R-117

Volumetric Computed Tomography (CT) of the Thymus and Parameters of Thymopoiesis in Adolescent and Adult Survivors of Perinatal HIV Infection

Paul Krogstad[1], Joseph Church[2], Marvin Belzer[2], Jaime Deville[1], Karin Nielsen[1], Christina Kitchen[3], Jason Lee[1], Stephanie Weston[1], Yongzhi Geng[1], and M Ines Boechat[1]

[1]David Geffen School of Medicine at UCLA, [2]Childrens Hospital and Keck School of Medicine at Univ of Southern California, Los Angeles, USA; and [3]Univ of California, Los Angeles, School of Public Health, USA

pkrogstad@mednet.ucla.edu Ph. 310 825 5235

Abstract

Background: Prior to 1996, potent ART was unavailable to children with perinatal infection. We hypothesized that unchecked HIV replication in early childhood may have lead to disruption of the thymic architecture that cannot be fully reversed by therapy. We therefore examined computed tomography (CT)-rendered thymic volume and parameters of thymopoiesis in clinically stable adolescents and young adults with HIV infection that was acquired perinatally, or by neonatal transfusion. Seronegative young adults served as controls for this analysis.

Methods: We enrolled 25 adolescents and adults with HIV infection acquired perinatally (n = 21) or by transfusion in infancy (n = 4); 15 (60%) had CDC class C disease. All were receiving HAART; 5 had plasma HIV RNA levels between 400 and 7500 copies/mL; and 20 had a viral load < 400 copies/mL for at least 1 year. Non-contrast CT of the thorax using 3-mm collimation was obtained, with volumetric analysis subsequently performed on a 3D imaging workstation. Whole blood antibody staining and flow cytometry were used to quantify CD4, CD8, and naïve CD45RA+CD27+CD4+ T-cell populations. T-cell receptor recombination excision circles (TREC) in total PBMC were quantified by real time PCR.

Results: The HIV-infected subjects (n = 25) were slightly younger than control subjects (n = 16) (mean age 18 [SD 1.4] vs 20 years [SD 1.4], p = 0.0015) but had very similar thymus volume (20.2 [SD 13.1] vs 15.4 [SD 6.09] mL (p = ns). CD4+ T-cell number and percentage were higher in the seronegative group (37.8%, 694 cells/µL vs 25.3%, 552 cells/µL), but the perinatally infected youths had mean TREC and naïve CD4+ T-cells values that were statistically indistinguishable from the control group. The number and percentage of total and naïve CD4 T-lymphocytes were highly correlated with thymic volume in the control group. In the HIV+ youth, TREC concentrations were strongly

Laboratory Methods

Cell Staining and Flow cytometry. Whole blood cell staining was performed using CD3-conjugated fluorescein isothiocyanate (FITC), CD8-conjugated phycoerythrin (PE), and CD4-conjugated allophycocyanin (APC) antibodies according to the manufacturer's (BD Biosciences, San Jose, CA) instructions. Flow cytometric analysis was performed using FACSCalibur flow cytometer and the results were analyzed using CellQuest Software (BD Biosciences).

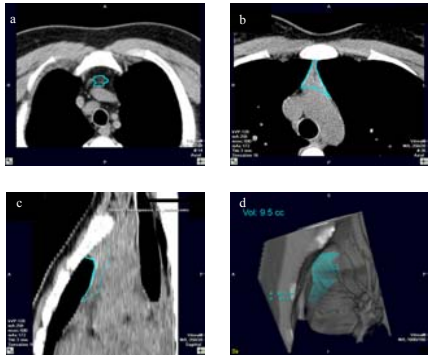
Quantitation of T cell receptor recombination excision circles (TREC). TREC were quantified by real time PCR (Halnon 2005, Pham 2003). A standard curve was established with 25 copies up to 1,000,000 copies of a plasmid containing the signal joint TREC fragment (provided by D. Douek). Cellular DNA was quantified by amplifying CCR5 sequences. Using an estimate of 8 µg of DNA per million
cells, TREC numbers are reported as TREC/million PBMC (or million purified CD4+ T cells).

HIV Viral Loads. HIV plasma RNA concentrations were measured at UCLA and CHLA using Amplicor HIV-1 Monitor testing, with a limit of sensitivity of 50 copies/ml (Roche Diagnostics, Indianapolis, ID).
Statistical Analysis. Analyses were conducted using SAS 8.2 (SAS, Inc., Cary, NC) software to examine data from the TREC assays on cell lysates from PBMC. Continuous variables were compared using a two-sided Wilcoxon Rank Sign Test. Categorical variables were compared using Fisher's exact test. Univariate analyses utilized SAS PROC CORR to calculate simple descriptive statistics and Pearson correlation coefficients for selected laboratory marker results. Ordinal logistic regression was used to analyze categorical outcomes.

Imaging Methods

Study Participants. We examined thymic volume and lymphocyte parameters in a subset of 25 HIV infected patients followed at the Adolescent Medicine Clinic at Children's Hospital Los Angeles (CHLA), the Clinical Immunology Service at CHLA, and the Care 4 Kids Clinic at UCLA (MCIC). These patients all had evidence of HIV infection since childhood by antibody methods, and had plasma HIV RNA levels of 10,000 copies/ml for at least one year, while receiving multidrug HAART. We also recruited healthy adolescents and young adults (n = 18) by advertising in the Los Angeles area, and confirmed that they were HIV uninfected by standard ELISA antibody screening methods. Informed consent for study participation was obtained in accordance with approvals from the Institutional Review Board at each institution.

Volumetric Imaging. Non-contrast helical CT of the chest was acquired from the thoracic inlet to the lung bases, with 3 mm collimation images provided for review. The CT slices containing the superior and inferior margins of the thymus were determined by two radiologists (J.C.L. and I.M.B.) who were blinded to the identity and HIV disease status of the patient. The thymic borders for these two CT slices and all intervening CT slices were determined and outlined using a free-hand region-of-interest (ROI) tool using the Vitrea 2 (Vital Images, Plymouth, MN) software running on special 3D imaging workstation. Surface rendering and Vitrea 2 built-in volume calculation were subsequently performed (1b-c).
As described by McCune et al., a thymic index categorizing overall thymic appearance was also scored to help determine the extent of true thymus and amount of fatty infiltration. It is based on a grading scale of 0 to 5:
0 – No soft tissue, with thymus entirely replaced by fat
1 – Minimal soft tissue, barely recognizable
2 – Minimal soft tissue, more obvious
3 – Moderate soft tissue
4 – Moderate soft tissue of greater extent, almost mass like



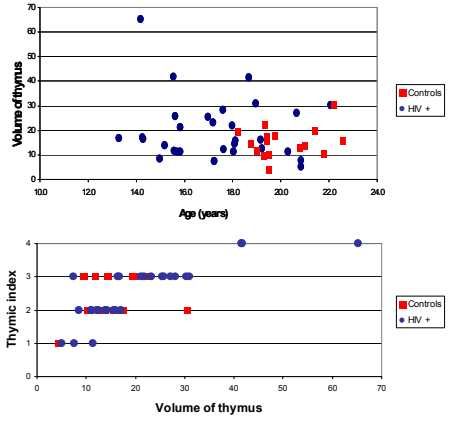
Sample Images.
a,b: Axial slices from upper thymus in a normal patient. The blue-colored outline traces the margin of the thymus.
c: Surface rendering is performed after region of interest is delineated in axial contiguous slices.
d: Volume calculation can be generated from Vitrea 2 after a surface is determined.

Patient Data

	Seronegative (n=16)	HIV Infected Youth* (n=25)	P value
Age	20.1 (1.3)	17.5 (2.5)	.0015
Gender	12 F/ 4M	10 F/15M	NS
% CD4+	37.9	26.1	.0003
CD4+ cells/µl	694	571	NS
% CD8+	442	853	.0005
CD8+ cells/µl	25.8	37.6	.0002
% CD8+ HLA-DR- CD38+	84.8 (7.3)	67.3 (20.1)	.0134
Thymus volume	15.4 (6.1)	20.3 (13.2)	NS
%/# Naïve CD4 (CD45RA+ CD27+)	44.1/320	49.0/304	NS/NS
TREC/million PBMC	10,403	12,126	NS

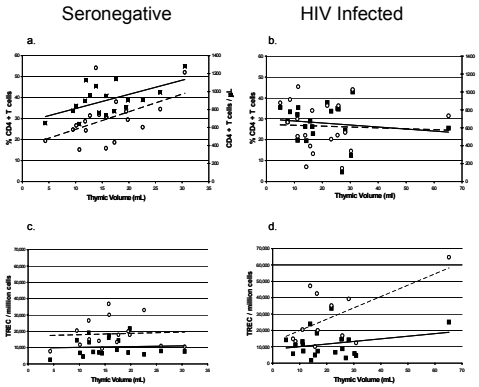
*This includes 20 patients who had had undetectable viral load (<400 copies/ml) for at least one year prior to study. All were receiving 3 or 4 drug antiretroviral therapy.

Thymic volume vs Age or Thymic Index



0 – No soft tissue, with thymus entirely replaced by fat
1 – Minimal soft tissue, barely recognizable
2 – Minimal soft tissue, more obvious
3 – Moderate soft tissue
4 – Moderate soft tissue of greater extent, almost mass

Thymic volume vs. TREC/ CD4 T cell Counts



a, b: circles represent the percentage of CD4+ lymphocytes, and squares the absolute number of CD4+ T lymphocytes in peripheral blood.
c,d. Squares: TREC/million CD4+ T cells. Circles: TREC/million peripheral blood mononuclear cells. Regression lines are shown for correlations for % CD4 T cells (broken line) and CD4 T cells/ml (solid line).
Significant correlations were noted between thymic volume and: CD4/µl (p=0.034) and %CD4 (p=0.012) in uninfected subjects, and between thymic volume and TREC/million CD4+ T cells in HIV infected (p=0.049).

Conclusions

Despite lifelong HIV infection, perinatally infected youth with successful and sustained ART may reach adulthood with essentially normal parameters of thymopoiesis.

There is a correlation between thymic volume and T cell counts in HIV negative young adults.

References

1. Steinmann GG. Changes in the human thymus during aging. Curr Top Pathol 1986;75:43-88.
2. Flores KG, Li J, Sempowski GD, Haynes BF, Hale LP. Analysis of the human thymic perivascular space during aging. J Clin Invest 1999;104:1031-9.
3. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature. 1998 Dec 17;396(6712):690-5
4. McCune JM, Loftus R, Schmidt DK, et al. High prevalence of thymic tissue in adults with human immunodeficiency virus-1 infection. J Clin Invest 1998;101:2301-8.
5. Vigano A, Vella S, Saresella M et al.Early immune reconstitution after potent antiretroviral therapy in HIV- infected children correlates with the increase in thymus volume. Aids 2000;14:251-61.
6. Haynes BF, Markert ML, Sempowski GD, Patel DD, Hale LP. The role of the thymus in immune reconstitution in aging, bone marrow transplantation, and HIV-1 infection. Annu Rev Immunol. 2000;18:529-60
7. Jamieson BD, Douek DC, Killian S, et al. Generation of functional thymocytes in the human adult. Immunity. 1999 May;10(5):569-75
8. Halnon NJ, Jamieson B, Plunkett M, Kitchen CM, Pham T, Krogstad P. Thymic function and impaired maintenance of peripheral T cell populations in children with congenital heart disease and surgical thymectomy. Pediatr Res. 2005 Jan;57(1):42-8.
9. Pham T, Belzer M, Church JA, et al. Assessment of thymic activity in human immunodeficiency virus-negative and -positive adolescents by real-time PCR quantitation of T-cell receptor rearrangement excision circles. Clin Diagn Lab Immunol. 2003 Mar;10(2):323-8.