Correlation Between CD4 Percent And Total Lymphocyte Count (TLC): TLC Thresholds Are Suboptimal For Initiation Of Antiretroviral Therapy In HIV-Infected Nigerian Children.

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Citation

Abstract

Total lymphocyte count (TLC) has been proposed by the world health organisation (WHO) as an alternative to percentage of CD4+ T-cell to indicate when antiretroviral therapy (ART) should be started in HIV-infected children in resource-poor setting.Objective: To evaluate the relationship between TLC and CD4 % in HIV-positive children in order to know when to initiation antiretroviral therapy (ART).Methods: A retrospective review of clinical and laboratory data of 120 infected children age 4 months to 15 years were selected at random, seen at paediatrics department of University of Maiduguri teaching hospital, Maiduguri from 2005 to 2008. Based on the base-line paired TLC-CD4%, degree of correlation between these two measures was determined taking into account of different age groups. The yield of the TLC cut-offs suggested in the WHO 2006 guideline for paediatric ART initiation were assessed. Also the reliability of different TLC cut-offs (above and below the WHO threshold for age group) was determined.Results: Data for 112 HIV-positive children with a mean age of 4.62± 2.87 years were completed for analysis. CD4% and the median TLC were higher in infant and younger children. An overall degree of positive correlation between CD4% and TLC was r= 0.68 (p=0.001). Two out of 5 (40%) children who were eligible for ART using CD4% were placed under incorrect immune categories by TLC thresholds. The reliability of shown wide ranges with sensitivity, specificity, PPV and NPV were maximally aggregated in age group ≤11 months at TLC 3500 for CD4% <25%, in age group 12-35 months at TLC 3500 for CD4% <20% and in age group ≥36 months TLC 2000 for CD4% <15%.Conclusion: Large number of HIV-infected children in developing countries required ART at presentation. Although good correlations exist between TLC and CD4%, the WHO TLC thresholds for initiating ART is suboptimal. More convenient and less expensive technologies are needed in resource-limited settings

INTRODUCTION

New cases paediatric HIV infection has been increasing in Nigeria over the years and the number of HIV positive children requiring ARV was estimated to be 98,040, of which less than 5% actually received ART [1]. Disease outcome is usually faster and more serious than in adults, resulting in high mortality and morbidity rates due to serious opportunistic infections[1,2]. For this reason, early prophylactic measures and specific antiretroviral therapy (ART) are mandatory and without treatment HIV-infected children are likely to die before the age of 3 years[1,2]. Absolute CD4 lymphocyte count and percentage and more recently, plasma viral load have been considered the most reliable markers of disease progression in HIV-infected patients[1-3] and have been the basis for indicating ART as well as prophylaxis against opportunistic infections. However, these tests require

resource and technical expertise both of which are not routinely available in many resource-poor settings such as Nigeria. CD4+ t-lymphocytes constitute at least 60% of the total lymphocyte count (TLC) in the blood. It is therefore reasonable to say that if CD4+ cell fall significantly as occur in HIV infection, it should reflect in concomitant fall in TLC thereby making absolute lymphocyte count a potential surrogate test for CD4 count[4]. The equipment and skills to perform total white blood cell count and differential are readily available in most hospitals and clinics in resource-poor countries, and performing a TLC costs less than US$1, a fraction of the cost of a CD4 cell measurement[5]. Several studies in children indicate that TLC independently predicts mortality and correlates with both CD4 percentage and total CD4 cell count[4,6,7]. However, because TLC is high in children <18 months of age, its reliability as a predictor of mortality in HIV-infected children varies with age and these

studies were done in developed countries [7]. Based on clinical studies of CD4 count and TLC such as the ones outlined above, the World Health Organization (WHO) recommended that health facilities without the ability to perform CD4 measurement should use TLC to guide decisions on when to start ART in patients who are mildly symptomatic[8]. We aimed to evaluation the relationship between CD4% and TLC and to assess thresholds of TLC at which ART should be considered in HIV-infected children in resource-poor setting, Nigeria.

SUBJECTS AND METHODS

A retrospective study of HIV-infected children, antiretroviral-naïve aged 4 months to 14 years, attending the paediatric infectious diseases unit at the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria, from July 2005 to June 2008 were enrolled. Ethical approval was obtained from the ethical committee of UMTH. Written consent for participation in the study was obtained from children's parents/caregivers. Their HIV status was confirmed by a Western blot test after an initial reactive enzyme-linked immunosorbent assay (ELISA). The polymerase chain reaction (PCR) for HIV-1 DNA was used to confirm HIV status in children aged less than 18 months.

On enrolment, clinical and laboratory data collected at baseline were analysed for all eligible children. Baseline haematological indices such as white blood cell (WBC) count and WBC differential count was obtained using an automated haematology counter (Advia-60, Advia) and CD4 lymphocyte count and % were obtained using the flow cytometry (the gold standard). Total lymphocyte count was calculated from the WBC count and the differential lymphocytes percentage (TLC is the WBC count multiplied by the lymphocyte percentage). Information obtained was entered into a data sheet and analysed for correlation between CD4% and TLC. For the purpose of this study, we used 2006 WHO age-specific immunological criteria (threshold) recommended for severe immunodeficiency and ART eligibility in children in resource-poor settings as the gold standard[8].

Data were analysed using SPSS statistical software version 11. Means (± standard deviation (SD)), medians and percentages were used to express data. Spearman correlation and statistical indices were used for comparison. Sensitivity (se), specificity (sp), positive predictive value (PPV) and negative predictive value (NPV) of various TLC cut-offs were determined based on age-specificCD4% (CD4% <25%

in ≤11 months infants, CD4% <20% in 12-35 months children, and for CD4% <15% in 36 months and above children), considered as gold standard for initiating ART in children (WHO). Tables were used for illustration where appropriate. A p-value of <0.05 was considered significant for all statistical comparisons.

RESULTS

A total of 120 HIV-infected, ART naive children with average baseline age of 4.62±2.87 years were recruited over the study period. Eight children were excluded because of incomplete data at the time of enrolment, leaving 112 children. They consisted of 66 males and 56 female with a male: female ratio of 1.2:1. A paired of TLC and CD4% was generated for each patient. Medians CD4% and TLC for age-specific groups is presented in table 1. Fifty four out of 112 were eligible for ART by age-specific CD4%, while 31 /122 were eligible by TLC. An overall degree of positive correlation between CD4% and TLC was r= 0.68 (p=0.001). Different degrees of positive correlation were noted between CD4% and TLC among the age groups as shown in table 1. Among the 54 children who were eligible for ART at presentation using CD4% threshold, only 3 in 5 (31/54 or %) would have been eligible using TLC threshold. In evaluating the age-specific TLC thresholds (cut-offs) proposed by WHO for initiating ART and various other TLC cut-offs predicting the appropriate CD4% cut-off, different levels of reliability were found (table 2). Sensitivity, specificity, PPV and NPV were maximally aggregated in age group ≤11 months at TLC 3500 for CD4% <25%, in age group 12-35 months at TLC 3500 for CD4% <20% and in age group ≥36 months TLC 2000 for CD4% <15%. The cost of a single CD4 count/% in Nigeria is approximately 35 US dollars, whereas the cost of a single full blood count is 3.4 US dollars and presently only five centres in the north-eastern Nigeria are equipped with a machine to carry out CD4 count/%, whereas TLC can be done at PHC centres level.

Figure 1

Table 1: Age-specific Correlation between median (IQR) CD4% and TLC in HIV-positive children

Age group (Months)	Median TLC (IQR)*	CD4% (IQR)	Correlation coefficient (r-value)	p- value
≤11	7032 (5152-8712)	26 (19-32)	0.37	0.060*
12-35	6249 (3840-8370)	13 (10-18)	0.32	0.096*
≥36	3297 (2201-4512)	11 (9-21)	0.23	0.104*

*IQR= inter-quartile range

*= Not significant

Figure 2

Table 2: Reliability of various TLC cut-offs as a predictor of CD4% for initiating ART for different age groups

TLC thresholds (cell/mm³)	Reliability			
	SE*%	SP*%	PPV*%	NPV*%
≤11 months				
4500	63	71	50	71
4000	45	86	53	78
3500	27	98	90	64
12-35 months				
3500	40	88	79	80
3000	25	92	93	68
2500	15	99	98	73
≥36 months				
3000	59	66	52	70
2500	49	87	65	58
2000	34	90	82	69

*SE- Sensitivity, SP- Specificity, PPV- Positive Predictive Value, NPV- Negative Predictive Value

DISCUSSION

As with the HIV paediatric prognostic marker collaborative study [9] and the WHO recommendations for initiating ART in infants and children[8], we found that, the baseline median CD4% is higher in infant and younger children, similarly, a decrease in median TLC was noted with increase in age in HIV-infected children. This signifies the importance of age-specific immunological criteria in initiating paediatric ART.

In 2005, there were more than 660,000 HIV positive children requiring ART, only 40,000 of them were receiving it and without treatment about 50% of them will succumb to HIV/AIDS before the age of 2 years (WHO/UNAIDS)[10]. Similarly, half of HIV-infected children in developing countries are eligible for ART at first presentation, likely due to late health seeking behaviour and the recent introduction

of free ART [11]. In this study similar proportion of children were eligible for ART using baseline CD4% criteria, underscores the need to scale up ART in developing countries.

In the present study after performing a regression analysis, we found a good positive correlation between CD4% and TLC (r=0.68), however when the correlation was tested among age-group, it slightly weakened. Similarly about 40% (2/5) of patient that were eligible for ART using CD4% were placed under incorrect immune categories by TLC thresholds thus delay ART initiation in these children. This is in consonance to study reported by Swaminathan et al[12] who evaluated cohort of HIV-infected Indian children and found a good correlation between TLC and CD4 count but about half of their patients were incorrectly categorised. Delay in ART initiation could lead to death in young infants who progress rapidly. Delayed ART initiation also impacts negatively on immunological recovery following ART initiation, as patients with a low CD4 lymphocyte nadir have a slower increase in CD4 count, prolonging the period at risk of opportunistic infections[13]. Furthermore, when ART is started in children with less than 5% CD4 percent, levels of CD4 rarely reach normal levels[14]. In contrast, HIV Paediatric Prognostic Marker Group who evaluated longitudinal data on 3,917 children[9]. The researchers compared their TLC thresholds for increased mortality and AIDS risk with the WHOs TLC and CD4 percentage thresholds for starting ART[9]. The study found that TLC weakly correlate with CD4 percentage but a powerful predictor of disease progression.

Total lymphocyte count cutoff point has a major impact on the sensitivity and specificity of this marker in predicting CD4 cell counts. The use of higher TLC cut-offs maximized sensitivity, but lowered specificity where as with lower TLC cut-offs, sensitivity decreases and specificity increases. Kassa et al[15] performed a study among Ethiopian patients, and found that TLC could not be a general surrogate marker for CD4 cell count, given the variability in sensitivity and specificity with different TLC cutoff points. Similar findings were made by Van der Ryst et al[16] among 2777 South African patients and found that, although a statistical correlation existed between TLC and CD4 count (r=0.704), the PPV and specificity of the TLC in predicting the CD4 cell count were poor. When the TLC cutoff was lowered, specificity increased, but sensitivity decreased substantially. The findings in our study that, WHO age-specific TLC cut-off values in predicting the appropriate CD4% were poor

complement these studies. This poor performance of the WHO TLC thresholds could be explained by the fact that current WHO criteria are based on US and European data, the underestimation of children eligible for ART based on TLC criteria may be even more pronounced in sub-Saharan African settings, as genetic and environmental factors, which may influence immunity, are currently not taken into account in the WHO classification[15-16]. Other studies have shown that West African have a physiological lymphocytosis that leads to TLC and CD4 cell counts that are higher than those of European patients[17]. Thus, consideration must be given to demographic differences and their effect on CD4 cell counts when interpreting studies and choosing TLC cutoff points.

In conclusion, a considerable proportion of HIV-infected children in developing countries required ART at presentation and age-specific CD4% remain the most appropriate immunological criterion for determine when to initiate ART in HIV-infected children. Although the correlation between TLC and CD4% is good and TLC is cheaper, available and simpler, the current WHO TLC thresholds for initiating ART is suboptimal in HIV-infected children, resulted in considerable misclassification and delayed treatment. Therefore, additional observational studies are needed that examine the value of TLC in predicting the CD4% and response to ART in different age groups, as well as in settings outside of North America and Europe in order to develop appropriate regional cut-offs for initiating ART . Also more convenient and less expensive technologies are needed as alternatives to currently available CD4 cell assays in resource-limited settings and political pressure in advocacy for reducing the cost of determining HIV disease stage and monitoring therapeutic outcomes.

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