

Predictors of immunologic long-term nonprogression in HIV-infected children: Implications for initiating therapy

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Background: Early markers that predict immunologic long-term nonprogression in infants with perinatally acquired HIV infection might assist in subsequent antiretroviral treatment decisions.

Objectives: We sought to identify early markers of immunologic long-term HIV disease nonprogression.

Methods: We analyzed immunologic and virologic characteristics at 1 and 2 months of age in HIV-infected children who were enrolled in the Women and Infants Transmission Study and born before 1995, comparing immunologic long-term nonprogressors (ILTNP; n = 10) with non-ILTNP (n = 127). ILTNPs were children who survived to 8 years or older with CD4 percentages of 25% or greater and counts of 500 cells/mm³ or more without receiving highly active antiretroviral therapy. Non-ILTNP were defined as all other HIV-infected children. Receiver operating characteristic curve analysis was used to assess combined sensitivity and specificity for each of these characteristics and to determine potential threshold values to discriminate between ILTNPs and non-ILTNP.

Results: Characteristics in the first 2 months of life associated with ILTNP status in univariate analysis included higher CD4 percentages, lower CD8⁺ percentages, lower CD8⁺HLA-DR⁺ percentages, and lower HIV-1 RNA PCR values. In receiver operating characteristic analysis CD8⁺HLA-DR⁺ percentage had the best combined sensitivity and specificity for discriminating between ILTNPs and non-ILTNP. CD8⁺HLA-DR⁺ percentages of 5% or less predicted ILTNP status with 80% sensitivity and 80% specificity. In multivariate analysis CD8⁺HLA-DR⁺ percentage of 5% or less remained a

significant predictor of ILTNP status after adjusting for CD3⁺CD4⁺ percentage and HIV-1 RNA PCR value (odds ratio, 15.4; 95% CI, 1.9-124.7).

Conclusion: CD8⁺HLA-DR⁺ T-lymphocyte percentage of less than 5% at 1 to 2 months of age might be predictive for ILTNP status but should not be used at this time to make treatment-deferral decisions. Immune activation in HIV-infected infants might herald more disease progression. Further study of the use of this subpopulation in early infancy to predict ILTNP status is warranted. (*J Allergy Clin Immunol* 2005;115:848-55.)

Key words: Human immunodeficiency virus, perinatal, immunologic long-term nonprogression, CD8⁺ T lymphocyte

There are numerous descriptive and cross-sectional studies of pediatric HIV-infected long-term survivors defined as perinatally infected children surviving beyond a range of 5 to 9 years.¹⁻⁶ However, in contrast to survival studies, reports of long-term nonprogressors (LTNP) in pediatric HIV are few in number and do not include comprehensive prospective studies. Two retrospective studies of long-term survivors include descriptions of small subgroups of true nonprogressors.^{7,8} Several additional studies have reported analyses of 1 or 2 virologic or host genetic variables in pediatric LTNP. One report suggested that functional and structural alterations in HIV *nef* genes correlate with pediatric LTNP.⁹ Several other

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Abbreviations used

Az:	Area score
HAART:	Highly active antiretroviral therapy
ILTNP:	Immunologic long-term nonprogressor
LTNP:	Long-term nonprogressor
OR:	Odds ratio
PCP:	<i>Pneumocystis jiroveci</i> -induced pneumonia
QA:	Quality assurance
ROC:	Receiver operating characteristic
WITS:	Women and Infants Transmission Study

studies reported disparate findings on the correlation of a CCR5-defective allele (CCR5 Δ 32) with long-term survival or nonprogression in HIV-infected children.¹⁰⁻¹⁴

Both viral load and CD4⁺ T-cell count have been used as independent predictors of risk for HIV disease progression in HIV-infected adults and children.¹⁵⁻¹⁷ The predictive thresholds of these 2 measures are not as well defined in children, particularly in young infants, as in adults.¹⁶ HIV RNA levels are difficult to use as predictors of risk for disease progression during the first year of life because levels are high, and there is marked overlap in levels between children with and without rapid disease progression.¹⁸⁻²⁰ However, very high RNA levels of greater than 100,000 copies/mL in infants have been associated with high risk for disease progression and mortality, especially if the CD4⁺ T-cell percentage is less than 15%.¹⁷

We used prospectively collected data from the Women and Infants Transmission Study (WITS) to look for a relationship between long-term nonprogression defined by immunologic parameters and lymphocyte subset phenotypes and viral load measured early in life. We evaluated data from a group of immunologic long-term nonprogressors (ILTNP) without regard to clinical symptoms because CD4⁺ levels are generally considered to be highly predictive of disease progression. In addition, we hypothesized that there might be a unique subgroup of pediatric LTNP who, without benefit of highly active antiretroviral therapy (HAART), do well clinically and immunologically after recovering from AIDS-defining illness early in life.²¹

METHODS

Patient population

WITS is an ongoing, longitudinal multicenter study of HIV-infected pregnant women and their infants that is sponsored by the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, and the National Institute on Drug Abuse. The study began in 1989 and is ongoing at medical centers in Puerto Rico (San Juan), Texas (Houston), Illinois (Chicago), Massachusetts (Boston and Worcester), and New York (Manhattan and Brooklyn). The institutional review board at each center approved the study protocol at each study site, and written informed consent was obtained from all participants before study enrollment.

Sample and data collection and definitions

Infants were enrolled at birth, and visits occurred routinely in infants within the first 7 days of life and at 1, 2, 4, 6, 9, 12, and 18 months of age and every 6 months thereafter. Clinical and laboratory evaluations were performed at each of these visits. Clinical evaluations included interview, physical examination, and chart review to collect interim events, including AIDS-defining conditions.

Infants were defined as uninfected for HIV if their peripheral blood mononuclear cultures or HIV DNA PCR test results were negative twice on separate visits after 1 month of age and the patient had no positive test results. Positive HIV DNA PCR results were confirmed by means of repeat HIV DNA PCR, HIV RNA PCR, or HIV culture.

The study was limited to children enrolled in the WITS who were born before or during 1994 to ensure sufficient follow-up time to identify LTNP. ILTNPs were defined as children living 8 years or longer without receipt of HAART and with CD4 levels of 25% or greater and counts of 500 or greater (CDC category 1) at age 8 years. HAART was defined as a treatment regimen including 3 or more drugs, one of which was a protease inhibitor or nonnucleoside analogue reverse transcriptase inhibitor, or the 3-drug combination of zidovudine (AZT), lamivudine (3TC), and abacavir. Children who received single or dual nucleoside therapy were not excluded from the ILTNP group. Non-ILTNPs were defined as all other HIV-infected children born in the same time period (before or during 1994).

Laboratory analysis

Routine blood tests were performed at hospital clinical laboratories that had been certified by the College of American Pathologists or other nationally recognized quality assurance (QA) programs. Flow cytometry and HIV culture were performed in WITS laboratories at each site. All sites participated in the laboratory QA program of the AIDS Clinical Trials Group for flow cytometry and the Virology Quality Assessment for HIV culture.²²⁻²⁴

For flow cytometry, samples were prepared for staining and analysis by means of whole-blood lysis, as previously published.²² The same reagents were used at all sites (Becton-Dickinson, San Jose, Calif) and consisted of an isotype control (IgG1 and IgG2a), CD45/CD14 (gating reagent), CD4/CD8 (compensation reagent), CD3/CD4 (CD4⁺ T lymphocytes), CD3/CD8 (CD8⁺ T lymphocytes), CD8/CD38 (activated CD8⁺ T lymphocytes), CD8/HLA-DR (activated CD8⁺ T lymphocytes), CD3⁻/CD16⁺CD56⁺ (natural killer cells), and CD3/CD19 (B lymphocytes), which were conjugated to FITC and phycoerythrin, respectively. The full protocol for flow cytometry has been previously described.¹⁹ By using this dual-color flow cytometric assay, all results are expressed as the percentage of total lymphocytes except for the CD8⁺DR⁺ T lymphocytes, which are expressed as the calculated subpopulation of CD8⁺ T lymphocytes. Conversion to a calculated subpopulation of CD8⁺ T lymphocytes was not possible for CD8/CD38 because of the high degree of error expected when setting the gate for the CD38 fluorochrome in dual-color flow cytometry.

Statistical analysis

A variety of methods were used to assess the ability of early lymphocyte phenotypic markers and viral load to discriminate between ILTNPs and non-ILTNPs. Preliminary analyses of discriminate validity included ANOVAs of the differences in mean lymphocyte counts, lymphocyte percentages, and viral load in the first 2 months of life by groups (ILTNP and non-ILTNPs). Because of the greater variability in absolute counts and the resulting lower power to detect differences, the relative (percentage) counts were used.²⁵

TABLE I. Univariate analysis of the lymphocyte markers and viral load by HIV status and survival classification

Markers at 2 mo	Classification	N	Mean	SD	95% CI	P value* (ILTNP vs non-ILTNP)
CD4 ⁺ %	HIV ⁻	561	49.7	7.9	49.0-50.3	.009
	HIV ⁺ , ILTNP	10	48.0	7.6	42.6-53.4	
	HIV ⁺ , non-ILTNP	102	38.4	11.1	36.2-40.6	
CD8 ⁺ %	HIV ⁻	556	20.3	6.1	19.8-20.8	.006
	HIV ⁺ , ILTNP	10	17.3	5.5	13.3-21.3	
	HIV ⁺ , non-ILTNP	101	26.3	9.9	24.4-28.3	
CD8 ⁺ HLA-DR %	HIV ⁻	525	4.3	4.6	3.9-4.7	<.0001
	HIV ⁺ , ILTNP	10	4.3	2.5	2.5-6.1	
	HIV ⁺ , non-ILTNP	92	11.8	7.7	10.2-13.4	
CD8 ⁺ CD38 ⁺ %	HIV ⁻	534	22.9	6.3	22.4-23.5	.03
	HIV ⁺ , ILTNP	10	23.0	5.9	18.8-27.2	
	HIV ⁺ , non-ILTNP	96	27.9	7.7	26.3-29.4	
CD16 ⁺ CD56 ⁺ %	HIV ⁻	564	11.7	4.5	11.3-12.1	.03
	HIV ⁺ , ILTNP	9	10.6	3.5	7.9-13.2	
	HIV ⁺ , non-ILTNP	105	13.9	9.2	12.1-15.7	
Log10 HIV-1 RNA	HIV ⁻	NA				.0005
	HIV ⁺ , ILTNP	10	4.8 [†]	0.5	4.4-5.2	
	HIV ⁺ , non-ILTNP	104	5.5 [‡]	0.6	5.4-5.6	

*Based on *t* test for difference in the mean values between ILTNPs and non-ILTNPs. Comparisons between groups for CD19 and CD3 percentages were not statistically significant.

[†]Median HIV-1 RNA of 118,716 copies/mL; interquartile range of 19,649 to 142,775 copies/mL.

[‡]Median HIV-1 RNA of 735,607 copies/mL; interquartile range of 152,922 to 864,655 copies/mL.

Receiver operating characteristic (ROC) plot analysis, which plots the sensitivity of a test versus its false-positive rate (1 – specificity) for all possible thresholds, was used to assess the diagnostic value of the 2-month lymphocyte percentages and viral load in predicting immunologic long-term nonprogression and determine the best threshold values for each marker. Area under the ROC curve, expressed in an area score (Az), provides a summary description of a continuous measure's combined sensitivity and specificity (ability) to discriminate throughout its entire range of possible thresholds. The Az parameter indicates how much the test improves discrimination over chance with higher Az scores, indicating greater combined sensitivity and specificity. Determining sensitivity and specificity rates for each marker entails choice of a threshold level for a continuous measure, therefore making the results dependent on the selected threshold. As suggested by Metz,²⁶ the ROC analysis was also used to identify a threshold at which sensitivity is highest at the lowest possible false-positive rate to overcome the problem of having several levels or selecting only one.

Logistic regression analysis was used to further assess the odds of a child being an ILTNP on the basis of combinations of lymphocyte thresholds identified by the ROC plot analysis. Cox proportional hazards models were used to examine the association between these thresholds and time to first immunosuppression, as defined by a CD4⁺ T-lymphocyte percentage of less than 25%.²⁷ This CD4⁺ T-lymphocyte percentage was used because this is the percentage that is the accepted cutoff in moving from immunologic class 1 (no immunosuppression) to immunologic class 2 (moderate immunosuppression), as defined by the Centers for Disease Control and Prevention. Children who did not achieve immunosuppression through June 2002 contributed to the censored observations. To remove the potential influence of antiretroviral therapy in extending survival times, we further censored follow-up at the first documented use of HAART and adjusted for other antiretroviral therapy (monotherapy and dual combination therapy) as time-dependent variables. Kaplan-Meier plots were used to show esti-

mates of immunosuppression-free survival stratified by the 2-month thresholds.

RESULTS

Subject characteristics at baseline

Of 190 HIV-infected children in the WITS database as of June 2002, 137 children who were born before or in 1994 were included in this analysis. Ten (7.3%) of the 137 children were ILTNPs.

There were no significant differences between ILTNPs and non-ILTNPs in terms of baseline characteristics, including sex, ethnicity, year of birth, prematurity, HIV positivity in the first 48 hours of life, or receipt of antiretroviral therapy in the first 18 months of life.

Characteristics of ILTNPs with clinical class C diagnosis

Four of the 10 ILTNPs had a clinical class C diagnosis. The AIDS-defining illnesses in these children were as follows: encephalopathy at 21.3 months; bacterial sepsis at 6.9 and again at 9.3 months; bacterial pneumonia and sepsis at 20 months, followed by bacterial sepsis at 31 months; and bacterial and *Pneumocystis jiroveci*-induced pneumonia (PCP) along with cytomegalovirus disease at 7.8 months and bacterial sepsis again at 48.8 months. Three of the 4 children had notably high CD4 levels throughout the duration of follow-up with CD4⁺ percentages ranging from 33% to 44% and absolute CD4⁺ counts ranging from 757 to 1479 at age 8 years. The remaining child, whose initial class C diagnosis was PCP

TABLE II. Best threshold lymphocyte percentages and RNA values for determining membership in the ILTNP category

Markers	Threshold*	N†	False negative‡	False positive§	Sensitivity	Specificity
CD4 ⁺ %	≥49	23/111 (21%)	5	19	0.50	0.81
CD8 ⁺ %	≤20	38/111 (34%)	1	29	0.90	0.71
CD8 ⁺ HLA-DR %	≤5	26/102 (25%)	2	18	0.80	0.80
CD8 ⁺ CD38 ⁺ %	≤25	50/106 (47%)	1	41	0.90	0.57
Log10 HIV-RNA	≤5.19	34/114 (30%)	2	26	0.80	0.75

*Thresholds determined on the basis of ROC analysis results.

†Number of infants with the specified threshold.

‡True ILTNPs missed under the criteria for threshold.

§Non-ILTNPs classified as ILTNPs under the criteria for threshold.

TABLE III. Sequential logistic regression for 2-month predictor of ILTNPs

Covariate	CD8 ⁺ HLA-DR ⁺ % ≤5*		CD8 ⁺ CD38 ⁺ % ≤25*	
	Adjusted odds ratios (95% CI)	P value	Adjusted odds ratios (95% CI)	P value
Model 1				
Main effect only	16.4 (3.2-84.2)	.0008	12.1 (1.5-99.0)	.02
Model 2				
Main effect	15.7 (2.6-93.7)	.002	9.4 (1.1-80.2)	.04
CD3 ⁺ CD4 ⁺ % ≥49	1.1 (0.2-5.3)	.90	2.3 (0.6-9.3)	.25
Model 3				
Main effect	15.4 (1.9-124.7)	.01	7.2 (0.8-67.4)	.08
CD3 ⁺ CD4 ⁺ % ≥49	0.8 (0.1-3.4)	.45	1.3 (0.3-6.4)	.71
Log10 HIV RNA ≤5.19	10.7 (1.6-70.2)	.01	8.1 (1.5-44.0)	.02

In the model (data not shown) in which both CD8⁺HLA-DR⁺ and CD8⁺CD38⁺ percentages were included as the main effects, only CD8⁺HLA-DR⁺ was associated with the odds for ILTNP.

*Main effect in each model.

and cytomegalovirus disease at 7.8 months, had lower CD4⁺ percentages (15% to 20%) but notably high absolute CD4⁺ counts (>1000) from age 1 to 5 years. This child received no therapy or only monotherapy through age 5.5 years. On dual nucleoside therapy for the remainder of the follow-up period, this child had improved CD4⁺ percentages (22% to 28%) and absolute CD4⁺ counts ranging from 429 to 774.

Profiles of lymphocyte phenotypes and viral load in ILTNPs and non-ILTNPs

In a preliminary analysis comparing trajectories of immunologic and virologic markers over the first 18 months of life in ILTNPs versus non-ILTNPs, differences between the 2 groups were most notable in CD4⁺ percentages, CD8⁺ percentages, CD8⁺HLA-DR⁺ percentages, and CD8⁺CD38⁺ percentages (see Fig E1 in the Journal's Online Repository at www.mosby.com/jaci). These differences appeared to manifest by 2 months of age. We thus compared mean levels of these immunologic and virologic markers at 2 months of life in HIV-positive ILTNPs (n = 10), HIV-positive non-ILTNPs (n = 127), and HIV-negative subjects (n = 561; Table I). At 2 months of life, ILTNPs had significantly lower CD8⁺ percentages and CD8⁺DR⁺ percentages and higher CD4⁺ percentages than the non-ILTNPs (*P* < .01 for each of the 3 comparisons). Furthermore, these values resemble the values from the enrolled HIV-uninfected children. In the univariate analysis lower HIV-1 RNA levels

were also associated with immunologic long-term non-progression.

Prognostic value of lymphocyte phenotypes and viral load for ILTNPs: Sensitivity, specificity, and determination of threshold levels

To assess the combined sensitivity and specificity of these 2-month markers for predicting immunologic long-term nonprogression and determine potential threshold values for each marker, we used ROC curve analysis with ILTNPs as the criterion (see Fig E2 in the Journal's Online Repository at www.mosby.com/jaci). Area under the ROC curve provided a summary measure of the diagnostic accuracy of each test. The ROC curves for CD4⁺ percentages, CD8⁺ percentages, CD8⁺CD38⁺ percentages, and CD8⁺HLA-DR⁺ percentages showed areas under the curve of 0.75, 0.75, 0.68, and 0.84, respectively. These results indicated that the 2-month marker with the best combined sensitivity and specificity for differentiating ILTNPs from non-ILTNPs was CD8⁺HLA-DR⁺ percentages; however, CD8⁺HLA-DR⁺ percentages had a significantly greater area under the curve than CD8⁺CD38⁺ percentages (Az, 0.84 vs 0.68; *P* = .03) but not CD4⁺ percentages (Az, 0.84 vs 0.75; *P* = .37) or CD8⁺ percentages (Az, 0.84 vs 0.75; *P* = .63).

The ROC curve analysis also allowed us to determine the best thresholds for predicting ILTNP status. The best threshold was defined as the level at which

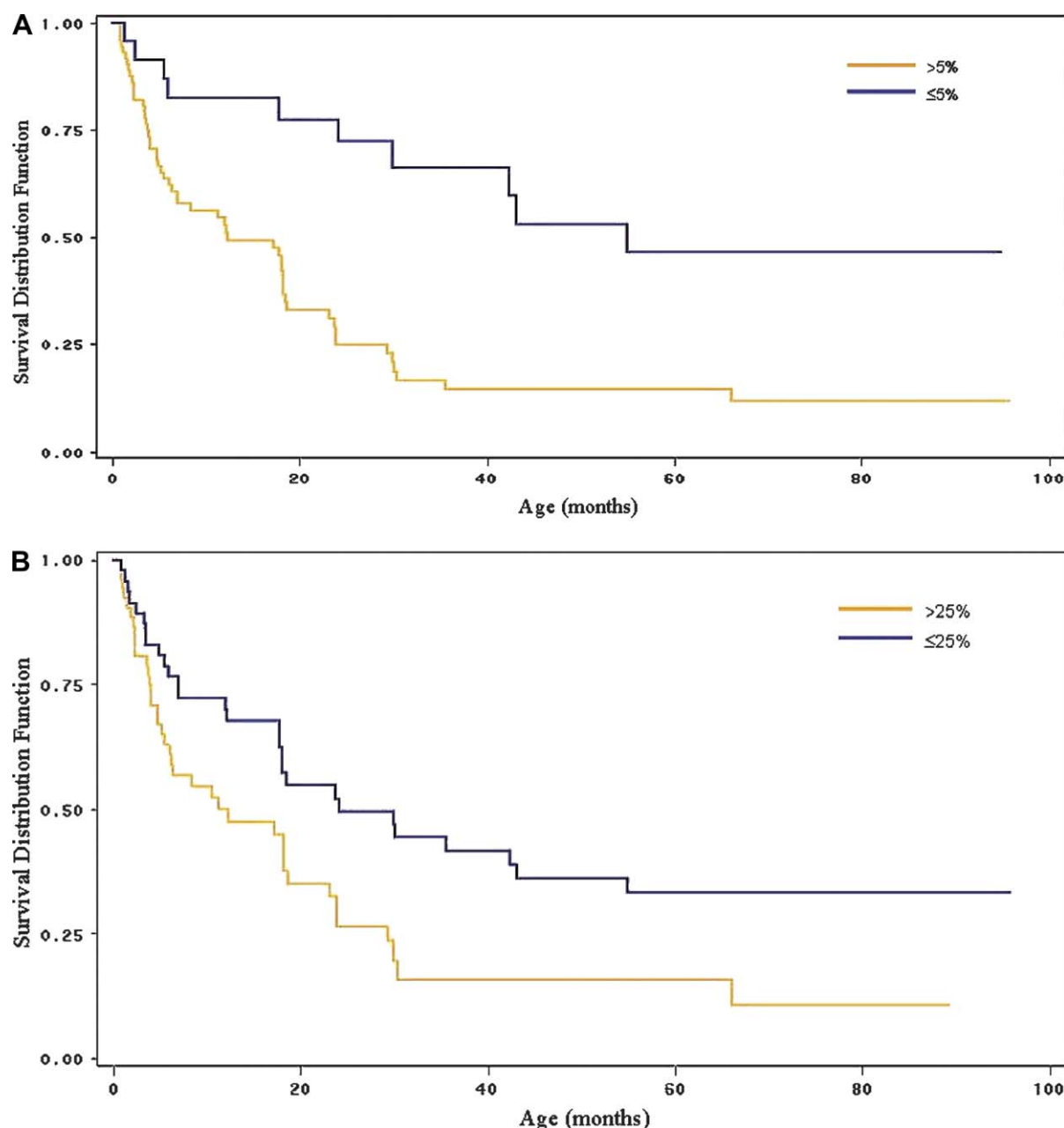


FIG 1. Kaplan-Meier curves of immunosuppression-free survival stratified by thresholds for CD8⁺HLA-DR⁺ (A) and CD8⁺CD38⁺ (B) percentages.

(Sensitivity + Specificity)/2 attains its highest value; that is, where the sum of false-positive results plus false-negative results is minimized. These best thresholds for each of the lymphocyte subsets are shown in Table II. Using the best threshold defined for CD8⁺HLA-DR⁺ percentages (≤5%), 2 of 10 ILTNPs were missed (20% false-negative rate), and 18 of 102 non-ILTNPs were misclassified as ILTNPs (20% false-positive rate). A threshold of a CD8⁺HLA-DR⁺ percentage of 5% or less thus predicted ILTNPs with 80% sensitivity and 80% specificity (Table II). Only 1 of 10 ILTNPs was missed when CD8⁺CD38⁺ percentages of 25% or less was used

as a threshold; however, the specificity (80%) of the CD8⁺HLA-DR⁺ percentage marker was much better than that of the CD8⁺CD38⁺ percentage marker (57%).

Predictive value of best threshold levels of lymphocyte phenotypes and viral load for ILTNPs

We tested the ability of these thresholds to differentiate ILTNPs from non-ILTNPs in univariate and sequential logistic regression models. The univariate models confirmed the prognostic value of markers

identified by means of ROC analysis (data not shown). Immunologic long-term nonprogression was associated with a CD8⁺HLA-DR⁺ percentage of 5% or less (odds ratio [OR], 16.4), a CD8⁺CD38⁺ percentage of 25% or less (OR, 12.1), a CD3⁺CD4⁺ percentage of 49% or greater (OR, 4.4), a CD3⁺CD8⁺ percentage of 20% or less (OR, 22.3), and a log10 HIV-1 RNA PCR value of 5.19 or less (OR, 12.1). There was a notably strong positive correlation between CD8⁺HLA-DR⁺ percentages and CD8⁺CD38⁺ percentages ($r=0.72$, $P=.0001$), indicating these activated CD8⁺ subsets might be associated with ILTNPs through related pathways. We thus used these 2 markers as the main effect in separate multivariate logistic regression models. In multivariate analysis (Table III) CD8⁺HLA-DR⁺ percentages of 5% or less remained a significant and independent predictor of ILTNP status after adjusting for CD3⁺CD4⁺ percentages and log10 HIV-1 RNA PCR values (OR, 15.4; 95% CI, 1.9-124.7).

Kaplan-Meier estimates of immunosuppression-free survival stratified by the 2-month thresholds for CD8⁺HLA-DR⁺ and CD8⁺CD38⁺ percentages showed evidence of prolonged survival without immunosuppression among children with CD8⁺HLA-DR⁺ percentages of 5% or less ($P=.0004$) and CD8⁺CD38⁺ percentages of 25% or less ($P=.011$). Median immunosuppression-free survival was 50 to 60 months for infants with CD8⁺HLA-DR⁺ percentages of 5% or less (Fig 1). Multivariate Cox proportional hazards analysis showed the independent predictive effect of early CD8⁺HLA-DR⁺ percentages. CD8⁺HLA-DR⁺ percentages of 5% or less in the first 2 months significantly decreased the risk of subsequent immunosuppression (risk ratio, 0.46; 95% CI, 0.21-0.82), even after controlling for antiretroviral therapy (time dependent) and HIV RNA PCR. In contrast, the association between CD8⁺CD38⁺ percentages and immunosuppression-free survival did not remain significant after controlling for potential confounders.

DISCUSSION

In this study evaluation for correlates of immunologic long-term nonprogression in a group of perinatally HIV-infected children showed that by 2 months of age ILTNPs had fewer activated CD8⁺ T lymphocytes, as measured on the basis of lower percentages of the CD8⁺HLA-DR⁺ subpopulation of CD8⁺ T lymphocytes than non-ILTNPs.

In studies of untreated adults, markers of T-cell activation predict the rate of HIV disease progression.²⁸⁻³⁶ The best described immunophenotypic marker of immune activation in HIV-infected adults is CD38 expression on T cells. Liu et al³⁶ showed that CD38 expression on CD8⁺ T cells was a better predictor of disease progression than other markers of activation, including combinations of HLA-DR and CD38 expression. However, CD38 expression on CD8⁺ T cells is also strongly correlated with other markers of cellular

activation, including HLA-DR.³⁷ In a recent study Deeks et al³⁸ showed that in untreated adults the CD8⁺ T-cell activation level reached early after primary infection was predictive for CD4⁺ T-cell decrease over time. CD8⁺ T-cell activation in this study was measured on the basis of density of CD38 expression; HLA-DR was not evaluated.³⁸

Subpopulations of CD8⁺ lymphocytes, including HLA-DR⁺, CD38⁺, and HLA-DR⁺CD38⁺ subsets, have been associated with more advanced disease progression in HIV-infected children.³⁹⁻⁴⁵ However, in early infancy CD38 expression is found on more than 95% of CD8⁺ T cells, and in infants CD38 is a marker of immaturity and of recent thymic emigrants in addition to being a marker of activation.^{46,47} CD38 expression is normally lost over time, and percentages of CD8 cells with the CD38 marker reach the adult normal level later in childhood. We found that CD8 HLA-DR was superior to CD8 CD38 in predicting immunologic long-term nonprogression when using dual-color flow cytometry in the early WITS cohort.

A limitation of this study is this use of dual-color flow cytometry. A similar study using 3- or 4-color flow cytometry to evaluate activated CD8⁺ T-cell subpopulations, especially CD8⁺CD38⁺HLA-DR⁺ T cells, is warranted. A second limitation is the inclusion of children who received single or dual nucleoside therapy in the group defined as ILTNPs because this use of antiretroviral therapy likely retarded the rate of CD4⁺ T-cell decrease for some patients. However, after adjusting for monotherapy and dual therapy, the association between CD8 HLA-DR and immunosuppression-free survival remained significant. Given the small number of ILTNPs, our finding needs to be investigated in other pediatric cohorts.

A recent meta-analysis of HIV disease progression in children showed that for children younger than 1 year of age, short-term risk of progression to AIDS remained high, even with CD4⁺ T-cell percentages of greater than 30%.¹⁶ The finding of AIDS-defining conditions in this cohort of ILTNPs mitigates our enthusiasm for recommending the use of a combination of HIV RNA PCR, CD4⁺, and CD8⁺HLA-DR⁺ T-lymphocyte measures for decisions regarding deferral of antiretroviral treatment for HIV-infected infants. However, the infants in the ILTNP group who had AIDS-defining conditions were born in the early 1990s and might not have received trimethoprim-sulfamethoxazole for PCP prophylaxis. It is known that the use of PCP prophylaxis in HIV-infected children has resulted in diminished instances of bacterial infections and PCP, which were the prominent diagnoses in 3 of the 4 ILTNP group children who had AIDS-defining conditions.

In summary, we find that CD8⁺HLA-DR⁺ T-lymphocyte percentages of less than 5% at 1 to 2 months of age might be predictive for ILTNP. If this finding is corroborated, measurement of activated CD8 cells at or around 2 months of age might be helpful in subsequent management of infants and might inform decisions about the timing and intensity of antiretroviral therapy, even though outright deferral of therapy does not appear warranted at

this time because of the clinical outcomes seen here (stage 3 events in 4 children). Early engagement of the activated CD8⁺ T-cell compartment would intuitively seem to be beneficial to the host infected with HIV. But here the exact opposite appears to be true where activation of the CD8⁺ T cells predicts the expected HIV disease progression rather than long-term nonprogression. This apparent immune paradox in HIV infection finds many parallel disease conditions in clinical immunology in which overreaching immunity contributes to disease progression.⁴⁸ Natural history studies of pediatric HIV infection have provided the unique opportunity of better understanding the immunopathogenesis of maternal to infant transmission in the context of a developing immune system in the neonate. This WITS study has focused the spotlight of investigation again on the very first months of life as being critical for the subsequent course of vertically acquired HIV infection. Further study of the use of the CD8⁺HLA-DR⁺ T-lymphocyte subpopulation percentage in early infancy as a way of predicting immunologic long-term nonprogression of HIV disease is clearly warranted.

REFERENCES

- Van de Pere P, Lepage P, Simonon A, Desgranges C, Hitimana DG, Bazubagira A, et al. Biological markers associated with prolonged survival in African children maternally infected by the human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* 1992;8:435-42.
- Grubman S, Gross E, Lerner-Weiss N, Hernandez M, McSherry GD, Hoyt LG, et al. Older children and adolescents with perinatally acquired human immunodeficiency virus infection. *Pediatrics* 1995;95:657-63.
- Martin N, Koup R, Kaslow R, Coffin J, Ammann A. Workshop on perinatally acquired human immunodeficiency virus infection in long-term surviving children: a collaborative study of factors contributing to slow disease progression. *AIDS Res Hum Retroviruses* 1996;12:1565-70.
- Patarca R, Sandler D, Maher K, Hutto C, Martin NL, Klimas NG, et al. Immunological correlates of disease severity in pediatric slow progressors with human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* 1996;12:1063-8.
- Nielsen K, McSherry G, Petru A, Frederick T, Wara D, Bryson Y, et al. A descriptive survey of pediatric human immunodeficiency virus-infected long-term survivors. *Pediatrics* 1997;99:E4.
- Chen Y, Winchester R, Korber B, Gagliano J, Bryson Y, Hutto C, et al. Influence of HLA alleles on the rate of progression of vertically transmitted HIV infection in children: association of several HLA-DR13 alleles with long-term survivorship and the potential association of HLA-A*2301 with rapid progression to AIDS. Long-term survivor study. *Hum Immunol* 1997;55:154-62.
- Salvini F, Scarlatti G, Bossi A, Pinzani R, Zibordi F, Giovanetti C, et al. Follow-up of vertically HIV-1-infected long-surviving children. *AIDS Patient Care STDs* 2001;15:59-65.
- Kline MW, Paul ME, Bohannon B, Kozinetz CA, Shearer WT. Characteristics of children surviving to 5 years of age or older with vertically acquired HIV infection. *Pediatric AIDS HIV Infect* 1995;6:350-3.
- Geffin R, Wolf D, Muller R, Hill MD, Stellwag E, Freitag M, et al. Functional and structural defects in HIV type 1 nef genes derived from pediatric long-term survivors. *AIDS Res Hum Retroviruses* 2000;16:1855-68.
- Rousseau CM, Just JJ, Abrams EJ, Casabona J, Stein Z, King MC, et al. CCR5del32 in perinatal HIV-1 infection. *J Acquir Immun Defic Syndr Hum Retrovirology* 1997;16:239-42.
- Bakshi SS, Zhang L, Ho D, Than S, Pahwa SG. Distribution of CCR5Δ32 in human immunodeficiency virus-infected children and its relationship to disease course. *Clin Diagn Lab Immunol* 1998;5:38-40.
- Misrahi M, Teglas JP, N'Go N, Burgard M, Mayaux MJ, Rouzioux C, et al. CCR5 chemokine receptor variant in HIV-1 mother-to-child transmission and disease progression in children. *JAMA* 1998;279:277-80.
- Buseyne F, Janvier G, Teglas JP, Ivanoff S, Burgard M, Bui E, et al. Impact of heterozygosity for the chemokine receptor CCR5-32-bp-deleted allele on plasma virus load and CD4 T lymphocytes in perinatally human immunodeficiency virus-infected children at 8 years of age. *J Infect Dis* 1998;178:1019-23.
- Romiti ML, Colognesi C, Cancrini C, Mas A, Berrino M, Salvatori F, et al. Prognostic value of a CCR5 defective allele in pediatric HIV-1 infection. *Mol Med* 2000;6:28-36.
- Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. Plasma viral load and CD4⁺ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126:946-54.
- Dunn D, HIV Paediatric Prognostic Markers Collaborative Study Group. Short-term risk of disease progression in HIV-1-infected children receiving no antiretroviral therapy or zidovudine monotherapy: estimates according to CD4 percent, viral load, and age. *Lancet* 2003;362:1605-11.
- Mofenson LM, Korelitz J, Meyer WA 3rd, Bethel J, Rich K, Pahwa S, et al. The relationship between serum human immunodeficiency virus type 1 (HIV-1) RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1-infected children. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *J Infect Dis* 1997;175:1029-38.
- Shearer WT, Quinn TC, LaRussa P, Lew JF, Mofenson L, Almy S, et al. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. *N Engl J Med* 1997;336:1337-42.
- Abrams EJ, Weedon J, Steketee RW, Lambert G, Bamji M, Brown T, et al. Association of human immunodeficiency virus (HIV) load early in life with disease progression among HIV-infected infants. New York City Perinatal HIV Transmission Collaborative Study Group. *J Infect Dis* 1998;178:101-8.
- Palumbo PE, Kwok S, Waters S, Wesley Y, Lewis D, McKinney N, et al. Viral measurement by polymerase chain reaction-based assays in human immunodeficiency virus-infected infants. *J Pediatr* 1995;126:592-5.
- Schrager LK, Young J, Fowler MG, Mathieson B, Vermund SH. Long-term survivors of HIV-1 infection: definitions and research challenges. *AIDS* 1994;8(suppl):S95-108.
- Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J. Guideline for flow cytometric immunophenotyping: A report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. *Cytometry* 1993;14:702.
- Paxton H, Kidd P, Landay A, Giorgi J, Flomenberg N, Walker E, et al. Results of the flow cytometry ACTG quality control program: analysis and findings. *Clin Immunol Immunopathol* 1989;52:68-84.
- Gelman R, Cheng S-C, Kidd P, Waxdal M, Kagan J. Assessment of the effects of instrumentation, monoclonal antibody and fluorochrome on flow cytometric immunophenotyping: a report based on two years of the NIAID DAIDS Flow Cytometry Quality Assessment Program. *Clin Immunol Immunopathol* 1993;66:150-62.
- Rich KC, Brambilla D, Pitt J, Moye J, Cooper E, Hillyer G, et al. Lymphocyte phenotyping in infants: maturation of lymphocyte subpopulations and the effects of HIV infection. *Clin Immunol Immunopathol* 1997;85:273-81.
- Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978;8:283-98.
- Centers for Disease Control and Prevention. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep* 1994;43(No. RR-12):1-10.
- Stites DP, Moss AR, Bacchetti P, Osmond D, McHugh TM, Wang YJ, et al. Lymphocyte subset analysis to predict progression to AIDS in a cohort of homosexual men in San Francisco. *Clin Immunol Immunopathol* 1989;52:96-103.
- Levacher M, Hulstaert F, Tallet S, Ullery S, Pocidalo JJ, Bach BA. The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value. *Clin Exp Immunol* 1992;90:376-82.
- Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K, Detels R. Elevated levels of CD38⁺CD8⁺T cells in HIV infection add to the

- prognostic value of low CD4+ T cell levels; results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J AIDS* 1993;6:904-12.
31. Giorgi JV, Ho HN, Hirji K, Chou CC, Hultin LE, O'Rourke, et al. CD8+ lymphocyte activation at human immunodeficiency virus type 1 seroconversion: development of HLA-DR+CD38-CD8+ cells is associated with subsequent stable CD4+cell levels. *J Infect Dis* 1994;170:775-81.
 32. Mocroft A, Bofill M, Lipman M, Medina E, Borthwick N, Timms A, et al. CD8+CD38+ lymphocyte percent: a useful immunological marker for monitoring HIV-1 infected patients. *J AIDS* 1997;14:158-62.
 33. Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N Engl J Med* 1990;322:166-72.
 34. Fahey JL, Taylor JM, Manna B, Nishanian P, Aziz N, Giorgi JV, et al. Prognostic significance of plasma markers of immune activation, HIV viral load and CD4 T-cell measurements. *AIDS* 1998;12:1581-90.
 35. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, Giorgi JV. CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. *J Acquir Immune Defic Syndr Hum Retrovirology* 1998;18:332-40.
 36. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter for AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *J Acquir Immune Defic Syndr Hum Retrovirology* 1997;16:83-92.
 37. Lederman MM, Valdez H. Immune restoration with antiretroviral therapies: implications for clinical management. *JAMA* 2000;284:223-8.
 38. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narvaez AB, et al. Immune activation set-point during early HIV infection predicts subsequent CD4+ T cell changes independent of viral load. *Blood* 2004; Epub ahead of print.
 39. Resino S, Galan I, Bellon JM, Navarro ML, Leon JA, Munoz-Fernandez MA. Characterizing the immune system after long-term undetectable viral load in HIV-1-infected children. *J Clin Immunol* 2003;23:279-89.
 40. Aldhous MC, Raab GM, Mok JY, Doherty KV, Bird AG, Froebel KS. CD4 and CD8 lymphocytes in diagnosis and disease progression of pediatric HIV infection. *Pediatr AIDS HIV Infect* 1996;7:20-30.
 41. Sherman GG, Scott LE, Galpin JS, Kuhn L, Tiemessen CT, Simmank K, et al. CD38 expression on CD8(+) T cells as a prognostic marker in vertically HIV-infected pediatric patients. *Pediatr Res* 2002;51:740-5.
 42. Paul ME, Shearer WT, Kozinetz CA, Lewis DE. Comparison of CD8(+) T-cell subsets in HIV-infected rapid progressor children versus non-rapid progressor children. *J Allergy Clin Immunol* 2001;108:258-64.
 43. McCloskey TW, Kohn N, Lesser M, Bakshi S, Pahwa S. Immunophenotypic analysis of HIV-infected children: alterations within the first year of life, changes with disease progression, and longitudinal analyses of lymphocyte subsets. *Cytometry* 2001;46:157-65.
 44. Than S, Kharbanda M, Chitnis V, Bakshi S, Gregersen PK, Pahwa S. Clonal dominance patterns of CD8 T cells in relation to disease progression in HIV-infected children. *J Immunol* 1999;162:3680-6.
 45. Plaeger-Marshall S, Isacescu V, O'Rourke S, Bertolli J, Bryson YJ, Stiehm ER. T-cell activation in pediatric AIDS pathogenesis: three-color immunophenotyping. *Clin Immunol Immunopathol* 1994;71:19-26.
 46. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* 2003;112:973-80.
 47. McCloskey TW, Cavaliere T, Bakshi S, Harper R, Fagin J, Kohn N, et al. Immunophenotyping of T lymphocytes by three-color flow cytometry in healthy newborns, children, and adults. *Clin Immunol Immunopathol* 1997;84:46-55.
 48. Shearer WT, Fleisher TA. The immune system. In: Adkinson NF Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER, editors. *Middleton's allergy: principles and practice*. 6th ed. Vol. I. St Louis: Mosby; 2003. p. 1-14.