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ORIGINAL RESEARCH ARTICLE

Plasma testosterone and arrhythmic events in male patients with arrhythmogenic right ventricular cardiomyopathy

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Abstract

Aims Arrhythmogenic right ventricular cardiomyopathy (ARVC) is associated with life-threatening ventricular arrhythmia and progressive ventricular dysfunction. Previous studies suggested that sex hormones play an important role in the onset and prognosis of ARVC. This study aimed to investigate the role of testosterone in predicting major adverse cardiac events in the Chinese ARVC cohort.

Methods and results Ninety-nine ARVC patients (median age, 40 years; 70.7% male) and 96 healthy controls (median age, 41 years; 62.5% male) were enrolled. The circulating levels of testosterone were measured by enzyme-linked immunosorbent assays (ELISA). The median follow-up time of all ARVC male patients was 17 months (interquartile range/IQR 9–29). Cox proportional hazards regression was used to analyse the effect of plasma testosterone and other well-described risk factors on malignant arrhythmic events in male ARVC patients. The male ARVC patients had significantly elevated levels of total testosterone [TT, 6.390 (4.438–8.768) ng/mL vs. 3.617 (2.073–4.479) ng/mL, $P < 0.0001$, data shown as the median with IQR], bioavailable testosterone [BT, 4.11 (1.990–6.545) ng/mL vs. 1.32 (0.7965–2.0350) ng/mL, $P < 0.0001$, median with IQR], and free testosterone [FT, 0.2055 (0.1000–0.4073) ng/mL vs. 0.0768 (0.0405–0.1105) ng/mL, $P < 0.0001$, median with IQR] than healthy male volunteer, whereas no differences were observed among female counterparts. There was no significant correlation between the baseline clinical characteristics and testosterone levels in male ARVC patients (Spearman's correlation test, $P > 0.05$). During the follow-up, the levels of testosterone were higher in male patients who experienced malignant arrhythmic events ($N = 22$) than in those who did not ($N = 25$) [TT, 9.034 (7.222–15.370) ng/mL vs. 4.633 (3.363–6.375) ng/mL, $P < 0.001$; BT, 7.485 (2.070–9.163) ng/mL vs. 3.300 (1.685–4.690) ng/mL, $P < 0.05$; FT, 0.453 (0.221–0.758) ng/mL vs. 0.161 (0.075–0.337) ng/mL $P < 0.05$, data expressed as median (IQR) and adjusted by Dunn's multiple comparisons test], whereas such distinction was not observed among patients with significant structural progression events ($N = 16$). Through multivariable adjustments, the Cox regression analysis showed the level of plasma total testosterone (HR = 1.325, 95% confidence interval = 1.171–1.498, $P < 0.001$) was an independent predictor for malignant arrhythmic events.

Conclusions The levels of plasma testosterone in ARVC male patients are higher than those in healthy males. Testosterone level, without relation to the baseline cardiac function and future significant structural progression events, is a strong predictor of future adverse arrhythmic events in male patients with ARVC. Therefore, our results suggest that testosterone may be a useful biomarker in arrhythmic risk prediction in the ARVC.

Keywords Arrhythmogenic right ventricular cardiomyopathy; Testosterone; Biomarker; Risk prediction; Outcome

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Introduction

Arrhythmogenic cardiomyopathy is an inherited disease that is associated with life-threatening ventricular arrhythmias, particularly during adolescence and early adulthood. It is one of the leading causes of sudden cardiac death in the young and athletes.[1] This disease primarily affects the right ventricle (RV), and therefore, it is often called arrhythmogenic right ventricular cardiomyopathy (ARVC). In advanced stages of the disease, the left ventricle (LV) may also be involved, resulting in progressive heart failure (HF).[1–3] The pathological hallmarks of ARVC are progressive loss of cardiomyocytes and fibrofatty infiltration from epicardial to endocardial.[4,5] Pathogenic mutations in genes encoding desmosomal proteins account for ~50% of ARVC patients, but at least 10 other genes have also been implicated in this disease, such as *PLN*, *DES*, *LMNA*, and *TMEM43*.[2,6]

Patients with ARVC can be clinically quiescent, with occasional periods of flares involving increased arrhythmic activities, also known as *hot phases*.[2,7] The most important objective in the management of ARVC patients was prevention of disease exacerbations for it puts the patients at high risk of sudden cardiac death (SCD).[2] Nowadays, as implantable cardioverter defibrillators (ICD) proved to be useful for the prevention of SCD in ARVC, the remodelling of the cardiac structure and progressive heart failure is becoming other essential threats for disease prognosis.[8] However, there has been little attention focused on the ventricular dysfunction progress in ARVC.[7] A transatlantic cohort provided definitive evidence, revealing that structural progression was also found to be associated with adverse outcomes in ARVC.[9,10] Depending on the trend of disease progression (the abnormalities of electrophysiology or ventricular dysfunction progression), different treatments should be taken to achieve good clinical efficacy. Thus, it is necessary to identify risk predictors that could specifically predict the patients susceptible to adverse HF events or malignant arrhythmic events during the study's follow-up period. Clinically, risk stratification in ARVC mainly depends on the measurement of structural dysfunction detected by cardiac imaging and electrocardiogram findings.[11] Besides, some genetic or molecular indicators were also found helpful for diagnosis or risk stratification. Some desmosomal mutations such as plakophilin-2 (*PKP2*) and desmoplakin (*DSP*) mutations were also known to be associated with poor clinical outcomes.[9,12]

Also, plasma anti-DSG2 antibodies showed a high specificity and sensitivity for the diagnosis of ARVC.[13] Moreover, ARVC patients with higher plasma levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) had higher arrhythmic risks.[14] Likewise, other biomarkers such as plasma bridging integrator 1 (BIN1),[15] galectin-3,[16] BNP[17] and C-reactive protein (CRP)[18] levels also indicated increased risks of arrhythmic events in ARVC patients. Nevertheless, affected by other complications such as heart failure, these established markers are not specific for predicting arrhythmic outcomes. Hence, in consideration of the ambiguous status of these biomarkers in outcome discrimination and few reported multicentre validation, new biomarkers pinpointing particular outcome of AVRC and contributing to clear risk stratification are also necessary. Recently, promising evidence suggested that sex hormones, in particular testosterone, may play an essential role in predicting arrhythmic risk in ARVC.[19]

Testosterone is the principal male sex hormone and circulates in the blood mostly bound to sex hormone-binding globulin (SHBG) or albumin, with only a small fraction in a free form. Epidemiological studies suggested that high levels of testosterone could exacerbate heart failure symptoms in patients with systolic dysfunction.[20] Animal studies also showed that high testosterone levels were associated with increased arrhythmic risk.[21,22] In ARVC patients, elevated testosterone levels were evidenced to be valuable predictors for adverse arrhythmic outcomes.[19] Nevertheless, publications clarifying role of testosterone in ARVC outcomes are limited yet, and the relationship between testosterone levels and cardiac dysfunction in ARVC is still obscure. Therefore, we investigated the reproducibility of these findings by studying the role of testosterone in predicting major arrhythmic events in the Chinese ARVC cohort and further explored the role of this hormone in predicting cardiac structural progression.

Methods

Study population and clinical data collection

The following formulas are used to calculate the sample size and power, respectively.[23]

$$n_A = \kappa n_B \text{ and } n_B = \left(1+\frac{1}{\kappa}\right)\left(\sigma\frac{Z_{1-\alpha/2}+Z_{1-\beta}}{\mu_A-\mu_B}\right)^2$$

$$1-\beta = \Phi\left(Z-Z_{1-\alpha/2}\right)+\Phi\left(-Z-Z_{1-\alpha/2}\right),\quad Z=\frac{\mu_A-\mu_B}{\sigma\sqrt{\frac{1}{n_A}+\frac{1}{n_B}}}$$

where $\kappa = n_A/n_B$ is the matching ratio, σ is a standard deviation, Φ is the standard normal distribution function, α is Type I error, β is Type II error. When the $1-\beta$ (power) was set to 0.9, α was 2.5%, κ was 1, the $n_{AC\ male}$ and $n_{normal\ male}$ was calculated to be 60.

Ninety-nine AC patients (68 male) and 96 healthy volunteers (61 male) were selected from the Chinese ARVC cohort by stratified random sampling. Between October 2015 and July 2018, 118 (81 men) unrelated probands diagnosed with ARVC [24] were included in the Chinese ARVC cohort. The ARVC diagnosis was made according to the 2010 revised Task Force Criteria and confirmed by cardiologist and radiologist through the medical history, histological examination, and clinical

examination including echocardiography, ECG, CMR, and 24 h Holter. All the ARVC probands were of Chinese Han nationality.

Supporting Information, *Table S1* shows the details of the 2010 Revised Task Force Criteria of the 68 male ARVC patients, which relied on the demonstration of structural, functional, and electrophysiological abnormalities. Given physical exercise could promote both structural progression and arrhythmic events in ARVC,[2] we excluded ARVC patients or healthy volunteers with competitive or endurance sport activity to avoid introducing associated cofounding factors. This made the physical activity levels between ARVC patients and control approximate comparable.

The control subjects did not have history of heart disease, hypertension, or metabolic disease. All patients underwent measurements of circulating levels of testosterone. The baseline demographics and medical details of ARVC patients were obtained retrospectively from patient charts at blood withdrawal and during follow-up by investigators blinded to the assay results. The follow-up started from blood collection and came to an end with observations of clinical endpoint events. Malignant arrhythmic events were defined as SCD, survived SCD, ventricular fibrillation (VF), sustained ventricular tachycardia (VT), and appropriate ICD discharge. ARVC patients with ventricular dysfunction had a high probability of undergoing heart transplantation or death during follow-up in the Fuwai cohort;[7] therefore, according to the previous literature,[8] we defined the significant structural progression event (SSPE) as a numerical reduction of 10% in LVEF based on echocardiography, heart transplantation, or death due to end-stage heart failure. The patients were followed-up for a median of 17 (interquartile ranges, IQR) months[9–29] in order to assess the role of plasma testosterone in survival outcome stratification. This study was approved by the Ethics Committee of Fuwai Hospital, Beijing, China. All patients provided written informed consent.

Measurements of plasma testosterone levels

For each hospitalized patient or outpatient, peripheral venous blood was collected in pyrogen-free EDTA tubes between 7 a.m. and 10 a.m. before they took heavy medication. The blood samples were immediately centrifuged at 2000 *g* for 20 min at 4°C to separate the plasmas and were stored at −80°C until assayed. Enzyme-linked immunosorbent assays (ELISA) were used to measure circulating levels of total testosterone (KGE010, R&D Systems, USA), SHBG (DSHBG0B, R&D Systems, USA), and albumin (EHALB, Thermo, UK) in plasma. All tests were performed according to the manufacturers' protocols. The free and bioavailable testosterone levels were measured using the calculator at http://www.issam.ch/freetesto.htm. The coefficient of variation (CV) values of the total testosterone, SHBG, and albumin essays were 5.6%, 7.6%, and 8.8%, respectively. And the limit of detection (LoD) was 0.041 ng/mL, 0.1 nmol/L, and 4.92 ng/mL, respectively.

Genetic screening

The whole-genome DNA was extracted from peripheral blood cells of ARVC patients using the DNeasy Blood and Tissue Kit (Qiagen, USA). Targeted next-generation sequencing was performed based on the Illumina Hi-seq2000 platform (Illumina, USA). The pathogenicity of the variant was filtered and evaluated by ACMG guidelines, and the mutations were identified as pathogenic, likely pathogenic, or a variant of uncertain significance (VUS); details were previously described.[25]

Statistical analysis

Continuous variables were expressed as the median with IQR, and categorical variables were presented as numbers and percentages. Categorical variables were analysed by χ^2 tests or Fisher's exact test. For continuous variables, the Mann–Whitney *U* test was used for comparisons between two groups, and the Kruskal–Wallis test was used for multiple groups (≥three groups, each *P* value is adjusted to account for the Dunn's multiple comparisons test). The correlations between testosterone level and clinical variables were analysed by Spearman's analysis. Malignant arrhythmic events-free survival rates were estimated by Kaplan–Meier curves and compared by the log-rank test. Cox proportional hazards regression was used to analyse the effect of plasma testosterone and other well-described risk factors on malignant arrhythmic events in male ARVC patients. Variables with significance level <0.1 were included in multivariate model. All statistical analyses were performed using SPSS Statistics, version 23.0 (IBM Corp, Armonk, NY, USA). Statistical charts were plotted using GraphPad Prism 7 (GraphPad Software Inc., CA, USA). A covariate with $P < 0.05$ was considered significant.

Results

Baseline characteristics and circulating testosterone

The clinical characteristics of the study population are presented in *Table 1*. At the time of blood collection, 44.79% of ARVC patients had suffered malignant arrhythmic events in medical history since onset of the disease. LV dysfunction, defined as LV ejection fraction (LVEF) $< 50\%$ by echocardiography, was rather common (34.34%), indicating advanced stages of disease in our ARVC cohort. The plasma levels of

Table 1 Baseline characteristics and the testosterone level of patients with ARVC and controls (N = 195)

Characteristics	ARVC (N = 99)	Control (N = 96)	P value
Male (%)	68/99 (70.7%)	61/96 (62.5%)	0.4543
Age (year)	40 (30–52)	41 (30–52)	0.3838
Weight (kg)	64 (55.5–75.0)	70 (60.0–77.0)	0.2857
Height (cm)	170 (164.3–175.0)	170 (165.0–175.0)	0.8140
Body mass index (kg/m^2)	22.76 (20.42–25.27)	23.83 (20.76–25.71)	0.1619
Systolic pressure (mmHg)	113 (100.0–124.3)		
Diastolic pressure (mmHg)	70 (64.25–80.00)		
MAE at baseline (%)	43/96 (44.79%)		
Age at onset of arrhythmia	27 (22.0–38.5)		
Diabetes	5/87 (5.75%)		
Hypertention	7/88 (7.95%)		
Smoking history	19/87 (21.84%)		
History of alcohol	18/87 (20.69%)		
IVS (mm)	9.0 (8.0–10.0)	8.3 (7.0–9.6)	0.1158
RVEDD (mm)	34.5 (26.75–44.25)	22.9 (20.70–24.60)	<0.0001
LVEDD (mm)	48.0 (45.0–55.0)	46.6 (45.1–48.4)	0.1379
LVEF (%)	51.50 (40.00–61.25)	60.75 (55.21–66.21)	0.0225
RVEF (%) (by CMR)	18 (12.5–27.0)		
NYHA			
I (%)	37/96 (38.54%)		
II (%)	19/96 (19.79%)		
III (%)	20/96 (20.83%)		
IV (%)	18/96 (18.75%)		
Epsilon waves (%)	12/53 (22.64%)	0/41	0.0009
QRS fragmentation (%)	37/53 (69.81%)	0/41	<0.0001
Negative T waves (%)	39/53 (73.58%)	1/41 (2.44%)	<0.0001
Medication			
ACEI/ARB (%)	41/83 (49.40%)		
Beta-blockers (%)	65/87 (74.71%)		
Spirolactone (%)	52/82 (63.41%)		
Amiodarone (%)	23/78 (29.49%)		
Glucose (mmol/L)	4.72 (4.310–5.075)		
HbAlc (%)	5.7 (5.3–6.3)		
Albumin (g/L)	43.5 (39.75–45.85)		
AST (IU/L)	20.5 (16–29)		
ALT (IU/L)	23 (16.00–29.25)		
Creatinine (μmol/L)	80 (68.30–90.78)		
BUN (mmol/L)	5.61 (4.508–6.925)		
Triglyceride (mmol/L)	1.105 (0.80–1.64)		
Cholesterol (mmol/L)	3.95 (3.17–4.57)		
NT-proBNP (pg/mL)	613.4 (304.7–2086.0)		
CK (IU/L)	71 (55.0–106.5)		
CK-MB (ng/mL)	10 (8–15)		
cTnI (ng/mL)	0.03 (0.0075–0.0870)		
Hs-CRP (mg/L)	1.295 (0.5425–2.7930)		
Big-ET (pmol/L)	0.53 (0.240–0.818)		
SHBG (nmol/L)	22.16 (10.54–41.31)	35.67 (21.03–54.42)	0.0785
Total testosterone (TT) (ng/mL)	4.622 (0.467–7.433)	1.988 (0.201–4.024)	<0.0001
TT in male (Dunn's multiple comprison)	6.390 (4.438–8.768)	3.617 (2.073–4.479)	<0.0001
TT in female (Dunn's multiple comprison)	0.3166 (0.1412–0.4392)	0.1359 (0.0514–0.2634)	>0.9999
Free testosterone (FT) (ng/mL)	0.106 (0.0134–0.271)	0.0400 (0.0032–0.0918)	<0.0001
FT in male (Dunn's multiple comprison)	0.2055 (0.1000–0.4073)	0.0768 (0.0405–0.1105)	<0.0001
FT in female (Dunn's multiple comprison)	0.0036 (0.0021–0.0125)	0.0018 (0.0008–0.0045)	>0.9999
Per cent of FT (%)	2.69 (1.44–4.12)	1.81 (1.41–2.573)	0.0012
Bioavailable testosterone (BT) (ng/mL)	2.21 (0.239–5.480)	0.7395 (0.0766–1.6280)	<0.0001
BT in male (Dunn's multiple comprison)	4.11 (1.990–6.545)	1.32 (0.7965–2.0350)	<0.0001
BT in female (Dunn's multiple comprison)	0.123 (0.0309–0.2370)	0.0412 (0.0162–0.0868)	>0.9999
Per cent of BT (%)	59.4 (36.5–77.5)	41.45 (24.63–57.88)	<0.0001

Data are expressed as median (IQR) or as number (percentage). For testosterone, 1 ng/mL = 3.467 nmol/L. Mann–Whitney test was used for comparison of continuous variables and Fisher's exact test for categorical variables. P values <0.05 were considered significant.
ACEI/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blockers; ALT, alanine aminotransferase; ARVC, arrhythmogenic right ventricular cardiomyopathy; AST, aspartate aminotransferase; Big-ET, big-endothelin; BUN, blood urea nitrogen; CK-MB, creatine kinase isoenzyme; CTnI, cardiac troponin I; HbAlc, glycosylated haemoglobin; Hs-CRP, high-sensitivity C-reactive protein; IVS, interventricular septal thickness; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MAE, malignant arrhythmic events; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; RVEDD, right ventricular end-diastolic diameter; RVEF, right ventricular ejection fraction; SHBG, sex hormone-binding globulin.

total testosterone (TT) [4.622 (0.467–7.433) vs. 1.988 (0.201–4.024), $P < 0.0001$, data expressed as median (IQR)], free testosterone (FT) [0.106 (0.0134–0.271) vs. 0.106 (0.0134–0.271), $P < 0.0001$, median with IQR], and bioavailable testosterone (BT) [2.21 (0.239–5.480) vs. 0.7395 (0.0766–1.6280), $P < 0.0001$, median with IQR] in ARVC patients were significantly higher as compared with those measured in healthy volunteers (*Table 1*). Although the sample size of normal controls was small, the normal value range for testosterone was consistent with previous reports.[26,27]

As shown in *Figure 1* and *Table 1*, significant deviations of TT, FT, and BT were between different genders. To exclude the effects of gender, a subgroup comparison was performed within female and male participants. The plasma concentrations of TT and BT had significant differences between the ARVC patients and healthy volunteers among male probands, whereas no differences were observed between patients and healthy subjects in female counterparts (*Figure 1*). Furthermore, we compared the testosterone levels among male patients with different genotypes. A majority of male patients (50/68) were genotype positive (Supporting Information, *Table S1*), including 11 patients with desmoglein-2 (*DSG2*), 10 patients with *PKP2*, 4 with *DSP*, 3 with desmocollin-2 (*DSC2*) mutations, and 1 with plakoglobin (JUP) mutation. Eight patients had more than one pathogenic variant (at least one desmosomal mutation each). The Kruskal–Wallis test showed that the levels of TT and BT had no significant association with the underlying genetic mutation. (*Figure 2A* and Supporting Information, *Figure S1A*).

Correlation analysis of testosterone in male ARVC patients.

The correlation analysis revealed that neither ventricular dilation (LV/RV end-diastolic diameters) nor cardiac dysfunction (LVEF, RVEF, and New York Heart Association class) at baseline had any association with TT or BT levels (*Table 2*, *Figure 2B*, Supporting Information, *Figure S1B*). These results suggested that testosterone levels were not indicative of cardiac structural or functional remodelling. There was no correlation between the baseline malignant arrhythmic events and testosterone levels. There were no significant correlations between testosterone levels and indices of hepatorenal function such as alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and creatinine. Likewise, testosterone levels were not related to the inflammatory factors, high-sensitivity C-reactive protein, and cardiac troponin T (*Table 2*). Some medications, such as amiodarone, spironolactone, and beta-blockers, have been reported to have an association with testosterone levels.[28,29] But our analysis (*Figure 2C–I* and Supporting Information, *Figure S1C,D*) demonstrated that there was no significant difference between the groups with or without drug use, cardiovascular

Figure 1 Plasma testosterone levels between the ARVC patients and healthy. The average level of testosterone in the female group was significantly lower than that of the male. The concentrations of plasma total testosterone (TT) and bioavailable testosterone (BT) had a significant difference between the male ARVC patients and healthy males, whereas no differences were observed in females (data shown as median with IQR, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$ using Kruskal–Wallis test, and each *P* value is adjusted to account for the Dunn's multiple comparisons test).

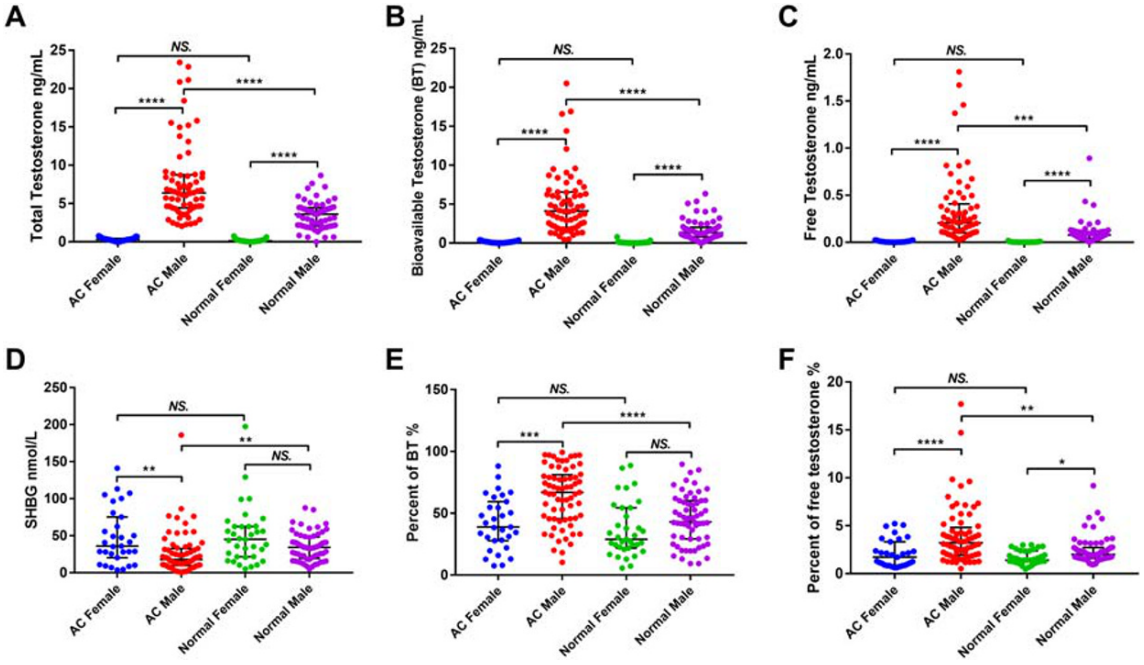
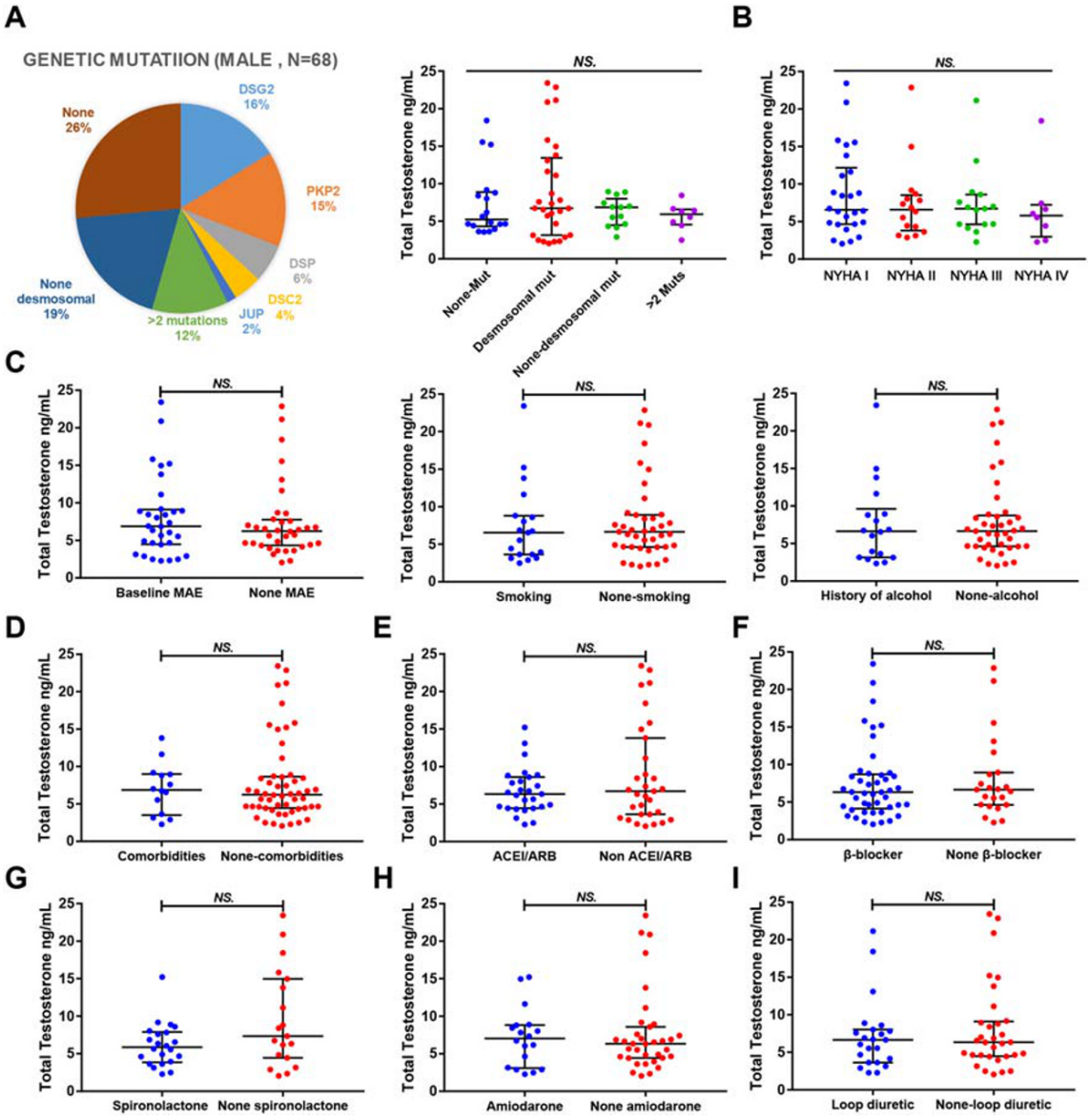


Figure 2 The association between TT levels and the baseline characteristics of the ARVC male patients (N = 68). (A) Genetic mutation status of male AC patients (*n* = 68). The plasma testosterone levels had no significant association with the underlying genetic mutation in male AC patients (using the Kruskal–Wallis test). (B) There was no significant difference in testosterone levels among groups with different NYHA. And the levels of testosterone also had no association with the baseline characteristics of the ARVC male patients, including the cardiovascular risk factors (C), co-morbidities (D), and heart failure medications (E–I).



risk factors (smoking, alcohol, etc.), and co-morbidities (diabetes, hypertension, etc.).

Circulating testosterone predicting malignant arrhythmic events

To evaluate the role of testosterone levels in predicting clinical adverse arrhythmic events, we performed a follow-up study among 68 ARVC male patients. During 17 months[9–29] of follow-up, 5 were withdrawn, 16 patients experienced SSPE, and 22 developed malignant arrhythmic events (*Table 3*). Amid two patients with both malignant arrhythmic events and SSPE, plasma testosterone levels (TT = 6.05 ng/mL, 4.90 ng/mL separately) were significantly lower than the average level in malignant arrhythmic events (TT = 11.73 ng/mL)—but were more similar with the expression levels in SSPE—and we included these patients in the malignant arrhythmic events group. The plasma levels of TT, BT, and FT in patients who experienced malignant arrhythmic events

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Table 2 Testosterone correlation with clinical characteristics in male ARVC patients ($N = 68$)

	Spearman correlation (TT)		Spearman correlation (BT)	
Variables	ρ	*P* value	ρ	*P* value
Height (cm)	0.315	0.013	0.327	0.010
Weight (kg)	0.125	0.343	0.112	0.393
Body mass index (kg/m^2)	0.032	0.801	0.001	0.991
Age (year)	−0.066	0.603	−0.119	0.345
Systolic pressure (mmHg)	0.054	0.668	0.078	0.535
Diastolic pressure (mmHg)	−0.030	0.815	0.001	0.995
Heart rate (per min)	0.082	0.518	0.110	0.381
Age at onset of heart disease (year)	−0.157	0.230	−0.209	0.108
Age at onset of arrhythmia (year)	−0.050	0.717	−0.142	0.307
Length of drinking history (year)	0.160	0.585	0.197	0.500
Length of smoking history (year)	−0.062	0.800	0.066	0.789
Number of precordial TWI	0.032	0.855	0.185	0.287
Number of precordial QRS fragmentation	0.046	0.792	0.116	0.506
LAID (mm)	−0.011	0.931	0.055	0.668
IVS (mm)	−0.008	0.951	0.056	0.659
LVEDD (mm)	−0.169	0.177	−0.028	0.822
LVEF (%)	0.100	0.430	0.048	0.706
RVEDD (mm)	0.038	0.765	−0.045	0.722
RVEF (%) (by CMR)	−0.099	0.631	−0.077	0.709
Hb (g/L)	−0.091	0.490	0.112	0.396
Albumin (g/L)	−0.141	0.269	0.001	0.998
Glucose (mmol/L)	0.020	0.879	0.078	0.550
HbAlc (%)	−0.163	0.267	−0.171	0.245
AST (IU/L)	−0.005	0.971	0.057	0.660
ALT (IU/L)	0.016	0.905	0.073	0.571
P (mmol/L)	0.273	0.037	0.260	0.047
Ca (mmol/L)	−0.074	0.578	0.048	0.716
Creatinine (μmol/L)	−0.066	0.608	−0.126	0.329
BUN (mmol/L)	−0.050	0.702	0.055	0.671
Triglyceride (mmol/L)	−0.001	0.992	0.092	0.486
Cholesterol (mmol/L)	−0.194	0.134	−0.041	0.756
LDL-Chol (mmol/L)	−0.153	0.248	−0.070	0.599
HDL-Chol (mmol/L)	−0.100	0.457	−0.045	0.737
FFA (mmol/L)	0.129	0.378	0.127	0.386
LP(a) (mg/L)	−0.046	0.731	−0.100	0.456
Apo A1 (g/L)	−0.121	0.399	−0.031	0.827
cTnI (ng/mL)	−0.064	0.771	0.202	0.356
Hs-CRP (mg/L)	0.147	0.261	0.207	0.113
NT-proBNP (pg/mL)	0.122	0.367	0.131	0.333

ALT, alanine aminotransferase; ARVC, arrhythmogenic right ventricular cardiomyopathy; AST, aspartate aminotransferase; BT, bioavailable testosterone; BUN, blood urea nitrogen; Ca, blood calcium; cTnI, cardiac troponin I; FFA, free fatty acid; Hb, haemoglobin; HbAlc, glycosylated haemoglobin; HDL-Chol, high density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; IVS, interventricular septal thickness; LAID, left atrium internal dimension; LDL-Chol, low density lipoprotein cholesterol; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; P, blood phosphorus; RVEDD, right ventricular end-diastolic diameter; TT, total testosterone; TWI, T wave inversion.

Table 3 The distribution of malignant arrhythmic events

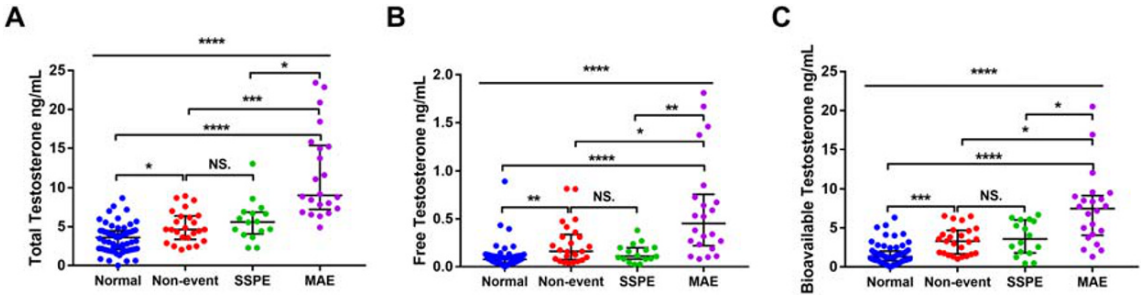
Malignant arrhythmic events	$N = 22$
Survived sudden cardiac arrest, *n* (%)	1 (4.55)
Sustained ventricular tachycardia, *n* (%)	11 (50.00)
appropriate ICD discharge, *n* (%)	10 (45.45)

Malignant arrhythmic events were defined as SCD, survived SCD, sustained ventricular tachycardia (VT), or appropriate ICD discharge.

were higher than those without (*P* value was adjusted by Dunn's multiple comparisons test) (*Figure 3*). It is worth noting that the concentrations of testosterones negatively correlated with follow-up time (Supporting Information, *Figure S2*), indicating that testosterone might be a predictor for malignant arrhythmic events. Sixteen patients had isolated SSPE, including 10 patients with transplants, 2 who died, and 4 with a numerical decrease over 10% in LVEF, but they did not have significantly higher plasma testosterone levels (*Figure 3*). Consistent with previous studies, our results indicate that arrhythmias and cardiac structural progression are different disease processes, even during initial short-time follow-up.[8] The results of Swiss cohort, with a different ethnic group, also proved that the testosterone level was associated with major arrhythmic cardiovascular events.[19] The similar conclusions within the Swiss cohort expanded the applicable population of this discovery.

The clinical characteristics of the ARVC male patients with malignant arrhythmic events, SSPE, and non-event groups

Figure 3 Circulating testosterone levels are associated with malignant arrhythmic events (MAE) in the follow-up. Plasma testosterone levels are significantly elevated in ARVC male patients with malignant arrhythmic events using the Kruskal–Wallis *U* test, whereas patients with significant structural progression event (SSPE) did not have significantly higher plasma testosterone levels (*P* values adjusted by Dunn's multiple comparisons test).



are presented in *Table 4*. Baseline characteristics and clinical data were roughly comparable among the groups, except for cardiac function (*SSPE* had worse HF symptoms). We observed that plasma testosterone level predicted a high risk of arrhythmia but not the SSPE for ARVC male patients. To further validate the relationship between plasma testosterone and arrhythmic events in male ARVC patients, we applied the receiver operating characteristic curve (ROC) to reveal the optimum cut-off value for TT, BT, and FT in discriminating malignant arrhythmic events positive and negative groups (TT = 6.722 ng/mL, AUC_{TT} = 0.902; BT = 4.650 ng/mL, AUC_{BT} = 0.803; FT = 0.257 ng/mL, AUC_{FT} = 0.794) (*Figure 4A–C*). By using the cut-off value as a grouping condition, it was demonstrated that there was a significant difference in malignant arrhythmic events-free survival rates between the groups (log-rank $p_{TT}<0.0001$; log-rank $p_{BT}<0.0001$; log-rank $p_{FT}<0.0001$) (*Figure 4D–F*).

For the male ARVC patients (n = 68), we further applied the Cox regression models to determine whether testosterone levels were associated with malignant arrhythmic events independently. Because all covariates reported in *Table 2* were insignificant, we included several clinically relevant variables that were directly or indirectly relevant to testosterone concentrations into the Cox model.[30,31] Given the highest AUC value (AUC_{TT} = 0.9024) of TT to malignant arrhythmic events, and the strong correlation among the expression levels of TT, FT, and BT (Pearson correlation, r_{TT-FT} = 0.727, $p_{TT-FT}<0.001$; r_{TT-BT} = 0.889, $p_{TT-BT} < 0.001$), we only included the TT into Cox regression analysis to eliminate collinearity. Based on the result of the multivariate Cox regression model, plasma TT level (HR = 1.325, 95% CI [1.171, 1.498]) was identified as an independent prognostic factor for malignant arrhythmic events (*Table 5*).

Discussion

In this study, we found that elevated levels of plasma testosterone are strong predictors of future adverse arrhythmic events in male patients with ARVC, supporting the hypothesis that sex hormone may play a role in the pathophysiology of the disease. Our findings in the Chinese ARVC cohort with a different genetic and race background and distinct baseline data are in line with previous observations reported by Akdis *et al.*[19] Furthermore, we also have several novel findings[1]: ARVC male patients showed higher levels of testosterone than normal males, while female counterparts did not.[2] The testosterone levels were not related to baseline cardiac function and future significant structural progression event but served as an independent predictor arrhythmia event.

Arrhythmogenic right ventricular cardiomyopathy may be associated with life-threatening ventricular arrhythmias in both early stage and subsequent progressive biventricular systolic dysfunction. Therefore, disease management should not only focus on the prevention and therapy of arrhythmias but also on halting or slowing down the progression of structural remodelling, in order to prevent heart failure.[9] Previous researches were committed to predict arrhythmic prognosis among ARVC patients based on imaging parameters or genotype testing.[11,32] Our study verified a new biomarker that can independently predict arrhythmia risk events in ARVC male patients without the influence of cardiac insufficiency or other factors, providing a new method for the prognosis assessment of ARVC male patients. Our ARVC cohort was considerably different from the previous Swiss cohort.[19] The Swiss cohort mainly consisted of ARVC patients with arrhythmias at an early or intermediate stage, while our cohort mainly enrolled ARVC patients at the advanced or terminal stage. It is reasonable that more severe phenotypes were observed in our ARVC cohort. In fact, as the National Centre for Cardiovascular Disease in China, Fuwai Hospital is also the biggest designated hospital for heart transplantation (more than 100 heart transplantations were performed annually in Fuwai). Hence, we have many referral patients from other hospitals, some of whom were in serious condition and required heart transplant. The blood testosterone can have high prediction efficiencies in both cohorts,

Table 4 Baseline characteristics of ARVC male patients in different defined groups

Characteristics	ARVC male patients (N = 68)	Non-event (N = 25)	SSPE (N = 16)	MAE (N = 22)	P value
Age (year)	40 (29–51)	33 (24–43)	42.00 (26.50–52.00)	43.50 (36.75–56.25)	0.062
Weight (kg)	69.5 (60.0–77.5)	66.00 (59.25–76.88)	63.65 (55.63–73.75)	75.00 (69.50–81.50)	0.021
Height (cm)	172 (168–176)	170.0 (165.5–175.0)	170.50 (167.25–174.50)	175.00 (170.00–179.00)	0.026
Body mass index (kg/m^2)	23.21 (21.03–26.02)	23.59 (21.19–26.44)	21.86 (18.93–24.65)	24.51 (22.82–26.14)	0.100
Systolic pressure (mmHg)	119.0 (100.0–129.5)	118.0 (100.0–130.0)	108.00 (95.00–120.00)	122.00 (108.25–130.00)	0.064
Diastolic pressure (mmHg)	73.0 (64.0–80.0)	70.0 (61.5–80.0)	73.50 (63.50–80.00)	76.00 (64.50–80.00)	0.666
Heart rate (per min)	69.0 (59.5–78.0)	67.5 (57.5–79.0)	68.50 (60.00–80.00)	69.50 (68.50–76.50)	0.893
Age at onset of heart disease (year)	26.00 (17.00–38.75)	24.0 (15.5–35.0)	30.00 (17.00–41.25)	29.00 (21.50–40.50)	0.266
Age at onset of arrhythmia (year)	27.82 (22.15–39.53)	24.56 (16.12–32.87)	30.36 (23.12–42.88)	34.90 (24.82–45.66)	0.049
LAAPD (mm)	33.00 (28.25–38.75)	33.00 (27.25–36.50)	40.50 (30.00–47.75)	33.00 (29.50–38.00)	0.111
IVS (mm)	9.0 (8.0–10.0)	9.00 (8.00–9.40)	9.00 (7.25–9.00)	9.50 (8.60–11.00)	0.291
RVEDD (mm)	33.00 (24.25–40.00)	33.00 (22.50–37.75)	36.50 (27.75–42.75)	28.00 (22.00–37.50)	0.203
LVEDD (mm)	48.00 (45.00–54.00)	47.50 (45.00–52.75)	54.50 (45.25–61.75)	47.50 (45.25–51.25)	0.210
LVEF (%)	58.00 (40.00–63.20)	58.0 (50.5–63.3)	38.50 (20.25–52.00)	62.00 (53.75–66.73)	<0.001
RVEDV (mL) $_{(CMR)}$	227.00 (173.85–376.78)	220.5 (204.7–270.05)	321.55 (185.10–475.58)	196.90 (166.40–382.40)	0.498
RVEF (%) $_{(CMR)}$	18.40 (10.87–32.50)	17.50 (10.75–37.50)	24.50 (10.87–28.25)	17.80 (10.00–34.00)	0.935
Gene mutation (%)	50/68	17/25	12/16	17/22	0.758
NYHA					<0.001
I (%)	27/66	10/24	2/16	13/22	
II (%)	16/66	7/24	1/16	7/22	
III (%)	15/66	6/24	7/16	1/22	
IV (%)	8/66	1/24	6/16	1/22	
PVC (%)	39/63	13/22	12/16	12/22	0.420
ICD (%)	20/60	3/22	6/16	11/22	0.0348
Hb (g/L)	147.50 (136.02–157.00)	149.5 (134.6–158.3)	151.00 (136.00–156.00)	144.00 (136.25–157.75)	0.931
Glucose (mmol/L)	4.75 (4.39–5.08)	4.53 (4.30–4.92)	5.05 (4.28–6.35)	4.80 (4.51–4.93)	0.210
HbAlc (%)	5.55 (5.20–6.18)	5.60 (5.40–6.20)	5.60 (5.20–6.50)	5.45 (5.09–6.15)	0.665
Albumin (g/L)	43.10 (39.20–45.70)	45.0 (40.0–46.0)	42.20 (37.25–45.50)	43.20 (39.38–45.00)	0.413
AST (IU/L)	21.50 (16.00–33.25)	20.0 (14.0–37.0)	22.50 (20.00–34.75)	21.50 (16.00–27.75)	0.411
ALT (IU/L)	23.00 (17.00–30.25)	23.0 (17.0–41.0)	23.00 (12.75–27.00)	25.50 (18.00–39.50)	0.492
P (mmol/L)	1.21 (1.09–1.37)	1.21 (1.08–1.36)	1.26 (1.06–1.37)	1.19 (1.12–1.40)	0.869
Creatinine (μmol/L)	82.48 (71.91–94.50)	80.00 (69.60–94.0)	86.66 (66.10–105.44)	81.61 (74.41–90.53)	0.683
BUN (mmol/L)	5.61 (4.45–6.86)	5.80 (4.40–6.85)	6.83 (4.56–8.29)	5.18 (4.20–5.92)	0.085
Triglyceride (mmol/L)	1.27 (0.88–1.71)	1.11 (0.71–1.66)	1.22 (0.84–1.78)	1.53 (1.08–1.84)	0.228
Cholesterol (mmol/L)	4.07 (3.19–4.56)	4.22 (3.10–5.00)	3.60 (2.82–4.33)	4.34 (3.56–4.73)	0.161
LDL-Chol (mmol/L)	2.49 (1.90–2.78)	2.49 (1.92–2.94)	2.45 (1.89–2.70)	2.51 (1.87–2.94)	0.744
HDL-Chol (mmol/L)	1.02 (0.89–1.23)	1.18 (0.87–1.38)	0.91 (0.66–1.14)	1.04 (0.93–1.19)	0.206
FFA (mmol/L)	0.42 (0.31–0.55)	0.36 (0.30–0.45)	0.51 (0.37–0.73)	0.43 (0.31–0.54)	0.140
LP(a) (mg/L)	120.35 (49.27–230.11)	152.46 (52.30–263.75)	107.86 (75.28–261.61)	106.27 (38.36–179.08)	0.667
Apo A1 (g/L)	1.15 (1.00–1.38)	1.28 (0.95–1.53)	1.16 (1.00–1.24)	1.12 (1.02–1.41)	0.585
CK-MB (ng/mL)	11.00 (8.00–16.00)	12.50 (9.75–17.00)	15.00 (10.00–23.00)	9.50 (7.25–11.75)	0.008
Hs-CRP (mg/L)	1.31 (0.64–2.88)	1.74 (0.44–3.07)	1.82 (0.95–4.78)	1.03 (0.46–2.36)	0.299

(*Continues*)

Table 4 (continued)

Characteristics	ARVC male patients (N = 68)	Non-event (N = 25)	SSPE (N = 16)	MAE (N = 22)	P value
cTnI (ng/mL)	4.50 (1.50–7.02)	5.90 (1.95–7.45)	3.00 (0.97–6.50)	7.00 (3.00–7.51)	0.612
NT-proBNP (pg/mL)	587.20 (260.80–1176.95)	623.90 (301.80–1068.00)	2023.00 (219.80–4563.80)	410.80 (144.53–976.53)	0.032
Total testosterone (ng/mL)	4.622 (0.467–7.433)	4.633 (3.363–6.375)	5.604 (4.069–6.857)	9.034 (7.222–15.370)	<0.001
Free testosterone (ng/mL)	0.106 (0.0134–0.271)	0.161 (0.075–0.337)	0.112 (0.080–0.200)	0.453 (0.221–0.758)	<0.001
Bioavailable testosterone (ng/mL)	2.21 (0.239–5.480)	3.300 (1.685–4.690)	3.590 (1.805–6.028)	7.485 (2.070–9.163)	<0.001

Data are expressed as Median (IQR) or as number (percentage). Kruskal–Wallis test was used for comparison of continuous variables (≥3 group). P values <0.05 were considered significant.

ALT, alanine aminotransferase; ARVC, arrhythmogenic right ventricular cardiomyopathy; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK-MB, creatine kinase isoenzyme; cTnI, cardiac troponin I; FFA, free fatty acids; Hb, haemoglobin; HbAlc, glycosylated haemoglobin; HDL-Chol, high density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; ICD, implantable cardioverter-defibrillator; IVS, interventricular septal thickness; LAAPD, left atrium anteroposterior diameter; LDL-Chol, low density lipoprotein cholesterol; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MAE, malignant arrhythmic events; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; P, blood phosphorus; RVEDD, right ventricular end-diastolic diameter; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; SSPE, significant structural progression event.

indicating its arrhythmia risk predictive ability for patients with ARVC in a different spectrum. The consistent high prediction efficiency of plasma testosterone in both Chinese and Swiss cohort revealed that testosterone could be a stable predictor for arrhythmia in ARVC patients despite racial differences.

It remains unclear why plasma testosterone levels are higher in ARVC male patients with severe arrhythmic events. We speculated that the ARVC patients with malignant arrhythmic events were in a state of more severe stress that promoted adrenal hormones secretion. Testosterone could exacerbate cardiomyocyte apoptosis and ion channels dysfunction. By using an ARVC patient-derived iPSC-cardiomyocyte model, it was demonstrated that testosterone could accelerate cardiomyocyte apoptosis and lipid accumulation due to defective canonical Wnt signalling pathway, which may be triggered by the abnormal activation of peroxisome proliferator-activated receptor-gamma (PPARγ) signalling.[33] By contrast, the testosterone did not intensify the lipogenesis and apoptosis in the healthy iPSC-CMs.[19] These results indicate that testosterone could specifically worsen those pathologies prompting the arrhythmic potential in ARVC.

Arrhythmogenic right ventricular cardiomyopathy, unlike other SCD-related diseases, has pathological hallmarks of progressive cardiomyocytes loss and fibrofatty infiltration. In a community-based study, Kumar Narayanan *et al*. found that higher testosterone levels were associated with lower sudden cardiac arrest in males.[34] This was probably because testosterone can also cause malignant clinical events by affecting ion channels. The testosterone-treated cardiomyocytes had higher androgen and β1-adrenergic receptor expressions that result in longer corrected QT intervals.[21] Moreover, some studies focused on the influences of testosterone on the myocardial contractile function regulated by Ca^{2+} handling. It was shown that testosterone modified the inward peak T-type Ca^{2+} currents, leading to prolonged depolarization, so that it may play a key role in the pathogenesis of many cardiovascular diseases.[35] It can be inferred that testosterone might participate in electrophysiological dysfunction and disease pathogenesis. And the influence of elevated testosterone in ARVC remains to be further elucidated.

We have to acknowledge that our study is limited by the fact that testosterone levels may show biological variability in the short-term and long-term. Moreover, even though all ARVC patients received standard treatment, given the limitations of sample size, we did not take the therapeutic drugs into account. We were also unable to adjust for all possible confounding variables related to the outcome. Additional investigations from multicentre cohorts are required for further validating the direct role of sex hormones in determining clinical outcomes in ARVC.

Figure 4 Plasma testosterone levels predict malignant arrhythmic events in ARVC male patients. (A–C) ROC curve to distinguish patients with or without Malignant arrhythmic events during follow-up. (D–F) Patients with above cut-off concentrations of testosterone showed significantly elevated malignant arrhythmic events during follow-up of 17 months[9–29] [log-rank (Mantel–Cox) test, $P < 0.0001$].

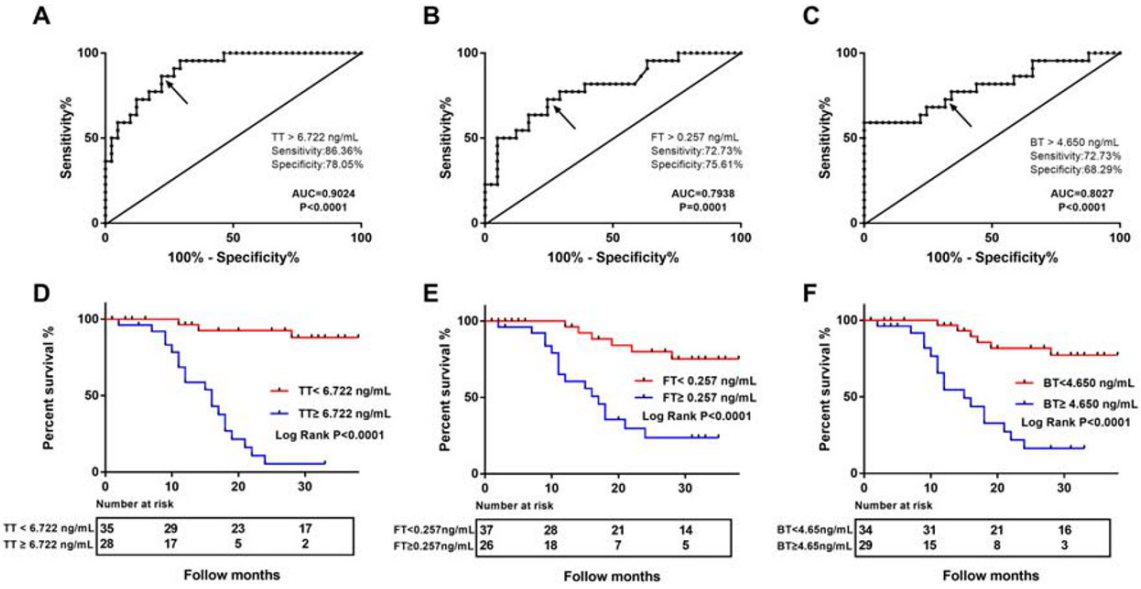


Table 5 Increasing serum testosterone is an independent predictor of malignant arrhythmic events in male ARVC patients (Cox regression analysis)

	Univariate Analysis		Multivariate Analysis	
Variables	HR (95% CI)	*P* value	HR (95% CI)	*P* value
TT	1.302 (1.193–1.421)	<0.001	1.325 (1.171–1.498)	<0.001
BMI	1.122 (0.997–1.263)	0.057	1.086 (0.921–1.280)	0.327
Age	1.026 (0.998–1.056)	0.072	1.008 (0.974–1.043)	0.643
MAE at baseline	3.448 (1.339–8.875)	0.010	1.448 (0.378–5.544)	0.589
LVEF%	1.00 (0.97–1.04)	0.836		
NYHA	0.787 (0.452–1.370)	0.397		
TWI	0.998 (0.776–1.282)	0.986		
NSVT	4.429 (0.595–32.969)	0.146		
RVEF ($_{CMR}$)	0.984 (0.941–1.029)	0.471		
NT-proBNP	1.000 (1.000–1.001)	0.593		
CK-MB	0.880 (0.777–0.997)	0.044	0.826 (0.671–1.016)	0.070
CTnI	1.188 (0.816–1.729)	0.368		
Hs-CRP	0.976 (0.804–1.185)	0.808		
Beta-blockers	1.805 (0.420–7.764)	0.427		
Amiodarone	2.180 (0.897–5.298)	0.085	1.497 (0.509–4.406)	0.464
Spironolactone	1.150 (0.487–2.712)	0.750		

BMI, body mass index; CI, confidence interval; CK-MB, creatine kinase isoenzyme; cTnI, cardiac troponin I; HR, hazard ratio; Hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; MAE, malignant arrhythmic events; NSVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; RVEF, right ventricular ejection fraction; TT, total testosterone; TWI, T wave inversion.

Conclusions

Plasma testosterone levels were higher in ARVC male patients than in those measured in healthy male patients and can predict future adverse arrhythmic events in male patients with ARVC but also not concerned with baseline cardiac function and follow-up SSPE. And similar results between the Chinses Hans and Caucasians expanded the applicable population of this discovery. Therefore, our results suggested that testosterone may be a promising biomarker in arrhythmic risk prediction in the ARVC.

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Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Clinical diagnosis of ARVC male patients (n = 68) based on Task Force Criteria in 2010

Table S2. Baseline characteristics of ARVC patients between Swiss and China cohort.

Figure S1. The levels of BT and FT were not affected by the baseline characteristics of ARVC male patients. **A**. there was no significant difference of BT and FT among the different types of mutations. **B-D**. The levels of BT and FT had no association with the baseline characteristics of the ARVC patients, including the cardiac function (**B**), cardiovascular risk factors **(C**) and heart failure medications (**D**).

Figure S2. The rate of malignant arrhythmic events was line with concentration of testosterones. **A-C**. The concentrations of testosterones held a negative relationship with follow-up time. The higher levels of testosterones predict the malignant arrhythmic events will become more soon.

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