Published in final edited form as:

Pediatr Infect Dis J. 2009 June ; 28(6): 493–497. doi:10.1097/INF.0b013e3181950b7f.

Total Lymphocyte Count and World Health Organization Pediatric Clinical Stage as Markers to Assess Need to Initiate Antiretroviral Therapy among Human Immunodeficiency Virus-Infected Children in Moshi, Northern Tanzania

Opemipo O. Johnson, BSⁱ, Daniel K. Benjamin, PhDⁱⁱ, Daniel K. Benjamin Jr., MD, PhD, MPHⁱ, Werner Schimana, MDⁱⁱⁱ, L. Gayani Tillekeratne, MDⁱ, John A. Crump, MB, ChB, DTM&H^{i,iii,iv}, Keren Z. Landman, MDⁱⁱⁱ, Grace D. Kinabo, MD, MMedⁱⁱⁱ, Blandina T. Mmbaga, MD, MMedⁱⁱⁱ, Levina J. Msuya, MD, MMedⁱⁱⁱ, John F. Shao, MD, MSc, PhD^{iii,iv}, Mark E. Swai, MDⁱⁱⁱ, and Coleen K. Cunningham, MDⁱ

ⁱDuke University Medical Center, Durham, North Carolina, USA

ⁱⁱClemson University, Clemson, South Carolina, USA

ⁱⁱⁱKilimanjaro Christian Medical Centre, Moshi, Tanzania

^{iv}Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania

Abstract

Background—The World Health Organization (WHO) has recommended the use of clinical staging alone and with total lymphocyte count to identify HIV infected children in need of antiretroviral therapy (ART) in resource-limited settings, when CD4 cell count is not available.

Methods—We prospectively enrolled children obtaining care for HIV infection at the Kilimanjaro Christian Medical Centre Pediatric Infectious Diseases Clinic in Moshi, Tanzania between March 2004 and May 2006 for this cohort study.

Results—192 (89.7%) of 214 children met WHO ART initiation criteria based on clinical staging or CD4 cell count. Several low-cost measures identified individuals who met WHO ART initiation criteria to the following degree: WHO stages 3 or 4 had 87.5% (95% CI: 82.8 – 92.1) sensitivity and, by definition, 100% (CI: 100 – 100) specificity; WHO recommended advance disease TLC cutoffs: sensitivity = 23.9% (95% CI: 17.3 – 30.5) specificity = 78.2% (95% CI: 67.3 – 89.1). Low TLC was a common finding, (50/214; 23%); however, it did not improve the sensitivity or specificity of clinical staging in identifying the severely immunosuppressed stage 2 children. Growth failure or use of total lymphocyte counts in isolation were not reliable indicators of severe immunosuppression or need to initiate ART.

Conclusion—The use of total lymphocyte count does not improve the ability to identify children in need of ART compared to clinical staging alone. Low absolute lymphocyte count did not correlate with severe immunosuppression based on CD4 cell count in this cohort.

Keywords

HIV/AIDS; ART; TLC; pediatrics; immunosuppression; CD4

Introduction

The urgent need to scale-up access to antiretroviral treatment (ART) among HIV-infected children and adults living in resource-limited countries has been acknowledged by the international community.^{1,2} Despite modest progress in providing ART to adults, there have been limited improvements in the access to ART for infants and children residing in these settings.² The limitations of scaling-up access to ART in pediatric populations include, but are not limited to: lack of affordable pediatric antiretroviral formulations; limited screening for HIV; and lack of simple, affordable laboratory diagnostics and monitoring technologies.²

In countries with substantial healthcare resources such as those in North America and Western Europe, HIV-infected children are monitored using clinical assessments in combination with laboratory assays including CD4-cell count and percentage^{3,4} and HIV-1 RNA measurements.⁵ While useful for monitoring of HIV-infected children, these laboratory assays are expensive and require sophisticated equipment and trained personnel; thus, they are not widely available in countries with limited resources.⁶ Recognizing these limitations, the World Health Organization (WHO) has emphasized the use of clinical assessment and low-cost laboratory markers particularly total lymphocyte count (TLC), in assessing the need to initiate ART among children residing in settings without CD4-cell count testing capabilities¹; however, data demonstrating the added utility of TLC in resource-limited settings are limited.

We therefore sought to determine the ability of TLC and other low cost measures to predict need to initiate ART and low CD4 count in HIV infected children in a low-resource setting, we conducted the study in Moshi, Tanzania.

Materials and Methods

The cohort

HIV-infected children receiving care at Kilimanjaro Christian Medical Center (KCMC) Pediatric Infectious Diseases Clinic in Moshi, Tanzania between March 2004 and May 2006 were offered enrollment into a prospective observational cohort. HIV infection was determined initially using Capillus HIV1/HIV2 Rapid Test (Trinity Biotech, Bray, Co Wicklow, Ireland) and confirmed using Determine HIV1/2 Rapid Test (Abbott Laboratories, Abbott Park, IL, USA). HIV infection among children 12 – 18 months was confirmed if the following circumstances were present; the child has known HIV/AIDS exposure, he/she displayed immunosuppressed CD4 cell count values or diagnosed with an AIDS defining illness. All enrolled subjects had adequate documentation of HIV infection status. All enrolled children with clinical assessment (staging), CD4 cell-count and TLC recorded prior to the initiation of ARV were included in this analysis. Additional data collected include growth indices (weight and height) and signs, symptoms and diagnosis that would allow WHO clinical staging. Children less than 12 months of age were excluded as there were only 4 children in this age group.

We utilized WHO's criteria for severe immunosuppression based on CD4 cell count and by age group; 11 months and younger - <25%, 12 – 34 months - <20%, 35 – 59 months - <15%, 60 months or older - <15% or <200cells/mm³. We also utilized WHO's recommended TLC cutoff values for initiating ART in stage 2 children residing in resource limited settings without CD4 testing capabilities; 11 months and younger - <4000mm³, 12 – 34 months - <3000mm³, 35 – 59 months - <2500mm³, 5 – 8 years - <2000mm³.¹

After review of all clinical data, including growth parameters, WHO clinical stage was assigned to each patient by a single pediatrician investigator using the 2005 WHO clinical grading system of HIV/AIDS for pediatric populations.⁷ Malnutrition can be defined through a series

of growth measurements or as z-score calculations. The WHO clinical stage classification adopted the Integrated Management of Childhood Illness criteria to identify malnutrition and growth failure. The criteria define children with Weight-for-Age z scores (WAZ) < -2SD as moderately malnourished: a clinical stage 3 defining condition. Children presenting with Weight-for-Height z scores (WHZ) < -3SD are severely wasted: a stage 4 defining illness. For the present study, we defined children with WAZ < -3SD as severely malnourished and classified it a stage 4 defining illness. The clinical practice at the site and in many international sites, is to assign clinical stage based on all clinical findings other than the growth parameters, presumably due to the perceived difficulty in calculating a z-score for every child, therefore, we assigned clinical stage to each subject in 2-different ways: first without using growth z-scores as defined by the Integrated Management of Childhood Illness (IMCI) guidelines in the classification ("clinical staging- no growth") and the second way, more consistent with WHO recommendations, uses all clinical data including growth z-scores ("clinical staging – with growth").

The protocol was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an Institutional Review Board of Duke University Medical Center.

The caregiver/guardian of each participant provided written informed consent before inclusion into study.

Laboratory and Statistical Analyses

The total number of lymphocytes per microliter of whole blood was obtained using Cell Dyn 1700CS Hematology Analyzer (Abbott Laboratories, Abbott Park, IL, USA). The lymphocyte subsets were determined by flow cytometry using automated FACS Count (Becton Dickinson, San Jose, California, USA). We calculated CD4 percentage values using CD4 cell count divided by TLC. We obtained samples for TLC and CD4 cell count on same day.

We defined severe immunosuppression based on CD4 cell count or percentage as published by WHO.¹ Table 1, (Supplemental Digital Content 1, <http://links.lww.com/A952>) shows the WHO CD4 count criteria for varying levels of immunosuppression and the TLC values which indicate need for ART initiation among children who are WHO stage 2 and residing in settings without CD4 testing capabilities. We analyzed the prognostic utility of several low-cost variables as alternative markers in identifying children in need of ART or with severely immunosuppressed CD4 values within our population.

We defined the WHO criteria for ART initiation among pediatric populations residing in resource-limited settings with CD4-cell count testing capabilities as the optimal measure of need for ART. Therefore, according to guidelines, all children presenting with advanced or severe disease (WHO clinical stage 3 or 4) or who display severe immunosuppression based on CD4 cell count or percentage meet treatment initiation criteria and should receive ART.¹ Using this definition as the gold-standard, we then compared select clinical and laboratory measures to identify these children for whom treatment is indicated. Growth indices were analyzed using WHO Height-for-Age z scores (HAZ), WAZ, and WHZ as defined by WHO.⁷

Data analyses were performed using JMP software (version 5.0.1, SAS Institute, North Carolina, USA).

Results

Two hundred fourteen patients with paired TLC and CD4 counts before the initiation of ART were identified. The median (range) age was 9.6 (1.12 to 15.90) years and 106 (49.5%) of 214 were female. The majority had advanced or severe disease as measured by WHO clinical staging and/or immunologic status and growth failure was not uncommon (Table 1, Supplemental Digital Content 1, <http://links.lww.com/A952>). Clinical staging done without incorporation of growth z-scores classified 145 (67.7%) children with advanced or severe (stage 3 or 4) disease whereas after correcting the clinical stage for growth z scores, an additional 23 children were classified with advanced or severe disease and therefore in need of treatment (Table 2).

Based on the WHO recommendations in resource-limited settings, all stage 3 and 4 children should be treated with antiretroviral therapy. The majority of the children in these stages had severe immunosuppression (131/168, 78%). However, a significant number of children classified as stage 1 or 2 also had severe immunosuppression (24/46; 52%) (Table 3). These severely immunosuppressed stage 1 and 2 children warrant treatment and therefore represent a failure of the use of clinical staging alone. Low TLC was a common finding, (50/214; 23%); however, it did not improve the sensitivity or specificity of clinical staging in identifying the severely immunosuppressed stage 2 children; among stage 2 children, only 4/34 (11%) had low TLC. Among the stage 2 children with severe immunosuppression, only 2/18 (11%) also had low TLC.

The prognostic utility of WHO recommended TLC cutoffs values for predicting children with low CD4 cell counts and percentages was poor for all age groups (Figure 1). TLC using the published WHO cutoffs or other potential thresholds for suppression showed little relationship to CD4 values (Table 4). There are no published TLC criteria for ART initiation for children 8 years and older; therefore, we analyzed several TLC values to determine their ability to predict severely immunosuppressed CD4 values among this age group. Although we observed better association of low TLC with low CD4 in this age group compared with younger age groups, the sensitivity and specificity measurements remain poor and clinically unreliable (Table 4).

Discussion

The WHO recommends the initiation of ART for all children presenting with clinical stage 3 or 4 disease. In resource limited settings without CD4 values available, the guidelines also recommend the use of TLC to guide decisions on ART initiation among children with stage 2 disease; with initiation of ART recommended if TLC values are below specified severe immunosuppression cutoffs values.¹ TLC is a measurement obtained from a simple, inexpensive complete blood count (CBC), which is widely available in most resource-limited settings. The use of TLC as a predictor of CD4-cell count and mortality has been observed among HIV-infected children living in the US⁴ and Europe^{3,4} as well as among HIV-infected adults residing in the United States,⁸ India⁹ and Africa.¹⁰ The validity of these recommendations among children residing in resource-limited settings is limited, a recently published meta-analysis of approximately 2500 untreated HIV-infected children in resource-limited settings suggests TLC is a poor mortality predictor.¹¹

Ideally, prognostic markers utilized should display high sensitivity for identifying a specific condition as well as reliable specificity to exclude individuals without the condition. With improvements in the access to ART among children in developing countries anticipated, it is imperative that adequate low-cost treatment-initiation markers are developed to identify individuals with greatest need of ART. In addition, because of scarcity in resources and toxicity

of ART, treatment guidelines should also aim to minimize the premature initiation of ART among infected children who do not yet need therapy.

Our findings demonstrate that use of clinical staging without incorporation of growth data (including z scores) leads to incomplete identification of children who meet criteria for stage 3 and 4 disease and therefore leaves children who require treatment unidentified. Despite the widespread use of WHO pediatric clinical stage as a prognostic marker to guide ART initiation decisions among health-care providers in resource limited settings, many do not calculate nor utilize growth z scores when assigning WHO clinical stage. In spite of its potential utility in improving classification of WHO clinical stage, the use of growth parameters alone had weak prognostic value for identifying children in need of ART, as noted below.

The utility of WHO pediatric clinical stage in predicting risk of HIV disease progression has been demonstrated in ART-naïve children residing in resource-limited settings,¹² and among infected children in developed nations.⁴ These, along with our findings, supported the WHO proposition to initiate treatment in children with WHO clinical stage 3 or 4. Children with stage 3 or 4 disease among our cohort were more likely to have severely immunosuppressed CD4 values compared to children with stage 1 or 2 disease. Furthermore, children presenting with WHO stage 3 or 4 disease met ART initiation criteria as practiced in developed countries with high sensitivity. This suggests WHO pediatric clinical stage 3 or 4 alone may serve as a reliable low-cost marker to guide decision in ART initiation among children residing in settings with inadequate access to CD4 counts measurements. Nevertheless, clinical staging will miss children who are stage 1 or 2 who have severely suppressed CD4 cell counts.

The prognostic utility of TLC in identifying disease progression, predicting mortality, guiding ART initiation decisions has been studied among HIV-infected children residing in developed countries³, its role in predicting mortality among children residing resource-limited setting was only recently published. The Cross Continent Collaboration for Kids meta-analysis of 2510 untreated HIV-infected children residing in resource-limited settings suggest TLC values are poor predictors of short-term mortality and only weakly associated with CD4 cell count.¹¹ In our study, the TLC did not improve the ability to identify children with severe immunosuppression among any stage of disease and specifically did not help to identify immunosuppressed children in clinical stage 2 in whom therapy is warranted. Our findings also demonstrated minimal improvements in identifying children in need of ART by using low TLC values in addition to WHO clinical stage 3 or 4 disease. Furthermore, in support of previous study findings,³ WHO recommended cut-offs for low TLC values was poorly associated with low CD4-cell count measurements among children in our cohort. We also analyzed the association of several different TLC cutoffs values to low CD4 cell counts and observed poor sensitivities and specificities across all age groups.

Low-cost laboratory and clinical markers including growth measures have shown positive association to HIV disease progression and/or mortality and morbidity.^{3,4,5} Growth failure has been significantly associated with HIV disease progression and an increased risk of mortality among HIV-infected children^{13, 14} and adults.¹⁵ Failure of weight gain is commonly defined as weight for age by gender less than the 3rd percentile for six consecutive months or decreased growth velocity without a documented alternative explanation.¹¹ Growth indices can be especially useful in monitoring of HIV disease in resource-constrained settings because of its low-cost and the ease of personnel training to obtain consistent, valid measurements. The prognostic utility of growth parameters in identifying HIV-infected children in need of ART in resource-limited settings has not been analyzed, to our knowledge. Among our cohort, the diagnosis of moderate or severe malnourishment and severe wasting alone as a prognostic marker had clinically unreliable sensitivity when identifying children in need of ART. These

findings parallel results from studies conducted on HIV-infected children residing in other resource-limited settings.¹⁶

The present study cannot determine if the difference in prognostic utility of CD4 cell count in children in developed countries compared to those in resource limited settings is due to actual biological differences; do the children actually have high TLC values even when CD4 cell counts are quite suppressed? Or is this a function of inadequate quality and consistency of the testing itself? However, the laboratory values obtained were those obtained in a clinical laboratory providing routine clinical test results for all patients in a large medical center and are therefore, comparable to clinical laboratories across Africa and other resource limited settings. Therefore, our study results reflect real-world data and are likely generalizable to other children obtaining care in similar settings. Our study cohort consisted mostly of children with stage 3 or 4 disease, with a limited number of children with stage 1 or 2 disease. We also did not include children 11 months of age and younger in our analysis; thus, our findings can not be reflected in that age group. This is a potential limitation to generalizing our findings among larger populations.

Based on our findings, TLC measurements offer minimal prognostic value in identifying children in need of ART in our cohort. Therefore, this test does not serve as an adequate low cost alternative to CD4 cell counts and percentages and is not useful in guiding treatment decisions for HIV-infected children in similar resource-limited setting. Further investigations into other low-cost clinical or laboratory markers are warranted. Alternatively, efforts may be placed on developing more affordable methods to accurately measure CD4 count and percent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We thank our study subjects and their families for their interest and participation. We thank the Kilimanjaro Christian Medical Center (KCMC) and Duke University collaboration, ISAAC project, for allowing us to conduct this study on site. We also thank the research nurses, Janeth U. Kimaro, Gertrude I. Kessy and Bona K. Shirima for their assistance and diligence. This presentation was made possible with help from the Duke University Center for AIDS Research (CFAR), an NIH funded program (P30 AI 64518). Dr. DK Benjamin Jr. received support from NICHD RO# HD42920-02. Opemipo O. Johnson was funded through a NIH Research Supplements for Underrepresented Minorities fellowship, grant # 5U01-AI27535-17S1

Funding sources

Duke University Center for AIDS Research (CFAR) - an NIH funded program (P30 AI 64518). Dr. DK Benjamin Jr. NICHD RO# HD42920-02. Opemipo O. Johnson an NIH Research Supplements fellowship, grant # 5U01-AI27535-17S1

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Figure 1.
Bivariate fit of TLC by CD4% measurements, among HIV-infected children in Pediatric Infectious Diseases clinic in Moshi, Tanzania. Red line denotes severe immunosuppression n = 214

Table 2

Sensitivity, Specificity, Positive Predictive value (PPV) and Negative Predictive value (NPV) for identifying children in need of ART based on WHO recommendations*. N = 214

Clinical Stage	# in cohort	# met WHO treatment criteria*	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value (%)	Negative predictive value (%)
WHO Clinical Stage with and without calculated growth z scores						
stage 1	15	9	4 (2–9)	72 (49–88)	60.0	8.0
stage 2	54	38	20 (15–26)	27 (12–50)	70.4	3.7
stage 3	124	124	65 (57–71)	100 (82–100)	100	24.4
stage 4	21	21	11 (7–16)	100 (82–100)	100	11.4
stages 3 & 4	145	145	76 (68–81)	100 (82–100)	100	31.9
WHO Clinical stage without growth	stage 1	12	3 (1–7)	73 (50–88)	50.0	7.9
	stage 2	34	9 (6–15)	27 (12–50)	52.9	3.3
	stage 3	146	76 (69–82)	100 (82–100)	100	32.4
	stage 4	22	11 (7–17)	100 (82–100)	100	11.5
WHO clinical stage + growth z scores	168	168	88 (82–92)	100 (82–100)	100	47.8

* Meeting WHO treatment criteria defined as those for whom ARV is indicated based on combined CD4 and clinical criteria (see methods)

Sensitivity, Specificity, Positive Predictive value (PPV) and Negative Predictive value (NPV) for severe immunosuppression as measured by TLC (Table 1) to identify children with severe immunosuppression based on CD4 values n = 214

Table 3

	#in cohort	#with severe suppression	Sensitivity,% (95% CI)	Specificity,% (95% CI)	Positive predictive value (%)	Negative predictivevalue (%)
WHO Clinical Stage with and without calculated growth z scores						
stage 1	12	6	4 (2-9)	90 (79-96)	50.0	26.2
stage 2	34	18	12 (7-18)	73 (60-83)	52.9	23.9
stage 3	146	113	73 (65-80)	44 (31-58)	77.4	38.2
stage 4	22	18	12 (7-18)	93 (83-98)	81.8	28.6
WHO stage + growth z scores	168	131	85 (78-90)	37 (25-51)	78.0	47.8

Table 4

Prognostic value of low TLC values to identify children with low CD4 in various age groups. N = 214

Age group	TLC values	#in cohort	#with low CD4*	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value (%)	Negative predictive value (%)
> 8 yrs, n = 96	TLC < 4000	81	60	82 (71–90)	9 (1–30)	74.1	13.3
	TLC < 3000	55	44	60 (48–71)	54 (33–74)	80.0	30.9
	TLC < 2500	40	33	45 (34–57)	70 (47–86)	82.5	28.6
	TLC < 2000	29	25	34 (24–46)	83 (60–94)	86.2	28.4
5–8 yrs, n = 56	TLC < 4000	43	26	70 (53–84)	10 (2–35)	60.5	15.4
	TLC < 3000	32	20	54 (37–70)	37 (17–61)	62.5	29.2
	TLC < 2500	20	13	35 (21–53)	63 (39–83)	65.0	33.3
	TLC < 2000	12	10	27 (14–44)	89 (69–98)	83.3	38.6
36–59 months, n = 34	TLC < 4000	22	12	54 (33–75)	17 (3–49)	54.6	16.7
	TLC < 3000	10	5	23 (9–46)	58 (29–84)	50.0	29.2
	TLC < 2500	7	3	14 (4–36)	67 (35–89)	42.9	29.6
	TLC < 2000	5	3	17 (4–36)	83 (51–97)	60.0	34.5
12–35 months, n = 26	TLC < 4000	6	3	14 (4–36)	25 (1–78)	50.0	5.0
	TLC < 3000	2	0	0 (0–18)	50 (9–91)	0	8.3
	TLC < 2500	1	0	0 (0–18)	75 (22–98)	0	12.0
	TLC < 2000	1	0	0 (0–18)	75 (22–98)	0	12.0

* See Methods section for WHO recommended low CD4 values by age group

*** WHO recommended TLC cutoffs values by age group