Thymic Dysfunction and Time of Infection Predict Mortality in Human Immunodeficiency Virus–Infected Infants


The effect of human immunodeficiency virus (HIV)–induced thymic dysfunction (TD) on mortality was studied in 265 infected infants in the CDC Perinatal AIDS Collaborative Transmission Study. TD was defined as both CD4 and CD8 T cell counts below the 5th percentile of joint distribution for uninfected infants within 6 months of life. The 40 HIV-infected infants with TD (15%) had a significantly greater mortality than did the 225 children without TD (44% vs. 9% within 2 years). Infants with TD infected in utero had higher mortality than did those infected intrapartum (70% vs. 37% within 2 years), while no significant difference was noted between infants without TD with either mode of transmission. The TD profile was independent of plasma virus load. Virus-induced TD by particular HIV strains and the time of transmission are likely to explain the variation in pathogenesis and patterns of disease progression and suggest the need for early aggressive therapies for HIV-infected infants with TD.

Thymopoiesis is the basic process for the production of mature naive T lymphocytes to populate the lymphoid system, and it is most active during the earlier parts of life [1]. Several investigators in our group recently reported [2] that 17 of 59 human immunodeficiency virus (HIV)–infected infants, who progressed to AIDS and died very rapidly, had an immunophenotypic profile early in life similar to that found in children with severe congenital thymic defect (DiGeorge anomaly) [3].

Our earlier report [2] examined a relatively small number of HIV-infected children from one medical center in Atlanta. The purpose of the present study was to extend this analytical approach to a larger population of 265 perinatally HIV-infected children prospectively followed by the 10 centers comprising the CDC Perinatal AIDS Collaborative Transmission Studies (PACTS) group. We also wished to refine the definition of the thymic dysfunction (TD) profile by determining the earliest age at which such infants could be reliably identified. In addition, the interrelationship of TD with other factors known to influence disease progression (timing of transmission [4] and virus load [5]) was also examined. Validating these observations could provide useful prognostic markers that may guide early therapy with intensive antiviral, and possible immune restoration, strategies.

Materials and Methods

Study population. The CDC PACTS group has enrolled and followed prospectively children born to HIV-infected mothers in centers located in New York City, Newark (New Jersey), Baltimore, and Atlanta since 1985. Of 1454 children enrolled by November 1996, 265 (18%) were found to be HIV-infected according to established criteria [6]. Polymerase chain reaction (PCR) test results in the first week of life, available for 113 of the infected children, were used to determine the presumptive timing of transmission. Only mortality associated with HIV, as determined by the clinical provider, was included in the analyses. All of the clinical and epidemiologic data were entered into a central computer at CDC, as described elsewhere [6].

Definition of the TD profile. Flow cytometric determinations of CD3, CD4, and CD8 T lymphocytes were obtained in the five laboratories associated with the PACTS group by use of conventional methods [7]. The joint distribution of CD4 and CD8 lymphocytes among the 1189 HIV-exposed but uninfected PACTS children, who had at least one immunophenotypic determination in the first 6 months of life, was used to establish the thresholds for TD characterization. As in the previous study, presence of the TD profile was defined as having both CD4 and CD8 cell counts below the 5th percentiles of the joint distribution graph in the 1189 HIV-exposed but noninfected control children. In case of multiple im-
munophenotypic studies from an individual available during this period, the last measurement of CD4 and CD8 T lymphocyte counts was used [2]. In addition, threshold values derived from joint distribution analyses from each of the first 6 months of life were also used to determine TD status at different ages and to ascertain the earliest time this approach might prove reliable for TD identification.

*HIV load assay.* Plasma HIV RNA was quantified by the nucleic acid sequence–based assay (Organon Teknika, Boxtel, Netherlands) following the manufacturer’s instructions.

*Statistical methods.* After censoring observations at loss to follow-up or non-HIV-related death, univariate times to HIV-related mortality were described with Kaplan-Meier estimates; corresponding comparisons between groups were made with generalized Wilcoxon test statistics. Cox proportional hazards modeling was used to evaluate the independent prognostic value of the TD profile, adjusted for early PCR positivity, year of birth, and sex. All *p* values are unadjusted for the number of comparisons made, but conclusions regarding associations are based on adjustment for this multiplicity. Standard descriptive statistics were used to characterize virus load, and differences between groups were compared by Kruskal-Wallis one-way analysis of variance.

**Results**

*Population characteristics.* On the basis of cut points established from figure 1 (1814/mm³ for CD4 and 904/mm³ for CD8 cells), 40 (15%; 95% confidence interval [CI], 11%–19%) of the HIV-infected children were classified as having TD and 225 (85%) as not having TD. There were no differences between HIV-infected children with or without the TD profile (*p* > .05) with regard to year of birth, race, sex, gestational age at birth, or proportion with available positive PCR results obtained within the first week of life. Likewise, maternal CD4 cell count and stage of maternal disease at the time of birth, as well as the proportion of mothers who received zidovudine during gestation, did not differ significantly between groups.

*Creditation of the TD profile with HIV-related mortality.* The risk of HIV-related death was 28% within 1 year and 44% within 2 years in infants with TD compared with 4% and 9%, respectively, in infants without TD (*p* < .001). To assess the relative prognostic significance of the CD4 and CD8 components, infants without TD were also compared (figure 2) according to whether they had both CD4 and CD8 cell counts

![Figure 1](image_url)  
Figure 1. Joint distribution of CD4 and CD8 T lymphocyte counts in first 6 months of life among 1189 HIV-exposed but uninfected infants. When result from >1 immunophenotypic test was available in first 6 months of life, last counts were used for comparison with previous study [5]. 5th percentiles for both CD4 and CD8 T cell counts are shown (1814/mm³ and 908/mm³, respectively).
Figure 2. Kaplan-Meier estimates of time (in years) to HIV-related death according to whether neither CD4 nor CD8 cell count dropped below cut point of 1814 or 904/mm³, respectively (A); only CD8 cell count dropped below cut point of 904/mm³ (B); only CD4 cell count dropped below cut point of 1814/mm³ (C); both CD4 and CD8 cell counts were below cut points obtained in first 6 months of life (D), i.e., TD+ profile.

>1814 and >904/mm³; CD8 cell counts <904 and CD4 cell counts >1814/mm³; and CD4 cell counts <1814 and CD8 cell counts >904/mm³. It can be noted that mortality within the first year was more closely related to decreases in CD8 lymphocytes than in CD4 lymphocytes. The mortality rate in the group with TD was much higher than that of the group with either CD4 or CD8 cell depression alone. The differences were even greater by 2 or 3 years of age. Thus, the method we used for identifying TD, which takes into account both CD4 and CD8 cell counts, confers a better prognostic marker of rapid death than either low CD4 or CD8 cell counts alone.

Table 1 presents in detail the 2-year Kaplan-Meier survival rates of infants according to the presence or absence of the TD profile during each of the first 6 months of life. On the basis of the 5th percentile values of noninfected infants tested at each month, the cut points of CD4 and CD8 cell counts for defining the TD profile were found to be very similar after the first postnatal month. The difference in the 2-year survival rates between the groups with and without TD were significant (P < .05) in all of the age groups older than 1 month.

Correlation of HIV-related mortality with PCR positivity in the first week of life. Of the 113 infected infants who had PCR testing within the first week of life, the result was positive for 47 and negative for 66. The risk of HIV-related death was 18% within 1 year and 24% within 2 years in the PCR-positive infants, compared with 5% and 10%, respectively, in PCR-negative infants (P = .02). Stratifying children with and without TD according to early PCR findings provided four subgroups of HIV-infected patients, whose survival is presented in figure 3. The worst prognosis occurred in children with TD who had positive early PCR results (1- and 2-year mortality of 52% and 70%, respectively), followed by the children with TD with negative early PCR results (1- and 2-year mortality of 25% and 37%, respectively). There was no significant difference observed between children without TD with positive or negative early PCR results. PCR-positive children without TD had a 2-year mortality of 10%, while PCR-negative children without TD had a 2-year mortality of 4%. Similar analysis based on CD4 cell counts indicates that lower counts (<1814/mm³) within 6 months of age were of greater prognostic value.
Thymic Dysfunction in HIV-Infected Infants

Figure 3. Kaplan-Meier plots of time (in years) to HIV-related death according to both presence of TD+ profile and polymerase chain reaction (PCR) results obtained within first week of life.

than were early positive PCR results (data not shown). The combination of low CD4 cell counts and early positive PCR results was associated with a 2-year mortality risk of 46%, which was significantly lower than that found in infants with TD and early PCR positivity (70%). Proportional hazards modeling allowed evaluation of the relative hazard of each of various factors on HIV-related mortality. The presence of TD was the most significant independent risk factor for HIV-related death (hazard ratio, 7.8; P < .001), followed by positive PCR test results in the first week of life (hazard ratio, 3.1; P = .01). When adjusted for PCR and TD profile, such variables as year of birth, sex, and gestational age did not have a significant impact on the risk of HIV-related death (P ≥ .05).

Virus load data were available for 32 samples from infected infants in the first week of life and 22 samples from infants between 1 and 2 months of life. No significant difference in HIV RNA copies/mL of plasma was detected between children with and without TD (P = .4 and P = .7, respectively).

Discussion

This report demonstrates, in a large cohort of prospectively followed HIV-infected children, that markers of TD are predictive of survival outcome in perinatal HIV infection. The cut points for CD4 and CD8 lymphocyte subsets for defining the TD profile, based on results obtained in five laboratories, were remarkably consistent with those derived from the previous study with a much smaller cohort [2]. TD was found to occur in 15% (95% CI, 11%–19%) of infants perinatally infected with HIV. This virus-induced defect, independent of virus loads, appeared to be largely responsible for the rapid progression to death in a subset of infected children previously noted by other investigators [8–11].

Our findings implicating the thymic involvement offer a convincing explanation for some of the differences in disease progression patterns between adult and pediatric AIDS. The previous suggestion that the immune system of a child is
immature, and thus cannot respond to the infection as vigorously as does the adult system, is not supported by data indicating initial strong cellular and humoral responses in many HIV-infected children [12] or in fetuses or newborns infected with many other agents [13]. The bimodal distribution of pediatric AIDS progression, indicating that many more children follow the adults’ pattern [14, 15] than follow the very rapid progression found in a subset of children [8–11], also casts doubt on age alone being the major factor. On the other hand, the single most significant difference between the pediatric and adult immune systems relates to the thymus. The fetal or neonatal thymus is pivotal in providing the developing lymphoid system with naive T cells, unlike the adult thymus, which is involuting after the lymphoid system has already been fully populated [1]. Thymic destruction would thus have a totally different impact on the integrity of the pediatric versus adult immune system. Whether the fetal, neonatal, or adult thymus is affected appears to depend on the particular HIV strains, which have been found to differ in their ability to infect the thymic epithelium and/or interfere with thymopoiesis [16–19]. Thymic infection also offers a likely explanation for the significantly different effects observed associated with the timing of transmission [4]. In utero infection caused by a virus with high affinity for the thymus would have the most impact on CD4 lymphocyte depletion, as it would have affected the thymus shortly after the initiation of thymopoiesis, around the 10th week of gestation. Since the human lymphoid system would have been partially populated with lymphocytes at birth, disruption of the thymic microenvironment by similar strains of HIV transmitted intrapartum would have less, though still severe, impact. HIV strains that do not affect the thymus, even if acquired in utero, would have only a slightly higher depletion of CD4 cells due to relatively longer exposure to virus-induced postthymic lymphocyte destruction, compared with intrapartum infection. Indeed, our data suggest that the effect of in utero transmission on disease progression is apparent only when the TD profile is also present. A model of HIV disease progression based on these observations has recently been developed [12].

The implications of the current study are of clinical and therapeutic significance. We have demonstrated that the combination of TD profile, determined as early as 2 or 3 months of age, with early positive PCR results obtained soon after birth offers the most powerful prognostic indicator for HIV-related mortality. Early aggressive combination therapies appear to be most needed for infants with such profiles or other markers [5, 20–22] predictive of rapid disease progression. Children with the TD profile may also represent prime candidates for immune restoration modalities, such as thymic transplantation [23].

### Table 1. Two-year survival by TD status in each month of life.

<table>
<thead>
<tr>
<th>Month</th>
<th>CD4/CD8 cell cutoffs</th>
<th>Group (n)</th>
<th>2-year survival</th>
<th>P*</th>
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<tr>
<td>1</td>
<td>1492/754 TD+ (5)</td>
<td>100%</td>
<td>.25</td>
<td></td>
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<tr>
<td></td>
<td>TD+ (90)</td>
<td>85%</td>
<td></td>
<td></td>
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<td>1967/1059 TD+ (12)</td>
<td>74%</td>
<td>.04</td>
<td></td>
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<td></td>
<td>TD+ (100)</td>
<td>91%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1845/936 TD+ (8)</td>
<td>50%</td>
<td>.02</td>
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<tr>
<td></td>
<td>TD+ (64)</td>
<td>84%</td>
<td></td>
<td></td>
</tr>
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<td>4</td>
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<tr>
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<td>TD+ (79)</td>
<td>96%</td>
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<tr>
<td>5</td>
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<td>61%</td>
<td>.001</td>
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<tr>
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<td>TD+ (79)</td>
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<td>6</td>
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<tr>
<td></td>
<td>TD+ (62)</td>
<td>96%</td>
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* Determined by Wilcoxon test.

### References