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Short communication

Hypogonadism in HIV-1-infected men is common and does not resolve during antiretroviral therapy

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Objectives: To assess the prevalence of abnormal testosterone and gonadotropin values in HIV-infected men before and after 2 years of combination antiretroviral therapy (cART).
Design: Multicentre cohort of HIV-infected adults.
Methods: We identified 139 Caucasian antiretroviral-naive male patients who started zidovudine/lamivudine-based cART that was virologically successful over a 2 year period. Ninety-seven were randomly chosen and plasma hormone determinations of free testosterone (fT) and luteinizing hormone (LH) at baseline and after 2 years of cART were evaluated.
Results: At baseline 68 patients (70%) had subnormal fT levels. In these, LH levels were low in 44%, normal in 47% and high in 9%. There was a trend for an association between lower $CD4^+$ T-cell counts and hypogonadism. Most participants had normal FSH levels. No significant changes of fT, LH and FSH levels were observed after 2 years of cART.
Conclusions: Low fT levels, mainly with normal or low LH levels and thus indicating secondary hypogonadism, are found in the majority of HIV-infected men and do not resolve during 2 years of successful cART.

Introduction

Changes in male sex hormones during HIV infection have been described since the beginning of the HIV epidemic [1,2]. Acquired hypogonadism, mostly hypogonadotropic, that being with low levels of luteinizing hormone (LH), is the disorder most frequently seen during the natural course of HIV infection. Little is known about the influence of combination antiretroviral therapy (cART) on the hypothalamic–pituitary–gonadal (HPG) axis. In particular, it is not known whether hypogonadism is reversible if successful cART is continued for several years.

The aim of this study was to describe the prevalence of abnormalities in free testosterone (fT), LH and follicle stimulating hormone (FSH) and their change during 2 years of successful cART in antiretroviral-naive HIV-infected men.

Materials and methods

The Swiss HIV Cohort Study

The Swiss HIV Cohort Study (SHCS, www.shcs.ch) is a prospective cohort study enrolling HIV-infected adults. Data are collected in seven study centres (Basel, Berne, Geneva, Lausanne, Lugano, St Gall and Zurich) according to a standardized protocol [3] at registration and all follow-up visits. Every 6 months blood plasma samples are obtained and stored at -80°C for future research projects. These

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plasma samples are not always taken in a morning fasting state.

Study design

We performed a study of ART-naive HIV-infected Caucasian men who started and continued a virologically successful zidovudine (AZT) and lamivudine (3TC)-based cART for 2 years. To be eligible for the study individuals had to remain on an unchanged cART. Patients with an acute opportunistic infection at the start of cART or androgen-treatment during the 2 years of study were excluded. Additional exclusion criteria were diabetes mellitus before starting cART and an uncontrolled AIDS-defining illness at the start of cART. Patients had to have undetectable plasma HIV RNA (<50 copies/ml) after 2 years of cART, as a marker for good adherence to the treatment. According to the SHCS dataset of July 2003, 139 participants fulfilled these criteria.

We randomly chose 100 participants and measured serum levels of LH, FSH and fT in the plasma stored before the start (baseline) and 2 years after unchanged cART. In one patient hormone levels could not be determined in the baseline sample. In addition, two patients were withdrawn from analysis because source data verification in patient charts revealed that they had been on antiretroviral drugs before entering the study. As a result, the study was performed with 97 patients.

Table 1. Characteristics of the study participants

Characteristics	*n*=97
Median age, years (IQR)	39 (35–45)
Duration of documented HIV infection, years (IQR)	1.2 (0.1–7.1)
HIV transmission mode, *n* (%)	
MSM	44 (45)
Heterosexual	30 (31)
IDU	22 (23)
Unknown/other	1 (1)
CDC stage, *n* (%)	
A	69 (71)
B	23 (24)
C	5 (5)
cART, *n* (%)	
PI based	61 (63)
NNRTI based	36 (37)
Before cART	
Median BMI, kg/m^2 (IQR)	23.4 (20.6–26)
Median CD4$^+$ T-cell count, cells/μl (IQR)	201 (102–331)
Median HIV RNA, $\log_{10}$ c/ml (IQR)	4.7 (3.3–5.2)
After 2 years of cART	
Median BMI, kg/m^2 (IQR)	24.4 (21.7–26.3)
Median CD4$^+$ T-cell count, cells/μl (IQR)	433 (277–673)

BMI, body mass index; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; IDU, intravenous drug use; IQR, interquartile range; MSM, men who have sex with men.

Data record

Date of birth, height, HIV-transmission mode and ethnicity are recorded at enrolment into the SHCS for every patient. Weight, plasma HIV RNA, CD4$^+$ T-cell count, stage of HIV infection, AIDS-defining conditions, all changes of antiretroviral treatment, the use of anabolic drugs and androgens are assessed prospectively every 6 months within the SHCS. The last recording before starting cART was used as baseline and stored plasma from this visit was used to determine baseline hormone levels. For data after 2 years of cART the recording nearest to 24 months after commencement of cART was used if it was at least 18 months after starting antiretroviral treatment.

Measurements of viral load and CD4$^+$ T-cells in the plasma

Plasma viral load was measured using the Roche Amplicor Monitor assay (Roche Diagnostics, Rotkreuz, Switzerland; limit of detection <50 copies/ml). CD4$^+$ T-cell counts were determined by flow cytometry at the reference laboratories of the SHCS.

Measurement of hormones in the plasma

FSH and LH were quantified by commercial microplate double-antibody ELISA methods (Easia®, MedGenix, Fleurus, Belgium).

Radio-immunoassay (Diagnostic Systems Laboratories, Webster, TX, USA) was used to measure fT. For all commercial assays, the manufacturer's protocols were followed. Initial assessment was carried out without dilution; in some cases, however, repetition in a diluted sample was necessary.

Hormone measurements after frozen storage have been validated [4]. All assays were performed in duplicate.

Age-adjusted normal values were used for the categorization of values within or outside the normal range.

Statistical analysis

A Wilcoxon signed-rank test and/or a two-sample Wilcoxon rank-sum (Mann–Whitney) test was used for numerical, χ^2 statistics for categorical data. A two-sided *P*-value of ≤0.05 was considered significant.

Results

The characteristics of the 97 studied participants are shown in Table 1. About two-thirds started a protease inhibitor (PI)-based regimen.

Hormone levels before and after 2 years of cART and the proportion of values measured below or above the age-adjusted normal levels at these two time points are given in Table 2.

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Table 2. Hormone levels in 97 HIV-infected men before and after 2 years of cART

	Before cART	After 2 years of cART	*P*-value
FSH			
Median hormone levels, IU/l (IQR)	3.0 (1.8–5.1)	3.1 (2.0–4.7)	0.5*
Low levels, *n* (%)	10 (10)	12 (12)	0.7†
High levels, *n* (%)	4 (4)	4 (4)	-
LH			
Median hormone levels, IU/l (IQR)	3.0 (1.7–5.0)	3.1 (2.0–5.7)	0.3*
Low levels, *n* (%)	33 (34)	25 (26)	0.15†
High levels, *n* (%)	11 (11)	11 (11)	-
fT			
Median hormone levels, pmol/l (IQR)	23.1 (17.6–30)	23.8 (18.0–31.2)	0.6*
Low levels, *n* (%)	68 (70)	61 (63)	0.2†
High levels, *n* (%)	1 (1)	1 (1)	-
Type of hypogonadism			
Hypogonadotropic hypogonadism, *n* (%)	30 (44)	23 (38)	1.0†
Normo-gonadotropic hypogonadism, *n* (%)	32 (47)	31 (51)	0.3†
Hypergonadotropic hypogonadism, *n* (%)	6 (9)	7 (11)	1.0†

*Two-sample Wilcoxon rank-sum test; †McNemar's χ^2 test. cART, combination antiretroviral therapy; FSH, follicle stimulating hormone; fT, free testosterone; IQR, interquartile range; LH, luteinizing hormone.

We did not find any factors significantly associated with hypogonadism (Table 3) except for a trend (P=0.1) for an association between low baseline CD4+ T-cell counts and fT levels below the age-adjusted normal limits.

At baseline, 70% of fT levels were below the age-adjusted lower normal levels. The LH level was high in six (9%), within the normal limits in 32 (47%) and low in 30 (44%) of these hypogonadal men.

There was no consistent effect on fT levels due to cART. After 2 years of cART, the proportion of men who showed low levels of fT had not changed significantly, with >60% remaining hypogonadal (Table 3). In 16 (24%) of the 68 initially hypogonadal patients, fT levels returned to normal during cART. Nine among them were and remained normogonadotropic, three changed from low to normal and one from normal to high LH levels. Nine (32%) of 29 men with initially normal fT levels developed hypogonadism and three men changed from normal to low LH levels. Therefore, the proportions of patients with hyper-, normogonadotropic or hypergonadotropic hypogonadism did not change significantly.

Changes in fT or LH levels did not differ with regard to whether the cART regimen included a PI or a non-nucleoside reverse transcriptase inhibitor.

Although the proportion of patients with low LH levels decreased after 2 years of cART, this did not reach statistical significance (P=0.15, McNemar's χ^2 test).

Discussion

Hypogonadism has repeatedly been described in HIV-infected men. HIV-related hypogonadism might or might not be related to an impaired HPG axis, as both hypogonodatropic [5,6] and hypergonadotropic [1,5,7] hypogonadism have been reported. Androgen deficiency has been found to be associated with low CD4+ T-cell counts, progression of illness, medication use and weight loss [8–10].

The proportion (70%) of men with hypogonadism observed in our study is the highest reported so far in a group of HIV-infected individuals not suffering from uncontrolled opportunistic diseases [1,10,11]. This might be explained in part by the age-adjusted normal range used to identify hypogonadism here. Moreover, some of the studies measured total testosterone levels [1], which might underestimate the frequency of hypogonadism in this population [10]. Therefore, the serum measurement of fT is still considered the gold standard for the diagnosis of hypogonadism [12]. The kit we used to measure fT is based on analogue tracer technology, which might show slightly different results compared with measurement by dialysis.

In the patients with low fT, LH levels were within or below the normal range in 91% and 89% before and after 2 years of cART, respectively (Table 3). In a functional HPG axis one would expect high LH levels as a response to low testosterone, so this finding points towards a mainly secondary hypogonadism in the studied population.

After 2 years of cART the fT levels did not increase significantly. This finding concurs with many other, mostly cross-sectional, studies, which found similar frequencies of androgen deficiency in HIV-positive patients with or without antiretroviral

Table 3. Logistic regression model of associations with low free testosterone levels

	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)	*P*-value
Plasma HIV RNA, per log_{10} copies/ml	0.61 (0.32–1.18)	0.60 (0.29–1.24)	0.2
CD4+ T-cell count at cART start, per 50 cells/μl increase	0.95 (0.84–1.06)	0.91 (0.80–1.03)	0.1
Duration of documented HIV infection, years	1.02 (0.93–1.21)	1.01 (0.91–1.12)	0.9
Body mass index increase, per unit	1.01 (0.89–1.15)	1.01 (0.88–1.18)	0.8
Age increase, per 10 years	0.99 (0.62–1.58)	0.91 (0.53–1.56)	0.7
HIV transmission by IDU as compared to heterosexual	1.46 (0.41–5.17)	1.20 (0.26–5.49)	0.8
HIV transmission by MSM as compared to heterosexual	0.83 (0.31–2.25)	0.82 (0.25–2.64)	0.7

The adjusted odds ratios are from a regression model that included all parameters shown in the table. cART, combination antiretroviral therapy; CI, confidence interval; IDU, intravenous drug use; MSM, men who have sex with men.

therapy [10,11]. One study, however, found a significant increase in testosterone levels in 15 patients during cART [13].

HIV infection has been shown to reduce semen quality in some studies [14–16], but this has been questioned [17]. In general, low semen quality is associated with pathological FSH levels. In our study the proportion of patients with pathological FSH levels was low and our findings do not suggest a uniform pathology of this hormonal pathway.

The structure of the SHCS allowed us to identify treatment-naive Caucasian HIV-infected men who started and continued a virologically successful cART on a constant nucleoside reverse transcriptase inhibitor backbone and to analyse fT and LH at the start of cART and after 2 years of successful treatment. This is a unique design to study the prevalence of endocrine abnormalities in HIV-infected men and the course of the abnormalities during cART, because prior antiretroviral therapy has been a major confounding factor in several other studies.

On the other hand, the use of stored plasma samples is a limitation of our study, because they were not all taken in a morning fasting state. Diurnal variations, with lower afternoon fT levels, might have led to a certain overestimation of the prevalence of hypogonadism. However, diurnal variations are mostly seen in young, healthy men and tend to disappear with increasing age [18,19].

In conclusion, our data show that a considerable proportion of treatment-naive HIV-positive men have abnormally low age-adjusted levels of fT, which is not reversible after 2 years of successful cART. This deficiency might have an impact on psychological issues such as depression, fatigue, lack of drive and decreased libido, but might also have long-term consequences including osteoporosis, loss of weight and anaemia. Looking for hypogonadism and, if necessary, testosterone supplementation after exclusion of contraindications might be indicated in these patients.

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