Association of hepatitis B infection in patients with HIV Encephalopathy


Received for publication, August 20, 2012
Accepted, December 15, 2012

PAUL MARINESCU¹, LOREDANA SABINA CORNELIA MANOLESCU²*
¹MD, PhD, assistant professor of virology, Public Health Direction, Giurgiu, Romania,
²MD, HIV/AIDS coordinator for Giurgiu County, Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology, Bucharest, Romania.
*Corresponding author: Loredana Sabina Cornelia Manolescu, MD PhD, Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology, 285 Mihai Bravu, Bucharest, 030304, Romania, tel: 0040723699253; fax:0040213242590, manolesculoredana@yahoo.com
Public Health Direction: (postal address: strada Bucuresti nr. 82, Giurgiu, Romania
Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology: postal address: 285 Mihai Bravu, Bucharest, 030304, Romania

Abstract

Objectives: Human immunodeficiency virus (HIV) can affect the central nervous system and determine HIV encephalopathy (HE). Evidence of hepatitis B virus (HBV) was found in cerebrospinal fluid in HIV co-infected patients. Here we assessed the degree of association between HBV infection and prognosis of HE in a large cohort of 462 HIV infected patients over a ten years period and the role of nadir CD4 cell count.

Materials and methods: HIV encephalopathy, HBV infections markers, HIV RNA and CD4 cell were measured and retrospectively analyzed.

Results: The prevalence of HE was 22.7%. More than half, 50.4% of the patients with HE presented HBV infection. Among the fifty three patients that presented at the same time HE and HBV infection and prognosis of HE in a large cohort of 462 HIV infected patients over a ten years period and the role of nadir CD4 cell count.

Materials and methods: HIV encephalopathy, HBV infections markers, HIV RNA and CD4 cell were measured and retrospectively analyzed.

Results: The prevalence of HE was 22.7%. More than half, 50.4% of the patients with HE presented HBV infection. Among the fifty three patients that presented at the same time HE and HBV infection, more than half, 66.03%, were first infected with HBV and then developed HE. It is possible that HBV infection is a risk factor for developing of HE. Further studies are needed to prove the HBV neurotropic potential.

Conclusions: The prognosis of HE was not significantly different in HBV presence or under antiretroviral treatment. Absolute CD4 nadir count and class C3 are proved to be strong predictors of HE in HIV infected patients even after several changes in antiretroviral therapy schemes.

Key words: Class C3, HBV infection, HIV, HIV encephalopathy (HE), nadir CD4

1. Introduction

Human immunodeficiency virus (HIV) can affect the central nervous system (CNS) in the early stages of disease and determine HIV encephalopathy (HE), a complex syndrome with cognitive, motor, and behavioral features (K. GOODKIN & al. [1], W.G. BRADLEY & al. [2]). Sometimes in untreated patients, HE is a part of the acute HIV syndrome. In children treated with highly active antiretroviral treatment (HAART), HE may be infrequent and largely reversible (K. GOODKIN & al. [1]. While the main risk factors associated with HE are well known and include low weight, anemia, low CD4+ count, high plasma HIV-RNA load, hepatitis C virus (HCV) infection and female gender (Y. STERN & al. [3], J.C. MCARTHUR & al. [4], X. LIU & al. [5]), no association with hepatitis B virus (HBV) infection was made.

Hepatitis B virus infection is an important cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide (W.H. SHENG & al. [6]). HBV was found in different extra hepatic sites and the presence of HBV-DNA in CSF of HIV co-infected patients was
previously reported (S. RUTA & al. [7]). Still there is little evidence regarding the neurotropic character of this virus.

There are 10,903 people living with HIV/AIDS in Romania, according to the data provided by Compartment for Monitoring and Evaluation of HIV/AIDS Data, National Institute of Infectious Diseases Prof. Dr. Matei Bals, as of 31st of December 2011. Currently, Romania has a large number of survivors, integrated in the 19-24 age group, who belong to the 1987-1990 cohort (≥6000). Almost 10% of them are currently living in the same region, the Giurgiu County (CNLAS [8]).

Here, we assessed the degree of association between hepatitis B virus infection and prognosis of HIV encephalopathy in a large cohort of HIV-infected patients with common epidemiological particularities which have experienced multiple therapeutic schemes over time. We undertook a critical review of the available evidence concerning whether the nadir CD4 cell count predicts neurocognitive impairment in HIV infected patients after several changes in antiretroviral therapy schemes.

2. Materials and methods

2.1. Ethics.

This research was approved by the ethical review board of the Infectious Disease Hospital from Giurgiu County.

2.2. Study participants.

Data from a cohort of 462 HIV infected individuals from Giurgiu County, Romania, was retrospectively analyzed at different points in time. These patients were born in poor families or abandoned in foster care during the period from 1987 to 1990 and were probably infected in the same way and probably during the same period of time, parenterally, by horizontal route. The main inclusion criteria for selecting these patients from Giurgiu Infectious Diseases Hospital’s database were HIV infection and the year of birth, making our cohort very homogeneous. The majority of patients were HIV diagnosed from 1989 to 1999 but there were 35 patients that were diagnosed later between 2000 and 2005. The HBV diagnosis was performed from 1996 to 2006. The patients were clinically evaluated in day clinic from Giurgiu Infectious Disease Hospital.

First CD4 and HIV RNA evaluations were performed in 1995 for those already diagnosed and which started at that time antiretroviral (ARV) therapy. Patients diagnosed after 1995 have been evaluated at the time of HIV diagnose and if eligible they started ARV therapy. As ARV drugs developed worldwide they were administered to Romanian patients so their ARV therapy changed along with the improvement of those drugs and according with the need of the patients. From the initial group 33.76% patients died until 2006.

2.3. Quantitative CD4 and HIV RNA Parameters.

HIV RNA and absolute CD4 cell count were measured in all the participants enrolled in the study. Absolute CD4 cell count has been performed at least twice a year and HIV RNA once a year up to now starting with 1995.

From 1995 and until 2005 absolute CD4 cell count was determined from fresh whole blood samples using the TRITEST three-color reagent CD4/CD8/CD3 with TRU-COUNT tubes (BECTON & DICKINSON, USA). Plasma HIV RNA levels were measured from fresh blood specimens using the ROCHE AMPLICOR, version 1.5 (dynamic range, 400–750 000 copies/mL) manufactured by ROCHE DIAGNOSTIC CORPORATION, USA.
Starting with 2005 absolute CD4 cell count was determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens using the COBAS AMPLIPREP/COBAS TAQMAN HIV-1 TEST (dynamic range, 20–10 000 000 copies/mL) manufactured by ROCHE DIAGNOSTIC CORPORATION, USA. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest. Because the dynamic ranges of these assays differed, for combined analyses, we assigned all HIV RNA measurements > 500 000 copies/mL as a value of 500 001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

2.4. **Criteria for HIV encephalopathy**

HE was defined as the presence, for at least 2 months, of at least one of the following progressive findings in a pediatric patient with no concurrent illness, other than HIV infection, that could explain the findings:
- Failure to attain, or loss of, developmental milestones or loss of intellectual ability verified by standard developmental or neuropsychological tests (Binet-Simon, Portage, Raven, Kids Scid and International HIV Dementia Scale)
- Acquired microcephaly as demonstrated by head circumference measurement or brain atrophy on serial computed tomography (CT) or magnetic resonance imaging (MRI) in children younger than 2 years
- Acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance (NOMENCLATURE [9]).

In our studied cohort HE was not diagnosed in adult patients.

2.5. **HBV Infection Markers**

The presence of hepatitis B surface antigen (HBsAg) and total antibody against hepatitis B core antigen (anti HBc) were tested by immunoenzymatic assays (MUREX BIOTECH LIMITED; KENT, ENGLAND) in all patients. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest.

2.6. **Statistical Analyses**

Differences in HIV RNA levels and absolute CD4 cell count was evaluated by Fisher's exact test analysis of contingency tables and chi-square test, using GraphPad Prism 5.0. Two-tailed $P$ values were reported. $P < .05$ was considered statistically significant. Mean values were compared using unpaired $t$ test. For scientific significance confidence intervals (CI) were established. $T$ test was performed for categorical variables and $F$ test for continuous variables.

3. **Results**

3.1. **Cohort Characteristics**

There were a total of 462 analyzed HIV infected patients. Out of them 156 deceased, median age at death 10 years (limits 7-16). These patients were not treated with any ARV regimen. 197 patients, median age at last investigation 17 years (limits 15-18), were followed up only until 2006 due to several reasons such as: change of residence, lack of adherence or enrollment in other AIDS centers. 109 patients, median age at last investigation 23 years (limits 21-24), were followed up until the end of 2011 (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic. The initial cohort was balanced with regard to sex but differed substantially with...
Starting with 2005 absolute CD4 cell count was determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens using the COBAS AMPLIPREP/COBAS TAQMAN HIV-1 TEST (dynamic range, 20–10,000,000 copies/mL), manufactured by ROCHE DIAGNOSTIC CORPORATION, USA. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest. Because the dynamic ranges of these assays differed, for combined analyses, we assigned all HIV RNA measurements > 500,000 copies/mL as a value of 500,001 copies/mL, and we assigned measurements < 400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

2.4. Criteria for HIV encephalopathy

HE was defined as the presence, for at least 2 months, of at least one of the following progressive findings in a pediatric patient with no concurrent illness, other than HIV infection, that could explain the findings:

- Failure to attain, or loss of, developmental milestones or loss of intellectual ability verified by standard developmental or neuropsychological tests (Binet-Simon, Portage, Raven, Kids Scid and International HIV Dementia Scale)
- Acquired microcephaly as demonstrated by head circumference measurement or brain atrophy on serial computed tomography (CT) or magnetic resonance imaging (MRI) in children younger than 2 years
- Acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance (NOMENCLATURE [9]).

In our studied cohort HE was not diagnosed in adult patients.

2.5. HBV Infection Markers

The presence of hepatitis B surface antigen (HBsAg) and total antibody against hepatitis B core antigen (anti HBc) were tested by immunoenzymatic assays (MUREX BIOTECH LIMITED; KENT, ENGLAND) in all patients. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest.

2.6. Statistical Analyses

Differences in HIV RNA levels and absolute CD4 cell count was evaluated by Fisher's exact test analysis of contingency tables and chi-square test, using GraphPad Prism 5.0. Two-tailed $P$ values were reported. $P < .05$ was considered statistically significant. Mean values were compared using unpaired $t$ test. For scientific significance confidence intervals (CI) were established. $T$ test was performed for categorical variables and $F$ test for continuous variables.

3. Results

3.1. Cohort Characteristics

There were a total of 462 analyzed HIV infected patients. Out of them 156 deceased, median age at death 10 years (limits 7-16). These patients were not treated with any ARV regimen. 197 patients, median age at last investigation 17 years (limits 15-18), were followed up only until 2006 due to several reasons such as: change of residence, lack of adherence or enrollment in other AIDS centers. 109 patients, median age at last investigation 23 years (limits 21-24), were followed up until the end of 2011 (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic.

The initial cohort was balanced with regard to sex but differed substantially with
Starting with 2005 absolute CD4 cell count was determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens using the COBAS AMPLIPREP/COBAS TAQMAN HIV-1 TEST (dynamic range, 20–10 000 000 copies/mL), manufactured by ROCHE DIAGNOSTIC CORPORATION, USA. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest. Because the dynamic ranges of these assays differed, for combined analyses, we assigned all HIV RNA measurements > 500 000 copies/mL as a value of 500 001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

2.4. Criteria for HIV encephalopathy

HE was defined as the presence, for at least 2 months, of at least one of the following progressive findings in a pediatric patient with no concurrent illness, other than HIV infection, that could explain the findings: - Failure to attain, or loss of, developmental milestones or loss of intellectual ability verified by standard developmental or neuropsychological tests (Binet-Simon, Portage, Raven, Kids Scid and International HIV Dementia Scale)
- Acquired microcephaly as demonstrated by head circumference measurement or brain atrophy on serial computed tomography (CT) or magnetic resonance imaging (MRI) in children younger than 2 years
- Acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance (NOMENCLATURE [9]).

In our studied cohort HE was not diagnosed in adult patients.

2.5. HBV Infection Markers

The presence of hepatitis B surface antigen (HBsAg) and total antibody against hepatitis B core antigen (anti HBc) were tested by immunoenzymatic assays (MUREX BIOTECH LIMITED; KENT, ENGLAND) in all patients. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest.

2.6. Statistical Analyses

Differences in HIV RNA levels and absolute CD4 cell count was evaluated by Fisher's exact test analysis of contingency tables and chi-square test, using GraphPad Prism 5.0. Two-tailed \( P \) values were reported. \( P < .05 \) was considered statistically significant. Mean values were compared using unpaired \( t \) test. For scientific significance confidence intervals (CI) were established. \( t \) test was performed for categorical variables and \( F \) test for continuous variables.

3. Results

3.1. Cohort Characteristics

There were a total of 462 analyzed HIV infected patients. Out of them 156 deceased, median age at death 10 years (limits 7-16). These patients were not treated with any ARV regimen. 197 patients, median age at last investigation 17 years (limits 15-18), were followed up only until 2006 due to several reasons such as: change of residence; lack of adherence or enrollment in other AIDS centers. 109 patients, median age at last investigation 23 years (limits 21-24), were followed up until the end of 2011 (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic.

The initial cohort was balanced with regard to sex but differed substantially with
regard to level of immune status and age. In the deceased group the majority, 62.87% were male participants. Many of the ARV treated patients have begun therapy since 1995 and experimented between one and six antiretroviral regimens until the end of 2011. The therapeutic regimens prescribed over time were: 2NRTIs (for at least 24 months), 3NRTIs (6 months), 2NRTIs+1NNRTI (17 months), 1NRTI+1NRTI+1PI (22 months), 2NRTIs+2PIs (48 months). All patients received regimens containing NRTIs (2 or 3) and PI (1 or 2), during the last 12 months and some patients received NNRTI along with NRTI and PI in their regimen. Administered regimens included drugs that penetrate CNS such as stavudine, abacavir, nevirapine, and zidovudine. About 40% of the studied patients received these drugs: 63.8% of the patients with HE and 33% of the patients without HE, \( p=0.0006 \).

Table 1. Cohort characteristics at last investigation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total of patients 462</th>
<th>Deceased ARV naive 156</th>
<th>Lost from evidence ARV treated 197</th>
<th>ARV treated 109</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females, no (%)</td>
<td>204 (48%)</td>
<td>58 (37.1%)</td>
<td>112 (56.8%)</td>
<td>61 (55.9%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17 (7-24)</td>
<td>10 (7-16)</td>
<td>17 (15-18)</td>
<td>23 (21-24)</td>
</tr>
<tr>
<td>CD4 count (cells/µL)</td>
<td>242 (0-1652)</td>
<td>73 (0-1040)</td>
<td>379 (5-1652)</td>
<td>495.5 (3-1250)</td>
</tr>
<tr>
<td>HIV RNA Level Log_{10}</td>
<td>3.31 (2.6-5.7)</td>
<td>5.5 (3.5-5.7)</td>
<td>2.9 (2.6-5.7)</td>
<td>2.6 (2.6-5.01)</td>
</tr>
</tbody>
</table>

NOTE. Values are medians (limits) unless otherwise listed.

\( a \) – \( P < .0001 \) by Fisher's and chi-square test.

\( b \) – Log_{10} (copies/mL): values truncated the narrowest dynamic range of 2.6–5.7.

Because the presence of HBsAg was assessed until the end of 2006 and in order to assure a constant number of studied patients for establishing a potential correlation between HBV presence and HE we decided to retrospectively evaluate all 462 patients only for a period of ten years: 1996-2006.

3.2. HE and HBsAg association

The prevalence of HE in our studied cohort over a period of ten years was of 22.7% (105 from 462 patients). Surprisingly more than half, 50.4% (53 from 105) of the patients with HE were HBV infected and presented HBsAg (Table 2). The median age of HE diagnosis was 10 years and the median absolute CD4 count at HE diagnosis was 75 cells/µL. In order to establish a possible correlation between HBsAg presence and HE we first reviewed and excluded the main risk factors associated with HE at the moment of diagnosis which include low weight, anemia, constitutional symptoms, low CD4 count, high plasma HIV-RNA viral load. No patient was infected with HCV. In the studied cohort, female gender, another known risk factor for HE previously mentioned in the introduction, did not influence the analysis, only 44.1% of the cohort was female gender.
regard to level of immune status and age. In the deceased group the majority, 62.87% were male participants.

Many of the ARV treated patients have begun therapy since 1995 and experimented between one and six antiretroviral regimens until the end of 2011. The therapeutic regimens prescribed over time were: 2NRTIs (for at least 24 months), 3NRTIs (6 months), 2NRTIs+1NNRTI (17 months), 1NRTI+1NRTI+1PI (22 months), 2NRTIs+2PIs (48 months). All patients received regimens containing NRTIs (2 or 3) and PI (1 or 2), during the last 12 months and some patients received NNRTI along with NRTI and PI in their regimen. Administered regimens included drugs that penetrate CNS such as stavudine, abacavir, nevirapine, and zidovudine. About 40% of the studied patients received these drugs: 63.8% of the patients with HE and 33% of the patients without HE, p=0.0006.

Table 1. Cohort characteristics at last investigation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total of patients 462</th>
<th>Deceased ARV naive 156</th>
<th>Lost from evidence ARV treated 197</th>
<th>ARV treated 109</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females, no (%)</td>
<td>204 (48%)</td>
<td>58 (37.1%)</td>
<td>112 (56.8%)</td>
<td>61 (55.9%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17 (7-24)</td>
<td>10 (7-16)</td>
<td>17 (15-18)</td>
<td>23 (21-24)</td>
</tr>
<tr>
<td>CD4 count (cells/µL)</td>
<td>242 (0-1652)</td>
<td>73 (0-1040)</td>
<td>379 (5-1652)</td>
<td>495.5 (3-1250)</td>
</tr>
<tr>
<td>HIV RNA Level $\log_{10}$ (copies/ml)</td>
<td>3.31 (2.6-5.7)</td>
<td>5.5 (3.5-5.7)</td>
<td>2.9 (2.6-5.7)</td>
<td>2.6 (2.6-5.01)</td>
</tr>
</tbody>
</table>

NOTE. Values are medians (limits) unless otherwise listed.

a – $P < .0001$ by Fisher's and chi-square test.

b – $\log_{10}$ (copies/mL): values truncated the narrowest dynamic range of 2.6–5.7.

Because the presence of HBsAg was assessed until the end of 2006 and in order to assure a constant number of studied patients for establishing a potential correlation between HBV presence and HE we decided to retrospectively evaluate all 462 patients only for a period of ten years: 1996-2006.

3.2. HE and HBsAg association

The prevalence of HE in our studied cohort over a period of ten years was of 22.7% (105 from 462 patients). Surprisingly more than half, 50.4% (53 from 105) of the patients with HE were HBV infected and presented HBsAg (Table 2). The median age of HE diagnosis was 10 years and the median absolute CD4 count at HE diagnosis was 75 cells/µL. In order to establish a possible correlation between HBsAg presence and HE we first reviewed and excluded the main risk factors associated with HE at the moment of diagnosis which include low weight, anemia, constitutional symptoms, low CD4$^+$ count, high plasma HIV-RNA viral load. No patient was infected with HCV. In the studied cohort, female gender, another known risk factor for HE previously mentioned in the introduction, did not influence the analysis, only 44.1% of the cohort was female gender.
STATISTICAL ANALYSES OF IMMUNE STATUS AND PROGRESSION TO AIDS IN NAIVE HIV INFECTED CHILDREN FROM GIURGIU COUNTY, ROMANIA

Abstract. In resource-limited settings absolute CD4 count is used as an alternative to initiate antiretroviral therapy. Here we assessed sex differences at the last absolute CD4 cell count and progression to AIDS in a cohort of 150 naive HIV-infected children. Absolute CD4 cell count was measured by flow cytometry. Statistical analyses were performed with GraphPad Prism 5.0. In cross-sectional analysis, CD4 count was higher in girls than in boys: 161.2±175.7 cells/µL vs 124.1±175.1 cells/µL, with no statistical significant differences, (P = 0.5088). When assessing progression to AIDS, the Kaplan-Meier analysis of the time to progression did not demonstrate a significant difference according to sex (P = 0.8842, log-rank test). The eligibility for therapy of boys and girls was equal on the basis of last CD4 count of less than 350 cells/µL. A linear regression model demonstrated that in girls the last CD4 value increased with the time until death while in boys decreased. In conclusion, in AIDS stage there is no significant sex-based difference in the CD4 count. CD4 count is a better predictor of mortality in girls. In resource limited settings treatment guidelines based only on CD4 count cannot lead to differences in eligibility for antiretroviral treatment according to sex.

Key words: HIV, sex differences, absolute CD4 cell count, progression to AIDS.

INTRODUCTION

The primary goal of antiretroviral therapy (ART) is to reduce HIV-associated morbidity and mortality. The CD4 count serves as the major laboratory indicator of immune function in patients who have HIV infection. It is one of the key factors in deciding whether to initiate ART and prophylaxis for opportunistic infections, and it is the strongest predictor of subsequent disease progression and survival according to clinical trials and cohort studies [4, 12]. All patients should have a baseline CD4 count at entry into care.

World Health Organization (WHO) Pediatric HIV Treatment Guidelines recommend initiation of ART for all HIV-infected children < 2 years of age...
(regardless of CD4 parameters), for children 2–5 years of age with an absolute CD4 cell count < 750 cells/µL, and for children >5 years of age with an absolute CD4 cell count < 350 cells/µL [1].

Several studies assessed sex differences correlated with the levels of absolute CD4 cell count in children naive to ART. They revealed significant sex differences in CD4 parameters present in HIV-infected children before the onset of puberty. The data suggested that intrinsic genetic differences between male and female individuals, unrelated to sex steroid hormone levels, influence CD4 parameters in HIV-infected individuals. A recent cross-sectional analysis of 670 ART naive HIV-infected African children aged 1 day to 18 years revealed that female children had significantly higher CD4 cell percentages than male children (median, 18% vs 15%; \( P < 0.0001 \)) and a trend toward higher absolute CD4 cell counts [11]. CD4 count is important for the current guidelines in the initiation of ART, which apply uniformly to women and men [11].

It was proved that viral load after HIV-1 seroconversion is an independent predictor of the risk of progression to the acquired immunodeficiency syndrome (AIDS) [2, 4, 5, 7, 8, 9, 10]. The relation between absolute CD4 count and the risk of progression to AIDS in children has not been studied.

In Romania there are 10,903 people living with HIV/AIDS, according to the data provided by the Compartment for Monitoring and Evaluation of HIV/AIDS Data, “Prof. Dr. Matei Balș” National Institute of Infectious Diseases, on 31 December 2011 [1]. A large number of these individuals were born between 1987–1990, (>6000). 10% of them were located in the same region, the Giurgiu County.

Here we assessed sex differences at the last absolute CD4 cell count and progression to AIDS in a cohort of 150 naive HIV-infected children that have lived in the same region and presented the same epidemiologic particularities and more important they belong to the same age group.

MATERIALS AND METHODS

ETHICS

This research was approved by the ethical review board of the Infectious Disease Hospital from Giurgiu County.

STUDY PARTICIPANTS

Data from an initial large cohort of 600 HIV infected individuals from Giurgiu County, Romania was analyzed. These patients were born in poor families or abandoned in foster care in the period from 1987 to 1990 and were probably infected in the same way and probably at the same period of time by horizontal route. The majority of patients were HIV diagnosed from 1989 to 2001. All
patients were clinically evaluated in day clinic from Giurgiu Infectious Disease Hospital. From this large cohort, 150 patients, 25%, had died in between years 1996–2004. These patients were admitted in day clinic evidence since 1995 up to 2001. We chose to study this population because it was naive at the time of death and the ART did not interfere with the natural evolution of HIV infection.

QUANTITATIVE CD4 PARAMETERS

Absolute CD4 cell count was measured in all 150 participants enrolled in the study once before death occurred. Absolute CD4 cell count was determined from fresh whole blood samples by flow cytometry using the Tritest Three-Color Reagent CD4/CD8/CD3 with Tru-Count Tubes (Becton & Dickinson, USA). The tests were conducted in “Professor Dr. Victor Babeş” Infectious Diseases Hospital in Bucharest.

STATISTICAL ANALYSES

GraphPad Prism 5.0 program was used for statistical analysis. Differences in absolute CD4 cell count was evaluated by paired t-test. Two-tailed P values were reported. P < 0.05 was considered statistically significant. Median values were compared using the Wilcoxon match-pairs signed rank test. Median values were used in all tables due to the fact that they are less affected by the presence of an outlier and are better and more reliable measures of central tendency. For scientific significance confidence intervals (CI) were established. The chi-square test was used for comparisons of categorical variables, with Fisher’s two-tailed exact test used when the sample was small. Linear regression models were developed with GraphPad Prism 5.0. A Kaplan–Meier analysis of the time from HIV diagnosis to AIDS according to sex was performed; the significance of the difference between the curves was assessed with the log-rank test. Analyses of baseline characteristics were performed with either a t-test for categorical variables or an F test for continuous variables, to determine whether there were sex differences. These analyses were performed separately for two groups, namely, HIV-infected naive boys and girls.

RESULTS AND DISCUSSION

COHORT CHARACTERISTICS

The group of 150 analyzed HIV infected patients was split into two subgroups; 56 girls and 94 boys (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic.
patients were clinically evaluated in day clinic from Giurgiu Infectious Disease Hospital. From this large cohort, 150 patients, 25%, had died in between years 1996–2004. These patients were admitted in day clinic evidence since 1995 up to 2001. We chose to study this population because it was naive at the time of death and the ART did not interfere with the natural evolution of HIV infection.

QUANTITATIVE CD4 PARAMETERS

Absolute CD4 cell count was measured in all 150 participants enrolled in the study once before death occurred. Absolute CD4 cell count was determined from fresh whole blood samples by flow cytometry using the Tritest Three-Color Reagent CD4/CD8/CD3 with Tru-Count Tubes (Becton & Dickinson, USA). The tests were conducted in “Professor Dr. Victor Babeş” Infectious Diseases Hospital in Bucharest.

STATISTICAL ANALYSES

GraphPad Prism 5.0 program was used for statistical analysis. Differences in absolute CD4 cell count was evaluated by paired t-test. Two-tailed \( P \) values were reported. \( P < 0.05 \) was considered statistically significant. Median values were compared using the Wilcoxon match-pairs signed rank test. Median values were used in all tables due to the fact that they are less affected by the presence of an outlier and are better and more reliable measures of central tendency. For scientific significance confidence intervals (CI) were established. The chi-square test was used for comparisons of categorical variables, with Fisher’s two-tailed exact test used when the sample was small. Linear regression models were developed with GraphPad Prism 5.0. A Kaplan–Meier analysis of the time from HIV diagnosis to AIDS according to sex was performed; the significance of the difference between the curves was assessed with the log-rank test. Analyses of baseline characteristics were performed with either a t-test for categorical variables or an \( F \) test for continuous variables, to determine whether there were sex differences. These analyses were performed separately for two groups, namely, HIV-infected naive boys and girls.

RESULTS AND DISCUSSION

COHORT CHARACTERISTICS

The group of 150 analyzed HIV infected patients was split into two subgroups; 56 girls and 94 boys (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic.
patients were clinically evaluated in day clinic from Giurgiu Infectious Disease Hospital. From this large cohort, 150 patients, 25%, had died in between years 1996–2004. These patients were admitted in day clinic evidence since 1995 up to 2001. We chose to study this population because it was naive at the time of death and the ART did not interfere with the natural evolution of HIV infection.

QUANTITATIVE CD4 PARAMETERS

Absolute CD4 cell count was measured in all 150 participants enrolled in the study once before death occurred. Absolute CD4 cell count was determined from fresh whole blood samples by flow cytometry using the Tritest Three-Color Reagent CD4/CD8/CD3 with Tru-Count Tubes (Becton & Dickinson, USA). The tests were conducted in “Professor Dr. Victor Babeş” Infectious Diseases Hospital in Bucharest.

STATISTICAL ANALYSES

GraphPad Prism 5.0 program was used for statistical analysis. Differences in absolute CD4 cell count was evaluated by paired t-test. Two-tailed \( P \) values were reported. \( P < 0.05 \) was considered statistically significant. Median values were compared using the Wilcoxon match-pairs signed rank test. Median values were used in all tables due to the fact that they are less affected by the presence of an outlier and are better and more reliable measures of central tendency. For scientific significance confidence intervals (CI) were established. The chi-square test was used for comparisons of categorical variables, with Fisher’s two-tailed exact test used when the sample was small. Linear regression models were developed with GraphPad Prism 5.0. A Kaplan–Meier analysis of the time from HIV diagnosis to AIDS according to sex was performed; the significance of the difference between the curves was assessed with the log-rank test. Analyses of baseline characteristics were performed with either a t-test for categorical variables or an \( F \) test for continuous variables, to determine whether there were sex differences. These analyses were performed separately for two groups, namely, HIV-infected naive boys and girls.

RESULTS AND DISCUSSION

COHORT CHARACTERISTICS

The group of 150 analyzed HIV infected patients was split into two subgroups: 56 girls and 94 boys (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic.
Our cohort is representative because the participants are from the same region, are probably infected in the same way and were subjected to the same behavioral and epidemiologic risk factors or socioeconomic disparities. The majority of patients came from HIV/AIDS uninfected parents. They were HIV infected by horizontal way of transmission.

Table 1

<table>
<thead>
<tr>
<th>Cohort characteristics</th>
<th>Total of patients</th>
<th>Girls (56)</th>
<th>Boys (94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>150</td>
<td>10 (7–16)</td>
<td>10 (7–16)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10 (7–16)</td>
<td>10 (7–16)</td>
<td>10 (7–16)</td>
</tr>
<tr>
<td>aCD4 count (cells/µL)</td>
<td>73 (0–1040)</td>
<td>92.5 (11–774)</td>
<td>59 (0–1040)</td>
</tr>
</tbody>
</table>

NOTE. Values are medians (limits) unless otherwise listed.

*P = 0.1064 by paired t-test.

SEX DIFFERENCES IN CD4 PARAMETERS EVALUATED BEFORE DEATH

In cross-sectional analysis, last absolute CD4 cell count before death was higher in girls than in boys. Mean values were calculated and compared with column statistical analysis from GraphPad Prism 5.0. The mean values for CD4 count in girls were 161.2±175.7 cells/µL compared with the values obtained in boys: 124.1±175.1 cells/µL, but the differences were not statistically significant (Fig. 1).

![CD4 cell count in girls compared with boys](image_url)

Fig. 1. Last CD4 cell count in girls compared with boys

(P = 0.5088 by Wilcoxon match-pairs signed rank test).
We undertook a critical epidemiological review of the available evidence concerning whether women have lower levels of human immunodeficiency virus (HIV) RNA than men at similar stages of HIV infection. The 13 studies included in this analysis reported viral load measurements in HIV-infected men and women at a single point in time (cross-sectional studies) or over time (longitudinal studies). Seven of the 9 cross-sectional studies demonstrated that women had 0.13–0.35 log10 (~2-fold) lower levels of HIV RNA than men, despite controlling for CD4+ cell count. Four longitudinal studies revealed that women had 0.33–0.78 log10 (2- to 6-fold) lower levels of HIV RNA than men, even when controlling for time since seroconversion. Adjustment for possible confounders of the relationship between sex and viral load, including age, race, mode of virus transmission, and antiretroviral therapy use, did not change this outcome. This finding is significant, because viral loads are frequently used to guide the initiation and modification of antiretroviral therapy.

Recent UNAIDS statistics report that women constitute 47% of adults living with HIV/AIDS worldwide. In the United States, the number of prevalent AIDS cases among women is steadily increasing; the Centers for Disease Control and Prevention [1] now estimate that 23% of the reported AIDS diagnoses occur in women. Women also represent the fastest-growing group with incident HIV infection, with the highest rates being among black and Hispanic women.

Recently, a volume of research has been undertaken to define survival, disease progression, access to care, and prognostic markers for HIV-infected women. Although early reports [2, 3] found that male sex appeared to confer a survival benefit in AIDS, later studies, which controlled for access to care, antiretroviral use, and disease stage, found similar rates of progression and survival for both sexes [4–7]. Several studies have reported that men and women differ in access to HIV care [8], with women being less likely to receive prophylaxis for opportunistic infections [9] or to start appropriate antiretroviral therapy (ART) [9, 10] (even in a single-clinic setting [11]). Given the clear survival benefit with current HIV therapeutics [12, 13], differential use of antiretrovirals for purely societal reasons may lead to survival disadvantages for women.

In terms of the biological rationale for differential, sex-based HIV therapeutics, current recommendations for initiating ART [14] stem from studies largely of male cohorts. In men, 2 key markers—the absolute count or relative percentage of CD4 lymphocytes and the number of plasma HIV RNA copies (viral load)—are used to prognosticate disease progression and mortality [15–19], direct timing of ART, and assess of therapeutic efficacy [15–17, 20–23]. Subsequent studies have examined differences in the prognostic marker of viral load among men and women [23–35]. Even after adjustment for CD4 cell count or time since seroconversion, some reports indicate that viral loads are lower in women than in...
Does Patient Sex Affect Human Immunodeficiency Virus Levels?

Monica Gandhi,1 Peter Bacchetti,1 Paolo Miotti,2 Thomas C. Quinn,3,4 Fulvia Veronese,2 and Ruth M. Greenblatt1

1Department of Medicine, Infectious Diseases Division, University of California, San Francisco; and 2Office of AIDS Research and 3National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, and 4Johns Hopkins University, Baltimore, Maryland

We undertook a critical epidemiological review of the available evidence concerning whether women have lower levels of human immunodeficiency virus (HIV) RNA than do men at similar stages of HIV infection. The 13 studies included in this analysis reported viral load measurements in HIV-infected men and women at a single point in time (cross-sectional studies) or over time (longitudinal studies). Seven of the 9 cross-sectional studies demonstrated that women had 0.13–0.35 log10 (~2-fold) lower levels of HIV RNA than men, despite controlling for CD4+ cell count. Four longitudinal studies revealed that women had 0.33–0.78 log10 (2- to 6-fold) lower levels of HIV RNA than men, even when controlling for time since seroconversion. Adjustment for possible confounders of the relationship between sex and viral load, including age, race, mode of virus transmission, and antiretroviral therapy use, did not change this outcome. This finding is significant, because viral loads are frequently used to guide the initiation and modification of antiretroviral therapy.

Recent UNAIDS statistics report that women constitute 47% of adults living with HIV/AIDS worldwide. In the United States, the number of prevalent AIDS cases among women is steadily increasing; the Centers for Disease Control and Prevention [1] now estimate that 23% of the reported AIDS diagnoses occur in women. Women also represent the fastest-growing group with incident HIV infection, with the highest rates being among black and Hispanic women.

Recently, a volume of research has been undertaken to define survival, disease progression, access to care, and prognostic markers for HIV-infected women. Although early reports [2, 3] found that male sex appeared to confer a survival benefit in AIDS, later studies, which controlled for access to care, antiretroviral use, and disease stage, found similar rates of progression and survival for both sexes [4–7]. Several studies have reported that men and women differ in access to HIV care [8], with women being less likely to receive prophylaxis for opportunistic infections [9] or to start appropriate antiretroviral therapy (ART) [9, 10] (even in a single-clinic setting [11]). Given the clear survival benefit with current HIV therapeutics [12, 13], differential use of antiretrovirals for purely societal reasons may lead to survival disadvantages for women.

In terms of the biological rationale for differential, sex-based HIV therapeutics, current recommendations for initiating ART [14] stem from studies largely of male cohorts. In men, 2 key markers—the absolute count or relative percentage of CD4 lymphocytes and the number of plasma HIV RNA copies (viral load)—are used to prognosticate disease progression and mortality [15–19], direct timing of ART, and assess of therapeutic efficacy [15–17, 20–23]. Subsequent studies have examined differences in the prognostic marker of viral load among men and women [23–35]. Even after adjustment for CD4 cell count or time since seroconversion, some reports indicate that viral loads are lower in women than in
sectional studies [28] did measure time since seroconversion and still found significantly lower viral loads for women. With a single exception [29], the cross-sectional studies analyzed participants in prospective cohorts, for which specimen sampling is usually performed over long time intervals. Thus, some of these analyses [23, 24, 26] used thawed plasma specimens that were frozen and stored for months to years before viral loads were determined. However, in all studies but one [26], freezing conditions, anticoagulants, and RNA quantification procedures were similar between sex groups. Any variability in viral load measurements between sexes should thus be randomly distributed and not bias the sex–viral load association. One study [26] compared viral loads in men and women by use of 2 separate cohorts with disparate plasma storage conditions and RNA quantitation assays. The authors attempted adjustment for these disparities by use of a translation formula to standardize the 2 cohorts’ HIV RNA assay results to a third method. Although different quantitation techniques could result in an apparent difference in median viral load between sexes, the use of serum samples that were stored longer before analysis in the men’s cohort (Multicenter AIDS Cohort Study) would have favored artifactually lower HIV RNA values for the men, contrary to observation. 

**Longitudinal studies.** Because CD4 cell count serves as a limited surrogate for disease stage, longitudinal studies allow comparisons between sex groups while controlling for duration of infection. Four of the studies [31–34] were longitudinal in design (i.e., serial viral load measurements were compared over time between sexes). These studies involved cohorts of patients at high risk of acquiring HIV infection, among whom observation began prior to seroconversion. The date of HIV infection for each subject could be determined within a time period between the last visit at which the subject was HIV seronegative and the first at which the subject was HIV seropositive. All 4 studies used consistent specimen processing techniques and HIV quantitation assays. One study followed a prospective cohort of HIV-infected Air Force members and compared viral load measurements at 3 time points over ~4 years [33]. At study entry (median CD4 cell count, 700 cells/μL), median HIV RNA levels were 0.52 log₁₀ (3.3 times) lower in women than in men (P = .04); at the second time point (median CD4 cell count, 500 cells/μL), levels were 0.69 log₁₀ (4.9 times) lower in women (P = .03); at the third time point (median CD4 cell count, 400 cells/μL), levels were 0.2 log₁₀ (1.5 times) lower in women (P = .11). None of the subjects were taking therapy for HIV infection at study entry. Time from seroconversion to study entry was, on average, 3 months shorter for the women than for the men. The second study found women to have mean viral loads that were 0.33 log₁₀ (2.13-fold) lower than those for men temporally proximate to seroconversion (P = .065) [31]. This comparison was adjusted for CD4 cell count, age, time since seroconversion, and injection drug use, but not for receipt of ART (although 60% of participants received treatment at some point during study follow-up). An analysis of viral loads a median of 3.5 years after seroconversion (and up to 11.3 years after seroconversion) revealed that women had 0.52 log₁₀ (3.3-fold) lower viral loads than did men (P = .012), an estimate unadjusted for CD4 cell count, age, transmission mode, or ART use.

Finally, Sterling et al. [32, 34] performed 2 analyses using 2 different subsets of men and women from the AIDS Linked to the Intravenous Experience (ALIVE) cohort [32, 34], a longitudinal study of injection drug users with or without HIV infection [68]. Because ~95% of participants of both sexes were African American, race was not a confounding variable in the 2 studies. Date of seroconversion was known to within <12 months for all participants. In the first report [32], a multiple regression analysis that adjusted for time since seroconversion and CD4 cell count revealed that the mean viral load for women was 0.78 log₁₀ (6.0-fold) lower than for men (P < .001). This difference in mean value decreased by 0.16 log₁₀ for each year following seroconversion (P = .002), with the viral load trajectories of men and women crossing 5.8 years after infection. Therefore, the rate of viral load increase over time in the 6 years after seroconversion was greater for women than for men, as women began with lower HIV RNA levels.

In the second report by Sterling et al. [34], none of the participants reported receipt of ART at the study visit after seroconversion. After adjustment for age, CD4 cell count at seroconversion, and time between estimated seroconversion date and the first viral load measurement, the initial median viral load was 0.5 log₁₀ (3.16 times) lower for women than it was for men (P = .001). Multivariate proportional hazards models stratified by sex and controlling for initial CD4 cell count and age showed no significant difference between men and women in the risk of progression to AIDS in ~5 years of follow-up, despite women starting with lower HIV RNA loads. Thus, although the relative viral load has a similar predictive value for progression to AIDS for men and women, the same absolute viral load seems to confer disparate risks for AIDS between the sexes.

Differences in access to health care between men and women could result in healthier outcomes and lower viral loads in one population over another. However, most of the 13 studies enrolled participants from longitudinal cohorts (Table 1), which provide some routine health care. Even if health care access differed according to sex, the expected bias would result in lower viral loads in men, secondary to more-frequent ART use among men than women at similar stages of infection [11]. Although most of the studies did not show lower viral loads in men, this access bias may have played a role in the 3 cross-
Global and national trends

Globally an estimated 2.5 million children are living with HIV/AIDS, 10,000 becoming infected daily and 2,60,000 deaths of children under 15 occur due to AIDS-related illnesses1. The estimated 2,60,000 [1,50,000-3,60,000] children who died from AIDS-related illnesses in 2009 were 19 per cent fewer than the estimated 3,20,000 [2,10,000-4,30,000] who died in 2004. This trend reflects the steady expansion of services to prevent transmission of HIV to infants and an increase (albeit slow) in access to treatment for children2. The United Nations General Assembly renewed its commitment to ‘accelerate progress towards the elimination of new child infections by 2015, reducing the number of new HIV infections among children by 90 per cent by the year 2015 and reducing mother-to-child transmission of HIV (MTCT) to 5 per cent’.
Immune reconstitution inflammatory syndrome (IRIS) is a paradoxical clinical deterioration caused by an exaggerated inflammatory immune response and may occur in the context of treated or untreated infections such as TB, cytomegalovirus (CMV), Herpes, etc., and typically presents during the period following ART initiation in the presence of a rising CD4 count and a decreasing HIV viral load.

**Monitoring and follow up of children on ART**

After commencing an infant or child on ART it is vital for frequent clinical monitoring at 2 wk after initiation and every 4 wks thereafter. These visits are to evaluate growth and development, immunization status and provide nutrition counselling. There is also an opportunity to educate the parents and care providers on adverse drug reactions, ART adherence and symptoms of common OIs.

**ARV drug toxicities**

The most common toxicities include the following:

- **Haematological**: with AZT (anaemia, neutropenia and, thrombocytopenia).
- **Mitochondrial dysfunction**: with other NRTI drugs: include lactic acidosis, hepatic toxicity, pancreatitis and peripheral neuropathy.
- **Lipodystrophy and other metabolic abnormalities**: more common with stavudine (d4T) and protease inhibitors, and to a lesser degree with other NRTI drugs. Abnormalities include fat maldistribution and body habitus changes, hyperlipidaemia, hyperglycaemia, insulin resistance, diabetes mellitus, osteopenia, osteoporosis and osteonecrosis.
- **Allergic reactions**: including skin rashes and hypersensitivity reactions. These are more common with the NNRTI drugs, but also seen with certain NRTI drugs, such as abacavir (ABC).
- **Hepatic dysfunction**: in children with hepatic dysfunction of any aetiology NVP requires careful consideration because of its potential life threatening hepatotoxicity.

**Discontinuation and drug substitution**

As a general principles mild toxicities do not require discontinuation of therapy or drug substitution, and symptomatic treatment may be given (e.g. antihistamines for a mild rash). Moderate or severe toxicities may require substitution with a drug in the same ARV class but with a different toxicity profile, or with a drug from a different class. These do not require discontinuation of all ART. Severe life-threatening toxicities require discontinuation of all ARV drugs, and the initiation of appropriate supportive therapy until the patient is stabilized and the toxicity is resolved.

**ART regimen failure**

Poor adherence, inadequate drug levels or primary drug resistance can all contribute to ARV treatment failure. Genetic differences in drug metabolism may also be important. When treatment failure is confirmed, switching to a new second-line regimen becomes necessary. Prior to switching therapy, it is essential to assess and address adherence issues.

**Clinical criteria**

The appearance of new WHO clinical stage 3 or 4 events in a child on ART may reflect disease progression and treatment failure provided the child is adherent to therapy.

**Immunological criteria**: It is characterized by a drop in the CD4 count to values at or below the age-dependent values, or a failure of the CD4 count to rise above these threshold values. It can be defined as a return of CD4 cell count to ‘pre-therapy baseline’ or below, after initial immune recovery, without any other concomitant infection to explain transient CD4 cell decrease or a greater than 50 per cent fall from ‘on therapy CD4 cells peak level’ without any other concomitant infection to explain transient CD4 cell decrease.

**Virolological criteria**: Virolological failure is recognized if the child is adherent to first-line ART regimen for more than 24 wk from initiation, and has a persistent viral load over 5000 copies/ml. In resource-limited settings it may not be feasible to perform viral load testing. Availability of viral load is not a prerequisite for initiation of ART or for determination of treatment failure.

**Choice of second-line regimen**: Children who fail the initial regimen of 2 NRTIs + 1 NNRTI, a regimen based on a protease inhibitor boosted with Ritonavir (PI/r) and combined with 2 NRTIs is recommended as the second-line treatment. The child should be referred to a setting where specialized HIV care is provided.

**Other issues**

**Psychosocial management of pediatric HIV**: HIV is a social disease and its management requires all aspects of physical, psychological, spiritual and social support...
Virologic and Immunologic Characterization of Long-Term Survivors of Human Immunodeficiency Virus Type 1 Infection

Yunzhen Cao, M.D., Lim Qiu, M.D., Linqiang Zhang, Ph.D., Jeffrey S nefrit, Ph.D., and David D. Ho, M.D.

Abstract Background. In most subjects infected with human immunodeficiency virus type 1 (HIV-1), clinical or laboratory evidence of immunodeficiency develops within 10 years of seroconversion, but a few infected people remain healthy and immunologically normal for more than a decade. Studies of these subjects, termed long-term survivors, may yield important clues for the development of prophylactic and therapeutic interventions against the acquired immunodeficiency syndrome.

Methods and Results. We studied 10 seropositive subjects who remained asymptomatic with normal and stable CD4+ lymphocyte counts despite 12 to 15 years of HIV-1 infection. Plasma cultures were uniformly negative for infectious virus. However, particle-associated HIV-1 RNA was detected in four subjects with a sensitive branched-DNA signal-amplification assay, whereas in five others the levels of HIV-1 RNA were too low to detect. Infectious HIV-1 was detected in peripheral-blood mononuclear cells (PBMC) of three subjects by standard limiting-dilution cultures, and infectious virus was recovered from another subject with use of a CD8-depleted culture. The other six subjects had no detectable infectious virus in their PBMC. A quantitative polymerase-chain-reaction assay revealed that all subjects had detectable but low titers of viral DNA in PBMC. Overall, the viral burden in the plasma and PBMC of long-term survivors was orders of magnitude lower than that typically found in subjects with progressive disease.

There was no in vitro evidence of resistance by host CD4+ lymphocytes to HIV-1 infection. However, long-term survivors had a vigorous, virus-inhibitory CD8+ lymphocyte response and a strong neutralizing-antibody response. In two subjects the kinetics of viral replication was consistent with the presence of a substantially attenuated strain of HIV-1.

Conclusions. Subjects who remain asymptomatic for many years despite HIV-1 infection have low levels of HIV-1 and a combination of strong virus-specific immune responses with some degree of attenuation of the virus. (N Engl J Med 1995;332:201-8.)

THE natural history and pathogenic processes of human immunodeficiency virus type 1 (HIV-1) infection are complex and variable, and they depend on a multitude of viral and host factors and their interactions. Host factors may result in a variable susceptibility to HIV-1 infection and its pathogenic effects, whereas variation in the virus may account for differences in virulence and disease progression. Although symptoms related to the acquired immunodeficiency syndrome (AIDS) or laboratory evidence of immunodeficiency develops in a majority of infected persons within 10 years of seroconversion, a small number (approximately 5 percent) of infected persons, termed long-term survivors or persons with long-term nonprogressive disease, have remained clinically healthy and immunologically normal for more than a decade. These long-term survivors have recently become the subject of intensive investigation, because they may yield important information on the determinants of nonprogression that may be useful in designing new interventional strategies to contain the disease.

To obtain a balanced view of the pathogenic processes in long-term survivors, we examined host, immunologic, and virologic factors in a cohort of 10 subjects who have remained asymptomatic with normal and stable CD4+ lymphocyte counts despite 12 to 15 years of HIV-1 infection.

Methods

Study Subjects

Ten HIV-1-seropositive subjects from the New York metropolitan area were referred to us because they met our working definition of long-term survivors of HIV-1 infection: they had no symptoms, normal and stable CD4+ lymphocyte counts, no prolonged use of anti-retroviral agents, and at least 12 years of infection. The general clinical characteristics of the cohort are summarized in Table 1. The subjects ranged in age from 38 to 47 years, and all but one were men. Seven were infected through homosexual contact, two were infected through intravenous drug use, and the one woman was infected heterosexually. Their CD4+ lymphocyte counts have been consistently in the normal range, with no decline over time. The duration of HIV-1 infection was documented by the date of seroconversion in three subjects (Subjects 1, 4, and 9) who participated in a prospective
VIROLOGIC AND IMMUNOLOGIC CHARACTERIZATION OF LONG-TERM SURVIVORS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

YUNZHEN CAO, M.D., LIMO QIN, M.D., LINQI ZHANG, PH.D., JEFFREY SAFRIT, PH.D., AND DAVID D. HO, M.D.

Abstract. Background. In most subjects infected with human immunodeficiency virus type 1 (HIV-1), clinical or laboratory evidence of immunodeficiency develops within 10 years of seroconversion, but a few infected people remain healthy and immunologically normal for more than a decade. Studies of these subjects, termed long-term survivors, may yield important clues for the development of prophylactic and therapeutic interventions against the acquired immunodeficiency syndrome.

Methods and Results. We studied 10 seropositive subjects who remained asymptomatic with normal and stable CD4+ lymphocyte counts despite 12 to 15 years of HIV-1 infection. Plasma cultures were uniformly negative for infectious virus. However, particle-associated HIV-1 RNA was detected in four subjects with a sensitive branched-DNA signal-amplification assay, whereas in five others the levels of HIV-1 RNA were too low to detect. Infectious HIV-1 was detected in peripheral-blood mononuclear cells (PBMC) of three subjects by standard limiting-dilution cultures, and infectious virus was recovered from another subject with use of a CD8-depleted culture. The other six subjects had no detectable infectious virus in their PBMC. A quantitative polymerase-chain-reaction assay revealed that all subjects had detectable but low titers of viral DNA in PBMC. Overall, the viral burden in the plasma and PBMC of long-term survivors was orders of magnitude lower than that typically found in subjects with progressive disease.

There was no in vitro evidence of resistance by host CD4+ lymphocytes to HIV-1 infection. However, long-term survivors had a vigorous, virus-inhibitory CD8+ lymphocyte response and a strong neutralizing-antibody response. In two subjects the kinetics of viral replication was consistent with the presence of a substantially attenuated strain of HIV-1.

Conclusions. Subjects who remain asymptomatic for many years despite HIV-1 infection have low levels of HIV-1 and a combination of strong virus-specific immune responses with some degree of attenuation of the virus. (N Engl J Med 1995;332:201-8.)

THE natural history and pathogenic processes of human immunodeficiency virus type 1 (HIV-1) infection are complex and variable, and they depend on a multitude of viral and host factors and their interactions.1 Host factors may result in a variable susceptibility to HIV-1 infection and its pathogenic effects, whereas variation in the virus may account for differences in virulence and disease progression. Although symptoms related to the acquired immunodeficiency syndrome (AIDS) or laboratory evidence of immunodeficiency develop in a majority of infected persons within 10 years of seroconversion,2,3 a small number (approximately 5 percent) of infected persons, termed long-term survivors or persons with long-term nonprogressive disease, have remained clinically healthy and immunologically normal for more than a decade.4-11 These long-term survivors have recently become the subject of intensive investigation, because they may yield important information on the determinants of nonprogression that may be useful in designing new interventional strategies to contain the disease.

To obtain a balanced view of the pathogenic processes in long-term survivors, we examined host, immunologic, and virologic factors in a cohort of 10 subjects who have remained asymptomatic with normal and stable CD4+ lymphocyte counts despite 12 to 15 years of HIV-1 infection.

Methods

Study Subjects

Ten HIV-1–seropositive subjects from the New York metropolitan area were referred to us because they met our working definition of long-term survivors of HIV-1 infection: they had no symptoms, normal and stable CD4+ lymphocyte counts, no prolonged use of antiretroviral agents, and at least 12 years of infection. The general clinical characteristics of the cohort are summarized in Table 1. The subjects ranged in age from 38 to 47 years, and all but one were men. Seven were infected through homosexual contact, two were infected through intravenous drug use, and the one woman was infected heterosexually. Their CD4+ lymphocyte counts have been consistently in the normal range, with no decline over time. The duration of HIV-1 infection was documented by the date of seroconversion in three subjects (Subjects 1, 4, and 9) who participated in a prospective study. The other seven subjects were identified through a retrospective seroconversion survey.
Sex Differences in HIV RNA Level and CD4 Cell Percentage During Childhood

Theodore D. Ruel,1 Brian C. Zanoni,2,3 Isaac Ssewanyana,4 Huyen Cao,5 Diane V. Havlir,5 Moses Kamya,6 Jane Achan,7 Edwin D. Charlebois,5 and Margaret E. Feeney1,2,3,8

1Department of Pediatrics, University of California, San Francisco, California; 2The Ragon Institute of MGH, MIT, and Harvard, Massachusetts General Hospital, Boston, Massachusetts; 3Sinikithemba Clinic and Philani Program, McCord Hospital, Durban, South Africa; 4Joint Clinical Research Centre, Kampala, Uganda; 5Department of Medicine, University of California, San Francisco, California; 6Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda; 7Department of Paediatrics, Makerere University College of Health Sciences, Kampala, Uganda; and 8Division of Experimental Medicine, Department of Medicine, University of California, San Francisco, California

Background. HIV-infected women have lower HIV RNA levels and higher CD4-cell counts than do men. This observation has been attributed to the immunomodulatory effects of sex steroid hormones, such as estrogen and progesterone. Limited data exist regarding potential sex differences in HIV RNA level and CD4 parameters among prepubertal children with untreated HIV infection.

Methods. We examined the relationship of sex to HIV RNA level and CD4 parameters among 670 perinatally HIV-infected, antiretroviral therapy–naive African children aged <18 years (median age, 4.8 years) using multivariate linear regression. In a subset of 188 children, we used longitudinal data to compare changes in HIV RNA levels and CD4 percentage over time. Levels of CD4 and CD8 T-cell activation (CD381HLA-DR1) were also compared between boys and girls.

Results. Female children had lower HIV RNA levels (P = .0004) and higher CD4 percentages (P < .0001), compared to male children. Multivariate linear regression demonstrated an independent association of sex with both HIV RNA level and CD4 percentage after controlling for other covariates. Multilevel mixed-effects linear regression analysis of longitudinal HIV RNA level and CD4 parameter data showed that sex differences persisted across all observed ages. Levels of T-cell activation did not differ between the sexes.

Conclusions. Significant sex differences in HIV RNA levels and CD4 parameters are present in HIV-infected children before the onset of puberty. These data suggest that intrinsic genetic differences between male and female individuals, unrelated to sex steroid hormone levels, influence HIV RNA level and CD4 parameters in HIV-infected individuals.
Sex Differences in HIV RNA Level and CD4 Cell Percentage During Childhood

Theodore D. Ruel,1 Brian C. Zanoni,2,3 Isaac Ssewanyana,4 Huyen Cao,5 Diane V. Havlir,5 Moses Kamya,6 Jane Achan,7 Edwin D. Charlebois,5 and Margaret E. Feeney1,2,3,8

1Department of Pediatrics, University of California, San Francisco, California; 2The Ragon Institute of MGH, MIT, and Harvard, Massachusetts General Hospital, Boston, Massachusetts; 3Sinikithemba Clinic and Philani Program, Mccord Hospital, Durban, South Africa; 4Joint Clinical Research Centre, Kampala, Uganda; 5Department of Medicine, University of California, San Francisco, California; 6Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda; 7Department of Paediatrics, Makerere University College of Health Sciences, Kampala, Uganda; and 8Division of Experimental Medicine, Department of Medicine, University of California, San Francisco, California

Background. HIV-infected women have lower HIV RNA levels and higher CD4-cell counts than do men. This observation has been attributed to the immunomodulatory effects of sex steroid hormones, such as estrogen and progesterone. Limited data exist regarding potential sex differences in HIV RNA level and CD4 parameters among prepubertal children with untreated HIV infection.

Methods. We examined the relationship of sex to HIV RNA level and CD4 parameters among 670 perinatally HIV-infected, antiretroviral therapy–naive African children aged <18 years (median age, 4.8 years) using multivariate linear regression. In a subset of 188 children, we used longitudinal data to compare changes in HIV RNA levels and CD4 percentage over time. Levels of CD4 and CD8 T-cell activation (CD38HLA-DR+) were also compared between boys and girls.

Results. Female children had lower HIV RNA levels (P = .0004) and higher CD4 percentages (P < .0001), compared to male children. Multivariate linear regression demonstrated an independent association of sex with both HIV RNA level and CD4 percentage after controlling for other covariates. Multilevel mixed-effects linear regression analysis of longitudinal HIV RNA level and CD4 parameter data showed that sex differences persisted across all observed ages. Levels of T-cell activation did not differ between the sexes.

Conclusions. Significant sex differences in HIV RNA levels and CD4 parameters are present in HIV-infected children before the onset of puberty. These data suggest that intrinsic genetic differences between male and female individuals, unrelated to sex steroid hormone levels, influence HIV RNA level and CD4 parameters in HIV-infected individuals.
Sex Differences in HIV RNA Level and CD4 Cell Percentage During Childhood

Theodore D. Ruel,1 Brian C. Zanoni,2,3 Isaac Ssewanyana,4 Huyen Cao,5 Diane V. Havlir,5 Moses Kamya,6 Jane Achan,7 Edwin D. Charlebois,5 and Margaret E. Feeney1,2,3,8

1Department of Pediatrics, University of California, San Francisco, California; 2The Ragon Institute of MGH, MIT, and Harvard, Massachusetts General Hospital, Boston, Massachusetts; 3Sinikithemba Clinic and Philani Program, Mccord Hospital, Durban, South Africa; 4Joint Clinical Research Centre, Kampala, Uganda; 5Department of Medicine, University of California, San Francisco, California; 6Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda; 7Department of Paediatrics, Makerere University College of Health Sciences, Kampala, Uganda; and 8Division of Experimental Medicine, Department of Medicine, University of California, San Francisco, California

Background. HIV-infected women have lower HIV RNA levels and higher CD4-cell counts than do men. This observation has been attributed to the immunomodulatory effects of sex steroid hormones, such as estrogen and progesterone. Limited data exist regarding potential sex differences in HIV RNA level and CD4 parameters among prepubertal children with untreated HIV infection.

Methods. We examined the relationship of sex to HIV RNA level and CD4 parameters among 670 perinatally HIV-infected, antiretroviral therapy–naive African children aged <18 years (median age, 4.8 years) using multivariate linear regression. In a subset of 188 children, we used longitudinal data to compare changes in HIV RNA levels and CD4 percentage over time. Levels of CD4 and CD8 T-cell activation (CD38HLA-DR+) were also compared between boys and girls.

Results. Female children had lower HIV RNA levels (P = .0004) and higher CD4 percentages (P < .0001), compared to male children. Multivariate linear regression demonstrated an independent association of sex with both HIV RNA level and CD4 percentage after controlling for other covariates. Multilevel mixed-effects linear regression analysis of longitudinal HIV RNA level and CD4 parameter data showed that sex differences persisted across all observed ages. Levels of T-cell activation did not differ between the sexes.

Conclusions. Significant sex differences in HIV RNA levels and CD4 parameters are present in HIV-infected children before the onset of puberty. These data suggest that intrinsic genetic differences between male and female individuals, unrelated to sex steroid hormone levels, influence HIV RNA level and CD4 parameters in HIV-infected individuals.

Adult women infected with HIV exhibit significantly lower viral loads, compared with men [1–3]. Sex disparities have been described in the manifestation of infection from other viruses, such as hepatitis C virus and hepatitis B virus, [4–6] and in the incidence and severity of autoimmune diseases [7]. However, the molecular mechanisms underlying sexual dimorphism in immune function remain unknown. Previous attempts to explain sex differences in HIV infection and other infectious diseases have focused on the numerous immunomodulatory effects of the major female sex steroid hormones estrogen and progesterone [8, 9]. Receptors for both estrogen and progesterone are expressed by most immune cell types, and levels of these hormones influence the expression of CCR5 by CD4 T cells and production of several cytokines [10, 11]. Exogenous administration of these hormones and their natural fluctuation during the ovulatory cycle have been shown to modulate innate and adaptive immune responses and may influence the rate of HIV replication [12]. The recent demonstration that production of interferon (IFN)–α by plasmacytoid dendritic cells in response to HIV-derived TLR ligands is higher in women than in
men and correlates with plasma progesterone levels has been suggested to be a potential biological mechanism for the observed sex differences in HIV load [13, 14].

Comparison of HIV RNA levels between prepubertal boys and girls provides a means to uncouple the impact of sex hormones from other sex-specific genetically determined effects. Two small studies in the United States and Europe described lower HIV RNA levels among girls than among boys [15, 16], but the majority of children in both cohorts were receiving combination antiretroviral therapy (ART). One study of ART-naive Kenyan infants detected no sex difference in viral load but included only a small number of participants [17]. Here, we assessed HIV RNA levels, CD4 parameters, and T-cell activation markers in a large cohort of HIV-infected children before the initiation of ART and found a significant sex disparity in both HIV RNA levels and CD4 percentage beginning in early childhood that was not associated with differences in indices of generalized T-cell activation.

**METHODS**

**Study Participants**

Data from 3 cohorts of HIV-infected ART-naive African children (<18 years of age) were analyzed. Cohorts 1 and 2 included children initiating HIV care at McCord Hospital in Durban, South Africa (May 2004–December 2008) and St. Mary’s Hospital in Marrianhill, South Africa (January 2007–December 2008), respectively. Cohort 3 consisted of 300 children enrolled from October 2005 through September 2006 in the Children with HIV and Malaria Project, an observational study based in Kampala, Uganda [18]. The participants in the study who did not meet criteria for initiation of ART at enrollment were followed up, with HIV RNA and CD4 levels measured every 12 weeks. In all cohorts, ART was initiated immediately for children determined to be eligible according to the country guidelines. This research was approved by the ethical review boards at each site, including McCord and St. Mary’s Hospitals, Massachusetts General Hospital, the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, and the University of California, San Francisco Committee on Human Research.

**Quantitative CD4 and HIV RNA Parameters**

CD4-cell counts and percentages were determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens. For cohort 3, all plasma HIV RNA levels were measured using the Roche Amplicor, version 1.5 (dynamic range, 400–750 000 copies/mL). For cohorts 1 and 2, the Roche Amplicor, version 1.5, assay was used for viral load determinations, but for some, the COBAS Amplicor/COBAS TaqMan HIV-1 Test (dynamic range, 25–3 000 000 IU/mL) or the NucliSens easyMAG (bioMérieux; dynamic range, 2.5–10 000 000 copies/mL) was used. Because the dynamic ranges of these assays differed, for combined analyses, we assigned all HIV RNA measurements >500 000 copies/mL as a value of 500 001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

**CD4 and CD8 Activation Markers**

For the participants in cohort 3, the proportions of CD4+ and CD8+ lymphocytes coexpressing the activation markers CD38 and HLA-DR were measured in freshly isolated peripheral blood mononuclear cells with use of the following antibodies: CD3 APC, CD8 PerCp-Cy5.5, HLA-DR FITC, and CD38 PE (as described elsewhere [19]). A minimum of 30 000 CD3+ cells per sample were acquired using a 4-color flow cytometer (FACS Calibur; BD Biosciences). CD4 and CD8 activation were defined as the percentage of CD3+ CD8- and CD3+ CD8+ lymphocytes, respectively, that coexpressed CD38 and HLA-DR. Data were analyzed using FLOWJO software (TreeStar).

**Statistical Analyses**

The Fisher’s exact and Mann-Whitney U tests were used for bivariate comparisons. Multivariate linear regression models were developed to assess the relationship of sex to HIV RNA level and CD4 percentage, adjusting for age, CD4 percentage, absolute CD4 count, cohort, and terms for the interaction of sex with age. The final models were chosen using backward variable selection, including only predictors that had a P value of =.05 in the full model. Because the truncation of HIV RNA levels >5.7 log copies/mL could violate the distributional assumptions underlying the linear regression model, each regression was repeated using bootstrap-based inference with 1000 repetitions. Similar models were developed to assess the relationship of sex to CD4 percentage. Frequencies of CD38+HLA-DR+ cells among CD3+ CD8- and CD3+ CD8+ lymphocytes were compared between male and female children with use of a multivariate linear regression that included sex, age, HIV RNA level, CD4 percentage, and absolute CD4-cell count.

To explore the association of HIV RNA level with sex over time, a multilevel mixed-effects linear regression model accounting for repeated measures was developed using longitudinal data from cohort 3. Mean changes in HIV RNA level with age were examined graphically by plotting observed measurements for each individual as a function of age. Mean trends for female and male children were estimated using locally linear regression smooths to allow visual inspection of observed differences without specifying a parametric model. Differences in age-related changes in HIV RNA level between sexes were evaluated using linear mixed effect regression models, accounting...
men and correlates with plasma progesterone levels has been suggested to be a potential biological mechanism for the observed sex differences in HIV load [13, 14].

Comparison of HIV RNA levels between prepubertal boys and girls provides a means to uncouple the impact of sex hormones from other sex-specific genetically determined effects. Two small studies in the United States and Europe described lower HIV RNA levels among girls than among boys [15, 16], but the majority of children in both cohorts were receiving combination antiretroviral therapy (ART). One study of ART-naive Kenyan infants detected no sex difference in viral load but included only a small number of participants [17]. Here, we assessed HIV RNA levels, CD4 parameters, and T-cell activation markers in a large cohort of HIV-infected children before the initiation of ART and found a significant sex disparity in both HIV RNA levels and CD4 percentage beginning in early childhood that was not associated with differences in indices of generalized T-cell activation.

METHODS

Study Participants
Data from 3 cohorts of HIV-infected ART-naive African children (<18 years of age) were analyzed. Cohorts 1 and 2 included children initiating HIV care at McCord Hospital in Durban, South Africa (May 2004–December 2008) and St. Mary’s Hospital in Marrianhill, South Africa (January 2007–December 2008), respectively. Cohort 3 consisted of 300 children enrolled from October 2005 through September 2006 in the Children with HIV and Malaria Project, an observational study based in Kampala, Uganda [18]. The participants in the study who did not meet criteria for initiation of ART at enrollment were followed up, with HIV RNA and CD4 levels measured every 12 weeks. In all cohorts, ART was initiated immediately for children determined to be eligible according to the country guidelines. This research was approved by the ethical review boards at each site, including McCord and St. Mary’s Hospitals, Massachusetts General Hospital, the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, and the University of California, San Francisco Committee on Human Research.

Quantitative CD4 and HIV RNA Parameters
CD4-cell counts and percentages were determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens. For cohort 3, all plasma HIV RNA levels were measured using the Roche Amplicor, version 1.5 (dynamic range, 25–3,000,000 IU/mL) or the NucliSens easyMAG (bioMérieux; dynamic range, 25–3,000,000 copies/mL). For combined analyses, we assigned all HIV RNA measurements >500,000 copies/mL as a value of 500,001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

CD4 and CD8 Activation Markers
For the participants in cohort 3, the proportions of CD4+ and CD8+ lymphocytes coexpressing the activation markers CD38 and HLA-DR were measured in freshly isolated peripheral blood mononuclear cells with use of the following antibodies: CD3 APC, CD8 PerCp-Cy5.5, HLA-DR FITC, and CD38 PE (as described elsewhere [19]). A minimum of 30,000 CD3+ cells per sample were acquired using a 4-color flow cytometer (FACS Calibur; BD Biosciences). CD4 and CD8 activation were defined as the percentage of CD3+ CD8- and CD3+ CD8+ lymphocytes, respectively, that coexpressed CD38 and HLA-DR. Data were analyzed using FLOWJO software (TreeStar).

Statistical Analyses
The Fisher’s exact and Mann-Whitney U tests were used for bivariate comparisons. Multivariate linear regression models were developed to assess the relationship of sex to HIV RNA level and CD4 percentage, adjusting for age, CD4 percentage, absolute CD4 count, cohort, and terms for the interaction of sex with age. The final models were chosen using backward variable selection, including only predictors that had a P value of ≤.05 in the full model. Because the truncation of HIV RNA levels >5.7 log copies/mL could violate the distributional assumptions underlying the linear regression model, each regression was repeated using bootstrap-based inference with 1000 repetitions. Similar models were developed to assess the relationship of sex to CD4 percentage. Frequencies of CD38+HLA-DR+ cells among CD3+ CD8- and CD3+ CD8+ lymphocytes were compared between male and female children with use of a multivariate linear regression that included sex, age, HIV RNA level, CD4 percentage, and absolute CD4-cell count.

To explore the association of HIV RNA level with sex over time, a multilevel mixed-effects linear regression model accounting for repeated measures was developed using longitudinal data from cohort 3. Mean changes in HIV RNA level with age were examined graphically by plotting observed measurements for each individual as a function of age. Mean trends for female and male children were estimated using locally linear regression smooths to allow visual inspection of observed differences without specifying a parametric model. Differences in age-related changes in HIV RNA level between sexes were evaluated using linear mixed effect regression models, accounting...
for within-individual correlations in repeated outcome measures. Regression models allowing sex-specific intercepts and slopes were fitted using restricted maximum likelihood with unstructured covariances. In addition, we examined the possibility of nonlinearities in age-related trends by including polynomial terms in the models. We performed the same analyses to evaluate the relationship of CD4 percentage with sex and age.

All statistical tests were 2-tailed, with a \( P \) value < .05 considered to be statistically significant and were performed using Stata statistical software (release 11.1; StataCorp) and R (version 2.11.1; R Foundation for Statistical Computing).

**RESULTS**

**Cohort Characteristics**

A total of 670 ART-naive HIV-infected African children aged 1 day to 18 years (median, 4.8 years) were studied. The cohorts were balanced with regard to sex but differed substantially with regard to age and level of immune compromise (Table 1) because of varying demographic characteristics and referral patterns. All children were black Africans. Consistent with the high HIV RNA levels known to occur during infancy and early childhood [20, 21], 36% of the children in our study had HIV RNA levels >5.7 log copies/mL. These children were significantly younger (median age, 1.56 vs 5.55 years; \( P < .0001 \)), had lower CD4 cell percentages (median, 15 vs 17; \( P = .02 \)), and were more likely to be male (40% vs 30%; \( P = .01 \)).

**Sex Differences in HIV RNA Levels**

In cross-sectional analysis, female children had significantly higher CD4 cell percentages than male children (median, 18% vs 15%; \( P < .0001 \), Figure 3) and a trend toward higher absolute CD4 cell counts (median, 602 vs 531 cells/μL; \( P = .053 \), Table 2). When stratified by age, CD4 cell percentages were significantly higher among female children >2 years of age (\( P < .0001 \)), but no difference was seen among younger children (Table 4). When stratified by HIV RNA level, there was a pronounced sex difference in CD4 cell percentages among children with HIV RNA levels in the quantifiable range (<5.7 log copies/mL; \( P < .0001 \)), but no difference was observed among children with HIV RNA levels exceeding the threshold for quantification (>5.7 log copies/mL; \( P = .63 \)). Female sex remained independently associated with CD4 cell percentage (\( P < .001 \)) after adjusting for CD4 cell count (\( P < .001 \), age (\( P < .001 \)), and HIV RNA level (\( P < .001 \) and cohort (\( P < .001 \)) in multivariate linear regression modeling. There was a statistically significant interaction between sex and HIV RNA level (\( P = .006 \)), with each 1 log copies/mL increase in HIV RNA level associated with a decrease of 0.6% in CD4 cell percentage among male children, but a 2.7% decrease among female children.

**Longitudinal Analyses**

To further evaluate the effect of sex on age-related changes in HIV RNA level, we developed a multilevel mixed-effects linear regression model with use of longitudinal data from cohort 3. A total of 88 HIV-infected children with a median of 10 HIV RNA measurements (range, 3–14) over a median of 756 days...
for within-individual correlations in repeated outcome measures. Regression models allowing sex-specific intercepts and slopes were fitted using restricted maximum likelihood with unstructured covariances. In addition, we examined the possibility of nonlinearities in age-related trends by including polynomial terms in the models. We performed the same analyses to evaluate the relationship of CD4 percentage with sex and age.

All statistical tests were 2-tailed, with a \( P \) value < .05 considered to be statistically significant, and were performed using Stata statistical software (release 11.1; StataCorp) and R (version 2.11.1; R Foundation for Statistical Computing).

**RESULTS**

**Cohort Characteristics**

A total of 670 ART-naive HIV-infected African children aged 1 day to 18 years (median, 4.8 years) were studied. The cohorts were balanced with regard to sex but differed substantially with regard to age and level of immune compromise (Table 1) because of varying demographic characteristics and referral patterns. All children were black Africans. Consistent with the high HIV RNA levels known to occur during infancy and early childhood [20, 21], 36% of the children in our study had HIV RNA levels >5.7 log copies/mL. These children were significantly younger (median age, 1.56 vs 5.55 years; \( P < .0001 \)), had lower CD4 cell percentages (median, 15 vs 17; \( P = .02 \)), and were more likely to be male (40% vs 30%; \( P = .01 \)).

**Sex Differences in HIV RNA Levels**

In cross-sectional analysis, HIV RNA levels were significantly lower in female than in male children (\( P < .001 \)) (Table 2). This difference was most pronounced among older children and those with higher CD4 percentages and absolute counts (Table 3). In multivariable regression analysis, sex remained a significant predictor of HIV RNA level (\( P = .026 \)) after adjustment for age (\( P < .001 \)) and CD4 percentage (\( P < .001 \)). When added to the multivariate model, the interaction term between sex and age did not achieve statistical significance (\( P = .37 \)). Confidence intervals (CIs) and \( P \) values with use of bootstrap-based inference were similar. To more specifically restrict our dataset to the prepubertal period, we repeated the multivariate analysis including only children aged ≤10 years (\( n = 612 \)) [20] and found that female sex remained significantly associated with lower HIV RNA level (\( P = .046 \)).

**Sex Differences in CD4 Parameters**

In cross-sectional analysis, female children had significantly higher CD4 cell percentages than male children (median, 18% vs 15%; \( P < .0001 \), Figure 3) and a trend toward higher absolute CD4 cell counts (median, 602 vs 531 cells/\( \mu L \); \( P = .053 \), Table 2). When stratified by age, CD4 cell percentages were significantly higher among female children ≥2 years of age (\( P < .0001 \)), but no difference was seen among younger children (Table 4). When stratified by HIV RNA level, there was a pronounced sex difference in CD4 cell percentages among children with HIV RNA levels in the quantifiable range (<5.7 log copies/mL; \( P < .0001 \)), but no difference was observed among children with HIV RNA levels exceeding the threshold for quantification (≥5.7 log copies/mL; \( P = .63 \)). Female sex remained independently associated with CD4 cell percentage (\( P < .001 \)), after adjusting for CD4 cell count (\( P < .001 \)), age (\( P < .001 \)), and HIV RNA level (\( P < .001 \)) and cohort (\( P < .001 \)) in multivariate linear regression modeling. There was a statistically significant interaction between sex and HIV RNA level (\( P = .006 \)), with each 1 log copies/mL increase in HIV RNA level associated with a decrease of 0.6% in CD4 cell percentage among male children, but a 2.7% decrease among female children.

**Longitudinal Analyses**

To further evaluate the effect of sex on age-related changes in HIV RNA level, we developed a multilevel mixed-effects linear regression model with use of longitudinal data from cohort 3. A total of 188 HIV-infected children with a median of 10 HIV RNA measurements (range, 3–14) over a median of 756 days

<table>
<thead>
<tr>
<th>Table 1. Cohort Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
</tr>
<tr>
<td>( n = )</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>CD4 percentage</td>
</tr>
<tr>
<td>CD4 count (cells/( \mu L ))</td>
</tr>
<tr>
<td>HIV RNA Level</td>
</tr>
</tbody>
</table>

**NOTE:** Values are medians (interquartile range) unless otherwise listed.

\( a \) Log_{10} (copies/mL): values truncated the narrowest dynamic range of 2.6–5.7.

\( b \) Log_{10} (copies/mL): values truncated the narrowest dynamic range of 2.6–5.7.
of follow-up were analyzed. We found that mean log viral loads were 2-fold higher among boys (mean difference, 0.31 log copies/mL; 95% CI, .16–.45 copies/mL; \( P \) < .001) across all ages tested (Figure 1). Multilevel mixed-effects linear regression analysis of CD4 cell percentage similarly showed that female patients had higher CD4 cell percentages (mean difference, 2.31; 95% CI, .65–3.97; \( P \) < .006) across all ages tested (Figure 2). Models allowing for different slopes for boys and girls or non-linearity in age-related changes did not significantly improve fits for either outcome.

**T Cell Immune Activation Levels**

T cell immune activation has been shown to be a strong independent predictor of HIV disease progression [22–25], and it has recently been reported that HIV-infected women exhibit higher levels of CD8 T-cell activation than do men after adjusting for viral load [13]. We compared T cell immune activation parameters among 100 female and 60 male ART-naive HIV-infected children (median age, 6.8 years) from cohort 3. The median level of CD38 and HLA-DR coexpression on CD4 T lymphocytes was similar between boys (13%; interquartile range [IQR], 8%–17%) and girls (12%; IQR, 8%–16%; \( P \) = .63). Levels of CD38 and HLA-DR coexpression on CD8+ T lymphocytes were also nearly equivalent, with 43% (IQR, 32%–52%) for male patients and 44% (IQR, 33%–52%) for girls (\( P \) = .93). In multivariate linear regression, CD4 activation levels were strongly associated with CD4 cell percentage (\( P \) < .001), but not sex (\( P \) = .74), absolute CD4 cell count (\( P \) = .10), age (\( P \) = .75), or HIV RNA level (\( P \) = .10). CD8 activation levels were associated with both CD4 percentage (\( P \) = .009) and absolute CD4 cell count (\( P \) = .022), but not sex (\( P \) = .31), age (\( P \) = .63), or HIV RNA level (\( P \) = .21).

**World Health Organization (WHO) Pediatric HIV Treatment Guidelines**

The 2010 WHO guidelines recommend initiation of ART for all HIV-infected children <24 months of age (regardless of CD4 parameters), for children 24–59 months of age with a CD4 cell percentage <25 or absolute CD4 cell count <750 cells/µL, and for children >5 years of age with an absolute CD4 cell count <350 cells/µL [26]. Because of the observed sex difference in CD4 cell percentages, we compared the proportion of boys and girls in our cohort who would qualify for ART under the current CD4-based guidelines. Among children ≥24 months of age, 109 (48%) of 225 female children were eligible for ART, compared with 141 (63%) of 224 male children (\( P \) = .0023).

**DISCUSSION**

Men and women are known to respond differently to a number of infections, but the relative contribution of sex hormones to this disparity has been unclear. Here, we revealed basic biologic differences in HIV load and CD4 cell dynamics between male and female individuals that are evident during early childhood, when levels of the major sex steroid hormones estrogen, progesterone, and testosterone do not appreciably differ between boys and girls [27]. These data suggest that intrinsic biologic differences in the immunologic response to HIV exist between boys and girls throughout childhood—a finding that has important implications for our understanding of the biology of HIV/AIDS • CID 2011:53 (15 September) • 595
antiviral immune response and potential implications for treatment guidelines.

Sex differences in HIV RNA levels among adults have been well established. During early HIV infection, plasma HIV RNA levels are ~0.5 log lower in adult women than in men, but this difference narrows as the disease progresses, becoming statistically insignificant when CD4 cell counts are ≥200 cells/mL [1–3, 28, 29]. CD4 cell counts are also higher among adult women with and without HIV infection [3]. Prior studies of HIV RNA levels and CD4 cell parameters among male and female children have been limited and have yielded conflicting results. One study of children receiving ART found HIV RNA levels to be 0.38 log lower in girls than in boys, with no difference in CD4 cell count or percentage [15]. Another group reported that HIV RNA levels were higher in girls at the peak of viremia but consistently lower after 4 years of age [16]. Of note, this group also reported CD4 cell counts to be significantly lower among HIV-infected girls than boys [30], in contrast to our findings of higher CD4 cell percentages among girls. However, these studies included few or no children who were naive to combination ART [15, 16, 30]. The only prior study to focus on ART-naive children was limited to 38 infants and found no difference in

Table 4: CD4 Cell Percentage Within Subgroups of Age, Absolute CD4 Cell Count, and HIV RNA Level

<table>
<thead>
<tr>
<th></th>
<th>n=</th>
<th>Females</th>
<th>Males</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>210</td>
<td>15 (10–21)</td>
<td>17 (11–21)</td>
<td>.692</td>
</tr>
<tr>
<td>2–6</td>
<td>200</td>
<td>20 (15–28)</td>
<td>14 (8–21)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>≥6</td>
<td>253</td>
<td>19 (10–26)</td>
<td>15 (9–22)</td>
<td>.021</td>
</tr>
<tr>
<td>CD4 Count (cells/µL)c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;350</td>
<td>197</td>
<td>9 (5–13)</td>
<td>8 (3–13)</td>
<td>.082</td>
</tr>
<tr>
<td>350–750</td>
<td>214</td>
<td>19 (13–25)</td>
<td>17 (13–21)</td>
<td>.045</td>
</tr>
<tr>
<td>≥750</td>
<td>232</td>
<td>23 (19–31)</td>
<td>21 (17–26)</td>
<td>.010</td>
</tr>
<tr>
<td>HIV RNA Log10 (copies/mL)d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>220</td>
<td>22 (13–29)</td>
<td>15 (9–22)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5.0–5.6</td>
<td>186</td>
<td>16 (11–23)</td>
<td>14 (10–22)</td>
<td>.109</td>
</tr>
<tr>
<td>≥5.7</td>
<td>232</td>
<td>15 (10–20)</td>
<td>15 (10–19)</td>
<td>.630</td>
</tr>
</tbody>
</table>

NOTE: Values are medians (IQR) unless otherwise listed.

a N = 663 children with CD4 percentage data.
b By Mann–Whitney U test.
c Data not available for 27 children.
d 36% had the truncated maximum value of 5.7 Log10 (copies/mL).

Figure 1. Loess-smoothed regression lines showing the relationship of mean HIV RNA levels (log10 copies/mL) to age for male and female children followed longitudinally in cohort 3. HIV RNA levels were lower among female children across all observed ages.

Figure 2. Loess-smoothed regression lines showing the relationship of CD4 cell percentage to age for male and female children followed longitudinally in cohort 3. CD4 cell percentages were higher among female individuals across all observed ages.
Sex differences in HIV RNA levels among adults have been well established. During early HIV infection, plasma HIV RNA levels are \( \sim 0.5 \) log lower in adult women than in men, but this difference narrows as the disease progresses, becoming statistically insignificant when CD4 cell counts are \( \geq 200 \text{ cells/mL} \) [1–3, 28, 29]. CD4 cell counts are also higher among adult women with and without HIV infection [3]. Prior studies of HIV RNA levels and CD4 cell parameters among male and female children have been limited and have yielded conflicting results. One study of children receiving ART found HIV RNA levels to be 0.38 log lower in girls than in boys, with no difference in CD4 cell count or percentage [15]. Another group reported that HIV RNA levels were higher in girls at the peak of viremia but consistently lower after 4 years of age [16]. Of note, this group also reported CD4 cell counts to be significantly lower among HIV-infected girls than boys [30], in contrast to our findings of higher CD4 cell percentages among girls. However, these studies included few or no children who were naive to combination ART [15, 16, 30]. The only prior study to focus on ART-naive children was limited to 38 infants and found no difference in

<table>
<thead>
<tr>
<th>Age (years)(^c)</th>
<th>n=</th>
<th>Females</th>
<th>Males</th>
<th>( P ) value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;2)</td>
<td>210</td>
<td>15 (10–21)</td>
<td>17 (11–21)</td>
<td>.692</td>
</tr>
<tr>
<td>(2–6)</td>
<td>200</td>
<td>20 (15–28)</td>
<td>14 (8–21)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>(\geq6)</td>
<td>253</td>
<td>19 (10–26)</td>
<td>15 (9–22)</td>
<td>.021</td>
</tr>
<tr>
<td>CD4 Count (cells/(\mu)L)(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;350)</td>
<td>197</td>
<td>9 (5–13)</td>
<td>8 (3–13)</td>
<td>.082</td>
</tr>
<tr>
<td>(350–750)</td>
<td>214</td>
<td>19 (13–25)</td>
<td>17 (13–21)</td>
<td>.045</td>
</tr>
<tr>
<td>(\geq750)</td>
<td>232</td>
<td>23 (19–31)</td>
<td>21 (17–26)</td>
<td>.010</td>
</tr>
<tr>
<td>HIV RNA Log(_{10}) (copies/mL)(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;5.0)</td>
<td>220</td>
<td>22 (13–29)</td>
<td>15 (9–22)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5.0–5.6</td>
<td>186</td>
<td>16 (11–23)</td>
<td>14 (10–22)</td>
<td>.109</td>
</tr>
<tr>
<td>(\geq5.7)</td>
<td>232</td>
<td>15 (10–20)</td>
<td>15 (10–19)</td>
<td>.630</td>
</tr>
</tbody>
</table>

\( ^a \) \( N = 663 \) children with CD4 percentage data.
\( ^b \) By Mann–Whitney \( U \) test.
\( ^c \) Data not available for 27 children.
\( ^d \) 36\% had the truncated maximum value of 5.7 Log\(_{10}\) (copies/mL).

**Table 4.** CD4 Cell Percentage Within Subgroups of Age, Absolute CD4 Cell Count, and HIV RNA Level

![Figure 1](image1.png)  **Figure 1.** Loess-smoothed regression lines showing the relationship of mean HIV RNA levels (log\(_{10}\) copies/mL) to age for male and female children followed longitudinally in cohort 3. HIV RNA levels were lower among female children across all observed ages.

![Figure 2](image2.png)  **Figure 2.** Loess-smoothed regression lines showing the relationship of CD4 cell percentage to age for male and female children followed longitudinally in cohort 3. CD4 cell percentages were higher among female individuals across all observed ages.
HIV RNA levels between male and female individuals [17]. For our study, we drew data from a large cohort of children, none of whom had received prior ART.

Multiple factors likely contribute to sex differences in the course and clinical manifestations of infectious diseases, including behavioral and epidemiologic risk factors, socioeconomic disparities, differences in gene expression, and levels of sex steroid hormones. Our data suggest that sex hormones are unlikely to be the major driver of the sex disparity in HIV RNA levels. Circulating levels of estrogen, progesterone, and testosterone remain low throughout the prepubertal years in both girls and boys, with the exception of the transient mini-puberty of infancy, which resolves within 4–6 months after birth. This brief postnatal hormone surge is believed to drive sexual differentiation in the brain [31] and could similarly imprint sex-based differences on immune cell populations. Alternatively, genetic differences between male and female individuals may drive sexually dimorphic immune responses. Experiments comparing transgenic XY and XX mice with a common gonadal type indicate that the XX sex chromosome complement, rather than female steroid hormones, predisposes female mice to autoimmune disease [32]. Sex-based differences in gene regulation and tissue-specific gene expression have been well established to impact human disease susceptibility and severity [27, 33]. Of the ~1000 human X chromosome genes that lack a homologue on the Y chromosome, ~15% escape X inactivation to some degree [34], potentially resulting in gene dosage differences between male and female individuals. Of note, many genes with key immune functions, including those encoding TLR7, TLR8, IRAK, CD40L, and FoxP3, reside on the X chromosome and could be subject to gene dosage effects [8].

The mechanisms by which sex-biased gene expression might ultimately result in plasma HIV RNA differences are not clear, but could include differences in target cell availability or permissiveness to infection, as well as differences in cytokine production or other innate or adaptive immune mechanisms. CD8+ T cell activation levels were recently shown to differ by sex and correlate with plasma progesterone levels in adults [13]. Levels of CD38 expression by T cells are naturally high in infants and decrease with age, making interpretation in the context of HIV infection more challenging [35]. However, we did not find a sex difference in CD4 or CD8 activation levels in our prepubertal children, suggesting that sex hormone-mediated changes in T-cell activation do not underlie the sex differences in plasma HIV RNA levels in children.

These results have potential implications for pediatric HIV treatment guidelines. HIV RNA levels and CD4 cell parameters are currently the key laboratory measures used to guide initiation and monitoring of ART. In our study, fewer girls would be eligible for ART on the basis of WHO pediatric guidelines, and other studies have suggested similar discrepancies for adult women [1–3]. The clinical significance of this disparity in eligibility is unclear, but 3 studies involving children have noted higher mortality rates among girls initiating ART [15, 16, 36]. Further study is indicated to determine whether initiation of ART at higher CD4 cell counts in female children might be needed to optimize outcomes.

Our analysis has several limitations. HIV RNA levels were truncated at 5.7 log copies/mL in more than one-third of study participants, limiting our ability to accurately quantify the effect size of sex at high HIV RNA levels. This limitation would be expected to conservatively bias our analysis toward the null hypothesis of no difference and may have decreased our sensitivity to detect sex-based differences among the younger or more severely immunosuppressed children who have high HIV RNA levels. It is also possible that our study population was vulnerable to a healthy survivor bias. If female individuals were more likely to have received poor care and died, we would have enrolled a healthier cohort of female persons than male persons. However, in that case, one would predict our study population to be disproportionately male, when in fact, the overall proportion was 50%. Furthermore, the fact that sex differences were stable over time in the longitudinal data analysis makes the influence of such bias less likely. Another potential limitation of our analysis is that data were combined from 3 cohorts that differed by age, stage of disease, and HIV RNA assay used; however, each cohort was balanced with regard to sex, and inclusion of cohort in the multivariate models did not alter the results.
HIV RNA levels between male and female individuals [17]. For our study, we drew data from a large cohort of children, none of whom had received prior ART.

Multiple factors likely contribute to sex differences in the course and clinical manifestations of infectious diseases, including behavioral and epidemiologic risk factors, socioeconomic disparities, differences in gene expression, and levels of sex steroid hormones. Our data suggest that sex hormones are unlikely to be the major driver of the sex disparity in HIV RNA levels. Circulating levels of estrogen, progesterone, and testosterone remain low throughout the prepubertal years in both girls and boys, with the exception of the transient mini-puberty of infancy, which resolves within 4–6 months after birth. This brief postnatal hormone surge is believed to drive sexual differentiation in the brain [31] and could similarly imprint sex-based differences on immune cell populations. Alternatively, genetic differences between male and female individuals may drive sexually dimorphic immune responses. Experiments comparing transgenic XY and XX mice with a common gonadal type indicate that the XX sex chromosome complement, rather than female steroid hormones, predisposes female mice to autoimmune disease [32]. Sex-based differences in gene regulation and tissue-specific gene expression have been well established to impact human disease susceptibility and severity [27, 33]. Of the ~1000 human X chromosome genes that lack a homologue on the Y chromosome, ~15% escape X inactivation to some degree [34], potentially resulting in gene dosage differences between male and female individuals. Of note, many genes with key immune functions, including those encoding TLR7, TLR8, IRAK, CD40L, and FoxP3, reside on the X chromosome and could be subject to gene dosage effects [8].

The mechanisms by which sex-biased gene expression might ultimately result in plasma HIV RNA differences are not clear, but could include differences in target cell availability or permissiveness to infection, as well as differences in cytokine production or other innate or adaptive immune mechanisms. CD8+ T cell activation levels were recently shown to differ by sex and correlate with plasma progesterone levels in adults [13]. Levels of CD38 expression by T cells are naturally high in infants and decrease with age, making interpretation in the context of HIV infection more challenging [35]. However, we did not find a sex difference in CD4 or CD8 activation levels in our prepubertal children, suggesting that sex hormone–mediated changes in T-cell activation do not underlie the sex differences in plasma HIV RNA levels in children.

These results have potential implications for pediatric HIV treatment guidelines. HIV RNA levels and CD4 cell parameters are currently the key laboratory measures used to guide initiation and monitoring of ART. In our study, fewer girls would be eligible for ART on the basis of WHO pediatric guidelines, and other studies have suggested similar discrepancies for adult women [1–3]. The clinical significance of this disparity in eligibility is unclear, but 3 studies involving children have noted higher mortality rates among girls initiating ART [15, 16, 36]. Further study is indicated to determine whether initiation of ART at higher CD4 cell counts in female children might be needed to optimize outcomes.

Our analysis has several limitations. HIV RNA levels were truncated at 5.7 log copies/mL in more than one-third of study participants, limiting our ability to accurately quantify the effect size of sex at high HIV RNA levels. This limitation would be expected to conservatively bias our analysis toward the null hypothesis of no difference and may have decreased our sensitivity to detect sex-based differences among the younger or more severely immunosuppressed children who have high HIV RNA levels. It is also possible that our study population was vulnerable to a healthy survivor bias. If female individuals were more likely to have received poor care and died, we would have enrolled a healthier cohort of female persons than male persons. However, in that case, one would predict our study population to be disproportionately male, when in fact, the overall proportion was 50%. Furthermore, the fact that sex differences were stable over time in the longitudinal data analysis makes the influence of such bias less likely. Another potential limitation of our analysis is that data were combined from 3 cohorts that differed by age, stage of disease, and HIV RNA assay used; however, each cohort was balanced with regard to sex, and inclusion of cohort in the multivariate models did not alter the results.
HIV RNA levels between male and female individuals [17]. For our study, we drew data from a large cohort of children, none of whom had received prior ART.

Multiple factors likely contribute to sex differences in the course and clinical manifestations of infectious diseases, including behavioral and epidemiologic risk factors, socioeconomic disparities, differences in gene expression, and levels of sex steroid hormones. Our data suggest that sex hormones are unlikely to be the major driver of the sex disparity in HIV RNA levels. Circulating levels of estrogen, progesterone, and testosterone remain low throughout the prepubertal years in both girls and boys, with the exception of the transient mini-puberty of infancy, which resolves within 4–6 months after birth. This brief postnatal hormone surge is believed to drive sexual differentiation in the brain [31] and could similarly imprint sex-based differences on immune cell populations. Alternatively, genetic differences between male and female individuals may drive sexually dimorphic immune responses. Experiments comparing transgenic XY and XX mice with a common gonadal type indicate that the XX sex chromosome complement, rather than female steroid hormones, predisposes female mice to autoimmune disease [32]. Sex-based differences in gene regulation and tissue-specific gene expression have been well established to impact human disease susceptibility and severity [27, 33]. Of the ~1000 human X chromosome genes that lack a homologue on the Y chromosome, ~15% escape X inactivation to some degree [34], potentially resulting in gene dosage differences between male and female individuals. Of note, many genes with key immune functions, including those encoding TLR7, TLR8, IRAK, CD40L, and FoxP3, reside on the X chromosome and could be subject to gene dosage effects [8].

The mechanisms by which sex-biased gene expression might ultimately result in plasma HIV RNA differences are not clear, but could include differences in target cell availability or permissiveness to infection, as well as differences in cytokine production or other innate or adaptive immune mechanisms. CD8+ T cell activation levels were recently shown to differ by sex and correlate with plasma progesterone levels in adults [13]. Levels of CD38 expression by T cells are naturally high in infants and decrease with age, making interpretation in the context of HIV infection more challenging [35]. However, we did not find a sex difference in CD4 or CD8 activation levels in our prepubertal children, suggesting that sex hormone–mediated changes in T-cell activation do not underlie the sex differences in plasma HIV RNA levels in children.

These results have potential implications for pediatric HIV treatment guidelines. HIV RNA levels and CD4 cell parameters are currently the key laboratory measures used to guide initiation and monitoring of ART. In our study, fewer girls would be eligible for ART on the basis of WHO pediatric guidelines, and other studies have suggested similar discrepancies for adult women [1–3]. The clinical significance of this disparity in eligibility is unclear, but 3 studies involving children have noted higher mortality rates among girls initiating ART [15, 16, 36]. Further study is indicated to determine whether initiation of ART at higher CD4 cell counts in female children might be needed to optimize outcomes.

Our analysis has several limitations. HIV RNA levels were truncated at 5.7 log copies/mL in more than one-third of study participants, limiting our ability to accurately quantify the effect size of sex at high HIV RNA levels. This limitation would be expected to conservatively bias our analysis toward the null hypothesis of no difference and may have decreased our sensitivity to detect sex-based differences among the younger or more severely immunosuppressed children who have high HIV RNA levels. It is also possible that our study population was vulnerable to a healthy survivor bias. If female individuals were more likely to have received poor care and died, we would have enrolled a healthier cohort of female persons than male persons. However, in that case, one would predict our study population to be disproportionately male, when in fact, the overall proportion was 50%. Furthermore, the fact that sex differences were stable over time in the longitudinal data analysis makes the influence of such bias less likely. Another potential limitation of our analysis is that data were combined from 3 cohorts that differed by age, stage of disease, and HIV RNA assay used; however, each cohort was balanced with regard to sex, and inclusion of cohort in the multivariate models did not alter the results.
In summary, we found substantial differences in HIV RNA level and CD4 cell percentages between HIV-infected boys and girls throughout childhood and before the onset of puberty. These data suggest that sex-specific mechanisms other than steroid hormones are important in the immunologic development and control of HIV infection in children. Our findings have broad implications for the understanding of sex differences in immune function and highlight the need for further study of the molecular mechanisms underlying this sexual dimorphism.

Acknowledgments

We thank the UCSF Clinical and Translational Science Institute Biostatistics Core and, in particular Steve Shiboski, for assistance with the longitudinal data analyses; and Holly Zanoni and Thuli Phungula for development and maintenance of the database of South African subjects.

Financial support. This work was supported by the Sullivan Family Foundation (Philani Program), the Elizabeth Glaser Pediatric AIDS Foundation (to M. E. F.), and the National Institute of Health (grants A151982 to D. H., K236045901A2 to T. D. R, and R01AI068497 to M. E. F.). M. E. F. is the recipient of the Jewels for Children Elizabeth Glaser Scientist Award.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgements section.

References

ANTIRETROVIRAL THERAPY FOR HIV INFECTION IN INFANTS AND CHILDREN: TOWARDS UNIVERSAL ACCESS
Recommendations for a public health approach

2010 revision
Clinical disease progression should be differentiated from IRIS. The worsening of disease after initial clinical improvement or the development of a new or recurrent OI soon after initiating ART in a child does not necessarily indicate treatment failure and is not always an indication to stop or switch ART (see Section 8.6 for further detail about IRIS).

11.5 Immunological definition of treatment failure

Immunological treatment failure can be identified by assessing the immunological response to ART in relation to baseline CD4. Treatment failure is characterized by a drop in the CD4 to values at or below the age-dependent values given in the box below (Box 11), or a failure of the CD4 count to rise above these threshold values. Recognition of treatment failure on the basis of immunological values relies on comparison with previous CD4 values, and underscores the need for CD4 measurement at the start of ART. Switching a regimen should be considered if CD4 values fall to <200 cells/mm³ (or <10%) for a child aged between 2 and 5 years or <100 cells/mm³ for a child aged 5 years or more (Box 11 and Table 15).

For any given CD4 threshold, the likelihood of disease progression or death is greater the younger the child. For infants and young children less than 2 years of age, the CD4 thresholds presented in Box 11 reflect very severe immunosuppression and should not be used. Specialist advice is needed to manage such cases.

Box 11: CD4 criteria suggesting immunological failure

<table>
<thead>
<tr>
<th>Immune failure is recognized as developing or returning to the following age-related immunological thresholds after at least 24 weeks on ART, in a treatment-adherent child:</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2 years to &lt;5 years of age</td>
</tr>
<tr>
<td>≥5 years of age</td>
</tr>
</tbody>
</table>

* Preferably, at least two CD4 measurements should be available.
  Use of %CD4+ in children <5 years and absolute CD4 counts in those ≥5 years of age is preferred.
  If serial CD4 values are available, the rate of decline should be taken into consideration.

11.6 Virological definition of treatment failure

Where regular access to viral load monitoring is available and affordable, it may be used to identify treatment failure. Viral load is the most sensitive way to detect viral replication. However, individual viral load values do not directly correlate with clinically relevant outcomes (death or disease progression).

Virological failure is recognized if the child is adherent to their (first-line) ART regimen, more than 24 weeks from initiation of ART, and has a persistent viral load over 5000 copies/ml. In resource-limited settings it may not be feasible to perform viral load testing. The availability of viral load is not a prerequisite for initiation of ART or for the determination of treatment failure.

11.7 Use of clinical and immunological findings for decision-making on switching ARV regimen

CD4 values supplement clinical findings when decisions are being made on switching therapy (Box 11 and Table 15). Switching a regimen should usually be considered only if two or more of the CD4 values...
11.9 Decision-making on switching ART using viral load measurement

Children with clinical failure and/or immunological failure may not all have virological failure, and may not need to switch to second-line therapy. However, a delay in switching therapy in a child with high levels of viral replication may lead to greater development of resistance and compromise the virological activity of standard second-line regimens. It is unclear whether this translates to compromised clinical outcomes. Therefore, in the context of accurately identifying treatment failure, measurement of viral load is useful. Currently, there are insufficient data to inform programmes on the best strategic approach to introducing viral load monitoring within ART programmes. Viral load is recommended where available to confirm clinical and/or immunological failure. WHO encourages further research and evaluation of approaches to the use of viral load monitoring [39, 120].

11.10 Use of other laboratory parameters for decision-making regarding switching ART

Total lymphocyte count should not be used for the evaluation of response to ART, because a change in TLC is a poor predictor of treatment success [121]. Up24 Ag testing is also not currently recommended to monitor the virologic treatment response. At present, testing for HIV drug resistance (HIV-DR) is not recommended as a routine part of HIV care in resource-limited settings and so is not considered further in these recommendations (see Chapter 18).