Brief Report

$CD4^+$ T-Lymphocyte Count/$CD8^+$ T-Lymphocyte Count Ratio: Surrogate for HIV Infection in Infants?

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Summary

Introduction: Early diagnosis and treatment is necessary to prevent HIV-infected infants progressing to AIDS. Antibody testing is not confirmatory before the age of 18 months and PCR not widely available in resource-poor settings. We studied the accuracy of $CD4^+$ T-lymphocyte count, CD4% and CD4/CD8 ratio as surrogate markers of infant HIV infection.
Methods: Two hundred and fifty-eight HIV-exposed Indian infants at a median age of 5 months (range 1–18) had DNA PCR and CD4, CD8 counts performed.
Results: Fifty five infants tested positive by HIV-1 DNA PCR whereas 203 were negative. Median CD4 count, CD4% and CD4/CD8 ratio were significantly lower in DNA PCR+ infants. Overall sensitivity and specificity of CD4/CD8 ratio <1.0 in predicting HIV was 91 and 92% with a negative predicted value (NPV) and positive predicted value (PPV) of 97 and 76%, respectively.
Conclusion: CD4/CD8 ratio <1.0 is a more sensitive surrogate marker of HIV infection in Indian infants than a CD4 count <1500 cells/μl or CD4% <25%.

Key words: HIV, infants, surrogate marker, CD4/CD8 ratio, diagnosis.

Introduction

Transmission of infection from mother to child perinatally is the most common route of human immunodeficiency virus (HIV) acquisition in children and was responsible for >370 000 (230 000–510 000) new infections in 2009, worldwide [1]. Without access to antiretroviral therapy (ART) and cotrimoxazole prophylaxis, about one third of infants will die by 1 year of age and 50% by 2 years of age [2, 3]. The Children with HIV early antiretroviral therapy trial demonstrated a reduction in mortality by 75% among infants initiated on early ART, compared with those in whom it was deferred [4]. The World Health Organization (WHO) recommends initiation of ART for all infants <24 months, highlighting the need to make an early diagnosis [5]. However, early diagnosis of HIV infection is a challenging task.

The gold standard is the HIV DNA PCR assay with 99% sensitivity and specificity at 6 weeks [6]. Recent reports suggest that the $CD4^+$ T-lymphocyte/$CD8^+$ T-lymphocyte (CD4/CD8) ratio could be used as an indicator of HIV infection in infants [3, 4]. With the scale up of ART worldwide, there is an increasing availability of flow-cytometric facilities to monitor response to treatment. We performed a comparative evaluation of the accuracy of the CD4 count, CD4% and CD4/CD8 ratio in predicting HIV infection among infants born to HIV-infected women at two sites in south India.

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Methodology

A prospective study was conducted at the National Institute for Research in Tuberculosis at Chennai and Madurai, India from January 2006 to October 2008, in collaboration with the government ART centres. Children born to HIV-infected women were investigated after pre-test counseling and informed consent from guardians. The study was approved by the Institutional Ethics Committee.

Two milliliter of whole blood was collected. Complete blood count was measured using COULTER® Ac•T™ 5diff Hematology Analyzer (Beckman CoulterInc, Miami, USA), CD4 and CD8 counts were determined by flow cytometry (Epics Altra flow cytometer, Beckman Coulter, USA) using a standard 4-colour staining protocol (CD45/14/4/8) and DNA PCR testing performed using Amplicor HIV-1 DNA v1.5 kit (Roche molecular

Diagnostics, NJ, USA). Children who tested positive were referred to government ART centres for treatment. SPSS software version 14.0 was used for statistical analysis. CD4, CD8 counts (number and percentage) and ratio was compared between infected and uninfected infants using the Kruskal–Wallis test and receiver operating characteristic curves plotted.

Results

Two hundred and fifty-eight HIV-exposed infants [128 females, 130 males, median age 5 months (1–18)] were referred for HIV testing. Fifty-five tested positive by DNA PCR (21F and 34 M) whereas 203 were negative (107F and 96 M). Median CD4 count, CD4% and hemoglobin were significantly lower in PCR positive infants compared with PCR negative infants whereas CD8 count and CD8% were higher. Table 1 shows the sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the CD4/CD8 ratio and different cutoffs of CD4 count and CD4%. The overall sensitivity and specificity of CD4/CD8 ratio <1.0 in predicting HIV infection was 91 and 92%, respectively, with PPV and NPV of 76 and 97%, respectively. The CD4/CD8 ratio had a higher sensitivity and NPV than CD4% or CD4 cell count in predicting HIV-1 infection. When stratified by age, CD4/CD8 ratio performed better in the 12–18 months age group than 0–11 months. Receiver operating characteristics (ROC) curves were plotted comparing CD4% and CD4/CD8 ratio (Fig. 1A) and CD4 count and CD4/CD8 ratio (Fig. 1B) against PCR. The area under the curve (AUC) was higher for the CD4/CD8 ratio (0.96) as

TABLE 1
Age wise comparison of efficiency of CD4/CD8 ratio, CD4% and CD4 count in predicting HIV infection according to DNA PCR result

Variables	0–11 months		12–18 months		0–18 months	
	PCR+	PCR−	PCR+	PCR−	PCR+	PCR−
CD4/CD8 ratio <1.0 (*n*)	35/39	14/168	15/16	2/35	50/55	16/203
Sensitivity, % (range)	89 (77–97)		94 (74–99)		91 (81–96)	
Specificity, % (range)	92 (89–93)		94 (85–97)		92 (90–94)	
PPV, % (range)	71 (62–77)		88 (70–94)		76 (68–80)	
NPV, % (range)	97 (94–99)		97 (88–99)		97 (95–99)	
CD4 <25% (*n*)	26/39	14/168	13/16	1/35	39/55	15/203
Sensitivity, % (range)	67 (53–79)		81 (61–87)		71 (60–80)	
Specificity, % (range)	92 (89–94)		97 (88–99)		93 (90–95)	
PPV, % (range)	65 (52–76)		93 (70–100)		72 (61–81)	
NPV, % (range)	92 (89–95)		92 (83–94)		92 (89–95)	
CD4 <20% (*n*)	22/39	6/168	11/16	0/35	33/55	6/203
Sensitivity, % (range)	56 (44–65)		68 (50–69)		60 (50–66)	
Specificity, % (range)	96 (94–98)		100 (91–100)		97 (94–99)	
PPV, % (range)	79 (61–90)		100 (73–100)		85 (71–93)	
NPV, % (range)	91 (88–92)		87 (80–88)		90 (88–92)	
CD4 < 1500 cell/μl (*n*)	24/39	16/168	12/16	3/35	36/55	19/203
Sensitivity, % (range)	62 (48–73)		75 (53–88)		65 (54–75)	
Specificity, % (range)	90 (87–93)		91 (82–97)		91 (88–93)	
PPV, % (range)	60 (46–72)		80 (57–94)		65 (54–75)	
NPV, % (range)	91 (88–94)		89 (79–95)		91 (88–93)	
CD4 < 1000 (*n*)	18/39	6/168	7/16	1/35	25/55	7/203
Sensitivity, % (range)	46 (34–55)		44 (25–50)		45 (36–52)	
Specificity, % (range)	96 (94–98)		97 (89–100)		97 (94–98)	
PPV, % (range)	75 (55–89)		88 (50–99)		78 (61–90)	
NPV, % (range)	89 (86–90)		79 (72–81)		87 (84–88)	

Values are represented as percentages (95% CI) unless otherwise specified.

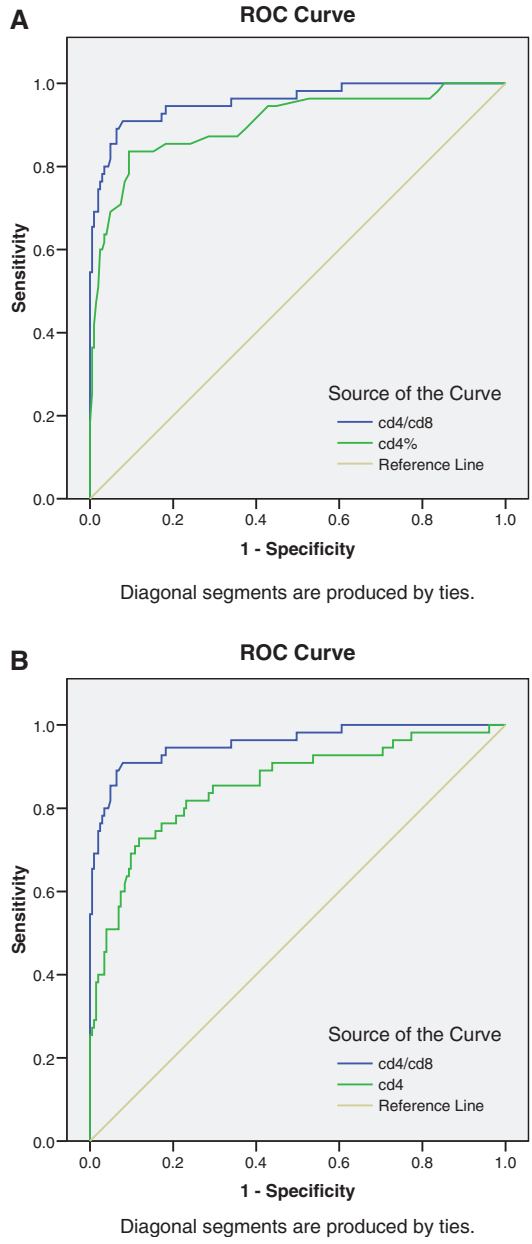

FIG. 1. **(A)** ROC curves plotting CD4% and CD4/CD8 ratio as predictors of HIV infection. **(B)** ROC curves plotting CD4 count and CD4/CD8 ratio as predictors of HIV infection according to DNA PCR status.

compared with the CD4% (AUC 0.90) or CD4 T-cell count (AUC 0.86), demonstrating that the CD4/CD8 ratio had better discriminative accuracy than the absolute CD4 count or CD4% in distinguishing between HIV-infected and uninfected infants.

Discussion

Our study results suggest that where facilities for virological diagnosis are not available but facilities for immunological assays exists; the CD4/CD8 ratio can play a valuable role in providing a presumptive diagnosis of HIV infection. Previous studies have shown that the ratio performed better than absolute CD4 counts in predicting HIV infection [7, 8]. With ratio, the errors in absolute counts and the use of different flow cytometric platforms would not affect the interpretation. The ratio performed better in the 12- to 18-month age group, possibly because of the higher variability of CD4 counts in younger infants. Even in the <12 month age group, the ratio was able to predict HIV with a sensitivity and specificity close to 90%. Although it cannot be used as a definitive diagnostic test, the value of a ratio <1.0 would alert physicians to the high probability of the infant being infected and need for ART. If the confirmatory test turned out negative, triple drug ART would have served as post-exposure prophylaxis and could be stopped later as the benefits of early treatment initiation in presumptively infected infants outweigh the risks; this approach needing validation in clinical settings.

Our study had some limitations. Although the sample size was reasonable, relatively high proportions (20%) of infants were HIV-positive, indicating some referral bias. The PPV and NPV would differ in a setting of varying HIV prevalence. Further, the diagnosis of HIV infection was confirmed by a single DNA PCR test, contrary to the usual practice of two tests. Since our laboratory participates in external quality assessment programs for HIV-1 DNA PCR and has been certified by the Virology Quality Assessment program, Rush University, Chicago, IL, USA, from 2007 to the present, we have a high degree of confidence in our results. Infants could not be followed for a second blood test as they were referred from different centres and went back to their primary care givers. We have observed that among infants born HIV-negative and who get infected through breast-feeding, the CD4/CD8 ratio was >1 initially and get reversed some time after infection [3], further demonstrating the utility of this marker.

Although infrastructure and capacity to undertake DNA PCR testing are being developed in many countries, the CD4/CD8 ratio could be used to make a presumptive diagnosis of HIV in exposed infants. This would permit quick referral, confirmatory testing and initiation of ART, thus avoiding unnecessary morbidity and mortality.

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