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ORIGINAL ARTICLE

Testosterone level and mortality in elderly men with systolic chronic heart failure

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Previous studies on the prognostic significance of serum levels of androgens in patients with chronic heart failure (CHF) have yielded conflicting results. The aim of this study was to examine the relationship between serum concentration of testosterone and mortality in men with systolic CHF. A total of 175 elderly men (age $\geqslant$ 60 years) with CHF were recruited. Total testosterone (TT) and sex hormone-binding globulin (SHBG) were measured, and estimated free testosterone (eFT) was calculated. The median follow-up time was 3.46 years. Of these patients, 17 had a TT level below 8 nmol l^{-1} (230 ng dl^{-1}), 27 had an eFT level below 0.225 nmol l^{-1} (65 pg ml^{-1}) and 12 had both. Using the age-specific tenth percentiles of TT and eFT in healthy men in our laboratory as cutoff points, the prevalences of TT and eFT deficiency was 21.7% (38/175) and 27.4% (48/175), respectively. Both TT and eFT were inversely associated with left ventricular ejection fraction (LVEF) and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) (all $P<0.01$). Kaplan–Meier curves for patients in low, medium and high tertiles according to TT and eFT level showed significantly different cumulative survival rate (both $P<0.01$ by log-rank test). However, after adjustment for clinical variables, there were no significant associations of either TT or eFT levels with survival time (OR=0.97, 95% CI: 0.84–1.12, $P=0.28$ and OR=0.92, 95% CI: 0.82–1.06, $P=0.14$, respectively). Our study showed that levels of TT and eFT are commonly decreased in elderly patients with systolic CHF and related to disease severity, but they are not independent predictors for mortality.

INTRODUCTION

Chronic heart failure (CHF) is a major health problem throughout the world.[1] In China, the prevalence of CHF was estimated to be 0.9% for the general population, 0.7% and 1.0% for the men and the women, respectively, and the prevalence of CHF increased substantially with age.[2] Previous studies have shown that androgen deficiency in men with CHF, and levels of testosterone, dehydroepiandrosterone and its sulfate have been reported to be decreased with increasing severity of heart failure.[3–6] It has been suggested that a low testosterone level may represent one of the factors contributing to the anabolic/catabolic imbalance characteristically present in many patients with advanced CHF,[4,6–9] and recent interventional studies have indicated that physiological testosterone supplementation increases functional capacity and muscle performance in both male[10–13] and female[14] patients with CHF. However, until now, only a few studies on the prognostic significance of serum levels of androgens in CHF patients have been published and yielded conflicting results.[15,16] The purpose of the present study was to evaluate prospectively the prevalence and prognostic consequences of androgens deficiencies in a cohort of elderly male patients with systolic CHF.

MATERIALS AND METHODS

Study population

From 1 March 2005 to 31 May 2007, consecutive elderly male patients with CHF and reduced left ventricular ejection fraction (LVEF) who attended the outpatient clinics in three cardiology departments (Chinese PLA Geneal Hospital, the 306th Hospital of Chinese PLA, and the Chinese PLA Air Force Hospital, Beijing, China) were enrolled in this prospective study. The criteria for study inclusion were: (i) age 60 years or over; (ii) a documented history of CHF for at least 6 months; (iii) LVEF of 45% or less as assessed by echocardiography; and (iv) clinical stability and unchanged medications for at least 1 month preceding the study. Patients with cirrhosis and alcoholism were excluded because these conditions can cause low testosterone concentrations.[17]

During the recruitment period, 181 patients who were suitable for the study and who agreed to participate were identified. In six patients, blood analyses on hormones were not feasible due to storage failure; thus, the current report refers to 175 subjects.

The study protocol was approved by the ethics committees of the participating hospitals and all patients gave written informed consent.

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Serum concentrations of androgens and other chemical measurements

Venous blood samples were taken in the morning between 07:00 and 08:00 following an overnight fast and after a supine rest of at least 15 min. After centrifugation, serum blood was collected and frozen at –70 °C until it was analyzed. Serum concentrations of total testosterone (TT) was measured by electrochemiluminescence (ACS:180; Bayer, Leverkusen, Germany). The inter-assay and intra-assay variability coefficients for TT were 6.5% and 7.0%, respectively. Serum level of sex hormone-binding globulin (SHBG) was measured with ELISA (BL, Hamburg, Germany), and the inter-assay and intra-assay variability coefficients were 5.2% and 3.0%, respectively, as described previously.[18] Serum level of estimated free testosterone (eFT) was calculated with the validated equation of Vermeulen *et al.*[19] Serum N-terminal pro-brain natriuretic peptide (NT-pro-BNP) was measured using an enzyme-linked immunosorbent assay microtiter plate (Roche Diagnostics, Basel, Switzerland). All measurements were performed at the Laboratory of the Endocrinology, Department of Chinese PLA General Hospital and by staff unaware of the clinical data. In our laboratory, the tenth percentiles of TT were 13.88 nmol l^{-1} (400.00 ng dl^{-1}) for healthy men aged 60–69 years and 12.35 nmol l^{-1} (356.00 ng dl^{-1}) for men aged 70 years or over, and the tenth percentiles of eFT were 0.22 nmol l^{-1} (6.20 ng dl^{-1}) for men aged 60–69 years and 0.19 nmol l^{-1} (5.80 ng dl^{-1}) for men aged 70 years or over.[18] Estimated glomerular filtration rate was calculated using the formula from the Modification of Diet in Renal Disease Study Group.[20] As comparison, we also used the criteria for initiation of testosterone treatment as proposed by the current recommendations[21,22] for data analysis.

Mortality data

Patient status (dead or alive) was ascertained between May and July 2010 by contacting the patient's family. Follow-up was 100% complete.

Statistical analyses

Data were presented as mean±s.d. when normally distributed, median (25th–75th percentile) when not-normally distributed, or number (frequency). Comparisons across groups were made using unpaired two-sided *t*-test, the χ^2 test, Mann–Whitney *U* test, Kruskal–Wallis test or analysis of variance, where appropriate. Correlations between serum level of TT or eFT and New York Heart Association (NYHA) class, LVEF or NT-pro-BNP were assessed by Spearman's correlation coefficient. The association of total and free testosterone levels with all cause mortality was determined by Cox proportional hazards regression.

In order to estimate the effect of the serum levels of TT and eFT on 5-year survival rates, Kaplan–Meier curves for cumulative survival were constructed for patients in low, medium and high tertiles according to TT and eFT levels, respectively. Differences in survival rates were tested with the Cox–Mantel log-rank test.

All tests were performed using the statistical software package SPSS (version 15.0.1; SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline clinical characteristics

Table 1 shows the baseline clinical characteristics of the entire cohort and of the cohort divided into tertiles based on the LVEF. Mean age at baseline was 68.5±5.4 years. Patients in lower tertiles of LVEF were characterized by older age, advanced NYHA class, decreased serum TT and eFT levels, increased SHBG concentration, decreased hemoglobin and plasma albumin concentration, decreased estimated glomerular filtration rate and increased serum NT-pro-BNP level. There were no significant differences among the three tertiles with regard to etiology (ischemic *vs.* no ischemic), mean systolic blood pressure, prevalence of comorbidities (atrial fibrillation, hypertension and diabetes) or medication use (angiotensin-converting enzyme inhibitor or angiotensin II receptor-1 blocker, β-blocker, digoxin and diuretics).

Prevalence of androgens deficiency

The median level of TT in all 175 patients was 20.3 nmol l^{-1} (25th–75th percentile: 17.0–27.2 nmol l^{-1}), and that of eFT was 0.28 nmol l^{-1} (25th–75th percentile: 0.21–0.35 nmol dl^{-1}) (**Table 1**). Using the age-specific tenth percentiles of TT and eFT in healthy men in our laboratory as aforementioned as cutoff points, the prevalences of TT and eFT deficiency were 21.7% (38/175) and 27.4% (48/175), respectively. Of the 175 patients, 17 had a TT level below 8 nmol l^{-1} (230 ng dl^{-1}), 27 had an eFT level below 0.225 nmol l^{-1} (65 pg ml^{-1}) and 12 had both a TT level below 8 nmol l^{-1} and an eFT level below 0.225 nmol l^{-1}. Using the criteria for initiation of testosterone treatment as proposed by the current recommendations,[21,22] that is, either a TT level below 8 nmol l^{-1} (230 ng dl^{-1}) or an eFT level below 0.225 nmol l^{-1} (65 pg ml^{-1}), regardless of age, 32% (56/175) of the patients would be suggested to have hypogonadism by laboratory diagnosis.

Serum androgen level and CHF severity and prognosis

To assess the relationship between serum androgen level and CHF severity, correlation coefficients between serum level of TT or eFT and NYHA class, LVEF and plasma NT-pro-BNP were computed. There was no significant correlation between TT or eFT level and NYHA class ($r=-0.12$, $P=0.14$ and $r=-0.09$, $P=0.24$, respectively), but both TT and eFT levels were significantly correlated with LVEF ($r=0.30$, $P<0.01$ and $r=0.31$, $P<0.01$, respectively) and NT-pro-BNP ($r=-0.21$, $P=0.005$ and $r=-0.23$, $P=0.003$, respectively).

During a median follow-up period of 3.46 years (25th–75th percentile: 3.01–3.90 years) for survivors, 54 patients (30.9%) died. The crude mortality risks in tertiles (from low to high) of free testosterone were 46.6%, 24.1% and 22.0%. **Figure 1** shows the Kaplan–Meier curves for cumulative survival rate for patients in low, medium and high tertiles according to TT and eFT levels ($\chi^2=11.82$, $P=0.003$ and $\chi^2=9.60$, $P=0.008$, respectively).

Using the age-specific criteria for TT and eFT deficiency defined in our laboratory, the crude mortalities in patients with and without TT deficiency were 39.5% (15/38) and 28.5% (39/137), respectively ($\chi^2=1.69$, $P=0.19$), and in patients with and without eFT deficiency, they were 47.9% (23/48) and 24.4% (31/127), respectively ($\chi^2=9.02$, $P=0.003$). In additional analysis, using the criteria for initiating testosterone treatment for males as proposed by the current recommendations,[21,22] the crude mortality in patients who had either a TT level below 8 nmol l^{-1} or an eFT level below 0.225 nmol l^{-1} was 42.9% (24/56), as compared the mortality of 25.2% (30/119) in those patients who met neither of the criteria ($\chi^2=5.56$, $P=0.023$).

Multivariable analyses

Table 2 shows the results of multivariable Cox regression, with survival time as the dependent variable, and confounding clinical and laboratory parameters listed in **Table 1** as independent variables. After adjustment for other variables, only age, LVEF and NYHA class

Table 1 Baseline characteristics of study patients

	All patients (n=175)	Tertiles of LVEF		
		Low (n=58)	Medium (n=58)	High (n=59)
Clinical variables				
Age, year	68.5±5.4	69.1±5.3	68.4±5.8	67.9±5.2
NYHA class, n (%)				
1–2	112 (64.0)	13 (11.6)	40 (35.7)	59 (52.7)
3–4	63 (36.0)	35 (55.6)	19 (30.2)	9 (14.3)
LVEF (%)	35.6 (2.4)	31.3 (3.1)	35.9 (1.9)	39.6 (2.4)
Systolic blood pressure, mmHg	120.2±14.8	119.4±15.4	118.6±14.5	122±14.6
Ischemic etiology, n (%)	96 (54.9)	29 (50.0)	33 (56.9)	34 (57.6)
Comorbidities, n (%)				
Atrial fibrillation	36 (20.6)	14 (23.7)	12 (20.6)	10 (17.2)
Hypertension	97 (55.4)	31 (53.4)	30 (51.7)	36 (61.0)
Diabetes	32 (18.3)	13 (22.4)	9 (15.5)	10 (16.9)
Medication, n (%)				
ACE inhibitor or ARB	158 (90.3)	53 (91.4)	55 (94.8)	50 (84.7)
β-blocker	119 (68.0)	40 (69.0)	38 (65.5)	41 (69.5)
Digoxin	62 (35.4)	21 (36.2)	20 (34.5)	21(35.6)
Diuretic	159 (90.9)	54 (93.1)	51 (87.9)	54 (91.5)
Laboratory variables				
Estimated GFR, (ml min^{-1} per 1.73 m^2)	66.0±22.3	62.6±23.5	66.3±22.3	69.2±21.2
NT-pro-BNP, pg ml^{-1}	1854 (386–3474)	2442 (648–4468)	1816 (474–3642)	1168 (288–2258)
Plasma albumin (g l^{-1})	41.4±4.6	40.5±5.5	41.6±3.8	41.8±4.1
Hemoglobin (g l^{-1})	13.4±1.9	13.2±2.0	13.5±1.9	13.6±1.9
TT (nmol l^{-1}), median (25th–75th percentile)	20.3 (17.0–27.2)	17.8 (11.6–22.8)	20.4 (14.2–26.8)	24.0(18.4–32.6)
eFT (nmol l^{-1}), median (25th–75th percentile)	0.28 (0.21–0.35)	0.25 (0.12–0.21)	0.28 (0.18–0.34)	0.31(0.22–0.44)
SHBG (nmol l^{-1}), median (25th–75th percentile)	56.4 (36.8–68.2)	61.2 (42.4–76.4)	54.8 (38.8–62.2)	48.8(34.6–56.0)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor-1 blocker; eFT, estimated free testosterone; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SHBG, sex hormone-binding globulin; TT, total testosterone.

were significantly associated with survival time. Although in univariable analyses or after adjustment for age, both serum TT and eFT levels were associated with patient survival time, after adjustment for the full variables, their prognostic significances were abolished (for TT, OR=0.97, 95% CI: 0.84–1.12, P=0.279; for eFT, OR=0.92, 95% CI: 0.82–1.06, P=0.135).

DISCUSSION

There were two major findings in our present study. First, in an unselected cohort of older men with systolic CHF, we found a relatively high prevalence of reduced serum concentrations of androgens. Serum TT and eFT levels were below the tenth percentile of their healthy peers in 21.7% and 27.4% of patients, respectively. Using the criteria for
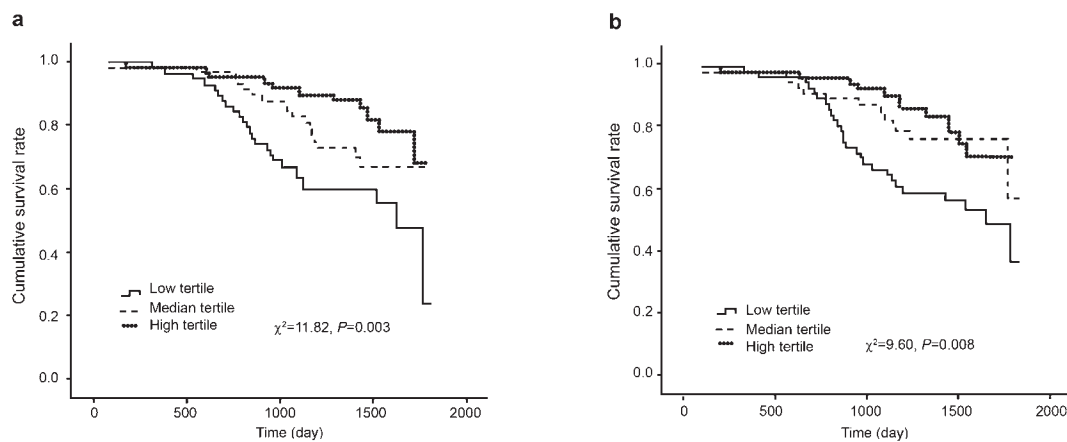

Figure 1 Kaplan–Meier curves reflecting 5-year cumulative survival rates in patients with systolic chronic heart failure according to tertiles of serum total testosterone (a) and serum free testosterone (b). (a) Tertiles of serum total testosterone were divided into low (<15.6 nmol dl^{-1}), medium (15.7–25.8 nmol dl^{-1}) and high (≥25.9 nmol dl^{-1}). There was significant difference in survival rate among tertiles of serum total testosterone (χ^2=11.82, P=0.003, by log-rank test). (b) Similarly, tertiles of serum free testosterone were divided into low (<0.21 nmol dl^{-1}), medium (0.21–0.35 nmol dl^{-1}) and high (≥0.36 nmol dl^{-1}). There was significant difference in survival rate among tertiles of serum free testosterone (χ^2=9.60, P=0.008, by log-rank test).

Table 2 Adjusted hazard ratios for all-cause mortality

	HR[a]	95% CI for HR	P value
Age, per year	1.07	1.01–1.13	0.034
NYHA class, per 1 advanced class	1.97	1.37–2.82	0.000
LVEF, per 1%	0.94	0.90–0.98	0.002
TT, per 1 nmol l^{-1}	0.97	0.84–1.12	0.279
eFT, per 0.01 nmol l^{-1}	0.92	0.82–1.06	0.135
SHBG, per 1 nmol l^{-1}	1.08	0.88–1.26	0.246

Abbreviations: CI, confidence interval; eFT, estimated free testosterone; HR, hazard ratio; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; SHBG, sex hormone-binding globulin; TT, total testosterone.
[a] HRs were adjusted for all variables listed in **Table 1**.

initiating testosterone treatment for males as proposed by the current recommendations,[21,22] nearly one-third of our patients would be considered to have laboratory diagnosed late-onset hypogonadism. In addition, both serum TT and eFT levels were correlated with LVEF and plasma NT-pro-BNP level, suggesting the relationship between androgen level and CHF severity; second, we had shown that in men with stable CHF and reduced LVEF, lower levels of serum TT and eFT were associated with poor prognosis. During the mean follow-up time of about 3 and half years, patients with testosterone deficiency, whether defined by our local laboratory criteria, or by current guideline criteria, had higher mortality than those patients without testosterone deficiency. However, these associations were not independent to other conventional clinical prognostic indicators.

Androgen deficiency is known to occur in several chronic disorders as well as in aging,[23,24] and may even play a role in the pathogenesis of cardiovascular disease.[25] Some studies have reported low plasma levels of testosterone in patients with CHF, whereas others have not.[6] A major problem in making comparisons between studies is the definition of testosterone deficiency. In recently published recommendations for diagnosis and treatment of late-onset hypogonadism, a TT less than 230 ng dl^{-1} was suggested as a lower normal range and a free testosterone level below 225 pmol l^{-1} (6.5 ng dl^{-1}) was suggested as thresholds when considering testosterone supplementation.[21,22] In the present study, we defined testosterone deficiency as below the tenth percentile for TT and eFT levels in age groups from a large population of 1080 healthy male subjects.[18] Because age is one of the major determinants of testosterone level in men,[18,23,24] we think that a definition of testosterone deficiency should not be based on threshold values that are constant across the entire age spectrum.

We found associations of decrease in serum TT and eFT levels with reduced LVEF or increased NT-pro-BNP, but not with NYHA class. These findings are in contrast with results reported by Jankowska et al.,[15] who found that serum TT and eFT levels correlated inversely with NYHA class in men with CHF, but neither TT nor eFT level correlated with echocardiographic or humoral indices of left ventricle function. As further comparisons, in patients with systolic and diastolic heart failure, Güder and colleagues[16] found that free testosterone, but not TT, was inversely associated with NYHA class and plasma NT-pro-BNP. The divergent and equivocal results, as well as the rather weak, although statistically significant correlations, suggest that serum androgen levels are not good surrogate of disease severity in CHF patients.

Until now, two studies have investigated the prognostic significance of androgens in heart failure. In a study of 208 men with CHF, Jankowska et al.[15] reported that TT and eFT, as well as DHEAS and IGF-1 levels, were predictive of mortality, independent of age, LVEF,

NYHA class, GRF and pro-BNP levels. More recently, Güder et al.,[16] in their multivariable analyses of 191 patients with CHF, found that after adjustment of variables that were associated with both the exposure and the outcome, neither TT nor eFT, DHEAS or SHBG represent independent predictors of mortality risk. The results of our present study are similar to the findings of Güder et al.[16] Although both serum TT and eFT levels were associated with patient survival time in univariable analyses, after adjustment for confounding factors, their prognostic significances were abolished. Our results, as well as those reported by Güder et al.,[16] suggest that androgens are unlikely to play a causal role in the progression of heart failure.

In spite of the uncertain role of testosterone in the pathophysiology of CHF, several clinical trials have examined the effect of testosterone therapy in CHF and have showed promising results. In an uncontrolled open-label trial, Tomoda found that treatment with oxymetholone improved cardiac function.[10] In a small double-blinded study, Pugh et al.[11] showed that intramuscular testosterone injections improved quality of life and functional capacity in CHF patients. As a follow-up to this pilot study, Malkin et al.[12] demonstrated similar effects of testosterone patches. More recently, in a double-blind, placebo-controlled, randomized trial in 70 elderly patients with CHF, Caminiti et al.[13] showed that intramuscular testosterone supplementation on top of optimal therapy improves functional exercise capacity, muscle strength, insulin and baroreflex sensitivity. These therapeutic effects were also observed in female patients.[14] Therefore, testosterone treatment may represent a new therapeutic approach in CHF that might have some potential.

Several limitations of the present study should be mentioned. First, the results are based on a single testosterone measurement in the study subjects, which may inadequately reflect the average sex hormone release. However, the distributions of both TT and eFT levels found in this study were consistent with our previous reports of healthy men; second, the modest sample size may have limited the power of the analyses; third, we used electrochemiluminescence for measuring serum testosterone levels, rather than the current mass spectrometry-based method as recommended by the Endocrine Society;[26] therefore, TT concentration might be overestimated in our study, as reflected in the relatively higher levels of TT and eFT compared to other published reports; fourth, our study population did not include all the consecutive patients who met the study criteria, and the information regarding patients who refused to participate had not been collected; this may possibly lead to bias in our analysis.

In summary, our present study showed that in men with systolic heart failure, androgen deficiency was relatively common and low TT or eFT levels were associated with disease severity and long-term mortality. However, the prognostic utility of TT and eFT depended on other clinical factors, suggesting that androgens are unlikely to play a causal role in the progression of heart failure, and the mechanisms of the reported beneficial effects of testosterone treatment for CHF patient remain to be elucidated.

AUTHOR CONTRIBUTIONS

HYW designed the study, performed the statistical analysis and drafted the manuscript. XFW and JHW participated in patient enrollment, collection of clinical data and helped to draft the manuscript. JYL participated in the study design and measurement of serum concentrations of androgens. All authors read and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declared no competing financial interests.

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