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Low Plasma Testosterone Is Associated With Elevated Cardiovascular Disease Biomarkers

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ABSTRACT

Background: The relation between testosterone (T) plasma concentration and cardiovascular (CV) risk is unclear, with evidence supporting increased risk in men with low and high T levels. Few studies have assessed CV risk as a function of plasma T levels using objective biomarkers.

Aim: To determine the relation between T levels and high-sensitivity CV risk biomarkers.

Methods: Ten thousand forty-one male patients were identified in the database of a commercial clinical laboratory performing biomarker testing. Patients were grouped by total T concentration and associations with the following biomarkers were determined: cardiac troponin I (cTnI), endothelin-1 (ET-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-17A, N-terminal pro—B-type natriuretic peptide (NTproBNP), high-density lipoprotein (HDL) cholesterol, high-sensitivity C-reactive protein (hs-CRP), hemoglobin A_{1c} (HbA_{1c}), and leptin.

Outcomes: Association of CV risk markers with levels of T in men.

Results: The median age of the cohort was 58 years (interquartile range = 48—68), and the median plasma T level was 420 ng/dL (interquartile range = 304—565); T levels did not vary with patient age. An inverse relation between plasma T levels and CV risk was observed for 9 of 10 CV markers: cTnI, ET-1, IL-6, TNF-α, NTproBNP, HDL cholesterol, hs-CRP, HbA_{1c}, and leptin. Even after adjusting for age, body mass index, HbA_{1c}, hs-CRP, and HDL cholesterol levels, the CV markers IL-6, ET-1, NTproBNP, and leptin were significantly associated with a T level lower than 250 ng/dL.

Clinical Implications: Men with low T levels could be at increased risk for increased CV disease as seen by increased CV risk markers.

Strength and Limitations: This study was performed in a group of 10,041 men and is the first study to examine CV risk associated with circulating T levels using a large panel of 10 objective biomarkers. This study is limited by an absence of clinical data indicating whether men had pre-existing CV disease or other CV risk factors.

Conclusion: Men with low plasma T levels exhibit increases in CV risk markers, consistent with a potential increased risk of CV disease. **Pastuszak AW, Kohn TP, Estis J, Lipshultz LI. Low Plasma Testosterone Is Associated With Elevated Cardiovascular Disease Biomarkers. J Sex Med 2017;XX:XXX—XXX.**

Key Words: Hypogonadism; Biomarkers; Cardiovascular Diseases; Troponin I; Endothelin-1; Interleukin-6; Tumor Necrosis Factor-α; Interleukin-17; Pro—Brain Natriuretic Peptide; Leptin

INTRODUCTION

Men begin to experience a decrease in testosterone (T) levels after 30 years of age.[1] This decrease in plasma T levels can be a harbinger of declining health; hypogonadal men have nearly twice the mortality risk of men with normal T levels.[2] Studies have found that hypogonadism is associated with an increased risk of cardiovascular disease (CVD).[3,4] The relation between T level and CV risk has been a controversial topic in recent years; several studies have shown that T therapy (TTh) is associated with an increase in CV events,[5—7] whereas most meta-analyses

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have demonstrated no association between T and CV events.[8–14] Although multiple studies have observed that TTh in hypogonadal men decreases the risk of all-cause mortality compared with untreated hypogonadal men,[2,15,16] other studies have found no relation between TTh and CVD.[17,18] Still other studies have supported the conclusion that untreated hypogonadal men can have an increased risk of CV events.[3,19,20]

Although most studies investigating the relation between T levels and CV risk have used CV outcomes such as myocardial infarction and stroke to determine risk, none have linked CV risk with a panel of high-sensitivity (hs) biomarkers. High-sensitivity immunoassays can quantify circulating levels of cardiac troponin I (cTnI), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-17A (IL-17A), and endothelin-1 (ET-1). Small increases in hs-cTnI levels, even in the absence of clinical symptoms, are a sign of underlying cardiomyocyte injury and cardiac disease.[21] Studies have shown that asymptomatic men and women with cTnI levels above the 99th percentile of the reference range have a significantly increased risk of CVD, coronary heart disease, and all causes of death.[22] Increased plasma IL-6 levels are observed in men with atherosclerosis and are associated with a threefold increased risk of death from CV causes.[23] Increased levels of TNF-α in asymptomatic men are associated with clinical and subclinical CVD and heart failure.[24] Another cytokine, IL-17A, mediates immune and inflammatory responses, and high levels in asymptomatic men are associated with worse atherosclerosis and vessel wall plaque instability.[23,25,26] Increased levels of ET-1 accelerate development of atherosclerotic disease by inducing smooth muscle cell hyperplasia[27] and are a predictor of increased 10-year mortality in otherwise asymptomatic individuals.[28]

In addition to these novel biomarkers, traditional CV risk biomarkers including N-terminal pro—brain natriuretic peptide (NTproBNP), high-density lipoprotein (HDL) cholesterol, high-sensitivity C-reactive protein (hs-CRP), leptin, and hemoglobin A1c (HbA_{1c}) can facilitate detection of subclinical heart disease including myocardial cell damage, vulnerable vessel wall plaque, and vascular inflammation.[29–33] Increased NTproBNP, a peptide released with myocardial stretching, can predict up to sixfold higher mortality and hospitalization for CV reasons in asymptomatic patients.[34] Low HDL cholesterol levels are a known health risk, and for every 10% decrease in HDL cholesterol, the risk of CVD increases by 13%.[35] Apparently healthy men with increased hs-CRP levels are at 1.5- to 3-fold increased risk of CV events.[36] HbA_{1c} also predicts future CVD. Every 1% increase in HbA_{1c} is associated with a 20% to 30% increase in CV events and all-cause mortality.[37] Together, these 10 biomarkers can facilitate determination of CV risk.

Several studies have found associations between these CV biomarkers and hypogonadism. Dhindsa et al[38] found an increase in TNF-α, CRP, and leptin in hypogonadal men. Increased IL-6 and CRP levels also have been reported in men with low T levels.[39] In a study of more than 3,000 men, Colangelo et al[40] observed that men with low T levels were at increased risk for diabetes mellitus and increased HbA_{1c} levels compared with eugonadal men. Although several studies have examined the relation between T levels and CV risk using individual biomarkers, in this study we present our findings examining the association between T levels and CV risk using a large panel of 10 objective biomarkers that have been linked to CV health.

METHODS

Study Design and Subject Identification

In collaboration with Singulex Clinical Laboratories (SCL; a laboratory certified by the Clinical Laboratory Improvement Amendments and accredited by the College of American Pathologists; Alameda, CA, USA), we accessed de-identified patient data from the SCL database from January 2013 through September 2014. Laboratory tests found in this database are marketed for patients with or at risk for CVD, as determined by their community-based physician. All men older than 18 years with data on circulating levels of total T, hs-cTnI, hs-IL-6, hs-TNF-α, and hs-IL-17A were included for analysis (N = 10,041). Biomarker results for leptin, NTproBNP, HDL cholesterol, hs-CRP, ET-1 and HbA_{1c}, were included when available (92%, 95%, 88%, 93%, 28%, and 75%, respectively). For each patient, all samples for all 10 biomarkers were drawn on a single day; 47% of tests were ordered by primary care providers, 22% of tests were ordered by cardiologists, 17% of tests were ordered by internists, 7% of tests were ordered by osteopathic providers, and 7% were ordered by other specialists.

Testing Methods

Immunoassays for hs-cTnI, hs-IL-6, hs-TNF-α, hs-IL-17A, and hs-ET-1 were used to quantify plasma concentrations. Leptin was measured using a plate-based sandwich immunoassay and a standard plate spectrophotometer. Total T, NTproBNP, HDL cholesterol, hs-CRP, and blood HbA_{1c} were measured on the Roche Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA). The total T reportable range was 2.5 to 7,500 ng/dL, functional sensitivity was 12 ng/dL, standardized by isotope dilution, gas chromatography, and mass spectrometry, repeatability CV range was 1.5% to 10.2%, and day-to-day CV range was 2.4% to 18.5% based on samples at 1,450 and 4.5 ng/dL, respectively.

Reference Limits

The hs-cTnI, hs-IL-6, hs-TNF-α, hs-IL-17A, hs-ET-1, and leptin assays were laboratory-developed tests with reference limits (RLs) determined as the 99th percentile from a reference population of apparently healthy subjects without CVD (95th percentile for leptin). The RL for T in the SCL, derived from internal standardization testing, is 250 ng/dL. The RLs for NTproBNP, HDL, hs-CRP, and HbA_{1c} were based on manufacturer-determined standards for the instrument.