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Free testosterone index and bioavailable testosterone as independent predictors of good coronary collateral circulation in CTO patients

Abdullah Yıldırım[1], Mustafa Lütfullah Ardıç[1], Emre Paçacı[2], Aslan Erdoğan[3], Akif Küçükcan[4], Mükremin Coşkun[1], Emre Sezici[1], Fadime Koca[5], Eyüp Özkan[3], Hazar Harbalıoğlu[1], Ömer Genç[3], İbrahim Halil Kurt[1]

[1]*Department of Cardiology, Adana City Training and Research Hospital, Adana, Turkiye*
[2]*Department of Cardiology, Osmaniye State Hospital, Osmaniye, Turkiye*
[3]*Department of Cardiology, Başakşehir Çam and Sakura City Hospital, İstanbul, Turkiye*
[4]*Department of Microbiology, Çukurova State Hospital, Adana, Turkiye*
[5]*Department of Cardiology, Çukurova State Hospital, Adana, Turkiye*

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ABSTRACT

Aims: Chronic total occlusion (CTO) represents a clinically important form of coronary artery disease in which collateral circulation plays a critical role in maintaining myocardial perfusion. Although testosterone has been suggested to influence vascular function and angiogenesis, the relationship between androgenic parameters and coronary collateral development remains uncertain.

Methods: This cross-sectional case-control study included 230 male patients diagnosed with CTO by coronary angiography. Coronary collateral circulation was graded using the Rentrop classified categorized as good CCF (score 2–3) or bad CCF (score 0–1). Serum levels of total testosterone, free testosterone, SHBG, dehydroepiandrosterone sulfate (DHEAS), and dihydrotestosterone (DHT) were measured, and bioavailable testosterone (BioT) and free testosterone index (FTI) were calculated. Associations between androgenic parameters and collateral circulation were analyzed using correlation analysis, logistic regression, and ROC curve analysis.

Results: Of the study cohort, 142 patients had good CCF and 88 had bad CCF. Patients with good CCF had higher levels of total testosterone ($p<0.001$), DHEAS ($p=0.048$), BioT ($p<0.001$), BioT percentage ($p<0.001$), and FTI ($p<0.001$), whereas SHBG was higher in the bad CCF group ($p<0.001$). Free testosterone and DHT did not differ between groups. In multivariable logistic regression, BMI (aOR=1.163, 95% CI:1.044 to 1.296, $p=0.006$) and FTI (aOR=1.573, 95% CI:1.345 to 1.841, $p<0.001$) were independently associated with good CCF. ROC analysis demonstrated strong predictive performance for FTI (AUC=0.815, 95% CI:0.727 to 0.884, $p<0.001$) and BioT (AUC=0.804, 95% CI:0.715 to 0.875, $p<0.001$), both superior to total or free testosterone.

Conclusion: Serum testosterone–related indices, particularly FTI and BioT, emerged as independent predictors of good coronary collateral circulation in male patients with CTO. Incorporating these parameters into clinical evaluation may provide additional prognostic information and help guide therapeutic strategies.

Keywords: Chronic total occlusion, coronary collateral circulation, testosterone, bioavailable testosterone, free testosterone index

INTRODUCTION

Chronic total occlusion (CTO) represents an important subgroup of coronary artery disease (CAD). In the presence of CTO, the preservation of myocardial viability and function requires the development of alternative blood flow pathways. This adaptive process is mediated by coronary collateral circulation. Coronary collaterals are anastomotic connections that form between different segments of the same artery or between different coronary arteries, and they can partially maintain myocardial perfusion.[1-3] Collateral vessel formation involves arteriogenesis, angiogenesis, and vasculogenesis, processes that are closely related to endothelial function, cellular proliferation, inflammatory responses, and various biochemical factors.[4]

Emerging evidence indicates that hormonal regulators may also play a significant role in collateral development. Testosterone, a steroid hormone with pleiotropic effects on the cardiovascular system, metabolism, and nervous system, has received particular attention.[5] Sex hormone–binding globulin

Corresponding Author: Abdullah Yıldırım, dr.yildirimabdullah@gmail.com

(SHBG), a key determinant of testosterone bioavailability, has been associated with several metabolic disorders.[6] Both experimental and clinical studies have suggested that testosterone may reduce myocardial ischemia, protect against reperfusion injury, improve endothelial function, exert anti-inflammatory effects, and promote angiogenesis.[7,8]

Nevertheless, findings regarding the association between testosterone levels and coronary collateral circulation have yielded conflicting results. Directly measured parameters such as total or free testosterone may not fully capture biological activity, whereas calculated indices such as bioavailable testosterone (BioT) and the free testosterone index (FTI) may provide a more accurate reflection of androgenic activity.

Therefore, the aim of this study was to investigate the relationship between testosterone and its related parameters—total testosterone, SHBG, BioT, and FTI—and coronary collateral circulation in male patients with CTO, and to evaluate the potential predictive value of BioT and FTI for the development of good collateral circulation.

METHODS

Ethics

The protocol was approved by the Çukurova University Clinical Researches Ethics Committee (Date: 02.10.2020, Decision No: 104-21), and written informed consent was obtained from all patients. The study complied with the Declaration of Helsinki, revised in 2013.

Study Population

This cross-sectional case-control study included 230 male patients who were admitted to our center between January 2018 and February 2020 and diagnosed with CTO on coronary angiography. Evidence of ischemia prior to angiography was confirmed by non-invasive tests such as myocardial perfusion scintigraphy and exercise testing. Patients with a history of coronary artery bypass surgery (n=103), history of percutaneous coronary intervention (n=106), heart failure (left ventricular ejection fraction <40% or NYHA class III/IV) (n=86), chronic kidney disease (defined as creatinine clearance <60 ml/min/1.73 m^2) (n=74), those receiving hormone therapy (n=10), patients with endocrine disorders (n=14), or active malignancy (n=30) were excluded. Since hormone levels may be affected by acute coronary syndromes, only patients with stable CAD were included in the study. **Figure 1** shows the flowchart of the study population.

Each patient's medical history, comorbidities, and taken medication data were obtained from national health registry systems (e-nabız), health records, and patient interviews. Basic demographic data including age, smoking history, body-mass index (BMI), and history of diabetes and hypertension were recorded. Diabetes mellitus was defined as a fasting blood glucose level ≥126 mg/dl and/or a previous diagnosis of diabetes with ongoing treatment. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or current use of antihypertensive medication.

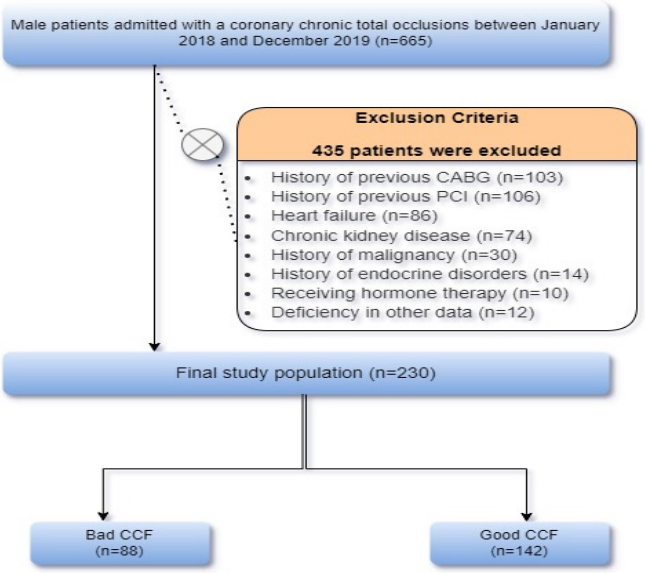

Figure 1. The flowchart illustrates the selection process of patients diagnosed with chronic total occlusion who underwent coronary angiography and were subsequently categorized into good or poor coronary collateral flow groups according to the rentrop classification

Echocardiographic examinations were performed during hospitalization by independent cardiologists in accordance with the recommendations of the European Association of Cardiovascular Imaging.[9] BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

CTO Definition, and Coronary Collateral Circulation

In all patients, coronary angiography was performed using a biplane angiography system (SIEMENS AXIOM Artis, Siemens Healthcare, Erlangen, Germany) via either the femoral or radial approach with 5–7 French catheters. During the procedure, each main coronary artery was imaged in at least two different projections. The coronary arteries were visualized in left and right oblique projections using cranial and caudal angulations. Measurements were obtained at end-diastole in the projection where the lesion was best visualized and showed the greatest lumen narrowing. Coronary flow was evaluated using the thrombolysis in myocardial infarction (TIMI) flow classification (grades 0 to 3).[10]

Coronary CTO was defined as TIMI grade 0 flow in an occluded segment with an estimated duration of more than 3 months. Patients with documented TIMI 0 flow at least 3 months prior to the index procedure or those with stable anginal symptoms persisting without change for at least 3 months were considered to have long-standing occlusion. The chronicity of lesions exceeding 3 months was confirmed by clinical history and angiographic findings.[3]

Collateral filling was evaluated angiographically using the Rentrop classification (0–3): Rentrop 0, no collateral filling; rentrop 1, very weak collateral flow without epicardial filling; rentrop 2, partial perfusion with opacification but incomplete filling of epicardial arteries; and rentrop 3, complete perfusion with full opacification of the epicardial vessels. Patients were

classified into two groups based on the rentrop score: "good CCF" (score 2–3) and "bad CCF" (score 0–1).[11]

All index cine coronary angiograms were analyzed at our center by consensus of two expert operators (one from the study team and one independent observer). In case of disagreement, an additional evaluation was performed by a third independent expert operator. SYNTAX score was calculated as previously defined.[12]

Laboratory Analysis

Laboratory analyses were performed using venous blood samples obtained from the antecubital vein at the time of admission. Samples were analyzed with the fully automated Beckman UniCel DXC 800 Synchron system (Beckman Coulter Inc., CA, USA). Serum high-sensitivity cardiac troponin I (hs-cTnI) levels were measured using the chemiluminescence method on a UniCel DXI 800 Synchron autoanalyzer (Beckman Coulter Inc., CA, USA). Estimated glomerular filtration rate (eGFR) was calculated using the creatinine-based modification of diet in renal disease (MDRD) equation. After at least 8 hours of fasting, blood samples obtained between 08:00 and 10:00 a.m. were used to measure serum total testosterone, free testosterone, dehydroepiandrosterone sulfate (DHEAS), and SHBG levels. Free testosterone was measured using the equilibrium dialysis method; total testosterone and DHEAS were determined by radioimmunoassay (RIA). SHBG levels were assessed using an electrochemiluminescence immunoassay. Directly measured parameters included SHBG, free testosterone, DHEAS, dihydrotestosterone (DHT), total testosterone, and albumin. Calculated parameters included BioT, the percentage of BioT, the free/total testosterone ratio, and the total testosterone/SHBG ratio.

BioT was estimated using the Vermeulen equation,[13] which incorporates total testosterone, SHBG, and albumin, and is conceptually defined as free testosterone plus albumin-bound testosterone.

The FTI was calculated as the ratio of total testosterone to SHBG multiplied by 100: (total testosterone/SHBG)×100.

The percentage of BioT was calculated as the proportion of BioT relative to total testosterone, using the formula: (BioT/total testosterone×100)×100.

The free/total testosterone ratio was defined as free testosterone relative to total testosterone, calculated as: free testosterone/total testosterone.

Statistical Analyses

The data analyses were performed using R statistical software (v4.5.2, Vienna, Austria). The normality of variables was assessed using the Kolmogorov–Smirnov test, supported by visual inspection of histograms and probability plots. Continuous variables were expressed as mean±standard deviation for normally distributed data and as median (interquartile range; IQR 25–75) for non-normally distributed data. Categorical variables were presented as numbers (percentages). Fisher's exact test or the Chi-square test (expected frequency >5) was used for comparisons of categorical variables, while independent Student's t-test or Mann–Whitney U test was applied for comparisons of continuous variables between groups

Predictors of the good collateral circulation were identified using logistic regression for each parameter. All clinical and angiographic characteristics were analyzed. Variables with $p<0.1$ in univariable analyses were included in the multivariable model. Odds ratio (OR), adjusted odds ratio (aOR), and 95% confidence interval (CI) were reported for all regression analyses. Multicollinearity was assessed using the variance inflation factor (VIF), with values >3 considered indicative of significant multicollinearity. The goodness-of-fit of logistic regression models was evaluated using the Hosmer–Lemeshow test. Receiver operating characteristic (ROC) curves and areas under the curve (AUC) were analyzed to assess the discriminative accuracy of total testosterone, FTI, BioT, and other parameters. Statistical comparisons of AUCs were conducted using the DeLong method. All analyses used two-sided tests, with a significance level set at 0.05.

RESULTS

The 230 patients included in the study were divided into two groups according to coronary collateral flow (CCF): bad CCF (n=88) and good CCF (n=142). The mean age was lower in the good CCF group compared with the bad CCF group (62.1±10.5 vs. 57.6±9.7 years, p=0.001). However, the mean BMI (27.4±3.6 vs. 28.5±3.7, p=0.024) and LVEF (49.5±9.6 vs. 54.8±9.5, p<0.001) were significantly higher in the good CCF group. In laboratory parameters, albumin (37.7±4.4 vs. 39.5±2.0, p<0.001), platelet count (241±87 vs. 277±105, p=0.007), HDL-C (41±9 vs. 46±9, p<0.001), total cholesterol (191±49 vs. 212±46, p=0.002), and triglycerides (183±128 vs. 244±168, p=0.005) were significantly higher in the good CCF group. White blood cell count (10.07±4.62 vs. 8.58±2.49, p=0.002) was higher in the bad CCF group. In terms of androgens and hormone levels, total testosterone (2.28±1.07 vs. 3.17±0.81, p<0.001) and DHEAS (120.1±49.4 vs. 156.9±75.2, p=0.048) were higher in the good CCF group. SHBG (39.1±18.6 vs. 30.8±9.5, p<0.001) was higher in the bad CCF group. Free testosterone and DHT levels were similar between the two groups (p=0.084 and p=0.307, respectively). BioT (88.4±40.4 vs. 150.2±43.8, p<0.001) and BioT percentage (40.3±10.6 vs. 47.7±8.1, p<0.001) levels were higher in the good CCF group. The free/total testosterone ratio was similar between the two groups (p=0.094). FTI (6.45±3.40 vs. 11.06±3.85, p<0.001) was significantly higher in the good CCF group (**Figure 2**). In terms of angiographic and procedural characteristics, there was no difference between the groups regarding the distribution of vessels with CTO lesions (p=0.269). The mean SYNTAX score was significantly lower in the good CCF group (31.1±10.6 vs. 27.2±10.2, p=0.007). Successful revascularization was also more frequent in this group (p=0.038) (**Table 1**). In correlation analysis, a strong negative correlation was observed between SYNTAX score and BioT (Spearman's rho=-0.253, 95% CI: -0.371 to -0.128, p<0.001; Kendall's Tau=-0.173, bootstrap 95% CI: -0.263 to -0.069, p<0.001) as well as between SYNTAX score and FTI (Spearman's rho=-0.277, 95% CI: -0.394 to -0.151, p<0.001; Kendall's Tau=-0.194, bootstrap 95% CI: -0.286 to -0.096, p<0.001) (**Figure 3A, 3B**).

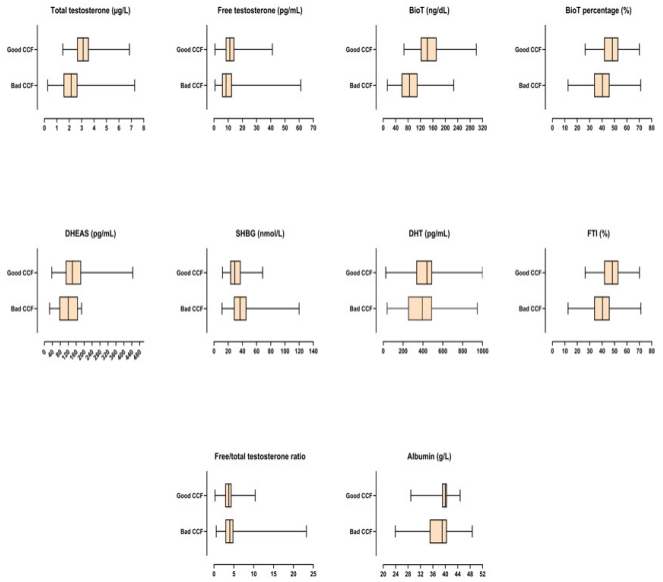

Figure 2. Comparison of testosterone-related parameters between patients with good and poor coronary collateral flow
BioT: Bioavailable testosterone, DHEAS: Dehydroepiandrosterone sulfate, DHT: Dihydrotestosterone, FTI: Free testosterone index, SHBG: Sex hormone–binding globulin

Table 1. Demographic, laboratory, and angiographic features of the overall population

	All population (n= 230)	Bad CCF (n= 88)	Good CCF (n=142)	p*
Basic characteristics and admission parameters				
Age, years	59.3±10.2	62.1±10.5	57.6±9.7	0.001
BMI, kg/m^2	28.1±3.7	27.4±3.6	28.5±3.7	0.024
LVEF, %	52.8±9.8	49.5±9.6	54.8±9.5	<0.001
Hypertension, n (%)	107 (46.5)	46 (52.3)	61 (43.0)	0.177
Diabetes mellitus, n (%)	119 (51.7)	40 (45.5)	79 (55.6)	0.205
Dyslipidemia, n (%)	65 (28.3)	23 (26.1)	42 (29.6)	0.573
Family history of CAD, n (%)	56 (24.3)	7 (7.9)	49 (34.5)	0.386
Smoking, n (%)	115 (50.0)	39 (44.3)	76 (53.5)	0.222
Laboratory findings				
Glucose, mg/dl	120 (99-158)	122 (102-171)	114 (96-155)	0.197
Urea, mg/dl	38.1±16.1	37.7±15.6	38.8±17.1	0.716
Creatinine, mg/dl	0.90±0.20	0.88±0.21	0.92±0.19	0.196
e-GFR, ml/min/1.73 m^2	92.2±19.2	90.8±17.4	94.9±15.1	0.059
CRP, mg/L	13.0 (5.1-32.9)	14.0 (4.8-36.1)	12.6 (5.0-31.7)	0.854
Albumin, g/L	38.8±3.2	37.7±4.4	39.5±2.0	<0.001
WBC, x10^3/µL	9.15±3.53	10.07±4.62	8.58±2.49	0.002
Hemoglobin, g/dl	13.5±1.6	13.6±1.7	13.4±1.5	0.536
Platelets, x10^3/µL	263±99	241±87	277±105	0.007
HDL-C, mg/dl	44±10	41±9	46±9	<0.001
LDL-C, mg/dl	119±34	120±36	118±33	0.705
Total-C, mg/dl	204±48	191±49	212±46	0.002
Triglycerides, mg/dl	220±156	183±128	244±168	0.005
Total testosterone, µg/L	2.82±1.01	2.28±1.07	3.17±0.81	<0.001
DHEAS, pg/ml	151.3±72.8	120.1±49.4	156.9±75.2	0.048
SHBG, nmol/L	33.9±14.2	39.1±18.6	30.8±9.5	<0.001
Free testosterone, pg/ml	11.14±6.53	10.18±8.26	11.73±5.12	0.084
DHT, pg/ml	420.4±225.6	403.8±231.5	451.1±214.1	0.307
BioT, ng/dl	126.5±52.0	88.4±40.4	150.2±43.8	<0.001
BioT percentage, %	44.9±9.8	40.3±10.6	47.7±8.1	<0.001
Free/total testosterone ratio	3.83 (2.86-4.58)	3.99 (2.87-4.88)	3.73 (2.84-4.40)	0.094
FTI, %	9.28±4.31	6.45±3.40	11.06±3.85	<0.001
Angiographic and procedural features				
CTO vessel, n (%)				0.269
LAD	71 (30.9)	25 (28.4)	46 (32.4)	
LCx	45 (19.6)	23 (26.1)	22 (15.5)	
RCA	99 (43.0)	35 (39.8)	64 (45.1)	
Two vessels	15 (6.5)	5 (5.7)	10 (7.0)	
Right coronary dominance n (%)	113 (45.3)	43 (48.9)	70 (49.3)	0.949
Revascularization, n (%)	102 (44.3)	71 (31.6)	71 (49.7)	0.038
Syntax II score	28.6±10.5	31.1±10.6	27.2±10.2	0.007

The table continues

Values are presented as n (%), median (interquartile range [IQR]$_{25-75}$), or mean± standard deviation (SD) * A p-value of <0.05 was considered statistically significant. Abbreviations; BioT: Bioavailable testosterone, CAD: Coronary artery disease, CRP: C-reactive protein, CTO: Chronic total occlusion, DHT: Dihydrotestosterone, e-GFR: Estimated glomerular filtration rate, FTI: Free testosterone index, HDL-C: High-density lipoprotein cholesterol, LAD: Left anterior descending artery, LCx: Left circumflex artery, LDL-C: Low-density lipoprotein cholesterol, LVEF: Left ventricular ejection fraction, RCA: Right coronary artery, Total-C: Total cholesterol, WBC: White blood cell

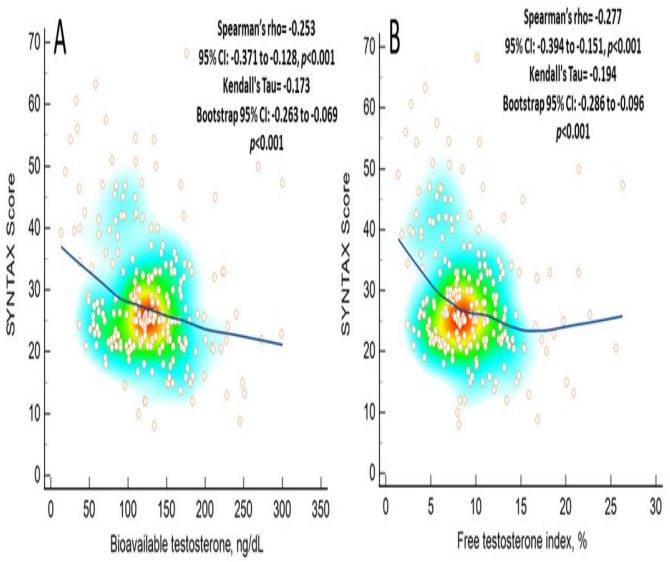

Figure 3. Correlation between SYNTAX score and testosterone-related indices. **A)** Scatter plot showing a negative correlation between SYNTAX score and BioT **B)** Scatter plot showing a negative correlation between SYNTAX score and FTI
BioT: Bioavailable testosterone, FTI: free testosterone index

Binary logistic regression analyses with covariate adjustment revealed the following associations: age (OR=0.955, 95% CI: 0.928 to 0.983, p=0.002), LVEF (OR=1.051, 95% CI: 1.021 to 1.081, p<0.001), FTI (OR=1.608, 95% CI: 1.403 to 1.843, p<0.001), SYNTAX score at admission (OR=0.954, 95% CI: 0.929 to 0.980, p<0.001), BMI (OR=1.091, 95% CI: 1.010 to 1.177, p=0.026), eGFR at admission (OR=1.019, 95% CI: 1.005 to 1.034, p=0.049), white blood cell count (OR=0.881, 95% CI: 0.808 to 0.961, p=0.004), platelet count (OR=1.004, 95% CI: 1.001 to 1.007, p=0.009), and total cholesterol (OR=1.010, 95% CI: 1.003 to 1.016, p=0.003) were independently associated with good CCF. In the multivariable model, BMI (aOR=1.163, 95% CI: 1.044 to 1.296, p=0.006) and FTI (aOR=1.573, 95% CI: 1.345 to 1.841, p<0.001) remained independently associated with good CCF (**Table 2**).

Comparison of Testosterone Levels for Coronary Collateral Flow Prediction

In ROC-curve analysis, FTI (AUC=0.815, 95% CI: 0.727 to 0.884, p<0.001), BioT (AUC=0.804, 95% CI: 0.715 to 0.875, p<0.001), total testosterone (AUC=0.743, 95% CI: 0.649 to 0.824, p<0.001), BioT percentage (AUC=0.730, 95% CI: 0.668 to 0.786, p<0.001), SHBG (AUC=0.671, 95% CI: 0.578 to 0.756, p<0.001), and free testosterone (AUC=0.733, 95% CI: 0.643 to 0.811, p<0.001) were independent predictors of good CCF (**Figure 4A, 4B**). When FTI was compared with BioT, it demonstrated a similar discriminative performance in predicting CCF (DBA=0.005, 95% CI: -0.020 to 0.030, p=0.705). However, when compared with free testosterone (DBA=0.227, 95% CI: 0.155 to 0.300, p<0.001) and total testosterone (DBA=0.074, 95% CI: 0.007 to 0.141, p=0.030), FTI demonstrated a significantly stronger discriminative performance (**Table 3**).

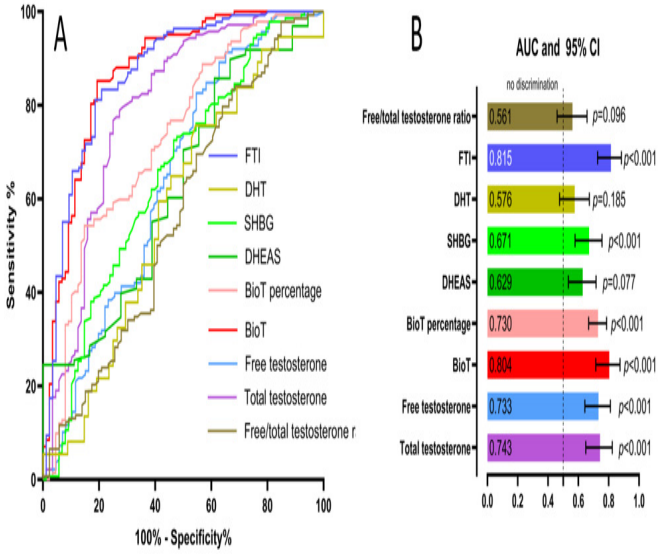

Figure 4. ROC curve analysis for predicting good coronary collateral flow. **A)** ROC curves for testosterone-related parameters including total testosterone, free testosterone, BioT, BioT percentage, FTI, SHBG, DHEAS, DHT, and the free/total testosterone ratio. **B)** AUC values with 95% CI for each parameter
AUC: Area under the curve, BioT: Bioavailable testosterone, CI: Confidence intervals, DHT: Dihydrotestosterone; DHEAS: Dehydroepiandrosterone sulfate, FTI: Free testosterone index, ROC: Receiver operating characteristic, SHBG: Sex hormone–binding globulin

Table 3. Pairwise comparison of AUC values between FTI and other testosterone-related parameters in predicting coronary collateral flow

	DBA[a]	95% CI[b]	Z statistic	p*
FTI vs. total testosterone	0.074	0.007 to 0.141	2.165	0.030
FTI vs. BioT	0.005	-0.020 to 0.030	0.378	0.705
FTI vs. free testosterone	0.227	0.155 to 0.300	2.681	<0.001

a The DeLong method was used to perform pairwise area under the curve comparisons of parameters to determine CCF, b Binomial exact, * A p-value of <0.05 was considered statistically significant. Abbreviations; BioT: Bioavailable testosterone, CI: Confidence interval, DBA: Differences between areas, FTI: Free testosterone index

DISCUSSION

The main findings of our single-center cross-sectional study were as follows: i) total testosterone, FTI, BioT, and SHBG levels were associated with good CCF, ii) the free/total testosterone ratio, DHEAS, and DHT levels were not associated with CCF, iii) a correlation was observed between SYNTAX score and both BioT and FTI, iv) in multivariable models, FTI remained independently associated with good CCF, and v) among patients with CTO, FTI showed a similar predictive performance to BioT but was superior to other androgenic indicators. These results suggest that, in addition to directly measured parameters such as total or free testosterone, FTI, BioT, and the percentage of BioT are associated with better coronary collateral circulation and greater cardiac reserve in patients with CTO.

Coronary collaterals are anastomotic connections that develop either between different segments of the same coronary artery or between different coronary arteries,

Table 2. Covariate and multivariable regression analyses to predict good coronary collateral flow

	Covariate			Model&		
	OR	95% CI	p*	a-OR	95% CI	p*
Age	0.955	0.928-0.983	0.002	0.987	0.940-1.036	0.593
LVEF	1.051	1.021-1.081	<0.001	1.011	0.969-1.054	0.618
SYNTAX score	0.954	0.929-0.980	<0.001	0.974	0.934-1.015	0.214
Estimated glomerular filtration rate	1.019	1.005-1.034	0.010	0.989	0.966-1.013	0.362
Body mass index	1.091	1.010-1.177	0.026	1.163	1.044-1.296	0.006
Free testosterone index	1.608	1.403-1.843	<0.001	1.573	1.345-1.841	<0.001
White blood cell count	0.881	0.808-0.961	0.004	0.965	0.857-1.088	0.561
Platelet count	1.004	1.001-1.007	0.009	1.001	0.997-1.005	0.515
Total cholesterol	1.010	1.003-1.016	0.003	1.007	0.999-1.015	0.104

* A p-value of <0.05 was considered statistically significant. & Model performance parameters: Nagelkerke R2=0.506, Brier score=0.086, AIC=122.18. Abbreviations; OR: Odds ratio, a-OR: Adjusted odds ratio, CI: Confidence interval, LVEF: Left ventricular ejection fraction

without the presence of an intervening capillary bed.[14] The development of coronary collaterals involves several mechanisms, including arteriogenesis, angiogenesis, and vasculogenesis.[15] Arteriogenesis refers to the opening and enlargement of pre-existing anastomotic channels in response to pressure gradients created by coronary stenosis or occlusion. Angiogenesis is defined as the formation of new vessels mediated by endothelial cell activation in response to cytokines released from ischemic and damaged myocardial cells. Collateral vasculogenesis, on the other hand, refers to the formation of new vessels through endothelial cell proliferation and remodeling processes.[16] Studies in aged mice have shown that androgens enhance progenitor cell production and mobilization, thereby improving ischemia-induced neovascularization.[17] Endothelial progenitor cell (EPC)–mediated vasculogenesis, a process that involves the production, mobilization, and recruitment of EPCs into new vessels, is critical for vascular repair and regeneration.[18] In ischemic heart disease, EPCs have been shown to contribute to the formation of the coronary collateral network. In patients with stable CAD, a positive correlation has been demonstrated between the number of circulating CD34+/CD133+ hematopoietic progenitor cells and the CCF index.[19]

In a cross-sectional study, levels of DHEA-S, total testosterone, free testosterone, and SHBG were found to be significantly lower in patients with CAD compared with controls, whereas estradiol and follicle-stimulating hormone levels were significantly higher; thus, androgenic hormone levels were suggested as potential predictors of CAD development in men.[20] In addition, reduced levels of circulating progenitor cells have been observed in hypogonadal men.[21] In a study of 115 patients with CTO, Erdoğan et al.[22] demonstrated that higher free and total testosterone levels predicted better collateral development. They also reported an association of DHEAS and SHBG levels with CCF, attributing these findings to the role of androgens in enhancing arteriogenesis and angiogenesis. Consistent with these findings, our study also demonstrated that higher androgen levels were associated with good coronary collateral circulation. This observation is in line with previous reports suggesting that androgens may enhance arterial blood flow, promote vasodilation, and contribute to angiogenesis.[23]

The higher prevalence of CAD in men compared with women has led to conflicting assumptions regarding whether serum testosterone levels may contribute to increased cardiovascular risk.[24,25] However, several studies have reported that the opposite is more likely.[25-28] Gururani et al.[8] reported a negative association between the GENSINI score, which reflects the severity of CAD, and both BioT and free testosterone levels. Köprülü et al.[29] found that low free testosterone levels were closely associated with higher SYNTAX scores in patients diagnosed with NSTEMI. Similarly, another study reported lower levels of DHEAS, free testosterone, and total testosterone in patients with CAD.[28] One of the most plausible mechanisms underlying this association is the conversion of testosterone to estrogen in peripheral adipose tissue via the aromatase enzyme, which may exert vasoprotective effects and slow the progression of atherosclerosis.[30] Another potential mechanism is the observed negative correlation between testosterone levels and insulin, fibrinogen, and plasminogen activator inhibitor in men with CAD.[8] The relative hypercoagulable state induced by low testosterone levels may also represent an important contributor to atherosclerosis development.[31,32] In contrast, another study found no association between SYNTAX score and either free or total testosterone levels.[25] Zeller et al.[33] reported that low testosterone concentrations were not associated with CAD or cardiac mortality in either men or women. These conflicting results may be explained by traditional confounding factors of cardiovascular disease such as age, hyperlipidemia, and genetic predisposition, as well as differences in study design and the relatively small sample sizes of previous investigations.[8,24,25,34] Another important consideration is that the biologically active forms of hormones differ from the total concentrations measured in circulation.[35] BioT and FTI are regarded as important androgenic parameters reflecting the biologically active forms. Malkin et al.[36] reported that BioT was more closely associated with CAD and mortality compared with total testosterone. Similarly, the NHANES study demonstrated an association between androgen levels and both CAD and mortality, and further reported that FTI was a more practical marker than BioT or total androgen levels. In addition, CRP levels and inflammatory burden were shown to be more closely correlated with these indices, which reflect active androgen levels, rather than with total androgen concentrations.[37] In line with these findings, our study also demonstrated that FTI and BioT showed a stronger association with good coronary collateral circulation.

The findings of this study demonstrate that coronary collateral circulation in patients with CTO is closely associated with androgenic hormone levels. In particular, calculated parameters such as FTI and BioT were found to be stronger predictors of collateral development than total or free testosterone alone. These observations provide important insights into the role of androgens in the pathophysiology of CAD. Our results suggest that hormonal profiles should be considered in the evaluation of coronary collateral circulation in future studies and may hold clinical relevance for the preservation of cardiac reserve.

Limitations

This study has several limitations. First, it was a single-center study with a relatively small sample size, which may limit the generalizability of the findings. Second, due to its cross-sectional design, causality between testosterone levels and coronary collateral circulation cannot be established. Third, as only male patients were included, the results cannot be extrapolated to women. Fourth, hormone levels were assessed at a single time point and therefore potential biological variability and circadian rhythm effects could not be accounted for. Fifth, detailed information on medication use (e.g., statins, ACE inhibitors, and hormone therapy) was not available, which may act as potential confounders.

CONCLUSION

As a result, serum testosterone levels are significantly associated with coronary collateral circulation in male

patients with CTO. In particular, FTI and BioT emerged as independent predictors of good collateral development. Incorporating these parameters into routine clinical evaluation may provide additional prognostic information and could help guide future therapeutic strategies.

ETHICAL DECLARATIONS

Ethics Committee Approval

The protocol was approved by the Çukurova University Clinical Researches Ethics Committee (Date: 02.10.2020, Decision No: 104-21).

Informed Consent

All patients signed a written informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Schaper W, Scholz D. Factors regulating arteriogenesis. *Thromb Vasc Biol*. 2003;23(7):1143-1151. doi:10.1161/01.ATV.0000069625.11230.96
2. Werner GS, Ferrari M, Heinke S, et al. Angiographic assessment of collateral connections in comparison with invasively determined collateral function in chronic coronary occlusions. *Circulation*. 2003; 107(15):1972-1977. doi:10.1161/01.CIR.0000061953.72662.3A
3. Sianos G, Werner GS, Galassi AR, et al. Recanalisation of chronic total coronary occlusions: 2012 consensus document from the EuroCTO club. *EuroIntervention*. 2012;8(1):139-145. doi:10.4244/EIJV8I1A21
4. Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res*. 2004;95(5): 449-458. doi:10.1161/01.RES.0000141145.78900.44
5. Kirby M, Hackett G, Ramachandran S. Testosterone and the heart. *Eur Cardiol*. 2019;14(2):103-110. doi:10.15420/ecr.2019.13.1
6. Sarkar M, VanWagner LB, Terry JG, et al. Sex hormone–binding globulin levels in young men are associated with nonalcoholic fatty liver disease in midlife. *Official J Am College Gastroenterol ACG*. 2019;114(5):758-763.
7. Soleimany A, Kavandi H, Khalili N, et al. The association between serum testosterone levels and coronary artery disease in men. *Shiraz E-Med J*. 2022;23(8):e116040. doi:10.5812/semj-116040
8. Gururani K, Jose J, George PV. Testosterone as a marker of coronary artery disease severity in middle aged males. *Indian Heart J*. 2016;68 Suppl 3(Suppl 3):S16-S20. doi:10.1016/j.ihj.2016.07.002
9. Baumgartner H, Hung J, Bermejo J, et al. Recommendations on the echocardiographic assessment of aortic valve stenosis: a focused update from the European Association of Cardiovascular Imaging and the American Society of Echocardiography. *Eur Heart J Cardiovasc Imaging*. 2017;18(3):254-275. doi:10.1093/ehjci/jew335
10. TIMI Study Group. The thrombolysis in myocardial ınfarction (TIMI) trial. Phase I findings. *N Engl J Med*. 1985;312(14):932-936. doi:10.1056/NEJM198504043121437
11. Rentrop KP, Cohen M, Blanke H, Phillips RA. Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol*. 1985; 5(3):587-592. doi:10.1016/s0735-1097(85)80380-6
12. Magro M, Nauta S, Simsek C, et al. Value of the SYNTAX score in patients treated by primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: the MI SYNTAXscore study. *Int J Cardiol Heart Vasc*. 2020;30:100643. doi:10.1016/j.ijcha.2020.100643
13. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666-3672. doi:10.1210/jcem.84.10.6079
14. Koerselman J, van der Graaf Y, de Jaegere PPT, Grobbee DE. Coronary collaterals: an important and underexposed aspect of coronary artery disease. *Circulation*. 2003;107(19):2507-2511. doi:10.1161/01.CIR.0000065118.99409.5F
15. Schaper W, Ito WD. Molecular mechanisms of coronary collateral vessel growth. *Circ Res*. 1996;79(5):911-919. doi:10.1161/01.res.79.5.911
16. Van Royen N, Piek JJ, Buschmann I, Hoefer I, Voskuil M, Schaper W. Stimulation of arteriogenesis; a new concept for the treatment of arterial occlusive disease. *Cardiovasc Res*. 2001;49(3):543-553. doi:10.1016/s0008-6363(00)00206-6
17. Lam YT, Lecce L, Yuen SC, et al. Androgens ameliorate impaired ischemia-induced neovascularization due to aging in male mice. *Endocrinology*. 2019;160(5):1137-1149. doi:10.1210/en.2018-00951
18. Tongers J, Roncalli JG, Losordo DW. Role of endothelial progenitor cells during ischemia-induced vasculogenesis and collateral formation. *Microvasc Res*. 2010;79(3):200-206. doi:10.1016/j.mvr.2010.01.012
19. Lambiase PD, Edwards RJ, Anthopoulos P, et al. Circulating humoral factors and endothelial progenitor cells in patients with differing coronary collateral support. *Circulation*. 2004;109(24):2986-2992. doi:10.1161/01.CIR.0000130639.97284.EC
20. Cao J, Zou H, Zhu BP, et al. Sex hormones and androgen receptor: risk factors of coronary heart disease in elderly men. *Chin Med Sci J*. 2010; 25(1):44-49. doi:10.1016/s1001-9294(10)60019-3
21. Foresta C, Caretta N, Lana A, et al. Reduced number of circulating endothelial progenitor cells in hypogonadal men. *J Clin Endocrinol Metab*. 2006;91(11):4599-4602. doi:10.1210/jc.2006-0763
22. Erdoğan A, Çakmak EÖ, Güler A, Kılıçgedik A, Kırma C. The relationship between levels of sex steroids and coronary collateral circulation in men patients with coronary artery disease. *Koşuyolu Heart Journal*. 2022;25(1):33-39.
23. Webb CM, McNeill JG, Hayward CS, De Zeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation*. 1999;100(16):1690-1696. doi:10.1161/01.cir.100.16.1690
24. D'Agostino RB Sr, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117(6):743-753. doi:10.1161/circulationaha.107.699579
25. Sezavar S, Hassanzadeh M, Samadanifard S, Zandi KF. Testosterone, coronary artery disease, and the syntax score in males: a case control study. *OA Text*. 2018;4:1-4. doi:10.15761/JIC.1000239
26. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. *Eur Heart J*. 2000;21(11):890-894. doi:10.1053/euhj.1999.1873
27. Kabakci G, Yildirir A, Can I, Unsal I, Erbas B. Relationship between endogenous sex hormone levels, lipoproteins and coronary atherosclerosis in men undergoing coronary angiography. *Cardiology*. 1999;92(4):221-225. doi:10.1159/000006977
28. Allameh F, Pourmand G, Bozorgi A, Nekuie S, Namdari F. The association between androgenic hormone levels and the risk of developing coronary artery disease (CAD). *Iran J Public Health*. 2016;45(1):14-19.
29. Köprülü D, Taşcanov MB. ST Elevasyonsuz Miyokart enfarktüsü geçiren erkek hastalarda serbest testosteron düzeyi ile koroner arter hastalığının ciddiyeti arasındaki ilişki. *Harran Üniversitesi Tıp Fakültesi Dergisi*. 2020;17(1):82-86. doi:10.35440/hutfd.671941
30. Nathan L, Shi W, Dinh H, et al. Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase. *Proc Natl Acad Sci U S A*. 2001;98(6):3589-3593. doi:10.1073/pnas.051003698

31. Phillips GB, Pinkernell BH, Jing TY. The association of hypotestosteronemia with coronary artery disease in men. *Arterioscler Thromb.* 1994;14(5):701-706. doi:10.1161/01.atv.14.5.701
32. Yang XC, Jing TY, Resnick LM, Phillips GB. Relation of hemostatic risk factors to other risk factors for coronary heart disease and to sex hormones in men. *Arterioscler Thromb.* 1993;13(4):467-471. doi:10.1161/01.atv.13.4.467
33. Zeller T, Appelbaum S, Kuulasmaa K, et al. Predictive value of low testosterone concentrations regarding coronary heart disease and mortality in men and women–evidence from the FINRISK 97 study. *J Intern Med*. 2019;286(3):317-325. doi:10.1111/joim.12943
34. Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile: the Framingham Study. *Am J Cardiol*. 1976;38(1):46-51. doi:10.1016/0002-9149(76)90061-8
35. Faix JD. Principles and pitfalls of free hormone measurements. *Best Pract Res Clin Endocrinol Metab*. 2013;27(5):631-645. doi:10.1016/j.beem.2013.06.007
36. Malkin CJ, Pugh PJ, Morris PD, Asif S, Jones TH, Channer KS. Low serum testosterone and increased mortality in men with coronary heart disease. *Heart*. 2010;96(22):1821-1825. doi:10.1136/hrt.2010.195412
37. Luo X, Wang Y, Wang L, Shen Y, Ren M. Association between female androgen levels, metabolic syndrome, and cardiovascular disease: an NHANES analysis (2013-2016). *Int J Womens Health*. 2024;16:2087-2101. doi:10.2147/ijwh.S475149