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Gonadal and sexual function in men living with HIV younger than 50 years old: focus on the crosstalk between sex steroids, health status and psychological issues

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INDEX

Chapter 1

Summary

(Riassunto)

SUMMARY

Background. Hypogonadism and sexual dysfunction are common in men living with HIV (MLWH). The relationship between sex steroids, sexual function and health status including psychological issues is poorly known in HIV.

Aim. To explore the impact on sexual and gonadal function of multiple determinants, including organic, relational and psychological components in MLWH younger than 50 years. Furthermore, we aimed at determining the prevalence and characterization of biochemical hypogonadism in MLWH aged<50 comparing liquid chromatography-tandem mass spectrometry (LC-MS/MS) with chemiluminescent immunoassay (CI).

Methods. A prospective, cross-sectional, observational study was conducted in MLWH <50 years. Sex steroids (serum total testosterone (TT), estradiol (E2), estrone, and dihydrotestosterone (DHT)) were measured by LC-MS/MS; TT and E2 were also assessed by CI. Free testosterone (cFT) was calculated by Vermeulen equation. Body composition was assessed by dual-energy X-ray absorptiometry and abdominal CT scan. Frailty was defined by a 37-item index. The questionnaire International Index of Erectile Function (IIEF)-15 was used to assess prevalence and degree of erectile dysfunction (ED). The Structured Interview of Erectile Dysfunction (SIEDY) was used to explore the organic (Scale1), relational (Scale2) and psychological (Scale3) components of ED.

Results. A total of 316 HIV-infected men aged 45.3±5.3 years were enrolled. Body fat parameters and frailty were significantly associated with sex steroids, being inversely related to cFT and TT, and directly related to estrone and E2/T ratio. The prevalence of biochemical hypogonadism was higher with LC-MS/MS than CI, both for TT (5.1% vs 3.2%, p<0.0001) or cFT (9.5% vs 7%, p<0.0001). Secondary form of hypogonadism was more prevalent than primary. With regard to sexual function, 187 patients (59.7%) had ED; sexual orientation, lack of stable relationship were major determinants for ED. Only 35 of 187 patients with ED (18.7%) reported the use of ED medications.

Conclusions. Our findings show that sexual dysfunctions and hypogonadism are common in

MLWH younger than 50 years old. Health status, frailty and body fat mass are strictly associated to each other and to sex steroids, concurring together to functional male hypogonadism in HIV. Within the multidimensional network of ED in MLWH, the psychological component was identified as a major determinant, highlighting the contribution of peculiar factors related to HIV psychological burden rather than gonadal status and other classical risk factors. In contrast to the high prevalence, only few patients reported the use of ED medications suggesting a general under-management of such issues. All the mentioned results were published in international scientific journal, giving a contribution in addressing sexual dysfunctions in MLWH through a tailored and multidisciplinary clinical approach.

RIASSUNTO

Introduzione. L'infezione da HIV è associata ad un'aumentata incidenza di co-morbidità, tra cui la disfunzione erettile (DE) ed il deficit di testosterone. La relazione tra steroidi sessuali, funzione sessuale e stato di salute, inclusa la componente psicologica, nell'HIV rimane ancora da chiarire.

Scopo. Definire l'impatto sulla funzione sessuale e gonadica della componente organica, relazionale e psicologica nei pazienti HIV con meno di 50 anni d'età. Inoltre, valutare la prevalenza e la caratterizzazione dell'ipogonadismo mediante l'impiego della metodica cromatografia liquida-tandem spettrometria di massa (LC-MS/MS) e chemiluminescenza (CI).

Metodi. Studio prospettico, osservazionale, trasversale, su pazienti con infezione da HIV e di età inferiore ai 50 anni. Gli steroidi sessuali (testosterone totale (TT), estradiolo (E2), estrone, diidrotestosterone (DHT)) sono stati misurati mediante LC-MS/MS; TT ed E2 sono stati misurati anche mediante CI. Il testosterone libero (cFT) è stato calcolato tramite la formula di Vermeulen. La composizione corporea è stata valutata mediante densitometria assiale a raggi X e tomografia computerizzata addominale. La fragilità è stata definita con un indice composto da 37 quesiti. L'indice internazionale della funzione erettile (IIEF)-15 è stato utilizzato per valutare la prevalenza e il grado di DE. È stata inoltre usata l'intervista strutturata sulla disfunzione erettile (SIEDY) al fine di valutare la componente organica, relazionale e psicologica del DE.

Risultati. Sono stati arruolati 316 pazienti HIV con età media di 45.3±5.3 anni. I parametri relativi al tessuto adiposo e la fragilità erano significativamente associati con gli steroidi sessuali, essendo inversamente relati a cFT e TT e direttamente relati a estrone e rapporto E2/T. La prevalenza di ipogonadismo era superiore mediante LC-MS/MS rispetto a CI, sia sulla base del TT (5.1% vs 3.2%, $p<0.0001$) e del cFT (9.5% vs 7%, $p<0.0001$). La prevalenza di ipogonadismo secondario era maggiore rispetto alla forma primitiva. 187 pazienti (59.7%) presentavano DE all'IIEF-15; di questi, solo 35 pazienti (18.7%) riferivano l'uso di farmaci per DE. L'orientamento sessuale e la mancanza di una relazione stabile erano i principali determinanti per DE.

Conclusioni. Il riscontro di ipogonadismo e disfunzione sessuale è frequente nei pazienti HIV di età

inferiore ai 50 anni. Lo stato di salute, la fragilità e la composizione corporea sono strettamente associati tra di loro ed è ipotizzabile che insieme concorrano a determinare una forma di ipogonadismo funzionale nei pazienti con HIV. Considerata la natura multifattoriale del DE, la componente psicologica sembrerebbe essere il principale determinante, suggerendo che nell'eziopatogenesi del DE nei soggetti HIV intervengano maggiormente fattori di rischio specifici legati all'HIV più che i fattori di rischio classici. Nonostante la prevalenza del DE sia così alta, solo pochi pazienti dichiarano di assumere una terapia appropriata per DE rivelando quanto le problematiche sessuali siano spesso sotto-gestite nella pratica clinica. Tutti i risultati sopra elencati sono stati pubblicati in riviste scientifiche internazionali, dando un contributo nella gestione le disfunzioni sessuali nell'HIV.

Chapter 2

General introduction

INTRODUCTION

Over the last decades the clinical presentation of men living with HIV (MLWH) has notably improved thanks to the advancements in medical management and the development of effective antiretroviral therapies (Guaraldi, Milic and Mussini 2019, Brothers et al. 2014). The life span increase of MLWH has led in turn to an increase of the prevalence of chronic non-infectious comorbidities due to both drug toxicity and persistence of viral infection (Guaraldi et al. 2019). This condition of multimorbidity, together with the associated poor health status and disabilities, all lead to HIV-related 'frailty' (Guaraldi and Rockwood 2017) occurring also in young to middle-aged patients (Martin and Volberding 2010, Guaraldi et al. 2011) similarly to older HIV-uninfected people (Clegg et al. 2013). Beyond the occurrence of several comorbidities, hypogonadism and sexual dysfunction are among the most prevalent in MLWH (Rochira et al. 2011, Samaras 2011, Monroe et al. 2014, Rochira and Guaraldi 2014). A complex multi-factorial network has been depicted to explain the underlying pathogenetic mechanisms of hypogonadism and sexual dysfunctions in this context. Indeed, in addition to traditional risk factors such as age, lifestyle, neurological, cardiovascular, and endocrine diseases, several HIV-related factors play a role in the pathogenesis of testosterone deficiency and sexual problems, including virus *per se*, antiretroviral therapies, and psychological distress. Nevertheless, in MLWH the relationship among comorbidities, health status, gonadal and sexual function has been explored by a very few studies hitherto (Erlandson et al. 2017, Rochira et al. 2015a).

From a clinical standpoint, signs and symptoms of low testosterone are non-specific, of mild-to-moderate degree, and often overlapping with those of other comorbidities in MLWH. For these reasons, hypogonadism can be underestimated in the absence of targeted laboratory blood examinations that may include testosterone, sex hormone-binding globulin (SHBG) and gonadotropins (Rochira et al. 2011, Rochira and Guaraldi 2014). At present, however, data on the prevalence of hypogonadism based on the combination of serum sex steroids measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), SHBG, and gonadotropins are lacking.

Indeed, the measurement of serum total testosterone by LC-MS/MS that is the recommended methodology has been performed only in a few studies, and none of them considered also estradiol, SHBG, and gonadotropins levels in order to obtain a comprehensive evaluation of the pituitary-gonadal axis; this does not allow a precise esteem of prevalence and a full characterization of hypogonadism in MLWH (Monroe et al. 2014, Blick 2013, Slama et al. 2016).

Aim of this thesis

The primary objective of this thesis is to explore the relationship between sex steroids, sex function, body composition and the overall health status (defined as multimorbidity and frailty) of MLWH younger than 50 years. Furthermore, secondary aims are the following: i) to report data about prevalence and characterization of hypogonadism in MLWH under 50; ii) to compare the performance of LC-MS/MS and chemiluminescent techniques in the evaluation of gonadal status in MLWH; iii) to investigate the prevalence of sexual dysfunctions and their possible determinants.

To this purpose, a unique prospective study has been carried out and subdivided in 3 chapters each focusing on a different objective among those listed above. **Chapter 3** is primarily aimed at investigating the relationship among gonadal function and general health status, defined as frailty and multimorbidity, in MLWH. Data presented in this chapter provide evidence on the strict interplay among sex steroids (both estrogens and testosterone), frailty, and body fat tissue, showing how all these factors are associated among each other and how they concur together to functional male hypogonadism in MLWH (De Vincentis et al. 2021a).

Chapter 4 focuses on the prevalence and characterization of hypogonadism in our cohort of MLWH; gonadal function is classified according to gonadotropins and testosterone values into eugonadism, primary hypogonadism, secondary hypogonadism, and compensated hypogonadism. Therefore, secondary aim of this chapter is to compare hormonal data obtained from LC-MS/MS and chemiluminescent immunoassay. Our findings confirm that testosterone deficiency is still a common finding in MLWH (De Vincentis et al. 2022). Notwithstanding the strong correlation

found between the two laboratory assays for testosterone measurement, the prevalence of male hypogonadism results underestimated when chemiluminescence is used compared to LC-MS/MS (De Vincentis et al. 2022). In clinical practice, SHBG for calculation of free testosterone and gonadotropins are essential for the detection of testosterone deficiency and classification of hypogonadism, revealing the real prevalence/form of male hypogonadism in MLWH (De Vincentis et al. 2022).

Finally, sexual dysfunctions are quite common and unrecognized condition in MLWH. The study presented in **Chapter 5** is aimed at determining the prevalence of sexual dysfunctions, especially erectile dysfunction, in MLWH under 50. Furthermore, it explores the impact on sexual function of multiple determinants, including organic, relational and psychological domains to discriminate major and minor risk factors in the onset of sexual dysfunction in HIV setting. These findings show that sexual dysfunctions are highly prevalent in HIV cohorts under 50. Within the multidimensional network of erectile dysfunction in MLWH, the psychological component is predominant, highlighting the contribution of peculiar factors related to HIV distress rather than gonadal status and other classical risk factors.

Studies presented in Chapters 3, 4, and 5 have already been published in international journals.

Chapter 3

Health status is related to testosterone, estrone and body fat: moving to functional hypogonadism in adult men with HIV

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ABSTRACT

Objective. Hypogonadism is common in HIV-infected men. The relationship between health status, sex steroids and body composition is poorly known in HIV. The aim was to investigate the association between health status (comorbidities/frailty), body composition, and gonadal function in young-to-middle-aged HIV-infected men.

Design. Prospective, cross-sectional, observational study.

Methods. HIV-infected men aged<50 years and ongoing Highly Active Antiretroviral Therapy were enrolled. Serum total testosterone (TT), estradiol (E2), estrone (E1) were measured by liquid chromatography-tandem mass spectrometry, LH and FSH by immunoassay. Free testosterone (cFT) was calculated by Vermeulen equation. Body composition was assessed by dual-energy X-ray absorptiometry and abdominal CT scan. Multimorbidity (MM) and frailty were defined as ≥3 comorbidities and by a 37-item index, respectively.

Results. A total of 316 HIV-infected men aged 45.3±5.3 years were enrolled. Body fat parameters were inversely related to cFT and TT, and directly related to E1 and E2/T ratio. Patients with MM had lower cFT ($p<0.0001$) and TT ($p=0.036$), and higher E1 ($p<0.0001$) and E2/T ratio ($p=0.002$). Frailty was inversely related to cFT ($R^2=0.057$, $p<0.0001$) and TT ($R^2=0.013$, $p=0.043$), and directly related to E1 ($R^2=0.171$, $p<0.0001$), E2 ($R^2=0.041$, $p=0.004$) and E2/T ratio ($R^2=0.104$, $p<0.0001$).

Conclusions. Lower TT and cFT, higher E1, E2/T ratio and visceral fat were independently associated to poor health status and frailty, being possible hallmarks of unhealthy conditions in adult HIV-infected men. Overall, MM, frailty and body fat mass are strictly associated to each other and to sex steroids, concurring together to functional male hypogonadism in HIV.

INTRODUCTION

Over the last decades the clinical presentation of patients with Human Immunodeficiency Virus (HIV) has improved thanks to advancements in medical management and the development of effective antiretroviral therapies (Guaraldi et al. 2019, Brothers et al. 2014). The price paid to obtain the life span increase of HIV-infected patients has been the development of chronic non-infectious comorbidities (NICMs) due to both drug toxicity and persistence of viral infection (Guaraldi et al. 2019), which represents a model of HIV-related accentuated aging (Martin and Volberding 2010, Guaraldi et al. 2011). This multimorbidity (MM) is the sum of more than 3 NICMs (Guaraldi et al. 2011, Brothers and Rockwood 2014). MM, together with the associated poor health status and disabilities, all lead to HIV-related 'frailty'(Guaraldi and Rockwood 2017) occurring also in young to middle-aged patients (Martin and Volberding 2010, Guaraldi et al. 2011) similarly to older HIV-uninfected people (Clegg et al. 2013). Beyond the occurrence of liver, renal, cardiovascular diseases, osteoporosis, endocrine metabolic disorders, and cancers (Guaraldi et al. 2019, Brothers et al. 2014, Guaraldi et al. 2011, Samaras 2011), hypogonadism is a common finding among HIV-infected men (Rochira et al. 2011, Samaras 2011, Monroe et al. 2014, Rochira and Guaraldi 2014). Circulating testosterone (T) declines with advancing age in HIV-uninfected men (Decaroli and Rochira 2017) and the same trend is observed also in men with HIV but at a younger age (Rochira et al. 2011, Rochira and Guaraldi 2014). Even though the underlying pathogenetic mechanisms of hypogonadism in these patients remains uncertain, available data suggests that both viral infection *per se* and undesired effects of highly active antiretroviral therapy (HAART) may be involved (Rochira and Guaraldi 2014).

A poor health status seems to be associated to low T in men (Hsu, Cumming and Handelsman 2018, Hyde et al. 2010, Travison et al. 2008, Rastrelli, Corona and Maggi 2020). In particular, low T levels are associated to several chronic diseases such as obesity (Kelly and Jones 2015), diabetes mellitus (Dhindsa et al. 2018), dyslipidemia , metabolic syndrome (Antonio et al. 2015) and several other systemic diseases (Rochira 2017). These forms of male hypogonadism are

considered as strictly related to poor health, secondary to other morbidities and partially reversible, and endocrinologists tend to classify them as a sort of functional hypogonadism (Grossmann and Matsumoto 2017, Corona et al. 2020, Rochira 2017). Besides, increased body fat is associated to low T in men, this association being bidirectional (Kelly and Jones 2015) and body fat, especially visceral fat, is associated with frailty both in HIV-uninfected (Kim et al. 2020) and HIV-infected men (Shah et al. 2012).

In HIV-infected men, the relationship among comorbidities, health status, and hypogonadism has been explored by a very few studies hitherto (Erlandson et al. 2017, Rochira et al. 2015a), leaving this association to be confirmed. With this in view, the aim of this study was to prospectively investigate the relationship between gonadal status, body composition, and overall health status, in a cohort of young to middle-aged HIV-infected men younger than 50 years. At this purpose, we primarily explored whether serum sex steroids (estrogens and androgens assayed by the recommended liquid chromatography-tandem mass spectrometry technique) were significantly associated with body fat parameters, HIV-related parameters, and general health status represented by NICMs and frailty.

MATERIALS AND METHODS

Study design and participants

A prospective, cross-sectional, observational study was carried out involving HIV-infected outpatients attending the Modena HIV Metabolic Clinic (MHMC) from May 2013 to December 2017.

Three hundred nineteen consecutive HIV-infected male patients were screened before enrolment in the study at the Unit of Endocrinology of the University of Modena and Reggio Emilia (Figure 1).

Inclusion criteria were men aged 18-50 years, with documented HIV-infection and ongoing HAART treatment.

Exclusion criteria were prior treatment (referred or documented) with androgens, sex steroids, antiandrogens, anabolic agents, GnRH agonists, psychotropic agents; documented hypothyroidism, known pituitary, testicular or adrenal diseases, a previous conventional pituitary surgery or radiotherapy; documented Acquired Immunodeficiency Syndrome (AIDS), active cancer, severe liver insufficiency or severe chronic renal failure (estimated glomerular filtration rate <30 mL/min). In order to avoid the overlap with hypogonadism due to aging, the limit of age for enrolment was per protocol fixed below or equal to 50 years. Biochemical hypogonadism was considered for serum total T below 10.4 nmol/L and serum free calculated T (cFT) below 220 pmol/L according to clinical guidelines (Bhasin et al. 2018).

At enrolment visit each patient was evaluated for inclusion and exclusion criteria through medical interview and adequate clinical work-up (Figure 1). Finally, 316 HIV-infected men met all abovementioned inclusion criteria and were enrolled in the study (Figure 1).

Main outcome measures

Laboratory analyses

Blood samples were collected at 8:00 am after an overnight fasting by an intravenous cannula inserted into an antecubital vein. The blood samples were centrifuged, and the serum was stored at -20° C until assayed.

Serum total T (Fanelli et al. 2011), E1 and E2 (Mezzullo et al. 2020) were measured by two validated isotopic dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) methods. Sensitivity was 0.1 nmol/L, 18.1 pmol/L and 36.0 pmol/L, intra-assay imprecision was <4%, <3% and <6%, inter-assay imprecision was <7%, <9% and <7% and accuracy ranged 97-100%, 83-111% and 92-108% for T, E1 and E2, respectively. T and E2 accuracy were verified through certified quality controls and multicenter comparison studies (Fanelli et al. 2011, Mezzullo et al. 2020, Büttler et al. 2015). Of the 316 enrolled patients, 71 participants were missing estrogens

assay data (for either E2 or E1). Serum E2 to total T ratio (E2/T) was computed considering E2 in pmol/L and total T in nmol/L.

Sex hormone binding globulin (SHBG) was assessed by chemiluminescent immunoassay (Architect, Abbott GmbH &Co, Germany) with both inter- and intra-assay coefficients of variation of 10.0%. cFT was calculated through the Vermeulen equation (Vermeulen, Verdonck and Kaufman 1999).

Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assayed by chemiluminescent immunoassay (Architect, Abbott GmbH & Co, Germany). The inter- and intra-assay coefficients of variation were ≤10%.

Serum prolactin (PRL) was assayed by chemiluminescent immunoassay (DxI 800, Beckman Coulter, U.S.A.). The inter- and intra-assay coefficients of variation were ≤10%.

Other biochemical parameters (e.g, glycaemia, glycated hemoglobin) were assessed by commercially available kits.

Anthropometric evaluation, body composition and bone mineral density assessment

Demographic characteristics and anthropometric measurements (weight, height, body mass index [BMI], waist, hip, and waist/hip ratio) were recorded at the time of the examination. Weight was measured after at least a 5-h fast by analogical balance and height by stadiometer. Waist and hip circumferences were measured as the average of 3 measurements. BMI was calculated as body weight (kilograms) divided by the square of height (square meters). A whole body composition assessment and measurement of bone mineral density (BMD) were both obtained by Dual-Energy X-ray Absorptiometry (DXA) (DXA, Hologic-QDR-2000 densitometer, Inc., Waltham, MA) according to standardized procedures (Park, Heymsfield and Gallagher 2002, Shepherd et al. 2015). BMD was measured at total body, lumbar spine (L1 to L4) and total hip. Precision error rates for the QDR 2000 were 1 % or less for BMD of 3% for the measurement of fat mass, and 1.5% for fat-free mass. BMD measured by DXA is expressed as an areal density in g/cm^2. For age <50 years a

condition of low BMD was defined for Z-score <2.0 at lumbar or femoral site. Adipose tissue distribution was studied by a CT scan, with a single-slice abdominal scan at L4 level as in standard protocols. Abdominal visceral adipose tissue (VAT) (Buvat et al.) and abdominal subcutaneous adipose tissue (SAT) were determined (Borkan et al. 1982), with total abdominal adipose tissue (TAT) being the sum of VAT and SAT.

NICMs and frailty

At the moment of the visit, each patient was investigated for assessing NICMs through a specific detailed checklist useful to standardize the case-report form filling. Patient self-reports, clinical history, data obtained at physical examination (e.g. obesity), and drug-tracing criteria obtained from patients' charts were used to identify the presence or absence of NICMs as previously described (Guaraldi et al. 2011). In addition, the results of biochemical (e.g. glycaemia for diabetes) and instrumental (e.g. DXA for osteoporosis) investigations were also used to assess the patients' NICMs. More in detail, the concomitant presence of the following diseases was investigated through the above-mentioned check list: hypertension (a blood pressure measurements >140/90 mmHg or current antihypertensive treatment), cardiovascular diseases (including all cardiovascular diseases different from hypertension), obesity (BMI ≥30 kg/m^2) and overweight (BMI ≥25.0 kg/m^2), chronic renal disease (estimated glomerular filtration rate 30-60 mL/min), malignancies, diabetes mellitus (fasting serum glucose levels ≥7.0 mmol/L documented twice), dyslipidemia, low BMD (history of non-traumatic bone fracture, Z-score <2.0 at DXA evaluation), vitamin D deficiency (serum 25(OH)vitamin D <75 nmol/L), chronic obstructive pulmonary disease (COPD) and chronic viral hepatitis. Any other disease not included in the checklist was reported when present. MM was defined as at least 3 comorbid conditions.

Frailty index (FI) is useful to predict future risk of adverse outcome and it represents the proportion of health deficits present out of a group of conditions. In the literature, different strategies to calculate frailty score have been proposed. In our study, FI was quantified through the 37-item

multimorbidity FI *ad hoc* validated for men with HIV infection in the Modena cohort (Guaraldi et al. 2015). This is based on the number of health deficits divided by the total number of deficits considered (range 0–1) (Guaraldi et al. 2015). Theoretically, scores can range from 0 (least frail) to 1 (most frail). The cut-off 0.21 was used to discriminate non-frail patients (FI ≤0.21) from frail patients (FI >0.21) (Guaraldi et al. 2015).

Ethics

The Institutional review board of Modena approved the protocol study (protocol n. 1446/15). This trial was registered in ClinicalTrials.gov (Identifier: NCT03747003).

Written informed consent has been obtained from each participant.

Statistical analysis

The non-parametric Mann-Whitney *U* test was used for comparisons of continuous variables since they resulted not normally distributed at the Kolmogorov-Smirnov test. Categorical variables were compared by Pearson's Chi-squared test.

Linear regression was used to examine the association between continuous variables; significant results were expressed through the R^2 coefficient.

Ordinal logistic regression was used to determine whether sex hormones levels were associated with an increased likelihood of greater frailty status and MM (Scott, Goldberg and Mayo 1997). Proportional odds models were used to estimate the odds ratio (OR) for the sex hormones quartiles simultaneously across the ordinal levels of the MM and frailty outcomes. Quartiles were defined according to the 25^{th}, 50^{th}, and 75^{th} percentile cut-points specified below. Cut-points for quartiles of total T: Q1, ≤17.4 nmol/L; Q2, 17.4 to <22.2 nmol/L; Q3, 22.2 to <27.4 nmol/L; and Q4, ≥27.4 nmol/L. Cut-points for quartiles of cFT: Q1, ≤294.6 pmol/L; Q2, 294.6 to <366.5 pmol/L; Q3, 366.5 to <451.8 pmol/L; and Q4, ≥451.8 pmol/L. Cut-points for quartiles of E2: Q1 ≤73.8 pmol/L; Q2, 73.8 to 90.7 pmol/L; Q3, 90.7 to 114.7 pmol/L; Q4, ≥114.7 pmol/L. Cut-points for quartiles of

E1: Q1, ≤69.8 pmol/L; Q2, 69.8 to <103.9 pmol/L; Q3, 103.9 to <138.3 pmol/L; and Q4, ≥138.3 pmol/L. Cut-points for quartiles of SHBG: Q1, ≤35.3 nmol/L; Q2, 35.3 to 47.8 nmol/L; Q3, 47.8 to 65.0 nmol/L; Q4, ≥65.0 nmol/L. Cut-points for quartiles of E2/T: Q1, ≤3.15; Q2, 3.15 to 3.99; Q3, 3.99 to 5.00; Q4, ≥5.00.

Ordinal logistic regression was further used to determine whether the presence of NICMs and MM was associated with an increased risk of hypogonadism, defined by low total T or low cFT or the combination of both. The incidence of NICMs was expressed as risk ratio of each comorbidity. Furthermore, data were combined considering different sub-groups based on the serum T levels and using random effects model, which provide a more conservative estimate of overall effect. Heterogeneity was calculated using the Q test and the I-squared statistic.

Statistical analyses were performed using the Statistical Package for the Social Sciences' (SPSS) software for Windows (version 26.0; SPSS Inc, Chicago, IL). For all comparisons, $p<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics, HIV-related parameters, hormonal measurement, DXA parameters, and the prevalence of NICMs of the entire cohort of 316 patients are summarized in Table 1.

The duration of HIV-infection was directly related to patients' age ($R^2=0.366$, $p<0.0001$), waist circumference ($R^2=0.032$, $p=0.002$), waist to hip ratio ($R^2=0.133$, $p<0.0001$), trunk fat mass ($R^2=0.013$, $p=0.044$), VAT ($R^2=0.072$, $p<0.0001$), TAT ($R^2=0.018$, $p=0.045$), and VAT/SAT ($R^2=0.044$, $p=0.002$).

Total T was above the normal range (>880 ng/dL) (Fanelli et al. 2011) in 44 patients who had median SHBG significantly higher than the remaining 271 patients (73.6 nmol/L *vs* 45.2 nmol/L, respectively) ($p<0.0001$) but with similar cFT (486.7 pmol/L *vs* 351.1 pg/mL), and both E2 (116.4 pmol/L *vs* 88.5 pmol/L) and E1 (106.5 pmol/L *vs* 103.2 pmol/L) within the normal range.

Sex steroids, HIV-related parameters, anthropometric variables and body composition

Total T and cFT were inversely related to patient's age and duration of HIV-infection (total T: R^2=0.021, p=0.009 for age; R^2=0.019, p=0.016 for duration of HIV-infection) (cFT: R^2=0.107, p<0.0001 for age; R^2=0.123, p<0.0001 for duration of HIV-infection), whereas only total T was inversely correlated with BMI (R^2=0.031, p=0.002). Total T and cFT were inversely related to all the parameters of body fat mass: total and trunk fat mass (total T: R^2=0.039, p<0.0001 for total fat mass; R^2=0.065, p<0.0001 for trunk fat mass) (cFT: R^2=0.018, p=0.019 for total fat mass; R^2=0.039, p<0.0001 for trunk fat mass), VAT (Figures 2a and 2b), TAT, and VAT/SAT (total T: R^2=0.057, p<0.001 for VAT; R^2=0.029, p<0.011 for TAT; R^2=0.024, p=0.021 for VAT/SAT) (cFT: R^2=0.046, p=0.001 for VAT; R^2=0.026, p=0.038 for TAT; R^2=0.018, p=0.047 for VAT/SAT).

E2 was directly correlated with BMI (R^2=0.160, p=0.022), while no correlation was found with age, duration of HIV-infection, total and trunk fat mass, total and trunk lean mass, VAT, SAT, and TAT.

E1 resulted directly correlated with duration of HIV-infection, trunk fat mass and VAT (R^2=0.289, p<0.0001; R^2=0.017, p=0.048; R^2=0.026, p=0.028, respectively) (Figure 2c).

SHBG was directly correlated with age and duration of HIV-infection (R^2=0.026, p=0.005 and R^2=0.045, p<0.001 respectively), but not with BMI. Besides, SHBG was directly related to total T, E2, and E1 (R^2=0.272, p<0.0001; R^2=0.120, p<0.0001; R^2=0.123, p<0.0001 respectively), and it was inversely related to cFT (R^2=0.068, p<0.0001), as expected.

E2/T was directly related to total fat mass (R^2=0.070, p<0.0001), trunk fat mass (R^2=0.089, p<0.0001), VAT (R^2=0.065, p=0.001) (Figure 2d), SAT (R^2=0.038, p=0.014), TAT (R^2=0.062, p=0.002), total lean mass (R^2=0.069, p<0.0001), and trunk lean mass (R^2=0.055, p=0.001).

Relationship between sex steroids and NICMs

Considering all NICMs, 91 patients (28.8%) had MM with at least 3 NICMs, while 225 patients (71.2%) had less than 3 NICMs: 33 patients (10.4%) had no NICMs, 109 patients (34.5%) had only one comorbidity and 83 patients (26.3%) had 2 NICMs; all these last three group were considered

as non-multimorbid (<3 NICMs). Patients with MM were significantly older than non-MM patients ($p<0.0001$) and they had a significantly longer duration of HIV-infection ($p<0.0001$) (Table 2).

Total T ($p=0.036$) and cFT ($p<0.0001$) were significantly lower in patients with MM (Table 2). E2 did not significantly differ between patients with and without MM ($p=0.300$) (Table 2). E1 ($p<0.0001$) and E2/T ($p=0.002$) were significantly higher in MM (Table 2).

Although no significant difference was found for BMI, waist circumference ($p=0.013$), waist/hip ratio ($p<0.0001$), trunk fat ($p=0.033$), VAT ($p=0.003$), and VAT/SAT ratio ($p=0.004$) were higher in patients with MM (Table 2). Considering glycemic balance, serum E1 correlated with both fasting glycemia ($R^2=0.103$, $p=0.001$) and glycated hemoglobin levels ($R^2=0.149$, $p<0.001$) and, similarly, E2 correlated with glycaemia ($R^2=0.077$, $p=0.008$) and glycated hemoglobin ($R^2=0.230$, $p<0.001$). cFT inversely correlated with both fasting glycemia ($R^2=0.026$, $p=0.030$) and glycated hemoglobin ($R^2=0.026$, $p=0.032$), whereas total T inversely correlated with fasting glycemia ($R^2=0.023$, $p=0.043$) but not with glycated hemoglobin.

In line with these findings, the mere sum of NICMs was inversely related to total T ($R^2=0.027$, $p=0.004$) and cFT ($R^2=0.024$, $p=0.005$), whereas it was directly related to E2 ($R^2=0.034$, $p=0.008$), E1 ($R^2=0.090$, $p<0.0001$), and E2/T ($R^2=0.098$, $p<0.001$). Accordingly, the prevalence of NICMs changed when the HIV-infected patients were compared according to quartiles of steroids levels. The fourth quartile (Q4) displayed the highest hormonal values and the first quartile (Q1) the lowest hormonal values. On average HIV-infected patients in the lowest quartile of cFT had a 3-fold increased likelihood risk of MM compared with patients in the highest quartile (Q1 *vs* Q4, OR 3.30; 95% confidence interval (CI), 1.59, 6.88; p for trend across quartiles <0.0001) (Table 3). Similarly, patients in the highest quartile of E2/T had a significantly increased odds of having MM compared with patients in the lowest quartile (Q1 *vs* Q4, OR 0.36; 95% CI 0.15, 0.85; p for trend across quartiles =0.031) (Table 3).

Considering the presence/absence of each NICM and MM, patients with MM had a 4-fold increased risk of hypogonadism on the basis of total T below 10.4 nmol/L (OR 4.23; 95% CI, 1.34, 13.33;

p=0.014; predictive accuracy 95.7%) and on the basis of low total T and/or low cFT (OR 4.30; 95% CI, 2.02, 9.17; p <0.0001; predictive accuracy 89.3%). Differently, hypertension was included in the model of logistic regression to predict cFT below 220 pmol/L, with patients suffering from hypertension having a 4-fold increased likelihood risk of having low cFT compared with patients without hypertension (OR 4.17; 95% CI, 1.79, 9.71; p=0.001; predictive accuracy 89.3%).The cumulative relative risk of having NCIMs was increased in HIV-infected men with biochemical hypogonadism when the NICMs were considered all together, with only a trend when the cut off of total T below 10.4 nmol/L was used for hypogonadism (p=0.07) (Figure 3a), but with significant outcome when hypogonadism was established considering cFT below 220 pmol/L (p=0.0003) (Figure 3b) or cFT below 220 pmol/L and/or total T below 10.4 nmol/L (p=0.0001) (Figure 3c). The following NICMs had a major weight in determining the overall effect on relative risk: hypertension, dyslipidemia, vitamin D deficiency, and chronic viral hepatitis (Figures 3b and 3c).

Differences in sex steroids according to the presence/absence of each NICMs (for details on NICMs prevalence see Table 1) are summarized in Table 4. When a significant difference was found it was consisting with lower levels of androgens and higher levels of estrogens in patients with compared to those without the comorbidity, with the exception of patients with chronic viral hepatitis where total T, E1 and SHBG were all increased (see Table 4 for details).

Relationship between sex steroids and frailty

According to the FI cut-off of 0.21, 207 patients (65.5%) were classified as frail and 109 patients (34.5%) as non-frail (Table 2). Frail patients were older than non-frail ones (p<0.0001) and they had a significantly longer duration of HIV-infection (p<0.0001) (Table 2). Moreover, frail patients had significantly higher BMI (p<0.0001), waist circumference (p<0.0001) and waist to hip ratio (p<0.0001) (Table 2). Frail patients had a greater amount of fat mass, both trunk (p<0.0001) and total (p=0.012) compared to non-frail patients (Table 2). Similarly, even VAT (p<0.0001), TAT

(p=0.002), and VAT/SAT (p<0.0001) ratio were greater in frail patients than non-frail ones (Table 2).

Total T (p<0.0001) and cFT (p=0.004) were significantly lower in frail than non-frail patients (Table 2).

At bivariate regression analysis between frailty and sex steroids, FI was inversely related to total T (R^2=0.013, p=0.043) (Figure 4a) and cFT (R^2=0.057, p<0.0001) (Figure 4b). On the contrary, FI was directly related to E1 (R^2=0.171, p<0.0001) (Figure 4c), E2 (R^2=0.041, p=0.004) (Figure 4d), E2/T (R^2=0.104, p<0.0001) (Figure 4e), and SHBG (R^2=0.029, p=0.003) (Figure 4f). FI was directly related to BMI (R^2=0.094, p<0.0001), trunk fat mass (R^2=0.151, p<0.0001), total fat mass (R^2=0.053, p<0.0001), VAT (R^2=0.223, p<0.0001) (Figure 4g), SAT (R^2 =0.036, p=0.004), TAT (R^2=0.142, p=<0.001), and VAT/SAT (R^2 =0.046, p=0.001), trunk lean mass (R^2=0.024, p=0.007), and total lean mass (R^2=0.024, p=0.007).

Furthermore, HIV-infected patients in the two lowest quartiles of total T had an almost 4-fold increased likelihood to be frail compared with those in the highest quartile (Q1 *vs* Q4, OR 3.74; 95% CI 1.87, 7.49; p for trend across quartiles =0.001) (Table 3). On average, HIV-infected patients in the two lowest quartiles of cFT had 2-fold increased likelihood risk to be frail compared with patients in the highest quartile (Q1 *vs* Q4, OR 2.12; 95% CI 1.09, 4.14; p for trend across quartiles =0.048) (Table 3). Patients in the second and third quartiles of E2/T had a significantly lower odds ratio of being frail compared with patients in the highest quartile (Q2 *vs* Q4, OR 0.39; 95% CI 0.16, 0.98; p for trend across quartiles =0.007) (Table 3). Conversely, no differences of frailty status were observed across quartiles of E2 (p=0.236) and E1 (p=0.495) (Table 3).

DISCUSSION

In this study low serum total T and cFT were independently associated to poor health measured as MM and frailty in young to middle-aged HIV-infected men, the strength of this association being more robust for cFT than for total T. In parallel, high serum E1 and E2/T resulted

also associated to both MM and frailty. Frailty was inversely related to T, especially cFT, while it was directly related to E1, E2, E2/T, SHBG, BMI, and body fat, particularly its visceral component. The results concerning estrogens and body fat represents a novel, interesting issue adding new insights on the complex network of health status, body composition, and circulating sex steroids in HIV-infected men. On one hand, cFT and total T were inversely related to total body fat and to visceral fat (trunk fat and VAT measured by DXA and CT) similarly to what happens in HIV-uninfected men (Kelly and Jones 2015, Gates et al. 2013, Antonio et al. 2015). On the other hand, E1 and the E2/T resulted directly related to the same abovementioned parameters of body fat. More worthy of mention, FI was directly related to total body fat and VAT according to previous findings coming from both HIV-infected (Shah et al. 2012) and HIV-uninfected men (Kim et al. 2020). This implies that body fat, especially visceral fat, is an important predictor of frailty and takes part to the interplay between sex steroids and overall health status, thus bridging the gap between poor health status and low T in HIV-related hypogonadism. Taken together all these data allow hypothesizing i) that increased body fat and in particular visceral fat may boost T decrease in HIV-infected men probably through an increase of T aromatization (Cohen 1999, Jasuja et al. 2013) or other mechanisms such as inhibition of gonadotropin secretion through leptin resistance or adipokynes release (Grossmann and Matsumoto 2017, Fernandez, Chacko and Pappachan 2019) as substantiated by the direct association between percentage of total and visceral fat and both E1 and E2/T and similarly to what happens in obese, hypogonadal men (Wu et al. 2010, Jasuja et al. 2013, Kelly and Jones 2015, Wu et al. 2018), ii) that body fat is linked to and/or is a biomarker of poor health status together with sex steroids. Accordingly, fat accumulation due to HIV-related lipodystrophy is associated to increased metabolic and immunological activity of the adipose tissue (Koethe et al. 2020) and we could speculate that, even in the absence of overt obesity, this hyperactivity may involve also endocrine pathways directly (e.g. changes in aromatase activity and expression) or indirectly (e.g. through immunological changes) affecting the hypothalamic-pituitary-gonadal axis function. It seems that increased body fat, increased E1 and E2/T, low

circulating T and poor health status in terms of both frailty and MM all take part to a sort of complex and multifactorial vicious circle resulting in HIV-related hypogonadism whose cause-effect relationships remain uncertain due to the bidirectional action exerted by these elements (Cohen 1999). As an example, low T levels can be at the same time the cause and the consequence of both changes in body fat and the onset of NICMs (Hsu et al. 2018, Grossmann and Matsumoto 2017, Kelly and Jones 2015, Handelsman 2011, Rochira and Guaraldi 2014, Rochira et al. 2015a, Rochira 2017). This study does not help establishing a cause-effect relationship among all these factors due to its cross-sectional design, but allows starting to move from the interpretation of low T in men with HIV as a condition of true hypogonadism (Monroe et al. 2014, Jasuja et al. 2018) to that of biochemical functional hypogonadism (Grossmann and Matsumoto 2017, Corona et al. 2020).

Similarly to previous retrospective studies (Rochira et al. 2015a, Erlandson et al. 2017), total and cFT were lower in MM and frail HIV-infected patients, the relative risk of having hypogonadism was higher in patients who were more comorbid and the overall sum of MM rather than each single NICM predicted. In addition, E1 and E2/T were increased in MM than non-MM and both MM and frailty were more prevalent in HIV-infected men with cFT and E2/T in the lowest and highest quartile respectively, while frailty was more prevalent also in men with total T in the lowest quartile. These findings resemble those coming from HIV-uninfected older men where lower T associated to comorbidities (Rastrelli et al. 2020, Rastrelli et al. 2015) to body fat (Wu et al. 2018) and the prevalence of hypogonadism was considerable in frail men (Hsu et al. 2018, Hyde et al. 2010, Travison et al. 2007, Wu et al. 2010, Connolly et al. 2017). These comorbidities, in fact, are highly prevalent in HIV-uninfected men with hypogonadism (15-17,20,26) and may concur to the so called functional hypogonadism as in older men (21,22,41,43). Altogether these results confirm that a poor health status is almost constantly linked to low T. Accordingly, the duration of HIV infection, which correlates with all the above-mentioned changes in sex steroids and body composition, is an indirect surrogate of patients' health, since patients with a longer duration are

expected to accumulate more comorbidities and to be frailer. Hence, the results of this study demonstrate that the more HIV-infected patients are frail and/or multimorbid, the more T is reduced, E1 and E2/T are increased, and visceral fat is augmented. Thus, low total T and cFT, high E1, and increased visceral fat may be consider as indirect biomarkers of poor health status in HIV-infected men. Likewise, elevated E1 and low T are both considered biomarkers of health status also in men with advanced liver disease (Sinclair et al. 2016) and E1 resulted associated with higher BMI and NICMs (Jasuja et al. 2013) as well as with a poor health status (Hsu et al. 2015) in older men. It is worthy to observe that most of correlations we found between duration of HIV infection and body composition were not so impressive (R^2 <0.1). This was probably due to the young age of our cohort that had beneficiated of better antiretroviral therapies, less associated with the risk of body composition changes such as lipodystrophy. In parallel, other factors, such as unbalanced sex steroids *milieu* and multimorbidity, may have concurred together to body composition modifications masking the role of HIV *per se*.

Aging is generally associated with decreased T, especially cFT, increased visceral fat and obesity, and increased SHBG (Wu et al. 2018, Jasuja et al. 2013, Handelsman 2011), as well as increased frailty and the number of NICMs (Hsu et al. 2018, Travison et al. 2007, Travison et al. 2008, Hyde et al. 2010, Rastrelli et al. 2020, Connolly et al. 2017). Also, E1 and E2 have been shown to increase with advancing age in older men (Wu et al. 2018, Jasuja et al. 2013), E1 increasing more than E2 (Jasuja et al. 2013) similarly to what happens in young to middle-aged HIV-infected patients of this study. Low T and other changes in circulating sex steroids were recorded notwithstanding i) the patients here investigated were per protocol relatively young (≤50 years) and ii) they were in a relatively good health status thanks to better efficacy and less toxicity of HAART resulting in reduced prevalence of NICMs than in the past (Ghosn et al. 2018). These findings confirm that hypogonadism occurs earlier in HIV-infected men (Rochira et al. 2011, Rochira et al. 2015a, Rochira and Guaraldi 2014, Erlandson et al. 2017, Monroe et al. 2014) and

support an accentuated aging in these patients (Guaraldi et al. 2019, Guaraldi and Rockwood 2017, Martin and Volberding 2010) consisting in a poor health status (MM and frailty) and concomitant functional hypogonadism as in older men (Hsu et al. 2018, Grossmann and Matsumoto 2017, Corona et al. 2020). Accordingly, T lower than normal is uncommon in HIV-uninfected men before the age of 50 in the absence of documented disease of the hypothalamus, the pituitary, and the testis (Kaufman and Vermeulen 1997).

In clinical practice, the approach to low T in an HIV-infected man without documented diseases of the hypothalamus-pituitary-testis axis should focus on the presence/absence of signs and symptoms of hypogonadism (Rochira and Guaraldi 2014, Bhasin et al. 2018, Corona et al. 2020) and on NICMs and the therapeutic approach should consider improving or reversing concomitant diseases through lifestyle changes (e.g. by reducing body weight) and/or pharmacological therapies rather than prescribing T therapy, as in functional hypogonadism (Rochira and Guaraldi 2014, Corona et al. 2020). Accordingly, in men living with HIV physical exercise seems to be effective in increasing serum T (Melo et al. 2019). Outside the context of T therapy for the AIDS wasting (Jasuja et al. 2018), in fact, T replacement therapy may be harmful especially in patients with a severe burden of NICMs (Basaria et al. 2010, Jasuja et al. 2018, Rochira and Guaraldi 2014). In the real life setting, exogenous T is frequently administered to HIV-infected men without an appropriate workup (i.e. even in cases where serum T was normal) (46). Indeed, in some countries, T therapy is even authorized to be given supraphysiological doses for the treatment of HIV-related wasting syndrome, independent of the baseline serum T value, based on the very different clinical expression of HIV disease that was evident historically prior to the advent of modern HAART in the late 1990s (11, 56). Besides, body image disorders are highly prevalent among HIV-infected men and are strongly associated to sexual dysfunction (Santi et al. 2014) and to the inappropriate use of anabolic steroids, including testosterone and other androgens (Griffiths et al. 2017, Ip et al. 2017). By considering all these aspects, the fact that T therapy is largely prescribed to men living with HIV raises concerns about the risk of overtreatment and safety in this clinical setting, as

already pointed out by our research group and other authors (Rochira and Guaraldi 2014, Haberlen et al. 2018). Caution, in fact, is needed before starting T therapy in HIV-infected men with low serum T and the possible risks and benefits should be carefully considered (Haberlen et al. 2018, Rochira and Guaraldi 2014) especially in comorbid HIV-infected men since unhealthy patients (Vigen et al. 2013, Finkle et al. 2014) and older men (Basaria et al. 2010) are at higher risk of undesired adverse events related to T administration. Our data indicate that, apart from a minority of patients with organic Leydig cell insufficiency and raised LH levels, most HIV-infected men having low T levels have functional gonadotropin insufficiency, for which evidence of safety and benefit of T supplementation is much less clear. Therefore, these men should be carefully evaluated and their treatment individualized in order to optimize non-gonadal comorbidities and thus avoid over-treatment, or even unnecessary treatment with T.

This study has strengths and limitations. This is a properly-designed study to investigate the gonadal function in HIV-infected men by measuring total T, E2, and E1 with the recommended analytical procedure (ID-LC-MS/MS) alongside SHBG in order to calculate free T that is recognized to be the most reliable biomarker to detect the gonadal status in these patients (Rochira 2017, Bhasin et al. 2018, Corona et al. 2020). The definition of general health status was performed by prospective data collection about NICMs and frailty, the latter assessed by *ad hoc* questionnaire validated for HIV-population (Guaraldi et al. 2015). Other points of strength are the detailed study of body composition and the mean age of our cohort, that allowed us to downgrade and avoid the influence of physiological aging on both T levels and body composition changes. A limit of this study is that morning total T has been determined on a single blood sample. The lack of a control group of HIV-uninfected men is a limit but it should be considered that the aim of the study was the identification of factors associated to low T in men living with HIV for which the cohort cross-sectional design is adequate.

In conclusion, this study provides evidence on the interplay among health status in terms of both NICMs and frailty, low T, high E1 and E2/ T ratio, and body fat mass and distribution in men

with HIV showing how all these factors are associated among each other. Overall HIV-infected patients with poor health status were prone to exhibit a worse gonadal function in the context of a form of functional male hypogonadism. Low T, high E1 and E2/T, and increased visceral fat may be considered hallmarks of frailty and unhealthy conditions in HIV-infected men. Further large studies like the Androgens in Men Study (AIMS) (Yeap et al. 2020) are needed in order to better define the cause-effect relationships among hypogonadism, sex steroids, health status, and body composition in men.

Table 1. Characteristics of the entire cohort and prevalence of each comorbidity. Data are expressed as median (minimum-maximum) and as a number (percentage) when categorical.

n 316	**Normal Range**	**Median (Min-Max)**
Age (years)	n.a.	47.0 (25-50)
Anthropometrical variables		
BMI (kg/m^2)	18-25	23.6 (16.33-38.74)
Waist/hip circumference ratio	<0.95	0.94 (0.72-1.19)
HIV-related parameters		
CD4 cell count nadir (cells/μL)	n.a.	251 (4-880)
CD4 absolute cell count (cells/μL)	>400	625 (173-1310)
Suppressed viral load (< 40 copies/mL) *n (%)*	n.a.	268 (84.8 %)
Years of HIV infection	n.a.	16.15 (1.13-35.40)
Years of exposure to HAART	n.a.	14.20 (0-33.78)
Ongoing HAART		
FI *n (%)*	n.a.	19 (6.01 %)
PI *n (%)*	n.a.	126 (39.87 %)
NNRTI *n (%)*	n.a.	160 (50.63 %)
NRTI *n (%)*	n.a.	264 (83.54 %)
II *n (%)*	n.a.	47 (14.87 %)
Hormonal measurements		
Serum LH (mIU/mL)	1-9	4.9 (0.01-43)
Serum FSH (mIU/mL)	1-12	5.6 (0.01-58.5)
Serum Total T (nmol/L)	10.4-30.5	22.2 (6.2-58.1)
Serum cFT (pmol/L)	>220	366.5 (43.9-942.8)
Serum SHBG (nmol/L)	13.5-71.4	47.8 (11.9-165.5)
Serum E2 (pmol/L)	<183.6	90.7 (31.2-258.1)
Serum E1 (pmol/L)	68.7-213.4	103.9 (28.5-443.5)
Serum E2/T	n.a.	3.99 (1.36-11.62)
Serum Prolactin (mIU/L)	63.8-276.6	157.4 (40.4-1327.6)
Bone parameters at DXA		
Body BMD (g/cm^2)	n.a.	1.21 (0.966-1.642)
Lumbar BMD (g/cm^2)	n.a.	0.959 (0.104-1.86)
Femoral neck BMD (g/cm^2)	n.a.	0.776 (0.695-1.389)
Femoral tot BMD (g/cm^2)	n.a.	0.84 (0.383-1.389)
Total lean mass (kg)	n.a.	52.94 (4.26-82.22)
Trunk lean mass (kg)	n.a.	25.48 (2.41-39.75)
Total fat mass (kg)	n.a.	17.28 (6.38-54.28)
Trunk fat mass (kg)	n.a.	8.430 (3.08-27.46)
Frailty Score	<0.21	0.25 (0.05-0.57)
NICMs		
Obesity (BMI ≥30) *n (%)*	n.a.	12 (3.8)
Overweight (BMI ≥25) *n (%)*	n.a.	98 (31.1)
Diabetes mellitus *n (%)*	n.a.	7 (2.2)
Hypertension *n (%)*	n.a.	53 (16.9)
Cardiovascular disease *n (%)*	n.a.	19 (6)
Chronic viral hepatitis *n (%)*	n.a.	57 (18.2)
HCV *n (%)*	n.a.	43 (13.7)
HBV *n (%)*	n.a.	19 (6.1)
Renal chronic failure *n (%)*	n.a.	1 (0.3)
Cancer *n (%)*	n.a.	22 (7.1)
Dyslipidemia *n (%)*	n.a.	153 (49.0)

Low BMD* *n (%)*	n.a.	43 (13.6)
Vitamin D deficiency** *n (%)*	n.a.	203 (64.2)
Chronic Obstructive Pulmonary Disease *n (%)*	n.a.	10 (3.2)

[Footnote to Table 1] *Abbreviations: HIV: Human Immunodeficiency Virus; HAART: Highly Active Antiretroviral Therapy; FI: fusion inhibitors; PI: protease inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; II: integrase inhibitors; BMI: Body Mass Index; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; E2: estradiol; E1: estrone, T: Testosterone; SHBG: Sex Hormone-binding Globulin; cFT: calculated free testosterone; DXA: Dual-energy X-ray Absorptiometry; BMD: Bone Mineral Density; NICMs: non-infectious comorbidities; HCV: Hepatitis C Virus; HBV: Hepatitis B Virus. *Low BMD was defined as Z-score <2.0 at lumbar or femoral site at DXA measurement. **vitamin D deficiency was defined as serum 25(OH)vitamin D levels <75 nmol/L.*

Table 2. Differences in clinical, hormonal and bone parameters comparing patients with MM (≥3 NICMs) to those without MM (<3 NICMs) and frail to non-frail patients. Only significant comparisons are in bold.

N	**Patients with MM (≥3 NICMs) 91 (28.8%)**	**Patients without MM (<3 NICMs) 225 (71.2%)**	**P value**	**Frail patients (FI>0.21) 207 (65.5%)**	**Non-frail patients (FI≤0.21) 109 (34.5%)**	**P value**
Frailty Score	0.324 (0.088-0.568)	0.235 (0.054-0.514)	**<0.0001**	0.297 (0.212-0.568)	0.162 (0.054-0.206)	**<0.0001**
Age (years)	49.3 (33.9-50)	45.7 (25.2-50)	**<0.0001**	48.1 (27-50)	44.2 (25-50)	**<0.0001**
Anthropometrical variables						
BMI (kg/m^2)	24.2 (16.9-38.7)	23.5 (16.3-35.5)	0.100	24.2 (16.3-38.7)	22.8 (18.8-29.3)	**<0.0001**
Waist circumference (cm)	88.5 (69.5-125)	86.0 (66.0-120.0)	**0.013**	89.0 (66.0-125.0)	83.5 (72.0-103.0)	**<0.0001**
Hip circumference (cm)	92 (79-128)	93 (68-118)	0.178	93.0 (68.0-128.0)	92.0 (79.0-109.0)	0.503
Waist/hip circumference ratio	0.97 (0.84-1.19)	0.94 (0.72-1.16)	**<0.0001**	0.96 (0.72-1.19)	0.90 (0.76-1.16)	**<0.0001**
Medical history						
Years of HIV infection	22.4 (3.9-34.0)	12.4 (1.1-35.4)	**<0.0001**	20.2 (1.5-35.4)	9.5 (1.1-29.5)	**<0.0001**
Years of exposure to HAART	17.5 (0-33.8)	10.8 (0-30.6)	**<0.0001**	16.8 (0-33.8)	7.2 (0-29.5)	**<0.0001**
Hormonal measurements						
Serum total T (nmol/L)	20.6 (6.6-58.1)	22.7 (6.2-54.7)	**0.036**	20.7 (6.2-58.1)	25.0 (7.1-47.7)	**<0.0001**
Serum cFT (pmol/L)	334.6 (43.9-601.2)	385.6 (59.2-942.8)	**<0.0001**	353.9 (43.9-909.4)	398.4 (144.8-942.8)	**0.004**
Serum SHBG (nmol/L)	51.8 (18.2-165.5)	46.8 (11.9-161.0)	0.321	46.6 (14.4-165.5)	49.6 (11.9-161.0)	0.149
Serum E2 (pmol/L)	91.0 (31.2-258.1)	90.3 (35.8-225.8)	0.300	88.8 (31.2-258.1)	95.8 (35.8-163.7)	0.252
Serum E1(pmol/L)	126.9 (38.5-443.5)	94.3 (28.5-308.5)	**<0.001**	106.3 (28.5-443.5)	102.1 (30.3-230.1)	0.487
Serum E2/T	4.46 (2.20-11.62)	3.91 (1.36-7.11)	**0.002**	3.99 (1.49-11.62)	4.02 (1.36-6.22)	0.196
Bone parameters and body composition at DXA						
Total lean mass (g)	53997 (39085-78444)	52764 (4258-82228)	0.118	53516 (4258-82228)	52001 (39085-65773)	0.075
Trunk lean mass (g)	25871 (18383-35433)	25240 (2413-39746)	0.095	25606 (2426-39746)	25081 (2413-31941)	**0.047**
Total fat mass (g)	17612 (6984-54284)	16738 (6376-43055)	0.987	17789 (6376-54284)	15523 (9801-32869)	**0.012**
Trunk fat mass (g)	9391 (3205-27457)	8143 (3084-26060)	**0.033**	9534 (3084-27457)	7121 (4113-17184)	**<0.0001**
Body composition at CT scan						
VAT (cm^2)	146 (25-429)	104 (23-460)	**0.003**	137.5 (23-460)	90 (24-395)	**<0.001**
SAT (cm^2)	132 (10-574)	134 (4-566)	0.820	137 (4-574)	128 (18-326)	0.231
TAT (cm^2)	284 (35-1003)	259 (31-1026)	0.116	287 (31-1026)	209.5 (67-537)	**0.002**
VAT/SAT	1.16 (0.31-6.09)	0.80 (0.22-9)	**0.004**	1.00 (0.22-9.00)	0.67 (0.22-6.09)	**<0.001**

[Footnote to Table 2] *Abbreviations: HIV: Human Immunodeficiency Virus; HAART: Highly Active Antiretroviral Therapy; MM: multimorbidity; NICMs: non-infectious comorbidities; F.I.: Frailty Index; BMI: Body Mass Index; T: Testosterone; E2/T: estradiol to Testosterone ratio; SHBG: Sex Hormone-binding Globulin; DXA: Dual-energy X-ray Absorptiometry; BMD: Bone Mineral Density.*

Table 3. Proportional odds ratio (95% CI) for frail status (FI≥0.21) and multimorbidity by quartiles of sex hormones.

	Q1	Q2	Q3	Q4	*P* value for trend
Multimorbidity					
Serum total T	1.93 (0.94, 3.98)	1.86 (0.91, 3.83)	1.68 (0.81, 3.50)	1.00 (referent)	0.276
Serum cFT	3.30 (1.59, 6.88)	2.88 (1.39, 5.98)	1.02 (0.45, 2.30)	1.00 (referent)	**<0.0001**
Serum SHBG	0.86 (0.45, 1.66)	0.38 (0.18, 0.79)	0.65 (0.33, 1.28)	1.00 (referent)	0.058
Serum E2	0.57 (0.24, 1.28)	0.58 (0.26, 1.31)	0.31 (0.12, 0.76)	1.00 (referent)	0.084
Serum E1	0.30 (0.13, 0.68)	0.34 (0.15, 0.77)	0.62 (0.29, 1.31)	1.00 (referent)	**0.012**
Serum E2/T	0.36 (0.15, 0.85)	0.37 (0.16, 0.87)	0.35 (0.15, 0.83)	1.00 (referent)	**0.031**
Frailty					
Serum total T	3.74 (1.87, 7.49)	2.75 (1.42, 5.32)	1.61 (0.85, 3.04)	1.00 (referent)	**0.001**
Serum cFT	2.12 (1.09, 4.14)	2.13 (1.10, 4.13)	1.21 (0.64, 2.28)	1.00 (referent)	**0.048**
Serum SHBG	2.01 (0.98, 4.13)	0.75 (0.39, 1.43)	0.70 (0.37, 1.34)	1.00 (referent)	**0.018**
Serum E2	1.77 (0.73, 4.30)	0.94 (0.42, 2.14)	0.71 (0.32, 1.60)	1.00 (referent)	0.236
Serum E1	0.90(0.39, 2.04)	0.58 (0.26, 1.30)	0.65 (0.29, 1.44)	1.00 (referent)	0.495
Serum E2/T	0.58 (0.23, 1.50)	0.39 (0.16, 0.98)	0.22 (0.09, 0.54)	1.00 (referent)	**0.007**

[Footnote to Table 3] *Abbreviations: Q: quartiles; T: testosterone; cFT: calculated free testosterone; SHBG: Sex Hormone-binding Globulin; E2: estradiol; E2/T: estradiol to testosterone ratio. Total T quartiles: Q1, ≤17.4 nmol/L; Q2, 17.4 to <22.2 nmol/L; Q3, 22.2 to <27.4 nmol/L; and Q4, ≥27.4 nmol/L. cFT quartiles: Q1, ≤294.6 pmol/L; Q2, 294.6 to <366.5 pmol/L; Q3, 366.5 to <451.8 pmol/L; and Q4, ≥451.8 pmol/L. E2 quartiles: Q1 ≤73.8 pmol/L; Q2, 73.8 to 90.7 pmol/L; Q3, 90.7 to 114.7 pmol/L; Q4, ≥114.7 pmol/L. E1 quartiles: Q1, ≤69.8 pmol/L; Q2, 69.8 to <103.9 pmol/L; Q3, 103.9 to <138.3 pmol/L; and Q4, ≥138.3 pmol/L. SHBG quartiles: Q1, ≤35.3 nmol/L; Q2, 35.3 to 47.8 nmol/L; Q3, 47.8 to 65.0 nmol/L; Q4, ≥65.0 nmol/L. E2/T quartiles: Q1, ≤3.15; Q2, 3.15 to 3.99; Q3, 3.99 to 5.00; Q4, ≥5.00.*

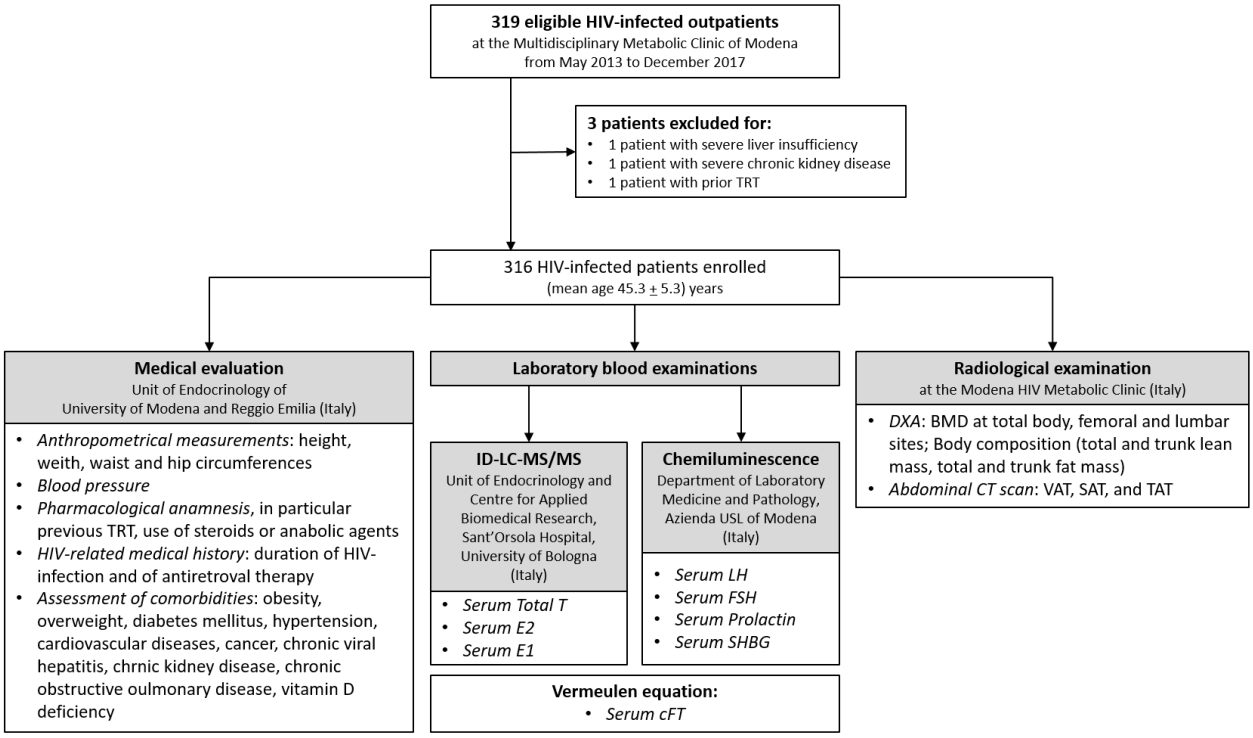
Table 4. comparison of sex hormones levels in HIV-infected patients according to the presence of each of the following NICMs: obesity, overweight, diabetes mellitus, hypertension, chronic viral hepatitis, cancer, dyslipidemia, low BMD, vitamin D deficiency, and COPD. Only significant comparisons are in bold.

NICMs		***n* (%)**	**Serum Total T (ng/dL)**	**Serum cFT (pg/mL)**	**Serum E2 (pg/mL)**	**Serum E1 (pg/mL)**	**Serum SHBG (nmol/L)**	**Serum E2/T**
Obesity	YES	12 (3.8)	17.7 (8.6-28.6)	311.4 (155.8-425.4)	120.4 (65-211.8)	138.0 (116.9-281.1)	44.6 (21.6-118.5)	5.17 (3.24-10.10)
	NO	304 (96.2)	22.2 (6.6-58.1)	371.2 (43.9-942.8)	90.3 (31.2-258.1)	102.1 (28.5-443.5)	47.9 (11.9-165.5)	3.99 (1.36-11.61)
		P value	0.062	**0.035**	0.062	**0.035**	0.074	**0.010**
Overweight	YES	98 (31.1)	20.2 (6.6-41.1)	350.9 (43.9-942.8)	95.8 (35.8-258.1)	111.7 (30.3-443.5)	43.1 (14.4-165.5)	4.46 (1.36-11.62)
	NO	218 (68.9)	23.0 (6.2-58.1)	374.2 (59.2-909.4)	89.6 (31.2-213.3)	99.5 (28.5-308.5)	50.4 (11.9-161.0)	3.80 (1.49-7.16)
		P value	**0.005**	0.269	**0.005**	0.269	0.264	0.253
Diabetes mellitus	YES	7 (2.2)	15.4 (12.4-28.7)	274.1 (111.1-361.9)	110.5 (84.1-194.2)	116.9 (69.5-206.0)	31.5 (30.3-127.6)	6.76 (6.59-6.82)
	NO	309 (97.8)	22.2 (6.6-58.1)	370.1 (43.9-942.8)	90.3 (31.2-258.1)	103.9 (28.5-443.5)	47.9 (11.9-165.5)	3.99 (1.36-11.62)
		P value	**0.015**	**0.037**	**0.015**	**0.037**	0.181	0.425
Hypertension	YES	53 (16.8)	21.2 (8.6-37.0)	350.9 (134.0-601.2)	101.0 (55.1-258.1)	126.5 (45.9-443.5)	59.6 (21.6-165.5)	4.59 (2.37-10.10)
	NO	263 (83.2)	22.4 (6.6-58.1)	373.8 (43.9-942.8)	89.2 (31.2-225.8)	99.1 (28.5-359.9)	46.6 (11.9-161.0)	3.93 (1.36-11.62)
		P value	0.518	0.059	0.518	0.059	0.060	**0.003**
Cardiovascular disease	YES	19 (6.0)	23.9 (8.7-32.6)	351.9 (134.0-535.9)	111.8 (85.9-258.1)	116.5 (51.4-341.4)	62.5 (18.2-126.7)	4.52 (3.78-8.99)
	NO	297 (94.0)	22.1 (6.6-58.1)	367.6 (43.9-942.8)	89.6 (31.2-225.8)	102.1(28.5-443.5)	47.2 (11.9-165.5)	3.98 (1.36-11.62)
		P value	0.758	0.499	0.758	0.499	**0.044**	0.086
Chronic viral hepatitis	YES	57 (18.0)	24.3 (6.6-58.1)	347.0 (43.9-909.4)	88.5 (57.3-258.1)	138.8 (38.5-443.5)	68.1 (18.5-165.5)	3.99 (1.49-11.62)
	NO	259 (82.0)	21.3 (7.1-54.7)	376.2 (111.1-942.8)	91.2 (31.2-163.7)	99.9 (28.5-273.3)	45.3 (11.9-137.1)	4.01 (1.36-7.95)
		P value	**0.014**	0.160	**0.014**	0.160	0.253	**0.006**
Cancer	YES	22 (7.0)	20.2 (7.1-37.9)	367.9 (144.8-600.0)	81.9 (35.8-180.2)	121.9 (45.9-267.0)	51.0 (11.9-85.9)	4.97 (1.78-7.11)
	NO	294 (93.0)	22.3 (6.6-58.1)	367.1 (43.9-942.8)	90.7 (31.2-258.1)	102.1 (28.5-443.5)	47.8 (14.4-165.5)	3.99 (1.36-11.62)
		P value	0.548	0.614	0.548	0.614	0.534	0.246
Dyslipidemia	YES	153 (48.4)	20.3 (6.6-58.1)	353.9 (43.9-942.8)	93.6 (31.2-258.1)	110.6 (31.4-359.9)	46.3 (16.4-137.1)	4.31 (1.90-11.62)
	NO	163 (51.6)	23.1 (7.1-47.7)	386.2 (111.1-889.2)	89.6 (35.8-169.2)	95.4 (28.5-443.5)	48.8 (11.9-165.5)	3.86 (1.36-7.16)
		P value	**0.006**	**0.025**	**0.006**	**0.025**	0.344	0.060
Low BMD	YES	43 (13.6)	22.5 (9.7-35.8)	354.1 (134.0-600.0)	82.6 (31.2-132.9)	100.8 28.5-219.3)	50.4 (18.5-128.5)	3.29 (1.78-6.79)
	NO	273 (86.4)	21.9 (6.2-58.1)	369.4 (43.9-942.8)	91.2 (35.8-258.1)	104.3 (30.3-443.5)	47.6 (11.9-165.5)	4.01 (1.36-11.62)
		P value	0.808	0.700	0.808	0.700	0.054	0.186
Vitamin D	YES	203 (64.2)	20.8 (6.2-58.8)	357.2 (43.9-889.2)	88.8 (31.2-213-3)	99.3 (30.3-443.5)	47.2 (14.4-165.5)	4.09 (1.36-11.62)

deficiency	NO	113 (35.8)	23.8 (7.1-44.9)	398.8 (134.0-942.8)	100.2 (35.8-258.1)	107.3 (28.5-341.4)	50.2 (11.9-136.3)	3.91 (1.49-8.99)
		P value	**0.005**	**0.020**	**0.005**	**0.020**	0.493	0.943
COPD	YES	10 (3.2)	22.1 (9.6-27.2)	347.8 (144.8-460.3)	98.6 (62.8-150.9)	226.3 (81.0-443.5)	59.7 (18.2-165.5)	4.00 (3.03-7.95)
	NO	306 (96.8)	22.2 (6.2-58.1)	368.7 (43.9-942.8)	90.7 (31.2-258.1)	103.0 (28.5-359.9)	47.8 (11.9-161.0)	3.99 (1.36-11.62)
		P value	0.471	0.095	0.498	0.151	0.646	0.705

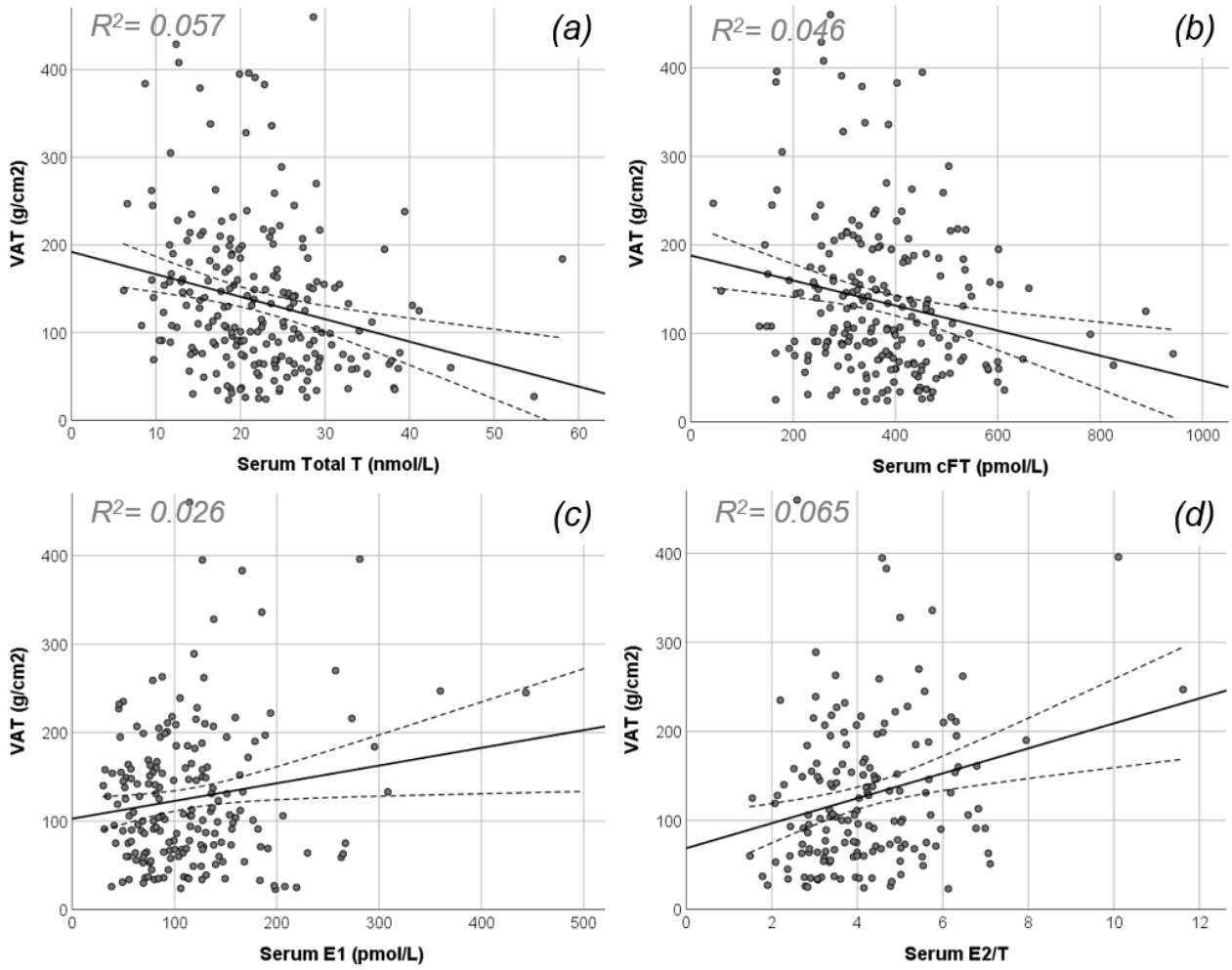
[Footnote to Table 4] *Abbreviations: HIV: Human Immunodeficiency Virus; NICMs: non-infectious comorbidities; BMI: Body Mass Index; BMD: Bone Mineral Density; COPD: chronic obstructive pulmonary disease; T: testosterone; cFT: calculated free testosterone; E2: Estradiol; E1: Estrone; SHBG: Sex Hormone-binding Globulin; E2/T: estradiol to testosterone ratio.*

Figure 1. Flow chart of the study design.



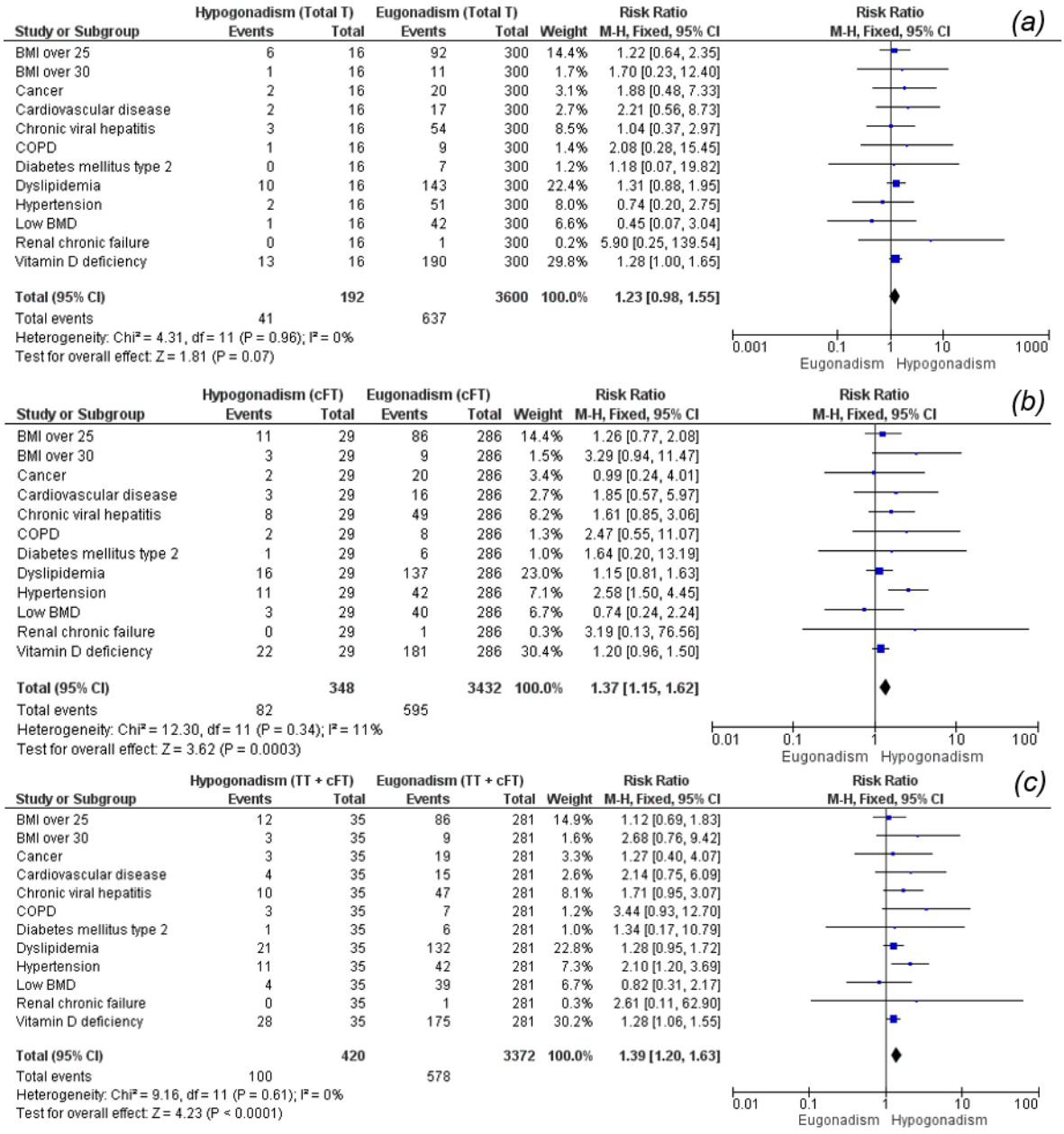
[Footnote to Figure 1] *Abbreviations: TRT: testosterone replacement therapy; ID-LC-MS/MS: isotopic dilution-liquid chromatography tandem mass spectrometry; T: testosterone; E1: estrone; E2: estradiol; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; SHBG: Sex Hormone-binding Globulin; cFT: calculated free testosterone; DXA: Dual-energy X-ray Absorptiometry; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; TAT: total adipose tissue.*

Figure 2. Bivariate linear regression between VAT and serum sex steroids: total testosterone **(a)**, calculated free testosterone **(b)**, estrone **(c)**, E2/T **(d)**. Dotted lines represent the 95% confidence interval.



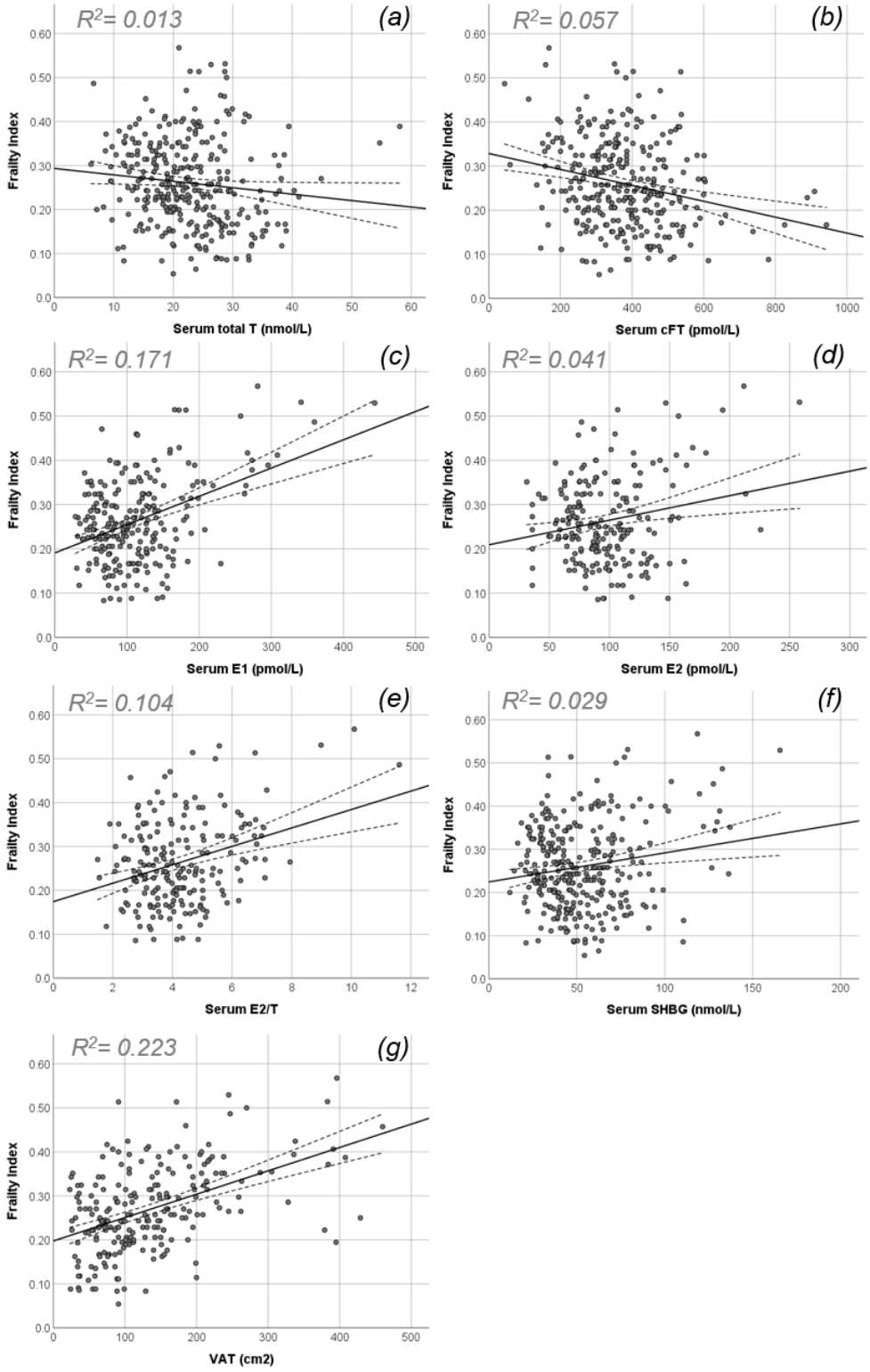
[Footnote to Figure 2] *Abbreviations: VAT: visceral adipose tissue; T: testosterone; E1: estrone; E2: estradiol; E2/T: estradiol to total testosterone ratio.*

Figure 3. Risk ratio (RR) of each NICMs in HIV-infected men with or without hypogonadism established on the basis of serum total T below 10.4 nmol/L (*a*), cFT below 220 pmol/L (*b*) or both (*c*). The diamond on the bottom indicates the cumulative RR of all NICMs in eugonadal and hypogonadal HIV-infected men that is increased in men with hypogonadism (p<0.001). 95% confidence intervals for each comorbidity are also illustrated.



[Footnote to Figure 3] *Abbreviations: NICMs: non-infectious comorbidities; CI: confidence interval; COPD: chronic obstructive pulmonary disease; BMD: bone mineral density; TT: serum total T; cFT: calculated free T.*

Figure 4. Bivariate linear regression between frailty index and serum sex steroids: total testosterone **(a)**, calculated free testosterone **(b)**, estrone **(c)**, estradiol **(d)**, estradiol to total testosterone ratio **(e)**, SHBG **(f)**, and VAT **(g)**. Dotted lines represent the 95% confidence interval.



[Footnote to Figure 4] *Abbreviations: T: testosterone; E1: estrone; E2: estradiol; E2/T: estradiol to total testosterone*

ratio; SHBG: Sex Hormone-binding Globulin; VAT: visceral adipose tissue.

Chapter 4

Primary, secondary and compensated male biochemical hypogonadism in people living with HIV (PLWH): relevance of sex hormone-binding globulin (SHBG) measurement and comparison between liquid chromatography-tandem mass spectrometry (LC-MS/MS) and chemiluminescent immunoassay for sex steroids assay

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ABSTRACT

Background: Data about classification of hypogonadism and estrogen deficiency in male people living with HIV (PLWH) are scanty.

Aim: To investigate the prevalence and characterization of biochemical hypogonadism and relative estrogen deficiency in male PLWH aged<50 comparing liquid chromatography-tandem mass spectrometry (LC-MS/MS) with chemiluminescent immunoassay (CI), and combining gonadotropin, sex hormone-binding globulin (SHBG) and serum estradiol (E2) measurements.

Methods: Prospective, cross-sectional, observational study. Serum total testosterone (TT), E2, gonadotropins, SHBG were measured by CI. TT and E2 were also assessed by LC-MS/MS. Free testosterone (cFT) was calculated by Vermeulen equation.

Results: A total of 316 PLWH (45.3±5.3 years) were enrolled. TT and cFT by LC-MS/MS were lower compared to CI ($p<0.0001$). The prevalence of biochemical hypogonadism was higher with LC-MS/MS than CI, both for TT (5.1% vs 3.2%, $p<0.0001$) or cFT (9.5% vs 7%, $p<0.0001$). The prevalence of hypogonadism (overt + compensated) was 17.1% for cFT using LC-MS/MS. Secondary form of hypogonadism was more prevalent than primary. The prevalence of relative estrogen deficiency was of 30.0% among hypogonadal patients and 15.5% among eugonadal.

Conclusions: The prevalence of male hypogonadism results underestimated by CI compared to LC-MS/MS in PLWH, both for TT and cFT. SHBG and gonadotropins are essential for detecting T deficiency.

Keywords: sex steroids, hypogonadism, testosterone, SHBG, HIV

INTRODUCTION

Hypogonadism is a frequent finding among young to middle-aged men with Human Immunodeficiency Virus (HIV) infection and seems to occur earlier in comparison with HIV-uninfected men (Rochira et al. 2011, Wong, Levy and Stephenson 2017, Rochira and Guaraldi 2014). Although the prevalence of male hypogonadism among people living with HIV (PLWH) has significantly lowered after the introduction of antiretroviral therapy (ART) (Santi et al. 2021), it remains high if compared with age-matched HIV-uninfected men, ranging from 13% to 40% in the age group of 20-60 years (Rochira and Guaraldi 2014, Lachâtre et al. 2017, Slama et al. 2016). Accordingly, in a recent meta-analysis we fixed the prevalence of male hypogonadism among PLWH to about 26% (Santi et al. 2021). Male hypogonadism in PLWH has multi-factorial nature and it is linked to the virus *per se*, to the therapeutic management, to HIV-related comorbidities and to changes in body fat distribution (Wong et al. 2017, Rochira and Guaraldi 2014, Rochira et al. 2011, Mirza, Luthra and Chirch 2018, De Vincentis et al. 2021a). Signs and symptoms of low serum testosterone (T) are non-specific, of mild-to-moderate degree, and often overlapping with those of infection *per se* in PLWH. For these reasons, hypogonadism can be underestimated in the absence of targeted laboratory blood examinations (Rochira et al. 2011, Rochira and Guaraldi 2014).

Immunoassay-based techniques are generally used in clinical practice for the assay of serum total T (TT), but they lack adequate accuracy and sensitivity in particular for values in the lowest normal range for men or below it (Rosner et al. 2007, Trost and Mulhall 2016, Wudy et al. 2018). Similarly, immunoassay-based techniques show poor accuracy also in the assessment of serum estradiol (E2), being quite unreliable especially at the low serum E2 concentration typical of men (Decaroli and Rochira 2017, Denver et al. 2019, Rochira and Carani 2017); for this reason, liquid chromatography tandem mass spectrometry (LC-MS/MS) represents the recommended methodology for the assessment of TT, E2 and, in general, of adrenal and sexual steroids (Trost and Mulhall 2016, Rochira and Carani 2017, Rosner et al. 2007, Adaway, Keevil and Owen 2015,

Fanelli et al. 2011, Decaroli and Rochira 2017, Simoni et al. 2012, Ohlsson et al. 2013b, Rosner et al. 2013, Demers et al. 2015, Handelsman et al. 2014). Moreover, serum T circulates mainly as protein-bound to sex hormone binding globulin (SHBG) and albumin, but only the small fraction of non-protein bound or free T (FT) is responsible for the biological activity of T (Rosner et al. 2007, Hammond, Wu and Simard 2012, Decaroli and Rochira 2017, Keevil and Adaway 2019). Abnormalities in SHBG levels can influence the serum TT reading (Laurent et al. 2016, Hammond et al. 2012), especially in systemic diseases associated to SHBG alterations (Rochira 2017). Thus, increased levels of SHBG that are typical of the HIV clinical setting may lead to normal levels of TT despite actually low FT levels, causing biochemical hypogonadism (Mirza et al. 2018, Rochira and Guaraldi 2014, Simoni et al. 2012). The importance of SHBG measurement in male PLWH has been largely emphasized by the guidelines on male hypogonadism of the Endocrine Society (Bhasin et al. 2018) as well as by recent reviews (Maffezzoni et al. 2020, Rochira and Guaraldi 2014), original studies (Pezzaioli et al. 2021b), and meta-analysis (Santi et al. 2021). At present, however, data on the prevalence of hypogonadism based on the combination of serum sex steroids measured by LC-MS/MS, SHBG and gonadotropins are lacking. In fact, the measurement of serum TT by the recommended methodology has been performed only in a few studies, and none of them considered also E2, SHBG, and gonadotropins levels in order to obtain a detailed and accurate evaluation of the pituitary-gonadal axis; this does not allow a precise esteem of prevalence and a full characterization of male hypogonadism (Monroe et al. 2014, Blick 2013, Slama et al. 2016). Furthermore, serum E2 levels in male PLWH have been evaluated by LC-MS/MS only in two studies, and neither aimed to investigate trends of serum E2 (Bedimo et al. 2016, De Vincentis et al. 2021a), thus leaving the issue of relative estrogen deficiency unexplored in this specific population.

The primary aim of this study was to investigate the prevalence of overt and subclinical male biochemical hypogonadism in a cohort of young to middle-aged PLWH. Gonadal status was evaluated not only by TT measurement but also by cFT levels calculated according to the validated Vermeulen formula (Vermeulen et al. 1999, Keevil and Adaway 2019). Thus, depending on

gonadotropins levels, hypogonadism was further classified into primary and secondary forms for a detailed characterization of the functionality of the whole hypothalamic-pituitary-gonadal axis. Therefore, secondary aim of this study was to compare the performance of LC-MS/MS technique and chemiluminescent immunoassay (CI) for serum TT assessment in HIV setting. Finally, another secondary aim was to investigate the prevalence of relative estrogen deficiency in our HIV cohort.

MATERIALS AND METHODS

Study design and participants

A prospective, cross-sectional, observational study was carried out involving male HIV-infected outpatients attending the Modena HIV Metabolic Clinic from May 2013 to December 2017. Three hundred nineteen consecutive male PLWH were assessed for suitability before enrolment in the study by the Unit of Endocrinology of the University of Modena and Reggio Emilia.

Inclusion and exclusion criteria have been previously detailed (De Vincentis et al. 2021a). Inclusion criteria were men aged 18-50 years, with documented HIV-infection and ongoing HAART treatment.

Exclusion criteria were prior treatment (referred or documented) with androgens, sex steroids, antiandrogens, anabolic agents, GnRH agonists, psychotropic agents; documented hypothyroidism, known pituitary, testicular or adrenal diseases, a previous conventional pituitary surgery or radiotherapy; documented Acquired Immunodeficiency Syndrome (AIDS), active cancer, severe liver insufficiency or severe chronic renal failure (estimated glomerular filtration rate <30 mL/min).

Only young to middle-aged (18-50 years) men were included to avoid the effects of aging on gonadal status and to exclude late onset hypogonadism (LOH) as a confounding factor (Corona, Rastrelli and Maggi 2013, Wu et al. 2008, Decaroli and Rochira 2017).

At enrolment visit each patient was evaluated for inclusion and exclusion criteria through medical interview and adequate clinical work-up. Finally, 316 male PLWH met all inclusion criteria and were enrolled in the study.

Main outcome measures

Laboratory analyses

After an overnight fasting an intravenous cannula was inserted into an antecubital vein at 8.00 am to collect a blood sample for serum and plasma assays. The blood samples were centrifuged, and the serum was stored at -20° C until assayed.

Serum TT (Fanelli et al. 2011), serum dihydrotestosterone and serum E2 (Mezzullo et al. 2020) were measured by two validated LC-MS/MS methods. Sensitivity was 19, 39.1 and 9.8 pg/mL, intra-assay coefficient of variation (CV) was <4%, <6% and <6%, inter-assay CV was <7%, <9% and <7% and accuracy ranged 97-100%, 81-112% and 92-108% for T, DHT and E2, respectively. T and E2 accuracy were verified through certified quality controls and multicenter comparison studies (Mezzullo et al. 2020, Fanelli et al. 2011, Büttler et al. 2015). Of the 316 enrolled patients, 71 participants were missing LC-MS/MS assay E2 data.

Serum TT and E2 were also assayed by CI assays: serum TT was measured by Architect 2nd Generation Testosterone (Abbott, USA), with an intra-assay CV < 10% and serum E2 was measured by Chemiluminescent Microparticle Immunoassay on the ARCHITECT platform (Abbott Laboratories). The sensitivity was 0.6 pg/mL with the lowest standard at 1.5 pg/mL, linearity to 150 pg/mL, and an ED50 of 20 pg/mL. The cross-reactivity with estrone and with less potent estrogens was less than 7 and 0.45%, respectively.

Serum SHBG was assayed by CI (Architect, Abbott GmbH &Co, Germany) with a sensitivity of 0.02 nmol/L. Inter- and intra-assay coefficients of variation of $\leq$10.0%, being lower in the mean normal range (around 5%) and higher for lowest and highest values (around 9%). SHBG levels were not available for 1 patient.

FT was calculated (cFT) by using the validated Vermeulen equation from both serum TT assessed with LC-MS/MS and serum TT assessed with CI (Vermeulen et al. 1999, Keevil and Adaway 2019).

According to the current Endocrine Society guidelines, diagnosis of biochemical hypogonadism was made when serum TT was below 320 ng/ml and/or cFT was below 64 pg/ml (Bhasin et al. 2018).

Serum LH and follicle stimulating hormone (FSH) were assayed by CI (Architect, Abbott GmbH & Co, Germany). The inter- and intra-assay CV were ≤10%.

Clinical parameters

At the time of the blood collection, demographic and anthropometric parameters (weight, height, body mass index [BMI]), together with the duration of HIV-infection and ART treatment and other risk factors and comorbidities (including the frailty index) were recorded, as previously described (De Vincentis et al. 2021a).

Ethics

The Institutional review board of Modena approved the protocol study (protocol n. 1446/15). This trial was registered in ClinicalTrials.gov (Identifier: NCT03747003).

Written informed consent has been obtained from each subject.

Statistical analysis

The non-parametric Mann-Whitney *U* test followed by the Dunn's multiple comparison *post hoc* test was used for comparisons of continuous variables since they resulted not normally distributed at the Kolmogorov-Smirnov test. Categorical variables were compared with Chi-square test.

Linear regression was used to examine the association between continuous variables; results were expressed through β and R^2 coefficients. Stepwise, linear, multiple regression analysis using a backward elimination method was applied to the data with $p<0.1$ as the criterion for a variable to enter the model.

The relationship and the agreement of the estimations between the two analytical techniques were investigated by calculating the concordance correlation coefficient (pc), the Passing and Bablok

regression (PB) (Passing and Bablok 1983) and by the Bland and Altman plot (BA) (Bland and Altman 1999), without exclusion of potential outliers. Comparisons were made assuming the LC-MS/MS assay as the reference method.

Statistical analyses were performed using the Statistical Package for the Social Sciences' (SPSS) software for Windows (version 26.0; SPSS Inc, Chicago, IL). Pc, PB, and BA analyses were performed using the MedCalc Software Version 15, 8 (© 1993–2015 MedCalc Software bvba, Belgium) (Bilić-Zulle 2011). For all comparisons, $p<0.05$ was considered statistically significant.

RESULTS

A total of 316 consecutive PLWH were enrolled, with a median age of 47 years (range 25.2-50.5 years) and a median duration of HIV-infection of 16.16 years (range 1.13-35.4 years). For comprehensive clinical characteristics of the 316 patients and hormonal outcomes, see our previous publication (De Vincentis et al. 2021a). Hormonal outcomes, grouped according to patients' gonadal status, are summarized in Table 1.

Comparison between CI and LC-MS/MS for T assessment

Serum TT and FT assessed with LC-MS/MS were directly related to serum TT (R^2=0.928, $p<0.0001$) and FT (R^2=0.896, $p<0.0001$) assessed with CI. For serum TT the PB regression did not exclude a linear relationship (p=0.380), notwithstanding proportional (slope: 1.173, 95% CI 1.138–1.213) and systematic (intercept: −35.995; 95% CI –58.657 to −15.948) errors were significantly present (Figure 1a). Similar results were obtained for cFT (slope: 1.226, 95% CI 1.179–1.276; intercept: −0.863; 95% CI –1.337 to −0.437) (Figure 1b).

However, an overestimation by CI was observed compared to LC-MS/MS. Both serum TT and cFT assessed by LC-MS/MS were significantly lower compared to serum TT (639 ng/ml vs 720 ng/ml, $p<0.0001$) and cFT (105.7 pg/ml vs 118.6 pg/ml, $p<0.0001$) assessed by CI. The BA plot

highlighted the presence of a significant mean difference between the two methods of 12.8% for TT (Figure 1c) and of 15.7% for cFT (Figure 1d).

In particular, 6 patients (1.9%) presented serum TT assessed by CI ≥ 320 ng/mL, but they resulted hypogonadal when serum TT was assessed by LC-MS/MS. Similarly, 9 patients (2.8%) presented cFT calculated from CI assessment ≥ 64 pg/mL, but they resulted hypogonadal when cFT was calculated from LC-MS/MS assessment.

Accordingly, by establishing LC-MS/MS as the reference method for TT assessment and the calculation of FT, CI presented high specificity for the diagnosis of hypogonadism considering both serum TT and cFT (100 % and 99.6%, respectively), but lacked high sensitivity considering both serum TT and cFT (62.5% and 70%, respectively) (Table 2).

Prevalence of hypogonadism

Prevalence of hypogonadism, comprised overt (primary and secondary) and compensated hypogonadism, according to serum TT or FT and laboratory assay, is reported in Table 3 and Figure 2. By using cFT on the basis of serum TT obtained by LC-MS/MS, the prevalence of hypogonadism (overt + compensated) was 17.1% (Table 3, Figure 2). The distribution of patients in relation to cFT and LH is represented in Figure 3. The prevalence of laboratory assessed (biochemical) hypogonadism was significantly higher when serum TT was measured by LC-MS/MS than by CI, both for hypogonadism defined considering serum TT (5.1% vs 3.2%, $p<0.0001$) and cFT (9.5% vs 7%, $p<0.0001$) (Table 3).

Prevalence of biochemical hypogonadism detected by cFT was higher compared to serum TT ($p<0.0001$), regardless of the hormonal assay used (Table 3).

Differences between eugonadal and hypogonadal patients

We classified patients in eugonadal and hypogonadal considering serum TT and/or cFT assessed by LC-MS/MS (Table 1). Comparing the 2 subgroups, hypogonadal patients were significantly older

(p=0.002) than eugonadal patients. Moreover, the duration of HIV infection was significantly longer in the hypogonadal subgroup (p<0.0001), as well as the duration of ART therapy (p<0.0001) (Table 1). Considering other hormonal parameters of the pituitary-gonadal axis, FSH levels (p=0.030) were significantly higher in the hypogonadal patients, while LH levels did not significantly differ between the 2 groups (p=0.057), although the mean levels were higher among hypogonadal patients; as expected, E2 was significantly lower in hypogonadal patients (p<0.0001) (Table 1). Finally, no significant differences were found for SHBG, DHT, and prolactin levels (Table 1).

Except for SHBG, similar differences were found also when patients were classified into eugonadism, primary hypogonadism, secondary hypogonadism and compensated hypogonadism on the basis of cFT (threshold 64 pg/mL) and serum LH (threshold 9 mIU/L) (Table 4).

Serum DHT did not change between hypogonadal and eugonadal PLWH (Table 1), also when different types of hypogonadism (primary, secondary, and compensated) were considered (Table 4).

Serum SHBG in HIV-infected patients

Serum SHBG did not differ between eugonadal and hypogonadal PLWH (Table 2). SHBG was significantly different among PLWH group according to different subtype of biochemical hypogonadism (compensated, primary, and secondary) (p<0.0001) (Table 4). At the post-hoc test, in fact, eugonadal PLWH had significanly lower levels of serum SHBG than PLWH with secondary hypogonadism (p=0.001), and PLWH with compensated hypogonadism (p=0.002); in addition, SHBG was higher in PLWH with secondary hypogonadism than in those with compensated hypogonadism (p<0.0001).

At linear regression analysis, serum SHBG was directly related to serum E2 (β=0.330, R^2=0.109; p<0.0001) (Figure 4a), serum TT (β=0.272, R^2=0.521; p<0.001) (Figure 4b), and serum LH

(β=0.223, R^2=0.050; p<0.001) (Figure 4c). In addition, also cFT resulted inversely related to LH (β=-0.242, R^2=0.058; p<0.0001) (Figure 4d).

The stepwise multivariate regression analysis showed that SHBG was associated with serum TT, viral liver co-infection and duration of HIV according to the following 3 different models: ***model 1***: only serum TT enter the model as a predictive variable (β= 0.527, R^2:0.278, p<0.0001); ***model 2***: serum TT (β=0.493) and viral liver co-infection (β=0.351) entered the model (R^2:0.400, p<0.0001); ***model 3***: serum TT (β=0.518), viral liver co-infection (β=0.286), and duration of HIV (β=0.159) entered the model (β=, R^2:0.421, p<0.0001). On the other hand, serum E2, LH, BMI, total alkaline phosphatase, smoke, alcohol and frailty index did not enter any model.

Serum E2 trends according to CI and LC-MS/MS

Serum E2 was significantly lower in hypogonadal than eugonadal male PLWH when assayed by CI, while did not differ when it was measured by LC-MS/MS (Table 1). The same results were obtained when different types of hypogonadism (primary, secondary, and compensated) were considered (Table 4).

Serum E2 assessed by LC-MS/MS was available for 204 patients out of 316. According to the lower reference limit of 12.4 pg/mL estimated with theranges of LC-MS/MS assay in a cohort of healthy unmedicated men, 10 patients (4.9%) elevated serum E2. According to the reference range of CI assay (n.v. < 50 pg/ml), 292 patients (92.7%) had normal serum E2 and 23 patients (7.3 %) had serum E2 above the upper limit of normal range.

The prevalence of relative estrogen deficiency, defined as serum E2 below 18 pg/ml, was of 8.5% (27 patients out of 316) with CI and of 16.1% (33 patients out of 204) with LC-MS/MS.

Among hypogonadal PLWH, 8 out of 30 patients had relative estrogen deficiency, defined as serum E2 measured by CI below18 pg/ml, with a prevalence of 26.7%, while 19 out of 286 eugondal PLWH had relative estrogen deficiency with a prevalence of 6.6%.

Among PLWH with serum E2 assessed by LC-MS/MS available, 3 out of 10 hypogonadal patients had relative estrogen deficiency, defined as serum E2 measured by LC-MS/MS below18 pg/ml, with a prevalence of 30.0%, while 30 out of 194 eugonadal PLWH had relative estrogen deficiency with a prevalence of 15.5%.

Serum E2 was significantly related to serum TT ($p<0.0001$, beta=0.461, $R^2=0.213$), cFT ($p=0.002$, beta=0.219, $R^2=0.048$) by LC-MS/MS, and SHBG ($p<0.0001$, beta=0.346, $R^2=0.120$).

Serum E2 assessed with LC-MS/MS was directly related to E2 assessed with CI ($R^2=0.498$, $p<0.0001$). Accordingly, the PB regression did not exclude a linear relationship ($p=0.500$), notwithstanding proportional (slope: 1.237, 95% CI 1.102–1.391) and systematic (intercept: 2.814; 95% CI –1.617 to 6.732) errors were significantly present (Figure 5a). As expected, E2 assessed by LC-MS/MS was significantly lower compared to CI (24.7 pg/ml vs 31 pg/ml, $p<0.0001$). The BA plot highlighted the presence of a significant mean difference between the two methods of 42.4% as well as a trend for increasing overestimation by the CI at lowering E2 levels (Figure 5b).

DISCUSSION

This study demonstrates that the measurement of serum TT by LC-MS/MS coupled with SHBG improves biochemical hypogonadism diagnosis in male PLWH. Besides, these results confirm that SHBG is a key diagnostic tool useful to identify biochemical hypogonadism in the context of HIV since prevalence of hypogonadism is about 1.9-fold increased using serum cFT rather than TT, regardless of the method used for TT measurement (CI or LC-MS/MS). This is in line with literature showing that cFT raises on average of 1.7 times the prevalence of biochemical hypogonadism in male PLWH (Pezzaioli et al. 2021b, Santi et al. 2021, Monroe et al. 2014, Slama et al. 2016) and fit with the evidence of significantly lower cFT in PLWH than HIV-uninfected men (Monroe et al. 2014, Slama et al. 2016), despite no difference in serum TT assessed by LC-MS/MS (Monroe et al. 2014). The importance of SHBG measurement in PLWH has been already largely emphasized (Pezzaioli et al. 2021b, Bhasin et al. 2018), but it has never been explored in

combination with gonadotropins and serum TT measured by LC-MS/MS (Monroe et al. 2014, Blick 2013, Slama et al. 2016). Serum SHBG is known to be altered in HIV infection and may interfere with the amount of unbound circulating sex steroids (Rochira and Guaraldi 2014). As in other studies (Pezzaioli et al. 2021b), the SHBG increase found in our study highly influences serum TT and E2 since it leads to compensatory LH increase in order to leave unchanged the unbound quote of circulating sex steroids as in compensated hypogonadism. Accordingly, SHBG was significantly higher in PLWH with secondary and compensated hypogonadism compared to eugonadal PLWH and was directly correlated with serum LH. In PLWH with secondary hypogonadism, SHBG was significantly higher than in those with compensated hypogonadism indicating that the hypothalamic-pituitary-gonadal axis was no more able to increase LH production for counteract T deficiency. In this study, serum TT, viral liver co-infection and duration of HIV infection resulted to be stronger predictor of the SHBG increase confirming that liver dysfunction and HIV infection may alter SHBG in these patients (Rochira and Guaraldi 2014, Quiros-Roldan et al. 2021).

The prevalence of overt biochemical hypogonadism in our cohort is higher with LC-MS/MS than with CI, both for serum TT (5.1% vs 3.2%) and cFT (9.5% vs 7.0%), thus confirming the superiority of LC-MS/MS over CI in unraveling mild forms of biochemical hypogonadism in HIV (Slama et al. 2016, Monroe et al. 2014) as in men without HIV infection (Fanelli et al. 2011, Bhasin et al. 2018). Serum TT in young to middle-aged male PLWH has been measured by LC-MS/MS only in two studies, the first showing a percentage of hypogonadism (24% for serum TT and 19% for cFT with cut-offs of <300 ng/ml and <50 pg/ml, respectively) higher than our study) (Blick 2013), the second showing a similar prevalence (9.3%) based on the cut-off of cFT <50 pg/ml (Monroe et al. 2014).

The novelty of this study lies in the comprehensive evaluation of pituitary-gonadal axis in PLWH <50 years based on the measurement of serum TT by LC-MS/MS coupled with the measurement of both gonadotropins and SHBG, which allows classifying biochemical hypogonadism as: secondary (6.0%), primary (3.5%), and compensated (7.6%), with hypogonadotropic hypogonadism as the

most common form of overt hypogonadism as previously described (Rochira et al. 2011, Mirza et al. 2018, Wong et al. 2017, Rochira and Guaraldi 2014, Dutta et al. 2017, Gomes et al. 2016, Pezzaioli et al. 2021a). Moreover, the pre-clinical condition of compensated hypogonadism, defined as normal serum T together with increased LH (Tajar et al. 2010), seems to occur as frequently as secondary hypogonadism in PLWH (Rochira et al. 2011, Rochira and Guaraldi 2014), similarly to what happens in older men (9.5% in men 40-79 years) where it is common and is considered a precursor of overt primary hypogonadism (Tajar et al. 2010, Rochira et al. 2011). Taken together overt and compensated forms biochemical hypogonadism is 17.1% in this study, confirming our (Rochira et al. 2011) and other authors' (Dutta et al. 2017, Pezzaioli et al. 2021b) previous results. With the exception of a recent study showing a prevalence of hypogonadism (4.4% with a TT cut-off of 348 ng/ml; 12.4% with a cFT cut-off of 70 pg/ml) similar to this study in PLWH <50 years (Lachâtre et al. 2017) the prevalence of male hypogonadism in PLWH in the ART era is highly heterogeneous, ranging from 13% up to 40% (Rochira and Guaraldi 2014, Santi et al. 2021), being of 33% on average, as settled by our recent meta-analysis (Santi et al. 2021). This heterogeneity is due to differences among studies in terms of serum TT assays, use of cFT or TT, use of different cut-offs, and mean age of patients (Santi et al. 2021, Rochira and Guaraldi 2014). Overall, it seems that the prevalence of male hypogonadism among PLWH has been decreasing in recent years thanks to improvement of their management and general health status (Santi et al. 2021), although the finding of compensated hypogonadism remains quite common in these patients. The low prevalence found in this study is close to that described among HIV-uninfected men of similar age groups (Vermeulen and Kaufman 2002, Araujo et al. 2004, Harman et al. 2001) and is in line with the above-mentioned decrease in last years. This low prevalence could be due to the younger age of our patients (inclusion criterion <50 years), to underestimation caused by exclusion of all patients of our cohort with previous or ongoing TRT(ruling out patients already diagnosed with overt hypogonadism), to the fact that almost all of them belong to HIV-cohorts that have beneficiated of last generation ART regimens (they have been enrolled since 2013) and infection contracted in

recent years (i.e. 7.7% of patients with length of disease <54 months) that avoided the exposure to older, more toxic drugs. Thus, the infection has had little time to determine its effects and complications including those on the hypothalamus-pituitary-gonadal axis (Rochira and Guaraldi 2014). There is growing evidence suggesting that male hypogonadotropic hypogonadism could be induced by an unhealthy status among PLWH as an adaptive mechanism to spare energy (Decaroli and Rochira 2017, De Vincentis et al. 2021a, Rochira et al. 2015a), similarly to aging men (Rochira and Guaraldi 2014, Corona et al. 2020, Rochira 2017). We have recently described the possible functional nature of hypogonadism in HIV, where low T could be considered more an epiphenomenon and a biomarker of poor health rather than a form of true clinical hypogonadism (Rochira et al. 2015a, De Vincentis et al. 2021a). Patients with a recent diagnosis of HIV, in fact, experience a better general health status and a lower incidence of comorbidities (Crane, Van Rompaey and Kitahata 2007, Guaraldi et al. 2014, Lagathu et al. 2019, Gelpi et al. 2019). To the best of our knowledge, no previous study has compared serum E2 measured with CI and LC-MS/MS in a cohort of male PLWH. Serum E2 measured with LC-MS/MS resulted significantly lower than with CI, thus confirming that immunoassays are not reliable in PLWH as well as in male population for the measurement of serum E2 in the male range (Ohlsson et al. 2013a, Demers et al. 2015, Rochira and Carani 2017), LC-MS/MS being the only reliable assay in men including PLWH (Adaway et al. 2015, Fanelli et al. 2011, Simoni et al. 2012, Ohlsson et al. 2013b, Rosner et al. 2013, Demers et al. 2015, Handelsman et al. 2014). Serum E2 measured by LC-MS/MS did not differ between hypogonadal and eugonadal PLWH, as in a recent study (Bedimo et al. 2016), but relative estrogen deficiency was almost frequent in PLWH with hypogonadism suggesting that when testosterone decreases serum E2 drops too even in PLWH (Decaroli and Rochira 2017, Rochira and Carani 2017), notwithstanding increased visceral adiposity and HIV lipodystrophy and related aromatase over-expression in adipose tissue (De Vincentis et al. 2021a, Santi et al. 2016, Rochira, Kara and Carani 2015b). Relative estrogen deficiency may contribute worsening some health conditions already common in male PLWH, such as osteoporosis, fat redistribution,

dyslipidaemia, and glucose metabolism alterations (Russell and Grossmann 2019, Rochira and Carani 2017).

This study has several strengths and limitations. Considering strengths, this is a properly-designed, prospective cohort study performed by using the reference method LC-MS/MS for serum TT measurement in association with the assessment of SHBG and gonadotropins, thus it provides a comprehensive view of the hypothalamus-pituitary-gonadal axis of male PLWH. In addition, the large recruitment of only young to middle-aged patients allowed us to avoid the effects of physiological aging on gonadal status and the possible forms of LOH. As regards to limitations, morning serum TT has been determined on a single blood sample rather than in two separate mornings as guidelines describe (Bhasin et al. 2018), however it was assessed by using two different methodologies, including the recommended LC-MS/MS. Moreover, this study lacks a specific control group of age-matched PLWH, but the prevalence of biochemical hypogonadism in HIV-uninfected men has been widely explored in literature allowing indirect comparison (Tajar et al. 2010, Vermeulen and Kaufman 2002, Araujo et al. 2004, Harman et al. 2001). Again, these data refer only to biochemical hypogonadism, not to a clinical diagnosis of the disease.In clinical practice well-validated immunoassays methods are adequate and sufficient for a baseline evaluation of serum TT and cFT, as supported by the good correlation between CI and LC-MS/MS in this study and overall acceptable sensitivity of commercially available CI (Simoni et al. 2012, Taylor, Keevil and Huhtaniemi 2015); this is not the case, however, for serum E2 (Rochira and Carani 2017). Mass spectrometric methods are superior and are becoming more available in clinical laboratories; thus, male gonadal function assessment will improve in the near future (Simoni et al. 2012, Taylor et al. 2015, Wudy et al. 2018, Denver et al. 2019). Our results suggest that SHBG should be considered mandatory in the clinical work-up for the diagnosis of male hypogonadism in PLWH in order to calculate cFT, independently from the methodology used for serum TT determination (Bhasin et al. 2018, Simoni et al. 2012, Rochira and Guaraldi 2014, Decaroli and Rochira 2017, Rosner et al. 2007), thus reinforcing available advice from Endocrine Society

guidelines and other studies (Lachâtre et al. 2017, Bhasin et al. 2018, Rochira and Guaraldi 2014, Pezzaioli et al. 2021b, Maffezzoni et al. 2020). Also serum gonadotropins are mandatory since they allow correctly classifying the type of hypogonadism and refining the diagnosis. At present, gonadotropins remain poorly considered in HIV research, especially in studies not designed by endocrinologists or experts in the field of hypogonadism (Rochira and Guaraldi 2014), thus preventing a wide and comprehensive use of gonadotropins in infectious diseases specialists' clinical practice for the diagnosis of male hypogonadism. This study suggests that clinicians should maintain close monitoring of gonadal function overtime in patients with compensated hypogonadism, a condition that is known to precede primary overt gonadal failure as also advised by other authors (Pezzaioli et al. 2021b). Finally, it should be remarked that the clinical diagnosis of hypogonadism cannot be based only on the finding of biochemical hypogonadism but needs to be coupled with signs and symptoms of hypogonadism (Pezzaioli et al. 2021b, Rochira and Guaraldi 2014, Rochira et al. 2011) as in HIV-uninfected men (Wu et al. 2010, Corona et al. 2020). However, coupling biochemical hypogonadism with clinical signs and symptoms remains challenging in male PLWH (Rochira and Guaraldi 2014) due to the high prevalence of some symptoms and signs such as bone loss and erectile dysfunction both in PLWH with and without hypogonadism (Pezzaioli et al. 2021b, Pezzaioli et al. 2021a, Santi et al. 2016, Rochira et al. 2011).

In conclusion, LC-MS/MS methodology is the best-performance methodology compared to CI in detecting the presence of serum T deficiency in young to middle-aged PLWH. SHBG levels should be assessed in PLWH allowing the calculation of cFT to not underdiagnose biochemical hypogonadism.

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"Gonadal Function in Human Immunodeficiency Virus (HIV)-Infected Men Assessed by Isotopic Dilution-Liquid Chromatography-Tandem Mass Spectrometry (ID-LC-MS/MS) and Chemiluminescent Immunoassay". Abstract has been selected for the "Endocrine Society Outstanding Abstract Award" in the amount of $750 and it has been published in the Journal of the Endocrine Society, Volume 3, Issue Supplement_1, April-May 2019, OR18-3, https://doi.org/10.1210/js.2019-OR18-3.

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Table 1. Differences between eugonadal and hypogonadal PLWH considering serum TT and/or cFT assessed by LC-MS/MS. Data are expressed as median (minimum-maximum).

	n.v.	**Eugonadal**	**Hypogonadal**	**p-value**
n 316		**281 (88.9%)**	**35 (11.1%)**	
Clinical characteristics				
Age (years)	-	46.75 (25.22-50.47)	49.63 (38.39-50.19)	**0.002**
BMI (Kg/m^2)	18.5-25	23.59 (16.33-38.74)	23.71 (18.29-36.52)	0.291
Years of HIV	-	14.64 (1.13-35.40)	22.85 (4.60-33.95)	**<0.0001**
ART Years	-	12.86 (0.00-33.78)	17.88 (1.94-30.78)	**<0.0001**
Hormonal measurements by CI (Architect 2nd generation, Abbott)				
LH (mIU/mL)	1-9	4.7 (1.1-43.0)	6.0 (0.0-35.6)	0.057
FSH (mIU/mL)	1-12	5.5 (1.2-42.0)	7.1 (0.0-58.5)	**0.030**
PRL (ng/mL)	2.1-17.7	7.4 (1.9-62.0)	7.4 (2.7-62.4)	0.304
TT (ng/dL)	>320	740.0 (350.0-1870.0)	410.0 (170.0-850.0)	<**0.0001**
cFT (pg/mL)	>64	125.6 (66.0-338.3)	59.8 (15.4-93.2)	<**0.0001**
E2 (pg/mL)	<50	32.0 (2.9-92.0)	25.5 (1.0-76.0)	**0.001**
SHBG (nmol/L)	13.5-71.4	47.3 (14.4-161.0)	60.5 (11.9-165.5)	0.160
Hormonal measurements by LC-MS/MS				
TT (ng/dL)	>320	662.65 (335.0-	339.45 (178.3-	<**0.0001**
cFT (pg/mL)	>64	111.0 (64.3-271.9)	54.6 (12.7-73.2)	<**0.0001**
E2 (pg/mL)	12.4-43.5	25.1 (8.5-70.3)	20.1 (9.8-57.7)	0.137
DHT (pg/mL)	165-679	382 (39-1804)	304 (39-672)	0.064

[Footnote to Table 1] *Abbreviations: PLWH: people living with HIV; n.v.: normal values; BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; ART: Antiretroviral Therapy; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; PRL: Prolactin; E2: Estradiol; TT: Total Testosterone; SHBG: Sex Hormone-Binding Globulin; cFT: Calculated Free Testosterone; CI: Chemiluminescent Immunoassay;* LC-MS/MS: *Liquid Chromatography Tandem Mass Spectrometry.*

Table 2. Diagnostic value of different methods used for the measurement of serum TT and cFT, taken LC-MS/MS as the reference method.

	Cut-off	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
TT (CI)	320 ng/mL	62.5	100	100	98	98.1
cFT (CI)	64 pg/mL	70	99.6	95.4	99.6	96.8

[Footnote to Table 2] *Abbreviations: TT: Total Testosterone; cFT: Calculated Free Testosterone; CI: Chemiluminescent Immunoassay;* LC-MS/MS: *Liquid Chromatography Tandem Mass Spectrometry; PPV: Positive Predictive Value; NPV: Negative Predictive Value.*

Table 3. Prevalence (expressed as number and percentage) of eugonadism, compensated hypogonadism, primary hypogonadism and secondary hypogonadism according to serum TT assessed by CI, serum TT assessed by LC-MS/MS, FT calculated by using TT assessed by CI and FT calculated by using TT assessed by LC-MS/MS.

	TT n (%)		**cFT n (%)**	
	CI	**LC-MS/MS**	**CI**	**LC-MS/MS**
Eugonadism	274 (86.7%)	268 (84.8%)	266 (84.4%)	261 (82.9%)
Impaired function of HPG axis	42 (13.3%)	48 (15.2%)	49 (15.6%)	54 (17.1%)
Compensated Hypogonadism	32 (10.1%)	32 (10.1%)	27 (8.6%)	24 (7.6%)
Overt Hypogonadism	10 (3.2%)*	16 (5.1%)*	22 (7.0%)**	30 (9.5%)**
- *Primary Hypogonadism*	3 (1.0%)	3 (1.0%)	8 (2.5%)	11 (3.5%)
- *Secondary Hypogonadism*	7 (2.2%)	13 (4.1%)	14 (4.4%)	19 (6.0%)

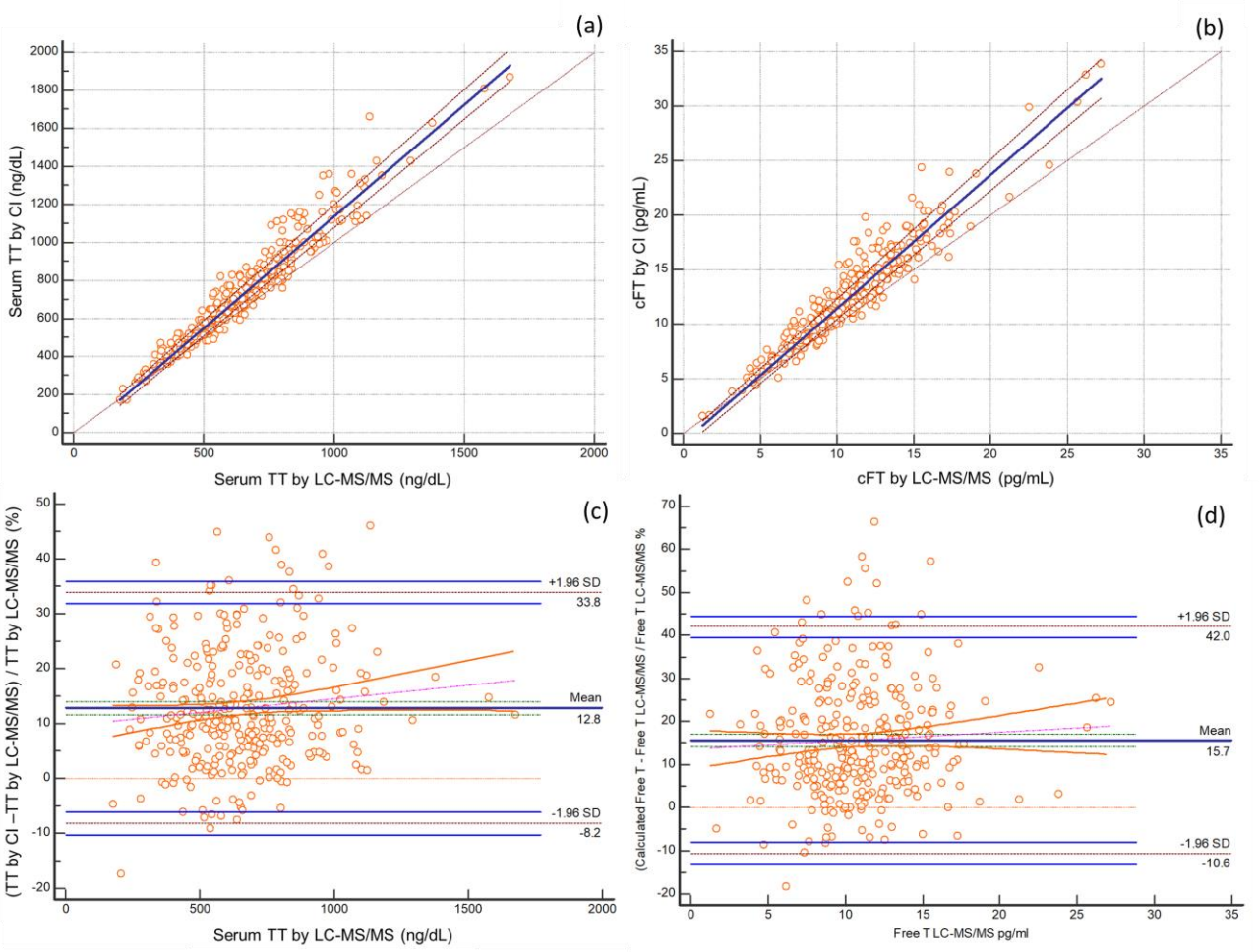
[Footnote to Table 3] *Abbreviations: TT: Total Testosterone; CI: Chemiluminescent Immunoassay;* LC-MS/MS: *Liquid Chromatography Tandem Mass Spectrometry; cFT: Calculated Free Testosterone; HPG: Hypothalamic-Pituitary-Gonadal. *p<0.0001; **p<0.0001.*

Table 4. Characteristics according to cFT in tandem with serum LH. Data are expressed as median (minimum-maximum).

	cFT ≥64 pg/mL		**cFT <64 pg/mL**		
	E	**CH**	**SH**	**PH**	
n 316	**261 (82.9%)**	**24 (7.6%)**	**19 (6.0%)**	**11 (3.5%)**	
Clinical characteristics					**p-value**
Age (years)	46.7 (25.2-50.5)	48.2 (36.5-50.3)	49.5 (38.6-50.0)	49.6 (43.7-50.0)	**0.004**
BMI (Kg/m^2)	23.6 (16.9-38.7)	22.8 (16.3-32.1)	25.8 (18.3-36.5)	22.8 (20.5-34.3)	0.210
Years of HIV	14.1 (1.1-35.4)	18.8 (3.4-34.0)	25.9 (10.2-34.0)	22.4 (15.5 (26.5)	**<0.0001**
ART Years	11.9 (0.0-33.8)	16.8 (1.3-29.9)	17.8 (1.9-30.5)	18.5 (13.4-24.5)	**0.001**
Hormonal measurements by CI (Architect 2nd generation, Abbott)					
LH (mIU/mL)	4.5 (1.1-9.0)	11.1 (9.1-43.0)	4.2 (0.0-8.6)	16.4 (11.6-35.6)	**<0.0001**
FSH (mIU/mL)	5.2 (1.2-37.9)	10.5 (3.1-42.0)	5.4 (0.0-12.3)	18.9 (6.6-58.5)	**<0.0001**
PRL (ng/mL)	7.4 (1.9-62.0)	6.6 (3.7-10.6)	8.1 (2.8-62.4)	6.7 (3.2-20.7)	0.355
TT (ng/dL)	730 (270-1810)	755 (410-1870)	440 (170-780)	440 (170-850)	**<0.0001**
cFT (pg/mL)	126.1 (63.2-	111.8 (80.7-	56.4 (15.4-70.4)	59.0 (16.2-76.7)	**<0.0001**
E2 (pg/mL)	32.0 (2.9-92.0)	31.5 (10.0-59.0)	26.0 (1.0-76.0)	22.0 (1.0-34.0)	**0.010**
SHBG (nmol/L)	46.5 (14.4-161.0)	62.1 (22.7-131.2)	75.5 (11.9	65.0 (31.0-129.7)	**<0.0001**
Hormonal measurements by LC-MS/MS					
TT (ng/dL)	655 (277-1578)	717 (392-1675)	399 (191-760)	342 (178-767)	**<0.0001**
cFT (pg/mL)	111.0 (64.3-	101.2 (65.9-	50.0 (12.7-62.0)	45.3 (17.1-58.3)	**<0.0001**
E2 (pg/mL)	25.1 (8.5-70.3)	24.3 (11.2-49.1)	20.3(9.8-57.7)	-	0.364
DHT (pg/mL)	376 (39-1804)	420 (196-893)	371 (39-672)	287 (94-416)	0.220

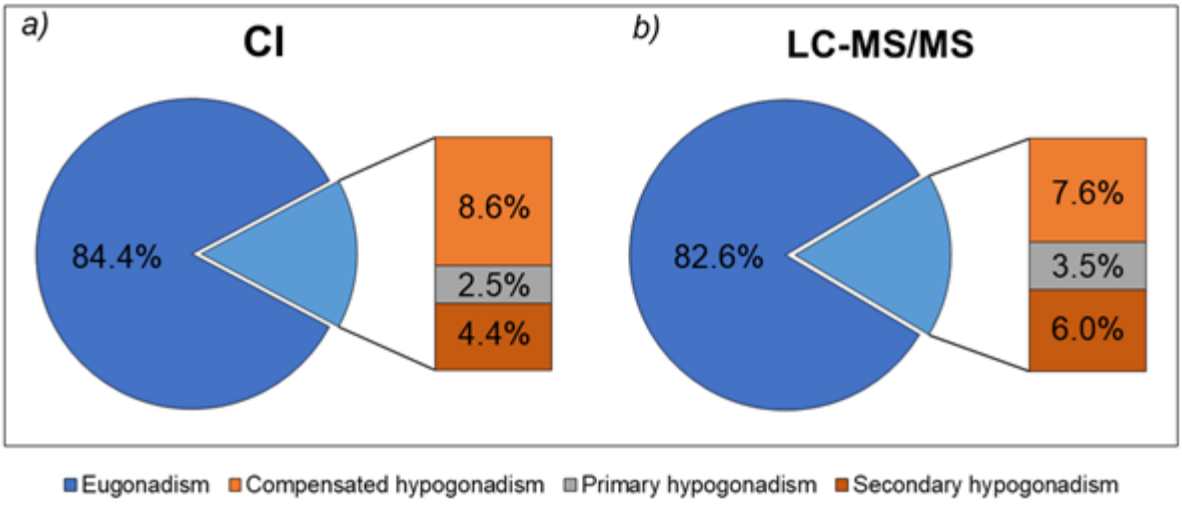
[Footnote to Table 4] *Abbreviations: BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; ART: Antiretroviral Therapy; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; PRL: Prolactin; E2: Estradiol; TT: Total Testosterone; SHBG: Sex Hormone-Binding Globulin; cFT: Calculated Free Testosterone; CI: chemiluminescent Immunoassay;* LC-MS/MS: *Liquid Chromatography Tandem Mass Spectrometry.*

Figure 1 Caption. Passing and Bablok regression between the CI and LC-MS/MS assays for serum TT **(a)** and cFT **(b);** Bland–Altman plot of serum TT **(c**) and cFT**(d).**



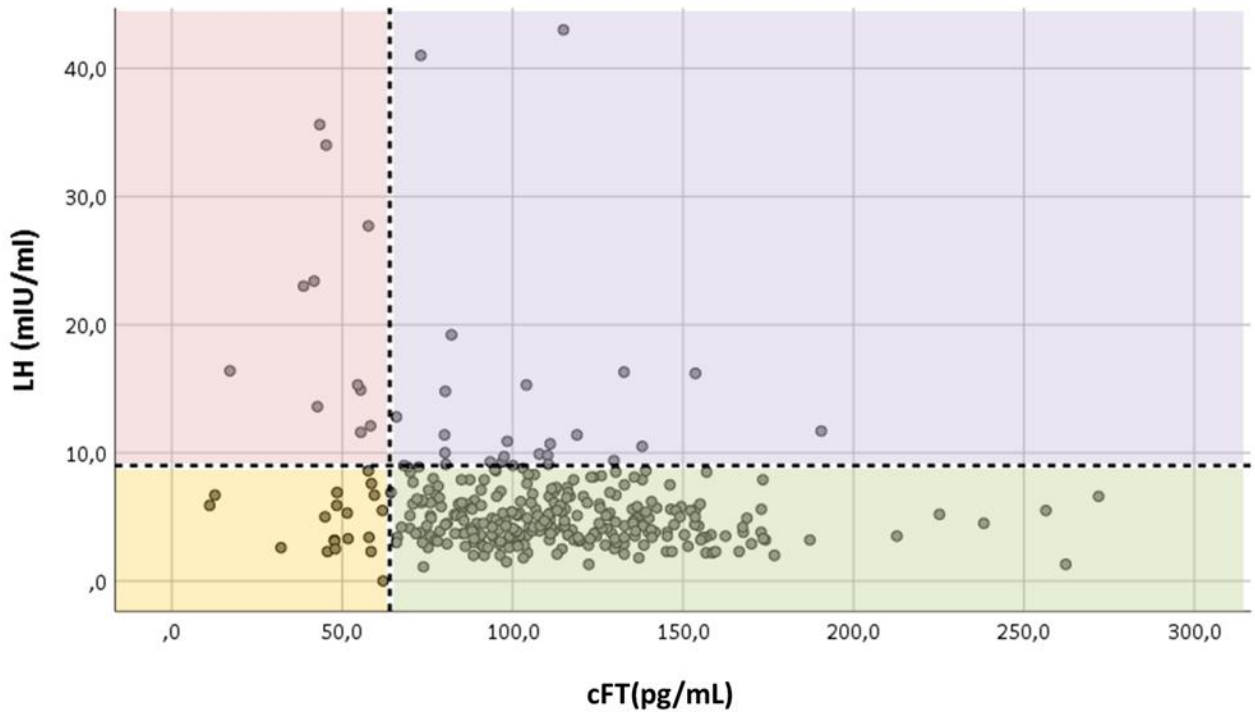
[Footnote to Figure 1] *Abbreviations: TT: total testosterone; CI: Chemiluminescent Immunoassay; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; cFT: calculated Free Testosterone.*

Figure 2 Caption. Prevalence of each category of gonadal status according to cFT levels calculated by using serum TT assessed by CI **(a)** and by LC-MS/MS **(b)**.



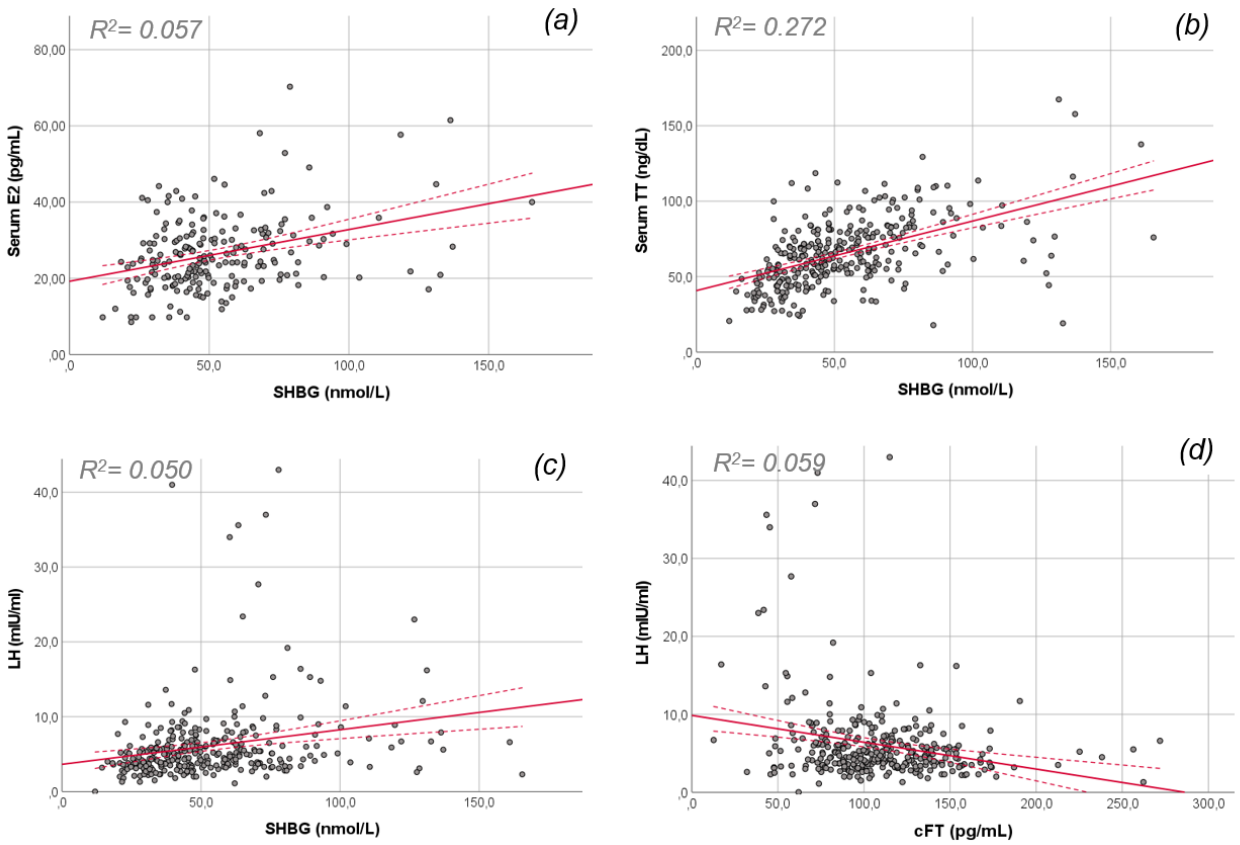
[Footnote to Figure 2] *Abbreviations: cFT: calculated Free Testosterone; TT: Total Testosterone; CI: Chemiluminescent Immunoassay; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry.*

Figure 3 Caption. Gonadal status of the entire cohort of PLWH according to cFT calculated from LC-MS/MS and serum LH levels. Green: eugonadism; Purple: compensated hypogonadism; Yellow: secondary hypogonadism; Pink: primary hypogonadism.



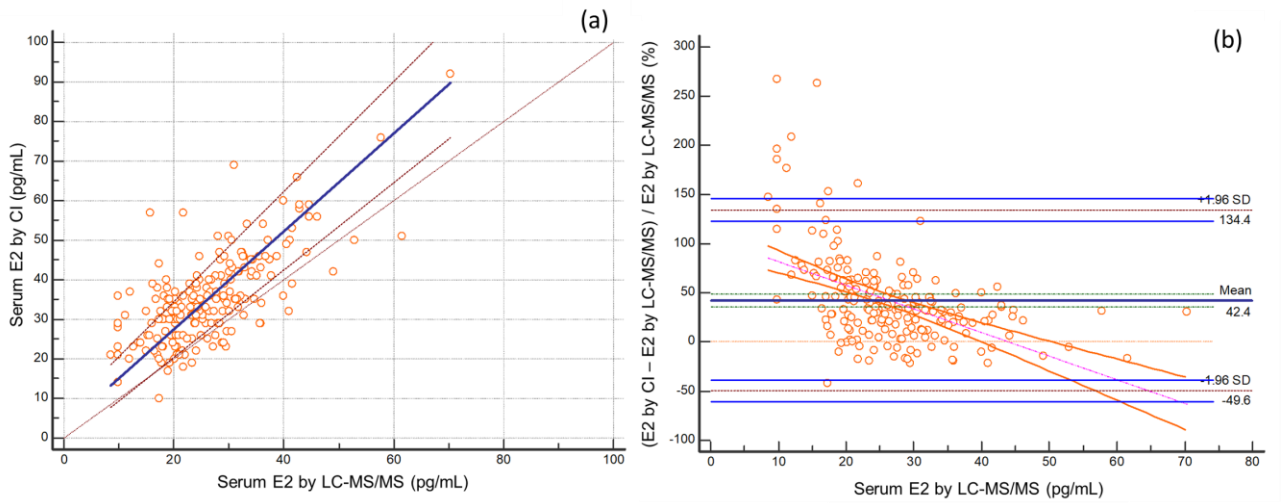
[Footnote to Figure 3] *Abbreviations: PLWH: people living with HIV; cFT: calculated Free Testosterone; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry, LH: Luteinizing Hormone.*

Figure 4 Caption. Linear regression between serum SHBG and serum E2 **(a)**, serum TT **(b)**, and serum LH **(c)**. In figure **4d** is reported the correlation between cFT and serum LH.



[Footnote to Figure 4] *SHBG: Sex Hormone Binding Globulin; E2: estradiol; TT: Total Testosterone; LH: luteinizing hormone; cFT: calculated free Testosterone.*

Figure 5 Caption. Passing and Bablok regression **(a)** and Bland–Altman plot **(b)** between the CI and LC-MS/MS assay for serum E2**.**



[Footnote to Figure 5] *Abbreviations: CI: Chemiluminescent Immunoassay; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; E2: estradiol.*

Chapter 5

Determinants of sexual function in men living with HIV younger than 50 years old: focus on organic, relational, and psychological issues

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ABSTRACT

Background: Sexual dysfunctions, particularly erectile dysfunction (ED), are common in men living with HIV (MLWH), whose organic and psychological components remain to be clarified. The aim of the study is to investigate the impact of risk factors of sexual dysfunctions, including organic, relational and psychological determinants of erectile function, in MLWH younger than 50 years old.

Methods: A cross-sectional, observational study was conducted in MLWH <50 years. The questionnaire International Index of Erectile Function (IIEF)-15 was used to assess prevalence and degree of ED. The Structured Interview of Erectile Dysfunction (SIEDY) was used to explore the organic (Scale1), relational (Scale2) and psychological (Scale3) components of ED. Total testosterone (TT), estradiol (E2) and dihydrotestosterone (DHT) were measured by liquid chromatography-tandem-mass spectrometry (LC-MS/MS); free testosterone (cFT) was calculated by Vermeulen equation.

Results: A total of 313 consecutive MLWH were prospectively enrolled (median age 47.0 years; median HIV-infection duration 16.2 years). 187 patients (59.7%) had ED, with higher prevalence non-heterosexual (138 out of 187, 73.8%) than heterosexual patients (p=0.003). Patients with ED showed a worse score of SIEDY scale 3 compared to patients without ED (p=0.025); IIEF-15 was inversely related to SIEDY scale 3 (p=0.042). No difference was found for sex steroids (TT, cFT, E2, DHT) between MLWH with and without ED. At the multivariate analysis sexual orientation, lack of stable relationship were major determinants for ED. Only 35 of 187 patients with ED (18.7%) reported the use of ED medications.

Conclusions: Within the multidimensional network of ED in MLWH, the psychological component is predominant, highlighting the contribution of peculiar factors related to HIV distress (e.g., fear of virus transmission, stigma) rather than gonadal status and other classical risk factors. In contrast to

the high prevalence, only few patients reported the use of ED medications suggesting a general under-management of such issues.

INTRODUCTION

Sexual dysfunction, consisting mainly of low sexual desire, erectile dysfunction (ED), is common in men living with HIV (MLWH) (Lamba et al. 2004, Santi et al. 2014, De Vincentis et al. 2021b). Since sexual health is a primary component of well-being (Laumann, Paik and Rosen 1999, World Health Organization 2002), it might significantly contribute to suboptimal quality of life (Andersson et al. 2020).

ED is a multidimensional condition defined as the inability to achieve or maintain a penile erection to be adequate enough for a satisfying sexual performance (Rochira, Carani and Granata 2022). The prevalence of overt ED in MLWH is 13-86% (De Vincentis et al. 2021b), higher to age-matched general population (Lamba et al. 2004, Zona et al. 2012, Santi et al. 2014, Dijkstra et al. 2018), reaching up to 70% in men over 70 years old and being uncommon before 50 years old (Corona et al. 2010, Feldman et al. 1994). In addition to traditional risk factors for ED such as age, lifestyle, neurological, cardiovascular, and endocrine diseases (i.e. hypogonadism) (Yafi et al. 2016, Rochira et al. 2022), several HIV-related factors play a role in the pathogenesis of ED in MLWH (Luo et al. 2017, Santi et al. 2014, De Vincentis et al. 2021b, Guaraldi et al. 2012), including HIV *per se* (Zona et al. 2012, Dijkstra et al. 2018), duration of HIV infection and antiretroviral therapy (ART)(Romero-Velez et al. 2014, Colson et al. 2002, Moreno-Pérez et al. 2010). Additionally, earlier onset of comorbidities, premature aging and frailty in MLWH may also be related to ED (Romero-Velez et al. 2014, Santi et al. 2014, De Vincentis et al. 2021b, Colson et al. 2002, Moreno-Pérez et al. 2010, Guaraldi et al. 2012).

In MLWH psychological domains deal with the fear of HIV transmission during sexual activity, social and cultural aspects of men who have sex with men (MSM), the stigma of the disease and the low satisfaction of body image (Rendina et al. 2012, Bourne et al. 2012, De Ryck et al. 2012,

Guaraldi et al. 2012, Baker, Lea and Lavakumar 2020). Nevertheless, the contribution of different risk factors to ED in MLWH and their relation with different ED components (organic, relational, psychogenic) have not been extensively investigated so far (Luo et al. 2017, Huntingdon et al. 2020).

In a physiological perspective erectile function, sexual desire, and morning erections are androgen-dependent (Granata et al. 1997, Rochira et al. 2022), the latter two being strongly dependent from circulating testosterone (Granata et al. 1997, Wu et al. 2010, Cunningham et al. 2015). Differently from HIV-uninfected men, erectile function does not associate with serum testosterone in MLWH in several retrospective studies, while the association remains for sexual desire even though weaker than expected (Rochira et al. 2011, Rochira and Guaraldi 2014, De Vincentis et al. 2021a, Santi et al. 2021, De Vincentis et al. 2022).

The primary aim of the study is to investigate through the use of validated questionnaires the prevalence of multiple sexual dysfunctions, especially ED and low libido, in MLWH younger than 50 years old. Secondary objective was to explore the association between sexual function and its different components (i.e. organic, relational and psychological), aiming at discriminating those determinants that had a major impact on the onset of sexual impairment.

METHODS

Study design and participants

This was a cross-sectional observational study of prospectively collected data from May 2013 to December 2017, previously described elsewhere (De Vincentis et al. 2021a), enrolling MLWH consecutively attending the Modena HIV Metabolic Clinic (MHMC).

Inclusion criteria were a documented HIV-infection on ART treatment, age 18-50 years and written informed consent. The choice to restrict the age range to less than 50 was made to limit all those factors involved in the pathogenesis of ED that are related to aging. Exclusion criteria were: severe liver or renal insufficiency, active malignancy, acquired immunodeficiency syndrome (AIDS),

endocrine disease (pituitary, thyroid, testicular diseases), any medication that could interfere with gonadal function as well as any previous treatment involving pituitary region (surgery, radiotherapy).

319 MLWH were assessed for eligibility, 6 were excluded from the study, and a total of 313 patients were enrolled.

Sexual function assessment

Sexual function was assessed through 2 validated questionnaires for ED: the International Index of Erectile Function-15 (IIEF-15) and the Structured Interview on Erectile Dysfunction (SIEDY) and 2 semi-structured interviews for other sexual function domains.

IIEF-15 is the most used tool in clinical practice to investigate the presence of ED (Rosen et al. 1997). IIEF-15 is a self-reported questionnaire that investigates the five domains of male sexual function in the last month: erectile function (EF), orgasmic function, sexual desire (SD), intercourse satisfaction, and overall satisfaction with sex life. Score is structured on a 5-point scale (from the worst 1, to the best score 5) and a cumulative score of EF domain below or equal to 25 is used to diagnose ED (Rosen et al. 1997, Cappelleri et al. 1999). According to the score of the EF domain, the EF was classified into the following four diagnostic categories: (i) no ED (EF score = 26–30); (ii) mild ED (EF score = 17– 25); (iii) moderate ED (EF score = 11–16); and (iv) severe ED (EF = 6–10). Sexual desire, orgasmic function, intercourse satisfaction and overall satisfaction were defined impaired with a score below 9 at the specific IIEF-15 domains (Rosen et al. 1997, Cappelleri et al. 1999).

SIEDY is a structured validated interviews submitted by the physician; it addressed four domains of male sexual function: i) organic ED (SIEDY scale 1); ii) relational ED (SIEDY scale 2); iii) psychogenetic ED (SIEDY scale 3); and iv) ED severity (SIEDY appendix A) (Corona et al. 2012). Scale 1 with a cut-off score >3.0 had a sensitivity of 67.9% and a specificity of 67.6% for identifying an organic component of ED (Petrone et al. 2003); a threshold score greater or equal

than 2 in Scale 2 predicts couple impairment with a sensitivity of 53% (Boddi et al. 2012); scale 3 with a threshold score greater or equal than 3 predicts psychological dimension impairment with an accuracy of 69.5% (Corona et al. 2012).

A decreased frequency of morning erections was evaluated during the medical interview and classified as follows: an estimated reduction of the frequency of 25% was considered as 'mild', 25-50% as 'moderate', 50-75% as 'severe', and >75% as 'extremely severe'.

Finally, an impairment of sexual desire (libido) was also explored with a non-validated semi-structured medical interview.

Covariates

Demographic and anthropometric variables

Demographic (age, sex) and anthropometric (weight, height, body mass index [BMI], waist, hip, and waist/hip ratio) variables were collected on the same day of the visit at MHMC.

Hormonal parameters

After an overnight fasting, an intravenous cannula was inserted into an antecubital vein at 8.00 am to collect a blood sample for serum and plasma assays. The blood samples were centrifuged, and the serum was stored at -20° C until assayed.

All biochemical and hormonal measurements have been performed as previously described.(De Vincentis et al. 2021a) In particular, sex steroids (total testosterone [TT], estradiol [E2], and dihydrotestosterone [DHT]) were assayed by validated LC-MS/MS (Fanelli et al. 2011, Mezzullo et al. 2020, Büttler et al. 2015). Of the 313 enrolled patients, 71 participants were missing E2 data. FT was calculated (cFT) by using the validated Vermeulen equation from both serum TT assessed with LC-MS/MS and serum TT assessed with CI (Keevil and Adaway 2019, Vermeulen et al. 1999).

SHBG, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and serum prolactin (PRL) were assessed by chemiluminescent immunoassay, as previously described (De Vincentis et al. 2021a).

According to the current Endocrine Society guidelines, diagnosis of biochemical hypogonadism was made when serum TT was below 320 ng/dL and/or cFT was below 64 pg/mL (Bhasin et al. 2018).

Biochemical measurements

The following biochemical parameters were assessed by commercially available kits: glycaemia, glycated hemoglobin (HbA1c), insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides.

Sexual orientation

Sexual orientation was explored with a non-validated semi-structured medical interview and patients were grouped into heterosexual and non-heterosexual. The latter were further subdivided into four subgroups: i) men who have sex with men (MSM) with a preferred active attitude (A>P); ii) MSM with a preferred passive attitude (P>A); iii) MSM with similar active or passive attitude (A=P); iv) bisexual (B).

HIV parameters

HIV-related variables included HIV duration, current and nadir CD4 cell count, and HIV RNA viral load.

C*omorbidities*

At the time of the visit, each patient was assessed for comorbidities as previously described (De Vincentis et al. 2021a). Comorbidities were defined using the European AIDS Clinical Society (EACS) guidelines (https://www.eacsociety.org/media/2017_guidelines_9.0-english_rev-20181024.pdf).

Ethics

The study protocol was approved by the North Emilia Vast Area (AVEN) Ethics Committee (protocol n. 1446/15), registered in ClinicalTrials.gov (Identifier: NCT03747003), and conducted in

accordance with the ethical standards of the Helsinki Declaration (1975, revised in 2013). Written informed consent has been obtained from each subject.

Statistical analysis

According to data distribution analyzed by the Kolmogorov–Smirnov test, comparisons of continuous variables were performed using the nonparametric Mann–Whitney test and Kruskal–Wallis test followed by the Dunn's multiple comparison *post hoc* test. Categorical variables were compared with Chi-square test.

Linear regression was used to examine the association between continuous variables; results were expressed through β and R^2 coefficients.

Bivariate logistic regressions were used to determine potential predictive factors for different domains of sexual function. Results were expressed through odds ratio (OR) and 95% confidence interval (CI). Thereafter, multivariate regression analysis using a backward elimination method was applied to the data with $p<0.1$ as the criterion for a variable to enter the model.

Statistical analyses were performed using the Statistical Package for the Social Sciences' (SPSS) software for Windows (version 27.0; SPSS Inc, Chicago, IL). For all comparisons, $p<0.05$ was considered statistically significant.

RESULTS

A total of 313 consecutive MLWH were enrolled, with a median age of 47 years (IQR 25.2-50.5) and a median duration of HIV-infection of 16.2 years (IQR 1.1-35.4). The clinical characteristics of the entire cohort are summarized in Table 1.

Supplementary table 1 summarized tools used to classify the 6 sexual function domains analyzed in this cohort which will be described separately. The prevalence of sexual dysfunctions according to scores of IIEF-15 specific domains and SIEDY scales were reported in Figure 1.

1. **Erectile function**

Out of 313 MLWH, 187 (59.7%) presented ED: in detail 59 had severe ED, 35 had moderate ED, and 93had mild ED (Figure 1, Table 1, Supplementary table 2).

Age, BMI and lifestyle were not associated with ED or its degree (Table 1, Supplementary table 2). Age was inversely related to IIEF-15 score (R^2=0.020, β=-0.140, p=0.013).

No significant difference was found for hormonal measurements (TT, cFT, E2, LH, FSH, DHT, SHBG, PRL) between MLWH with and without ED (Table 1). However, among patients with ED, serum TT and cFT were significantly lower in patients with severe ED compared to those with mild ED (Table 2). Among MLWH with ED identified as having an organic component by SIEDY both serum TT and cFT resulted to be protective for ED (Table 2) indicating that *vice versa* low serum T was at higher risk for organic component. The prevalence of ED in hypogonadal and eugonadal MLWH did not significantly differ (p=0.172); ED was found in 24 hypogonadal patients out of 34 (70.6%) presented ED, and in 162 eugonadal patients out of 279 (58.4%). However, IIEF-15 score of erectile function was significantly lower in hypogonadal patients compared to eugonadal (19.5 [IQR 5.5-27] vs 24 [IQR 15-28], p=0.034).

Comorbidities did not differ in MLWH with and without ED for (Table 1).

ED was more prevalent in non-heterosexual than heterosexual MLWH (p<0.001) (Table 1). In detail, 41 patients with ED out of 187 (21.9%) and only 4 patients without ED out of 126 (3.2%) were MSM with a more frequent passive attitude (p<0.0001) (Table 1). The univariate logistic regression showed that non-heterosexual MLWH had a two-fold increased likelihood to present ED compared to heterosexual MLWH (OR=2.045; 95% CI 1.264-3.308; p=0.004). Furthermore, MSM patients with a more frequent passive than active attitude had an 11-fold increased likelihood to present ED compared with heterosexual men (OR=11.087; 95% CI=3.70-33.23; p<0.0001); similarly, bisexual men had a 9-fold increased likelihood to present ED compared with heterosexual men (OR=9.74; 95% CI=1.12-79.67; p<0.0001).

The use of PDE-5i was more common among MLWH with ED than MLWH without ED (p=0.008). Of note, only 35 patients up to 187 with ED (18.7%) reported the use of PDE-5i. The use of PDE-5i was more common in hypogonadal (10 out of 34, 29.4%) than eugonadal MLWH (35 out of 244, 14.3%) (p=0.008), whereas no difference was found in relation to the sexual orientation (p=0.908).

ED was further analyzed in its 3 components according to the SIEDY scales (Table 2).

Organic component of ED

According to SIEDY scale 1, 99 MLWH out of 313 (31.6%) presented an impaired organic component: of them, 64 had already developed ED, whereas 35 have not (Table 1, Figure 1). Overall, MLWH with altered organic component had an increased likelihood to present ED (OR 1.13; 95% CI: 1.01-1.27; p=0.028).

Logistic analysis was performed to detect predictors of organic ED. Among demographic and anthropometric variables, age, BMI and W/H ratio were associated with a higher risk of organic ED (Table 2). LH and FSH were positively associated with organic ED, while serum TT and cFT were inversely associated with organic ED (Table 2). As expected, insulin, HbA1c, hypertension, cardiovascular disease, and dyslipidemia were related to a greater risk of organic ED (Table 2). Among HIV variables, HIV duration only was significantly associated with organic ED (Table 2). As anticipated, use of PDE-5i was associated with organic ED (Table 2).

Relational component of ED

The presence of a stable relationship (i.e. lasting at least 2 months) was explored through the item n. 5 of SIEDY. A total of 77 out of 102 heterosexual (75.5%) and 119 out of 211 non-heterosexual (56.9%) MLWH declared to be in a stable relationship, with a significant difference among the 2 subgroups (p<0.0001). ED was more frequent among MLWH without a stable relationship (86 out of 117 patients, 73.5%) than those with a stable relationship (101 out of 196 patients, 51.5%) (p<0.0001).

According to SIEDY scale 2, 42 out of 196 MLWH with a stable relationship (21.4%) presented a relational impairment: of them, 25 had developed ED, whereas 17 have not (Table 1, Figure 1). Age, hypertension and use of PDE-5i were associated with higher risk of relational ED in univariate analysis (Table 2).

Psychological domains of ED

According to SIEDY scale 3, 141 out of 313 MLWH (45.0%) presented a psychological component for ED: of them, 92 had already ED, whereas 49 had not (Table 1, Figure 1). Patients with ED had a significantly higher score of SIEDY scale 3 compared to patients without ED (p=0.025) (Table 1).

IIEF-15 score of erectile function domain was inversely related to SIEDY scale 3 (R^2=0.013, β=-0.115, p=0.042). Accordingly, MLWH with compromised psychological component of erection had nearly 1.5-fold increased likelihood to present ED (OR=1.2, 95% CI: 1.03-1.31; p=0.017).

Smoking, MSM sexual orientation and use of PDE-5i were associated with higher risk of psychological ED, while HDL and being in a stable relationship had a protective effect on psychological ED (Table 2).

Multivariate analysis

A multivariate logistic regression analysis was performed to identify risk factors for ED and its sub-domains (data now shown for relational and psychological sub-domains not contributing for clinical interpretation). Co-variates were chosen including variables that at univariate analysis displayed p-value <0.10. Figure 3 depicts the Forest-Plot of significant variables. The multivariate analysis showed that a reduced frequency of morning erections (OR=2.15; 95% CI: 1.21-3.80; p=0.009), MSM sexual orientation (OR=2.42; 95% CI: 1.27-4.60; p=0.007), and, marginally, use of PDE-5i (OR=2.36; 95% CI: 1.00-5.59; p=0.051) were risk factors for ED, whereas being in a stable relationship had a protective effect on ED (OR=0.29; 95% CI: 0.16-0.52; p<0.001) (Figure 3A). On the contrary, HIV-related variables lost the statistical significance (Figure 3A). Similarly,

dyslipidemia (OR=4.36; 95% CI: 2.01-9.43; p<0.001) and the use of PDE-5i (OR=3.44; 95% CI: 1.42-8.32; p=0.006) were risk factors for organic ED (Figure 3B).

2. Sexual desire

Considering IIEF-15 specific domain, 239 patients out of 313 (77.9%) presented reduced sexual desire, while 68 patients (22.1 %) had normal desire (Figure 1). Patients with ED reported higher rates of reduced libido compared to patients without ED (Table 1).

No difference was found for hormonal parameters (TT, cFT, E2, DHT) between patients with low libido and those not complaining a reduction of sexual desire. However, considering the gonadal status as a categorical variable (i.e hypogonadism *vs* eugonadism), 31 hypogondal patients out of 34 (93.9%) reported a reduction of sexual desire, whereas 208 eugonadal patients out of 276 (75.9%) had a reduction of sexual desire, with a significant difference (p=0.018).

The prevalence of low libido did not differ in relation to the sexual orientation (p=0.615). Indeed, at the logistic regression, the only parameters which reduced sexual desire were age and smoking (Table 3).

3. Morning erections

A total of 104 MLWH (33.2%) reported a reduction of morning erections. A reduction of morning erections was more frequent among patients with ED (p=0.004) (Table 1).

Patients with reduced morning erections had significantly lower serum TT and cFT compared to patients not complaining a reduction of morning erections (TT: 584 ng/dL [IQR 467-748] vs 674 ng/dL [IQR 540-802], p=0.006; cFT: 99 pg/mL [IQR 77-125] vs 110 pg/mL [IQR 88-125], p=0.006). Accordingly, the reduction of morning erection was more frequent among hypogonadal patients than eugonadal (61.8% vs 36.9%, respectively; p=0.005). At the logistic regression, increased LH, low serum TT and low cFT were associated with a higher risk of morning erections (Table 3).

Furthermore, age, opioids use, diabetes, dyslipidemia, and HIV duration were positively associated with reduced morning erections (Table 3).

4. Orgasmic function

According to IIEF-15 domain specific for orgasmic function, 193 (61.7%) MLWH presented an orgasmic function impairment (Figure 1). None of explored variables was found as a significant risk factor for impaired orgasmic function at the logistic regression (Table 3).

Finally, 227 MLWH (73.9%) reported an overall satisfaction impairment, according to the specific domain of IIEF-15 (Figure 1).

DISCUSSION

This study provides evidence that sexual dysfunction, particularly ED, in young MLWH is a highly prevalent multidimensional disorder related to sexual orientation and psychological domains. In our cohort the prevalence of sexual dysfunctions was 60% for ED and up to 78% for reduced sexual desire, in line with previous studies on ED (Bernal et al. 2019, Fumaz et al. 2017, Romero-Velez et al. 2014, Pérez et al. 2013, Zona et al. 2012, Guaraldi et al. 2012, Vansintejan et al. 2013, Pinzone et al. 2015, Dijkstra et al. 2018) and reduced sexual desire (Lallemand et al. 2002). ED prevalence is 3-time higher than age-matched (30-50 years) Italian general population (22.4%.) (Parazzini et al. 2000).

Previous studies have explored the relationship between depression and/or anxiety and ED in MLWH (Dijkstra et al. 2018, Pérez et al. 2013, Crum-Cianflone et al. 2007, Moreno-Pérez et al. 2010, Wang et al. 2013, Hart et al. 2015, Fumaz et al. 2017, Pinzone et al. 2015) focusing on the mere association between each of these diseases or the use of anxiolytic/antidepressant and ED. Our study is the first to investigate psychological domain in MLWH using the SIEDY scale 3, which is the only tool that specifically explores the presence of psychological impairment (not specific for a psychological disorder) leading to ED (Corona et al. 2012, Petrone et al. 2003).

The results are, however, conflicting and no study has evaluated the psychological component intrinsic to ED in MLWH. Indeed, psychological domains in MLWH goes beyond the diagnosis of anxiety/depression and it is linked to several factors including stigma, fear of HIV transmission and use of condom (Baker et al. 2020, Santi et al. 2014, De Vincentis et al. 2021b, Huntingdon et al. 2020).

This study adds information also on the relational component of ED which is often neglected (Huntingdon et al. 2020) and suggests that the lack of a stable relationship is associated to ED in MLWH.

Another major determinant of ED is sexual orientation since MSM and bisexual MLWH have an eleven-fold and nine-fold, respectively, increased likelihood to present ED compared to heterosexual men, in line with most of previous studies (Sollima et al. 2001, Zona et al. 2012, Huntingdon et al. 2020, Barbonetti et al. 2019, Cheng 2022). To explain the higher prevalence of ED in MSM, it should be considered that MSM deal with additional/potentiated psychological issues, such as social stigma, importance of body image, and sexual behavior (e.g. casual sex group, anal penetration requiring a better erection in terms of both duration and rigidity), including a major interest in passive activity and less importance given to erection. All together these issues could further compromise sexual life and self-perceived impairment of sexual function (De Vincentis et al. 2021b, Santi et al. 2014, Cheng 2022, Baker et al. 2020). The fact that ED was associated with passive sexual attitude in MLWH confirms previous data and raises concerns about causality since it is difficult to establish if the choice of a passive sexual attitude prompts ED or *vice versa* (Huntingdon et al. 2020).

Psychological component of ED and impaired sexual desire and psychological issues are strictly interrelated in MLWH. Several emotional status and psychological issues are peculiar of MLWH and/or MSM and may strongly impact on sexual behavior. Among them: i) the stigma and ii) changes in body image may be related to self-feeling of being less desirable and to fear to be rejected by the partner (when disclosing HIV status or when the latter is incidentally discovered);

iii) the fear of HIV transmission has possible direct impact on all components of sexual behavior; iv) the obligatory use of condom may impact on quality of erection; v) peculiar sexual behavior in MSM. All these psychological and emotional aspects are well recognized in MLWH and MSM literature (Santi et al. 2014, De Vincentis et al. 2021b, Huntingdon et al. 2020, Baker et al. 2020, Cheng 2022) and need to be introduced among clinicians bringing them to the attention of both the specialist in infectious disease and the andrologist/expert in sexual medicine.

Other HIV-related factors such as duration of infection and ART exposure contribute to increased risk of ED in these patients, but their impact is lost in the multivariate analysis. This is in line with most previous studies (Guaraldi et al. 2012, Huntingdon et al. 2020, Crum-Cianflone et al. 2007).

In our study classical risk factors of ED, especially cardiometabolic parameters (i.e. cardiovascular disease, hypertension, and dyslipidemia), resulted associated with organic ED and reduced morning erections, but their role was less evident in the multivariate analysis for ED where non-organic components prevailed. Differently from HIV-uninfected patients for whom classical risk factors are almost constantly associated with ED (Rochira et al. 2022, Yafi et al. 2016, Corona et al. 2010, Feldman et al. 1994, Allen and Walter 2019), the strength of this association is weaker or absent in MLWH (Guaraldi et al. 2012, Romero-Velez et al. 2014, Moreno-Pérez et al. 2010, Pérez et al. 2013). This means that MLWH patients affected by ED/reduced morning erections presented more vulnerability and health deficits in terms of sum of classical risk factors (Shacham et al. 2017, Dijkstra et al. 2018), but each of them has a minor role when taken alone in comparison to other non-quantifiable factors that are mainly related to ED in MLWH (De Vincentis et al. 2021a). This is also the case for hypogonadism that is so much prevalent in MLWH (De Vincentis et al. 2022, Rochira and Guaraldi 2014, Rochira et al. 2011, Santi et al. 2021, Pezzaioli et al. 2021b) that is advised in guidelines of male hypogonadism (Bhasin et al. 2018, Isidori et al. 2022). Anyhow, when deepening the association between sex steroids and ED, both serum TT and cFT did not differ between MLWH with and without ED and they did not correlate with all IIEF-15 erectile function score, except for patients identified as having an organic component of ED at SIEDY. Besides, ED

prevalence did not change between eugonadal and hypogonadal MLWH. This confirms that gonadal status has a less determinant impact on ED, the latter being mainly related to androgen-independent aspects in MLWH. This is in line with previous studies obtained by us (Guaraldi et al. 2012, Rochira et al. 2011) and by other research groups (Crum-Cianflone et al. 2007, Moreno-Pérez et al. 2010). Differently from HIV-uninfected patients where serum testosterone is almost constantly related to erectile function (Wu et al. 2010, Cunningham et al. 2015), the lack of interrelationship between ED and androgen status seems to be a peculiar hallmark of ED in MLWH, notwithstanding the increased prevalence of hypogonadism in these patients (Rochira et al. 2011, Rochira and Guaraldi 2014, De Vincentis et al. 2021a, Santi et al. 2021, Pezzaioli et al. 2021b, De Vincentis et al. 2022). Sexual desire, a well-known androgen-dependent issue was not associated to both serum TT and cFT in this study, thus reinforcing the role of psychological component in MLWH since sexual desire is also strongly dependent from the psychological status (Rochira et al. 2022). Morning erections were the only parameters that resulted to be related to total testosterone and cFT in MLWH, confirming their strong testosterone dependency and total independency from psychological issues (Granata et al. 1997). From a clinical standpoint, morning erections should be considered a useful surrogate of the organic (hormonal and cardiovascular) component that could be easily explored during the medical evaluation of sexual heath.

The high prevalence of sexual dysfunctions in our cohort clashes with the low reported use of PDE5-i (14.4%). Although this data was obtained from a direct investigator's question to patients leading to a possible underestimation of the real use of PDE5-i, this is unlikely to be explained only by a methodological bias and suggests that ED is an undertreated and undermanaged condition in MLWH. Accordingly, this issue results to be overlooked in sexual medicine research and in the clinic both in MSM (Cheng 2022) (who represent the 67% of patients in our MLWH cohort) and MLWH in general (Santi et al. 2014, De Vincentis et al. 2021a, Huntingdon et al. 2020, Baker et al. 2020).

This study has strengths and limitations. The principal strength is that is the first study to explore sexual dysfunction in MLWH using different validated questionnaires (IIEF-15, SIEDY) to study both prevalence and components. Another strength is represented by the measurement of sex steroids with the gold standard LC-MS/MS. A possible limitation is the use of the IIEF questionnaire that was constructed and validated in heterosexual men. We did not use the modified IIEF for MSM (Coyne et al. 2010) since only the English version is available and MLWH were enrolled regardless of their sexual preference, as other previous studies (Romero-Velez et al. 2014, Fumaz et al. 2017). Furthermore a researcher was available during questionnaire filling in case of doubts. For all these reasons, the authors do not believe that the use of IIEF-15may have increased significantly the risk of bias. The cross-sectional study design study that does not allow to define cause-effect relationship. However, concerning the psychological component of ED the use of an *ad hoc* validated questionnaire useful to clarify the origin of ED allows inferring about the role of psychological issues on ED with a good strength of evidence. The lack of control group is another limit concerning the increased rate of prevalence of sexual dysfunction in MLWH compared to age-matched HIV-uninfected population, but our data confirm a well-established issue.

In conclusion, our findings point out that HIV-related factors, particularly the psychological component, are predominant in the onset of ED in MLWH. A tailored clinical approach focusing the multidimensional domains of sexual dysfunction may improve sexual health in MLWH.

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Table 1. Characteristics of the entire cohort and comparison between patients with and without ED according to IIEF-15 erectile function domain score. Continuous variables are reported as median (IQR).

		Patients with ED (IIEF-15 ≤ 25)	**Patients without ED (IIEF-15 > 25)**	***p*-value**
n 313	**n.v.**	187 (59.7%)	126 (40.3%)	
Age (years)	-	47.4 (42.9-50)	46.7 (42.4-49.2)	0.130
Sexual orientation				
Heterosexual	-	49 (15.7%)	53 (16.9%)	**<0.0001**
MSM and bisexual	-	138 (44.1%)	73 (23.3%)	
MSM (A>P)	-	25 (8.0%)	28 (8.9%)	
MSM (A=P)	-	63 (20.1%)	40 (12.8%)	
MSM (A<P)	-	41 (13.1%)	4 (1.3%)	
Bisexual	-	9 (2.9%)	1 (0.3%)	
Anthropometric variables				
BMI (kg/m^2)	18.5-25	23.8 (22.1-25.6)	23.6 (21.8-25.5)	0.634
W/H circumference ratio	< 0.95	0.95 (0.90-0.98)	0.95 (0.90-0.99)	0.858
Sexual function parameters				
Impaired SIEDY scale 1 (> 3)	-	64 (34.4%)	35 (27.8%)	0.217
Impaired SIEDY scale 2 (≥ 2)*	-	25 (17.9%)	17 (24.8%)	0.242
Impaired SIEDY scale 3 (≥ 3)	-	92 (49.5%)	49 (38.9%)	0.066
Reduced sexual desire	-	155 (84.2%)	84 (68.3%)	**0.001**
Reduced morning erections	-	74 (39.6%)	30 (23.8%)	**0.004**
Hormonal measurements				
LH (mIU/mL)	1.4-8.9	4.7 (3.5-6.8)	5.0 (3.6-6.6)	0.830
FSH (mIU/mL)	1.7-6.9	5.6 (4.1-8.4)	5.6 (3.6-8.0)	0.261
PRL (ng/mL)	2.1-17.7	7.5 (5.7-10.4)	7.2 (5.2-9.3)	0.092
Serum E2 (pg/mL)	<50	24.4 (19.4-30.4)	25.6 (20.3-33.6)	0.145
Serum TT (ng/dL)	>320	632.9 (498.4-781.3)	647.6 (509.8-797.5)	0.577
E2/TT		0.036 (0.030-0.047)	0.040 (0.031-0.047)	0.144
SHBG (nmol/L)	13.5-71.4	46.6 (35.1-64.3)	49.6 (35.5-68.0)	0.734
cFT (pg/mL)	>64	104.1 (82.7-134.0)	108.0 (85.8-128.5)	0.760
DHT (pg/mL)	165-679	385.7 (279.0-535.1)	343.2 (245-520.3)	0.215
Biochemical measurements				
Fasting glucose (mg/dl)	70-100	92 (86-97.5)	93 (87-98.5)	0.460
Insulin (mIU/ml)	2-23	7.95 (5.18-12.02)	7.40 (5.00-13.40)	0.872
HOMA Index	< 2.5	1.69 (1.14-2.97)	1.76 (1.17-3.02)	0.854

HbA1c (mmol/mol)	20-38	34.00 (31.15-36.71)	34.00 (31.00-36.00)	0.633
Total Cholesterol (mg/dl)	< 200	186 (162-214)	190 (169-222.5)	0.269
LDL Cholesterol (mg/dl)	< 100	116 (97-139.5)	124 (98-144)	0.237
HDL Cholesterol (mg/dl)	> 45	46 (36-56)	46 (38-52)	0.867
Tryglicerides (mg/dl)	< 180	122 (86.5-183)	134 (87.5-199.5)	0.390
Lifestyle and drug use				
Smoking	-	77 (41.4%)	46 (36.5%)	0.386
Alcohol use (moderate/intense)	-	99 (52.9%)	69 (54.8%)	0.751
Opioids use	-	39 (20.9%)	25 (19.8%)	0.827
PDE-5i use	-	35 (18.7%)	10 (7.9%)	**0.008**
Psychotropic drugs use	-	25 (13.4%)	10 (7.9%)	0.135
Comorbidities and frailty				
Diabetes	-	5 (2.7%)	2 (1.6%)	0.524
Hypertension	-	37 (19.8%)	16 (12.7%)	0.101
Cardiovascular disease	-	13 (7.0%)	6 (4.8%)	0.426
Dyslipidemia	-	93 (49.7%)	60 (48.0%)	0.764
Frailty index	< 0.21	0.25 (0.20-0.33)	0.24 (0.17-0.32)	0.270
HIV parameters				
HIV duration (years)	-	16.6 (8.9-23.8)	14.1 (7.5-23.7)	0.266
ART exposure (years)	-	14.5 (7.5-19.6)	13.2 (4.8-20.1)	0.140
Nadir CD4 (cells/μL)	-	252 (82-352)	250 (220-350)	0.453
Current CD4 (cells/μL)	>400	625 (507-803)	624 (514-880)	0.898
HIV undetectability (<40 copies/mL)	-	143 (76.5%)	103 (81.8%)	0.874

[Footnote to Table 1] *Abbreviations: IQR: interquartile range; n.v.: normal values; MSM: men who have sex with men; A>P: MSM with a preferred active attitude; A=P: MSM with similar active and passive attitude; P>A: MSM with a preferred passive attitude; B: bisexual. BMI: Body Mass Index; W/H: Waist/hip circumference ratio; ART: Antiretroviral Therapy; PDE-5i: phosphodiesterase 5-inhibitors; IIEF: validated International Index of Erectile Function; SIEDY: Structured Interview of Erectile Dysfunction; EF: erectile function; ED: erectile dysfunction; SD: sexual desire; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; PRL: Prolactin; E2: Estradiol; TT: Total Testosterone; SHBG: Sex Hormone-Binding Globulin; cFT: calculated Free Testosterone; DHT: Dihydrotestosterone; HOMA: Homeostasis Model Assessment; HbA1c: Glycated Hemoglobin; HDL: high density lipoprotein; LDL: low-density lipoprotein; Mann-Whitney test and Chi-square test were used to compare continuous and categorical variables, respectively. *This analysis was restricted to only patients reporting a stable relationship (196 out of 313).*

Table 2. Univariate logistic regressions with different components of ED (organic, relational and psychological) as outcomes. Results are reported as odds ratio [95% CI]. Significant results ($p<0.05$) are shown in bold.

			ED with impaired organic component		**ED with impaired relational component**		**ED with impaired psychological component**	
			OR [95% CI]	*p-value*	*OR [95% CI]*	*p-value*	*OR [95% CI]*	*p-value*
Organic determinants	Demographic and anthropometric parameters	Age	**1.085 [1.017-1.158]**	**0.013**	**1.146 [1.014-1.297]**	**0.030**	0.981 [0.940-1.024]	0.375
		BMI	**1.154 [1.058-1.258]**	**0.001**	1.053 [0.928-1.195]	0.423	0.989 [0.919-1.065]	0.773
		W/H	**1768 [23-135499]**	**<0.001**	67.801 [0.148-31149]	0.178	0.431 [0.014-13.231]	0.630
	Hormonal measurements	LH	**1.056 [1.009-1.104]**	**0.018**	1.026 [0.966-1.090]	0.404	1.011 [0.969-1.054]	0.624
		FSH	**1.064 [1.022-1.108]**	**0.003**	0.991 [0.924-1.062]	0.791	1.010 [0.976-1.042]	0.562
		Prolactin	1.012 [0.975-1.049]	0.537	0.994 [0.929-1.063]	0.861	1.013 [0.981-1.047]	0.432
		SHBG	1.007 [0.997-1.017]	0.164	1.001 [0.986-1.017]	0.860	0.998 [0.990-1.007]	0.738
		Serum TT	**0.861 [0.753-0.985]**	**0.030**	1.044 [0.875-1.245]	0.632	0.923 [0.831-1.025]	0.133
		cFT	**0.890 [0.818-0.969]**	**0.007**	1.062 [0.957-1.178]	0.258	0.981 [0.921-1.044]	0.537
		Serum E2	1.021 [0.984-1.058]	0.268	0.998 [0.949-1.048]	0.923	0.979 [0.950-1.010]	0.185
		DHT	1.239 [0.291-5.281]	0.772	0.720 [0.766-7.859]	0.787	1.030 [0.323-3.282]	0.961
	Biochemical measurements	Fasting glucose	1.011 [0.998-1.025]	0.101	1.005 [0.985-1.026]	0.607	0.986 [0.967-1.005]	0.139
		Insulin	**1.041 [1.009-1.074]**	**0.011**	1.022 [0.987-1.059]	0.222	1.010 [0.983-1.037]	0.467
		HOMA Index	1.089 [0.995-1.192]	0.065	1.045 [0.959-1.139]	0.318	1.001 [0.931-1.081]	0.935
		HbA1c	**1.076 [1.024-1.132]**	**0.004**	1.038 [0.969-1.112]	0.287	0.979 [0.938-1.022]	0.341
		Total Cholesterol	0.996 [0.989-1.004]	0.330	0.996 [0.986-1.006]	0.513	0.995 [0.989-1.001]	0.134
		LDL Cholesterol	0.999 [0.991-1.007]	0.835	0.996 [0.984-1.008]	0.549	0.997 [0.991-1.004]	0.455
		HDL Cholesterol	0.980 [0.960-1.001]	0.065	0.991 [0.959-1.025]	0.600	**0.981 [0.964-0.986]**	**0.030**
		Tryglicerides	1.000 [0.998-1.002]	0.991	1.001 [0.998-1.004]	0.515	1.001 [0.999-1.003]	0.276
	Lifestyle	Smoking	1.155 [0.661-2.019]	0.612	1.202 [0.509-2.839]	0.674	**2.000 [1.250-3.201]**	**0.004**
		Alcohol use (moderate/intense)	1.053 [0.606-1.827]	0.855	1.741 [0.713-4.250]	0.224	0.896 [0.566-1.418]	0.639
		Opioids use	1.555 [0.820-2.946]	0.176	2.186 [0.891-5.631]	0.088	0.844 [0.474-1.500]	0.563
	Comorbidities and frailty	Diabetes	3.012 [0.657-13.814]	0.156	1.740 [0.187-16.221]	0.627	0.272 [0.032-2.284]	0.230
		Hypertension	**2.975 [1.564-5.660]**	**<0.001**	**3.893 [1.574-9.630]**	**0.003**	1.346 [0.739-2.451]	0.332
		Cardiovascular diseases	**2.547 [1.001-6.483]**	**0.050**	2.455 [0.617-9.716]	0.202	1.228 [0.479-3.146]	0.669
		Dyslipidemia	**4.523 [2.407-8.501]**	**<0.001**	1.029 [0.442-2.397]	0.947	1.746 [0.580-1.453]	0.645
		Frailty index	**24.845 [1.513-408]**	**0.024**	17.246 [0.473-400.079]	0.076	3.819 [0.163-18.710]	0.258
	HIV parameters	HIV duration (years)	**1.051 [1.018-1.086]**	**0.003**	1.050 [0.999-1.102]	0.053	1.005 [0.979-1.032]	0.694
		ART duration (years)	**1.048 [1.010-1.086]**	**0.012**	**1.055 [1.000-1.113]**	**0.048**	0.993 [0.964-1.023]	0.641
		Nadir CD4	1.000 [0.997-1.003]	0.976	0.997 [0.993-1.002]	0.262	1.001 [0.998-1.004]	0.646
		Current CD4	0.999 [0.997-1.001]	0.410	0.998 [0.996-1.001]	0.278	1.000 [0.998-1.002]	0.685
		HIV undetectability (<40 copies)	0.343 [0.094-1.257]	0.106	0.429 [0.048-3.848]	0.449	0.275 [0.089-2.137]	0.219
Relational	Sexual	MSM and bisexual	1.107 [0.619-1.977]	0.732	1.505 [0.648-3.498]	0.342	**1.902 [1.137-3.181]**	**0.014**

determinants	orientation						
	PDE-5i use	**2.855 [1.446-5.635]**	**0.002**	**2.778 [1.037-7.440]**	**0.042**	**2.378 [1.254-4.509]**	**0.008**
	Stable relationship	0.842 [0.480-1.476]	0.548	n.a.	n.a.	**0.383 [0.238-0.617]**	**<0.001**
Psychological determinants	Antidepressant drugs use	1.157 [0.410-3.263]	0.783	0.552 [0.069-4.440]	0.576	2.114 [0.883-5.060]	0.093
	Benzodiazepines use	0.971 [0.313-3.011]	0.959	2.037 [0.399-10.488]	0.392	0.891 [0.345-2.331]	0.811

[Footnote to Table 2]: *Abbreviations: CI: confidence interval; ED: erectile dysfunction; BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; W/H: Waist/hip circumference ratio; ART: Antiretroviral Therapy; MSM: men who have sex with men; PDE-5i: phosphodiesterase 5-inhibitors; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; PRL: Prolactin; E2: Estradiol; TT: Total Testosterone; SHBG: Sex Hormone-Binding Globulin; cFT: calculated Free Testosterone; DHT: Dihydrotestosterone; HOMA: Homeostasis Model Assessment; HbA1c: Glycated Hemoglobin; HDL: high density lipoprotein; LDL: low-density lipoprotein.*

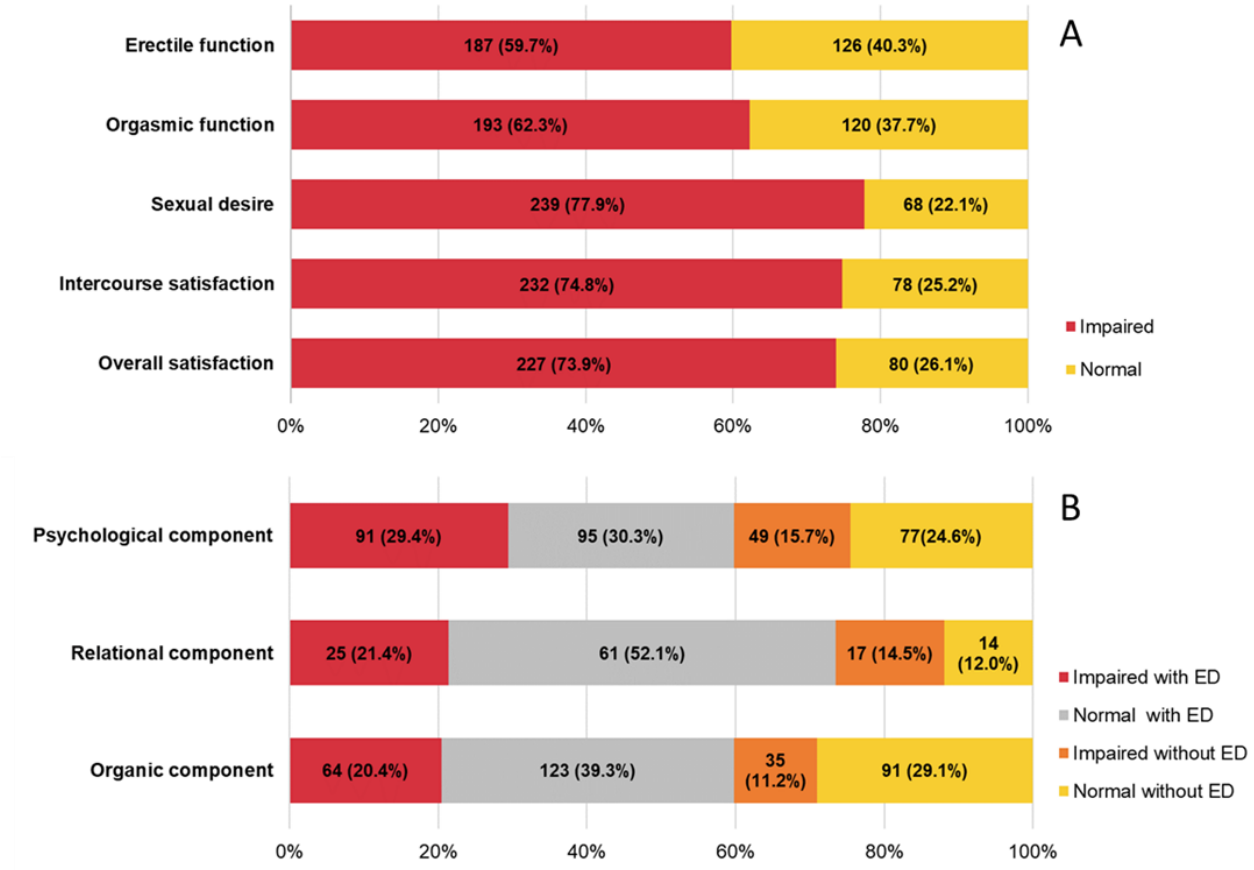
Table 3. Univariate logistic regressions with reduced sexual desire, impaired orgasmic function, and reduced morning erections as outcomes. Results are reported as odds ratio [95% CI]. Significant results ($p<0.05$) are shown in bold.

			Reduced sexual desire		**Impaired orgasmic function**		**Reduced frequency of morning erections**	
			OR [95% CI]	*p-value*	*OR [95% CI]*	*p-value*	*OR [95% CI]*	*p-value*
Organic determinants	Demographic and anthropometric parameters	Age	**1.060 [1.010-1.111]**	**0.018**	0.971 [0.928-1.016]	0.199	**1.084 [1.032-1.139]**	**0.001**
		BMI	0.965 [0.887-1.050]	0.412	0.963 [0.895-1.036]	0.314	1.068 [0.992-1.149]	0.079
		W/H	0.338 [1.006-19.500]	0.600	0.037 [0.001-1.177]	0.062	27.408 [0.883-850.972]	0.059
	Hormonal measurements	LH	1.037 [0.971-1.107]	0.282	0.995 [0.954-1.039]	0.824	**1.065 [1.013-1.120]**	**0.013**
		FSH	1.046 [0.986-1.111]	0.137	0.989 [0.955-1.024]	0.524	1.021 [0.986-1.057]	0.247
		Prolactin	0.977 [0.944-1.011]	0.184	0.978 [0.946-1.012]	0.204	0.997 [0.964-1.031]	0.850
		SHBG	1.006 [0.995-1.017]	0.319	0.998 [0.989-1.007]	0.626	1.004 [0.995-1.013]	0.395
		Serum TT	0.987 [0.877-1.112]	0.832	0.939 [0.848-1.039]	0.222	**0.863 [0.774-0.962]**	**0.008**
		cFT	0.969 [0.903-1.040]	0.389	0.990 [0.931-1.053]	0.752	**0.887 [0.828-0.951]**	**<0.001**
		Serum E2	1.007 [0.973-1.042]	0.706	0.995 [0.966-1.025]	0.742	1.010 [0.981-1.040]	0.499
		DHT	1.308 [1.323-5.925]	0.707	0.767 [0.237-2.488]	0.659	1.195 [0.376-3.776]	0.762
	Biochemical measurements	Fasting glucose	1.002 [0.986-1.018]	0.829	0.995 [0.983-1.008]	0.468	1.005 [0.993-1.018]	0.416
		Insulin	0.991 [0.961-1.023]	0.590	0.999 [0.972-1.027]	0.955	1.022 [0.994-1.051]	0.127
		HOMA Index	1.000 [0.914-1.093]	0.993	1.006 [0.932-1.087]	0.874	1.040 [0.962-1.125]	0.320
		HbA1c	1.004 [0.956-1.054]	0.869	0.992 [0.952-1.033]	0.696	1.034 [0.992-1.778]	0.113
		Total Cholesterol	1.002 [0.995-1.009]	0.567	0.999 [0.993-1.005]	0.789	1.000 [0.994-1.006]	0.908
		LDL Cholesterol	1.007 [0.998-1.015]	0.126	1.001 [0.994-1.008]	0.708	1.004 [0.997-1.010]	0.314
		HDL Cholesterol	1.007 [0.987-1.027]	0.512	1.014 [0.997-1.031]	0.110	0.988 [0.972-1.004]	0.141
		Tryglicerides	0.999 [0.997-1.001]	0.399	1.000 [0.998-1.002]	0.886	1.000 [0.999-1.002]	0.726
	Lifestyle	Smoking	**1.869 [1.037-3.368]**	**0.037**	1.682 [1.038-2.725]	0.035	1.439 [0.906-2.287]	0.123
		Alcohol use (moderate/intense)	1.388 [0.809-2.382]	0.234	1.073 [0.677-1.699]	0.765	1.309 [0.829-2.068]	0.248
		Opioids use	1.613 [0.771-3.374]	0.204	0.902 [0.512-1.590]	0.722	**1.881 [1.080-3.273]**	**0.026**
	Comorbidities and frailty	Diabetes	-	0.999	1.529 [0.292-8.012]	0.615	**9.692 [1.152-81.520]**	**0.037**
		Hypertension	0.825 [0.412-1.653]	0.587	1.349 [0.719-2.530]	0.351	1.476 [0.814-2.675]	0.200
		Cardiovascular diseases	1.451 [0.407-5.166]	0.566	0.823 [0.321-2.111]	0.686	1.780 [0.702-4.514]	0.225
		Dyslipidemia	1.624 [0.939-2.810]	0.083	1.565 [0.983-2.490]	0.059	**1.989 [1.254-3.155]**	**0.003**
		Frailty index	9.728 [0.558-169.507]	0.119	1.512 [0.140-16.316]	0.733	**31.976[2.900-352.558]**	**0.005**
	HIV parameters	HIV duration (years)	1.009 [0.978-1.041]	0.586	0.990 [0.946-1.016]	0.435	**1.051 [1.023-1.080]**	**<0.001**
		ART duration (years)	0.995 [0.962-1.030]	0.794	0.984 [0.956-1.013]	0.285	**1.042 [1.012-1.074]**	**0.006**

		Nadir CD4	0.999 [0.996-1.001]	0.308	1.009 [0.990-1.030]	0.347	0.997 [0.994-1.000]	0.060
		Current CD4	1.000 [0.998-1.002]	0.765	1.011 [0.995-1.027]	0.189	0.999 [0.997-1.001]	0.503
		HIV undetectability (<40 copies)	0.502 [0-105-2.388]	0.386	-	0.998	0.199 [0.039-1.005]	0.051
Relational determinants	Sexual orientation	MSM and bisexual	1.163 [0.646-2.092]	0.615	0.739 [0.455-1.202]	0.223	0.995 [0.613-1.614]	0.984
	PDE-5i use		0.897 [0.416-1.932]	0.780	0.856 [0.447-1.641]	0.640	3.355 [1.734-6.491]	<0.001
	Stable relationship		1.043 [0.599-1.817]	0.881	0.800 [0.495-1.291]	0.360	0.999 [0.625-1.596]	0.996
Psychological determinants	Antidepressant drugs use		3.014 [0.686-13.231]	0.144	0.867 [0.358-2.095]	0.751	1.313 [0.549-3.139]	0.541
	Benzodiazepines use		1.659 [0.472-5.838]	0.430	1.135 [0.439-2.932]	0.794	1.032 [0.409-2.602]	0.947

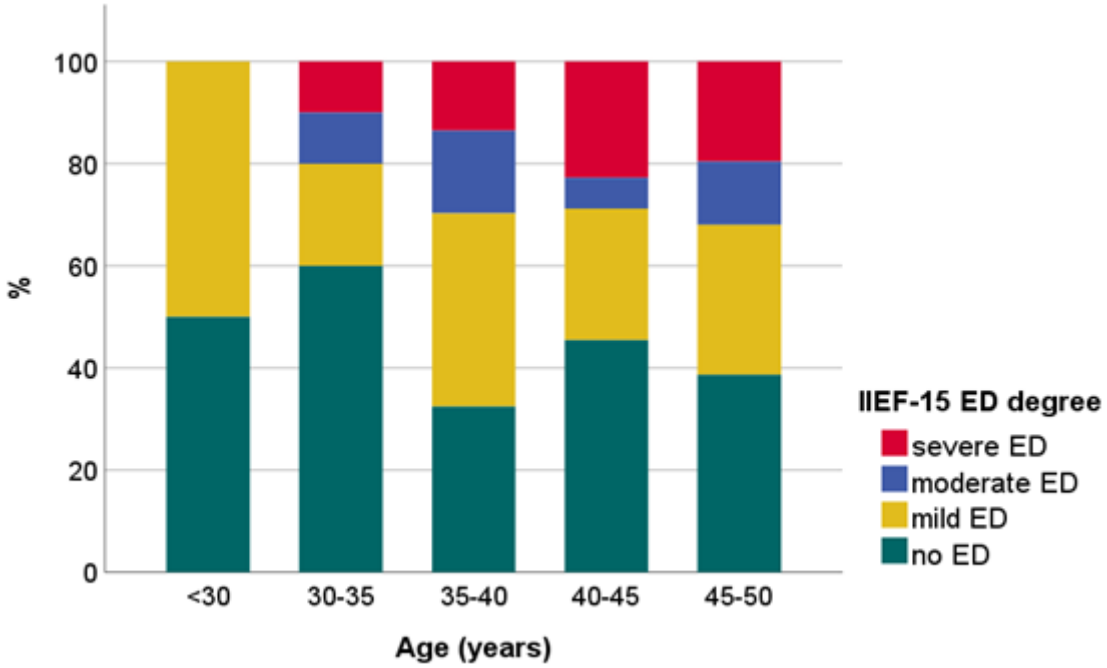
[Footnote to Table 3]: *Abbreviations: CI: confidence interval; ED: erectile dysfunction; BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; W/H: Waist/hip circumference ratio; ART: Antiretroviral Therapy; MSM: men who have sex with men; PDE-5i: phosphodiesterase 5-inhibitors; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; PRL: Prolactin; E2: Estradiol; TT: Total Testosterone; SHBG: Sex Hormone-Binding Globulin; cFT: calculated Free Testosterone; DHT: Dihydrotestosterone; HOMA: Homeostasis Model Assessment; HbA1c: Glycated Hemoglobin; HDL: high density lipoprotein; LDL: low-density lipoprotein.*

Figure 1. Prevalence of sexual dysfunctions according to IIEF-15 domains (A) and to SIEDY scale (B).



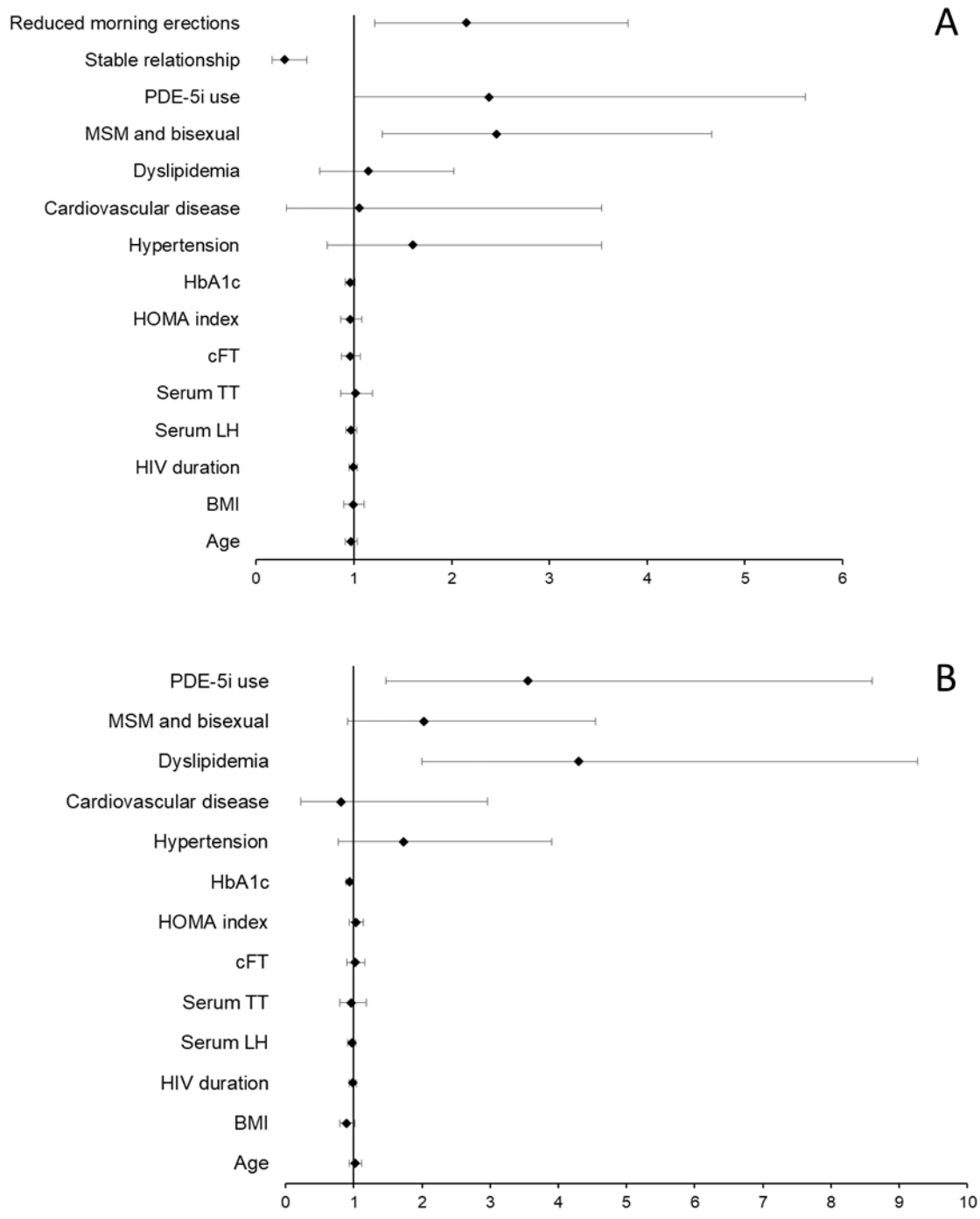
[Footnote to Figure 1] *Abbreviations: IIEF: validated International Index of Erectile Function; ED: erectile dysfunction; SIEDY: Structured Interview of Erectile Dysfunction.*

Figure 2. Distribution of ED severity according to patients' age range.



[Footnote to Figure 2] *Abbreviations: IIEF: validated International Index of Erectile Function; ED: erectile dysfunction.*

Figure 3. Forest-Plot for the multivariate analysis performed to identify significant determinants of ED (A) and organic ED (B).



[Footnote to Figure 3] *Abbreviations: ED: erectile dysfunction; MSM: men who have sex with men; BMI: Body Mass Index; LH: Luteinizing Hormone; TT: Total Testosterone; cFT: calculated Free Testosterone; HOMA: Homeostasis Model Assessment; HbA1c: Glycated Hemoglobin; PDE-5i: phosphodiesterase 5-inhibitors.*

Supplementary table 1. Tools used to classify the 6 sexual function domains analyzed: 1. erectile dysfunction with its organic, relational and psychological components; 2. reduced sexual desire; 3. impaired orgasmic function; 4. reduced frequency of morning erections.

1. Erectile dysfunction IIEF-15	1.1 Organic determinants	SIEDY scale 1
	1.2 Relational determinants	SIEDY scale 2
	1.3 Psychological determinants	SIEDY scale 3
2. Reduced sexual desire		IIEF-15 sexual desire domain Semi-structured medical interview
3. Impaired orgasmic function		IIEF-15 orgasmic function domain
4. Reduced frequency of morning erections		Semi-structured medical interview

[Footnote to Supplementary Table 1] *Abbreviations: PDE-5i: phosphodiesterase 5-inhibitors; IIEF: validated International Index of Erectile Function; SIEDY: Structured Interview of Erectile Dysfunction.*

Supplementary table 2. Comparison of subgroups of patients with ED in relation to ED degree according to IIEF-15 erectile function domain score. Continuous variables are reported as median (IQR).

	n.v.	MLWH with mild ED (IIEF-15 17-25)	MLWH with moderate ED (IIEF-15 11-16)	MLWH with severe ED (IIEF-15 6-10)	*p*-value
n 187		93	35	59	
Age (years)	-	46.8 (41.5-49.8)	48.6 (43.7-50.0)	47.4 (44.1-50.0)	0.377
MSM and bisexual		70 (75.3%)	28 (80.0%)	40 (67.8%)	0.387
Anthropometrical variables					
BMI (kg/m^2)	18.5-25	23.5 (21.7-25.4)	24.1 (22.5-25.8)	23.6 (22.1-25.5)	0.300
W/H circumference ratio	< 0.95	0.93 (0.88-0.99)	0.95 (0.93-0.98)	0.95 (0.91-0.98)	0.253
Sexual function parameters					
Impaired SIEDY scale 1 (> 3)		30 (32.6%)	12 (34.3%)	22 (37.3%)	0.840
Impaired SIEDY scale 2 (≥ 2)*		14 (26.4%)	2 (10.5%)	9 (31.0%)	0.252
Impaired SIEDY scale 3 (≥ 3)		44 (47.8%)	18 (51.4%)	30 (50.8%)	0.906
Reduced libido		71 (78.9%)	30 (85.7%)	54 (91.5%)	0.113
Reduced morning erections		46 (49.5%)	17 (48.6%)	28 (47.5%)	0.971
Hormonal measurements					
LH (mIU/mL)	1.4-8.9	4.7 (3.4-6.5)	4.3 (3.5-6.3)	5.3 (3.6-8.1)	0.380
FSH (mIU/mL)	1.7-6.9	5.4 (3.9-8.3)	5.4 (4.2 -7.2)	6.3 (4.3-10.8)	0.377
PRL (ng/mL)	2.1-17.7	7.3 (5.7-9.5)	8.5 (5.0-12.3)	7.8 (5.9-10.3)	0.649
Serum E2 (pg/mL)	<50	26.4 (20.6-31.1)	24.8 (18.1-30.5)	21.3 (17.3-29.2)	0.161
Serum TT (ng/dL)	>320	689 (545-802)	612 (407-804)	594 (464-745)	**0.043**
E2/TT		0.035 (0.029-0.043)	0.038 (0.029-0.047)	0.038 (0.027-0.050)	0.824
SHBG (nmol/L)	13.5-71.4	46.6 (37.1-63.2)	46.8 (32.1-62.4)	46.5 (34.3-73.3)	0.556
cFT (pg/mL)	>64	111.8 (94.5-138.0)	96.0 (75.2-135.9)	95.1 (69.2-125.4)	**0.015**
DHT (pg/mL)	165-679	411 (294-518)	356 (261-612)	403 (256-546)	0.743
Biochemical measurements					
Fasting glucose (mg/dl)	70-100	92 (87-96)	92 (87-97)	94 (85-101)	0.555
Insulin (mIU/ml)	2-23	6.8 (4.6-11.9)	8.5 (5.7-13.5)	8.9 (5.3-14.0)	0.111
HOMA Index	< 2.5	1.54 (0.99-2.78)	1.81 (1.25-3.24)	2.13 (1.17-3.07)	0.069
HbA1c (mmol/mol)	20-38	33.0 (31.0-35.8)	34.0 (32.0-36.6)	34.4 (32.8-37.7)	**0.025**
Total Cholesterol (mg/dl)	< 200	186 (161-214)	1585 (154-208)	189 (163-221)	0.351
LDL Cholesterol (mg/dl)	< 100	117 (93-143)	110 (93-126)	118 /101-145)	0.258
HDL Cholesterol (mg/dl)	> 45	49 (38-60)	42 (34-55)	43 (34-55)	0.153
Tryglicerides (mg/dl)	< 180	111 (76-166)	141 (105-205)	139 (91-188)	**0.025**
Lifestyle and drug use					
Smoking		31 (33.7%)	15 (42.9%)	31 (52.5%)	0.071

Alcohol use (moderate/intense)		49 (52.7%)	21 (60.0%)	29 (49.2%)	0.594
Opioids use		18 (19.4%)	4 (11.4%)	17 (28.8%)	0.118
PDE-5i use		20 (21.5%)	7 (20.0%)	8 (22.6%)	0.462

[Footnote to Supplementary Table 2] *Abbreviations: IQR: interquartile range; n.v.: normal values; BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; W/H: Waist/hip circumference ratio; ART: Antiretroviral Therapy; MSM: men who have sex with men; PDE-5i: phosphodiesterase 5-inhibitors; IIEF: validated International Index of Erectile Function; SIEDY: Structured Interview of Erectile Dysfunction; EF: erectile function; ED: erectile dysfunction; SD: sexual desire; LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; PRL: Prolactin; E2: Estradiol; TT: Total Testosterone; SHBG: Sex Hormone-Binding Globulin; cFT: calculated Free Testosterone; DHT: Dihydrotestosterone; HOMA: Homeostasis Model Assessment; HbA1c: Glycated Hemoglobin; HDL: high density lipoprotein; LDL: low-density lipoprotein; HBV: hepatitis B virus; HCV: hepatitis C virus. Mann-Whitney test and Chi-square test were used to compare continuous and categorical variables, respectively.*

Chapter 6

Conclusions

CONCLUSIONS

The studies presented in this thesis show overall the high prevalence of gonadal and sexual dysfunctions in MLWH, as a result of complex interplays between organic and psychological factors, with the latter being the predominant. Knowing the relations among main determinants of testosterone deficitency or erectile dysfunction and how to detect them is necessary for an adequate management in clinical practice.

In detail, data reported in **Chapter 3** provide evidence on the strict interplay between health status (assessed through multimorbidity and frailty), body composition, and sex steroids in MLWH. Although it is not possible to establish a cause-effect relationship among all these factors due to the cross-sectional design of the study, these findings allow starting to move from the interpretation of low testosterone in MLWH as a condition of true hypogonadism to that of biochemical functional hypogonadism.

The study in **Chapter 4** shows that testosterone deficiency is still a common finding in MLWH under 50, confirming the process of premature aging related to the infection *per se* and to the antiretroviral agents. Notwithstanding the strong correlation found between the two laboratory assays for testosterone measurement, the prevalence of male hypogonadism results underestimated when chemiluminescence is used compared to mass spectrometry. In clinical practice, the presence of testosterone deficiency should be adequately detected through the measurement of SHBG and gonadotropins to screen/rule out even forms of compensated hypogonadism.

Lastly, **Chapter 5** shows that sexual dysfunctions are very common in MLWH younger than 50 years old, being present in more than half of patients. Within the multidimensional network of erectile dysfunction in MLWH, the psychological component was identified as a major determinant, highlighting the contribution of peculiar factors related to HIV psychological burden (e.g., disclosure interactions, stigma) rather than gonadal status and other classical risk factors. From a clinical standpoint, this chapter also highlights a general under-management of sexual issues in the daily clinical practice. Since sexual health is a primary component of well-being, these findings

serve as a reminder of the importance of addressing sexual dysfunctions in MLWH through a tailored and multidisciplinary clinical approach aiming at improving quality of life.

As a general conclusion, it can be said that the clinical conditions of MLWH have been notably changed over the last decades thanks to medical advances. Beyond the management of the mere viral infection, other unmet medical neeeds are emerging deserving attention in this population. Both gonadal and sexual dysfunctions represent two of the most common endocrine HIV-related diseases even in young adults. Furthemore, hypogonadism and sexual dysfunction, especially erectile dysfunction, could play as hallmarks of an impaired general health status. For this reason, awareness about sexual health should be reinforced to adequatly address it in the daily clinical practice aiming at improving the well-being of MLWH and, in turn, the overall quality of life.

Chapter 7

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