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Androgen levels in men with acute and chronic coronary syndrome

Thesis summary

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The dissertation contains 174 pages including 58 tables and 16 figures. 382 references are cited.

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Abbreviations used:

1. CAD – coronary artery disease
2. bioT – bioavailable testosterone
3. GnRH – gonadotropin releasing hormone
4. ER – estrogen receptor
5. ERβ – estrogen receptor β
6. DHT - dehydrotestosterone
7. DM - type 2 diabetes mellitus
8. ICH – ischemic heart disease
9. BMI – body mass index
10. C/D – cortisol to DHEA-S ratio
11. CRH - corticotropin releasing hormone
12. MS - metabolic syndrome
13. UAP - unstable angina pectoris
14. ACS- acute coronary syndrome
15. T - testosterone
16. tT – total testosterone
17. fT – free testosterone
18. TG - triglycerides
19. TRT - testosterone replacement therapy
20. E -17ß – estradiol
21. fE – free estradiol
22. tE – total estradiol
23. CVD - cardiovascular disease
24. HR- heart rate
25. EF - ejection fraction
26. LOH - late-onset hypogonadism
27. CCS - chronic coronary syndrome
28. HPA - hypothalamic-pituitary-adrenal axis
29. HPG - hypothalamic-pituitary-gonadal axis
30. AR - androgen receptor
31. ARE- androgen response elements
32. cAMP- cyclic adenosine monophosphate
33. DHEA-S – dehydroepiandrosterone sulfate
34. ERK- extracellular signal-regulating protein kinase
35. FSH – follicle-stimulating hormone
36. GPER - G-protein coupled estrogen receptor
37. LH – luteinizing hormone
38. NSTEMI – non-ST-elevation myocardial infarction
39. PI3K - phosphatidylinositol 3-kinase
40. SHBG - Sex hormone binding globulin
41. STEMI – ST-elevation myocardial infarction

I. Introduction

The problem about androgen levels in men with acute and chronic coronary syndrome is highly relevant. The role of the so-called "major risk factors" has proven to be insufficient for both the overall elucidation of CHD pathogenesis and its prevention. In this regard, other factors playing a role in the genesis of this socially important disease with an increasing incidence worldwide are being sought.

A body of evidence has accumulated implicating sex hormones in the pathogenesis of CHD. Despite the empirical evidence that men are more likely to be affected than women in the pre-climacteric period and the suggestion that androgens are one of the likely causes, no firm scientific evidence has been presented and the question remains open. Regarding the effect of testosterone on the cardiovascular system, in patients with coronary pathology, there is conflicting data. (Gencer & Mach, 2016) Observational studies have linked hypotestosteronemia to higher CHD mortality, but there are also doubts about the safety of androgen replacement therapy. (Snyder et al., 2018)

In the context of CHD, low testosterone levels have been associated with both better adaptation, resp. survival, by some investigators and worse prognosis by others. (Gencer et al., 2021; Pesonen et al., 2016). Besides T, other androgens (DHEA-S) and estrogens are also relevant to the cardiovascular system. Their involvement in the pathogenesis of atherosclerotic cardiovascular disease has been suggested. (Wu & von Eckardstein, 2003) Therefore, a method allowing the simultaneous consideration of hormones that are assumed to be functionally dependent is being explored. Such an attempt to objectify hormonal interactions and balance may be represented by hormone ratios, i.e. an index that is considered as an indicator of the balance between two endocrine systems. (Maninger et al., 2009) Despite the potentially valuable information from the mentioned indices, their value in certain clinical situations as well as the most appropriate mathematical methods for their application have not yet been conclusively proven.

II. Aim and tasks

Aim:

Investigating the role of androgen hormones in adaptation to acute coronary syndrome and in the development of cardiovascular disease in men with acute and chronic coronary syndrome.

Tasks:

1. To investigate the pituitary-gonadal axis by measuring the values of total testosterone, calculated free testosterone, luteinizing and follicle-stimulating hormone in patients with acute and chronic coronary syndrome and in healthy controls.
2. To investigate differences and look for correlations in DHEA-S/cortisol; testosterone/LH; testosterone/estradiol ratios in healthy controls and in patients with acute and chronic coronary syndrome.
3. In patients with ischaemic heart disease, investigate differences in hormonal indices between subgroups with and without diabetes mellitus.
4. To follow the dynamics of sex hormones after the onset of ACS.
5. To investigate the relationship between the severity of acute coronary syndrome and other clinical, anthropometric and paraclinical indices, and steroid hormone levels in the serum of patients with acute coronary syndrome, in the acute period.
6. To screen for hypogonadism combined with anxiety and depression, and erectile dysfunction, at the onset of ACS and at the sixth month of the event.

III. Contingency, methods and definitions

1. Contingency

Patient recruitment: patients were recruited among hospitalized men - 105 patients, mean age 56.75 years (36-70). Of these, 72 with ACS and 32 with CCS. During the study period, 35 controls with a mean age of 54.22 (44-68) were included. The distribution of some clinical, paraclinical parameters, comorbidities and risk factors are presented in Tables 1-4.

The distribution of patients by group is presented in Figure 1

Figure 1. Distribution of patients and controls by groups

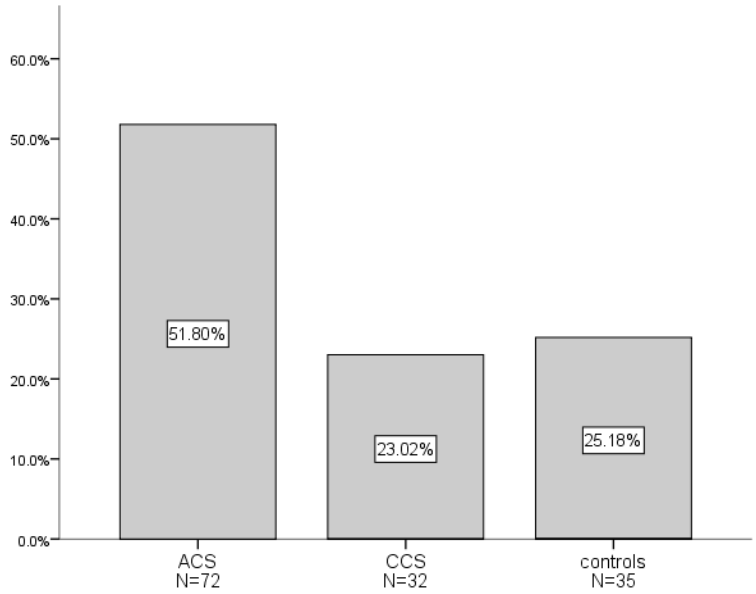
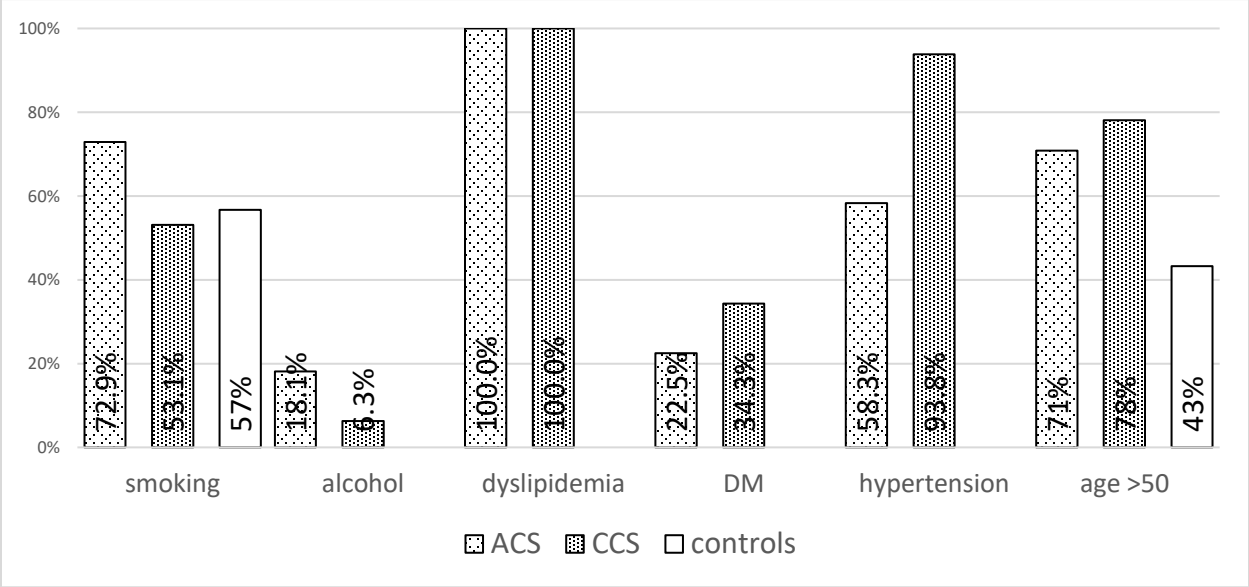


Figure 2 shows the distribution of some modifiable and non-modifiable cardiovascular risk factors in the three groups (ACS, CCS and control group).

Figure 2. Distribution of CV risk factors by groups.



The enrolled patients were divided into the following five categories:

1. Patients with acute coronary syndrome including STEMI, NSTEMI, UAP - "ACS".
2. Patients with stable ischemic heart disease presenting to one of the cardiology clinics for diagnostic clarification with coronary angiography - "CCS" group.
3. Combined ACS and CHD in one composite group - "IHD" group. This group is composed by the common sign of the presence of arterial coronary disease, regardless of whether the coronary syndrome is acute or chronic.
4. Patients from the ACS group followed up for at least 6 months after the acute cardiovascular event.
5. The control group consist of patients without evidence of cardiovascular disease.

2. Inclusion and exclusion criteria:

Male patients with acute coronary syndrome (STEMI, NSTEMI, unstable angina) were included in the ACS group.

Exclusion criteria for the ACS group are history of congenital or acquired causes of primary or secondary hypogonadism; chemotherapy and radiotherapy in the last 3 months; hormone therapy. As the comorbidities in the study cohort of patients were significant, additional exclusion criteria were severe decompensated organ failure unrelated to acute coronary syndrome; history of uncompensated endocrinopathies ; concomitant severe acute illness; acute or recent (previous 90 days) COVID-19 infection; undergone surgery within the last 6 months; history of proven psychiatric illness and psychotropic medication intake.

For the CHD group, inclusion requirements were proven ischemic heart disease. The exclusion criteria overlapped with those for CHD with the addition of ACS in the past 6 months (180 days) relative to the time of inclusion.

Controls were patients aged between 18 and 70 years without evidence of previous ACS and concomitant CVD. Exclusion criteria included all those mentioned for the ACS and CHD groups. Additional exclusion criteria were history of CHD, history of arterial hypertension and type 2 diabetes mellitus.

3. Methods

Observing the circadian rhythm and preanalytical conditions of blood sampling, the following parameters were studied: albumin (Siemens Advia 1800); cortisol (ADVIA Centaur CP system); DHEA-S (IMMULITE 2000 DHEA-SO4); testosterone (Siemens IMMULITE 2000); estradiol (Siemens IMMULITE 2000); LH, SHBG (IMMULITE 2000).

Survey methods:

Three survey methods were used in this study: androtest, HADS, and IIEF-5 (Corona et al., 2006) (Rosen et al., 1999) (Stern, 2014)

Statistical methods:

SPSS 19 statistical package was used. Statistical methods: Analysis of variance; Parametric analysis - Student's t - criterion for dependent (Paired t-test) and independent samples (Independent t-test); Non-

parametric Mann-Whitney analysis; Analysis of variance (ANOVA) with post-hoc Sheffe and Games-Howell tests; Non-parametric Kruskal-Wallis analysis, with Mann-Whitney post hoc serial tests with Bonferroni correction; Spearman and Pearson correlation analysis ; Univariate and multivariate regression analysis - Graphical analysis

For the hormone ratios shown, the following units of measurement were used: cortisol/DHEA-S - nmol/l to nmol/l; testosterone/estradiol - nmol/l to nmol/l; testosterone to LH and FT/LH - nmol/l to mIU/ml;

4. Definitions

Myocardial infarction: acute myocardial injury in clinical evidence of acute myocardial ischemia and evidence of a rise and/or fall in troponin with at least one value above the 99th percentile of reference values and one other of the following features: Symptoms of myocardial ischemia, new ischemic ECG changes; development of an abnormal Q wave; imaging evidence of new loss of viable myocardium or new local disturbances in cardiac wall kinetics in a manner typical of ischemic etiology; identification of thrombus angiographically. The presence or absence of ST-segment elevation ≥2 mm on 12-lead ECG sectioned patients into those with or without ST-elevation ST-elevation myocardial infarction (STEMI) or non-ST-elevation myocardial infarction (NSTEMI), respectively (Thygesen et al., 2018)Patients with typical clinical symptomatology and serially negative markers of myocardial necrosis were classified as having UAP.

When defining (hypogonadism) hypotestosteronemia we used a criterion based on the recommendations for good clinical practice of the Bulgarian Society of Endocrinology and the "Endocrine Society" in the USA (Bhasin et al., 2018; Bulgarian Society of Endocrinology, 2019) A value above 9.2 nmol/l was taken as the lower limit of normal. In terms of free testosterone, a lower limit of 0.220 nmol/l or 220 pmol/l has been used in defining "hypogonadism" (Wu et al., 2010)

Time in ischemia: the time from the onset of angina complaints to the time of stenting according to the angiographic protocol.

IV. Results

1. Characteristics of study subjects by groups:

With a view of structuring subgroups by individual indicators, the statistical processing of the data is presented in Tables 1 to 5.

Tables 1 to 5 present the population structure by some characteristics and the differences between the different groups and their statistical significances.

Table 1 Distribution and differences by age, BMI, marital status, smoking and alcohol use.

		ACS (n=72)	**CCS (n=32)**	**controls (n=35)**	
age	mean ± SD (95% CI)	56.12 ±9.73 (53.73-58.51)	58.51±8.41 (55.33-61.72)	54.22 ±7.23 (51.61-56.83)	χ^2=3.622 p=.163
BMI		28.54±4.37 (27.47-29.62)	31.7±5.25 (29.70-33.69)	28.98 ±3.52 (27.7-30.25)	**χ^2=8.135 p=.017**
marital status	married	N=42 95.5%	N=13 92.9%%	N=35 100%	
	not maried	N=2 4.5%	N=1 7.1%	n/a	
smoking	yes	N=51 72.9%	N=17 53.1%	N=21 60%	χ^2=3.171 p=0.074
	no	N=19 27.1%	N=14 43.8%	N=14 40%	
alcohol	yes	N=13	N=2	n/a	**χ^2=126.25**

	18.1%	6.3%		**p<.001**
no	N=54	N=28	N=35	
	75%	87.5%	100%	
no data	N=3	N=1	n/a	
	6.8%	3.1%		

Table 2. Post-hoc Mann-Whitney test to detect between-group differences in BMI.

indicator	groups	U value	p value
BMI	ACS : CCS	615	p=.006
	ACS : controls	960	p=.469
	CCS : controls	323	p=.042

Statistical significance was observed with Bonferroni correction for 3 groups p=.0167.

There was a statistically significant difference between the three groups for BMI: χ^2 (2)=8.135 p=.017,but not for age and χ^2(2)=3.622 p=.163. (Table 1) From the post-hoc analyses of the differences in BMI, this was only found between the ACS and CCS groups (U=615, p=.006). (Table 2).

In patients in the ACS group, the majority had ST-elevation myocardial infarction (72.2%). The other 2 subgroups had significantly lower percentages (NSTEMI- 12.5% and UAP- 15.8%). The comorbidities differed significantly between the ACS group and the CCS group. The percentage of patients aware of the presence of IHD was significantly higher in the CCS group than that of the ACS patients. The difference was 78.1% versus 38.9% at χ^2=14.233, p<.001. The trend for history of type 2 diabetes mellitus was the opposite. There was a higher relative proportion of this population in the CCS group (χ^2=11.778, p=.003).

In summary of the results presented in Tables 1 and 2:

- Statistically significant higher BMI was found in the CCS group compared to the other two. On the other hand, for age no statistically significant difference is found among the three groups.

- The populations of patients with ACS and CCS differ from each other in terms of the relative proportion of diabetics as well as the relative number of those with known ACS or IHD at admission.

Patient characteristics and between-group differences related to cardiovascular disease and cardiovascular risk are presented in Tables 3 to 5.

Of all patients in the ACS group, 71.2% were stented earlier than the twelfth hour. For 76.1% of them it was the first acute cardiovascular event.

The results of a study of the clinical characteristics of patients with ACS are presented in Table 3.

Table 3 Results of the study of clinical parameters in the subgroups of ACS admission (acute period)

		ACS in total (n=72)	STEMI (n=52)	NSTEMI (n=9)	UAP (n=11)	F/ χ^2 p
Acute HI	yes	n=15 21.1%	n=14 27.5%	n=1 11.1%	n/a	χ^2=4.712 p=.03
	no	n=56 78.9%	n=37 72.5%	n=8 88.9%	n=11 100%	
Killip class	1	n=67 93.1%	n=48 92.3%	n=8 88.9%	n/a	χ^2= 2.259 p=.555
	2	n=2 2.8%	n=2 3.8%	n/a	n/a	
	3	n=3 4.2%	n=2 3.8%	n=1 11.1%	n/a	
ejection fraction		49.67 ±10.5 46.65-52.69	48.11 ±9.48 45.47-50.75	43.89 ±10.63 35.72-52.06	53.73 ±13.63 44.56-62.88	F=2.343 p=0.103
Grace score		94.38 ±25.89	99.42 ±20.27	86.77 ±35.24	79.27 ±18.7	F=4.288

	86.95- 101.82	93.66-105.18	59.68-105.18	66.71-91.83	**p=0.017**
systolic BP	138.70 ±28.04 (132.06- 145.34)	141.92 ±27.67 134.14- 149.7	128.89 ±24.59 109.98- 147.79	131.81 ±31.64 110.56- 153.08	χ^2=3.25 p= 0.197
diastolic BP	83.92 ±15.83 (79.37- 88.47)	84.27 ±14.45 80.21- 88.33	75 ±13.69 64.47- 85.53	75.45 ±14.4 65.78- 85.13	**χ^2=6.7 p=0.035**
HR	77.34 ±18.27 70.91- 83.78	80.04 ±21.31 73.98- 86.1	66.1 ±10.81 57.80- 74.42	73.36 ±14.46 63.64- 83.08	χ^2=4.28 p= 0.118
CK	413.67 ±716.96 214.07-613.27	493.02 ±788.79 244.05-741.99	150.50 ±177.26 131.55-432.55	99.29 ±32.23 69.48-129.10	**χ^2=10.253 p<.001**
CK-MB	56.85 ±103.51 32.35-81.36	70.06 ±115.97 37.77-102.34	35.09 ±62.23 16.94-87.11	10.28 ±6.27 6.07-14.50	**χ^2=17.375 p<.001**
Tr-1	58.96 ±69.18 42.71-75.22	80.81 ±70.03 61.32-100.31	3.42 ±4.33 .09-6.74	1.11 ±2.52 -.58-2.81	**χ^2=36.203 p<.001**

A statistically significant difference was observed in the incidence of acute cardiac decompensation between subgroups within the ACS. (χ2(2)=4.712, p=.03) However, the difference in ejection fraction was not significant between NSTEMI, and UAP subgroups.

GRACE score, systolic and diastolic arterial pressure showed statistically significant difference between groups. (Correspondingly F=4.288, p=0.017 and χ^2=6.7 p=0.035).

In relation to the differences found presented in Table 3, additional Scheffe post-hoc tests and three consecutive Mann-Whitney tests with Bonferroni correction were performed. These post-hoc tests are described in Table 4.

Table 4 Scheffe's post-hoc test for indicators with statistical significance between the means of the three groups (STEMI, NSTEMI, UAP) Mann-Whitney test for comparison between the mean diastolic BP values in the three groups (STEMI, NSTEMI, UAP).

		mean difference between groups	p-value	95% CI	
Grace score	STEMI: NSTEMI	12.642	p=.303	-7.65	32.93
	STEMI: UAP	**20.147**	**p=0.031**	**1.49**	**38.81**
	NSTEMI: UAP	7.505	p=.758	-17.68	32.69

	U value	p-value

diastolic BP	STEMI : NSTEMI	-1.912	0.056
	STEMI : UAP	-2.048	0.040
	NSTEMI: UAP	-0.038	0.968

From the post-hoc test conducted by Scheffe, there was a difference in GRACE score between the STEMI and UAP groups but not between NSTEMI and the other two. For diastolic BP, there was no statistically significant difference between the groups.

The differences in biochemical parameters between the ACS and CCS groups are investigated. The results are presented in Table 5.

The results of comparing biochemical parameters between groups ACS and CCS.

			ACS (n=72)	CCS (n=32)	t/U value; p-value
Lipid profile	HDL	mean ± SD (95% CI)	1.153 ±0.3411 1.072-1.234	1.093 0.3434 0.965-1.221	U=965.000 p=.457
	LDL		3.46 ±1.23 3.170-3.754	2.41 ±0.94 2.063-2.763	t=4.167 p<0.001
	total cholesterol		5.454 ±1.5323 5.094-5.814	4.159 0.9716 3.796-4.521	U=513.500 p<0.001
	TG		1.9097 ±1.00329 1.6669-2.1526	1.7124 ±.80234 1.4072-2.0176	U=895.000 p=0.473
blood count	HB		146.09 ±14.26 142.69-149.49	145.567 ±10.1427 1141.78-149.35	t=0.180 p=0.857
	ER		4.9265 ±0.57244 4.7890-5.0640	4.9020 ±0.40762 4.7498-5.0542	t=0.21 p= 0.832
	LEU		10.8749 ±3.95853 9.9310-11.8187	8.0077 ±2.09253 7.2263-8.79	U=576.500 p<0.001
	TR		231.886 ±71.5 214.8-248.9	219.667 65.7 195.1-244.2	0.801 p= 0.424
	HTC		0.43911 0.040134	0.44357 0.028285	t= -0.55 p= 0.58

		0.42954-.44868	0.433-0.454	
biochemistry	AST	24.99 ±6.88 22.89-27.08	24.334 ±7.8795 20.536-28.131	U=375.500 p=0.524
	ALT	27.804 13.4125 23.909-31.698	29.862 8.1524 26.047-33.677	U=351.000 p=.082
	creatinine	94.956 29.0682 88.125-101.786	86.032 18.4248 79.152-92.912	U=938.500 p=0.299
	eGFR	85.38 22.211 80.16-90.59	89.03 16.059 83.04-95.03	U=992.000 p=0.518
	glucose	8.1790 4.14330 7.2054-9.1527	7.5313 3.83551 6.0991-8.9635	U=901.000 p=0.188
	albumin	42.09 3.8167 41.188-42.982	42.21 4.4983 40.588-43.831	t=-.145 p=.885

There is a statistically significant difference in LDL-cholesterol and total cholesterol levels between the groups of patients with ACS and those with stable angina (t=4.167 p<0.001; U=513.500 p<0.001). A statistically significant difference is found between leukocyte values in the two groups. (U=576.500 p<0.001). There is no statistically significant difference between ACS and CCS groups in terms of other biochemical parameters, blood count parameters, HDL and triglyceride levels.

2. Hormonal parameters in the ACS, CCS and control groups

When comparing the hormone levels between the three groups, a statistically significant difference was found in terms of tT (F=3.928, p=.022), bioT in nmol/l (χ^2= 13.968, p=.001), fT in nmol/l (F=3. 458, p=.034) DHEA-S (χ^2= 18.269, p<.001), cortisol (F=5.786, p=.004), LH (χ^2= 6.886,

p=.032), C/D ratio (χ2= 20.295, p<. 001), the tT/tE ratio (χ^2= 13.304, p=.001), fT/fE (χ^2= 6.876, p=.032) and the tT/LH ratio (χ^2= 7.165, p=.028) (Table 6).

Table 6 Differences in hormonal parameters between the study groups.

	ACS (N=72)	CCS (N=32)	controls (N=35)	F/ χ^2-value p-value
testosterone	8.97 ±4.11	10.06 ±3.92	11.1 ±2.38	**F=3.928 p=.022**
SHBG	30.71 ±10.88	35.63 ±15.52	31.55 ±10.46	χ^2= 1.370 p=.504
estradiol	185.34 ±76.04	192.81 ±89.89	164.49 ±39.58	χ^2= 1.141 p=.565
DHEA-S	3.25 ±2.23	2.48 ±1.80	4.81 ±2.45	**χ^2= 18.269 p<.001**
cortisol	529.29 ±159.76	442.21 ±148.92	441.17 ±122.4	**F=5.786 p=.004**
LH	3.52 ±1.86	3.31 ±1.35	4.34 ±1.83	**χ^2= 6.886 p=.032**
fT	0.18993 ±0.09	0.191 ±0.065	.22±.04	**F=3.458 p=.034**
fT %	2.146 ±.45	1.93 ±.49	2.05±.35	χ^2= 3.645 p=.162
bioT	4.41 ±2.15	4.44 ±1.62	5.60±.89	**χ^2= 13.968 p=.001**
bioT %	48.57 ±12.55	45.36 ±13.28	50.88±10.46	χ^2= 2.437 p=.296
C/D	.219 ±.152	0.264 ±.152	.108±.058	**χ^2= 20.295 p<.001**
tT/tE	51.7 ±29.151	63.176 ±35.348	71.55 ± 25.682	**χ^2= 13.304 p=.001**
fE	4.854 ±2.1	4.58 ±1.81	4.34±.96	χ^2= .676 p=.713
fE%	2.63 ±.26	2.54 ±.40	2.63 ±.26	χ^2= .902 p=.637
fT/fE	.045 ±.026	.046 ±.022	.044±.026	**χ^2= 6.876 p=.032**
tT/LH	2.949 ±1.966	3.714 ±1.924	2.936±1.153	**χ^2= 7.165 p=.028**
fT/LH	0.059 ±0.035	0.061 ±0.027	0.059 ±0.020	χ^2= 1.996 p=.369

Because of the differences found in hormonal parameters, post-hoc analyses were performed to identify between-group differences. The results are presented in Tables 7 and 8.

Table 7 Post-hoc tests to detect between-group differences in total T, cortisol and free T.

		mean group difference	p-value	95% CI	
Games-Howell post-hoc for total T	ACS : CCS	-1.08326	.419	-3.1377	.9711
	ACS : controls	**-2.12631**	**.003**	-3.6328	-.9244
	CCS: controls	-1.04305	.412	-3.0105	.9244
Scheffe post-hoc test for cortisol	ACS : CCS	**87.07664**	**.030**	6.7152	167.4381
	ACS : controls	**88.11771**	**.028**	9.5916	166.6438
	CCS : controls	1.04107	1.000	-92.1025	94.1847
	ACS : CCS	-.000639	.999	-.03810	.03682
	ACS : controls	**-.039988**	**.017**	-.07390	-.00607

Games-Howell post-hoc test for free testosterone	CCS : controls	**-.039349**	**.029**	-.07531	-.00339

The results presented in Table 7 demonstrate a difference in the level of tT between the ACS group and the control group. No statistically significant difference is found between the CCS group with ACS as well as between CCS and controls. Our data on fT levels were similar. From the Games-Howell post-hoc analysis, it is clear that there is a difference in tT levels between the CCS and controls and between ACS and control, whereas there is no difference in values between the CCS and ACS groups. The cortisol levels in the ACS group are statistically significantly higher compared to those in the CCS group and to the controls.

Table 8 Post-hoc tests to detect between-group differences in DHEA-S, LH, bioT, C/D, tT/tE, fT/fE, tT/LH.

parameter	groups	U-value	p-value
DHEA-S	ACS: CCS	881.500	.085
	ACS: controls	**602.500**	**.001**
	CCS: controls	**194.000**	**<.001**
LH	ACS: CCS	1083.000	.878
	ACS: controls	809.500	.190
	CCS: controls	350.500	.020
bioT	ACS: CCS	1131.500	.885
	ACS: controls	**777.000**	**.001**
	CCS: controls	**273.500**	**<.001**
C/D	ACS: CCS	911.000	.424
	ACS: controls	**537.000**	**<.001**
	CCS: controls	**214.500**	**<.001**
tT/tE	ACS: CCS	888.000	.101
	ACS: controls	**541.000**	**<.001**
	CCS: controls	377.500	.103
fT/fE	ACS: CCS	1022	.499
	ACS: controls	**776.0**	**.008**
	CCS: controls	384.0	.124
tT/LH	ACS: CCS	**776.500**	**.010**
	ACS: controls	1018.000	.284
	CCS: controls	401.000	.096

DHEA-S values in the three groups show statistically significant difference among them (χ^2= 18.269, p<.001). (Table 6) Differences are found between the ACS and control groups (U=602. 5 p=.001), CCS and controls (U=194.0 p<.001), but not between CCS and ACS groups (U=881.5 p=.085). (Table 8) BioT also shows a difference between groups (χ^2= 13.968, p=.001). Post-hoc analysis, just as with DHEA-S reveal differences between the ACS group and controls (U=777.0 p=.001), CCS and controls (U=273.5 p<.001), but not between the CCS and ACS groups (U=1131.5 p=.885). (Table 8) The results are similar when examining differences in C/D ratio values. Kruskal-Wallis analysis reveal a statistically significant difference (χ^2= 20.295, p<.001). (Table 6) After conducting three Mann-Whitney tests, it is clear that, as with the previous two indicators, there is a difference between the ACS group and the controls (U=537.0 p<.001), between CCS group and controls (U=214.5 p<.001), but not between the CCS and ACS groups (U=911. 0, p=.424). (Table 8) For LH and for the tT/tE ratio, there is also a difference between the ACS and CCS groups on the one hand and the controls on the other hand. (Table 6) Similarly for tT/tE, fT/fE also demonstrated a statistically significant difference in mean values between the ACS and control groups. (U=776.0 p=.008) (Table 8)

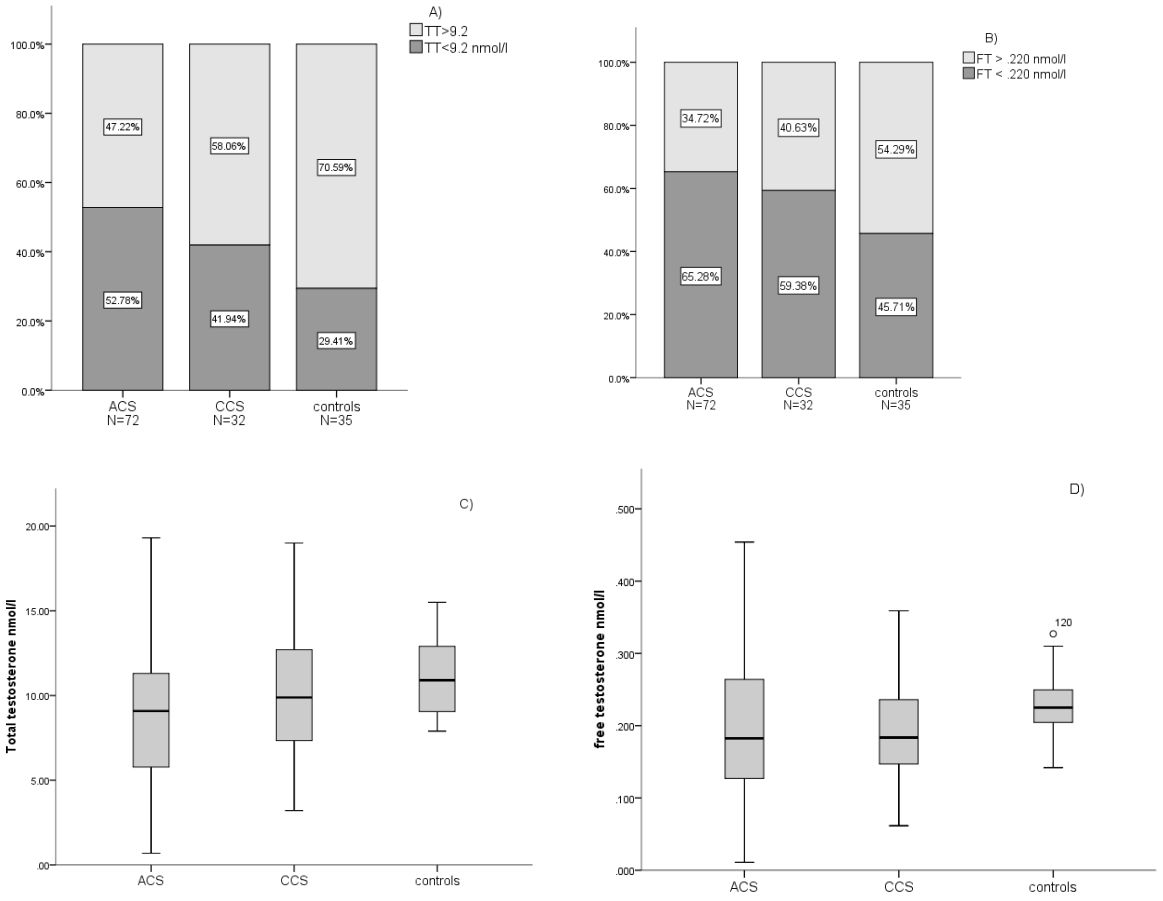
For three of the parameters (DHEA-S, bioT, C/D, fT), there appear to be a difference between the ACS group with that of the controls and the CCS with that of the controls, but not between the two patient groups. These differences place the above-mentioned values in two separate groups -the cardiac patients and the healthy controls.

When a post-hoc test for the T/LH ratio is performed, a statistically significant difference is found between the ACS and CCS groups (U=776.5 p=.010) (Table 8).

Using a threshold value for tT of 9.2nmol/l we found that 52.78% of patients in the ACS group would have been classified as having hypotestosteronemia. Correspondingly for the CCS and control groups this was 41.94% and 29.41% respectively. (Figure 3A) Using free testosterone <.220nmol/l as the criteria then 65.28% of patients in the ACS group would have been classified as having hypotestosteronemia. In the CCS and control groups 59.38% and 45.71% respectively. (Figure 3C) There was a statistically significant difference in the incidence of hypotestosteronemia

between the CCS and control groups using both oT ($\chi^2(1)=5.089$, p=.024). (Figure 3B) Using fT as criteria the difference was of borderline statistical significance. ($\chi^2(1)=3.723$, p=.05). (Figure 3D)

Figure 3. Percentage distribution of men with low and normal testosterone and difference between groups.



In summary:

1. Controls had significantly higher values of tT and fT, bioT and DHEA-S compared to the other two groups (CCS and ACS).

2. Controls had significantly lower C/D ratio compared to the other two groups.

3. The ACS group had a significantly lower value of tT/tE and fT/fE ratios compared to the controls group.

4. The ACS group had significantly higher morning serum cortisol than the other two groups

5. The ACS group had a significantly lower tT/LH ratio compared to the CCS group.

3. Acute coronary syndrome (ACS)

3.1 Comparison of hormonal parameters depending on the type of incident.

Comparison of hormonal values between patients according to the subtype of stricture coronary syndrome reveals statistically significant differences in terms of tT (χ^2=7.79 p=.02), fT (χ^2=7.311 p=.026), bioT (χ^2=8.582 p=.014), and tT/tE (χ^2=6.724 p=.035), presented in Figures 4 and 5.

Figure 4

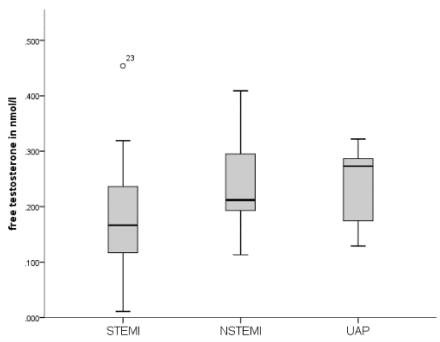
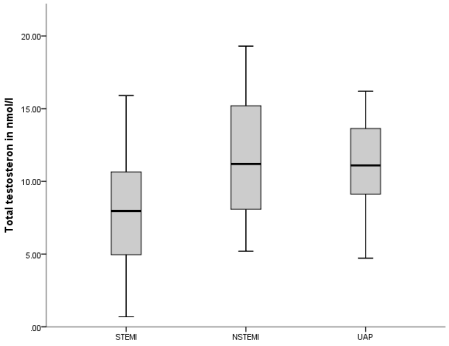


Figure 5



Based on differences in hormonal parameters, STEMI differentiates as a separate subgroup (relative to NSTEMI and UAP) Therefore, we examined differences in hormonal parameters between the STEMI subgroup and controls. The differences between the groups are shown in Table 9.

Table 9 Differences in hormonal indices between patients with STEMI and controls.

parameter	STEMI (n=52)	controls (n=35)	U-value, p-value
age	55.52 ±9.144 (52.67-58.37)	54.21 ±7.413 (51.39-57.03)	U=776 p=.246
BMI	28.27 ±4.15 (26.98-29.56)	28.9 ±3.31 (27.64-30.16)	U=686.5 p=.423
tT	8.33 ±3.35 (7.29-9.37)	10.98 ±2.17 (10.16-11.81)	**U=454.5 p<.001**
SHBG	30.59 ±11.42 (27.03-34.15)	29.9 ±9.99 (26.11-33.7)	U=877 p=.775
tE	183.68 ±78.99 (159.07-208.30)	163.49 ±40.08 (148.24-178.73)	U=788 p=.685
DHEA-S	3.54 ±2.23 (2.85-4.24)	5.11 ±2.43 (4.19-6.03)	**U=596.5 p=.013**
cortisol	529.7 ±170.87 (476.45-582.95)	441.64 ±123.1 (394.82-488.47)	**U=501 p=.002**
LH	3.29 ±1.50 (2.82-3.76)	4.2 ±1.8 (3.51-4.88)	**U=532.5 p=.006**
FT	.180 ±.083).154-.205)	.223 ±.038 (.209-.238)	**U=514.5 p=.001**
FT %	2.18 ±.52 (2.02-2.34)	2.07 ±.36 (1.93-2.21)	U=806 p=.368
bioT	4.15 ±2.07 (3.51-4.80)	5.63 ±.90 (5.29-5.97)	**U=445 p<.001**
bioT %	49.56 ±12.78 (45.58-53.54)	51.24 ±10.73 (47.16-55.33)	U=787 p=.287
C/D	.198 ±.113 (.162-.233)	.106 ±.055 (.086-.127)	**U=453 p=.001**
TT/TE	47.82 ±26.48 (39.57-56.07)	71.71 ±25.98 (61.83-81.59)	**U=371.5 p<.001**
FE	4.79±2.22 (4.10-5.48)	4.33 ±.96 (3.97-4.69)	U=786 p=.672
FE%	2.62 ±.29 (2.53-2.71)	2.64 ±.26 (2.54-2.74)	U=811 p=.847
FT/FE	.044 ±.027 (.036-.053)	.055 ±.020 (.047-.063)	**U=490 p=.002**
TT/LH	2.95 ±1.72 (2.42-3.49)	2.99 ±1.19 (2.54-3.44)	U=706 p=.170
FT/LH	.063 ±.037 (.052-.075)	.061 ±.022 (.052-.069)	U=713 p=.297

In the STEMI group, mean age and BMI does not differ from controls. Differences that emerg in the present analysis are with respect to T levels. All three of its fractions have a higher mean value in the control group compared to STEMI patients (TT: U=454.5 p<.001; FT: U=514.5 p=.001 and bioT: U=445 p<.001). Percentages of bioT and FT are not significantly different between the two groups. A statistically significant difference with a higher mean in the control

group is also found for DHEA-S (U=596.5 p=.013) and LH (U=532.5 p=.006). Cortisol is higher in the STEMI subgroup compared to the control group.(U=501 p=.002) The C/D ratio is lower in the control group (U=453 p=.001), while TT/TE and FT/FE are higher in the control group.(U=371.5 p<.001 and U=490 p=.002, respectively). In summary of the presented data, the differences between ACS and controls on the one hand and STEMI and controls on the other hand overlap to a large extent. In both cases we observ statistically significant differences in TT, DHEA-S, cortisol, bioT, C/D and TT/TE levels. When comparing STEMI with controls, additional differences besides those already mentioned are found, namely in FT levels and FT/FE ratio.

3.2 Differences between groups according to ST-elevation.

When examining the group of ACS patients according to the presence of ST-elevation at admission, some differences in hormonal indices are found between the groups, presented in Table 10.

Table 10 Differences in hormonal indices between groups with and without ST-elevation.

параметър	Със ST-елевация (STEMI)	Без ST-елевация (NSTEMI+НАП)	U-стойност p-стойност
BMI	28.4 4.54 (27.1-29.7)	28.9 4.02 (26.75-31.04)	U=359.5 p=.620
age	56.11 ±9.05 (53.61-58.62)	55.94 ±10.69 (50.63-61.62)	U=472.5 p=.953
TT	8.1674 ± 3.8 (7.12-9.22)	10.94 ± 4.13 (8.89-12.99)	**U=306 p=.024**
SHBG	30.651 ±10.8 (27.67-33.63)	30.18 ±11.31 (24.56-35.81)	U=438.5 p=.611
TE	185.87 ±81.56 (163.4-208.4)	182.94 ±61.47 (152.37-213.51)	U=461 p=.833
DHEA-S	3.51 ±2.16 (2.9-4.12)	2.64 ±2.33 (1.48-3.80)	**U=314 p=.048**
cortisol	538.65 ±169.2 (492-585.3)	511.6 ±128.61 (447.65-575.56)	U=431 p=.543
LH	3.4 ±1.8 (2.9-3.91073)	3.76 ±2.05 (2.71-4.82)	U=401.5 p=.650
FT	.176 ±.09 (.151-.200)	.226 ±.078 (.187-.265)	**U=317 p=.034**
FT %	2.16 ±1.72 (4.51-6.22)	2.13 ±.36 (1.95-2.31)	U=451.5 p=.736
bioT	4.05 ±2.2 (3.44-4.65)	5.36 ±1.72 (4.51-6.22)	**U=289 p=.013**
bioT %	48.93 ±11.96 (45.64-52.23)	48.04 ±14.66 (40.75-55.33)	U=421.5 p=.463
C/D	.197 ±.145 (.157-.238)	.267 ±.153 (.188-.346)	U=315 p=.077

TT/TE	46.91 ±28.96 (38.93-54.89)	64.18 ±26.41 (51.052-77.314)	U=290.5 p=.014
FE	4.86 ±2.26 (4.23-5.48)	4.85 ±1.64 (4.04-5.66)	U=457.5 p=.797
FE%	2.62 ±.27 (2.55-2.70)	2.66 ±.233 (2.55-2.78)	U=419.5 p=.447
FT/FE	.042 ±.028 (.035-.050)	.051 ±.022 (.04-.062)	U=344 p=.079
TT/LH	2.82 ±1.89 (2.301-3.344)	3.34 ±2.25 (2.187-4.498)	U=377 p=.314
FT/LH	.058 ±.036 (.048-.068)	.061 ±.034 (.0428-.079)	U=384 p=.724

For TT, FT and bioT levels, a statically significant difference is found between groups using Mann-Whitney analysis (respectively: U=306 p=.024; U=317 p=.034 and U=289 p=.013). For all three parameters, the mean value for each of the two samples is lower in the ST-elevation subgroup. For DHEA-S, there is also a statistically significant difference with a higher value in the group of patients with ST-elevation (U=314 p=.048). This is against the background of statistically insignificant difference in age and BMI. For the TT/TE ratio, as with testosterone, a statistically significant higher value is found in the group of patients without ST-elevation.

3.3 Diabetes mellitus and ACS

Considering the group of patients with ACS according to the presence of diabetes mellitus, some differences are found between the two subgroups, presented in Table 11.

Table 11 Differences in hormonal parameters according to the presence of DM in the group of ACS.

	DM (n=16)	without DM (n=55)	t/U, p-value
AGE	62.19 ±7.350 (58.27-66.10)	54.27 ±9.261 (51.77-56.78)	t=-3.138 p=.003
testosterone	7.77 ±4.64 (5.3-10.24)	9.25 ±3.93 (8.19-10.31)	t=1.268 p=.209
SHBG	27.19 ±9.73 (22.01-32.38)	31.54 ±11.09 (28.54-34.54)	t=1.416 p=.161
estradiol	177.31 ±89.17 (129.79-224.82)	187.05 ±73.13 (167.28-206.8)	U=375 p=.371
DHEA-S	1.85 ±1.32 (1.15-2.56)	3.66 ±2.31 (3.03-4.3)	U=196.5 p=.001
cortisol	548.02 ±125.35 (481.23-614.8)	521.64 ±169.58 (475.79-567.48)	t=-.577 p=.566
LH	3.62 ±1.39 (2.78-4.46)	3.50 ±1.98 (2.96-4.03)	U=312.5 p=.483
FT	.172 ±.091 (.124-.221)	.194 ±.09 (.170-.219)	t=.851 p=.398
FT %	2.28 ±.5 (2.02-2.55)	2.11 ±.44 (1.99-2.23)	U=343 p=.182
bioT	3.88 ±2.04 (2.79-4.97)	4.55 ±2.19 (3.96-5.14)	t=1.090 p=.280

bioT %	50.76 ±9.28 (45.82-55.71)	48.13 ±13.4 (44.51-51.75)	U=365.5 p=.305
C/D	.377 ±.202 (.265-.489)	.175 ±.101 (.148-.203)	U=137 p<.001
TT/TE	50.371 ±36.99 (30.66-70.08)	51.741 ±27.018 (44.437-59.045)	U=377 p=0.386
FE	4.82 ±2.41 (3.54-6.10)	4.86 ±2.04 (4.30-5.41)	t=.060 p=.952
FE%	2.73 ±.23 (2.61-2.85)	2.61 ±.26 (2.54-2.68)	U=324 p=.110
FT/FE	.042 ±.030 (.026-.058)	.046 ±.026 (.039-.053)	U=363 p=.289
TT/LH	1.994 ±1.25 (1.323-2.66)	3.22 ±2.074 (2.654-3.786)	U=262 p=.017
FT/LH	.046 ±.027 (.031-.061)	.062 ±.037 (.052-.073)	U=316 p=.266

Using a Student's t-test we found a statistically significant higher mean age for the diabetic group (t=-3.138 p=.003). For both DHEA-S and TT/LH ratio we found a statistically significant lower value in the diabetic group using the Mann-Whitney non-parametric analysis (respectively U=196.5, p=.001 and U=262, p=.017). The C/D ratio is higher in the diabetic group. (U=137, p<.001) In stepwise regression analysis, the presence of DM became a statistically insignificant determinant of DHEA-S after adjustment for age. (β=-.170, p=.119) Therefore, due to the different mean ages, we cannot conclusively conclude whether the presence of DM influences DHEA-S levels in the first hours after ACS.

Examining the TT levels against the lower limit of 9.2 nmol/l, we find no difference in the incidence of hypogonadism in patients with ACS according to the presence of DM (χ^2= 1.92, p=. 165) This trend was also true for the STEMI subgroup-there is no statistically significant difference in the incidence of hypogonadism (defined as TT<9.2nmol/l) in diabetic patients and patients with normal glucose tolerance. (χ^2=.225, p=.724).

In summary of the differences between patients with and without DM:

- No significant difference is found in T levels and its fractions in patients with and without diabetes mellitus in the ACS group on the background of different mean ages.

- The difference in DHEA-S levels between patients with and without DM2 was not significant after adjustment for age.

- In the group of diabetic patients with ACS, a lower TT/LH ratio and higher C/D were found compared with patients without DM.

3.4 Associations between hormonal and clinical parameters

The correlations of the hormonal parameters examined up to the 48th hour of ACS with some clinical parameters examined during hospitalization are presented in Table 12 - 14.

Table 12 Correlations of hormonal indices with presence of DM, history of CVD and age in the ACS group.

	DM	history for IHD	history for hypertension	age
TT	r_s=-.164 p=.173	r_s=.201 p=.096	r_s=.117 p=.334	r=0.120 p=.314
SHBG	r_s=-.169 p=.160	r_s=.094 p=.440	r_s=.017 p=.890	**r= 0.367 p=0.002**
DHEAS	**r_s=-.392 p=.001**	**r_s=-.512 p<001**	**r_s=-.296 p=.014**	**r_s=-0.506 p<0.001**
LH	r_s=.086 p=.487	**r_s=.315 p=.009**	r_s=.194 p=.112	r_s= 0.186 p=0.125
FT	r_s=-.093 p=.441	r_s=.144 p=.236	r_s=.095 p=.433	r= -0.016 p=0.893
FT%	r_s=.160 p=.184	r_s=-.081 p=.506	r_s=-.011 .928	**r_s=-0.298 p=0.011**
bioT	r_s=-.120 p=.319	r_s=.159 p=.187	r_s=.094 p=.441	r= -0.052 p=0.663
bioT%	r_s=.123 p=.309	r_s=-.129 p=.287	r_s=-.075 p=.539	**r_s=-0.428 p<0.001**
E2	r_s=-.107 p=.375	r_s=.105 .385	**r_s=.253 p=.034**	r_s=0.175 p=0.142
FE	r_s=-.060 p=.619	r_s=.085 p=.483	**r_s=.244 p=.042**	r= 0.203 p=0.087
FE%	r_s=.191 p=.111	r_s=-.053 p=.661	r_s=.050 p=.682	**r_s=-0.260 p=.028**
cortisol	r_s=.094 p=.437	r_s=-.182 p=.132	r_s=-.100 p=.412	r= -0.055 p=0.647
C/D	**r_s=.473 p<.001**	**r_s=.417 p<.001**	**r_s=.253 p=.037**	**r_s=0.459 p<0.001**
TT/TE	r_s=-.104, p=.390	r_s=.154, p=.204	r_s=.031, p=.801	r_s=-0.066 p=0.580
FT/FE	r_s=-.127, p=.293	r_s=.046 p=.704	r_s=-.146 p=.227	r_s=-0.180 p=0.130
TT/LH	**r_s=-.286 p=.016**	r_s=-.119 p=.332	r_s=-.064 p=.604	r_s=-0.002 p=0.989
FT/LH	r_s=-.137 p=.269	r_s=-.143 p=.251	r_s=.011 p=.931	r_s=-0.068 p=0.581

Table 13 Correlations of hormonal indices with type of event, myocardial necrosis enzymes, and presence of ST-elevation in the ACS group Type of event - STEMI, NSTEMI, or UAP.

	type of accident	CK-MB	CK	troponin	number of coronary arteries with stenosis	ST-elevations
TT	**r_s=.327 p=.005**	r_s=-.135 p=.263	r_s=-.210 p=.135	r_s=-.217 p=.067	r_s=.055 p=.651	**r_s=-.270 p=.023**
SHBG	r_s=-.023 p=.847	r_s=-.089 p=.462	r_s=-.075 p=.595	r_s=.050 p=.675	r_s=.203 p=.091	r_s=.061 p=.614
albumin	r_s=.182 p=.126	r_s=-.180 p=.132	r_s=-.120 p=.395	r_s=-.105 p=.380	r_s=-.086 p=.479	**r_s=-.265 p=.026**
estradiol	r_s=.098 p=.413	**r_s=-.378 p=.001**	**r_s=-.322 p=.020**	r_s=-.044 p=.711	r_s=-.202 p=.094	r_s=-.025 p=.834
DHEA-S	**r_s=-.262 p=.028**	r_s=.026 p=.830	r_s=.026 p=.853	r_s=.149 p=.219	**r_s=-.340 p=.004**	**r_s=.240 p=.047**
LH	r_s=.119 p=.331	r_s=-.210 p=.085	r_s=-.213 p=.137	r_s=-.176 p=.148	r_s=-.033 p=.794	r_s=-.055 p=.654
cortisol	r_s=-.091 p=.448	r_s=.100 p=.404	r_s=.041 p=.774	r_s=.077 p=.520	**r_s=-.236 p=.049**	r_s=.073 p=.547
FT	**r_s=.318 p=.007**	r_s=-.094 p=.434	r_s=-.215 p=.126	**r_s=-.266 p=.024**	r_s=-.032 p=.794	**r_s=-.253 p=.033**
FT%	r_s=.043 p=.718	r_s=.063 p=.599	r_s=.016 p=.910	r_s=-.114 p=.340	r_s=-.151 p=.212	r_s=-.040 p=.739
bioT	**r_s=.344 p=.003**	r_s=-.120 p=.319	r_s=-.248 p=.077	**r_s=-.293 p=.012**	r_s=-.036 p=.769	**r_s=-.297 p=.012**
bioT %	r_s=.056 p=.638	r_s=.020 p=.866	r_s=-.021 p=.880	r_s=-.111 p=.354	r_s=-.229 p=.056	r_s=-.088 p=.467
C/D	**r_s=.260 p=.030**	r_s=-.075 p=.538	r_s=-.012 p=.932	r_s=-.148 p=.223	**r_s=.295 p=.015**	r_s=-.214 p=.077
TT/TE	**r_s=.264 p=.025**	r_s=.133 p=.270	r_s=.094 p=.509	r_s=-.118 p=.325	r_s=.129 p=.289	**r_s=-.295 p=.013**
FE	r_s=.099 p=.410	**r_s=-.355 p=.002**	**r_s=-.301 p=.030**	r_s=-.064 p=.596	**r_s=-.264 p=.027**	r_s=-.031 p=.799
FE %	r_s=.054 p=.654	r_s=.076 p=.527	r_s=-.007 p=.963	r_s=-.081 p=.497	r_s=-.217 p=.071	r_s=-.091 p=.451
FT /FE	r_s=.183 p=.125	r_s=.139 p=.246	r_s=.097 p=.492	r_s=-.137 p=.251	r_s=.146 p=.227	r_s=-.210 p=.079
TT / LH	r_s=.116 p=.336	r_s=.029 p=.812	r_s=.016 p=.910	r_s=.025 p=.838	r_s=.056 p=.646	r_s=-.121 p=.318
FT / LH	r_s=.041 p=.740	r_s=.083 p=.504	r_s=.060 p=.684	r_s=.003 p=.983	r_s=-.033 p=.791	r_s=-.043 p=.727

Table 14. Correlations of hormonal indices with statin intake, order of type of incident, Killip class, time in ischemia, and smoking history in the ACS group.

	statin use	number if accidents	smoking	GRACE	EF%
TT	r_s=.159 p=.186	**r_s=.240 p=.044**	r_s=.139 p=.251	r=-.083 p=.494	r=.058 p=.626
SHBG	r_s=-.005 p=0.965	r_s=.062 p=.608	**r_s=.289 p=.015**	**r=.312 p=0.008**	r=-.034 p=.779
albumin	r_s=.044 p=.713	r_s=.003 p=.979	r_s=-.071 p=.560	**r_s=-.352 p=0.003**	r_s=0.216 p=.073
TE	r_s=.161 p=.181	r_s= .201 p=.092	r_s=.018 p=.881	**r_s=-.330 p=0.003**	r_s=.130 p=.277
DHEA-S	**r_s=-.243 p=.044**	**r_s=-.404 p=.001**	r_s=-.223 p=.067	r_s= 0.082 p=0.509	r_s=0.048 .693
LH	r_s=.135 p=.272	r_s=.180 p=.142	r_s=.005 p=.967	r=-0.191 p=.114	r=.113 p=.344
cortisol	r_s=-.201 p=.093	r_s=-.189 p=.114	r_s=-.198 p=.100	r_s= -0.203 p=.091	r_s=.094 p=.432
FT	r_s=.119 p=.325	r_s=.182 p=.129	r_s=.022 p=.855	**r_s= -.262 p=.028**	r_s=.143 p=.230
FT%	r_s=.000 p=.998	r_s=-.044 p=.719	**r_s=-.308 p=.010**	**r_s= -.301 p=.011**	r_s=0.100 p=.405
bioT	r_s=.161 p=.179	r_s=.205 p=.087	r_s=.008 p=.948	r_s=-0.089 p=.463	**r_s=.249 p=.035**

bioT%	r_s=-.027 p=.824	r_s=-.091 p=.452	r_s=**-.259 p=.030**	r=-0.026 p=.830	**r=.254 p=.031**
C/D	r_s=.154 p=.206	**r_s=.291 p=.015**	r_s=.153 p=.213	r_s=-0.2 p=.096	r_s=0.076 p=.528
TT/TE	r_s=.080 p=.507	r_s=.079 p=.511	r_s=.113 p=.352	r=-0.064 p=.601	r=-0.005 p=.965
FE	r_s=.156 p=.194	r_s=.179 p=.135	r_s=-.037 p=.759	**r_s=0.295 p=.015**	r_s=-0.175 p=.147
FE %	r_s=.038 p=.756	r_s=-.036 p=.768	**r_s=-.323 p=.006**	r_s=-0.159 p=.188	r_s=-0.079 p=.512
FT/FE	r_s=-.001 p=.996	r_s=-.002 p=.988	r_s=.056 p=.643	r_s=-0.172 p=.154	r_s=-0.066 p=.584
TT/LH	r_s=.063 p=.603	r_s=.008 p=.948	r_s=.052 p=.671	r_s= -0.193 p=.113	r_s= .046 p=.705
FT/LH	r_s=.042 p=.738	r_s=.004 p=.973	r_s=-.130 p=.297	**r_s= -.251 p=.042**	r_s= .113 p=.358

From the results presented, there is a correlation of TT with type (r_s=.327 p=.005) and incident order (r_s=.240 p=.044), a weak negative correlation with the presence of ST-elevation (r_s=-.270 p=.023).(Table 13, 14) When adjusted for the presence of ST-elevation, incident order does not reach statistical significance as a predictor of TT level (p=.857)

FT in molar value was similarly associated with type of incident (r_s=.318,p=.007) and presence of ST-elevation (r_s=-.253 p=.033). (Table 13) However, unlike TT, FT is also negatively correlated with troponin value (r_s=-.266 p=.024). (Table 13) Except for this, similar correlations to those of FT are reported: weak positive correlation with incident type (r_s=.344, p=.003) and presence of ST-elevation (r_s=-.297, p=.012). (Table 13) BioT correlats with troponin with a higher correlation coefficient compared to FT with troponin (r_s=-.293 p=.012). The absolute value of bioT is also negatively associated with GRACE score (r_s= -.262, p=.028). (Table 14) This is against a moderate Spearman's correlation coefficient (r_s=-330, p=.005) for association between GRACE score and albumin levels.

The percentage of FT shows some negative associations with smoking history (r_s=-.308, p=.01), androtest score (r_s=-.346 p=.042) and age (r_s=-0.298 p=0.011) (Table 12, 14).

Percentage bioT correlates with GRACE score (r_s=-.301, p=.011), smoking history (r_s=-.259, p=.030) and androtest score (r_s=-.377, p=.025). There is also a moderate, negative association with age (r_s=-0.428 p<0.001) (Table 12, 14).

In the ACS group, SHBG correlates with age (r=-0.367 p=0.002), smoking (r_s=.289 p=.015) and GRACE (r=.312 p=0.008). (Table 12, 14) Since bioavailable and FT are a function of SHBG level

calculated according to A. Vermeulen (Vermeulen et al, 1999) this could explain the association between individual testosterone fractions and the factors age, smoking and GRACE score.

In the ACS group, there is no correlation between cortisol and any of the other parameters examined apart from a weak negative correlation with the number of arteries with stenosis (r_s=-.236, p=.049) The location of arterial thrombosis is not associated with any of the hormonal values.

In the group of patients with ACS, there is a statistically significant negative correlation between DHEA-S level on the one hand and history of DM (r_s=-.392 p=.001), arterial hypertension (r_s=-.296 p=. 014), CHD (r_s=-.512 p<001) and age (r_s= -0.506 p<0.001). (Table 12) DHEA-S is also associated with the type of event (r_s=-.262, p=.028) as well as the number of affected arteries (r_s=-.340, p=.004) ST-elevation (r_s=.240 p=.047) (Table 13).

Linear regression analysis for the ACS group reveales that the predictors age and history of CAD determine the DHEA-S value in 30.9% (R^2=.309, F=16.18, p<.001). Using both predictors, age is found to have the relatively greater weight (β=-.492, p<.001) with the presence of DM not reaching statistical significance (β=-.17, p=.119). After adjustment for age, the variables history of CHD or history of arterial hypertension, number of affected branches and presence of ST-elevation are not statistically significant predictors in the multiple regression model. (β=-.226, p=.055; β=-.075, p=.525; β=-.433 p=.135; β=-.124, p=.082, respectively).

DHEA-S also demonstrates a moderate correlation with incident order (r_s=-.404, p=.001) and GRACE score (r_s=-.352 p=0.003).(Table 13, 14) There is no statistically significant association between DHEA-S value and the results of the completed questionnaires (IIEF5, androtest, HADS).

Estradiol is associated with the presence of known arterial hypertension at the onset of ACS (r_s=.253 p=.034) and with a weak positive correlation with ejection fraction (r_s=.249, p=.035).(Table 12, 14)

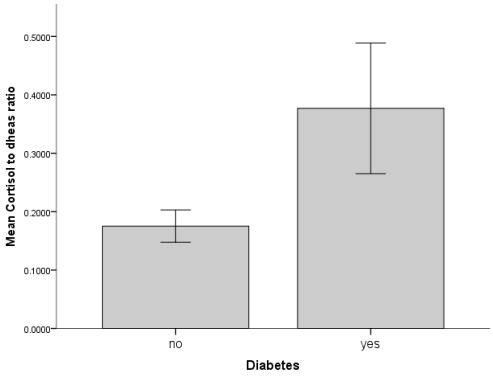
The TT/TE ratio correlates negatively with BMI (r_s=-.277 p=.024) and the presence of ST-elevation (r_s=-.295 p=.013) and type of incident (rs=.264 p=.025) with low strength. (Table 13) After adjusting for BMI, the presence of ST-elevation remains negatively associated with TT/TE (β=-.296, p=.013), whereas the type of incident loses its statistical significance (β=-.296, p=.399)

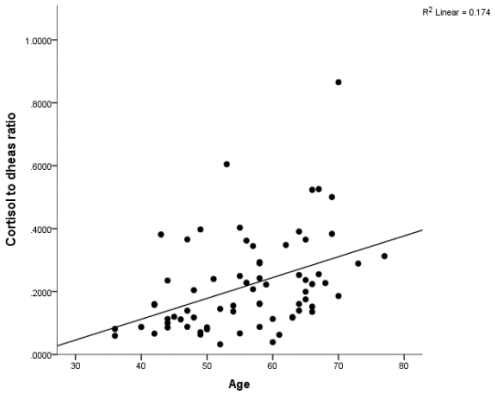
Another derived coefficient is the ratio of FT to FE. In the ACS group, a trend forms between FT/FE and BMI as the relationship did not reach statistical significance (rs=-.241, p=.051).

In the group of ACS, no correlation of TT/LH and FT/LH ratios with any of the other clinical parameters are found.[1]

An examination of the C/D ratios presented in the above tables reveals similar relationships as DHEA-S, with history of DM (r_S=.473 p<. 001) (Figure 7), CHD (r_S=.417 p<.001), arterial hypertension (r_S=.253, p=.037), age (r_S=0.459, p<0.001) (Figure 6), incident sequence (r_S=.291, p=.015) and GRACE score (r_S=0.295, p=.015). However, in contrast to DHEA-S, there is a moderate correlation of the C/D ratio with the androtest score.(r_S=.357, p=.038) Cortisol as a single measurement shows the described correlation.(r_S=.104, p=.552)

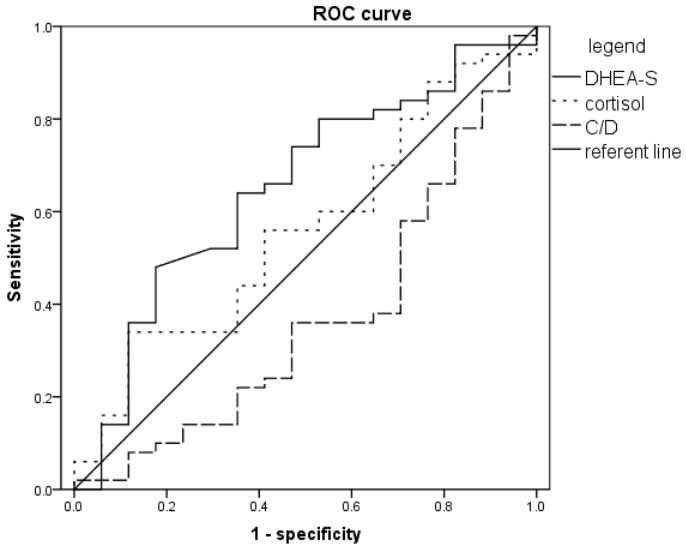
Figure 6 and 7





[1] For better presentation, the described correlations between the studied parameters are divided into three tables.

Figure 8.ROC curve for predictors of ST-elevation



DHEA-S has the largest area under the curve at 65.1%.For cortisol and C/D, the area under the curve is significantly less, .560 and .369, respectively. At a value for DHEA-S of 1.25 mkmol/l se=.940, sp=.176.At a value of 6.32 mkmol/l se=.140, sp=.941. At a value above 6.32 mkmol/l, the presence of ST-elevation in a patient with AMI could be excluded in 94.1%. While at a value below 1.25mkmol/l ST-elevation was evidenced in 94% of patients. Using the lower reference limit of 1.17 mkmol/l, a sensitivity of 70.0% and a specificity of 52.9% are found.

3.5 Correlations between hormonal and biochemical parameters

Tables 15 and 16 present the correlation coefficients and their statistical significance for the associations between hormonal values and some biochemical parameters studied in the ACS group.

Table 15 Correlation between hormonal and biochemical parameters.

	HDL	troponin	eGFR	TG	AST	ALT
TT	r_s=.222 p=.063	r_s=-.217 p=.067	r_s= 0.126 p=.292	r_s=-.09 p=.459	r_s=-.003 p=.987	r_s=-.179 p=.224
SHBG	r_s=.182 p=.130	r_s=.050 p=.675	r_s= -.155 p=.192	r_s=-.223 p=.068	r_s=-.036 p=.816	**r_s=-.507 p<.000**
albumin	r_s=.114 p=.345	r_s=-.105 p=.380	r_s=.071 p=.555	**r_s=.240 p=.049**	r_s=-.010 p=.949	r_s=.021 p=.887
estradiol	r_s=.133 p=.269	r_s=-.044 p=.711	r_s=.034 p=.779	r_s=.119 p=.332	r_s=-.246 p=.107	r_s=-.177 p=.229
DHEA-S	r_s=.056 p=.650	r_s=.149 p=.219	**r_s=.472 p<.001**	r_s=.027 p=.830	r_s=-.097 p=.532	r_s=-.017 p=.908
LH	r_s=-.224 p=.067	r_s=-.176 p=.148	r_s= -.052 p=.669	r_s=.124 p=.322	r_s=-.230 p=.142	r_s=.011 p=.944
cortisol	r_s=.089 p=.461	r_s=.077 p=.520	r_s= -.037 p=.760	r_s=.113 p=.361	r_s=-.219 p=.154	r_s=-.038 p=.798
FT	r_s=.149 p=.214	**r_s=-.266 p=.024**	r_s=.220 p=.064	r_s=-.047 p=.705	r_s=-.076 p=.624	r_s=-.038 p=.800
FT %	r_s=-.132 p=.273	r_s=-.114 p=.340	r_s=.168 p=.159	r_s=.066 p=.593	r_s=-.028 p=.856	**r_s=.417 p=.003**
bioT	r_s=.188 p=.116	**r_s=-.293 p=.012**	r_s=.200 p=.091	r_s=.025 p=.840	r_s=.006 p=.967	r_s=-.032 p=.831
bioT %	r_s=-.081 p=.503	r_s=-.111 p=.354	r_s=.180 p=.130	r_s=.180 p=.141	r_s=-.048 p=-.758	**r_s=.447 p=.001**
C/D	r_s=-.153 p=.209	r_s=-.148 p=.223	**r_s=-.394 p=.001**	r_s=-.122 p=.328	r_s=-.135 p=.389	r_s=-.020 p=.892
TT/TE	r_s=.143 p=.235	r_s=-.118 p=.325	r_s=.227 p=.055	r_s=-.204 p=.095	**r_s=.296 p=.05**	r_s=.016 p=.912
FE	r_s=.087 p=.468	r_s=-.064 p=.596	r_s=.084 p=.484	r_s=.173 p=.159	r_s=-.224 p=.144	r_s=-.079 p=.593
FE %	r_s=-.099 p=.410	r_s=-.081 p=.497	r_s=.231 p=.051	r_s=.145 p=.239	r_s=.043 p=.784	**r_s=.408 p=.004**
FT/FE	r_s=.076 p=.528	r_s=-.137 p=.251	r_s=.173 p=.145	r_s=-.175 p=.153	r_s=.146 p=.343	r_s=.090 p=.544
TT/LH	**r_s=.422 p<.001**	r_s=.025 p=.838	r_s=.173 p=.145	**r_s=-.248 p=.043**	**r_s=.328 p=.032**	r_s=-.130 p=.385
FT/LH	**r_s=.420 P<.001**	r_s=.003 p=.983	r_s=.233 p=.055	**r_s=-.296 p=.017**	**r_s=.308 p=.050**	r_s=.019 p=.903

Таблица 1 Correlations between hormonal parameters and some blood count, and biochemical parameters in the ACS group.

	hemoglobin	LEU	thrombocytes	HTC	CK-MB	CK
TT	r_s=.126 p=.298	r_s= -.126 p=.300	r_s= -.099 p=.414	r_s=.091 p=.455	r_s=-.135 p=.263	r_s=-.210 p=.135
SHBG	r_s=.003 p=.980	r_s= -.041 p=.735	**r_s= -.249 p=.038**	r_s=.013 p=.912	r_s=-.089 p=.462	r_s=-.075 p=.595
albumin	r_s= -.090 p=.459	r_s= -.023 p=.848	r_s=.175 p=.148	r_s= -.129 p=.288	r_s=-.180 p=.132	r_s=-.120 p=.395

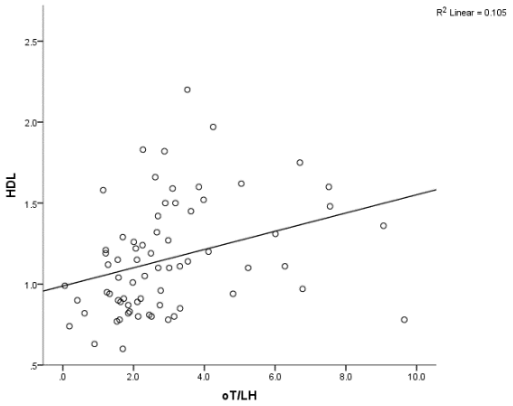
TE	r_s=.058 p=.633	**r_s= -.273 p=.022**	r_s= -.131 p=.281	r_s=.035 p=.773	**r_s=-.378 p=.001**	**r_s=-.322 p=.020**
DHEA-S	**r_s=.260 p=.032**	r_s=.020 p=.875	**r_s=.279 p=.021**	r_s=.099 p=.421	r_s=.026 p=.830	r_s=.026 p=.853
LH	**r_s= -.244 p=.046**	r_s=.008 p=.949	r_s= -.075 p=.544	**r_s= -.255 p=.037**	r_s=-.210 p=.085	r_s=-.213 p=.137
cortisol	r_s= -.184 p=.127	r_s=.077 p=.524	r_s=.214 p=.075	**r=-.253 p=.035**	r_s=.100 p=.404	r_s=.041 p=.774
FT	r_s=.128 p=.289	r_s= -.088 p=.471	r_s=.011 p=.930	r_s=.080 p=.508	r_s=-.094 p=.434	r_s=-.215 p=.126
FT%	r_s=.059 p=.626	r_s=.031 p=.796	**r_s=.249 p=.038**	r_s=.029 p=.812	r_s=.063 p=.599	r_s=.016 p=.910
bioT	r_s=.107 p=.378	r_s= -.083 p=.494	r_s=.007 p=.955	r_s=.044 p=.716	r_s=-.120 p=.319	r_s=-.248 p=.077
bioT %	r_s=.028 p=.816	r_s=.037 p=.764	**r_s=.264 p=-.027**	r_s= -.002 p=.989	r_s=.020 p=.866	r_s=-.021 p=.880
C/D	**r_s= -.244 p=.045**	r_s=.056 p=.653	r_s= -.168 p=.170	r_s= -.153 p=.212	r_s=-.075 p=.538	r_s=-.012 p=.932
TT/TE	r_s=.086 p=.477	r_s=.054 p=.658	r_s= -.109 p=.369	r_s=.092 p=.451	r_s=.133 p=.270	r_s=.094 p=.509
FE	r_s=.058 p=.631	**r_s=-.258 p=.031**	r_s=-.093 p=.442	r_s=.043 p=.724	**r_s=-.355 p=.002**	**r_s=-.301 p=.030**
FE %	r_s=.049 p=.688	r_s=-.035 p=.774	r_s=.167 p=.166	r_s=.039 p=.748	r_s=.076 p=.527	r_s=-.007 p=.963
FT / FE	r_s=.073 p=.547	r_s=.170 p=.159	r_s=.118 p=.331	r_s=.031 p=.800	r_s=.139 p=.246	r_s=.097 p=.492
TT / LH	**r_s=.295 p=.014**	r_s=-.131 p=.282	r_s=-.086 p=.483	r_s=.231 p=.057	r_s=.029 p=.812	r_s=.016 p=.910
FT / LH	r_s=.238 p=.054	r_s=-.102 p=.416	r_s=-.078 p=.536	r_s=.154 p=.217	r_s=.083 p=.504	r_s=.060 p=.684

There is a negative correlation between DHEA-S levels and serum creatinine (r_s=-.383 p<.001) and a moderate positive correlation with estimated glomerular filtration rate (r_s=.472 p<.001) (Table 15).

In the course of our study, there was a negative association between SHBG levels and ALT value (r_s=-.507 p<.000). Correlation analysis also demonstrated a positive association of FT%, bioT% and FE% with ALT. (Table 15) We performed stepwise regression analysis controlling for ALT value. Relative to liver function, age, smoking history and GRACE score lost their statistical significance (p>.051)

There was a moderate positive correlation between HDL and TT/LH and FT/LH ratios (r_s=.422 p<.001 and r_s=.420 P<.001) (Figure 9) (Table 15).

Figure 9.



After controlling for statin intake, the positive correlation between TT/LH and HDL (r=.435, p<.001) and FT/LH and HDL (r=.397, p=.001) persists. Statistical analysis also demonstrated the association of TT/LH and FT/LH with triglycerides. (Table 15) After controlling for statin intake, both TT/LH and FT/LH become nonsignificant predictors of TG levels. (p>.05) Correlations with LDL and total cholesterol are not found in the ACS group.

Bioavailable and free T demonstrate a weak negative correlation with troponin values.(r_S=-.293, p=.012 and r_S=-.266, p=.024, respectively).(Table 15) Regarding estrogens, both total and free estradiol demonstrate a moderate strength inverse relationship with CPK and CK-MB values.(Table 16)

In order to examine the DHEA-S relationships in more detail, multiple linear regression is performed. It is apparent that eGFR retained its predictive value with respect to age. (β=-.024, p=.04) Both parameters determined the value of DHEA-S in 33.6% (R2=.336, F=16.923, p<.001)

3.6 Hormonal parameters

Tables 17 - 19 present the correlations between hormonal parameters in the ACS group.

Table 17. Correlations between hormonal indices in the ACS group.

	TT	SHBG	albumin	TE	DHEA-S	LH	cortisol	FT
TT	1	**r=.364 p=.002**	r=-.004 p=.975	**r_s=.369 p=.001**	r_s=.028 p=.815	**r_s=.327 p=.006**	r=-.184 p=.121	**r=.901 p<.001**
SHBG		1	r=-.122 p=.309	r_s=.135 p=.257	r_s=-.231 p=.054	r_s=.116 p=.342	r=-.078 p=.517	r=.034 p=.779
albumin			1	r_s=.043 p=.721	r_s=.185 p=.126	r_s=-.159 p=.192	**r=.248 p=.035**	r=.014 p=.905
TE				1	r_s=.092 p=.448	r_s=.092 p=.453	r_s=-.079 p=.508	**r_s=.327 p=.005**
DHEA-S					1	r_s=-.114 p=.359	r_s=.148 p=.221	r_s=.136 p=.261
LH						1	r_s=-.045 p=.713	**r_s=.308 p=.010**
cortisol							1	r=-.144 p=.227
FT								1

Table 18 Correlations between hormonal parameters in the ACS group.

	FT %	bioT	bioT%	C/D	TT/TE	FE
TT	r_s=-.083 p=.488	**r_s=.897 p<.001**	r_s=-.131 p=.273	r_s=-.025 p=.837	**r_s=.636 p<.001**	**r=.291 p=.013**
SHBG	**r_s=-.901 p<.001**	r_s=-.029 p=.811	**r_s=-.903 p<.001**	r_s=**.228 p=.050**	**r_s=.253 p=.032**	r=-.064 p=.596
albumin	r_s=-.030 p=.802	r_s=.119 p=.317	**r_s=.316 p=.007**	r_s=-.227 p=.059	r_s=-.040 p=.741	r=.097 p=.416
TE	r_s=-.046 p=.702	**r_s=.360 p=.002**	r_s=-.052 p=.667	r_s=-.092 p=.450	**r_s=-.303 p=.010**	**r_s=.974 p<.001**
DHEAS	**r_s=.240 p=.045**	r_s=.132 p=.276	**r_s=.307 p=.010**	**r_s=-.856 p<.001**	r_s=-.046 p=.704	r_s=.115 p=.344
LH	r_s=.004 p=.971	**r_s=.254 p=.035**	r_s=-.053 p=.666	r_s=.219 p=.075	**r_s=.251 p=.037**	r_s=.078 p=.523
cortisol	r_s=-.003 p=.981	r_s=-.133 p=.266	r_s=.132 p=.269	r_s=.173 p=.153	r_s=-.116 p=.332	r=-.112 p=.350
FT	**r_s=.246 p=.038**	**r_s=.973 p<.001**	r_s=.180 p=.131	r_s=-.106 p=.382	**r_s=.557 p<.001**	**r=.353 p=.002**
FT %	1	**r_s=.247 p=.036**	**r_s=.897 p<.001**	r_s=-.185 p=.126	r_s=-.164 p=.169	**r=.353 p=.002**
bioT		1	r_s=.231 p=.051	r_s=-.145 p=.231	**r_s=.517 p<.001**	**r_s=.368 p=.001**
bioT %			1	**r_s=-.280 p=.019**	r_s=-.186 p=.117	r_s=.113 p=.344
C/D				1	r_s=.044 p=.717	r_s=-.121 p=.319
TT/TE					1	**r_s=-.349 p=.003**
FE						1

Table 19 Correlations between hormonal parameters in the ACS group.

	FE %	FT/FE	TT/LH	FT / LH
oT	**r_s=-.246 p=.037**	**r_s=.587 p<.001**	**r_s=.494 p<.001**	**r_s=.365 p=.002**
SHBG	**r_s=-.920 p<.001**	r_s=-.006 p=.963	r_s=.198 p=.098	r_s=-.137 p=.265
albumin	r_s=.153 p=.199	r_s=-.012 p=.919	r_s=.151 p=.207	r_s=.116 p=.345
TE	r_s=-.065 p=.588	**r_s=-.414 p<.001**	**r_s=.303 p=.010**	**r_s=.318 p=.008**
DHEA-S	r_s=.203 p=.091	r_s=.061 p=.618	r_s=.103 p=.398	r_s=.154 p=.218
LH	r_s=-.141 p=.249	**r_s=.262 p=.030**	**r_s=-.543 p<.001**	**r_s=-.535 p<.001**
cortisol	r_s=.058 p=.626	r_s=-.118 p=.323	r_s=-.136 p=.258	r_s=-.044 p=.723
FT	r_s=.064 p=.592	**r_s=.619 p<.001**	**r_s=.433 p<.001**	**r_s=.441 p<.001**
FT %	**r_s=.824 p<.001**	r_s=.201 p=.091	r_s=-.101 p=.402	r_s=.248 p=.041
bioT	r_s=.107 p=.370	**r_s=.574 p<.001**	**r_s=.469 p<.001**	**r_s=.472 p<.001**
bioT %	**r_s=.826 p<.001**	r_s=.149 p=.211	r_s=-.086 p=.473	r_s=.214 p=.080
C/D	r_s=-.221 p=.067	r_s=-.018 p=.885	r_s=-.189 p=.119	r_s=-.201 p=.106
TT/TE	r_s=-.174 p=.144	**r_s=.763 p<.001**	**r_s=.251 p=.035**	r_s=.112 p=.363
FE	r_s=.131 p=.273	**r_s=-.439 p<.001**	**r_s=.251 p=.035**	**r_s=.334 p=.005**
FE %	1	r_s=-.077 p=.521	r_s=-.115 p=.338	r_s=.221 p=.070
FT / FE		1	r_s=.205 p=.086	r_s=.161 p=.190
TT / LH			1	**r_s=.912 p<.001**
FT / LH				1

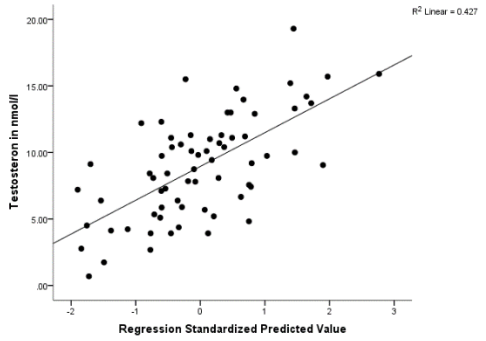
*Results are presented by indicator and are therefore not presented under each table.

There is a medium strength correlation between total testosterone levels with TE (r_s=.369, p=.001), TT with LH (rs=.327, p=.006) (Table 17) and a weak correlation between TT and FE in absolute value (r_s=. 297, p=.011) (Table 18) and in percentage - FE% - (r_s=-.246, p=.037). (Table 19) These correlations are in the background of the correlation of TT with SHBG (r=.364 p=.002). (Table 17).

Given the correlations described, we performed multiple linear regression using the variables LH, TE and SHBG. The model is statistically significant, with hormone values predicting TT in 31.9% (R^2=.319, F=10.003, p<.001). To assess the effect of ST-elevation on T values, we performed a multiple regression stepwise analysis including TE, SHBG, LH and ST-elevation values. When controlling for hormone levels, the regression coefficient for ST-elevation (β=-.330, p=.001) remains statistically significant. The addition of ST-elevation improves the predictive value of the

model by 10.8%. Including all four variables in the regression model, we conclude that the value of TT is determined in 42.7% of the mentioned variables (Figure 10).

Figure 10



In terms of bioT, there is a weak positive correlation with LH levels (r_S=.254, p=.035). (Table 18) This correlation is weaker compared to the correlations of TT with LH. BioT correlates with TE, with a correlation coefficient rho comparable to that between TT and TE (r_S=.360, p=.002). (Table 17) On the other hand, the correlation of bioT with TE (r_S=.368, p=.001) was stronger than that between TT and TE. This is attributed to the fact that both values (of bioT and FE) directly derive from that of SHBG.

The percentage value of bioT was associated with the value of DHEA-S (r_S=.307, p=.010) without this being due to a statistically significant correlation with SHBG. A weak correlation was also observed with the C/D ratio (r_S=-.280, p=.019) (Table 18).

FT demonstrates correlation coefficients similar to those of TT. FT similar to bioT correlates moderately with FE (r_S=.330, p=.005). The FT % value, like bioT % correlated weakly with DHEA-S (rs=.240, p=.045) (Table 18)

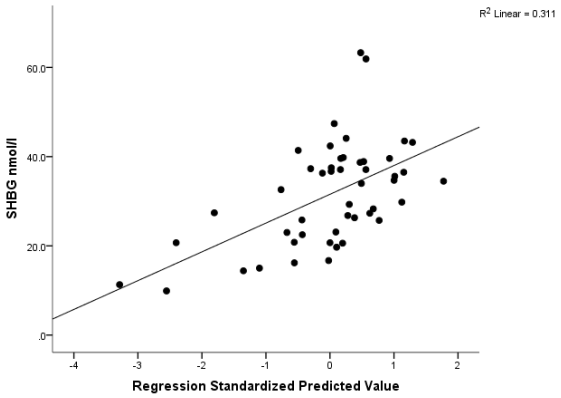
In the ACS group, there is a moderate positive correlation between SHBG and TT (r=.364, p=.002) and a weak one with the TT/TE ratio (r_S=.253, p=.032). No correlation with TE was observed in this group (Table 18).

There was a moderate positive correlation between SHBG and GRACE score. Thus, higher SHBG values are related to a worse patient risk profile. After adjusting for DHEA-S level, SHBG loses

its statistical significance in determining GRACE levels (β=-.335, p=.055). A stepwise linear regression analysis revealed that adding DHEA-S and albumin to SHBG could predict 22.9% of the variance in GRACE score (F=7.627, p<.001). In that model SHBG had borderline statistical significance with β=.221 and p=.053. We find a correlation of borderline statistical significance of SHBG with the C/D ratio (r_s=.228, p=.050) and SHBG. (r_s=-.231, p=.054).

Linear regression analysis was performed to assess the effect of TT and age on SHBG level. It reveals that both TT (β=.859, p=.003) and age (β=.121, p=.003) are significant predictors of SHBG level in early ACS. These two parameters explained 23.9% of the variance in SHBG (R^2=.239, F=10.809, p<.001). After adding ALT to the regression model then the value of age as a predictor of SHBG levels became statistically insignificant (β=.255, p=.093). The multiple linear regression model including the ALT value and the level of TT explained 31.1% of the variance in the SHBG value (R2=.311, F=10.156, p<.001) (Figure 11)

Figure 11.



We also examined the association between SHBG and GRACE score. There is a moderate positive correlation between the two parameters and SHBG alone is responsible for 9.7% of the variation in GRACE. Thus, higher SHBG values is associated with a worse patient risk profile.

Stepwise linear regression analysis revealed that adding DHEA-S and albumin to SHBG achieved a better prognostic model than SHBG alone and could predict 22.9% of GRACE score variations ($F=7.627$, $p<.001$). In that model, the SHBG value has borderline statistical significance with $\beta=.221$ and $p=.05$.

In the ACS group, LH correlates weakly with the value of TT/TE ratio ($r_s=.251$, $p=.037$) and FT/FE ($r_s=.262$, $p=.03$) (Table 18, 19) In the ACS group, TE correlates positively with the FT/LH ratio ($r_s=.305$, $p=.012$). Free E demonstrates a stronger correlation with the same ratio ($r_s=0.335$, $p=.005$) (Table 19).

3.7 Dynamics of androgens after ACS.

In order to test the effect of time on hormone levels, a dependent t-test is used, given the normal distribution of the data. The results are presented in Tables 20 and 21.

It was found that there is no statistically significant effect of time for the studied parameters (Table 20).

Modeling of the results was carried out using bootsraping technique in SPSS (Table 21). As a result, some differences gained statistical significance.

Table 20 Results of Student's t-test for the studied parameters in the followed patients.

	Paired Differences					t	df	Sig. (2-tailed)	cohen's d
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
ИТМ	**.4533333**	**2.3733240**	**.6127896**	**-.8609697**	**1.7676363**	**.740**	**14**	**.472**	**0.191**
Testosteron	-1.2058824	3.6165938	.8771528	-3.0653633	.6535986	-1.375	16	.188	-0.333
SHBG	.6500000	8.2966660	2.0741665	-3.7709812	5.0709812	.313	15	.758	0.078
свTestosterone	-.0001938	.0862024	.0215506	-.0461278	.0457403	-.009	15	.993	-0.002
свТ %	.1687500	.4827681	.1206920	-.0884989	.4259989	1.398	15	.182	0.350
Био Т	-.40313	2.15330	.53833	-1.55054	.74429	-.749	15	.466	-0.199
Био Т %	.2125000	13.3965605	3.3491401	-6.9260232	7.3510232	.063	15	.950	0.016
DHEA-S	.7237500	1.7486752	.4371688	-.2080532	1.6555532	1.656	15	.119	0.414
ejection fraction	-5.313	10.799	2.700	-11.067	.442	-1.968	15	.068	-0.492

Table 21. Results of t-test for dependent samples after bootstrapping for the hormonal parameters examined during follow-up.

			Bootstrap				
					Sig.	95% CI	
	mean	Bias	Std. Error	t	(2-tailed)	upper	lower
TT	-1.3241379	.0231931	.6733096	-1.966	.057	-2.6196201	-.0476356
SHBG	.3413793	.0682034	1.5473014	0.220	.655	-2.7577447	3.3307717
FT	.0050310	-.0002287	.0153151	0.328	.990	-.0266490	.0350982
FT%	.2000000	-.0049569	.0876888	**2.281**	**.038**	.0318555	.3744477
bioT	-.31138	-.00630	.38762	-0.803	.290	-1.10637	.42645
bioT %	.6862069	-.1152862	2.4616281	0.279	.928	-3.7779087	5.6822330
DHEA-S	.6706897	.0065210	.3229835	**2.076**	**.024**	.1127761	1.3611428

The percent change in free testosterone in the baseline sample did not reach statistical significance - t(15)=1.398, p=.182, d=-0.333. After modeling, the difference reaches statistical significance at t=2.281, p=.038 with a moderate effect size of d=.389. After modeling, a difference is also found for DHEA-S level. A baseline Student's t-test is performed with t=-1.077, p=.290 and effect size d=0.414. After modelling, the difference gaines statistical significance with t=2.076 at p=.024 and similar effect size d=0.421. No statistical significance is found for total testosterone after bootstrapping either, but a trend for change over time emerges (t=-1.974, p=.057, d=-.339).

In summary of patient follow-up:

1. There is statistically significant difference in hormonal indices at follow-up in some of the patients; there is a trend for increasing TT values.

2. When modeling the results, there is a statistically significant increase in FT% with time and a decrease in DHEA-S.

4. Chronic coronary syndrome

4.1 Diabetes mellitus and chronic coronary syndrome (CCS)

Studying the CCS patients according to the presence of DM, some differences are found as presented in Table 22.

Table 22. Differences in hormonal parameters depending on the presence of DM in the HCS group.

	DM n=11	without DM n=16	t/ U p-value
age	59.55±7 (54.84-64.25)	58.38 ±9.458 (53.34-63.41)	t=-.801 p=.430
TT	7.64 ±3.07 (5.58-9.7)	12.24 ±3.55 (10.34-14.13)	**t=2.827 p=.008**
SHBG	25.51 ±9.57 (19.08-31.94)	42.36 ±15.08 (34.32-50.39)	**t=3.042 p=.005**
TE	176.4 ±67.73 (130.9-221.89)	182.45 ±83.55 (137.93-226.97)	U=96 p=.583
DHEA-S	2.29 ±1.56 (1.24-3.34)	2.95 ±1.98 (1.89-4.01)	U=109 p=.815
cortisol	462.94±104.88 (392.48-533.4)	409.33 ±177.58 (314.71-503.95)	U=-.568 p=.574

LH	3.37 ±1.65 (2.26-4.48)	3.41 ±.99 (2.88-3.93)	t=-.171 p=.865
FT	.172 ±.079 (.118-.225)	.211 ±.048 (.185-.237)	t=1.202 p=.239
FT %	2.25 ±.35 (2.01-2.48)	1.78 ±.33 (1.61-1.95)	**t=-2.989 p=.006**
bioT	4.05 ±1.90 (2.78-5.33)	4.92 ±1.34 (4.21-5.64)	t=.979 p=.335
bioT %	53.61 ±10.32 (46.68-60.54)	41.74 ±10.49 (36.15-47.33)	**t=-2.812 p=.009**
C/D	.299 ±.183 (.176-.422)	.213 ±.167 (.124-.302)	U=81 p=.438
TT/TE	47.02 ±19.572 (33.876-60.173)	74.875 ±31.209 (58.245-91.505)	U=63 p=.054
FE	4.84 ±1.81 (3.62-6.06)	4.39 ±1.77 (3.45-5.33)	t=-.599 p=.554
FE%	2.76 ±.22 (2.62-2.91)	2.43 ±.27 (2.29-2.57)	**U=47 p=.008**
FT/FE	.038 ±.017 (.027-.050)	.054 ±.020 (.043-.064)	t=1.438 p=.161
TT/LH	2.871 ±1.751 (1.694-4.047)	3.735 ±1.113 (3.142-4.328)	t=1.864 p=.072
FT/LH	.061 ±.031 (.040-.082)	.066 ±.023 (.054-.078)	t=-.034 p=.973

The differences in TT and SHBG levels are statistically significantly higher in the group of patients without DM (t=2.827 p=.008 and t=3.042 p=.005, respectively). Using Student's t-test, we also report a statistically significant difference in the level of FT% and bioT%, with higher values for both parameters in the patients with DM. A difference in the levels of FE% is observed between the two subgroups. (U=47 p=.008) From the hormonal ratios we find no statistically significant difference. The TT/TE and TT/LH ratios shows a trend with U=63, p=0.54 and t=1.864 p=.072 respectively. The differences described are against the background of statistically indistinguishable mean age. For DHEA-S also no difference was found between the two groups.

4.2 Clinical indicators

Statistically significant correlations between hormonal and clinical parameters are presented in Table 23.

Table 23. Correlation of hormonal indicators with the presence of DM, FI, BMI and age.

	DM typ 2	EF %	BMI	age
TT	**r_s=-.490 p=.005**	r=.276 p=.141	**r_s=-.491 p=.008**	r=0.0615 p=.742
SHBG	**r_s=-.501 p=.004**	r=.089 p=.638	r=-.241 p=.216	r=0.217 p=.240
albumin	r_s=.218 p=.232	r=-.242 p=.190	r=.106 p=.583	r=-0.274 p=.130
TT	r_s=-.106 p=.572	r_s=-0.091 p=.632	r_s=.074 p=.709	r_s=-.201 p=.279
DHEA-S	r_s=-.046 p=.801	r_s=.158 p=.396	r_s=-.169 p=.382	r_s=-.283 p=.117
LH	r_s=-.046 p=.801	r=-.009 p=.960	r=-.052 p=.787	**r=-.479 p=.006**
cortisol	r_s=.060 p=.747	r=-.345 p=.062	r=.034 p=.864	r=-0.0003 p=.998
FT	r_s=-.303 p=.092	r=.341 p=.061	r=-.280 p=.141	r=0.26957 p=.143

FT %	**r_s=.460 p=.008**	r=-.145 p=.437	r=.285 p=.134	r=-0.0979 p=.594
bioT	r_s=-.260 p=.150	r=.244 p=.186	r=-.245 p=.200	r=-0.2295 p=.206
bioT %	**r_s=.460 p=.008**	r=-.212 p=.252	r=.268 p=.160	r=-0.1916 p=.294
C/D	r_s=.153 p=.428	r_s=-.181 p=.356	r_s=.162 p=.428	r=-0.309 p=.085
TT/TE	r_s=-.354 p=.051	**r_s=.410 p=.024**	**r_s=-.554 p=.002**	r_s=.243 p=.188
FE	r_s=.092 p=.629	r=-.057 p=.769	**r=.394 p=.042**	r=-0.2161 p=.251
FE%	**r_s=.475 p=.007**	r_s=-.191 p=.311	**r_s=.410 p=.030**	**r_s=-.383 p=.033**
FT/FE	r_s=-.264 p=.152	**r=.366 p=.047**	**r=-.502 p=.006**	r=0.2526 p=.170
TT/LH	**r_s=-.381 p=.031**	r=.277 p=.131	r=-.269 p=.159	r=-0.0878 p=.633
FT/LH	r_s=.052 p=.785	r=.137 p=.477	r=-.084 p=.672	r=-0.093 p=.626

In the CCS group, there is a mean negative correlation between TT and the presence of DM (r_s=-.490, p=.005). A moderate negative correlation is also reported between TT and BMI (r_s=-.491, p=.008). The absolute value of FT and bioT shows no linear correlation with the patient characteristics studied. For FT, there is a trend of association with EF, without it reaching statistical significance. (r=.341 p=.061) However, the percentage of FT% and bioT% is associated with history of DM, similar to TT (for FT%: r_s=.460, p=.008 and for bioT%: r_s=.460, p=.008, respectively). These observations are in the context of a strong association of SHBG with the presence of DM (r_s=-.501, p=.004) (Table 23).

Free E, but not total E, correlates positively with BMI (r=.394 p=.042). Free estradiol in percentage, like TT% and FT% correlated with the presence of diabetes mellitus in the CCS group. (r_s=.475 p=.007) These correlations are mainly attributed to the strong correlation between SHBG and DM in the same sample.

In the CCS group, ejection fraction shows a correlation with TT/TE ratio (r_s=.410, p=.024)and FT/FE (r=.366, p=.047). TT/TE also correlates negatively with BMI (r_s=-.554, p=.002). Another coefficient is the ratio of FT to FE. It reached statistical significance with BMI (r=-.502, p=.006) with correlation coefficients similar to those for the TT/TE ratio. In the CCS group, the T/LH ratio demonstrated a moderate correlation with the presence of DM (r_s=-.381, p=.031) (Table 23).

Cortisol showed a correlation only with the depression score from the HADS (r_s=-.691, p=.013) in the CCS group. In the group of patients with stable coronary artery disease, there was also a statistically significant correlation of cortisol value with the HADS depression score (r_s=-.691 p=.013). IIEF-5, androtest and HADS anxiety score did not show statistically significant correlation

with cortisol. The IIEF-5 score showed a very strong negative correlation with androtest (r_s=-790; p=0.020).

4.3 Biochemical parameters

Several statistically significant correlations with paraclinical parameters are found in the CCS group using Spearman correlation as presented in Table 24.

Table 24. Correlations between hormonal and biochemical indicators in the HCS group.

	HDL	LDL	total cholesterol	TG	ALAT	Hв	LEU	thrombocytes	hematocrit
TT	**r_s=.402**	**r_s=.390**	r_s=.228	r_s=-.354	**r_s=-.593**	r_s=.129	r_s=-.343	r_s=-.332	r_s=.070
	p=.031	**p=.036**	p=.234	p=.064	**p=.008**	p=.504	p=.068	p=.078	p=.716
SHBG	r_s=.304	r_s=.324	r_s=.150	**r_s=-.385**	r_s=-.354	r_s=-.162	r_s=-.289	r_s=-.358	r_s=-.191
	p=.109	p=.087	p=.438	**p=.043**	p=.138	p=.402	p=.129	p=.057	p=.320
albumin	**r_s=-.514**	r_s=-.355	r_s=-.349	r_s=.355	r_s=.066	r_s=-.120	**r_s=.435**	**r_s=.372**	r_s=-.159
	p=.004	p=.054	p=.059	p=.059	p=.784	p=.527	**p=.016**	**p=.043**	p=.403
TE	r_s=-.037	r_s=.361	r_s=.247	r_s=.004	r_s=.236	**r_s=.375**	r_s=.105	r_s=.015	**r_s=.381**
	p=.849	p=.055	p=.196	p=.985	p=.330	**p=.045**	p=.586	p=.939	**p=.041**
DHEA-S	r_s=-.124	r_s=-.047	r_s=-.140	r_s=.008	r_s=-.184	r_s=.068	r_s=.014	r_s=.134	r_s=-.039
	p=.515	p=.807	p=.461	p=.969	p=.438	p=.721	p=.943	p=.481	p=.839
LH	r_s=.097	r_s=-.162	r_s=-.162	r_s=-.055	**r_s=-.454**	r_s=.031	r_s=.294	r_s=-.152	r_s=.053
	p=.612	p=.393	p=.392	p=.776	**p=.045**	p=.869	p=.114	p=.422	p=.780
cortisol	r_s=-.002	r_s=.041	r_s=.022	r_s=-.060	r_s=.353	r_s=-.015	r_s=.189	r_s=.213	r_s=.033
	p=.991	p=.832	p=.909	p=.763	p=.139	p=.940	p=.327	p=.267	p=.865
FT	r_s=.330	r_s=.247	r_s=.150	r_s=-.191	r_s=-.382	r_s=.282	r_s=-.178	r_s=-.117	r_s=.249
	p=.075	p=.189	p=.428	p=.322	p=.097	p=.131	p=.347	p=.539	p=.184
FT %	r_s=-.299	r_s=-.243	r_s=-.158	r_s=.357	r_s=.134	r_s=.130	r_s=.283	**r_s=.380**	r_s=.161
	p=.108	p=.195	p=.404	p=.057	p=.574	p=.492	p=.130	**p=.038**	p=.395
bioT	r_s=.202	r_s=.158	r_s=.032	r_s=-.107	r_s=-.409	r_s=.191	r_s=-.024	r_s=.008	r_s=.173
	p=.283	p=.404	p=.866	p=.580	p=.073	p=.311	p=.898	p=.966	p=.360
bioT %	**r_s=-.389**	r_s=-.337	r_s=-.244	**r_s=.401**	r_s=.102	r_s=.108	**r_s=.375**	**r_s=.428**	r_s=.116
	p=.034	p=.069	p=.194	**p=.031**	p=.670	p=.569	**p=.041**	**p=.018**	p=.541
C/D	r_s=.221	r_s=.054	r_s=.163	r_s=-.040	**r_s=.494**	r_s=-.059	r_s=-.016	r_s=.002	r_s=.048
	p=.259	p=.784	p=.407	p=.842	**p=.032**	p=.767	p=.936	p=.991	p=.808
TT/TE	**r_s=.381**	r_s=.055	r_s=.046	r_s=-.330	r_s=-.364	r_s=-.115	**r_s=-.475**	r_s=-.335	r_s=-.174
	p=.041	p=.777	p=.811	p=.087	p=.126	p=.554	**p=.009**	p=.075	p=.367
FE	r_s=-.250	r_s=.253	r_s=.089	r_s=.120	r_s=.084	r_s=.329	r_s=.309	r_s=.201	**r_s=.374**
	p=.191	p=.185	p=.645	p=.542	p=.734	p=.081	p=.102	p=.297	**p=.046**
FE %	r_s=-.357	r_s=-.277	r_s=-.168	**r_s=.428**	r_s=.073	r_s=.122	**r_s=.398**	**r_s=.431**	r_s=.133
	p=.058	p=.146	p=.383	**p=.023**	p=.766	p=.528	**p=.032**	**p=.019**	p=.491
FT/FE	**r_s=.381**	r_s=.019	r_s=.056	r_s=-.282	r_s=-.387	r_s=-.079	**r_s=-.471**	r_s=-.253	r_s=-.136
	p=.042	p=.922	p=.771	p=.146	p=.102	p=.686	**p=.010**	p=.186	p=.481
TT / LH	r_s=.258	**r_s=.384**	**r_s=.364**	r_s=-.296	r_s=.281	r_s=.220	**r_s=-.476**	r_s=-.191	r_s=.194
	p=.168	**p=.036**	**p=.048**	p=.120	p=.230	p=.244	**p=.008**	p=.311	p=.304
FT / LH	r_s=.142	r_s=.301	r_s=.257	r_s=.113	r_s=-.169	r_s=.162	r_s=-.172	r_s=.207	r_s=.138
	p=.471	p=.119	p=.187	p=.575	p=.504	p=.410	p=.382	p=.290	p=.484

The main androgen studied, TT, correlates positively with moderate strength with HDL, LDL (r_S=.402, p=.031 and r_S=.390, p=.036, respectively) and negatively with ALT.(r_S=-.593, p=.008). FT correlated positively with platelet count (r_S=.380, p=.038). After adjusting for statin use, the correlation of LDL with TT is maintained (r=.389, p=.041). Of the androgens, bioT% demonstrates the highest associations with HDL (r_S=-.389 p=.034); TG (r_S=.401, p=.031); leukocyte count (r_S=.375 p=.041) and platelets (r_S=.428, p=.018). The statistically significant correlations of bioT, almost completely overlaps with those of albumin, which in turn determines the bioavailable fraction of T (Table 24). After adjusting for statin use, the statistical significance of the correlation between bioT% and HDL is maintained (r=-.490, p=.007).

Our results showed that estradiol correlates positively with hemoglobin (r_S=.375, p=.045) and hematocrit (r_S=.381 p=.041). Free estradiol also correlates with hematocrit (r_S=.374 p=.046).

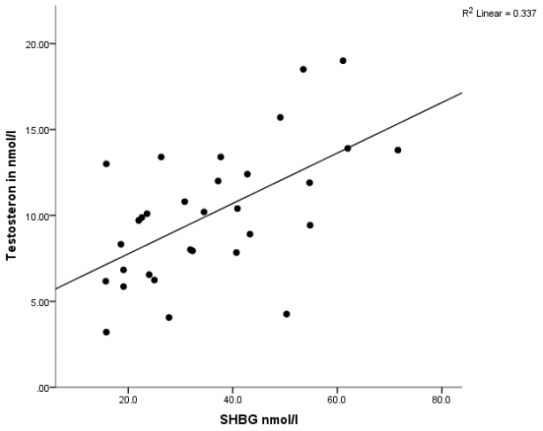
Hormonal ratios are also associated with some of the paraclinical parameters. C/D correlates moderately, positively with ALT value (r_S=.494, p=.032) The ratios of TT/TE and FT/FE show similar coefficients of moderate positive correlation with HDL and leukocyte count. (Table 24) After adjusting for statin intake, the statistically significant correlation between FT/FE ratios and HDL (r=.369, p=0.50) and between TT/TE and HDL (r=.484, p=.009) remain.

TT/LH correlated positively with lipid profile parameters (LDL r_S=.384, p=.036 and total cholesterol r_S=.364 p=.048) and negatively with leukocytes (r_S=-.476, p=.008). After adjusting for statin use, the correlation between TT/LH on the one hand and LDL and total cholesterol on the other loses its statistical significance.

4.4 Hormonal parameters

Using Pearson's correlation we found a strong positive correlation between TT and SHBG (r=.580, p=.001). (Figure 12) In addition, TT is also associated with the percentage of FE (r_S=-.560 p=.001). Percent FT and bioT correlated positively with DHEA-S (for FT%: r_S=0.378 p=0.032; for bioT%: r_S=0.377 p=0.034). These associations are on the background of a moderate negative correlation between SHBG and albumin: r=-.460, p=.009.

Figure 12



In the CCS group, bioT in absolute value correlates with FE (r_s=.372, p=.043) As in other analogous correlations, this one is explained by their common determinant - SHBG. BioT% correlated with DHEA-S value with a correlation coefficient of r_s=0.377, p=.034. No significant correlations were found in the CCS group with FT in absolute value, but its percentage expression, like bioT% was associated with DHEA-S levels (r_s=0.378, p=0.032) In the CCS group for FE% a moderate negative correlation was found with the TT/LH ratio (r_s=-0.426, p=.017).

5. Ischaemic hearth disease (IHD)

5.1 Distribution of hormone values between the control group and all IHD patients.

When comparing the three groups, several common differences were found between the ACS and CCS groups on the one hand and the controls on the other. This also led to investigating the differences between healthy (controls) and IHD patients (the combined group of ACS and CCS in common with the designation ischemicx hearth disease (IHD). The differences are presented in Table 25.

Table 25 The table shows the differences in hormonal parameters between IHD patients and healthy controls.

	IHD (ACS+CCS) (n=104)	controls (n=35)	T/U-value p-value
age	56.75 ±9.12 (54.96-58.54)	54.22 ±7.23 (51.61-56.83)	t=1.651 p=.101
BMI	29.51 ±4.85 (28-521-30.5)	28.98 ±3.52 (27.7-30.25)	U=1474.5 p=.800
TT	9.3 ±4.07 (8.50-10.09)	11.1 ±2.38 (10.26-11.93)	**t=-2.444 p=.016**
SHBG	32.19 ±12.59 (29.73-34.65)	31.55 ±10.46 (27.95-35.14)	U=1795 p=.971
TE	187.59 ±80.08 (171.94-203.24)	164.49 ±39.58 (150.2-178.76)	U=1447 p=.298
DHEA-S	3.01 ±2.12 (2.59-3.42)	4.81 ±2.45 (3.97-5.66)	U=984.5 p<.001
cortisol	503.08 ±160.93 (471.63-534.54)	441.17 ±122.4 (385.30-485.3)	**t=2.308 p=.024**
LH	3.46 ±1.71 (3.12-3.79)	4.34 ±1.83 (3.69-4.99)	**U= 978.0 p=.013**
FT	.190 ±.083 (.174-.206)	.22±.04 (.21-.24)	**t=-2.639 p=.009**
FT %	2.08 ±.474 (1.99-2.17)	2.05±.35 (1.93-2.18)	U=1709 p=.590
bioT	4.42 ±1.99 (4.03-4.80)	5.60±.89 (5.28-5.92)	**t= -3.566 p=0.001**
bioT %	47.58 ±12.81 (45.09-50.07)	50.88±10.46 (47.10-54.65)	U=1571 p=.227
C/D	.232 ±.165 (.199-.265)	.108±.058 (.087-.129)	U=**751.5 p<.001**
TT/TE	55.154 ±31.417 (49.014-61.294)	71.55 ±25.68 (62.291-80.81)	U=**1018.5 p=.001**
FE	4.77 ±2.01 (4.38-5.17)	4.34±.96 (4.00-4.69)	U=1526 p=.580
FE%	2.60 ±.31 (2.54-2.66)	2.63 ±.26 (2.54-2.72)	t=-.518 p=.607
FT/FE	.045 ±025 (.04-.05)	.044±.026 (.038-.051)	**U=1160 p=.012**
T/LH	3.187 ±1.976 (3.187-2.8)	2.936±1.153 (2.520-3.351)	U=1673 p=.893
cвT/LH	.0596 ±.033 (.053-.066)	0.059 ±0.020 (.052-.067)	U=1486 p=.487

1. The ICH group had significantly higher cortisol and C/D levels compared to controls (U=581.500 p<0.001).

2. The IHD group had significantly lower levels of TT (t=-2.824 p=0.006), FT (t=-2.898 p=0.005), bioT (U=908.5 p=0.001), DHEA-S (U=796.5 p<0.001), LH (U= 978, p=0.013), TT/TE (U=869.500 p=0.003), FT/FE (U=1000.0 p=0.025) compared to controls.

5.2 Diabetes mellitus and ACD

In the ACD group, there was a significantly higher age in the diabetes mellitus group (t= -3.485 p=.001) as well as a higher body mass index (U=542.5, p=.006).

Higher mean age and BMI in the DM subgroup showed significantly lower ot (t=2.35 p=.021), DHEA-S (U=620 p=. 004), SHBG (U=656.5 p=.007) and bioT%.(U=715 p=.020) In the DM group, higher mean svT% (U=697.5 p=.014) and svE%(U=639.5 p=.005) were found. The C/D ratio was higher in the DM group (U=471 p<.001), while ot/LH was higher in the non-DM2 group (U=658 p=.007).

5.2 Clinical characteristics

In the IHD group, we found a correlation between TT and the presence of DM (rs=-.243 p=.014) and between TT and BMI (rs=-.267 p=.009).

The absolute value of FT and bioT is negatively related to BMI (FT: r_s=-.235, p=.022; bioT: r_s=-.220, p=.033) The percentage value of FT on the other hand is associated with the presence of diabetes mellitus (r_s=.244 p=.013), age (r_s=-.290 p=.003). The same correlations are also characteristic for the percentage of bioT. (r_s=.231 p=.019 and r_s=-.382 p<.001).

SHBG levels are associated with the presence of diabetes mellitus (r_s=-.269 p=.006) and with age (r_s=.306 p=.002). In multiple linear regression analysis, we found that both diabetes mellitus (β=-.395, p<.001) and age (β=.426, p<.001) were significant predictors of SHBG level (R2=.237, F=15.367, p<.001). Controlling for age and presence of diabetes mellitus, only ALT value (β=-.296, p<.001) but not HDL (p=.317) and TG (p=.146) remained statistically significant predictors of SHBG level. A prognostic model including ALT levels, history of DM and age explained 32.3% of the variance in SHBG values (R^2=.323, F=9.997, p<.001).

There was a statistically significant correlation between androtest and DHEA-S values (r_s =-.292 p=.046) and androtest and C/D ratio (r_s =.375 p=.011). IIEF-5 showed a strong negative

correlation with androtest score. (r_s =-.557, p<.001) The association of HADS for depression with albumin was assumed to be random and subject to interpretation.

An association between TE and history of IHD was found (r_s =.210, p=.036). Free estradiol showed the same correlations as total estradiol.

In the group of IHD patients (ACS+CCS), correlations similar to those in the acute coronary syndrome group are observed. DHEA-S demonstrateds an association with the presence of DM (r_s=-.291 p=.003), history of CHD (r_s =-.408 p<.001), arterial hypertension (rs-.276 p=.006), age (rs-.459 p<.001) and stenting sequence (rs=-.203, p=.043). In contrast to the ACS group, there was also a weak correlation with the structured androtest interview score here (r_s=-.292 p=.046).

TT/TE correlates negatively with BMI (r_s= -.310, p=.002). Another derived coefficient is the ratio of FT to FE. It is of moderate strength and reaches statistical significance (r_s=-.306, p=.003) with correlation coefficients similar to those for the TT/TE ratio.

The TT/LH ratio demonstrated a weak correlation with the presence of DM (CCS: r_s=-.381, p=.031; IHD: r_s=-.268 p=.007). It was also associated with incident sequence in the IHD group (r_s=-.213, p=.032). The FT/LH ratio also shows an association with the GRACE score (r_s=-.251 p=.042).

5.3 Biochemical indicators

In the IHD group, nonparametric analysis demonstrated a weak positive association of HDL with TT (r_s=.251, p=.012), SHBG (r_s=218, p=.029), TT/TE and a moderate one with TT/LH (r_s=.374, p=.000) and FT/LH ratios (r_s=.357, p<.001).

Triglyceride level correlated negatively with SHBG (r_s=-.260, p=.011), FT/FE ratio (r_s=-.201, p=.050), TT/LH (r_s=-.258, p=.011), and FT/LH (r_s=-.219, p=.036). There was a weak positive association with the percentage of bioavailable testosterone (r_s=.234, p=.021). ALT values, were negatively associated with TT (r_s=-.282, p=.021) and SHBG (r_s=-.467, p<.001) levels. In view of the direct relationship between free hormone fractions and SHBG then FT%, bioT% and FE% also correlate with ALT.

TT/LH is positively associated with hemoglobin (r_s=.247, p=.014) and hematocrit (r_s=.212, p=.035), and negatively associated with leukocyte count (r_s=-.268, p=.007). Hematocrit usually concidered a surrogate marker of testosterone action does not correlate with any of the T fractions, in absolute value or percentage.

DHEA-S correlates positively with hemoglobin level with a weak correlation coefficient (r_s=.211, p=.037) On the other hand, for DHEA-S we find a moderate association with estimated glomerular filtration rate – eGFR - (r_s=.393, p<.001) The C/D ratio, where DHEA-S participates as denominator, was moderately negatively associated with eGFR (r_s=-.317, p=.001). The eGFR was also weakly correlated with the TT/TE (r_s=.206, p=.039) and TT/LH (r_s=.203, p=.042) ratios.

5.4 Hormonal parameters

Androtest is associated with DHEA-S (r_s=-.292 p=.046). As in the ACS group, a moderate correlation is found between C/D ratio value and androtest score in the pooled group of IHD patients (r_s=.375, p=.011). It is greater than that characteristic of DHEA-S alone.

A negative association was found between the total testosterone value and the free estradiol percentage value (r_s=-0.346, p<0.001).

Initial analyses and comparisons revealed some similarities and differences between the groups which necessitated further investigation:

Using Spearman's non-parametric test, there is a statistically significant association between the total testosterone value with that of SHBG (r_s=.407 p<.001) and TE (r_s=.319 p=.001). With FE, TT showed a moderate negative correlation with Spearman's coefficient r_s=-.346, p<.001.

Similarly to the other two groups, we performed stepwise regression analysis to examine the correlations of TT with other hormonal and anthropometric parameters in more detail. We found that BMI does not reach statistical significance when adjusted for TE, LH and SHBG (p=.346). A multiple linear regression model involving the variables LH and SHBG was statistically significant (R^2=.270, F=16.486, p<.001), determining 27% of the TT variance.

Free testosterone has a weak positive correlation with TE (r_s=.279 p=.004), FE (r_s=.294, p=.003) and LH (r_s=.231, p=.020). The percentage of FT on the other hand correlates with DHEA-S (r_s=.294, p=.003) and the percentage of bioT with DHEA-S with a correlation coefficient of r_s=.331 at p=.001. Percentage bioT in the IHD group correlates weekly, negatively with the C/D ratio (r_s=-.228, p=.023)

In the IHD group, there is also a moderate strength correlation of SHBG with TT (r_s=.407, p<.001) and a weak negative correlation with DHEA-S (r_s=-.260, p=.009). SHBG, possibly due to the previously described association with TT also demonstrates weak positive correlations with TT/TE (r_s=.269, p=.006) and TT/LH (r_s=.245, p=.013) ratios.

In the ACS group, LH correlates weakly with the value of the oT/oE ratio (r_s=.251, p=.037). and FT/FE (r_s=.262, p=.03) The same association was found in the ACS group (r_s=.200, p=.046).

In the IHD group, TE correlates with a weak positive coeficinet with both TT/LH (r_s=.295, p=.003) and FT/LH (r_s=.263, p=.009). The FT/LH ratio in the IHD group also correlates with the FE value (r_s=.283, p=.005). The percentage of FE correlates negatively with the TT/LH ratio (r_s=-.203, p=.041).

A weak negative correlation is also found between SHBG and DHEA-S (r_s=-.260, p=.009). Being derivatives of SHBG level, the percentage fractions of testosterone (free and bioavailable percentage) correlated positively with DHEA-S (r_s=.294, p=.003; r_s=.331, p=.001, respectively). There is no correlation between LH and cortisol on the one hand and testosterone, albumin total estradiol as well as different fractions of T for the group IHD.

6. Control group

Spearman's and Pearson's coefficients were used to examine correlations in the control group. There was a moderate positive association between estradiol and age (r=0.4365 p=0.023). Other correlations with age we found were of DHEA-S (r=-0.397 p=0.029), bioT% (r=-.42 p=.021),

FE% (r_s=-0.411 p=.024). The correlation of C/D with age (r_s=.359) reached statistical significance at p=.066.

V. Discussion

1. Testosterone

Since testosterone is the main steroid in males and is widely used as a marker of gonadal function it is of central interest in the present study.

In summary, our observations show that, against a background of similar BMI and age between groups suggest that ACS is associated with lower values of T and its fractions relative to healthy subjects. Such dynamics during acute coronary syndrome is a phenomenon described in various studies. (Gencer et al., 2019; Glueck et al., 1993; Pesonen et al., 2016; Pugh et al., 2002b; Tripathi & Hegde, 1998; Wang et al., 2018b) It remains unclear whether the T decline is solely due to the acute illness or whether hypothypotestosteronemia precedes it as a sign of CVD.

We also found a statistically significant difference between the number of patients with hypogonadism in each of the three groups using a cut-off value of 9.2 nmol/l for TT or 0.220 nmol/l for FT. These values were adopted for the general population, but in this case we study patients with a mean age of 56.3 yr. This is an age at which late onset hypogonadism is expected, decline in TT levels influenced by diseases, environmental factors, the aging process and other hormonal systems. In this regard, prospective studies have been conducted and age-adjusted reference ranges have been proposed. (Feldman et al., 2002; Kelsey et al., 2014) These observations as well as the variable clinical presentation of male hypogonadism make its characterization with a clear biochemical marker difficult.

1.1 Testosterone depending on the type of ACS

We found that the ACS group is not homogeneous with respect to TT levels. Correlation analysis reveals an association with the type and sequence of the event and the presence of ST-elevation. For this reason, we further examine the ACS group according to the type of incident (STEMI, NSTEMI, or UAP), depending on the presence of ST-elevation.

Considering the different types of ACS (STEMI, NSTEMI, or UAP), there was a statistically significant difference between them in terms of all T fractions, with the lowest values for the STEMI subgroup. There was no difference in SHBG levels, therefore the differences in FT and bioT are entirely on basis on total testosterone levels. To our knowledge, a similar difference has only been described in one other publication by a Chinese collective in 2011 (Hu et al., 2011) When categorizing the ACS group according to the presence of ST-elevation, similar differences were found as when comparing STEMI, NSTEMI and UAP. This largely reflects the division of the ACS group into STEMI on the one hand and NSTEMI+UAP on the other. We also looked for an explanation of this fact in the relationship between the severity of coronary atherosclerosis and the T value. We were unable to find such that depends on the number of affected coronary branches, time in ischemia, presence or absence of acute cardiac decompensation. In contrast, FT and bioT correlate negatively with the size of the infarct zone (increased troponin). This correlation should be interpreted considering the sensitivity of the laboratory method used to measure troponin, which is up to a maximum of 180 ng/ml. This observation gives reason to believe that FT and bioT better reflect the severity of ACS. A similar association but of TT with troponin was also found by Gencer et al. (Gencer et al., 2019; Sapin et al., n.d.).

In contrast to our study, a correlation between the severity of coronary atherosclerosis and TT levels has been demonstrated in other studies. In one of them, this was done for both stable IHD and ACS using angiographic parameters. (Hu et al., 2011) In three others, differences between patients with stable ACS and controls were investigated, also demonstrating a correlation between the severity of ACS and T levels. (G. B. Phillips et al., 1994; Rosano et al., 2007) In the mentioned studies, severity was estimated as the number of affected branches and by sum of angiographic indices. One of these angiographyc scores - SYNTAX was not used in our study. In a Bulgarian study by Semerdjieva, on the other hand, no association was found between either TT or DHEA-S values and troponin levels or SYNTAX score (Semerdjieva, 2015).

In summary: we assume that the lack of traditional associations (as TT with BMI) is due to the decline in T following the occurrence of ACS. This could account for the disruption of commonly observed associations of TT. (e.g. androtest)

1.2 Testosterone and DM in the groups ACS and CCS

Due to the availability of literature data on the difference in testosterone levels in patients with and without impaired glucose metabolism, we examined differences between groups based on this trait. Testosterone levels do not differ depending on the presence of diabetes mellitus in the ACD group. We also found no difference in the incidence of hypogonadism between the diabetic group and patients without glucose abnormalities within the ACD group. In this case, the threshold value of 9.2 nmol/l was chosen for hypogonadism. In the pooled group of ischemic patients (ACS + CCS), there was a statistically significant difference in TT levels in the DM group.

Within the STEMI subgroup, there is no statistically significant difference between T levels in patients with and without impaired glucose metabolism. A difference in the incidence of hypogonadism between diabetics and non-diabetics has been demonstrated by other investigators. (Wang et al., 2018a) In the aforementioned article (Wang et al., 2018a) a higher incidence of patients with TT <300 ng/dl (or 10.4 nmol/l) was found in the group with newly diagnosed type 2 DM.

In the context of our data, if a threshold value of 10.4 nmol/l for hypogonadism is adopted, i.e. if the criterion of Wang. et al. is applied, again no difference in the incidence of hypogonadism was found between the two groups (with and without DM) within the ACS group ($\chi^2(1)=1.658$, p=.198). Because only patients with AMI were included in that study, we repeated the analysis at a threshold value for oT of 10.4 nmol/l only for the subgroup of patients with myocardial infarction (STEMI + NSTEMI). Again, we find no correlation between the incidence of hypogonadism depending on diabetes mellitus in our sample ($\chi^2(1) =2.191$, p=.139).

Some differences in study design should also be noted. Whereas Wang et al. include patients with newly diagnosed DM, in our study all patients with diabetes mellitus had a diagnosis present at inclusion, they were treated with either oral therapy or insulin. Patients with decompensated DM (blood glucose above 20 mmol/l and positive urine or blood ketone bodies) were not included.

The exemplary analysis we performed presents the main limitations when compared with all previous studies on the subject. There are no common cut-off values defining the low level of oT, and there are no common variables (e.g. GRACE scoring, etc.). In view of the differences we have pointed out between different subgroups with ACS, the inclusion of patients with a different type of ACS (STEMI, NSTEMI, or UAP) also plays an important role.

In contrast to the ACS group, the group of CCS and the pooled group of IHD (ACS+CCS group) differed statistically significantly between patients with and without impaired glucose metabolism. This observation was against a background of similar mean age and BMI between the two groups. This finding is also supported by epidemiological studies on patients with type 2 diabetes mellitus. (Cheung et al., 2015; Dandona & Dhindsa, 2011) It is suggested that this is a result of a disturbance in the hypothalamus-pituitary-gonadal axis, insulin resistance and obesity. (Cheung et al., 2015) (Gencer & Mach, 2016) It is hypothesized that patients with impaired glucose metabolism have more sensitive regulation of the hypothalamus-gonadal axis, combined with an inability to adapt to stress and reduced LH secretion. Insulin resistance is associated with a reduced response to LH in Leydig cells, resulting in decreased T secretion (Araujo et al., 2007).

In the context of acute physiological stress in ACS, it has been suggested that insulin resistance in the setting of stress at the onset of ACS suppresses LH secretion and/or action to a greater extent in patients with type 2 diabetes mellitus (Smit & Romijn, 2006a) (WANG et al, 1978a) This thesis is not new and was proposed as early as 1979 by Wang et al. It is possible that this is a manifestation of one of the underlying pathophysiological mechanisms explaining the relationship between low T levels in diabetics, insulin resistance and cardiovascular disease. In view of our data, we assume such a pathogenetic link in all patients with CCS. To further clarify this issue, the TT/LH ratio was also investigated.

An additional difference we found between the ACS and CCS groups is in their correlation with BMI. Traditionally, hypotestosteronemia is associated with clinical features such as age, BMI, and endocrine disease. However, no such association is found in our study. In the ACS group, T levels do not show the usual correlations with age, body mass index and age. The explanation of this fact is probably the strong influence of the acute stress event on HPG axis. However, in other studies, even in the AMI group, a correlation between TT and BMI has been described. (Pesonen et al., 2016) In contrast, a moderate positive correlation of TT with BMI is found in patients with chronic coronary syndrome (r_s=-.491 p=.008).

The difference between the two groups in the association of ot with BMI, DM (absent in ACS and moderate in CHD) further supports the hypothesis that additional factors influencing testosterone concentration are present in the setting of myocardial ischemia.

1.3 Testosterone follow-up

One of the objectives of our study was to track the short-term dynamics in T levels.

There was no statistically significant change in any of the androgens over time. The increasing trends in FT% and DHEA-S that we describe in modelling the results, although not representative, are consistent with the literature data already mentioned (Niccoli et al., 2014; Pugh et al, 2002a; WANG et al., 1978b) Favorable dynamics in oT levels after ACS have been described by other authors after the onset of ACS. (Pesonen et al., 2016; Wang et al., 2018a) Such data for DHEA-S are not available. In modelling, we found a trend for increasing levels of free testosterone percentage and reduction in DHEA-S after a 6-month time period. In the model thus described, the change in oT value also increased with time, but this change did not reach statistical significance, probably because of the small number of patients in the sample. The trend of increasing TT over time demonstrated by our model as well as the lack of correlation of TT with correlated BMI, age, or EF suggests the decline in TT induced by the acute cardiovascular event. A lower value of testosterone levels in diabetics as well as an association of TT with BMI was reported only in the CHD and CCS groups but not in the ACS group.

2. DHEA-S

2.1 DHEA-S and cardiovascular risk

The negative correlation between coronary artery disease and DHEA-S has been described. (Zhang, Xiao, Liu, et al., 2022) Lower DHEA-S levels have also been identified as an independent risk factor for the development of CHD in a prospective follow-up of men aged 40-70 (Feldman et al., 2001) as well as in older (69-81 years) men (Tivesten et al., 2014) In support of this, a significantly lower DHEA-S level is found in both subgroups (ACS and CCS) of IHD compared to controls. The association between DHEA-S level and patients' risk characteristic, i.e. history of previously known CHD, ACS, GRACE score and age, was confirmed in the more detailed study of patients with IHD. On the other hand, no association was found with the severity of ACS. It is possible that this is partly a result of the sensitivity of the troponin measurement method-maximal serum troponin values were up to 180 ng/ml. This is in line with the literature, as there is no evidence of dynamics in DHEA-S levels by analogy to T.

On closer examination, after controlling for age, we found it to be the main predictor of DHEA-S levels, superior to the other GRACE scorers and incident sequence mentioned.

Based on the literature review, we also looked at the association of DHEA-S levels with the presence of diabetes mellitus, another proven cardiovascular risk factor. Such an association has not been conclusively demonstrated in the literature. In a cross-sectional study conducted in Italy, no association between DM and DHEA-S was found. (Ravaglia et al., 2002) There are reports that describing a negative correlation between DHEA-S and glucose levels. (Thomas et al., 1999) In another prospective study, a reduction in DHEA-S levels was associated with the development of DM. Serum levels were also significantly lower in the group with glucose abnormalities, but as in our study and in the Japanese cohort, this relationship proved to be insignificant after controlling for age. (Kameda et al., 2005)

We also found a statistically significant difference between the DHEA-S levels of patients with ACS depending on the presence of diabetes mellitus. However, this difference was at the expense

of a higher mean age in the group of diabetics with ACS compared to patients without glucose disorders and ACS. When controlling for age, the presence of diabetes mellitus, as with the other risk factors, did not acquire statistical significance; the presence of DM became an insignificant predictor of DHEA-S level. One other limitation that did not allow full comparison with literature data was the lack of insulin studied. For this reason, data on the relationship between insulin resistance and DHEA-S levels were not available.

The two cited studies were performed in stable patients, whereas the sample described above was of patients with ACS. When the same analyses were repeated in the CCS group, there was no statistically significant difference in DHEA-S levels between patients with and without DM. However, it should be noted that, unlike the ACS or IHD groups, there is no statistically significant difference in age between the subgroups with and without DM within the CCS group. In other words, we can assume, despite the small numbers (11 patients with DM and 21 without), that despite the presence of abnormalities in glucose metabolism at similar age and cardiovascular status, we observe similar DHEA-S levels.

It is not surprising to conclude that DHEA-S is lower in the IHD group, given the associations with classic cardiovascular risk factors (one of which is age). However, the direction of the association and the underlying pathogenetic mechanism could not be clarified with our current observations and the data at hand.

From the ROC analysis performed, we found that values above 6.3 mkmol/l DHEA-S virtually excluded the presence of STEMI, whereas values below 1.25 were 95% consistent with ST-elevation. This represents the upper half of the reference limit for DHEA-S. The lower reference limit for DHEA-S has a sensitivity of 70.0% and a specificity of 52.9% for the presence of ST-elevation. It is clear from the analysis conducted that DHEA-S levels below the lower reference limit cannot reliably predict the presence of ST-elevation, in analogy to cortisol (Separham et al., 2017).

This further suggests that low DHEA-S levels are the result of a chronic condition rather than a consequence of ACS. In view of the associations described above, we can speculate that DHEA-S

levels are an expression of general health status, and at most a function of age, rather than a link in the pathogenesis of cardiovascular disease.

2.2 DHEA-S and renal function

We found that age is a significant factor for DHEA-S levels in IHD patients relative to the presence of arterial coronary disease, severity of vascular event, or the presence of abnormal glucose metabolism. This relationship was stable in all groups considered. This fact makes it unlikely that ischaemic heart disease or acute stress (ACS) is the cause of the lower DHEA-S level or that it is a link in the pathogenetic mechanism leading to the development of cardiovascular disease. Our study, found a positive association between renal function and serum DHEA-S levels. This is the first time such a relationship has been found in a population of patients with ACS.

Our results also confirmed a positive correlation between DHEA-S levels and glomerular filtration rate. When adjusted for age, both retained their significance. However, such an association was found for the first time, to our knowledge, in a population of patients with ACS based on the literature review.

In the literature, basic research supports the thesis of a correlation between androgens and renal function. Conflicting data are available on both the benefit of androgen environment (Carrero et al., 2012) and the improvement of some renal parameters after male rat castration (Fortepiani et al., 2003; Garate-Carrillo et al., 2020; Verzola et al., 2004)

A negative association between creatinine clearance and DHEA-S has been described in healthy males in the general population (Tomaszewski et al., 2009) as well as in a mixed population (healthy and CKD patients). (Yamaguchi et al., 2021) Low DHEA-S and oT values have also been found in dialysis patients against a background of increased cardiovascular risk. (Shemies et al., 2020; van der Burgh et al., 2022) There have been reports of an association of hypogonadism with progression of CKD, with proterinuria in DM2 and with increased risk of CKD in DM2.(Amiri et al., 2020) DHEA-S may be considered as one of the precursors for testosterone synthesis.(Ma et al., 2022) (Zhang, Xiao, Li, et al., 2022)

In our data, correlation of DHEA-S with glomerular filtration rate was not found in the CCS group. The reason for this could not be clarified with the present data. One possible explanation is the small number of patients in this group. Another possibility, which we assume, is that acute coronary syndrome and the pathophysiological changes associated with it could account for the association between glomerular filtration rate and DHEA-S. It is possible that a different incidence of comorbidities, for example diabetes mellitus, could account for this difference. In the IHD group we find a similar relationship to that in the ACS group. Due to the fact that two thirds of the IHD group are ACS patients we assume the possibility that the relatively higher percentage of ACS patients is the reason for the correlation between eGFR and DHEA-S.

Given that glomerular filtration rate and DHEA-S decrease with age, an association between low DHEA-S levels and eGFR seems likely. However, the direction of the relationship remains incompletely understood. The wide variations in DHEA-S levels described, against a background of different causes of renal injury, make elucidation of the direction of the relationship even more difficult. Further exploration of these issues would require a study focused on renal function and pathology in the aforementioned patient cohorts.

3. SHBG

In our study, we found similar mean SHBG values in patients with acute, chronic coronary syndrome and in healthy controls. Despite similar absolute values in the acute and chronic coronary syndrome groups, different associations of SHBG with biochemical and clinical parameters were found. Literature evidence on the association between SHBG levels, mortality and cardiovascular pathology is somewhat mixed, with both low and high levels associated with increased CV risk. (Yeap et al., 2022)(Yeap et al., 2014)

3.1 SHBG and ACS

In the OCS group, the most significant factors determining SHBG variation are TT and ALT. Age, GRACE score and presence of DM became statistically insignificant factors after adjusting for TT and ALT in the stepwise linear regression analysis. The fact that ot demonstrated a moderate correlation with SHBG is a logical consequence of the fact that SHBG is the protein that binds T

and serves to form the plasma pool of the latter. The ALT value, on the other hand, as a marker of liver injury, is also related to SHBG levels. Liver enzyme levels are associated with hepatic nonalcoholic steatohepatitis in SM and DM independent of alcohol intake. This association makes them, as well as the presence of DM and insulin resistance, a potential marker of cardiovascular risk (Lioudaki et al., 2011).

In the ACS group, there is also no difference in SHBG levels between patients with and without DM on the background of different mean ages (t=1.416 p=.161). There was also no correlation of TT levels with the presence of DM (r_s=-.169 p=.160). Also, as for TT, where the association between DM and hypotestosteronemia has been demonstrated in the literature, lower levels of the protein were found in SHBG in DM patients. (Liu et al., 2020) The inflammatory response and insulin suppress SHBG production. (Mohammed et al., 2018) However, whether this type of SHBG upregulation plays a role in the context of ACS and whether it could be accomplished at all within 36-48 h is not known. As with TT, this could be attributed this unexpected observation to ACS and its concomitant pathophysiological processes. For this reason, there are currently no data on SHBG dynamics immediately after the occurrence of ACS. Despite the weak negative correlation of SHBG with troponin, there was no difference in its mean value between groups with and without ACS and controls.

In stable coronary artery disease, we found some known associations of SHBG with a worse metabolic profile of the patient, namely the presence of diabetes mellitus and higher triglycerides. On the other hand, in ACS in combination with DHEA-S and albumin, SHBG is a determinant of GRACE score. All these associations highlight SHBG as an additional risk factor for cardiovascular disease in men both in period immediately after the onset of ACS and in stable CHD.

Some differences were also found depending on the presence of diabetes mellitus. In the CCS group, in contrast to the ACS group, we found a higher SHBG value in the group without diabetes mellitus.

As in the ACS, IHD and control groups, in the CCS group TT correlates strongly, positively with SHBG for reasons discussed in the previous chapter.

It should be noted that in the CCS group age does not show a statistically significant correlation with SHBG. This observation contradicts the literature data. It may be due to the smaller number of patients compared to those in the ACS. The correlation between SHBG and age in the control group also did not reach statistical significance.

Nevertheless, the correlation in the CCS group of SHBG and TG is confirmed in studies by other authors in the general population. (Aribas et al., 2021) In the cited study in 3264 men and women, age, T and TG were shown to be the most significant predictors of SHBG value. Previous studies have also shown a positive association between higher SHBG levels and a more favorable cardiovascular risk profile in men (Canoy et al., 2014; Firtser et al., 2012) These facts suggest a greater cardiovascular risk in patients with lower SHBG levels. However, whether SHBG is an independent predictor of CV risk or a sign of endocrine or metabolic dysfunction cannot be assessed with the current data.

In view of our observations, it would be appropriate to add SHBG to the already known CVD risk factors. This is supported by the correlation of SHBG with GRACE score in the ACS group.

4. Cortisol

Regarding cortisol, we found significant differences between the groups, with the highest mean value in the group of patients with ACS. This observation is not surprising given the physiological role of the hormone. The same finding has been confirmed in other recent prospective studies. (Aladio et al., 2021) In the referenced study difference in cortisol levels depending on the presence or absence of ST-elevation, the Killip class of heart failure. The association with acute heart failure class was not confirmed in our data. This is probably due to the small number of patients with acute cardiac decompensation (two patients with class 2 and three with class 3 Killip). The long-known association between infarct size, expressed by troponin value, and cortisol levels was also not confirmed in our study. (STUBBS et al., 1999) We attribute this to the fact that the sensitivity of the laboratory method for troponin reaches up to 180 ng/ml.

5. Albumin

We also found a negative correlation between albumin levels and GRACE score. We could speculate that lower albumin concentrations are associated with a riskier patient profile. This assumption is supported by literature data. Serum albumin is one of the major proteins in plasma and is involved in the processes of chronic inflammation. (Nicholson et al., 2000; Quinlan et al., 2005) Kurtul demonstrated that albumin is associated with SYNTAX scoring and complication rates within ACS. (Kurtul et al., 2016)

6. Hormonal ratios

Hormone ratios are a method to simultaneously assess the effect of two interrelated hormones, often interpreted as the balance between two systems. However, to date the meaning and interpretation of hormone ratios has not been fully elucidated. In view of our data on the studied TT/LH, FT/LH, TT/TE; FT/FE; C/D ratios.

6.1 Total testosterone to LH (TT/LH)

6.1.1 TT/LH and diabetes mellitus

The oT/LH ratio is seen as a marker of the functional status of the negative feedback exerted by TT on the pituitary. We hypothesize that a changes in the ratio corresponds to an altered balance in the HPG axis influenced by the presence of diabetes mellitus or by ACS. Which exact pathogenetic factor would lead to such a change could not be answered by the present study. A decrease in the TT/LH ratio could be due to either a decrease in oT or an increase in the denominator value. A third possibility is a divergent change in both values.

In our study, we found a significant difference in the TT/LH ratio between the diabetic and euglycemic groups within the patients with ACS at the expense of higher values in the group without DM. The same trend was found in the chronic coronary syndrome group.

On the other hand, we found no difference in the TT/LH ratio between controls and patients (IHD group) and between patients with ACS and controls. The relationship between TT/LH and the presence of diabetes mellitus is supported by the moderate negative correlation between them for the ACS group. (r_s=-.286 p=.016) This observation suggests that dysfunction in the HPA axis is more closely associated with the presence of diabetes mellitus in the setting of acute physiological stress. Since no relationship was found between TT and DM and between LH and DM we assume that the relationship between the two hormones provides more information than the analysis of each variable separately. It should be mentioned that the mean age in the group of patients with ACS and DM was statistically significantly higher compared to the group of patients with ACS without DM. Although there is no statistically significant difference in LH levels between the two groups, an increase in LH and a change in the functional status of the HPG axis is associated with age and has been described in other studies. (Feldman et al., 2002) In this situation, an age-related difference in TT/LH cannot be excluded. The fact that there is no significant difference in the value of the TT/LH ratio between ACS and controls against the background of similar age supports this assumption.

The reason for the different TT/LH ratio may be the different sensitivity of the HCG axis in patients with impaired glucose metabolism. A more sensitive HPG axis would result in an inability to respond adequately to stimulation by gonadotropins, with or without a change in LH secretion. Insulin resistance is associated with a reduced Leydig cell response to LH. (Pitteloud et al., 2005a) In a study by a US collective using a hypersinusinemic euglycemic clamp technique, a direct association between T secretion and insulin sensitivity was found, while LH levels remained unchanged. The findings are consistent with previous data in which acute stress causes a decrease in serum testosterone, although LH levels may be normal or elevated. The mechanism is unclear, but potentially involves suppression by inflammatory cytokines of Leydig cells. (Langouche & van den Berghe, 2014b) It is likely that in the presence of DM, insulin resistance is further enhanced following the acute inflammatory response in the early phase of AMI, (Smit & Romijn, 2006b)

reducing the ability of the testis to respond to LH. Thus, the results suggest a pathophysiological mechanism that may support our hypothesis regarding the relationship between testosterone, insulin resistance and CVD.

In the ACS group, the value of the TT/LH ratio did not differ according to ST-elevation, although it is the presence of ST-elevation that is one of the factors associated with lower oT (the numerator in the ratio). This was at similar mean age between the subgroups with and without ST-elevation. Thus, the association of oT/LH with diabetes mellitus comes to the fore. For this reason, we could assume that ACS alone does not lead to hypothalamic dysfunction (expressed by altered TT/LH) without the presence of an underlying disorder (consequent to DM)

Comparison of TT/LH in the CCS group revealed no difference between patients with and without DM at similar ages. It could be assumed that the lack of acute decompensation of the CCS does not cause the manifestation of hypothalamic dysfunction conditioned by DM, or that it was the result of the similar mean age of the patients.

6.1.2 TT/LH and lipid profile

An association of TT/LH ratio with lipid parameters was also found. Both TT/LH and FT/LH in the ACS group were negatively associated with triglycerides and positively associated with HDL cholesterol. The relationship persisted after controlling for statin intake. Our observation between elevated triglycerides and TT/LH ratio shifted towards the denominator are in agreement with literature data. There is evidence that insulin resistance is associated with reduced Leydig cell response to LH. Dyslipidaemia and hypertriglyceridaemia in particular is one of the determinants of insulin resistance. Lipid infusion has been shown experimentally to result in suppression of gonadotropic secretion. (Chosich et al., 2017) A similar effect has been demonstrated in vitro - suppression of gonadotropic gene expression by free fatty acids.

In the CCS group, a positive association of oT/LH with LDL and total cholesterol is found, but no association with HDL. After controlling for statin intake the aforementioned correlations lost their statistical significance. LH also showed no statistically significant associations with lipid profile parameters. Other studies in men with stable coronary artery disease have found a positive

correlation between LH and HDL, but not with LDL or total cholesterol. (J. K. Wranicz et al., 2005) In the context of our data, we have no strong evidence to support an association of the TT/LH ratio and lipid profile in CCS patients.

Although the effects of lipids are likely more complex than suppression of pituitary function (McDonald et al., 2022) our study supports the hypothesis of an interaction between abnormalities in fat metabolism and HPG axis dysfunction in the context of arterial coronary disease. A ratio shifted in favor of the numerator (TT or FT) was associated with a more favorable lipid profile (higher HDL). The correlation of TT/LH with the presence of diabetes mellitus, on the other hand, suggests a disturbance in the HPG axis in the context of glucose disorders. A more detailed investigation of the relationship between diabetes mellitus, dyslipidemia that often accompanies DM, and HPG axis dysfunction should be the subject of another study with a design dedicated to this purpose.

6.2 Cortisol to DHEA-S ratio (C/D ratio)

Cortisol and DHEA-S are products of adrenal steroidogenesis, and it can be assumed that their relative proportion is relevant to stress response. The fact that the C/D ratio predicts health outcomes better than either hormone level alone, (Butcher et al., 2005) and that it predicts all-cause mortality (Boscarino, 2008) is the theoretical basis for examining it in the context of arterial coronary disease. DHEA-S concentration is more constant over time due to its long half-life. (Klinge et al., 2018) In other models of acute stress, its slow decline has been described (Beishuizen et al., 2002)In view of these data, we assume that DHEA-S levels are studied soon enough after the onset of acute coronary syndrome to reflect its stable concentration, not yet affected by acute ilness.

In contrast to DHEA-S, the C/D ratio was also associated with the presence of diabetes mellitus and smoking even after controlling for age in the ACS group. We could not attribute this effect to an association between age, diabetes mellitus, and cortisol as none was found in our cohort. Cortisol values showed no statistically significant difference between patients with and without diabetes mellitus. Therefore, we can conclude that the value of the C/D ratio gives us additional

information that is not available when analyzing only the baseline variables (in this case, cortisol and DHEA-S). Proceeding from this situation, in this case the value of the ratio is a sign of the imbalance between two systems, it is not the absolute values of the hormones that gain significance but their relative values to each other. In this case, the imbalance between two metabolic pathways in the adrenal cortex. The higher value of the ratio is in the direction of cortisol synthesis and secretion, while the lower values of the ratio - in favor of DHEA-S synthesis and secretion.

In the ACS group, higher values were reported in diabetics and in elderly patients, a group of patients with a higher incidence of metabolic abnormalities. Evidence for the association of higher C/D ratio with elements of the metabolic syndrome has been reported by other investigators as well. (El-Zawawy et al., 2022) (A. C. Phillips et al., 2010).

Other studies have suggested that the imbalance between decreased DHEA-S and increased cortisol in acute illness could predict survival in sepsis. (Beishuizen et al., 2002)(Tsai et al., 2017) The change in the ratio in favor of cortisol could be interpreted as an adaptive response to stress. The C/D ratio could also be considered as a predictor of condition after the acute incident as it was in patients with ischemic stroke. (Blum et al., 2013)

Unlike the ACS group in CCS after adjustment for age, diabetes mellitus becomes a non-significant predictor of C/D. One possibility is that this is a consequence of the smaller number of patients in this group, and the small number of patients with diabetes mellitus (n=11). For this reason, we could not draw conclusions based on these results.

Differences in C/D between the ACS and CCS groups will not be interpreted, it is assumed that they are a function of change in cortisol level, characteristic for the acute condition.

6.3 Estradiol and total testosterone/total estradiol ratio (T/E)

The oT/oE ratio reflects the relationship between sex hormones and certain risk characteristics of patients with CHD. Imbalance of testosterone and estrogens is associated with disturbances in

lipid profile independent of statin intake. The T/E ratio, rather than absolute estrogen levels, is more relevant in modulating the effect of androgens on CHD risk factors. This provides new directions for the study of the pathogenesis of coronary artery disease.

Estradiol as one of the major sex steroids, and in the context of the available data, is seen as a product of the aromatization of testosterone. In view of this, it is of central interest not only as a stand-alone measurement but also relation to T levels.

We found a negative association between estradiol levels and CPK and CK-MB. The same association was found for free estradiol. Despite this association, we found no difference in estradiol levels between the three groups or between the subtypes of acute coronary syndrome. In a publication by N. Semerzhieva, a positive correlation between TE and markers of myocardial necrosis was described. Such a relationship has also been described by another author between the number of affected coronary branches and TE. (GUO et al., 2014) A possible explanation for this discrepancy between the literature data and our observation of the association between TE and the severity of ACS could be to consider the decrease in T in the course of ACS as a decreased substrate for TE synthesis.

We therefore examined the TT/TE relationship in more detail to look for an imbalance between T and E in the context of acute myocardial ischemia. We examined the T/E ratio as a marker for aromatization of androgens in peripheral tissues. A negative association between BMI and TT/TE and FT/FE ratios is found. In all groups, the correlation coefficient with TT/TE is greater compared with that of TT/TE for the same group. Accordingly, we can conclude that TT/TE is a better indicator of aromatization in these groups relative to FT/FE. Based on this observation, we could derive a recommendation that investigation of TT/TE is preferable over FT/FE in future studies. This is supported by the fact that the measurement of TT and TE is also more accessible.

In the group ACS, TT/TE correlated weakly with BMI, whereas in the CCS the correlation was strong. Considering the main difference between the two groups, acute ischemia, we assume that the altered hormonal balance reduces the otherwise strong association between the two parameters. This is supported by the fact that, after controlling for BMI in the ACS group, ST-elevation was a significant predictor of the level of TT/TE. (β=-.296, p=.013) A similar difference in

TT/TE was found when comparing STEMI with non-ST-elevation ACS (NSTEMI and UAP) - with a higher aromatization index in STEMI. Because there was no statistically significant difference in TE or FE levels between the ST-elevation ACS (STEMI) and non-ST-elevation ACS (NSTEMI+UAP) groups , we assumed that the differences in TT/TE between the two groups were primarily accounted for by a relative change in E versus T and a change in the absolute value of T. On the basis of these observations, we can assume an increase in aromatization in more severe myocardial injury leading to a decrease in the TT/TE ratio. Another possibility is that the relatively greater decrease in TT in STEMI is responsible for the shift in the ratio in favor of TE. It is possible that both causes contribute to varying degrees to the shift in the TT/TE ratio.

Estrogens have beneficial effects on the neovascularization of ischemic tissues, have been shown to play a role in the recruitment of endothelial progenitor cells, and improve the process of myocardial recovery after ischemia/reperfusion. (Hamada et al., 2006; Yuan et al., 2018) In view of these effects, increasing aromatization makes physiological sense. In a 2006 study, a U.S. collective provided evidence of an increase in peripheral aromatization of androgens in the setting of acute systemic disease.(Spratt et al., 2006) In this case, the study was done in patients undergoing aorto-coronary bypass grafting, but it also found a decline in TT levels, as in our cohort.

Our observations, supported by the literature review, suggest that the increased TT/TE ratio may be a specific response to acute physiological stress (ACS) rather than a casual observation. However, the cause of the increased aromatization could not be indicated with our available data.

In the CCS group, TT/TE was positively associated with HDL (rho=.381, p=.041) and negatively associated with leukocytes (rho=-.475, p=.009). An opposite relationship has been found by other investigators, a negative association of HDL with T/E . Our results suggest a beneficial effect of a more androgenic environment, whereas the study by Zheng et al. (Zheng et al., 2012) suggests a more favorable effect of a more estrogenic environment. Due to the fact that dyslipidemia is one of the major cardiovascular risk factors the influence of sex hormones is essential. An important caveat in comparing the two studies is the fact that the article described did not specify the intake of lipid-lowering agents. Although in our study 26.8% of the patients with CHD were on statin

treatment, after adjustment for statin intake the TT/TE ratio was still a significant predictor of HDL levels in the CHD group.

7. Survey method

There was no association between androtest and any of the T fractions in patients with ACS and CHD. We could assume that the lack of such a correlation lies precisely in the peculiarity of the population - the presence of ACS or stable IHD.

In the ACS group, we found a negative association between androtest and DHEA, and a positive one with the C/D ratio. Such an association was described for the first time in the literature, the significance could not be clarified with the available data. We could speculate that, as a sign of general health status, low DHEA-S is also associated with a higher incidence of sexual dysfunction. This conclusion is supported by other studies (El-Sakka, 2018)

VI. Conclusion

In the course of the study, changes in sex hormone levels were found after the onset of ACS. Given the incidence of cardiovascular complications, the short- and long-term effects on hormone homeostasis are of interest because of their impact on general health status. The role of hormonal factors and regulation of endocrine axes in the occurrence and development of CVD is also a question not sufficiently understood.

In this regard, the present study appears to be relevant, seeking answers to some of the issues raised.

We observed the expected T dynamics in the acute period after the onset of myocardial ischemia. Additionally, we found lower T levels in the subgroup of patients with ST-elevation. The observed dynamics violated the usual associations with body mass index, in contrast to the group of patients with chronic coronary syndrome. This suggests that in the context of ACS, the usual risk factors for hypothyroidism give way to the stronger influence of myocardial ischemia.

Myocardial ischemia affects the gonadal axis by mechanisms other than activation of the CHA axis that could not be indicated by the present study.

For HDEA-S, on the other hand, an association of glomerular filtration rate and age with DHEA-S emerged, independent of patients' risk characteristics or severity of ACS. For this reason, in agreement with other investigators, we assumed that DHEA-S is an expression of general health status or plays a role in the pathogenesis of ischemic cardiac pathology, but its low levels are not a consequence of the occurrence of ACS.

SHBG is associated with some well-established risk characteristics of patients with ACS and CHD. In our opinion, this makes it suitable as an additional cardiovascular risk marker and simultaneously allows a more detailed hormonal assessment (determination of FT and bioT).

Both absolute hormone values and hormone ratios demonstrated correlations with clinical and biochemical parameters. The TT/LH ratios differed in patients with and without diabetes mellitus, suggesting hypothalamic dysfunction exacerbated in the course of ACS. Aromatization index expressed by TT/TE ratio was better associated with cardiovascular risk factors better than either of the indices individually.

These observations of ours support the original hypothesis that examining the hormonal axes in their direct interaction with each other yields more information relative to each indicator alone.

Based on this, we could add the oT/LH ratio, TT/TE as well as albumin value to the usual risk characteristics given the associations of the indicators in question with lipid profile and GRACE score. The C/D ratio as an expression of the balance between the functional status of different areas in the adrenal cortex also showed an association with metabolic abnormalities and risk characteristic.

All these associations represent an opportunity to further define the risk profile of the patient with ACS. They provide the basis for further prospective studies that could elucidate some important aspects in the regulation of gating function and the extracardiac effects of steroid hormones in the context of ischemic heart pathology.

VII. Conclusions

Task 1: To investigate the pituitary-gonadal axis by measuring the values of total testosterone, calculated free testosterone, luteinizing and follicle-stimulating hormone in patients with acute and chronic coronary syndrome and in healthy controls.

1. Lower values of total, free and bioavailable testosterone and a higher incidence of hypotestosteronemia are found in patients with ACS compared to controls.
2. Patients with ST-elevation ACS (STEMI) is associated with significantly lower levels of TT, FT and bioT compared to patients with non-ST-elevation ACS (NSTEMI and UAP) against a background of similar age, SHBG and BMI.
3. There are no difference in LH levels between the three groups studied (ACS, CCS, controls)

Task 2: To investigate differences and look for correlations in DHEA-S/cortisol; testosterone/LH; testosterone/estradiol ratios in healthy controls and in patients with acute and chronic coronary syndrome.

1. Aromatization index depends on both BMI and the presence of ST-elevation, it is lower in the STEMI group.
2. Testosterone to estradiol ratio, rather than the absolute androgen or estrogen levels, correlates with lipid parameters even after adjustment for statin use in the CCS group.
3. Patients with DM in both the ACS and CCS groups were found to have a lower TT/LH ratio compared with patients without impaired glucose metabolism.
4. In the ACS group, a higher TT/LH ratio is associated with a higher HDL cholesterol value even after adjustment for statin intake.
5. Higher C/D ratio was associated with worse risk profile of patients with ACS (presence of DM, older age).

Task 3: In patients with ischemic heart disease, investigate differences in hormonal indices between subgroups with and without diabetes mellitus.

1. Testosterone levels and incidence of hypotestosteronemia do not differ according to the presence of diabetes mellitus in the ACS group at different BMIs and age.

2. Lower TT levels were found in patients with CCS and diabetes mellitus compared to those without impaired glucose metabolism.

3. DHEA-S levels are not affected by the presence of DM in acute and chronic coronary syndrome.

4. In the CCS group, lower SHBG levels are associated with a worse metabolic profile of patients, namely the presence of DM2 and elevated triglycerides.

Task 4: To follow the dynamics of sex hormones after the onset of ACS.

1. In mathematical modeling, we found a trend for increasing levels of free testosterone in percentage and decreasing DHEA-S over a 6-month period after the onset of ACS.

2. There was a tendency rise of TT value over time after the onset of ACS.

Task 5: To investigate the relationship between the severity of acute coronary syndrome and other clinical, anthropometric and paraclinical indices, and steroid hormone levels in the serum of patients with acute coronary syndrome, in the acute period.

1. Free and bioavailable testosterone better reflect the size of the ischemic zone (expressed by troponin) relative to total testosterone.

2. The difference between the two groups in the association of TT with BMI (absent in ACS and moderate in CCS) supports the hypothesis that there are additional factors influencing testosterone concentration in myocardial infarction.

3. The major determinants of DHEA-S were age and glomerular filtration rate in the ACS and IHD groups, whereas for the CS group it was age alone.

4. There is an association between SHBG and TT levels, liver enzymes, age and triglyceride levels in the ACS group.

5. SHBG is an appropriate parameter reflecting and complementing the risk characteristics of patients with ACS.

6. A negative correlation is found between albumin levels and patients' cardiovascular risk expressed by GRACE score.

7. By SHBG, DHEA-S and albumin values in the first 48 h after the onset of ACS, 22.9% of the variation in GRACE score could be determined.

Task 6: To screen for hypogonadism combined with anxiety and depression, and erectile dysfunction, at the onset of ACS and at the sixth month of the event.

1. In the ACS group, we a higher androtest score is associated with lower DHEA-S levels as well as at higher cortisol/DHEA-S ratio levels. Such an association was described for the first time in the literature, the significance could not be clarified with the available data.

2. The IIEF-5 score demonstrates a negative association with HADS score for anxiety and a positive association with androtest in patients with ACS.

VIII. Contributions

Contributions of scientific and practical nature:

1. For the first time, the cortisol/DHEA-S ratio is investigated in patients with acute coronary syndrome.
2. For the first time, the total testosterone/LH ratio has been shown to differ in patients with acute coronary syndrome according to the presence of diabetes mellitus.
3. Based on the results, additional risk characteristics in patients with ACS were determined.

Contributions of confirmatory nature:

(Related to inconclusive statements in the literature)

1. The association of DHEA-S with coronary artery disease was confirmed.
2. Confirmed the changes in total testosterone levels in the first days after the onset of acute coronary syndrome.
3. Confirmed the higher incidence of hypotestosteronemia in patients with acute coronary syndrome compared to controls.

List of publications related to the dissertation topic

1. Physiological and pathophysiological role of dehydroepiandrosterone in cardiovascular disease. S. Shishkov, M. Boyadjieva; Varna Medical Forum, Vol. 11, June 2022, Issue 1

2. Testosterone and cardiovascular disease-a literature review; S. Shishkov, M. Boyadzhieva Scripta Scientifica Medica, 2022;54(1):9-18

3. "Total testosterone in men with acute coronary syndrome." S. Shishkov, K. Hristozov, M. Boyadzhieva, s. Slavcheva - Scripta scientifica medica (in press).

Participation in scientific forums:

"Levels of androgens in men with acute and chronic coronary syndrome" S. S. S. Shishkov, S. Hristozov, presented at the National Symposium of the Bulgarian Society of Endocrinology, Plovdiv, 6-8.10.22.

Scientific projects related to the dissertation:

"Dynamics in serum levels of male sex steroids in men after acute coronary syndrome" - funded by the Science Fund of MU - Varna (research project № 19017 from 2020).