ORIGINAL RESEARCH—ENDOCRINOLOGY

The Determination of Total Testosterone and Free Testosterone (RIA) are not Applicable to the Evaluation of Gonadal Function in HIV-Infected Males

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ABSTRACT

Introduction. Hypogonadism is common in human immunodeficiency virus (HIV)-infected men; the high concentration of sex hormone binding globulin (SHBG) in this population, induces a "false increase" in total testosterone (TT) values.

Aims. To validate the determination of TT and measured free testosterone (FT [radioimmunoassay {RIA}]) for hypogonadism diagnosis in an HIV-infected population using calculated free testosterone (CFT) as reference method; and also to determine the prevalence and identify the risks factors of hypogonadism.

Methods. Cross-sectional, observational study. Ninety HIV-infected males (42 ± 8.2 years), not HCV coinfected, antiretroviral therapy (ART)-*naive* (14 patients), on current ART with enhanced protease inhibitor (PI) (39 patients), or patients on PI-*naive* ART (NN) (37 patients).

Main Outcome Measures. CFT was calculated by determining TT, SHBG, and albumin (Vermeulen's formula); hypogonadism was defined as CFT <0.22 nmol/L (reference range for young healthy males in our laboratory); sensitivity of TT and FT (RIA) for hypogonadism diagnosis was calculated.

Results. Twelve patients (13.3%, 95% confidence interval [CI] 7.8–21.9) by CFT presented hypogonadism. TT and FT (RIA) presented a sensitivity of less than 30% in the diagnosis of hypogonadism. Logistic regression multivariate analysis confirmed an independent association between hypogonadism, the patient's age per decade, odds ratio (OR) 6.9 (CI 1.9–24.8; $P = 0.003$), and longer duration of HIV infection per decade, OR 13.1 (CI 1.3–130.6; $P = 0.02$). Hypogonadism was associated with erectile dysfunction.

Conclusions. TT and FT (RIA) are not useful in the differential diagnosis of hypogonadism in HIV-infected males. There is a significant prevalence of hypogonadism in HIV-infected males, with the patient's age and duration of the disease being the only identifiable risk factors. **Moreno-Pérez O, Escoín C, Serna-Candel C, Portilla J, Boix V, Alfayate R, González-Sánchez V, Mauri M, Sánchez-Payá J, and Picó A. The determination of total testosterone and free testosterone (RIA) are not applicable to the evaluation of gonadal function in HIV infected males. J Sex Med 2010;7:2873–2883.**

Key Words. Hypogonadism; Calculated Free Testosterone; SHBG; HIV; Screening; Risk Factors

Introduction

Hypogonadism is a common endocrine disorder in men infected by the human immunodeficiency virus (HIV) [1,2]; it affects 17–25% of men receiving highly effective antiretroviral therapy (ART) [1–3]. These rates of gonadal dysfunction are greater than those reported in the general adult population [4].

Hypogonadism in males is characterized by the incapacity of the testes to produce physiological quantities of testosterone (androgenic deficiency) and a normal number of spermatozoids due to the interruption at one or more levels of the hypothalamic-hypophyseal-gonadal axis [5]. Clinical guidelines recommend making a diagnosis of androgen deficiency only in men with consistent symptoms and signs and unequivocally low serum testosterone levels [5]; however, many symptoms are nonspecific and overlap with their own chronic diseases, such as fatigue, loss of energy, low mood, irritability, and reduced intellectual ability. Both primary hypogonadism by testicular failure and secondary by hypothalamic-pituitary failure, with acquired or congenital origin, have been associated with HIV infection and may appear jointly in these patients. Various etiopathogenic mechanisms in HIV-infected men, such as systemic diseases, opportunistic infections, malnutrition, weight loss, and cachexia, have been suggested [6,7]. However, many young men in a stable clinical state with none of these risk factors develop hypogonadism. HIV infection may play a role in testicular function, either directly or indirectly mediated by cytokines [8]. Antiretroviral treatment may also have a role. Protease inhibitors (PI) appear to inhibit the cytochrome P450 (CYP3A4) mediated testosterone metabolism in vitro in human hepatocyte microsomes [9], although no effects have been seen in vivo.

Hypogonadism may contribute to erectile dysfunction (ED) in HIV-infected patients, although its actual role has not been clearly established [1,10]. Otherwise, sex steroids are involved in the regulation of a large number of physiological processes, such as endothelial function, metabolism of lipoproteins, insulin homeostasis, and body composition [11,12]. Hypogonadism, ED, obesity, insulin resistance, and metabolic syndrome often coexist in the same subjects; it should be emphasized that testosterone seems to exert a favorable effect upon vascular reactivity, inflammation, cytokine production, adhesion molecule expression as well as on serum lipid concentration and hemostatic factors, suggesting a protective role against the development of atherosclerosis and its clinical complications [13]. Low serum testosterone concentrations have been proposed for the list of cardiovascular risk factors in the general population [14]. Early diagnosis of hypogonadism in HIV-infected males is important due to its impact on quality of life and its possible repercussion on cardiovascular function.

The diagnosis of hypogonadism is based on the finding of low serum testosterone concentrations. Serum total testosterone (TT) concentrations, representing the sum of unbound and protein-bound testosterone in circulation, are measured by (radioimmunoassay) RIA, immunometric assays, or liquid chromatography tandem mass spectrometry. Most of the circulating testosterone is bound to sex hormone binding globulin (SHBG) and to albumin; only 0.5–3% of circulating testosterone is unbound or "free." The term "bioavailable testosterone" (BT) refers to unbound testosterone plus testosterone bound loosely to albumin. In adult males of general population, free testosterone (FT) determined by direct techniques (RIA) and the FT index are not adequate measures of FT [15,16]. In the HIV-infected population, the high concentration of SHBG induces an increase in TT values that can lead to underestimation of hypogonadism diagnosis. The *Infectious Diseases Society of America* recommends determining FT with reliable assay (such equilibrium dialysis) if alterations in binding proteins are suspected [17]. Although apparent free testosterone concentration (AFTC) can be measured accurately by equilibrium dialysis and BT by ammonium sulfate [5], these accurate and reliable reference assays are expensive, laborious, and usually are not available in local laboratories, and are not useful in everyday clinical practice [15]. However, mathematical equations which take into consideration the kinetics of the bond between FT and albumin and SHBG provide results practically identical to those of the reference techniques (correlation coefficient 0.87–0.98). Calculated free testosterone (CFT) and calculated BT (CBT) are good surrogate markers of FT in plasma [15,18]. So, the *Endocrine Society* recommends determining FT and BT by reference assays, or also be calculated from TT, SHBG, and albumin using published algorithms, as screening tests of hypogonadism in conditions associated with alterations in SHBG concentrations, like in HIV-infected males [5]. Clinicians should use the lower values of the normal range for healthy young men established by their reference laboratory [5].

The diagnostic approach for hypogonadism in HIV-infected males should include CFT or CBT [5,8]. So far, no study using this approach has been published on HIV-infected males; TT and FT (RIA) are the only parameters used.

Aims

The main objective of this study was to validate TT and FT (RIA) determinations for hypogonadism diagnosis in HIV-infected men, using CFT as reference method. Secondary objectives were: to determine the prevalence of hypogonadism in HIV-infected males whose clinical state was stable, using CFT for diagnosis, and to try to find possible risk factors as the duration of HIV infection, type and time of exposure to ART, CD4 level, and lipodystrophy. Finally, the validity of screening questionnaires in the diagnosis of hypogonadism in this population and the association of hypogonadism with ED was studied.

Materials and Methods

A cross-sectional observational study was carried out in the Infectious Diseases Unit of a tertiary hospital and was approved by the hospital's Ethics Committee for Clinical Research. Men undergoing follow-up in the Unit and who fulfilled the following inclusion criteria were sequentially sampled: HIV infection, age ≥18 years, informed consent given, ART-*naive* or on effective ART (<50 copies RNA/mL) and no changes in the previous 6 months. Patients on treatment were included only provided that they were being treated with 2–3 nucleoside reverse transcriptase inhibitors (NRTI) plus an enhanced PI or—if they have never been treated with PIs—with 2–3 NRTIs and a non-NRTI. Patients suffering from chronic hepatitis C (RNA-HCV$^+$), active disease defining AIDS, or diabetes mellitus were excluded. Active drug use, psychiatric illness or cognitive deficit (which make it impossible for the patient to understand the study), irregular follow-up, or therapeutic noncompliance (impossibility of having accurate information about the history of exposure to different antiviral drugs) were also causes of exclusion. Any use of prior androgen replacement therapy (either by prescription or self-use) or concomitant treatment with ganciclovir, ketoconazole, megestrol acetate, spironolactone, thiazide diuretics, anti-androgen, or estrogen therapy, which may alter the plasma testosterone concentrations, was not permitted.

Main Outcome Measurements

Outcome Variables

Primary

The sensitivity and specificity of serum FT (RIA) (reference range, rr 0.031–0.163 nmol/L; 9–47 pg/mL) (RIA, Coat-A-Count, [Siemens]) and TT (rr 10.4–34.7 nmol/L; 3–10 ng/mL) (chemoluminescent immunoassay, UniCell DXI 800 [Acess Testosterone Bekman Coulter]; intra-assay coefficient of variation [CV] is 2.6% for 29.4 nmol/L, 2.3% for 16.6 nmol/L, and 4.3% for 2 nmol/L; inter-assay CV is 5.4% for 26 nmol/L, 3.9% for 16.6 nmol/L, and 7.1% for 2 nmol/L; lower limit of sensitivity 0.35 nmol/L) for the diagnosis of hypogonadism were determined in our population. Hypogonadism was defined as CFT <0.22 nmol/L (<6.36 ng/dL), lower than the normal range for healthy young males. It was calculated by determining TT, SHBG (nmol/L; rr 4–72) (chemoluminescent immunometric assay, IMMULITE 2000, [Siemens]), and albumin (rr 480–803 μmol/L; 3,170–5,300 mg/dL) (kinetic nephelometry, immunochemical systems IMMAGE [Bekman Coulter]), from the equation Free Testosterone = ([Testosterone] – (N × [Free Testosterone]))/(Kt{SHBG – [Testosterone] + N [Free Testosterone]}), where Kt (10^9 L/mol) is the association constant of SHBG for T, and N = (Ka [3.6×10^4 L/mol, association constant of albumin for T] × Ca [albumin concentration, g/L]) + 1, described by Vermeulen et al. [16]. All hormone serum samples were collected between 0800 and 0900 h AM. In order to establish the rr of CFT values for young healthy males in our reference laboratory, samples were taken from 127 eugonadal healthy young men between 18 and 30 years old, body mass index (BMI) <30 kg/m^2, with no HIV infection who in addition fulfilled the exclusion criteria of our study. rr in our laboratory of CFT was defined as 0.22–0.77 nmol/L (6.36–22.2 ng/dL). The sample size calculation for the validation of CFT in our reference laboratory was established using a confidence level of 95%, an accuracy of 20% and an expected standard deviation of 1.07. The sample size was estimated on at least 110 individuals. For the age, distribution percentages were applied to men with regard to the existing general population for each year of age, obtained from the municipal register.

Secondary

Prevalence and Type of Hypogonadism. The follicle stimulate hormone (FSH) (U/L; rr 1–8), luteinizing hormone (LH) (U/L; rr 2–11.2), (electro-

chemiluminescent immunoassay, Modular Analytics E170, [Roche Diagnostics]) and prolactin plasma concentration (μg/L; rr 4.6–21) (electrochemiluminescent immunoassay, Modular Analytics E170, [Roche Diagnostics]) were determined. Secondary hypogonadism was defined as FSH or LH concentrations below or equal to the normal concentrations.

ED. Each patient completed the International Index of Erectile Function (IIEF-15) sexual dysfunction questionnaire [19]. ED was defined as an erectile function score of ≤25 [20]. Hypogonadism questionnaires: each patient completed two questionnaires—the *Androgen Deficiency in Aging Men Questionnaire* (ADAM) and the *Aging Male's Symptoms Scale* (AMS) [21,22].

Explanatory Variables

Sociodemographic and lifestyle variables were studied as well as variables associated with HIV infection and with ART. Nadir and current $CD4^+$ lymphocyte count (cells/mm^3 and percentage; flow cytometry; FASCalibur, Becton Dickinson Biosciences, San Jose, CA, USA), plasma viral load (PVL-HIV) (copies RNA/mL, logarithmic units; lower detection limit 39 copies/mL; COBAS TaqMan HIV-test Roche Diagnostics). Patients were classified into three groups according to their history of ART, *naive* (*naive group*), current ART with enhanced PI (PI group), or current ART based on non-NRTI and no history of previous PI treatment (NN group). Current medication, prior use of other antiretroviral drugs, and duration of exposure to these drugs were recorded.

Variables associated with findings on physical examination, metabolism, and lipodystrophy: somatometry, BMI, waist–hip ratio and genital examination determining testicular volume with a Prader orchidometer. Lipodystrophy was determined using a standard questionnaire specific for lipodystrophy based on physical examination [23].

Statistical Analysis

Qualitative variables were expressed as absolute and relative frequencies in percentages. Quantitative variables were expressed as means and standard deviation when the distribution was parametric and as medians and 25th and 75th percentiles when the distribution was nonparametric. In order to evaluate the validity of TT, FT (RIA), and the ADAM and AMS questionnaires for the diagnosis of hypogonadism, using CFT as the standard, the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and diagnostic likelihood ratio were calculated. The prevalence of hypogonadism was calculated with its 95% confidence interval (95% CI), and the association between the various clinical characteristics of the patients and hypogonadism was studied (chi-squared test for qualitative variables; Student's *t* test or Mann–Whitney test for quantitative variables). In order to quantify the strength of the association, the prevalence odds ratio (OR) was calculated with its 95% CI. An unconditional logistic regression multivariate analysis was done in which the explanatory variables with a statistical significance of $P < 0.10$ were included, together with those considered clinically relevant. In all cases, values of $P < 0.05$ were considered statistically significant. The SPSS 10.1 program (SPSS, Chicago, IL, USA) was used.

Results

One hundred nine men fulfilled the inclusion criteria, 19 refused to give their written consent to participate in the study, clinical characteristics of those patients who refused did not differ from the included population, so 90 patients were finally included (Table 1).

The mean age was 42 years (range, 25–68) (patients distribution by age: 32 [25–39 years], 43 [40–49 years], 15 [>50 years]); 18.9% were in clinical stage C; 72.2% of the patients had an undetectable viral load, median PVL-HIV 39 copies/mL [P_{25}–P_{75}, 39–103.5]. The median $CD4^+$ count was 465 cells/mm^3 and only two patients had a $CD4^+$ count below 200 cells/mm^3. Seventy-six patients (84.4%) were receiving ART. In the NN group (N = 37), there was a longer duration of the disease progression (10.1 ± 5.5 vs. 7.3 ± 5.2 years, $P = 0.026$), a longer duration of exposure to ART (81.6 ± 46.7 vs. 50.4 ± 30.4 months, $P = 0.001$), and a longer duration of current ART (24 [16–43.5] vs. 16 [10–30] months, $P = 0.009$) than in the PI group (N = 39). There were no differences between the groups in age, nadir $CD4^+$ count, current $CD4^+$ count, and PVL-HIV.

Prevalence of Hypogonadism

Twelve patients fulfilled the criteria for hypogonadism by CFT (13.3%, CI 7.8–21.9), 10 (83.3%) of hypothalamic-hypophyseal origin and two of gonadal origin (Table 2). Hypogonadism distribution by treatment groups was: six in the PI group and six in the NN group. The CFT concentration

Table 1 Clinical characteristics (N = 90)

	% (N)	Mean ± SD	Median [P_{25}–P_{75}]
Age, years	—	42 ± 8.2	—
CDC Stage		—	—
A	58.9 (53)		
B	22.2 (20)		
C	18.9 (17)		
Duration HIV—years	—	7.8 ± 5.6	—
Nadir $CD4^+$, cells/mm^3	—	—	205 [125–287]
Current $CD4^+$, cells/mm^3	—	—	465 [365–676]
Viral load RNA/mL	—	—	39 [39–104]
Group		—	—
Naive	15.5 (14)		
NN	41.1 (37)		
PI	43.3 (39)		
Duration exposure, months	—		—
Total ART		66.4 ± 42.4	
Non-NRTI		35.9 ± 26.7	
NRTI		110.8 ± 89.8*	
PI		54.7 ± 39.5	
Current exposure to			
ART	(38)		
NRTI	(17)		
Tenofovir	(35)		
Azidothymidine	(25)		
Lamivudine	(8)		
Emtricitabine	(15)		
Didanosine	(9)		
Abacavir			
Stavudine	(34)		
Non-NRTI	(3)		
Efavirenz			
Nevirapine	(23)		
PI	(10)		
Lopinavir	(3)		
Atazanavir	(3)		
Fosamprenavir			
Tipranavir			
Alcohol consumption			—
No	56.6 (51)		
Yes	43.3 (39)		
SDU		8.6 ± 7.7	
Smoker		—	—
No	26.7 (24)		
Ex-smoker	7.8 (7)		
Yes	65.6 (59)		
Sedentary lifestyle		—	—
No	42.7 (35)		
Yes	57.3 (47)		
BMI, kg/m^2	—	24.7 ± 3.4	—
WHR	—	0.95 ± 0.1	—
Testicular volume (mL)	—	20.9 ± 4.8	
Lipodystrophy		—	—
No	67.9 (87)		
Yes	32.1 (28)		
TT, ng/mL	—	5.1 ± 1.4	—
CFT, ng/dL	—	9.8 ± 3.3	—
FT (RIA), pg/mL	—	13.5 ± 3.9	—
FTI, %	—	47.5 ± 19.8	—
LH, U/L	—	—	6 [4–8]
FSH, U/L	—	—	5 [3–8]
PRL, ng/mL	—	13.2 ± 8.3	—
SHBG, nmol/L		43.1 ± 18.3	
ED (IIEF-15)		—	—
No	46.6 (41)		
Yes	53.4 (47)		
AMS		—	—
No	37.1 (33)		
Yes	62.9 (56)		
ADAM		—	—
No	36.0 (32)		
Yes	64.0 (57)		

*NRTI recorded as sum of exposure. To convert to International System of Units: TT (ng/mL × 3.467) nmol/L; CFT (ng/dL × 0.03467) nmol/L; FT (pg/mL × 0.003467) nmol/L.

SD = standard deviation; % = percentage; N = number of patients; NN = current antiretroviral therapy (ART) based on 2–3 nucleoside reverse transcriptase inhibitors (NRTI) plus a non-nucleoside reverse transcriptase inhibitor (non-NRTI) and never received treatment with protease inhibitors (PI); PI = current ART based on 2–3 NRTI plus an enhanced PI; SDU = standard drink unit per week; BMI = body mass index; WHR = waist-hip-ratio; TT = total testosterone; CFT = calculated free testosterone; FT = free testosterone; FTI = free testosterone index; ED = erectile dysfunction; AMS = Aging Male's Symptoms Scale; ADAM = Androgen Deficiency in Aging Men; HIV = human immunodeficiency virus.

Table 2 Risk factors for hypogonadism

Factor	Hypogonadism N = 12	Eugonadism N = 78	OR (95% CI)	*P* value
Age, years (mean ± SD)	49 ± 5.5	40.1 ± 8	3.47 (1.48–8.1)†	0.004*
CDC stage %(n)				
A	75 (9)	56.4 (44)	1	
B	8.3 (1)	24.4 (19)	0.26 (0.3–2.17)	0.21
C	16.6 (2)	19.2 (15)	0.65 (0.13–3.36)	0.61
Duration HIV, years (mean ± SD)	11.1 ± 5.6	7.3 ± 5.5	3.21 (1.07–9.66)†	0.038*
Nadir $CD4^+$, cells/mm^3 median [P_{25}–P_{75}]	221.5 [124–243]	201 [124–304]	0.99 (0.99–1.002)	0.31
Current $CD4^+$, cells/mm^3 median [P_{25}–P_{75}]	460.5 [372–584]	467.5 [354–683]	0.99 (0.99–1.002)	0.48
Viral load, RNA/mL median [P_{25}–P_{75}]	39 [39–39]	39 [39–151]	0.99 (0.98–1.01)	0.64
Tt° group, % (N)				
Naïve	0 (0)	17.9 (14)	1	
ART (NN + PI)	100 (12)	82.1 (64)	Infinite	0.99
Duration exposure, years (mean ± SD)				
Total ART	5.7 ± 3.6	5.5 ± 3.5	1.083 (0.93–1.26)	0.31
non-NRTI	2.5 ± 1.9	3 ± 2.2	1.031 (0.79–1.33)	0.82
NRTI	11 ± 6.9	8.9 ± 7.5	1.017 (0.85–1.21)	0.85
PI	5.9 ± 3.4	4.3 ± 3.2	1.1 (0.93–1.31)	0.25
Alcohol consumption, % (N)				
Yes	41.7 (5)	43.6 (34)	0.92 (0.27–3.17)	0.9
SDU	8 ± 5.6	8.7 ± 8.1	0.99 (0.86–1.12)	0.83
Smoker, % (N)				
Ex-smoker	8.3 (1)	7.7 (6)	1.17 (0.1–13.36)	0.9
Yes	66.7 (8)	65.4 (51)	1.1 (0.26–4.55)	0.89
Sedentary lifestyle, % (N)				
Yes	58.3 (7)	57.1 (40)	1.05 (0.3–3.63)	0.94
BMI, Kg/m^2 (mean ± SD)	25 ± 5.1	24.7 ± 3.1	1.03 (0.86–1.22)	0.76
WHR, mean ± SD	0.97 ± 0.01	0.95 ± 0.07	42.9 (0.01–179399)	0.37
WL >102 cm, %(n)	16.7 (2)	6.6 (5)	2.84 (0.48–16.64)	0.25
Testicular volume, mL (media ± DE)	21 ± 4.2	20.9 ± 4.9	1.002 (0.9–1.13)	0.98
Lipodystrophy, % (N) yes	45.5 (5)‡	29.5 (23)	1.99 (0.55–7.19)	0.29
ED (IIEF-15), % (N) si	100 (12)	46.1 (35)‡	NA	NA
AMS, % (N) yes	66.7 (8)	62.3 (48)‡	1.21 (0.33–4.4)	0.77
ADAM, % (N) yes	75.0 (9)	62.3 (48)‡	1.81 (0.45–7.25)	0.4

*<0.05.
†For every 10 years.
‡Two patients did not complete the whole IIEF questionnaire, one patient with hypogonadism did not complete the lipodystrophy questionnaire and another patient did not complete the AMS/ADAM questionnaires.
SD = standard deviation; % = percentage; n = number of patients; OR = odds ratio; CI = confidence interval; Tt° = treatment; NN = current antiretroviral therapy (ART) based on 2–3 nucleoside reverse transcriptase inhibitors (NRTI) plus one non-nucleoside reverse transcriptase inhibitor (non-NRTI) and never received treatment with protease inhibitors (PI); PI = current ART based on 2–3 NRTI plus an enhanced PI; SDU = standard drink unit per week; BMI = body mass index; WHR = waist-hip-ratio; WL = waistline; ED = erectile dysfunction; AMS = Aging Male's Symptoms Scale; ADAM = Androgen Deficiency in Aging Men; NA = not applicable; HIV = human immunodeficiency virus; IIEF = International Index of Erectile Function.

was 0.18 ± 0.034 nmol/L (5.3 ± 0.65 ng/dL) in patients with hypogonadism vs. 0.36 ± 0.1 nmol/L (10.4 ± 2.9 ng/dL) in the rest. The SHBG concentration was 53.16 ± 17.5 nmol/L in patients with hypogonadism vs. 41.56 ± 18 nmol/L in the rest ($P = 0.04$). Patients with hypogonadism had normal prolactin plasma levels.

The median SHBG concentration of HIV-infected patients less than or equal to 40 years old (N = 36) and our reference population of eugonadal healthy men 18–30 years old (N = 127) was 39 nmol/L (P25 31–P75 53) and 27 nmol/L (P25 22–P75 33), respectively (Mann–Whitney *U*-test, $P < 0.001$).

Factors Associated with Hypogonadism

The factors associated with hypogonadism in the univariate analysis are shown in Table 2. Hypogonadism prevalence was similar in the PI as compared with the NN group (OR = 0.94, $P = 0.92$). A detailed study of the more experienced subgroup of patients, those in the upper interquartile range (>P_{75}) of exposure to ART, non-NRTI, NRTI, or PI, showed no greater risk of developing hypogonadism. Exposure to antiretroviral drugs was not associated with hypogonadism; however, patients with hypogonadism had been exposed to lopinavir for a longer time (39.5 ± 3.4 vs. 23.5 ± 20.4 months; $P = 0.001$) and to ritonavir as an enhancer (57.8 ± 19.7 vs. 33.3 ± 22.9 months; $P = 0.03$). Multivariate analysis confirmed an independent association in our population between age (per decade; OR 6.9, CI 1.9–24.8, $P = 0.003$) and longer time of HIV infection (per year; OR 13.1, CI 1.3–130.6, $P = 0.02$) with hypogonadism (Figure 1).

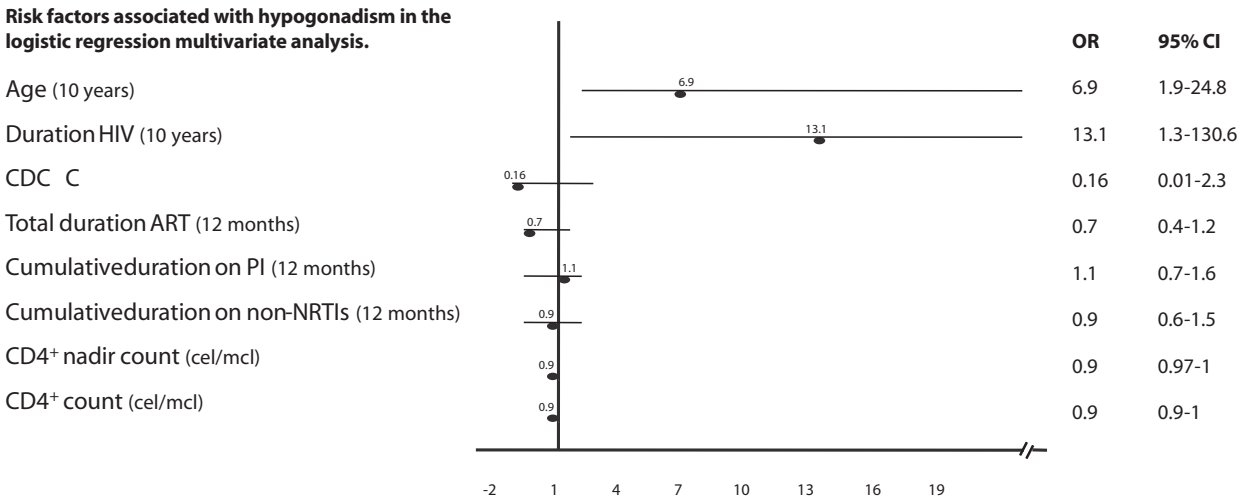

Figure 1 Risk factors associated with hypogonadism in the logistic regression multivariate analysis. OR = odds ratio (error bars are 95% confidence interval [CI]); non-NRTI = no nucleoside reverse transcriptase inhibitors; PI = protease inhibitors.

Usefulness of TT, FT (RIA) in its Diagnosis

The TT concentration was 12.5 ± 3.1 nmol/L (3.6 ± 0.97 ng/mL) in patients with hypogonadism versus 18.4 ± 4.5 nmol/L (5.3 ± 1.3 ng/mL) in the eugonadal subpopulation ($P < 0.001$). A TT concentration below the normal range for our reference laboratory was found in only three of the patients with hypogonadism (Table 3). The measured FT concentration was 0.034 ± 0.009 nmol/L (9.7 ± 2.71 pg/mL) in patients with hypogonadism vs. 0.049 ± 0.013 nmol/L (14.2 ± 3.7 pg/mL) in the eugonadal subpopulation ($P < 0.001$). Although the determination of plasma FT showed a correlation $P = 0.65$ ($P < 0.001$) with the CFT, it only identified four of the 12 patients with hypogonadism.

Usefulness of Questionnaires for the Diagnosis of Hypogonadism

Sensitivity and specificity of ADAM and AMS for hypogonadism diagnosis using CFT as the gold standard are presented in Table 3. The ADAM and AMS questionnaires were consistent with hypogonadism in 64% and 62.9% of the subjects, respectively. Questionnaires showed a specificity less than 38% for hypogonadism diagnosis defined by a CFT <0.22 nmol/L.

ED

All the patients with hypogonadism suffered from ED as opposed to 46.1% of the eugonadal patients (relative risk 2.2, $P < 0.01$). Otherwise, 25.5% of the patients with ED had hypogonadism, whereas all the population without ED was eugonadal.

Discussion

Hypogonadism is common in HIV-infected males, with a prevalence of 13.3% in our population. These data are similar to those previously reported [1–3], even that hypogonadism diagnostic criteria are not homogeneous and do not include CFT as

Table 3 Usefulness of TT, FT (RIA) and hypogonadism questionnaires in the diagnosis of hypogonadism in HIV-infected male

	Sensitivity	Specificity	NPV	PPV	R(+)	R (–)	Diag. OR
TT	25.0%	100%	89.6%	100%	Infinite	0.75	Infinite
FT(RIA)	33.3%	96.2%	90.4%	57.1%	8.76	0.69	12.7
ADAM	75.0%	37.7%	90.6%	15.8%	1.2	0.66	1.8
AMS	66.7%	37.7%	87.9%	14.3%	1.07	0.88	1.2

Calculated free testosterone (CFT) <0.22 nmol/L (6.36 ng/dL) used as the reference for diagnosis of hypogonadism.
NPV = negative predictive value; PPV = positive predictive value; R(+) = positive likelihood ratio; R(–) = negative likelihood ratio; Diag. OR = diagnostic likelihood ratio; TT = diagnosis of hypogonadism as total testosterone <10.4 nmol/L (3 ng/mL); FT (RIA) = diagnosis of hypogonadism as measured free testosterone <0.031 nmol/L (9 pg/mL); ADAM = Androgen Deficiency in Aging Men Questionnaire with positive result; AMS = Aging Male's Symptoms Scale with positive result; HIV = human immunodeficiency virus.

the gold standard in this population [5,16]. Characteristics of our population, with HIV infection males with stable clinical condition, with less than 20% were in clinical stage C, more than 70% had an undetectable viral load, and only two patients had a $CD4^+$ count below 200 cells/mm^3, could explain why the prevalence of hypogonadism in our population, using a more sensitive test (CFT), was similar than in other reported HIV cohorts with most advanced clinical situation, but where it is used much less sensitive methods of determination of testosterone as a diagnostic key of hypogonadism. According to our findings, neither TT nor FT determined by direct techniques (RIA) are good surrogate markers of FT in HIV-infected males compared with CFT. TT and FT (RIA) could not identify more than 30% of the patients with hypogonadism, which makes them useless as screening tests for hypogonadism in this population.

In the general population, the most widely accepted parameters to establish the presence of hypogonadism is the measurement of serum TT. There are no generally accepted lower limits of normal. A recent consensus among the major societies of andrology and urology in the field suggests that TT level above 12 nmol/L (3.5 ng/mL) does not require substitution and levels below 8 nmol/L (2.3 ng/mL) will usually benefit from testosterone treatment [24]. If the serum TT level is between 8 and 12 nmol/L, or in diseases in which the high concentration of SHBG induces an increase in TT values that can lead to underestimation of hypogonadism, repeating the measurement of TT with SHBG to calculate FT or FT by equilibrium dialysis may be helpful; in these cases, a FT level below 0.225 nmol/L (6.5 ng/dL) can provide supportive evidence for testosterone treatment [24]. Despite the fact that the *Endocrine Society* reports the existence of different lower limits of the normal range in healthy young men in several reference laboratories (TT, 10.4 nmol/L [3 ng/mL]; FT, 0.17 nmol/L [5 ng/dL]) [5], both consensus are in agreement given that there are known variations among assay methods; it is imperative that the practitioners utilize reliable laboratories and are acquainted with the rrs for TT and FT (CFT or by equilibrium dialysis) from their local laboratory [5,24].

Our study identified age and time of HIV infection as the only predictors of hypogonadism. The association between age and hypogonadism is well known in the general population and has also been identified as a risk factor for hypogonadism in HIV-infected males [1]. The production of testosterone decreases by 1% per year [4,25]. According to the Massachusetts Male Aging Study [4], this decrease is accompanied by an annual increase of around 1.2% in SHBG. This increase in SHBG appears to accelerate in HIV-infected males and with an increase of over 50% in their SHBG concentrations over the expected for their age. This means that TT decreases with age to a lesser extent than is shown by the FT and BT concentrations and does not result in a reduction of testicular secretory function [26,27]. Quantification of CFT as an indirect approximation to bioactive testosterone eliminates the bias produced by the rise in SHBG. In our study, the difference found in SHBG level among HIV-infected males and the reference population of eugonadal healthy men (39 nmol/L and 27 nmol/L, respectively) support this highest annual increase in SHBG levels in HIV-infected patients. Although there were differences in the mean age and age distribution between the two groups compared (HIV-infected males vs. non-infected healthy males), the rise of SHBG associated with age [4] does not justify this large difference, and supports the use of the CFT in this population.

The absence conditions associated to HIV infection in the patients studied, as opportunistic infections or malnutrition, suggest that other factors, like an excess of cytokines (alpha tumoral necrosis factor [TNF-α], interleukin 1 [IL-1], interleukin 6 [IL-6]) affecting testicular steroidogenesis, interfering in the gonadotropin releasing hormone secretion, FSH/LH, or in its peripheral action, are involved in the pathogenesis of hypogonadism in HIV infection [8,28,29].

The design of our study makes it possible to interpret the effect of ART and limits possible confounding factors. The type of ART and duration of exposure are not associated with hypogonadism. Although the patients with hypogonadism were exposed for a longer period to lopinavir and ritonavir—as an enhancer—exposure to any antiretroviral was not associated with an increased risk of hypogonadism. These findings suggest that ART does not play a direct role on the etiopathogenesis of hypogonadism [1–3,30].

The ADAM and AMS self-evaluation screening questionnaires were positive in more than 60% of the population studied, but with a very low specificity (<38%) and positive predictive value (<16%) for hypogonadism by CFT. The low specificity of these questionnaires is well known in general population, and similar findings have reported in HIV-infected males [1], and exclude them as screening tests for hypogonadism in this population.

The prevalence of ED in HIV-infected males is greater than in the general population and its pathogenesis is not clear; 53.4% of our patients presented ED, which is similar to the data obtained using the IIEF questionnaire [1,31]. The prevalence of androgenic insufficiency in the general population with ED is estimated to vary between 5% and 35% [32,33]. In our population, all the patients with hypogonadism presented ED, and in a quarter of the patients with ED, it was associated with hypogonadism. Given the prevalence of hypogonadism in HIV-infected males, the role of androgens in erectile physiology [34–36], and the results obtained in our study, it seems necessary to rule out androgenic insufficiency in all HIV-infected males with ED [8,17].

Hypogonadism, ED, insulin resistance, and metabolic syndrome often coexist in the same subjects. This cluster of abnormalities is associated with an increased risk of diabetes and cardiovascular diseases, affecting not only quality of life but also life expectancy [13]. Theoretically, since these alterations are pathogenetically linked though bidirectional relationships, testosterone replacement therapy should be able to induce a reduction of insulin resistance besides the improvement of sexual function [13]. This therapy could be beneficial if started at the early stages of atherogenesis, but their effects are lost in the context of an advanced atherosclerotic disease [37].

Our study has some limitations. The size of the sample is not large, although a detailed record of the patients' medical history and pharmacological treatment was available. There was no control group; however, many data in the literature on the general population are available which allows us to draw conclusions. A major concern in our study is the absence of validation of CFT compared to the AFTC determinate by equilibrium dialysis in HIV patients; however, CFT levels and AFTC were almost identical in sera of non-HIV populations with SHBG capacities varying from low to extremely high as in hyperthyroidism [15,38], mimicking the high concentration of SHBG present in sera of HIV-infected males, validating our findings. Ours was a cross-sectional study; prospective studies may be useful to examine the incidence of hypogonadism and establish the temporal risk factors.

Conclusions

Hypogonadism is common in HIV-infected males. Early diagnosis of hypogonadism and substitutive hormonal therapy started early on are important due to the impact of this disorder on quality of life, the greater risk of depression, ED, and the possible repercussions on the patient's future cardiovascular health. To the best of our knowledge, this is the first study in HIV-infected males that uses CFT to diagnose hypogonadism in this population and confirms that TT and FT (RIA) are not useful in this population. Age and duration of HIV infection are the only predictors of hypogonadism in this population. The absence of other clinical markers associated with hypogonadism and the prevalence of hypogonadism in initial stages justify early routine screening of hypogonadism in HIV-infected males using CFT.

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The authors have nothing to disclose.

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References

1 Crum-Cianflone NF, Bavaro M, Hale B, Amling C, Truett A, Brandt C, Pope B, Furtek K, Medina S, Wallace MR. Erectile dysfunction and hypogonadism among men with HIV. AIDS Patient Care STDS 2007;21:9–19.

2 Rietschel P, Corcoran C, Stanley T, Basgoz N, Klibanski A, Grinspoon S. Prevalence of hypogonadism among men with weight loss related to human immunodeficiency virus infection who were receiving highly active antiretroviral therapy. Clin Infect Dis 2000;31:1240–4.

3 Crum NF, Furtek KJ, Olson PE, Amling CL, Wallace MR. A review of hypogonadism and erectile dysfunction among HIV-infected men during the pre- and post-HAART eras: Diagnosis, pathogenesis, and management. AIDS Patient Care STDS 2005;19:655–71.

4 Araujo AB, O'Donnell AB, Brambilla DJ, Simpson WB, Longcope C, Matsumoto AM, McKinlay JB. Prevalence and incidence of androgen deficiency in middle-aged and older men: Estimates from the Massachusetts Male Aging Study. J Clin Endocrinol Metab 2004;89:5920–6.

5 Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM. Testosterone therapy in adult men with androgen deficiency syndromes: An endocrine society clinical practice guideline. J Clin Endocrinol Metab 2006;91:1995–2010.

6 Cohan GR. HIV-associated hypogonadism. AIDS Read 2006;16:341–54.

7 Grinspoon S. Androgen deficiency and HIV infection. Clin Infect Dis 2005;41:1804–5.

8 Moreno-Perez O, Pico A, Portilla J. Hypogonadism, erectile dysfunction and endothelial dysfunction among HIV-infected men. Med Clin (Barc) 2009;132:311–21.

9 Inaba T, Fischer NE, Riddick DS, Stewart DJ, Hidaka T. HIV protease inhibitors, saquinavir, indinavir and ritonavir: Inhibition of CYP3A4-mediated metabolism of testosterone and benzoxazinorifamycin, KRM-1648, in human liver microsomes. Toxicol Lett 1997;93:215–9.

10 Cofrancesco J Jr, Whalen JJ 3rd, Dobs AS. Testosterone replacement treatment options for HIV-infected men. J Acquir Immune Defic Syndr Hum Retrovirol 1997;16:254–65.

11 Turgeon JL, Carr MC, Maki PM, Mendelsohn ME, Wise PM. Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: Insights from basic science and clinical studies. Endocr Rev 2006;27:575–605.

12 Foresta C, Caretta N, Lana A, De Toni L, Biagioli A, Ferlin A, Garolla A. Reduced number of circulating endothelial progenitor cells in hypogonadal men. J Clin Endocrinol Metab 2006;91:4599–602.

13 Corona G, Mannucci E, Forti G, Maggi M. Hypogonadism, ED, metabolic syndrome and obesity: A pathological link supporting cardiovascular diseases. Int J Androl 2009;32:587–98.

14 Basaria S, Dobs AS. Testosterone making an entry into the cardiometabolic world. Circulation 2007;116:2658–61.

15 Aberg JA, Kaplan JE, Libman H, Emmanuel P, Anderson JR, Stone VE, Oleske JM, Currier JS, Gallant JE. Primary care guidelines for the management of persons infected with human immunodeficiency virus: 2009 update by the HIV medicine association of the infectious diseases society of America. Clin Infect Dis 2009;49:651–81.

16 Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.

17 Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: Utility, limitations, and pitfalls in measuring testosterone: An Endocrine Society position statement. J Clin Endocrinol Metab 2007;92:405–13.

18 Giton F, Urien S, Born C, Tichet J, Guéchot J, Callebert J, Bronsard F, Raynaud JP, Fiet J. Determination of bioavailable testosterone (non sex hormone binding globulin [SHBG]-bound testosterone) in a population of healthy French men: Influence of androstenediol on testosterone binding to SHBG. Clin Chem 2007;53:2160–8.

19 Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A. The International Index of Erectile Function (IIEF): A multidimensional scale for assessment of erectile dysfunction. Urology 1997;49:822–30.

20 Cappelleri JC, Rosen RC, Smith MD, Mishra A, Osterloh IH. Diagnostic evaluation of the erectile function domain of the International Index of Erectile Function. Urology 1999;54:346–51.

21 Morley JE, Charlton E, Patrick P, Kaiser FE, Cadeau P, McCready D, Perry HM 3rd. Validation of a screening questionnaire for androgen deficiency in aging males. Metabolism 2000;49:1239–42.

22 Moore C, Huebler D, Zimmermann T, Heinemann LA, Saad F, Thai do M. The aging males' symptoms scale (AMS) as outcome measure for treatment of androgen deficiency. Eur Urol 2004;46:80–7.

23 HIV Lipodystrophy Case Definition Study Group. An objective case definition of lipodystrophy in HIV-infected adults: A case-control study. Lancet 2003;361:726–35.

24 Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, Kaufman JM, Legros JJ, Lunenfeld B, Morales A, Morley JE, Schulman C, Thompson IM, Weidner W, Wu FC. Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA and ASA recommendations. Eur Urol 2009;55:121–30.

25 Beck AT, Steer RA, Garbin MG. Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. Clin Psychol Rev 1988;8:77–100.

26 Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR, Baltimore Longitudinal Study of Aging. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2001;86:724–31.

27 Wagner GJ, Rabkin JG. Testosterone therapy for clinical symptoms of hypogonadism in eugonadal men with AIDS. Int J STD AIDS 1998;9:41–4.

28 Martin ME, Benassayag C, Amiel C, Canton P, Nunez EA. Alterations in the concentrations and binding properties of sex steroid binding protein and corticosteroid-binding globulin in HIV+ patients. J Endocrinol Invest 1992;15:597–603.

29 Sellmeyer DE, Grunfeld C. Endocrine and metabolic disturbances in human immunodeficiency virus infection and the acquired immune deficiency syndrome. Endocr Rev 1996;17:518–32.

30 Mylonakis E, Koutkia P, Grinspoon S. Diagnosis and treatment of androgen deficiency in human immunodeficiency virus-infected men and women. Clin Infect Dis 2001;33:857–64.

31 Wunder DM, Bersinger NA, Fux CA, Mueller NJ, Hirschel B, Cavassini M, Elzi L, Schmid P, Bernasconi E, Mueller B,

Furrer H, Swiss HIV Cohort Study. Hypogonadism in HIV-1-infected men is common and does not resolve during antiretroviral therapy. Antivir Ther 2007;12:261–5.
32 Ende AR, Lo Re V 3rd, DiNubile MJ, Mounzer K. Erectile dysfunction in an urban HIV-positive population. AIDS Patient Care STDS 2006;20:75–8.
33 Cofrancesco J Jr, Whalen JJ 3rd, Dobs AS. Testosterone replacement treatment options for HIV-infected men. J Acquir Immune Defic Syndr Hum Retrovirol 1997;16:254–65.
34 Mikhail N. Does testosterone have a role in erectile function? Am J Med 2006;119:373–82.
35 Latini DM, Penson DF, Wallace KL, Lubeck DP, Lue TF. Clinical and psychosocial characteristics of men with erectile dysfunction: Baseline data from ExCEED. J Sex Med 2006;3:1059–67.
36 Traish AM, Guay AT. Are androgens critical for penile erections in humans? Examining the clinical and preclinical evidence. J Sex Med 2006;3:382–407.
37 Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. Science 2005;308: 1583–7.
38 DeVan ML, Bankson DD, Abadie JM. To what extent are free testosterone (FT) values reproducible between the two Washingtons, and can calculated FT be used in lieu of expensive direct measurements? Am J Clin Pathol 2008;129:459–63.