Predictive Value of Quantitative Plasma HIV RNA and CD4+ Lymphocyte Count in HIV-Infected Infants and Children

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Context.—Pediatric human immunodeficiency virus (HIV) infection has unique viral pathogenetic features that preclude routine extrapolation from adult studies and require specific analysis.

Objectives.—To evaluate the prognostic value of 2 key laboratory markers—plasma RNA and CD4+ lymphocyte count—for HIV disease progression in infants and children and to establish targeted values for optimal outcome.

Design.—Data from a cohort of 566 infants and children who participated in a randomized, placebo-controlled trial of nucleoside reverse transcriptase inhibitors (ACTG 152) were analyzed. The trial was conducted between 1991 and 1995 and enrolled a heterogenous cohort of antiretroviral therapy–naïve children (age, 3 months to 18 years); patients had a median follow-up of 32 months.

Main Outcome Measures.—The trial clinical end points consisted of time to first HIV disease progression (growth failure, decline in neurologic or neurodevelopmental function, opportunistic infections) or death.

Results.—Baseline plasma RNA levels were high (age group medians, 5 × 10^4 to > 10^6 copies/mL), and both baseline RNA and CD4+ lymphocyte count were independently predictive of subsequent clinical course. Risk reduction for disease progression between 49% and 64% was observed for each log_{10} reduction in baseline RNA and CD4+ cell count. Marker values of less than 10,000 copies/mL for plasma RNA and greater than 500 × 10^6 copies/mL (<6.5 years of age) or greater than 200 × 10^6 copies/mL (>6.5 years) for CD4+ cell count were associated with a 2-year disease progression rate of less than 5%.

Conclusions.—Two key laboratory markers—plasma RNA and CD4+ lymphocyte count—are independent predictors of clinical course among HIV-infected infants and children. The linear, age-independent relationship between log_{10} plasma RNA and relative risk of disease progression strongly supports therapeutic efforts to achieve plasma virus levels as low as possible.

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THE TREATMENT of human immunodeficiency virus (HIV) infection with antiretroviral compounds has recently undergone rapid progress. In parallel, the quantitation of plasma viral RNA and CD4+ lymphocytes has become the foundation on which prediction of clinical course and response to therapy is based. Most of this rapid change has resulted from clinical studies conducted in adult populations. Aggressive, multi-drug regimens are currently being introduced into pediatric populations both in clinical practice as well as in controlled trials. While plasma RNA and CD4+ lymphocyte quantitation data are being generated in children, it is unclear whether the experience and guidelines developed for adults will be applicable to infants and children. Special issues that underscore this concern include the presence of a developing immune system at the time of infection followed by high levels of proliferating target cells and immune activation and persistently high levels of plasma virus for extended periods in young children.

This article focuses on analyses using the large plasma RNA and CD4+ lymphocyte database obtained before and during therapy in 566 infants and children who participated in a large treatment trial, ACTG 152. Plasma RNA and CD4+ lymphocyte levels associated with a low risk of disease progression in HIV-infected infants and children are described.

METHODS

ACTG 152 Trial Design

ACTG 152 was a randomized, double-blind, placebo-controlled study that enrolled symptomatic, HIV-infected infants and children between the ages of 3 months and 18 years who were antiretroviral therapy naïve or experienced 6 weeks or fewer of previous therapy. Participants were stratified by age (3-30 months and 30 months to 18 years) and randomized to 1 of 3 treatment arms: zidovudine monotherapy; didanosine monotherapy; or combination therapy (zidovudine plus didanosine). Primary end points were entirely clinical and consisted of time to first HIV disease progression (growth failure; decline in neurologic or neurodevelopmental function; opportunistic infections) or death, occurring on or off study therapy. The zidovudine treatment arm was prematurely unblinded in the spring of 1995 following an interim analysis (data collected through November 16, 1994). The
other 2 treatment arms continued in a blinded fashion through August 31, 1995.

Laboratory Assays

Plasma HIV RNA quantitation was performed using the NASBA HIV-1 RNA QT Amplification System (Organa Teknika Corp, Durham, NC). Plasma samples, which were collected at baseline and every 24 weeks during therapy, were batch tested after study termination. The linear range of the assay, using 100 µL of plasma and calibrators diluted 10-fold, was from 10^2 to 10^6 (or more) copies/mL. Samples in the undetectable range below 10^6 copies/mL were assigned a value of 500 for the analyses. Quantitation of CD4+ lymphocytes was accomplished in real time using standard flow cytometric methods.

Statistical Methods

Baseline characteristics were compared between the analysis cohort and the general trial population by using the Wilcoxon rank sum test for continuous variables and the χ² test for categorical variables. Two-year disease progression-free survival (PFS) rates were estimated using the Kaplan-Meier method. Cox proportional hazards regression models with stratification by study treatment arm were used to assess the prognostic value of RNA concentrations, CD4+ lymphocyte count, and age. Analyses using continuous measures of the markers were undertaken after log₁₀ transformation. Quadratic terms were used to test for departures from linearity in the Cox models. Age was included as a continuous covariate when testing for interaction with marker effect. Clinical follow-up data through study closure (August 31, 1995) were used for the didanosine and combination treatment arms. For the zidovudine monotherapy arm, data collected through November 16, 1994, prior to unblinding, were used. All P values are 2-sided and unadjusted for multiple comparisons.

RESULTS

Baseline Characteristics

This article focuses on an analysis of plasma RNA and CD4+ lymphocyte data collected before and during antiretroviral therapy in 566 (68%) of the 831 infants and children who participated in the ACTG 152 clinical trial. The 265 subjects not participating in this analysis were excluded because baseline plasma specimens were not available. A total of 33 children (6%) received 6 or fewer weeks of zidovudine monotherapy or prophylaxis prior to study entry. There were no significant differences between the analysis cohort and the general trial population for any variable evaluated, including age, sex, median CD4+ lymphocyte count at baseline, and randomized treatment group. The baseline data (Table 1) are presented in 4 age groups for comparison with age-specific normal ranges and with previously published results for the entire cohort. The median plasma RNA concentration at baseline ranged from 1.4 million copies/mL for the 134 children younger than 30 months to 48,000 copies/mL for the 134 children older than 6 years. An age-dependent downward trend in plasma RNA was observed in children aged from 3 months through 6 years, at which age a plateau was reached of between 50,000 and 100,000 copies/mL (Figure 1). Although a relatively large range of plasma RNA values was observed for any given level of CD4+ lymphocyte count, there was a modest but significant correlation between the 2 variables (Spearman correlation range, −0.21 to −0.42).

Prognostic Value of Baseline RNA and CD4+ Lymphocytes

Two-year PFS was assessed within different baseline RNA groups by Kaplan-Meier analyses for the entire cohort and for subsets older than and younger than 30 months (Table 2). The age groupings were chosen to conform with the ACTG 152 trial stratification, while the baseline plasma RNA subdivisions were selected to approximate separation of the data into quartiles. Of the entire cohort with baseline plasma RNA of 50,000 (or fewer) copies/mL, 154 (94%) had no disease progression after 2 years. While the median baseline RNA value for the infants younger than 30 months was 10-fold higher than for older children, similar 2-year PFS rates were observed for a given level of plasma RNA within the range of overlap (Table 2 and Figure 2). Regardless of age group analyzed, a steady decline in PFS was observed with increasing baseline RNA. Cox proportional hazards modeling was used to assess the significance of baseline RNA (log₁₀-transformed) as a predictor of time to clinical disease progression or death in children of all ages. Baseline RNA was a highly significant predictor (P<.001). Age was not a significant predictor in the model with baseline RNA, and no sig-
significant departure from linearity was seen for the RNA effect.

Baseline CD4+ lymphocyte counts were divided into age-specific categories in accordance with current guidelines and practice and to conform with previous ACTG 152 analyses.13,14 Prediction of 2-year PFS by baseline CD4+ lymphocyte count revealed threshold effects that varied with age (Figure 3, A-D). Among the oldest children, 52 (92%) had 2-year PFS for baseline CD4+ cell counts of between 200 and 500 $\times 10^6$/L and 54 (99%) for counts greater than 500 $\times 10^6$/L, compared with 28 children (44%) with baseline counts less than 200 $\times 10^6$/L. For the 30 months to 6 years and 12 to 30 months age groups, high 2-year PFS rates of 97% to 100% (n=101) and 88% to 94% (n=108), respectively, were observed for CD4+ cell counts of more than 500 $\times 10^6$/L, before sharp rate drops occurred. Finally, for the youngest age group, rates of 76% to 82% were observed for baseline CD4+ cell counts higher than 1000 $\times 10^6$/L (n=94), below which 2-year PFS plummeted. In a multivariate Cox proportional hazards model, baseline CD4+ cell count (log10 transformed) and age were both strong predictors of time to disease progression or death ($P<.001$).

Prognostic Value of Laboratory Markers After 24 Weeks of Therapy

Similar analyses of the predictive value of both plasma RNA and CD4+ lymphocyte count for 2-year PFS after 24 weeks of antiretroviral therapy were performed. Since the cohort had aged 6 months from study entry, age groupings for this analysis were increased accordingly (younger than 36 months and 36 months or older) to effectively track the groups established at baseline. A greater than 90% 2-year PFS was observed if plasma RNA fell to less than 10 000 copies/mL, regardless of age group (Table 3). Similar 2-year PFS rates were observed for a given level of plasma RNA within the range of overlap (Figure 2). Week 24 log10 RNA was a significant predictor of time to clinical progression or death ($P<.001$), an effect that was linear and independent of age.

The cohort was separated into 3 groups (9-36 months; 3-6.5 years; and $>6.5$ years), based on the age attained after 24 weeks of therapy, for the analysis using the CD4+ lymphocyte count. Increased risk correlated with decreasing levels of CD4+ lymphocytes after 6 months of therapy, with threshold effects varying with age: 500 $\times 10^6$/L for the 2 youngest age groups and 200 $\times 10^6$/L for children older than 6.5 years. In a multivariate Cox proportional hazards model, the CD4+ cell count (log10 transformed) attained after 24 weeks of therapy and age were both highly significant predictors of time to clinical progression or death ($P<.001$). In addition, the association between age and relative risk was demonstrated to be nonlinear ($P<.001$).

Combined Use of Plasma RNA and CD4+ Lymphocyte Count to Predict Clinical Course and Response to Therapy

Cox proportional hazards models were used to estimate the independent significance of plasma RNA and CD4+ lymphocyte count when considered jointly. Table 4 summarizes the results of 2 models evaluating the laboratory variables at baseline and after 24 weeks of therapy within 2 age strata. All models support the strong, independent predictive value of both plasma RNA levels and CD4+ lymphocyte counts. Risk reductions for disease progression or death of 49% and 64% for infants younger than 30 months and children 30 months or older, respectively, were demonstrated for each log10 decrease in baseline plasma RNA. Similar risk reductions were observed for RNA levels after 24 weeks of therapy. Substantial risk reductions (>50%) were also documented for CD4+ lymphocyte levels of more than 200 $\times 10^6$/L when compared with those less than 200 $\times 10^6$/L (Table 4).

Given that both plasma RNA and CD4+ lymphocyte number had independent predictive value concerning disease progression, their combined use was formally evaluated (Table 5). Threshold values for baseline CD4+ lymphocyte count were chosen based on the previous univariate Kaplan-Meier analyses and were age dependent. Baseline plasma RNA levels were chosen based on proximity to median values for each age group and ranged from 1 000 000 copies/mL for the youngest group to 50 000 copies/mL for the oldest children. The children in the “best” quadrant for each age group, comprising those with the highest CD4+ lymphocyte counts and the lowest plasma RNA levels, had 2-year PFS from 89% to 100% (Table 5). In contrast, 2-year PFS rates from 34% to 57% were observed for children in the highest-risk quadrants.

A similar analysis was performed for children who had received 24 weeks of an-
tiretroviral therapy without reaching an end point. Three discrete age groups were evaluated (Table 6) with a threshold CD4+ lymphocyte count of $500 \times 10^6/L$ chosen for the 2 youngest groups and $200 \times 10^6/L$ for the oldest group. Plasma RNA values of 10 000 copies/mL or less were considered to be “low risk” for children of all ages based on the univariate analyses and previously reported adult guideline figures. This classification resulted in 2-year PFS rates of 96% to 97% for children in the lowest-risk quadrants (lower 95% confidence interval, 88%-92%).

COMMENT

It has not been possible to extrapolate findings and guidelines from adult natural history and therapeutic trials to the pediatric population for 2 important reasons: high plasma levels of virus in infancy persist through much of early childhood, and infection is established in the context of a developmentally immature immune system undergoing differentiation and stimulation. This study delineates risks related to disease progression based on RNA values and CD4+ lymphocyte counts in a pediatric population evaluated prospectively. The baseline RNA results for the 566 infants and children participating in ACTG 152 corroborate and extend previous findings of high, persistent levels of plasma virus. It was not until approximately 6 years of age that members of this cross-sectional cohort reached a group steady state of between 50 000 and 100 000 copies/mL of plasma RNA at baseline. These values are at least 10-fold lower than those observed in infancy and are comparable with established steady-state adult levels.

Studies performed in HIV-infected adults have clearly demonstrated the independent clinical prognostic value of plasma RNA measurements (relative to other immunologic and virologic variables) and have resulted in a succession of clinical guidelines. Most, but not all, of these studies have also documented predictive value for CD4+ lymphocyte enumeration and, most importantly, that the combined use of plasma RNA and CD4+ lymphocyte data is a more powerful approach than using either variable alone. Significant risk reduction for development of the acquired immunodeficiency syndrome or death with decreasing baseline plasma RNA levels have been demonstrated within a series of adult studies. Some of the larger include the ACTG 175 substudy (83% risk reduction per log10 decrease in baseline plasma RNA), the ACTG 241 analysis (56% risk reduction per log decrease in baseline RNA), and the Multicenter AIDS Cohort Study (36% risk...
...cyte count. This is remarkably consistent with a recent study of an intravenous immunoglobulin trial in children in whom the long-term risk of death was reduced 64% for each log₁₀ baseline plasma RNA decrease.¹¹

The unique nature of the evolution of plasma RNA levels throughout infancy and early childhood, compared with the natural history of viral RNA levels in adults, has created uncertainty as to whether the predictive value of RNA levels would be age specific in younger populations. This study has documented a linear relationship between plasma log₁₀ RNA and relative risk for disease progression within defined pediatric age groups. Most interestingly, however, is the observation that the risk associated with a given RNA level is age independent, i.e., driven by the RNA value and not the age of the child. This is consistent with the observation that HIV-infected infants experience a high disease progression rate. Within large pediatric clinical trials, such as ACTG 152 and 300,¹²,¹³ that are composed of a broad range of ages at entry (1-3 months to 18 years), approximately two thirds of clinical endpoints occur disproportionately in the youngest subset of the cohort, specifically those younger than 2.5 to 3 years.¹⁴ The markedly elevated plasma RNA levels observed in infancy, which only gradually decline, are a major risk factor for this increased disease progression rate. The need for investigation into additional virologic and host factors that may influence risk for disease progression is recognized, as there is significant overlap in plasma RNA values between groups of children experiencing disease progression or death and those with PFS.

The linear relationship between plasma log₁₀ RNA level and relative risk for disease progression has 2 related clinical implications. First, it is difficult to assign targeted threshold plasma RNA values below which disease progression falls sharply. Second, the lower the plasma RNA value, either before or after a therapeutic intervention, the lower the relative clinical risk. This study supports the growing concept that antiretroviral drug regimens in children should have the goal of achieving nondetectable viral RNA levels. This analysis was conducted within a treatment study that used modestly effective nucleoside reverse transcriptase inhibitors, reducing viral load a mean of 1.0 log₁₀ or less, resulting in relatively few children with nondetectable plasma RNA.²⁴ Nonetheless, risk for disease progression within 2 years of below 7% was documented for children whose plasma RNA levels were 10,000 (or fewer) copies/mL after 24 weeks of therapy. Clinical reality dictates that not all children will be capable of achieving such low levels of plasma virus, in which case risk can be estimated. The feasibility and clinical outcome of an aggressive pursuit of viral load suppression in children awaits much anticipated clinical trials using potent antiretroviral combinations.

As with the majority of adult studies and 1 pediatric study,¹¹,¹₉,²₁ CD₄⁺ lymphocyte count was documented to possess a realistic challenge for clinicians caring for HIV-infected children, that is, suppressing viral replication to less than...
10,000 copies/mL—ideally to nondetectable levels. Achieving nondetectable RNA levels has a reasonable chance of maintaining an intact immune system. Given the relationship between plasma RNA and risk, as well as its independence from age, this approach to risk assessment is likely to be valid regardless of the therapeutic regimen used. The increasing antiviral potency of drug combinations being used by clinicians and under going evaluation in trial settings will undoubtedly achieve better control, if not complete suppression, of viral replication than what was achieved in ACTG 152. Validation of and modifications to the targeted values delineated here will be necessary as data from future prospective studies become available.

The first 2 authors, Dr Palumbo and Ms Raskino, contributed equally to this article.

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