Gonadal status in males with acute coronary syndrome and the extent of coronary artery disease: a comparative cross-sectional analysis

Hladiny pohlavných hormónov u mužov s akútnym koronárnym syndrómom a rozsah koronárneho postihnutia: porovnávacia prierezová štúdia

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Abstract. *Introduction and aims:* Association between coronary artery disease (CAD) and male gonadal status has been previously suggested, but still remains inconclusive. The aims of our study in males with recent acute coronary syndrome (ACS) were to evaluate the prevalence of hypogonadism, to compare their gonadal status with non-ACS matched controls and to evaluate gonadal status by the extent of CAD.

Subjects and methods: A cross-sectional retrospective study included 96 males with recent ACS and age and 49 BMI matched non-ACS controls. Basic demography and standard CAD risk factors were recorded as well as fasting serum levels of total testosterone (TT), sex hormone binding globulin (SHBG), estradiol, follicle-stimulating and luteinizing hormone (FSH and LH) and calculated free androgen index (FAI). First, we evaluated the prevalence of hypogonadism (TT< 7 nmol/l and FAI<24%). Second, we compared patients and control group for ACS associated factors on univariate and multivariate analysis. Third, using the same analysis in patients undergoing coronary angiography we aimed at identifying factors associated with CAD extent (0-1 vessel vs. 2-3 vessel disease).

Results: 96 patients and 49 controls were included. Hypogonadism was confirmed in 20.8% of patients. Patients had lower levels of TT (12.638 ±6.138 vs. 15.702±4.903, p<0.01), FAI (30.292 ±12.489 vs. 36.902 ±9.816, p<0.01) and higher levels of LH (5.58 IQR 4.4-8.21 vs. 5.0 IQR 3.64- 6.35, p=0.04) compared with controls. In multivariate analysis, smoking, hypertension, dyslipidemia and FAI were identified as factors independently associated with ACS. 73 patients underwent coronary angiography, 43 had findings of 2-3 vessel disease. Hypertension, BMI and FAI were found as associated factors with CAD extent and FAI as its single independent risk factor.

Conclusion: Lower gonadal status was found common (20.8%) in males with recent ACS. It was independent on standard ACS risk factors and was independently associated with CAD extent. Fig. 1, Tab. 4, Ref. 50, online full text (Free, PDF), www.cardiologyletters.sk

Key words: acute coronary syndrome – testosterone – free androgen index

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Abstrakt. *Úvod a ciele*: Vzťah medzi koronárnou chorobou srdca (CAD) a hladinami mužských pohlavných hormónov bol predmetom viacerých štúdií s nejednoznačnými výsledkami. Cieľom našej práce bolo porovnať hladiny pohlavných hormónov u mužov s recentným akútnym koronárnym syndrómom (ACS) a kontrol bez ACS a porovnať hormonálne hladiny s rozsahom koronárneho postihnutia.
Pacienti a metódy: Prierezová retrospektívna štúdia analyzovala 96 mužov s recentným ACS a 49 kontrol bez ACS. U všetkých pacientov boli zaznamenané štandardné rizikové faktory kardiovaskulárnych ochorení a v rannom sére boli analyzované hladiny celkového testosterónu (TT), „sex hormonebinding globulínu" (SHBG), estradiolu, folikulostimulačného a luteinizačného hormonónu (FSH a LH) a kalkulovaný bol „freeandrogen index" (FAI). Uvedené parametre sme porovnali u pacientov a kontrol v modeli univariantnej aj mutlivariantnej analýzy. U pacientov, ktorí absolvovali koronarografiu, sme skúmali faktory súvisiace s rozsahom koronárneho postihnutia (0 – 1-cievne versus 2 – 3-cievne postihnutie).
Výsledky: Analyzovaných bolo 96 pacientov a 49 kontrol. Pacienti mali v porovnaní s kontrolami nižšie hodnoty TT (12,638 ± 6,138 vs. 15,702 ± 4,903, p < 0,01), FAI (30,292 ± 12,489 vs. 36,902 ± 9,816, p < 0,01) a vyššie hodnoty LH (5,58 IQR 4,4 – 8,21 vs. 5,0 IQR 3,64 – 6,35, p = 0,04). V modeli multivariantnej analýzy fajčenie, hypertenzia, dyslipidémia a FAI boli nezávisle asociované s ACS. Pacienti s 2 – 3-cievnym postihnutím mali významne nižšie hladiny FAI ako pacienti s 0 – 1-cievnym postihnutím. FAI bol jediný nezávislý faktor spojený s rozsahom koronárneho postihnutia.
Záver: Pacienti s prekonaným ACS majú oproti kontrolám nižšie hladiny mužských pohlavných hormónov. FAI je jediným faktorom spojeným negatívne s rozsahom koronárnej choroby srdca. Obr. 1, Tab. 4, Lit. 50, online full text (Free, PDF), www.cardiologyletters.sk
Kľúčové slová: akútny koronárny syndróm – koronárna choroba srdca – testosterón – freeandrogen index

The relationship between sex hormones and cardiovascular diseases in men is still controversial. Although large prospective trials documented higher total or cardiovascular mortality in males with lower levels of testosterone (1, 2, 3, 4, 5), testosterone replacement therapy (TRT) in elderly males had controversial results (6, 7, 8, 9). Testosterone stimulates erytropoesis (10) and TRT was associated with polyglobulia (6, 7) and thrombosis in predisposed patients (11). However, several smaller studies showed protective effect of TRT on coronary perfusion (12, 13, 14). There are several mechanisms as to how testosterone could impact the cardiovascular system. First, in the pathogenesis of atherosclerosis, testosterone stimulates progenitor cells responsible for endotel reparation (15), decreases proinflamatory cytokines TNFα, IL1, IL6 and increases the activity of anti-inflamatory IL10 (16, 17). Second, testosterone influences on cardiovascular risk factors, especially adiposity and insulin resistance. In dozens of studies TRT was associated with increased lean body mass, decreased fat body mass (18, 19, 20, 21) and improved insulin sensitivity (22, 23, 24). Finally, it seems that testosterone can affect vascular reactivity and cause vasodilatation by non-genomic effects (25).

There are reports that testosterone levels are lower in males with coronary artery disease (CAD) (26, 27, 28) and hypogonadal CAD patients have higher mortality than eugonadal cases (29) . However, results of prospective trials which were focused on cardiovascular events in males with lower testosterone levels were inconsistent (2, 30, 31, 32). There are only few studies evaluating the relationship of testosterone and the severity of CAD on coronary angiography using total testosterone and free testosterone as markers of gonadal status (27, 28, 33, 34). Some proved the relationship for total testosterone and some only for free testosterone.

Therefore the aims of our study were to evaluate the prevalence of hypogonadism in males with recent ACS, to compare their gonadal status with non-ACS matched controls and to identify a possible association between gonadal status and extent of coronary artery disease on coronary angiography.

Subjects and methods

96 males hospitalized with recent ACS during the inclusion period (2013-2016) from the Department of Internal Medicine, University Hospital Bratislava and the Department of Cardiology, National Institute of Cardiovascular Diseases in Bratislava, were included in the study. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki and ethical approval was obtained from the University Hospital Bratislava Ethics Committee**.** Informed consent was obtained from all individual participants included in the study.

Inclusion criteria for the patients group were male gender, age between 40-90 years, recent acute coronary syndrome, absence of the history of hypogonadism and testosterone substitution, absence of cancer and serious chronic disease, absence of other acute severe disease (except for recent acute coronary syndrome). Acute coronary syndrome included ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) and non-stable angina (NAP) and was defined according to ESC 2012 guidelines. Recent ACS was defined as at least 7 days up to 3 months following acute coronary syndrome. Inclusion criteria for

the control group were no history of ACS and angina (no history of STEMI, NSTEMI, NAP and stable angina), other inclusion and exclusion criteria were identical to the patients group. 49 controls were matched to patients according to age and BMI.

A complete medical history containing standard cardiovascular risk factors e.g. hypertension, dyslipidemia, smoking and diet was obtained in all subjects. Basic anthropometric measures of height, weight, waist circumference, and body mass index (BMI) were assessed at study inclusion. Blood samples were taken between the 7thday and 12th week after ACS. At the time of blood sampling patients had to be in a stable clinical condition without angina or symptoms of heart failure. All samples from both centers were analyzed in a single laboratory. Blood sampling was performed at fasting state in the morning from 6:00-7:00 a.m. for total testosterone (TT), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2). Samples were assessed by the electrochemiluminiscence method using commercial kits (Roche, Basel, Switzerland). Free androgen index (FAI) was calculated using this formula: FAI= 100 x (TT/SHBG). Hypogonadism was defined as TT< 7 nmol/l and FAI<24%. All subjects were evaluated for lipid spectrum and diabetes mellitus. This included assessment of low density lipoprotein (LDL), high density lipoprotein (HDL), triacylglycerides (TAG) and fasting morning glucose by standard laboratory analyzers, as well as patient's medication for diabetes mellitus and dyslipidemia.

We examined coronary angiography findings from the National Institute of Cardiovascular Diseases in 73 patients (23 patients did not attend coronary angiography). Significant stenosis was defined as more than 50% stenosis, according to the American College of Cardiology taskforce 2011. All cases undergoing coronary angiography were evaluated for CAD extent. The non-severe CAD group was defined as 0 and 1 vessel disease, the severe CAD group was defined as 2 and 3 vessel disease. Cases with extended coronary stenosis requiring CABG were also assigned into the severe CAD group.

Statistical analysis

We compared sex hormones in ACS patients and controls. The Kolmogorov-Smirnov test was used to evaluate data distributions. Normally distributed values are given as mean±standard deviation and were compared by unpaired T test. Parameters with non-normal distribution are given as median and interquartile range and were compared by Mann-Whitney test. Categorical parameters were compared by Chi-square test. For both multivariate models the dependent variable was either the presence of ACS or coronary angiography findings of severe CAD. All parameters significantly different between the defined groups were entered as independent variables into stepwise multivariate logistic regression which identified independently associated factors with any of the events (ACS, severe CAD). All other variables were excluded from the model.

Results

96 consecutive patients were enrolled in the patients group and 49 cases were included in the control group. In the patients group 24 patients had STEMI, 46 patients had NSTEMI and 26 patients had unstable angina.

Table 1 Summary statistics for patients and control groups and univariate analysis of parameters associated with ACS in males

	Patients group (n=96)		Control group (n=49)		
	mean/median	SD/IQR	mean/median	SD/IQR	p
Age (years)	64	59.000 to 74.000	63	55.000 to 68.000	ns
Body height (m)	1.75	1.700 to 1.800	1.78	1.730 to 1.823	ns
Body weight (kg)	83.5	76.500 to 96.000	90	75.750 to 99.250	ns
BMI (kg/m^2)	28.535	±4.6815	28.384	±4.4136	ns
BMI>25 (%)	76		75.5		ns
Waist circumference (cm)	102.3	±14.621	102.63	±11.4652	ns
Type 2 diabetes (%)	38.5		22.4		ns
Hypertension (%)	86.5		55.1		**<0.0001**
Dyslipidemia (%)	79.2		49		**<0.001**
Smoking (%)	33.3		12.2		**<0.01**
Estradiol (pmol/l)	104.1	81.513 to 132.950	96.8	70.350 to 130.600	ns
FSH (IU/l)	5.78	3.855 to 10.008	5.41	3.800 to 8.780	ns
LH (IU/l)	5.58	4.400 to 8.217	5	3.643 to 6.350	**0.04**
Testosterone (nmol/l)	12.638	±6.138	15.702	±4.9032	**0.003**
SHBG (nmol/l)	38.62	30.115 to 52.700	44.2	31.200 to 55.625	ns
FAI (%)	30.292	±12.4892	36.902	±9.8163	**0.002**

BMI – body mass index, FSH – follicle stimulating hormone, LH – luteinizing hormone, SHBG – sex hormone binding globuline, FAI – free androgen index

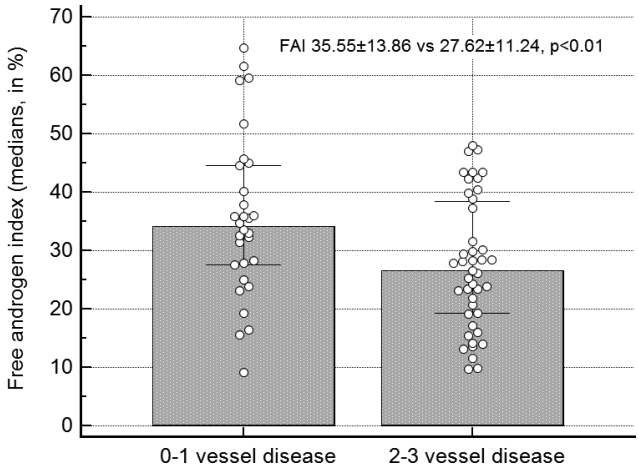

Figure 1 Comparison of FAI levels in 0-1 vessel disease versus 2-3 vessel disease

20 cases from the patients group (20.8%) had newly diagnosed hypogonadism: 7 were hyper-gonadotropic and 13 with normal LH levels. Hyperprolactinemia was excluded in all these subjects.

The patients group had significantly lower levels of TT (12.638±6.138 vs. 15.702±4.903, p<0.01), FAI (30.292 ±12.489 vs. 36.902±9.816, p<0.01) and significantly higher levels of LH compared with controls (5.58 IQR 4.4-8.21 vs. 5.0 IQR 3.64-6.35, p=0.04). There were no differences in FSH and estradiol levels. All measured parameters for both groups are displayed in **Table 1**. As expected, patients group had a higher prevalence of obvious risk factors of cardiovascular diseases (type 2 diabetes mellitus, arterial hypertension, dyslipidemia and smoking). In the stepwise multivariate logistic regression of parameters associated with ACS smoking, hypertension, dyslipidemia and FAI were independently associated with ACS **(Table 2)**.

73 cases underwent coronary angiography with the following findings: 7 cases with no significant stenosis, 23 cases with one vessel disease, 23 cases with 2 vessel disease and 20 cases with 3 vessel disease or extended stenosis requiring CABG. 30 patients were assigned to the 0-1 vessel disease group (non-severe CAD) and 43 patients were assigned to the 2-3 vessel disease group (severe CAD). Comparison of all parameters showed that patients with 0-1 vessel disease had significantly higher FAI (35.55% vs. 27.62%, p<0.01) **(Figure 1)**, lower prevalence of hypertension and lower BMI

Table 2 Results of stepwise multivariate logistic regression of parameters associated with the risk of ACS in males

Variable	Coefficient	Std. Error	Wald	Odds ratio	95% CI	p
Smoking	1.18385	0.53595	4.8792	3.2669	1.1427 to 9.3400	0.03
Hypertension	1.45323	0.46318	9.8441	4.2769	1.7253 to 10.6021	<0.01
Dyslipidemia	1.1564	0.4299	7.2357	3.1785	1.3686 to 7.3817	<0.01
FAI	-0.046498	0.018294	6.4606	0.9546	0.9209 to 0.9894	0.01

Variables entered: smoking, hypertension, statin therapy, type 2 diabetes, LH and FAI
Variables not included in the model: LH, type 2 diabetes
FA – free androgen index

Table 3 Univariate analysis of parameters associated with severe CAD on coronary angiography (2-3 vessel disease) in males

	0-1 vessel disease (n=30)		2-3 vessel disease (n=43)		
	mean/median	SD/IQR	mean/median	SD/IQR	p
Age (years)	62	57.000 to 66.000	65	59.250 to 71.000	ns
Body height (m)	1.75	1.700 to 1.800	1.72	1.692 to 1.800	ns
Body weight (kg)	80	74.000 to 94.000	92	78.250 to 99.500	ns
BMI (kg/m^2)	27.169	±4.7788	29.769	±4.4847	**0.02**
BMI>25 (%)	70		83.7		ns
Waist circumference (cm)	97.143	±14.2875	102.385	±14.5164	ns
Type 2 diabetes (%)	26.7		46.5		ns
Hypertension (%)	80		95.3		**0.04**
Dyslipidemia (%)	73.3		88.4		ns
Smoking (%)	30		37.2		ns
Estradiol (pmol/l)	103.6	73.600 to 147.500	96.32	74.950 to 124.425	ns
FSH (IU/l)	5.43	3.000 to 10.040	5.69	3.990 to 9.910	ns
LH (IU/l)	5.795	3.600 to 7.100	5.545	4.400 to 9.990	ns
Testosterone (nmol/l)	14.154	±6.2687	11.888	±6.5553	ns
SHBG (nmol/l)	38.4	30.330 to 49.130	37.97	29.535 to 52.450	ns
FAI (%)	35.552	±13.8564	27.619	±11.2379	**<0.01**

BMI – body mass index, FSH – follicle stimulating hormone, LH – luteinizing hormone, SHBG – sex hormone binding globulin, FAI – free androgen index

Table 4 Results of stepwise multivariate logistic regression of parameters associated with the severity of CAD on coronary angiography in males

Variable	Coefficient	Std. Error	Variable	Odds ratio	95% CI	p
FAI	-0.051732	0.020832	FAI	0.9496	0.9116 to 0.9892	0.013
Constant	1.98454	0.70629				0.005

Variables entered: age, BMI, type 2 diabetes, hypertension, FAI
Variables not included in the model: age, BMI, type 2 diabetes, hypertension
FAI – free androgen index

compared with the severe CAD group, otherwise there were no differences between the groups **(Table 3)**. Stepwise multivariate logistic regression of parameters associated with the extent of CAD identified FAI as a single independent factor associated with severe CAD **(Table 4)**.

Discussion

The principle findings of our study are that 20.8% of males with recent ACS have undiagnosed hypogonadism. Furthermore, ACS males had lower levels of TT and FAI and higher levels of LH compared with non-ACS controls and that FAI was associated with ACS independently of obvious CAD risk factors. Finally, FAI was a single independent factor associated with the extent of CAD.

Among the patients' group we confirmed previously undiagnosed hypogonadism in 20 (20.8%) patients (defined as TT< 7 nmol/l and FAI<24%), 7 of whom were hypergonadotropic (elevated LH). This fact might suggest that hypogonadism was not a direct consequence of the stress induced by the ACS itself, but rather a preexisting condition. Our results are consistent with the study of Malkin et al. where 24% hypogonadal patients were found among 930 patients with CAD (29). We assume that the prevalence of hypogonadism in our group reflects its epidemiology. Although hypogonadal patients in our study were older and had higher BMI compared to eugonadal subjects, we would consider it appropriate to evaluate sex hormones in males with CAD.

There are conflicting results about sex hormones levels in patients with documented CAD. In some studies testosterone – total or free – was lower in patients with positive coronary angiography compared with those with negative (26, 27, 28). Different results were documented in large prospective trials: in some, low levels of testosterone were not associated with higher incidence of cardiovascular events (30, 31, 32) but in the large meta-analysis there was inverse correlation between total and free testosterone and the incidence of cardiovascular events in men over 70 years old (35). Only one study confirmed "J-shaped" association between total testosterone and occurrence of ischemic attacks (stroke or myocardial infarction) (36) and no other study showed that patients with coronary heart disease have high levels of androgens. On the other hand only a few studies confirmed changes of LH levels in patients with coronary heart disease. In some studies, high-normal LH levels were associated with higher mortality (37), overall mortality (38) and higher prevalence of cardiovascular events in the follow-up (39). In patients with any chronic or acute illness LH levels decreased or stay unchanged (40, 41, 42, 43). In this study LH levels in patients differ from low normal to high levels in hypergonadotropic hypogonadism. However, in comparison with healthy controls the levels in the study group were higher, similar to the study by Rosano et al. (28).

Few studies evaluated sex hormones according to the severity of coronary angiography with unequivocal results (27, 28, 34, 44). Most of them documented the inverse relationship between the severity of CAD only for total testosterone, though some of them documented it also for free testosterone (33, 45). In one study TT inversely correlated with severity of ACS (44). In contrast, in Multi-Ethnic Study of Atherosclerosis, patients with lower free testosterone had a higher coronary artery calcium score, but lower carotid intima-media thickness (46). In addition, a recent prospectively designed study showed that patients with low-normal testosterone levels in the acute phase of ACS had better survival in the follow-up, assuming this may indicate better adaptation to stress (47). Ours showed that unlike TT, values of FAI as surrogates of bioavailable testosterone declined with the severity of coronary atherosclerosis. One of the possible explanations is the fact that total testosterone is affected by concentrations of binding globulins, especially SHBG. SHBG rises with age and decreases in patients with hyperinsulinemia which is typically found in obese patients with type 2 diabetes. In this study TT was significantly inversely associated with BMI and adiposity. In fact, lower levels of SHGB consequently lead to lower levels of TT but not free testosterone (FAI or bioavailable testosterone) (48) as confirmed in several reports in cases with metabolic syndrome (49, 50). Therefore, it appears that FAI could be more accurate as a surrogate of gonadal status in males with various common comorbidities. However, as the design of our study was cross sectional, it did not allow us to conclude whether low testosterone is the cause or the consequence of CAD. The final answer to the question as to whether low gonadal status is just a secondary marker of stress induced by CAD or is indeed associated upstream with pathogenesis

of coronary atherosclerosis still remains unclear. Our finding of 7 cases of undiagnosed hypergonadotropic hypogonadism in the patients' group and no such case in the age-matched control group would at least suggest some pathogenetic role of hypogonadism on ACS. Larger prospective and therapeutic trials would bring more light into causal relationship between hypogonadism and CAD.

This study has several strengths. It has included consecutive cases with cross-sectional design with the control group carefully matched for age and BMI. Multivariate analysis included in the model all standard risk factors of CAD. A high percentage of cases underwent coronary angiography which enabled the extent of CAD analysis.

Our study has several limitations. Blood sampling was carried out in the late acute phase of ACS which could have a negative effect on gonadal status. However, we consider this limitation as hardly avoidable due to the character and morbidity of the patients group. Later blood sampling could definitely lead to a higher los of follow-up rate and would lead to selection bias. Nevertheless, when comparing results from blood samples of patients sampled 7 days post ACS with patients sampled 3 months post ACS we found no differences between sex hormone levels (results not shown). Another limitation of our study is single blood sampling and lack of free testosterone assessment.

In conclusion

We found a high prevalence (20.8%) of previously undiagnosed hypogonadism (both hypergonadotropic and hypogonadotropic) among males with recent ACS. We confirmed that patients with recent ACS have lower levels of TT, FAI and higher levels of LH compared with controls and FAI was associated with ACS independently on standard CAD risk factors. Finally, FAI was also independently associated with CAD extent on coronary angiography.

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