surrogate marker for CD4 counts in HIV-1 infected children in Kenya. BMC Infect Dis 2011; 11:259.
50. Oudenhoven HP, Meijerink H, Wisaksana R, *et al.* Total lymphocyte count is a good marker for HIV-related mortality and can be used as a tool for starting HIV treatment in a resource-limited setting. Trop Med Int Health 2011; 16:1372–1379.
51. Sreenivasan S, Dasegowda V. Comparing absolute lymphocyte count to total lymphocyte count, as a CD4 T cell surrogate, to initiate antiretroviral therapy. J Glob Infect Dis 2011; 3:265–268.
52. Maclennan CA, Dzumani F, Namarika A, *et al.* Affordable pediatric CD4 counting by flow cytometry in Malawi. Cytometry B Clin Cytom 2008; 74 (Suppl 1):S90–S97.

53. Mbopi-Keou FX, Mion S, Sagnia B, Belec L. Single-platform, volumetric, CD45-assisted pan-leucogating Auto flow cytometer for absolute and percentage CD4 T cell counting in resource-constrained settings: validation in Cameroon. Clin Vaccine Immunol 2012; 19:609–615.

54. Mbopi-Keou FX, Sagnia B, Ngogang J, *et al.* Validation of a single-platform, volumetric, flow cytometry for CD4 T cell count monitoring in therapeutic mobile unit. J Transl Med 2012; 10:22.

55. Rodriguez WR, Christodoulides N, Floriano PN, *et al.* A microchip CD4 counting method for HIV monitoring in resource-poor settings. PLoS Med 2005; 2:e182.

56. Thairu L, Katzenstein D, Israelski D. Operational challenges in delivering CD4 diagnostics in sub-Saharan Africa. AIDS Care 2011; 23:814–821.

57. ■ Sitoe N, Luecke E, Tembe N, *et al.* Absolute and percentage CD4+ T-cell enumeration by flow cytometry using capillary blood. J Immunol Methods 2011; 372:1–6.

This study evaluated the use of capillary blood rather than venous blood as a means of decreasing costs associated with CD4 testing in resource-limited settings. Study authors found supporting evidence that capillary blood sample results were comparable to venous blood results and contributed significantly to decreasing costs associated with testing.

58. ■ WHO. Global health sector strategy on HIV/AIDS 2011–2015. 2011. http://www.who.int/hiv/pub/hiv_strategy/en/index.html. [Accessed 12 March 2012]

This WHO report provides an updated summary of HIV/AIDS goals and strategies for implementation.

59. WHO. 2009 WHO ART Guidelines Review. 2009. http://www.who.int/hiv/topics/treatment/ART_cost_estimates.pdf. [Accessed 12 March 2012]