Gender and race do not alter early-life determinants of clinical disease progression in HIV-1 vertically infected children

European Collaborative Study*

Objective: To identify early life predictors of clinical progression before and beyond age 1 year.

Design: Prospective follow-up of 161 vertically HIV infected children in the ongoing European Collaborative Study provided data from birth over 16 years.

Methods: Kaplan–Meier and Cox regression procedures were used to assess the predictive value of first available laboratory and clinical markers for progression defined as serious disease or death. We investigate gender and race effects on associations and the optimal threshold for longitudinal CD4+ percentage measurements after age 6 months for predicting disease progression.

Results: Earliest (during the first 6 months) measurements of CD4+ percentage below 20% [three-fold increased risk ($P = 0.041$)] and absolute lymphocytes (AL) (reduction of risk of three-quarters for a one log increase ($P = 0.014$)) were independently associated with overall and rapid disease progression during the first year. Persistent lymphadenopathy (or hepatomegaly) in early life was also additionally associated with overall disease progression, and after age 1 year [greater than doubling of risk, ($P = 0.040$)], but not with rapid progression. Associations were not significantly dependent on gender or race. CD4+ percentage of 10% was the best prognostic cut-off.

Conclusions: Early clinical markers are strongly predictive of disease progression after 1 year of age into adolescence. However, rapid progression is less straightforward to predict, probably due largely to early progression during the first few months in such individuals. The independently predictive value of AL measurements suggest they could be used alone in the management of children in resource-poor settings.

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Introduction

Without effective antiretroviral therapy (ART), a quarter of vertically infected children born in developed countries progress to serious disease or death in the first year of life, rising to half by 5 years of age [1]. Immunological and virological factors [2,3] are associated with progression, as are clinical symptoms such as hepatomegaly, splenomegaly, and lymphadenopathy in early life [2,4]. Prematurity may also be associated

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with rapid disease progression in the first year of life [5]. Whether factors associated with early disease progression differ from those associated with disease progression after age 1 year is unknown.

Although age-related virological and immunological levels and patterns in children differed by gender and race in the European Collaborative Study (ECS) [6,7], reported rates of clinical progression in vertically infected children are similar for boys and girls [8]. Whether the risk of progression for specific values of laboratory markers varies by gender or race remains unclear.

Prospective data from the ECS on children born to HIV-infected women, provide a unique opportunity to identify early-life predictors of clinical progression in the first year and thereafter over 16 years, and to address whether differences in laboratory markers by gender and race result in differential risks of progression to serious disease.

Methods

The ECS is an ongoing prospective study with almost 17 years follow-up of a representative cohort of children born to HIV-infected women. Detailed clinical and laboratory information from 11 paediatric centres in eight European countries is collected according to a standard protocol. Infected children are seen at birth, 3 and 6 weeks, 3, 4.5, 6, 9, 12, 18 and 24 months and at least twice yearly thereafter [9]. Parental consent is obtained; the study is approved by local ethics committees. HIV infection status was identified by the onset of AIDS, detection of virus or antigen in at least two separate blood samples and/or persistence of antibody beyond 18 months of age.

Severe disease progression is defined by CDC category C, or death [10]; rapid disease progression is defined as occurring within the first year of life. Children were classified as severely premature if born at or before 34 weeks of gestation, moderately premature between 34 and 37 weeks and full-term beyond 37 weeks. Antiretroviral treatment was categorized into monotherapy or combination (two or more) ART. Categories were updated at each visit, allowing children to be reassigned if the number of drugs prescribed increased. Black children in this cohort are born to mothers from sub-Saharan Africa.

Laboratory tests were carried out locally. Venous blood specimens were anticoagulated using ethylenediaminetetraacetic acid or heparin and processed within 24 h. An automated haemocytometer was used to obtain absolute lymphocyte (AL) counts and subtypes of white blood cells. Tests were based on flow cytometry (FACSCAN) with Becton-Dickinson (BD Biosciences, San Jose, California, USA) antibodies. CD4+, CD8+ cell count and AL measurements were $\log_{10}$ transformed. CD4+ cell counts were also expressed as a percentage of AL count. From exploratory analysis of the distribution of CD4+ percentage, values above or below 20% gave the best categorization in the first 6 months, with lower values being appropriate for later in life.

With the exceptions of an investigation of gender- and race-specific effects and assessment of a threshold for CD4+ percentage over all ages preceding disease progression, this article chiefly explores the early-life determinants of serious clinical disease progression or death. The predictive values of first available, pre-disease progression, log CD4+ cell count, AL count and CD4+ percentage, presence of hepatomegaly, splenomegaly and lymphadenopathy (and specifically axillary node enlargement) at two or more visits during the first 6 months of life were estimated. The main effects of gender, prematurity, race, neonatal prophylactic ART on risk of progression, and modification by gender and race on the effects of the early life biological indicators were assessed. Further, as the difference in CD4+ count levels by gender increases from age 1 year onward, measurements at age two (above or below 500 cells [10]) were assessed for effects on disease progression specific to boys and to girls; similarly, as levels of CD4+ percent reportedly differ consistently for black and white children after early life, measurements above or below 15% [10] were assessed for race-specific effects. In this observational study, the effect of ART given before disease progression could not be assessed, but use was accounted for throughout.

With widespread use of neonatal zidovudine and initiation of ART early in life (for prevention of vertical transmission and disease progression respectively) in recent years, disease progression and HIV-related death in the ECS has been almost entirely restricted to those born before 1997 [1]. We therefore consider only children born before 1997 but include follow-up information until September 2002. HIV-RNA viral load assays have only become routinely available since 1997, and consequently, there are few available measurements early in life for those who have progressed.

Statistical methods

The overall survivor function was obtained by Kaplan–Meier analysis. Cox proportional hazards modelling [11] was performed to estimate survival allowing for covariates. ART treatment was included as a time-dependent variable. Candidate terms for the multivariable model included ART treatment and factors reaching significance at the 15% level in univariate models. Comparisons between models were performed

using likelihood ratio tests to identify the optimum model. The relative predictive capabilities of the various terms in the final model were determined by changes in the deviance, excluding each in turn, where a large difference indicates substantial contribution to the model. All survival analyses were performed using STATA (STATA Version 7.0; College Station, Texas, USA).

In a separate analysis, classification trees [12] were used to detect the most predictive threshold for longitudinal CD4+ percentage measurements at any age after the first 6 months of life prior to disease progression. This was carried out using S-PLUS 2000 (Insightful, Seattle, Washington, USA) in a Windows environment.

Results

Laboratory measurements and clinical information from 5209 visits were available for all 161 infected children in the ECS born before 1997 (Table 1). By 1 year of age, an estimated 17.7% [95% confidence interval (CI), 12.6–24.6%] of children will have progressed to serious disease or death; increasing to 36.3% (95% CI, 29.2–44.4%) by age 5, and 44.1% (95% CI, 36.3–52.7%) by 10 years. Overall, similar numbers of girls [33 of 76, (43%)] and boys [32 of 85 (38%)] progressed. There were some differences by race with 52 of 113 (46%) white, 10 of 32 (31%) black and only three of 16 (23%) of the other (mainly Asian) children progressing ($P = 0.065$), which reflects later enrolment of children of non-white ethnicity when ART use was increasingly widespread [9].

Overall disease progression and early immunological and clinical measurements

First laboratory measurements were taken at a mean age of 1.5 months (range, birth–5.8 months). The median first CD4+ percentage for rapid progressors was 35.0% [interquartile range (IQR), 24.6–43.5], substantially lower than the 43.1% (range, 36.5–54.5%) for children progressing to serious disease or death beyond 1 year of age. Univariably, first available log AL count and first CD4+ percentage (Fig. 1) predicted subsequent progression at any point during follow-up, but first available log CD4+ and CD8+ cell count did not (Table 2). The risk of progression to serious disease or death was associated with hepatomegaly and lymphadenopathy observed at two or more occasions, but not with axillary node enlargement or with splenomegaly. Progression did not significantly differ by gender, race, prematurity or neonatal ART prophylaxis (Table 2).

As maternal CD4+ cell counts were only recorded routinely in participating centres since 1995, there was a limited subset [24 of 161 (15%)] of mothers with available measurements at delivery and multivariable analysis was thus not possible. In univariable analysis a log increase in maternal CD4+ count was compatible with a 75% reduction in risk of progression for the child ($P = 0.171$).

Table 1. Laboratory and clinical data on the first 6 months of life for 161 infected children born before 1997.

Factor	Progressors n = 65 (40%)	Non-progressors n = 96 (60%)	Total n = (161)
First available log CD4+ cell count median (IQR)	3.32 (3.18–3.51)	3.41 (3.28–3.5)	3.39 (3.25–3.51)
First available log CD8+ cell count median (IQR)	3.21 (3.08–3.29)	3.15 (2.97–3.32)	3.19 (2.99–3.30)
First available absolute lymphocyte count median (IQR)	3.74 (3.66–3.88)	3.81 (3.70–3.90)	3.78 (3.68–3.89)
First available CD4 percentage median (IQR)	41.7 (29.0–49.9)	41.4 (32.8–50)	41.4 (30.2–50.0)
Hepatomegaly (observed at two or more visits within first 6 months)			
Yes	49 (75.4%)	83 (86.5%)	132 (82.0%)
No	16 (24.6%)	13 (13.5%)	29 (18.0%)
Splenomegaly (observed at two or more visits within first 6 months)			
Yes	58 (89.2%)	85 (88.5%)	143 (88.8%)
No	7 (10.8%)	11 (11.5%)	18 (11.2%)
Lymphadenopathy (observed at two or more visits within first 6 months)			
Yes	53 (81.5%)	85 (88.5%)	138 (85.7%)
No	12 (18.5%)	11 (11.5%)	23 (14.3%)
Enlarged axillary nodes (observed at two or more visits within first 6 months)			
Yes	53 (81.5%)	79 (82.3%)	132 (82.0%)
No	12 (18.5%)	17 (17.7%)	29 (18.0%)
ART treatment ever before progression			
No	46 (70.8%)	22 (22.9%)	68 (42.2%)
Monotherapy	16 (24.6%)	7 (7.3%)	23 (14.3%)
Combination	3 (4.6%)	67 (69.8%)	70 (43.5%)
Median age (years) at last visit (IQR)	3.7 (1.4–7.1)	9.5 (6.4–11.7)	7.1 (3.1–11.0)
Median number of visits (IQR)	13 (7–80)	20 (13–69)	18 (11–80)

IQR, inter-quartile range; ART, antiretroviral therapy.

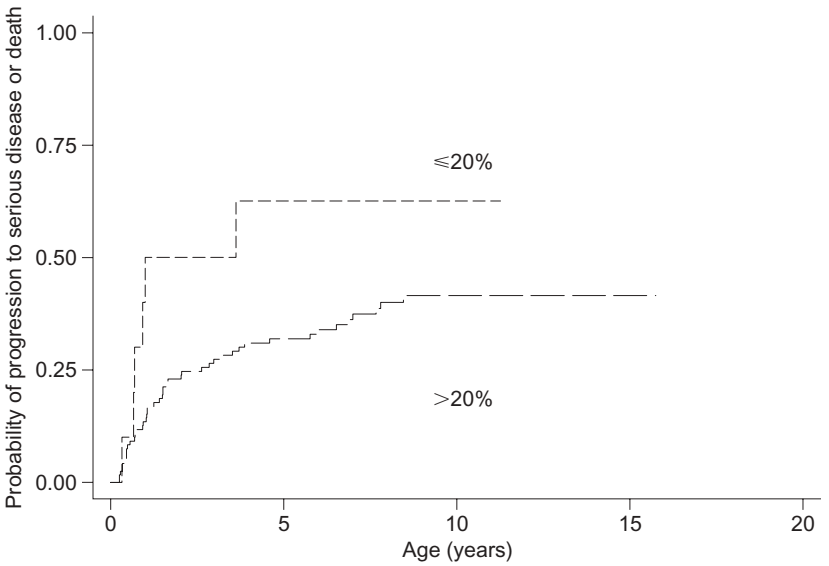

Fig. 1. Kaplan–Meier survival plot of progression to Centers for Disease Control clinical category C or death by CD4+ percentage category in the first 6 months of life.

Table 2. Overall progression to serious disease or death by infant and maternal factors.

	Univariable		Multivariable[a]	
Factor	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Gender				
Girls	Reference			
Boys	1.18 (0.73, 1.91)	0.506		
Prematurity				
Full-term	Reference			
Moderately premature	1.32 (0.71, 2.43)	0.377		
Severely premature	1.44 (0.67, 3.09)	0.353		
Race				
White	Reference			
Black	0.67 (0.34, 1.31)	0.240		
Zidovudine administered neonatally[b]				
Yes	0.19 (0.03, 1.37)	0.100		
No	Reference			
First available log CD4+ cell count (per log increase)	0.44 (0.11, 1.79)	0.253		
First available log CD8+ cell count per log increase)	0.96 (0.29, 3.18)	0.953		
First available log absolute lymphocyte count (per log increase)	0.31 (0.10, 0.99)	0.049	0.233 (0.074, 0.741)	0.014
First available CD4 percentage				
< 20%	2.15 (0.85, 5.48)	0.107	2.79 (1.04, 7.46)	0.041
⩾ 20%	Reference		Reference	
Hepatomegaly (observed at two or more visits)[b]				
No	Reference			
Yes	1.83 (1.07, 3.12)	0.028		
Splenomegaly (observed at two or more visits)				
No	Reference			
Yes	1.05 (0.48, 2.31)	0.896		
Lymphadenopathy (observed at two or more visits)				
No	Reference			
Yes	1.76 (0.99, 3.13)	0.052	1.883 (1.015, 3.493)	0.045
Enlarged axillary nodes (observed at two or more visits)				
No	Reference			
Yes	1.27 (0.67, 2.39)	0.460		
Treatment				
No	Reference		Reference	
Monotherapy	1.75 (0.93, 3.32)	0.084	1.495 (0.732, 3.054)	0.270
Combination	0.61 (0.16, 2.29)	0.466	0.504 (0.121, 2.091)	0.345
Maternal CD4+ count at delivery(per log increase)	0. 274 (0.043, 1.746)	0.171		

[a]Final model: $\chi^2 = 17.51$; $P = 0.0036$. [b]Candidate for final model but not subsequently significant. CI, confidence interval.

Gender- and race-specific effects of clinical and immunological markers

In analyses of models including terms representing interactions of gender and race with each of the clinical and immunological measurements, effects on disease progression of early immunological markers and clinical indicators were not modified by gender or race ($P > 0.3$, all tests for gender, $P > 0.5$, all tests for race). In the subset of 22 children who had not progressed to serious disease or death by age 2 years and had available CD4+ measurements at that age, a CD4+ count below 500×10^6 cells/l at age 2 years was associated with a greater risk of clinical progression for boys than for girls although the difference was not statistically significant. [hazard ratio (HR) = 3.65 and 2.62, respectively, $P = 0.521$]. A CD4+ percentage of less than 15% was associated with greater progression risk in black children than white, but again, not statistically significantly so (HR = 8.88 and 1.31, respectively, $P = 0.213$).

Simultaneous predictive value of early clinical and immunological variables

In multivariable analysis allowing for ART (Table 2), children with a first CD4+ percentage below 20% had nearly a three-fold increased risk of progression to serious disease or death compared with those with a value above 20% ($P = 0.041$). A log (ten-fold) increase in first AL count independently reduced risk of progression by 77% ($P = 0.014$) and lymphadenopathy at two or more visits within the first 6 months of life increased risk by 88% ($P = 0.045$). A CD4+ percentage above or below 20% was substantially more predictive of disease progression than AL count or lymphadenopathy (deviance differences = 81.4, 5.2 and 3.2, respectively). For example, keeping other variables constant [no early persistent lymphadenopathy and an AL count of 6000 cells (3.78 in log units), say] and accounting for treatment, compared with a child with an early CD4+ percentage above 20% (with a risk of progression by age 5 years of 25%), one with a CD4+ percentage value below 20% were 2.79 times more likely to progress (with a risk of progression by age 5 years of 70% ($2.79 \times 25\%$). Hepatomegaly (HR = 1.885; $P = 0.035$; Model $\chi^2 = 17.32$; $P = 0.0039$) and lymphadenopathy had similar and interchangeable, but not independent predictive effects. Allowing for trimethoprine sulfamethoxazol *Pneumoncystis carinii* pneumonia (PCP) prophylaxis and for the use of intravenous immunoglobulin before disease progression did not significantly alter estimates.

Threshold for CD4+ percentage

Using all clinical status and CD4+ percentage data beyond 6 months of age, classification tree analysis was used to investigate the optimal value for splitting the CD4+ percentage measurements into two groups in terms of predicting subsequent disease progression. At any age, the threshold value of 10% for CD4+ percentage (HR = 4.83; 95% CI, 2.63–8.86; $P < 0.0001$) was found to best predict progression to serious disease or death.

Rapid progression

Of the 65 children who progressed to serious disease or died, 28 (43%) did so within the first year of life. The majority (19 of 28, 68%) had opportunistic infections, mostly PCP (12 of 19, 63%); four had encephalopathy; two serious bacterial infections and the remaining three died at home probably of an undiagnosed opportunistic infection. The predictive value of a log increase in first CD4+ cell count was significant and reduced rapid progression risk by 89% (HR = 0.11; 95% CI, 0.02–0.71; $P = 0.021$); a first CD4+ percentage below 20% was associated with a four-fold increase in risk (HR = 4.16; 95% CI, 1.66–10.39; $P = 0.002$). The reduction in risk of rapid progression with a log increase in first AL measurement was 81% (HR = 0.19; 95% CI, 0.04–0.87; $P = 0.003$). Prematurity was not associated with rapid disease progression ($P = 0.813$). In a multivariable model also including ART, first CD4+ percentage below 20% and log AL count were independently predictive of rapid disease progression or death (HR = 6.62; 95% CI, 2.35–18.66; $P < 0.001$ and HR = 0.11; 95% CI, 0.39–98, $P = 0.012$, respectively). Neither gender nor race modified effects of any early laboratory or clinical markers. Adjusting for TMP-SMX and intravenous immunoglobulin prophylaxis did not alter risk estimates.

Progression to serious disease after 1 year of age

The remaining 37(57%) children progressed to serious disease after age 1 year: 10 (27%) with encephalopathy, nine (24%) with serious recurrent bacterial infections, 13 (35%) with opportunistic infections (only three of which were PCP); four with another C-defining illness and one died of unspecified HIV-related causes. In univariable analysis, early persistent hepatomegaly (HR = 2.22; 95% CI, 1.11–4.46; $P = 0.024$) and early persistent lymphadenopathy (HR = 2.16; 95% CI, 1.01–4.61; $P = 0.048$) were associated with progression beyond the first year. Severe prematurity was marginally associated with late disease progression (HR = 2.24; 95% CI, 0.91–5.54; $P = 0.080$). In multivariable analysis, adjusting for ART, persistent hepatomegaly (HR = 2.15; 95% CI, 1.03–4.47; $P = 0.040$) was the single significant independent indicator of disease progression after age 1 year. Separately, persistent lymphadeopathy was associated with a doubled, but not statistically significant risk (HR = 2.01; 95% CI, 0.90–4.46; $P = 0.087$). Adjustment for TMP-SMX and IVIG did not impact on estimates.

Discussion

In this cohort of children born and followed up in

Europe, early life measures of CD4+ percentage below 20% and a ten-fold increase in AL counts were independently associated with clinical progression throughout childhood, and with rapid disease progression or death within the first year of life. Early persistence of lympadenopathy and hepatomegaly additionally predicted subsequent overall progression to serious disease and progression beyond 1 year of life, but not rapid progression. CD4+ percentage was more informative than any other laboratory or clinical indicator. However, for a given value of clinical or immunological marker early in life or around age 2 years, disease progression did not differ by gender or race. Less than half of this cohort received combination therapy before disease progression, but differentiating between double and other combination therapies in the treatment adjustment had no bearing on the results (data not shown). With adjustment for ART, results inform knowledge about underlying mechanisms of vertically-acquired disease progression and thus remain relevant in the HAART era.

These findings enhance previous work addressing the prognostic value of early markers of disease progression and death in the early years in life. However, earlier studies used measurements relatively close to progression [13,14], or cross-sectional data from clinical trials [15–17]. Studies with longitudinal data from birth have been limited to shorter follow-up [3,18,19], have focused on limited laboratory determinations [2,20–23] or have involved too few children to draw reliable conclusions [22]. Whether predictive values of given markers vary by gender or race has not been previously explored. We have been able to consider an extensive period of follow-up, providing a largely natural account of both short- and long-term paediatric disease progression and predictors.

Although clinical evidence of infection in the first 6 months of life, such as lymphadenopathy and hepatomegaly, are not associated with subsequent rapid progression before age 1 year, they are predictive of long-term prognosis. Hepatomegaly was the only factor associated with disease progression beyond age 1 year when assessed separately. These findings appear to contradict those of Rich *et al* [2] who found early presence of lymphadenopathy, hepatomegaly or splenomegaly with CD4+ percentage predicted rapid progression. However, their approach differed methodologically: first, rapid progression was defined as occurring during the first 6 months of life only; second, clinical progression preceding laboratory determinations in some children was nonetheless included and third, their statistical methods involved only logistic models with presence or absence of progression as a binary response. That early immunological factors were predictive of progression before 1 year of age but not beyond in our cohort is consistent with earlier observations of levels reflecting current status more than long-term survival and wellbeing [3].

We recently described significant differences in immunological patterns over 12 years by gender and race [6]. However, these differences were small in the first year, which agrees with our findings here that effects of early life markers on overall disease progression are not dependent on gender or race. Despite the magnitude of differences in immunological patterns becoming larger as children get older [7], we did not find gender differences in progression for a given CD4+ count or race differences in progression for a given CD4+ percentage at age 2 years. This may have been due to small numbers.

A CD4+ percentage cut-off of 10% best identified the risk of disease progression beyond 6 months of age, which is lower than the 15% threshold in the CDC categories below which children are classified as severely immunosuppressed [10]. The CDC clinical and immunological classification systems have been shown to be in poor agreement [1,25]. The high degree of overlap in lymphocyte measurements in uninfected and infected children [6] could explain the necessity for more extreme limits in predicting serious progression.

Because of limited numbers of infected children and of mothers with relevant data, we were unable to investigate effects of other factors possibly associated with disease progression in vertically infected children such as maternal ART use [5,20,26], timing of transmission [23], p24 antigen [17], maternal viral load [2,27], and vitamin A [2]. Our findings of higher maternal CD4+ lymphocytes associated with lower risk of disease progression, although not significant, are compatible with findings of others [2,28].

We were also unable to assess the child's HIV RNA viral load as a marker for disease progression directly here, but the association between clinical indicators such as lymphadenopathy and hepatomegaly and viral activity indicates that early viral load measurements, when available, would be useful prognostic indicators, as has been shown by others [3,17,19]. It has been suggested that virus load is the optimal predictor of paediatric HIV progression [3], however, the relative stability of immunological markers, especially CD4+ percentage, compared to the highly variable HIV RNA viral load levels [7], may make them as clinically relevant in predicting serious disease progression. We were unable to confirm an association of either overall or rapid disease progression with prematurity [5] which could be due to small numbers of infants born very prematurely.

The findings here and elsewhere [29–31] imply that in

the absence of specific CD4+ cell assays, AL measurements alone would provide sufficient insight to inform management of individual children after the first few months of life, which could be particularly relevant in less developed countries where laboratory resources may be limited.

Generally in this cohort, early progression was due to opportunistic infections whereas progression later in life was more dominated by encephalopathy and bacterially-related illnesses. Our findings indicate that CD4+ percentages and AL counts could inform prevention management of PCP and similar morbidities, whereas occurrence of early persistent hepatomegaly could alert the need for measures to prevent bacterial infections and encephalopathy. This is in line with findings from an American birth cohort study which suggested encephalopathy was more likely following development of early symptoms of HIV [32].

This extension of knowledge about the associations of early-life clinical and immunological factors with rapid and long-term progression to serious disease or death informs the understanding of the dynamics of vertically-acquired HIV infection.

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References

1. European-Collaborative-Study, Gray L, Newell ML, Thorne C, Peckham C, Levy J. **Fluctuations in symptoms in human immunodeficiency virus-infected children: the first 10 years of life.** *Pediatrics* 2001; **108**:116–122.
2. Women and Infants Transmission Study Group, Rich K, Fowler MG, Mofenson LM, Abboud R, Pitt J, *et al*. **Maternal and infant factors predicting disease progression in human immunodeficiency virus type 1-infected infants.** *Pediatrics* 2000; **105**:e8.
3. Women and Infants Transmission Study Group, Kalish LA, McIntosh K, Read JS, Diaz C, Landesman SH, *et al*. **Evaluation of human immunodeficiency virus (HIV) type 1 load, CD4 T cell level, and clinical class as time-fixed and time-varying markers of disease progression in HIV-1-infected children.** *J Infect Dis* 1999; **180**:1514–1520.
4. European Collaborative Study. **Children born to women with HIV-1 infection: natural history and risk of transmission.** *Lancet* 1991; **337**:253–260.
5. de-Souza RS, Gomez-Marin O, Scott GB, Guasti S, O'Sullivan MJ, Oliveira RH, *et al*. **Effect of prenatal zidovudine on disease progression in perinatally HIV-1-infected infants**. *J Acquir Immune Defic Syndr* 2000; **24**:154–161.
6. European Collaborative Study. **Are there gender and race differences in cellular immunity patterns over age in infected and uninfected children born to HIV-infected women?.** *J Acquir Immune Defic Syndr* 2003; **33**:635–641.
7. European Collaborative Study. **Level and pattern of HIV-1-RNA viral load over age: differences between girls and boys?** *AIDS* 2002; **16**:97–104.
8. Morris CR, Araba-Owoyele L, Spector SA, Maldonado YA. **Disease patterns and survival after acquired immunodeficiency syndrome diagnosis in human immunodeficiency virus-infected children**. *Pediatr Infect Dis J* 1996; **15**:321–328.
9. European Collaborative Study. **HIV-infected pregnant women and vertical transmission in Europe since 1986.** *AIDS* 2001; **15**:761–770.
10. Centers For Disease Control. **1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age.** *MMWR* 1994; **43**(RR12):1–10.
11. Andersen P, Gill R. **Cox's regression model for counting processes, a large sample study.** *Ann Stat* 1982; **10**:1100–1120.
12. Breiman L., Friedman J.H., Olshen R.A., Stone CJ. *Classification and Regression Trees*. Monterey: Wadsworth and Brooks/Cole, 1984.
13. 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: a meta-analysis.** *Lancet* 2003; **362**:1605–1611.
14. CASCADE Collaboration. **Short-term risk of AIDS according to**

the current CD4 count and viral load in antiretroviral naive individuals and those treated in the monotherapy era. *AIDS* 2004; **18**:51–58.
15. Palumbo PE, Raskino C, Fiscus S, Pahwa S, Fowler MG, Spector SA, *et al.* **Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children.** *JAMA* 1998; **279**:756–761.
16. Dickover RE, Dillon M, Gillette SG, Deveikis A, Keller M, Plaeger-Marshall S, *et al.* **Rapid increases in load of human immunodeficiency virus correlate with early disease progression and loss of CD4 cells in vertically infected infants.** *J Infect Dis* 1994; **170**:1279–1284.
17. Mofenson LM, Harris DR, Rich K, Meyer WA, Read JS, Moye JJ, *et al.* **Serum HIV-1 p24 antibody, HIV-1 RNA copy number and CD4 lymphocyte percentage are independently associated with risk of mortality in HIV-1-infected children. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group.** *AIDS* 1999; **13**:31–39.
18. Tetali S, Bakshi S, Than S, Pahwa S, Abrams E, Romano J, *et al.* **Plasma virus load evaluation in relation to disease progression in HIV-infected children.** *AIDS Res Hum Retroviruses* 1998; **14**:571–577.
19. 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**:101–108.
20. Kuhn L, Abrams EJ, Weedon J, Lambert G, Schoenbaum EE, Nesheim SR, *et al.* **Disease progression and early viral dynamics in human immunodeficiency virus-infected children exposed to zidovudine during prenatal and perinatal periods.** *J Infect Dis* 2000; **182**:104–111.
21. Women and Infants Transmission Study Group, Shearer WT, Quinn TC, LaRussa P, Lew JF, Mofenson L. *et al.* **Viral load and disease progression in infants infected with human immunodeficiency virus type 1.** *N Engl J Med* 1997; **336**:1337–1342.
22. Salvatori F, Masiero S, Giaquinto C, Wade CM, Brown AJ, Chieco-Bianchi L, *et al.* **Evolution of human immunodeficiency virus type 1 in perinatally infected infants with rapid and slow progression to disease.** *J Virol* 1997; **71**:4694–4706.
23. Dickover RE, Dillon M, Leung KM, Krogstad P, Plaeger S, Kwok S, *et al.* **Early prognostic indicators in primary perinatal human immunodeficiency virus type 1 infection: importance of viral RNA and the timing of transmission on long-term outcome.** *J Infect Dis* 1998; **178**:375–387.
24. Hall AJ, Yee LJ, Thomas SL. **Life course epidemiology and infectious diseases.** *Int J Epidemiol* 2002; **31**:300–301.
25. The French Pediatric HIV Infection Study Group and European Collaborative Study, Blanche S, Newell ML, Mayaux MJ, Dunn DT, Teglas JP, *et al.* **Morbidity and mortality in European children vertically infected by HIV-1.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **14**:442–450.
26. The Italian register for HIV Infection in Children. **Rapid disease progression in HIV-1 perinatally infected children born to mothers receiving zidovudine monotherapy during pregnancy.** *AIDS* 1999; **13**:927–933.
27. New York City Perinatal HIV Transmission Collaborative Study Group, Lambert G, Thea DM, Pliner V, Steketee RW, Abrams EJ, *et al.* **Effect of maternal CD4+ cell count, acquired immunodeficiency syndrome, and viral load on disease progression in infants with perinatally acquired human immunodeficiency virus type 1 infection.** *J Pediatr* 1997; **130**:890–897.
28. Diaz C, Hanson C, Cooper ER, Read JS, Watson J, Mendez HA, *et al.* **Disease progression in a cohort of infants with vertically acquired HIV infection observed from birth: the Women and Infants Transmission Study (WITS).** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **18**:221–228.
29. Kumarasamy N, Mahajan AP, Flanigan TP, Hemalatha R, Mayer KH, Carpenter CC, *et al.* **Total lymphocyte count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India and other resource-constrained countries.** *J Acquir Immune Defic Syndr* 2002; **31**:378–383.
30. Mofenson L, Harris DR, Bethel J, Moye J, Read J, Meyer W, *et al.* **Second tier surrogate markers for use in resource-limited settings: association of total lymphocyte count and immune-complex dissociated p24 antigen with mortality in HIV-infected children.** *Tenth Conference on Retroviruses and Opportunistic Infections*, Boston, February 2003.
31. Badri M, Wood R. **Usefulness of total lymphocyte count in monitoring highly active antiretroviral therapy in resource-limited settings.** *AIDS* 2003; **17**:541–545.
32. Women and Infants Transmission Study Group, Cooper ER, Hanson C, Diaz C, Mendez H, Abboud R, *et al.* **Encephalopathy and progression of human immunodeficiency virus disease in a cohort of children with perinatally acquired human immunodeficiency virus infection.** Women and Infants Transmission Study Group. *J Pediatr* 1998; **132**:808–812.

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