STATISTICAL ANALYSES OF IMMUNE STATUS AND PROGRESSION TO AIDS IN NAIVE HIV INFECTED CHILDREN FROM GIURGIU COUNTY, ROMANIA

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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 signifiant 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

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(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 suggestted 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 1day 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 Compartment for Monitoring and Evaluation of HIV/AIDS Data, "Prof. Dr. Matei Balş" 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.

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

Cohort characteristics

Number of patients	Total of patients 150	Girls 56	Boys 94
Age (years)	10 (7–16)	10 (7–16)	10 (7–16)
Admitted to day clinic	1995–2001	1995–2001	1995–2000
HIV diagnosis date	1989–2001	1989–2001	1989–2000
[a]CD4 count (cells/μL)	73 (0–1040)	92.5 (11–774)	59 (0–1040)
Date of death	1996–2004	1996–2004	1996–2004

NOTE. Values are medians (limits) unless otherwise listed.
[a]$P = 0.1064$ 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).

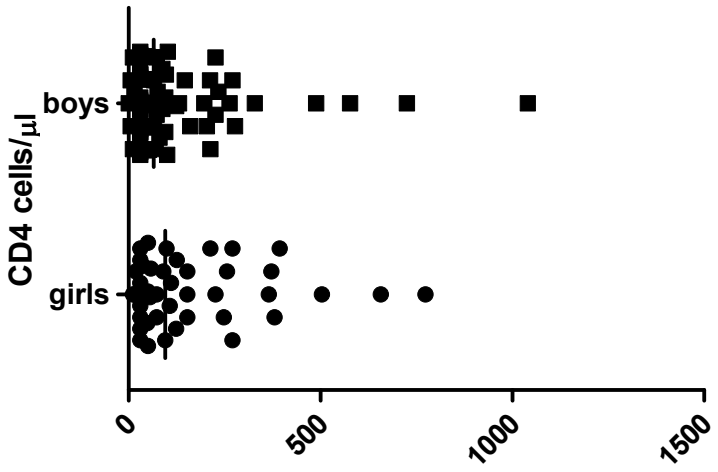

Fig. 1. Last CD4 cell count in girls compared with boys ($P = 0.5088$ by Wilcoxon match-pairs signed rank test).

When stratifying by age group (Table 2) we referred only to ages ranged 8–14, we could not compare other ages due to lack of children. The median values of last absolute CD4 count were higher in female participants almost in all tested ages, except for age 14, where boys presented higher CD4 levels. The model of linear regression based on CD4 mean values showed identical overall slopes, but the differences between slopes were not significantly different. Overall the elevations were identical and not statistically significant ($F = 1.45$; $P = 0.2582$) (Fig. 2).

Table 2

Last absolute CD4 count stratified by age group

Age	CD4 count (cells/L) girls	CD4 count (cells/L) boys	*P* value
8	372 (73–774)	124 (44–263)	0.06
9	153 (30–380)	75 (5–489)	0.04
10	73 (30–394)	47 (30–577)	0.06
11	102.5 (30–270)	44 (30–1040)	0.62
12	78.5 (49–504)	30 (5–124)	0.12
13	55 (11–107)	30 (30–277)	0.75
14	50 (50–124)	160 (50–212)	0.50

NOTE. Values are medians (limits) unless otherwise listed.
P value was calculated with Wilcoxon match-pairs signed rank test

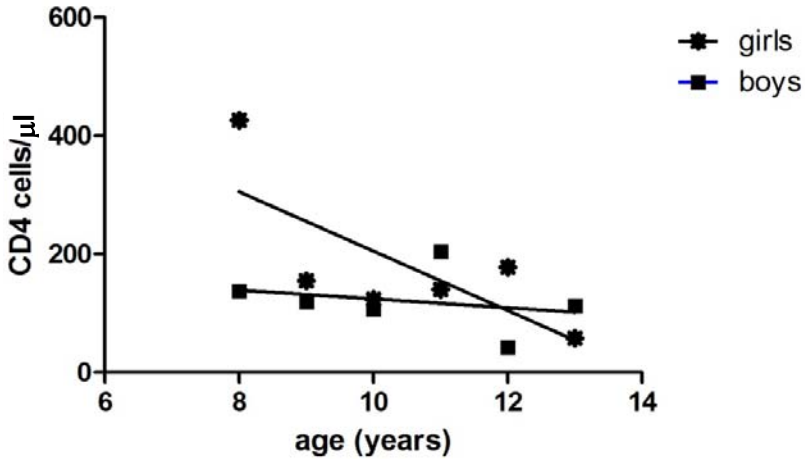

Fig. 2. Linear regression lines showing the relationship of CD4 means (cells/µL) to age in male and female children by age group; differences were not statistically significant.

PROGRESSION TO AIDS ACCORDING TO SEX

All the participants in this study progressed to AIDS and died at the median age of 10 (limits: 7–16 years). Even if the median death age was the same for boys

and girls a statistically significant higher number of boys died ($P < 0.0001$). The time from the first visit to the Day Clinic to death was evaluated for girls and boys separately (Table 3). We found no differences between girls and boys regarding median age at the moment of HIV diagnosis, median age at entry in Day Clinic and median interval from HIV diagnosis to death, all measured in years. The time of follow-up was statistically higher for girls than for boys.

Table 3

Age characteristics of the 150 participants

Characteristic	Girls (56)	Boys (94)	[a]*P* value
Median age at HIV diagnosis (years)	5.5 (1–12)	5 (1–13)	0.1949
Median age at entry in Day Clinic	8 (6–12)	8 (7–13)	0.4212
Median follow up (years)	2 (0–7)	1 (0–8)	0.0001
Median interval from HIV diagnosis to death (years)	4.5 (1–14)	5 (0–13)	0.0837

[a] Wilcoxon Signed Rank Test, *P* value (two tailed)

HIV infection progressed to AIDS in 94 boys and 56 girls; a Kaplan-Meier analysis of the time to progression did not demonstrate a significant difference according to sex ($P = 0.8842$ by the log-rank test) (Fig. 3). In addition, a Cox proportional-hazards model of the time to a diagnosis of AIDS, in which sex was a covariate, showed that the risk of AIDS was not significantly greater for girls than for boys (hazard ratio for girls, 1.029; 95% CI: 0.1822 to 1.618 ; $P = 0.3056$). The relative proportions of AIDS-defining diagnoses did not differ according to sex.

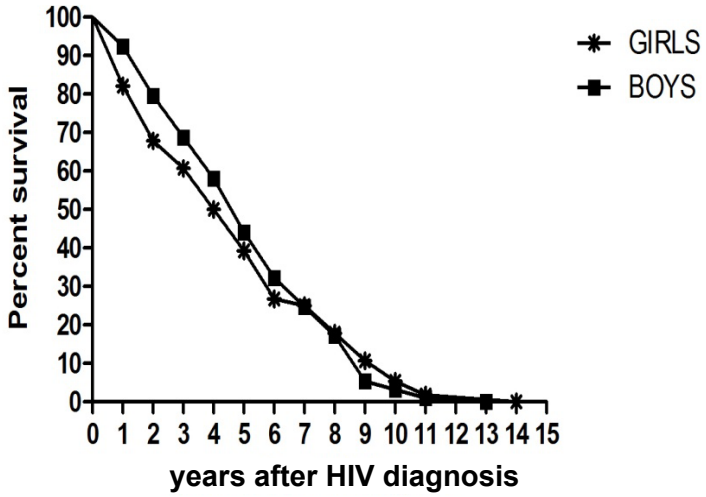

Fig. 3. Kaplan-Meier estimates of survival with progression to AIDS according to sex. The curves represent the percentages of patients surviving without AIDS during the fourteen years after seroconversion.

The numbers of boys and girls at risk during each 12-month interval are given in Table 4. There was no significant difference between men and women in the risk of progression to AIDS ($P = 0.8842$ by the log-rank test).

Table 4

Number of boys and girls at risk of AIDS progression

Age	0	1	2	3	4	5	6	7	8	9	10	11	13	14
Girls	56	56	46	38	34	28	22	15	14	10	6	3	0	1
Boys	94	94	86	74	64	54	41	30	23	16	5	3	1	

To determine whether the last CD4 count correlated with the time until death we used Pearson correlation calculation model. The result turned out negative, the time to death could not be correlated with CD4 count either in girls, $P = 0.061$ or in boys $P = 0.072$. A linear regression model (Fig. 4) was constructed also and the differences between the slopes were significant, ($F = 4.013$, $P = 0.047$). We noticed that in girls the last CD4 value increased with time until death while in boys it decreased, fact that correlates with the mean CD4 values. This fact suggests that absolute CD4 cell count may be a better predictor of mortality in girls than in boys.

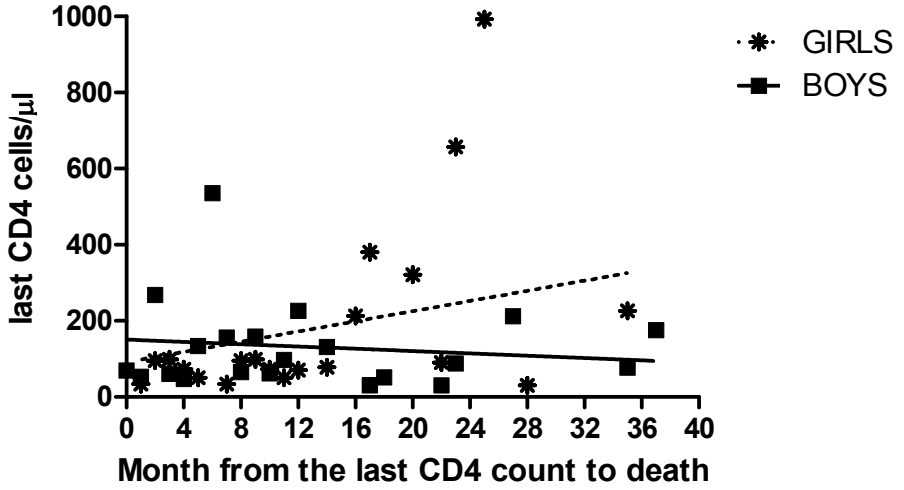

Fig. 4. Linear regression lines showing the relationship of last CD4 levels (cells/µl) to time (month) from the last determination to death in male and female children.

ELIGIBILITY OF GIRLS AND BOYS FOR ART ON THE BASIS OF CURRENT TREATMENT GUIDELINES

To assess the effect of our findings with respect to the World Health Organization (WHO) Pediatric HIV Treatment Guidelines [12], we compared the proportions of boys and girls who would have been eligible for antiretroviral

therapy (Table 5). We considered eligible for ART all children with CD4 count level < 350 cells/μL.

Table 5

Eligibility of girls and boys for ART on the basis of current guidelines

Age (years)	Girls (56)		Boys (94)		*P** value
	number	% eligible	number	% eligible	
7	1	100	3	100	NA
8	10	40	18	100	0.6332
9	14	77.7	18	92.3	0.5442
10	9	88.8	20	94.4	1.0000
11	8	100	10	85.7	0.4667
12	6	75	11	100	0.3077
13	3	100	4	100	NA
14	3	100	4	100	NA
15	1	100	4	100	NA
16	1	100	3	66.6	1.0000

**P* values were determined with the Chi-square and Fisher's exact test.
NA = In this situation, chi-square analysis was impossible.

There was no significant difference in the proportions of boys and girls who would have been eligible for therapy solely on the basis of last absolute CD4 lymphocyte count of less than 350 cells/μL. Some investigators suggested, in contrast, that the cut-off values for CD4 count used in the current guidelines may lead to premature use of antiretroviral therapy [6]. The major goal of HIV therapy is to maintain the long-term health of the patient while avoiding drug-related toxicity and preserving viable future treatment options. Early, aggressive therapy often prematurely exposes patients to risks for medication-related side effects and resistance. Viral load is the basis for the current guidelines for the initiation of antiretroviral therapy, which apply uniformly to boys and girls. In resource-limited settings it is not always feasible to perform viral load testing very often. The absolute CD4 count is used as an alternative to initiate ART. At this moment differences between sexes with regard to CD4 count are debated in many studies.

CONCLUSIONS

Here we analysed differences of immune status and AIDS progression between sexes in a unitary cohort of 150 HIV infected patients never exposed to ART while evaluating the natural HIV infection. Girls presented increased values of absolute CD4 count levels at the last evaluation before death at all ages tested compared with boys. We conclude that later in the course of infection, when the patients reached the AIDS stage, there is no significant sex-based difference in the

CD4 count. Even if the median interval from HIV diagnosis to death was higher in boys (5 years *vs* 4.5 years in girls) the rates of progression to AIDS turn out to be similar. Last CD4 count did not correlate with the time until death but there were significant differences in boys compared with girls suggesting that absolute CD4 cell count may be a better predictor of mortality in girls.

Although we did not determine the optimal time for initiating therapy, these factors suggest that the cut-off values in the current guidelines should be reassessed in the light of the equal risk of disease progression for boys and girls and their equal eligibility for therapy if the absolute CD4 count is the initiation criterion.

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