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Total Lymphocyte Count: not a surrogate marker for risk of death in HIV infected Ugandan children

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Abstract

Objectives—To determine the utility of Total Lymphocyte Count (TLC) in predicting the 12 month mortality in HIV infected Ugandan children; to correlate TLC and CD4 cell %.

Design—This is a retrospective data analysis of clinical and laboratory data collected prospectively on 128 HIV infected children in the HIVNET 012 trial.

Methods—TLC and CD4 cell % measurements were obtained at birth, 14 weeks and 12, 24, 36, 48, and 60 months of age and assessed with respect to risk of death within 12 months.

Results—Median TLC/ul (CD4 cell %) were 4150 (41%) at birth, 4900 (24%) at 12 months, 4300 (19%) at 24 months, 4150 (19 %) at 36 months, 4100 (18%) at 48 months and 3800 (20%) at 60 months. The highest risk of mortality within 12 months was 34–37% at birth and declined to 13–15% at 24 months regardless of TLC measurement. The correlation between CD4 cell % and TLC was extremely low overall (r = 0.01).

Conclusion—The TLC did not predict a risk of progression to death within 12 months and therefore TLC alone may not be a useful surrogate marker for determining those children in greatest need for antiretroviral therapy in HIV infected Ugandan children.

Keywords

Total Lymphocyte Count; HIV; Africa; children

INTRODUCTION

Approximately 2.3 million children are living with HIV infection and over 90% reside in sub Saharan Africa [1]. Despite breakthroughs in prevention of mother to child HIV transmission (PMTCT), an estimated 540,000 children become infected through mother to child transmission (MTCT) each year [1]. Most of these new infections are in resource constrained

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settings where the implementation of PMTCT programs is limited and access to care and treatment inadequate. The escalating disease burden and the high mortality rate of HIV infected children from developing countries make the need for antiretroviral therapy (ART) even more urgent. By two years of age, 40–50% of perinatally infected children in these settings will have died compared to only 18% in developed countries before ART was available [2,3]. Multiple studies including those from adults and children in Africa have shown that viral load and CD4 cell count are independently associated with HIV disease progression and death [4–6]. HIV RNA is considered the gold standard for follow up of patients on treatment but in resource limited settings the CD4 cell count has been the most feasible method [4]. However in view of the limited availability and costs of these tests in regions of the world where most infected children reside, evaluation of alternative markers to predict disease progression and mortality risk is needed.

Simple laboratory tests such as Total Lymphocyte Count (TLC), hemoglobin estimation, albumin, and HIV p24 antigen have been identified as possible surrogate markers for monitoring disease progression of HIV infected adults and children in developing countries [4,7]. The TLC is an inexpensive and useful marker for staging disease and predicting AIDS progression or death in HIV infected adults [8,9]. Previous studies in adults have documented the correlation between TLC and CD4 cell count but with mixed interpretation of clinical utility [10–13]. Some of the adult studies have demonstrated clear correlation of TLC and CD4 cell count with high predictive values for AIDS defining opportunistic infections and death when the TLCs are less than 1500 cells/ul [14–18]. However, the data from children are limited and mainly from the developed world. Based largely on U.S. and European data, the World Health Organization (WHO) recommends using TLC <4000, <3000, and <2500 cells/ul and clinical findings to help guide decisions on starting ART for children <12 months, 12–35 months and greater than 35 months of age, respectively when CD4 cell % is not available [19]. However, it is well known that lymphocyte counts vary with age and tend to be higher in African children [20,21]. Therefore, the need for data from Africa to validate the utility of the TLC in predicting death within 12 months and informing decisions on antiretroviral treatment initiation is crucial.

The HIVNET 012 study provides a unique opportunity to assess the role of TLC as a predictor of death among HIV infected African children [22]. A retrospective review of the five year longitudinal data available from infected children in the HIVNET 012 study was conducted to determine the risk of HIV disease progression to death within a 12 month period. The TLC, CD4 cell %, and viral load thresholds were used to determine which factors would be most predictive of the risk of mortality in these Ugandan children. The correlation between TLC and CD4 cell %, as well as the sensitivity, specificity, and positive and negative predictive value of TLC using the WHO CD4 cell % thresholds as the gold standard were assessed.

METHODS

HIVNET 012 Study Design

The HIVNET 012 study design, methods and results for infants through age 18 months are published elsewhere [22,23]. In brief, 645 HIV infected pregnant women from Uganda were enrolled into a randomized perinatal HIV prevention clinical trial comparing intrapartum/neonatal zidovudine (ZDV) or Nevirapine (NVP) for PMTCT between November 1997 – April 1999. The study was approved by the AIDS Research Committee, Uganda and the Johns Hopkins Institutional Review Board, Baltimore MD USA. Informed consent was obtained from all participants and/or guardians.

Study population and procedures

Children born to HIV infected mothers in the HIVNET 012 trial were prospectively followed for 18 months to determine drug safety, HIV infection rate, and mortality [22,23]. Continued long term follow up (LTFU) to document safety of neonatal exposure to NVP or ZDV was undertaken with a protocol amendment to extend follow-up through age 60 months. Enrollment into the extended follow-up occurred after counseling the mother/guardian about the study extension and obtaining additional informed consent. This analysis will focus mainly on the laboratory parameters and survival of the subset of HIV infected children. The majority of the children were ART naïve as ART was not routinely available at the study site until the last year of the study. Only 8/48 (17%) children in the > 35 months age group were started on highly active ART (HAART) during the study period.

Qualitative HIV-1 RNA PCR assays were done at age 1–3 days, 6 weeks, 14 weeks and 12 months for HIV diagnosis. Infants were defined as HIV-1 infected based on a positive qualitative HIV-1 RNA PCR assay confirmed on a second sample by either a positive qualitative HIV-1 RNA PCR, a quantitative HIV-1 RNA PCR, or an HIV culture, or by a positive HIV–1 enzyme immunoassay (EIA) and western blot for HIV-1 antibody if ≥ 18 months old. In case of an infant death where there was only one positive HIV-1 RNA assay on the sample preceding death, the infant was considered HIV positive. Once a child was identified as HIV infected, quantitative HIV-1 RNA assays were done at each subsequent scheduled blood draw and at all LTFU scheduled visits. HIV infected children had complete blood counts (including total lymphocyte counts) and CD4 cell counts and percents done at birth, 14 weeks, and subsequently every 6 months from 12 to 60 months of age. HIV uninfected children had laboratory evaluations done only up to 18 months with only clinical evaluations during the LTFU.

Laboratory Tests

All laboratory tests were performed in the Makerere University-Johns Hopkins University Research Collaboration (MUJHU) laboratory in Kampala, Uganda. This laboratory conforms to US Clinical Laboratory Improvement Act of 1988 regulations for the assays used in this study and participated in proficiency testing programs for these assays. The CD4 cell counts and CD4 cell % were done using standard flow cytometry and were performed within 24 hours of obtaining the blood sample. During the first 3 months of the study these were measured using a Coulter T540 hematology analyzer and the EPIC Profile II flow cytometer (Coulter Corporation, Miami FL, USA). After February 1998, the CD4 cell count was measured using a fluorescence-activated cell sorting instrument (Becton-Dickinson, San Jose CA, USA). Qualitative and quantitative HIV-1 RNA PCR testing were done using the Roche HIV-1 Amplicor MONITOR assay v1.0 with additional primers or the v1.5 kit, (Roche Diagnostics, Indianapolis IN, USA) on plasma separated from whole blood and frozen at −70°C within 24 hours of collection. An EIA for HIV-1 antibody was done on infants at 18 months of age with all reactive specimens confirmed with a western blot.

Statistical Analysis

Survival Analysis—Partly conditional survival methods were used to model the relationship between survival time and CD4 cell %, TLC, and HIV RNA laboratory measurements [24]. Each marker value contributed one unit of observation to the model, so one or more observations for each individual child were included in the analysis. The survival time scale was set to zero for each new marker measurement. If a child was alive one year after the measurement was taken, the survival time was censored at 365 days. A Cox proportional hazards regression model (SAS PHREG) was fit to estimate the risk of death within one year by each marker. The models were adjusted for age at the time of the measurement. Confidence intervals for hazard ratios and probabilities of death were calculated by the percentile method

from 10,000 bootstrap samples. Re-sampling was done on the population of individual children rather than the marker values. Probabilities of death within one year were calculated by estimating the baseline survival function by a nonparametric maximum likelihood method from the predicted survival probabilities.

Receiver Operating Curves (ROC) for Markers of Disease Progression and Mortality

Time dependant ROC curves [25] were estimated to evaluate the ability of CD4 cell %, TLC, and HIV RNA to identify death within one year from the time of the measurement. Confidence intervals for the area under the curve (AUC), sensitivity and specificity were calculated by the percentile method based on 1000 bootstrap samples. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the WHO TLC thresholds relative to WHO CD4 cell % thresholds used for determining severe immune suppression were calculated for the following age groups: < 12 months, 12–35 months, and > 35–60 months. The following WHO thresholds for CD4 cell % were the gold standard: < 25% for children aged less than 12 months, < 20% for children between 12 and 35 months, and < 15% for children >35 – 60 months. The corresponding WHO thresholds for TLC were < 4000 cells/ul, < 3000 cells/ul, and < 2500 cells/ul for the three age groups, respectively.

RESULTS

In the HIVNET 012 study, there were 128 HIV positive children, with 52% female. Forty two HIV infected children (33%) died within the first 18 months of life and 11 children died between 18 months and the first LTFU visit at 24 month. Of the remaining 75 eligible children, 65 (86%) were enrolled in the LTFU study. There were six children who were not enrolled in the LTFU study but were known to have died after 24 months of age. An additional 11 children died during the LTFU study for a total of 70 deaths over the 5 year follow-up period (55%). Of the 499 HIV negative children, 51% were female and 26 (5%) and 35 (7%) infants died by 18 months and 5 years of age respectively.

At least one TLC, CD4 cell %, and HIV-1 RNA laboratory measurement after detection of HIV infection was available from 122 of the 128 infected children (95%). There were 594 pairs of TLC and CD4 cell % measurements available for evaluation from birth through 60 months of age. One hundred and eight children contributed 183 pairs of TLC and CD4 cell % measurements before 12 months of age. Eighty nine children had 189 pairs of measurements between 12 and 35 months of age, and 58 children had 222 TLC and CD4 cell % measurements from 35 – 60 months of age.

Figures 1a & b present the TLC and CD4 cell % values at birth and during follow up for infected children and for the first 18 months for uninfected children. In the first 18 months the median TLC in infected children ranged from 4150 – 5800 cells per ul, which was similar to HIV negative children whose median TLC range was 4200 – 5600 cells per ul. In contrast, the CD4 cell % of HIV infected children dropped more rapidly from a median of 41% to 19% compared to HIV negative children whose median CD4 cell % remained above 38% throughout this time period. The proportion of infected children with at least one CD4 cell % value of < 25% in the < 12 month age group was 51% (55/108); one CD4 cell % < 20% in the 12–35 month age group was 55% (47/85); and one CD4 cell percent < 15% in the >35–60 months age group was 45% (26/58). For the same age categories respectively, the proportion of infected children with at least one TLC < 4000 cells/ul was 58% (67/115); one TLC < 3000 cells/ul was 31% (27/87); one TLC < 2500 cells/ul was 25% (15/59). The proportion of infected children with a TLC < 2000 cells/ul at any time point was very low, ranging from 0 – 9%.

The risk of death within one year was assessed using the WHO recommended age specific TLC threshold values. Table 1 shows the estimated probability of death within 12 months for

six-month age intervals from birth to 3.5 years for TLC levels in 500 cell/ul intervals from 2000 to 4500 cells/ul. The risk of mortality was highest for the youngest children at any given TLC threshold; for example, the 12-month risk of mortality at a TLC threshold of 3000 cells/ul was 29% for infants aged 6 months compared to 11% at age 2.5 years. The table also shows that for any age interval, the 12-month risk of death did not significantly vary by TLC threshold.

The hazard ratios (HR) from the partly conditional survival models, adjusted for age, for the three markers of CD4 cell %, TLC, and HIV RNA are presented in Table 2. CD4 cell % and HIV RNA were both significantly associated with the risk of death at a 95% confidence level. For example, decrease in CD4 cell % of 10% was associated with a 1.68 fold increase in risk of death and a one log increase in HIV RNA was associated with a 2.21 fold increase in risk of death. However, decreases of 1000 cells/ul in TLC were associated with only a 1.06 fold increase in risk of death, and this HR was not significant.

ROC curves were calculated for TLC, CD4 cell %, and HIV-1 RNA as surrogate markers for one year mortality (Figures 2a & b). TLC and CD4 cell % were poor predictors of one year mortality for children less than 12 months. The area under the curve (AUC) for TLC, CD4 cell %, and HIV-1 RNA were 0.50 (95% CI 0.41, 0.60); 0.63 (0.52, 0.73); and 0.61(0.50, 0.72) respectively. For children < 12 months of age, a TLC threshold of 4000 cells/ul had 38% sensitivity and 67% specificity for mortality within the next 12 months. In the older children aged 12–35 months, the AUC for TLC, CD4 cell % and HIV-1 RNA were 0.65 (95% CI 0.52, 0.76); 0.67 (0.54, 0.80); and 0.73 (0.61, 0.82) respectively. A TLC threshold of 3000 cells/ul had 34% sensitivity and 87% specificity for subsequent mortality. In contrast to the TLC threshold for children aged 12–35 months, a CD4 threshold of 20% for the same age group had 59% sensitivity and 63% specificity for 12-month mortality.

The age specific WHO TLC thresholds relative to the gold standard of the WHO CD4 cell % age group threshold for initiation of pediatric ART are shown in table 3. A TLC threshold of 4000 cells/ul weakly distinguished children < 12 months who had a CD4 cell count < 25% with 29% sensitivity, 37% positive predictive value (PPV), and 67% specificity. For children aged >35–60 months, a TLC threshold of 2500 cells/ul as a surrogate for a CD4 cell count threshold of 15% had 27% sensitivity, 63% PPV, and 94% specificity. The correlation between CD4 cell % and TLC was extremely low overall (r = 0.01) and by age groups; < 12 months r = − 0.30; 12–35 months r = 0.03; > 35–60 months r = 0.22.

DISCUSSION

In most developing countries the need for ART far outweighs its availability. Therefore, identifying which HIV infected children are in urgent need of ART is a critical priority. Many children in resource limited settings who need ART are only identified using WHO clinical staging. The Total Lymphocyte Count (TLC) has been identified as an easier and less expensive potential marker of short-term risk of death in HIV infected pediatric cohorts in the U.S. and Europe [4,26]. These studies indicate that while the absolute correlation of CD4 cell count and TLC was not high, TLC was a useful independent marker for disease progression and death [4,26]. Data relating TLC to CD4 cell counts, disease progression and survival among African children are limited. Rouet F et al reported that the CD4 cell count in infected and uninfected children from West Africa were significantly different at 3 and 6 months of age but the TLC at the same time points had no significant difference measurable [27]. This analysis using HIVNET 012 trial data is one of the first studies evaluating the utility of these TLC threshold values using longitudinal data from a cohort of perinatally HIV infected children from Africa.

Children under one year of age had significantly higher CD4 cell % and TLC than older children with an extremely poor (r = − 0.30) correlation between them, as has been reported by others

[4,21]. The high CD4 cell counts in infancy and the rapid decline over the subsequent 12–24 months may contribute to the lack of close correlation. In the Women and Infants Transmission Study Group (WITS), low CD4 cell % in the first two months of life was associated with HIV disease progression by age 6 months [27]. However, the factors that independently predicted infant progression by 18 months of age were only progression to CDC category B by age 6 months and mean viral load before age 6 months. In this Ugandan study, the TLC was a less sensitive marker for progression to death than CD4 cell % in these children with the highest risk of dying. Our study highlights the high risk of early infant mortality regardless of CD4 cell % or TLC and thus the need for aggressive identification and treatment of HIV infected infants.

The high TLC noted in this study have been reported previously in other African children and probably relates to genetic variation and host responses to multiple inter-current infections [20]. The differences between Africa and the developed world may be related to lower total white counts but higher TLC seen in African children. In healthy West African children between 12–23 months of age, lower CD4 cell percents were reported despite having higher absolute lymphocyte counts compared to U.S children [21]. The majority (84%) of HIV infected children in our study had a TLC above 2000 cells/ul throughout the study period. Taha Taha et al also reported no significant differences in median TLC measurements between HIV infected and uninfected children in Malawi [28]. Children under five years of age from sub-Saharan African have a high rate of malnutrition and multiple intercurrent infections associated with a high mortality regardless of HIV infection.

In this study a TLC ≤ 2000 cells/ul did not suggest an increased risk of progression to death in HIV infected children across all age groups. However, Mofenson demonstrated a 12 month risk of death to exceed 15% when the TLC was less than 3.8×10^{-9} cells/L in HIV infected children < 2 years of age and less than 2.3×10^{-9} cells/L in HIV infected children > 2 years of age in data from the NICHD Intravenous Immunoglobulin Study [4]. In the HIV Pediatric Prognostic Markers Collaborative Study, children over 2 years of age with a TLC < 1500 − 2000/ul had a sharp increase in the 12 month risk of death and AIDS [26]. We had less than fifteen children with a TLC < 1500 cells/ul, which was inadequate to include in the analysis for disease progression to death. The risk of death was highest for the younger age groups and declined with age, with 34% and 12% risks for mortality at birth and two years respectively for the same TLC. Gibb et al in their analysis of over 2000 children from one Brazilian and ten African sites also documented that the TLC was a weak predictor of mortality with CD4 cell count and percent being the strongest predictors [29]. Decisions to initiate HAART should be based on all the available surrogate markers and clinical judgment since the TLC alone may not predict those children at risk of dying if HAART was not initiated.

In older children (12–35 months), the AUC for TLC (0.65) approached that for CD4 cell % (0.67) suggesting better correlation between TLC and CD4 cell % in this age group. However, this close correlation did not extrapolate into a specific TLC thresholds predicting short term mortality risk. Those children > 35 months with a TLC > 2500 had a high specificity of 94% for CD4 cell count of 15%, suggesting that children with TLC counts > 2500 are less likely to die in the subsequent 12 months. The lack of utility of TLC in guiding whether to initiate pediatric treatment is consistent with findings by van der Ryst et al who reported the limitations of using TLC to guide treatment decisions among HIV infected adult patients in a South African study [30].

The TLC had a high specificity and negative predictive value when compared to CD4 cell count suggesting that children with higher TLC are at less risk of dying in the next 12 months. However, because of its low sensitivity and PPV, HIV infected children at highest risk of dying are not identified by TLC alone.

One of the limitations of these data is that the number of data points in the different age subgroups is relatively small. There were also fewer data points for older children; limiting the power to detect an association between TLC and mortality. This analysis also did not differentiate between asymptomatic and symptomatic children or WHO clinical staging or risk of progression to AIDS defining opportunistic infections. In addition, these results from Ugandan HIV infected children may not be generalizable to infected children from other resource limited settings.

One major strength of this analysis is that longitudinal data were available from a large prospective cohort of HIV infected African children. Another strength is that laboratory data were collected under rigorous clinical trial conditions with ongoing quality controls.

Conclusion

The findings from this analysis underscore the limited utility of the TLC alone as a surrogate marker to predict mortality in HIV infected children in resource limited settings such as Uganda. Analyses of larger data sets of African children should be undertaken to confirm these findings and identify cost effective mortality markers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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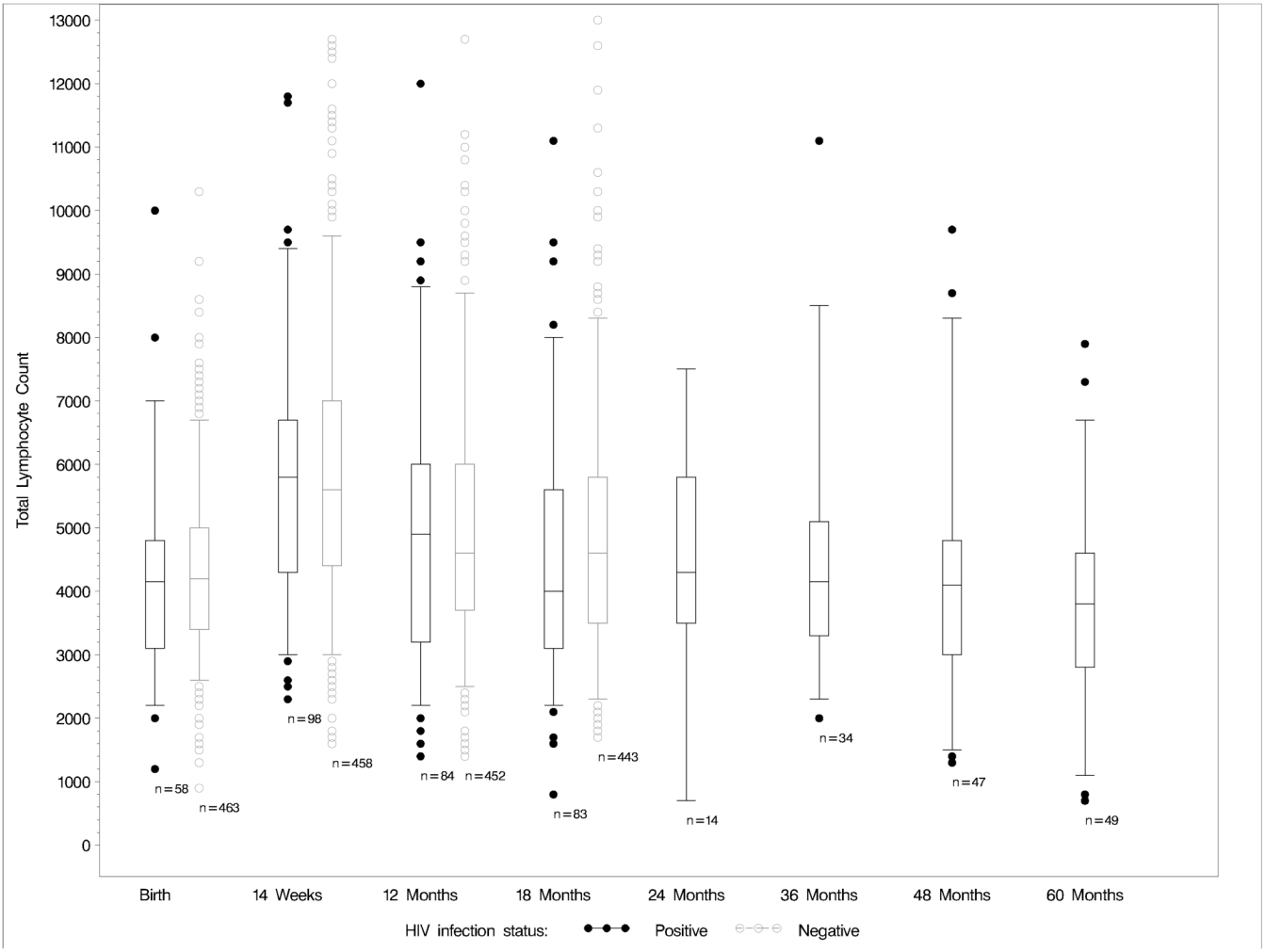

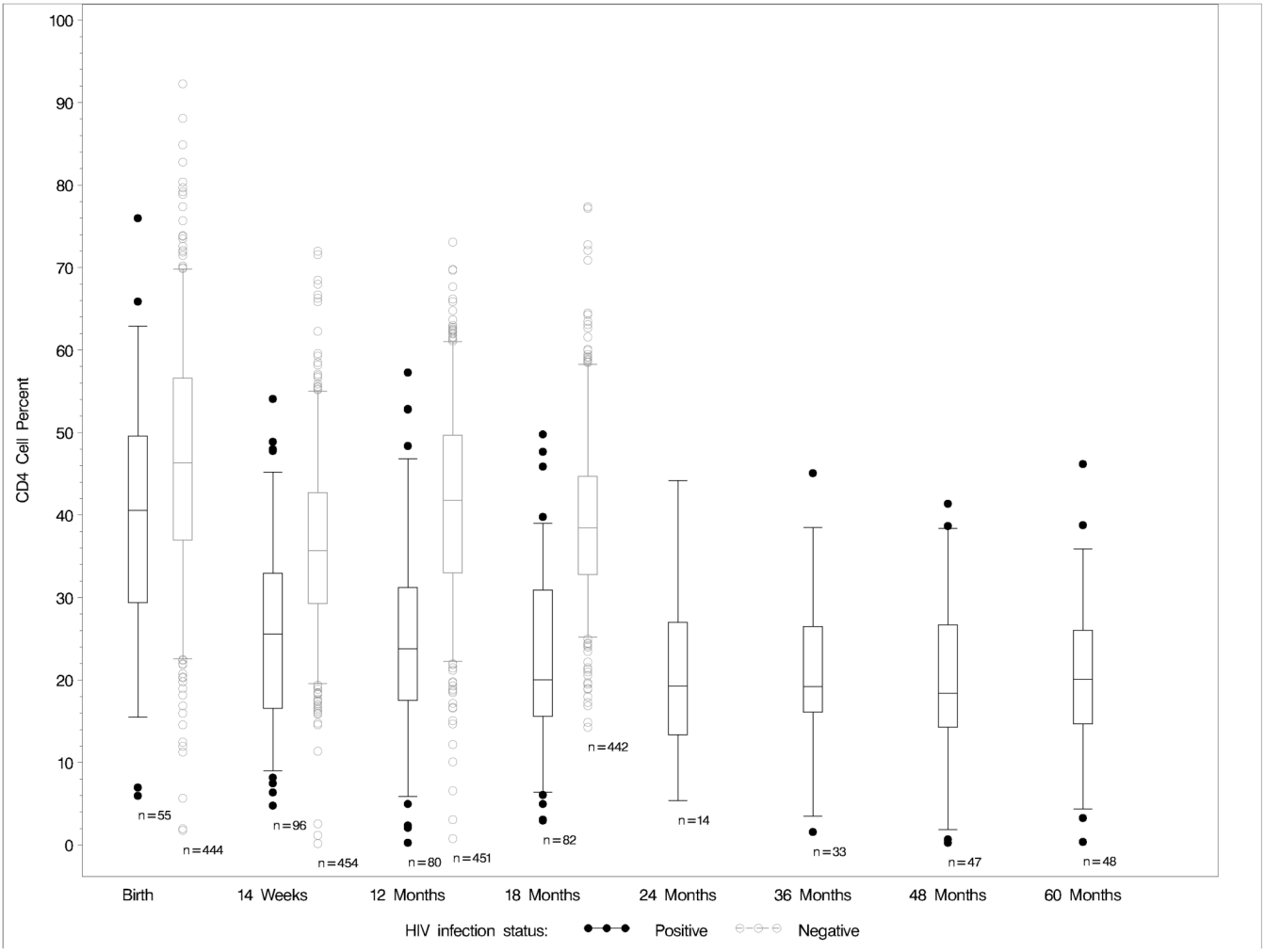

Figure 1.

1a. Total Lymphocyte count in HIV infected (birth – 60 months) and HIV negative (birth – 18 months) children.

1b. CD4 cell percent in HIV infected (birth – 60 months) and HIV negative (birth – 18 months) children

Figure 2a

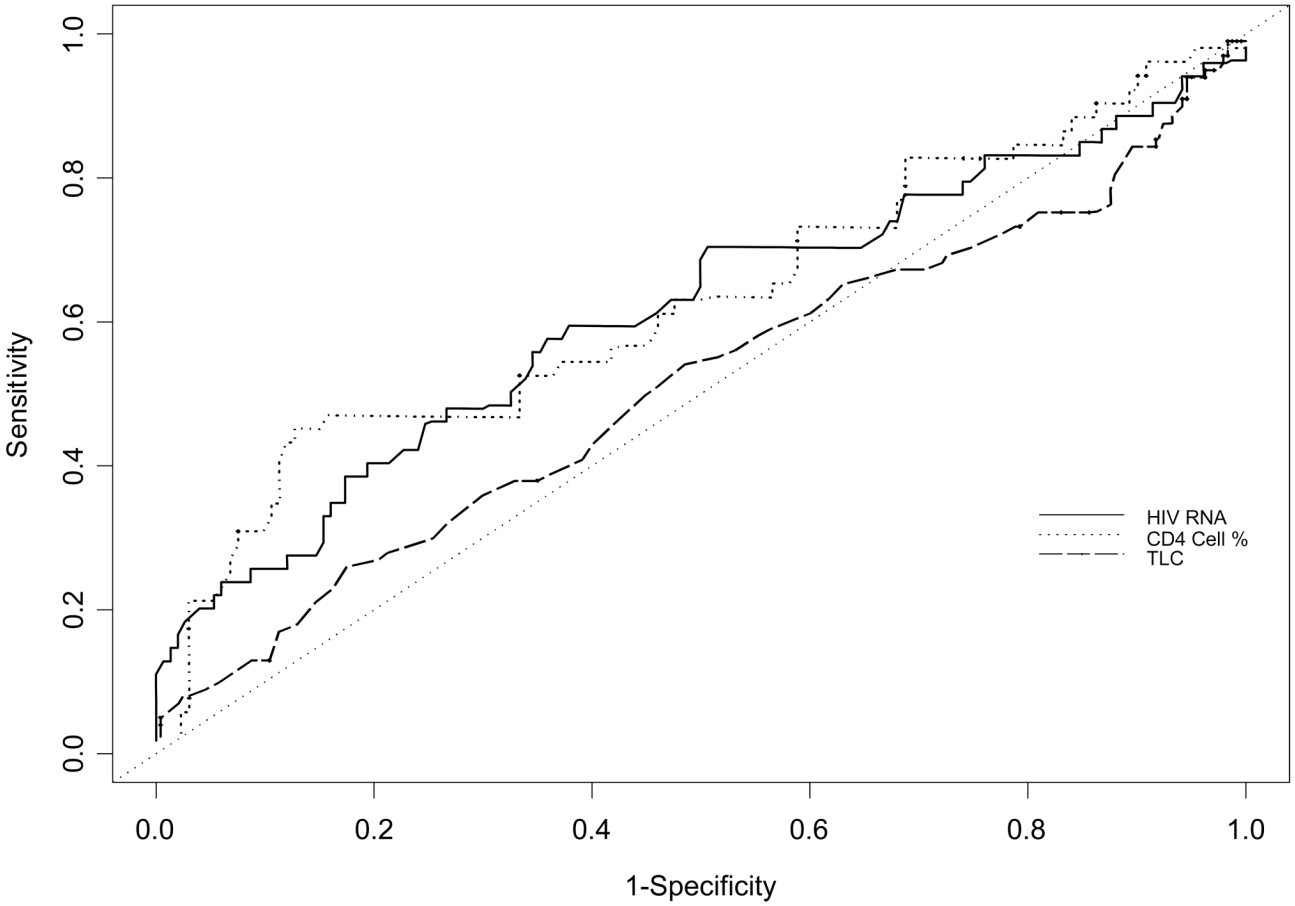

Figure 2b

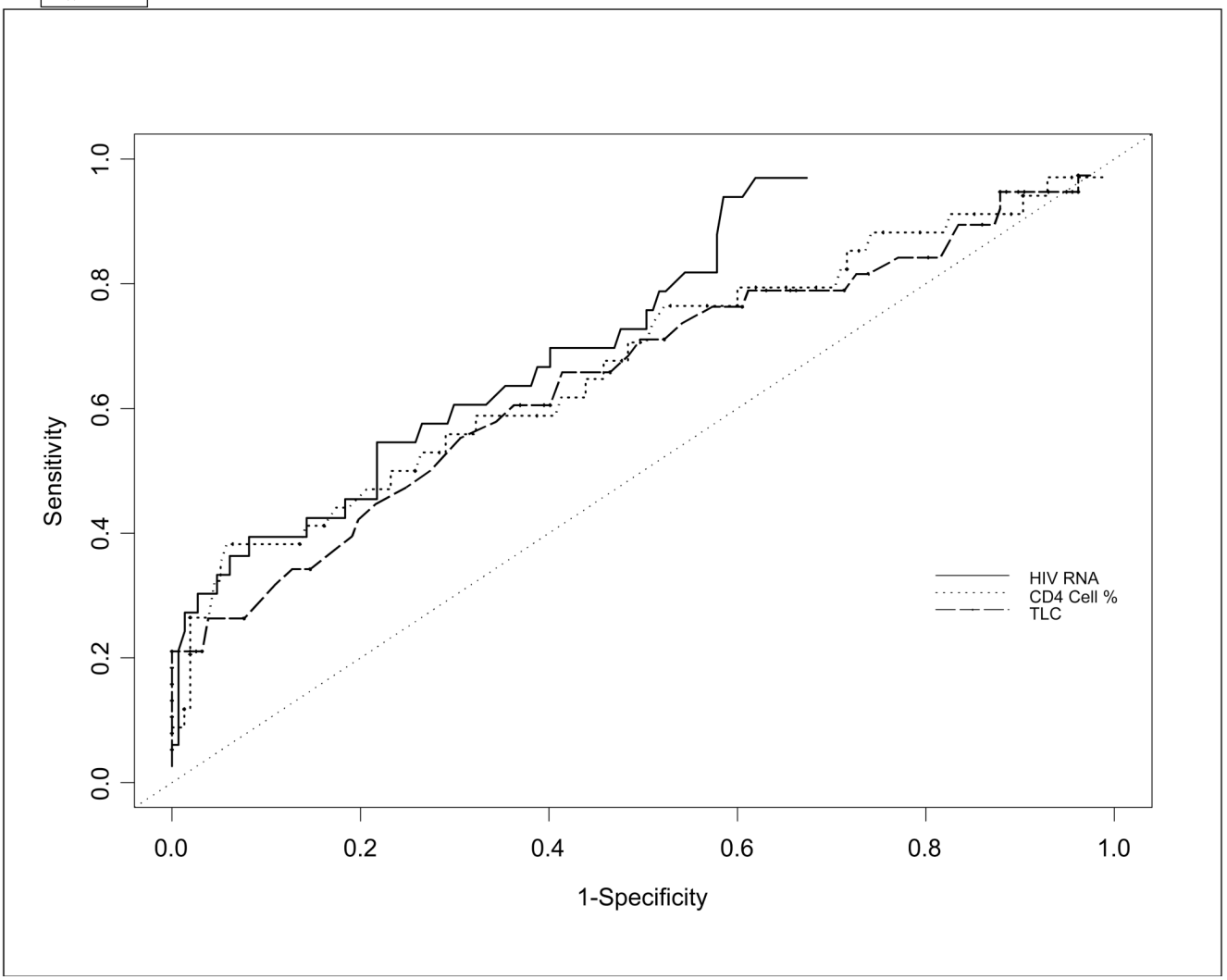

Figure 2.

2a. ROC curves of HIV RNA, CD4 cell %, and TLC predicting short term risk of death within 1 year for children < 12 months of age

2b ROC curves of HIV RNA, CD4 cell %, and TLC predicting short term risk of death within 1 year for children 12–35 months of age.

Table 1

Probability of death within one year by Total Lymphocyte Count and age

	2000		2500		3000		3500		4000		4500	
Age	**Prob.**	**95% CI**	**Prob.**	**95% CI**	**Prob.**	**95% CI**	**Prob.**	**95% CI**	**Prob.**	**95% CI**	**Prob.**	**95% CI**
Birth	0.37	(0.26, 0.53)	0.37	(0.26, 0.50)	0.36	(0.26, 0.48)	0.35	(0.26, 0.46)	0.34	(0.26, 0.44)	0.34	(0.25, 0.43)
6 months	0.30	(0.21, 0.43)	0.30	(0.21, 0.40)	0.29	(0.21, 0.38)	0.28	(0.21, 0.36)	0.28	(0.21, 0.34)	0.27	(0.21, 0.33)
1 year	0.24	(0.16, 0.36)	0.24	(0.17, 0.33)	0.23	(0.17, 0.31)	0.23	(0.17, 0.29)	0.22	(0.17, 0.28)	0.22	(0.17, 0.27)
1.5 years	0.19	(0.12, 0.29)	0.19	(0.12, 0.27)	0.18	(0.13, 0.25)	0.18	(0.13, 0.24)	0.17	(0.13, 0.23)	0.17	(0.12, 0.22)
2 years	0.15	(0.09, 0.24)	0.15	(0.09, 0.22)	0.14	(0.09, 0.21)	0.14	(0.09, 0.20)	0.14	(0.09, 0.19)	0.13	(0.09, 0.18)
2.5 years	0.12	(0.06, 0.20)	0.12	(0.07, 0.19)	0.11	(0.06, 0.18)	0.11	(0.06, 0.17)	0.11	(0.06, 0.16)	0.11	(0.06, 0.15)

Table 2

Risk of death for CD4 cell %, Total Lymphocyte Count, and viral load

	Hazard Ratio	95% CI
CD4 Cell % (−1%)	1.053	(1.026, 1.087)
CD4 Cell % (−5%)	1.296	(1.139, 1.519)
CD4 Cell % (−10%)	1.681	(1.297, 2.308)
Total Lymphocyte Count (−100 cells/mm^3)	1.005	(0.994, 1.022)
Total Lymphocyte Count (−250 cells/mm^3)	1.014	(0.986, 1.056)
Total Lymphocyte Count (−500 cells/mm^3)	1.027	(0.973, 1.116)
Total Lymphocyte Count (−750 cells/mm^3)	1.041	(0.959, 1.179)
Total Lymphocyte Count (−1000 cells/mm^3)	1.055	(0.946, 1.245)
log HIV RNA (+1 log)	2.211	(1.440, 3.531)

Table 3

TLC sensitivity, specificity, PPV and NPV for CD4 cell percent

CD4 Cell %	WHO TLC cut off	Sensitivity	Specificity	PPV	NPV
25%	< 12 mos: < 4000 cells/mm^3	0.29	0.67	0.37	0.59
20%	12–35 mos: < 3000 cells/mm^3	0.19	0.86	0.50	0.60
15%	≥ 35 mos: < 2500 cells/mm^3	0.27	0.94	0.63	0.76