Copeptin, IGFBP-1 and cardiovascular prognosis in patients with type 2 diabetes during and after acute myocardial infarction

A report from the DIGAMI 2 trial

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TABLE OF CONTENTS

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Background – Cardiovascular disease, e.g. coronary artery disease, is the main cause of death globally. The prognosis after acute myocardial infarction (AMI) is more severe for patients with type 2 diabetes (T2DM) than without. This is not completely explained by hyperglycemia and traditional CV-risk factors. Hence the search for novel risk factors and/or riskmarkers is important. In this report two riskmarkers: Copeptin, a marker for vasopressin, and IGFBP-1, one of IGF binding proteins, are investigated.

Methods – Copeptin and IGFBP-1 were analyzed in patients (age: 70, male: 70%) with T2DM and AMI participating in the DIGAMI2 trial. Samples were analyzed at admission (n=393), at discharge (n=309) and after three months (n=288). The primary endpoint was cardiovascular events (cardiovascular death, AMI, and stroke) (CVevent).

Results – The median copeptin levels at admission were 21.8 pmol/l, at discharge 8.5 pmol/l and after three months 8.40 pmol/l. There were significant correlations between copeptin at admission, discharge and after three months. In an unadjusted Cox Regression Hazard analysis, both biomarkers independently predicted CVevent at all occasion. In a multiple model including both biomarkers, copeptin was a predictor of all events apart from non-fatal reinfarction and stroke at discharge while IGFBP-1 did not remain as an independent predictor of cardiovascular event. In the final multiple model, including age and creatinine clearance, copeptin remained an independent predictor for all events at admission and after three months but not at discharge.

Conclusions – Copeptin levels were elevated at admission, then decreased relatively fast and stabilized. There was a relationship between copeptin and IGFBP-1 throughout the study period. Furthermore, copeptin was a stronger predictor for cardiovascular events, both at admission and after three months. These findings may have implications for the understanding of the association between diabetes and cardiovascular disease and could be a potentially pharmacological target.

ABBREVIATIONS

ACS – Acute coronary syndrome

AMI – Acute Myocardial Infarction

AVP – Arginine vasopressin

BMI – Body mass index

CAD – Coronary artery disease

CVD – Cardiovascular Disease

DIGAMI 2 – Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction 2 trial

ECG – Electrocardiograph

IGFBP-1 – Insulin Growth Factor Binding Protein 1

NSTEMI – Non-ST-segment elevation myocardial infarction

OGTT – Oral glucose tolerance test

STEMI – ST-segment elevation myocardial infarction

T2DM - Type 2 Diabetes Mellitus

WHO – World Health Organization

INTRODUCTION

Cardiovascular disease

Cardiovascular disease (CVD), a group of diseases that involve the heart or blood vessels, is the main cause of death globally [1, 2]. In the European Union, CVD causes about 2 million deaths each year and accounts for the largest number of premature deaths before the age of 75 or 35% of all deaths in men and 38% in women. The World Health Organizations (WHO) predicted that by 2030, almost 23.6 million people will die from CVD and CVD is projected to remain as the globally leading mortality cause. The incidence of CVD is increasing quickly in developing countries as a result of radical lifestyle changes and aging populations. Furthermore CVD imposes a considerable economic burden with for example an estimated annual cost in the European states of €193 billion representing an average annual per capita cost of € 391[1]. As a reference, the total EU-budget for 2013 is €150,9 billion.

Coronary artery disease (CAD) is characterized by inflammation, endothelial dysfunction and build-up of lipids, calcium and cellular debris within the intima of the vessel wall [3]. CAD can be asymptomatic or symptomatic (stable angina pectoris or acute coronary syndrome (ACS)). ACS includes ST-segment elevation myocardial infarction (STEMI), non-STEMI or unstable angina depending on the presence or not of electrocardiographic (ECG) changes and troponin release [4].

The Interheart study, a case-control study of AMI, enrolling 15.152 patients with AMI and 14.820 controls in 52 countries from all continents, showed that 90% of the population attributable risk for AMI in men and 94% in women relates to nine modifiable risk factors [5].

These are smoking, increased blood lipids, hypertension, diabetes, abdominal obesity, a poor psychosocial environment, too low consumption of fruits and vegetables, too much alcohol and lack of regular physical activity.

About 20% of patients with AMI have previously known diabetes [6]. In 2002, Norhammar et al. reported on a high prevalence of abnormal glucose regulations in patients with AMI and no previously known diabetes [7]. When investigated with an oral glucose tolerance test (OGTT) 31% of the patients had undiagnosed diabetes and 35% impaired glucose tolerance (IGT). Similar findings were repeated in Euro Heart Survey (n=4961) extending the findings to patients with stable CAD [8, 9]. The awareness of the glucometabolic state in patients with CAD is important since it contributes to a more dismal prognosis [8].

Diabetes Mellitus Type 2

In 2011 an estimated 355 million people in the world had diabetes with a predicted increase to 438 million by the year of 2030 [10]. The majority (90%) of them, have type 2 Diabetes Mellitus (T2DM). T2DM is a chronic metabolic disease that is characterized by peripheral insulin resistance combined with a relative or absolute insulin deficiency resulting in hyperglycemia [11]. There are several reasons for the increasing prevalence of T2DM, predominantly a windespread sedentary lifestyle leading to obesity and an increased average lifespan with pancreatic beta-cell function decreasing with age.

Patients with T2DM often lack symptoms of hyperglycemia and remain undiagnosed for years. Notably several complications due to the undetected hyperglycemia may develop if treatment is not initiated at an early stage.

Chronic hyperglycemia is associated with dysfunction of small vessels (i.e microvascular complications) and failure of different organs, such as the retina, kidneys and nerves. In addition, patients with T2DM are at a two to three times increased risk for macrovascular complications, i.e. cardiovascular, cerebrovascular and peripheral artery diseases [11]. This has prognostic implications. A Finnish study demonstrated that patients with T2DM without a history of a MI had as high risk of MI as patients without T2DM with a previous MI[12]. Furthermore the prognosis after a cardiovascular event is more severe for patients with T2DM than without[13].

Several epidemiological studies have shown a correlation between high glucose levels and macrovascular complications. The four main hypotheses on why hyperglycemia may induce vascular complications. The first is related to increased intracellular oxidative stress., the second involves modification of proteins involved in gene transcription causing cellular dysfunction, the third is the activation of protein kinase C causing endothelial dysfunction and the fourth increased expression of transforming growth factor-1 and plasminogen activator inhibitor. These four pathways seem to be interlinked through activation of oxidative stress [14].

Other mechanisms, common among patients with diabetes and paving the way for the development of atherosclerosis and macrovascular complications, are endothelial dysfunction and increased susceptibility to inflammation. In addition, patients with T2DM have an increased risk for thrombotic occlusion and plaque rupture due to decreased endogenous fibrinolysis, a pro-thrombotic state and increased smooth muscle cell apoptosis. Disturbances in the myocardial energy production in T2DM may increase the propensity to develop myocardial dysfunction. This involves a shift from glucose oxidation to the more oxygen demanding beta oxidation which is further enhanced during stress i.e. AMI due to catecholamine release [11].

Hyperglycemia during hospitalization for ACS is associated with a negative prognostic impact in patients with and without T2DM [15, 16]. High glucose levels in this situation may be an expression for an underlying glucometabolic disturbance. It can, however, be the consequence of stress induced catecholamine release leading to mobilization of free fatty acids from the adipose tissue and a decreased insulin release in combination with increased insulin resistance and glycogenolysis causing hyperglycemia [11].

The increased cardiovascular risk and the impaired prognosis after an AMI in patients with T2DM are not completely explained by hyperglycemia and traditional cardiovascular risk factors such as hypertension, obesity or dyslipidaemia. Thus, the search for novel risk factors and/or riskmarkers linking T2DM to CVD is important for our understanding of the pathophysiological process. In the present project two biomarkers will be reviewed and their association with patients with T2DM and AMI explored.

Copeptin

The hormone arginine vasopressin (AVP) has numerous physiological functions in the human body, including vasoconstriction, anti-diuresis. It is suggested to be involved in glucose homeostasis and platelet aggregation [17].

AVP is synthesized as the precursor preprovasopressin in magnocellular nuclei of the hypothalamus to be stored in vesicles in the posterior lobe of the pituitary gland. The release of AVP is mainly regulated by high plasma osmolality and decreased blood volume. Once in the circulation, AVP acts via three distinctive G protein-coupled receptors.

The V_{1a} receptor mediates strong arteriolar vasoconstriction while through antidiuretic effects are initiated via the V_2 receptors. V_{1b} receptors are endocrinologically active but their exact mechanism of action is still not known. It is suggested that these receptors (V_{1b}) modulate ACTH secretion from the anterior pituitary gland, as well as glucagon and insulin secretion from the pancreas [17, 18].

Further knowledge on the AVP secretion may contribute further knowledge on the importance of AVP in the pathogenesis of cardiovascular disorders. However, AVP has a relatively short half-life and most of the circulating AVP is bound to platelets, which makes it difficult to measure. After synthesis AVP is subjected to an enzyme cascade to attain its bioactive conformation. Copeptin, also known as the AVP-associated glycopeptide or CT-proAVP, is a 39 amino acid glycopeptide that comprises the C-terminal degradation part of the prehormone and is released in equimolar doses to AVP [18]. Unlike AVP, copeptin is stable and therefore a good marker for the AVP release[19].

AVP is a stress hormone and it has been hypothesized that this system is activated by an AMI. High copeptin levels are indeed a negative prognostic marker in such conditions [17, 20, 21] There are several mechanisms by which activation of the AVP system may be harmful during and after an AMI. Among them are increasing left ventricular after- and preload due to vasoconstriction and reabsorption of water in the collecting ducts of the kidneys respectively or by cardiac remodeling. Furthermore elevated levels of AVP seem to increase the risk of developing diabetes [22, 23] and are strongly associated with stroke, sudden death and combined cardiovascular events in hemodialysis patients with T2DM[24]. These associations are, however not fully understood and in need of further investigations.

IGFBP-1

Insulin Growth Factor Binding Protein 1 (IGFBP-1) is one of six insulin growth factor (IGF) binding proteins. The binding prolongs the half-life of IGF and prevents the activation of receptor signaling. IGFBP-1 is mainly produced by the liver. The production is up-regulated in response to pro-inflammatory cytokines, physiological stress and is mainly down-regulated by inhibitory effects of insulin [25].

In a general population low levels of IGFBP-1 are related to increased risk of diabetes and possibly to increased cardiovascular risk. However, during the development of T2DM the IGFBP-1 concentrations decreases, indicating increased hepatic insulin resistance. On the contrary, high levels of IGFBP-1 in patients with AMI and T2DM have been related to increased cardiovascular mortality and morbidity [26]. This is potentially caused by a decreased insulin production due to beta-cell dysfunction.

There seems to be a connection between the IGF and the AVP hormonal systems. In a study of 14 patients with diabetes insipidus the IGFBP-1 levels increased when the AVP analogue desmopressin was infused [27]. This relationship was confirmed during the acute phase of myocardial infarction in an epidemiological report of the Diabetes Mellitus Insuline-Glucose Infusion in Acute Myocardial Infarction 2 trial (DIGAMI 2) trial in which both IGFBP-1 and copeptin were independent prognostic predictors of cardiovascular events (cardiovascular death, myocardial infarction, and stroke) [28]. There was a statistical correlation between the two hormone systems. Copeptin was, however, a stronger predictor of event. Further studies are required to fully understand the connection between copeptin and IGFBP-1 and an interesting aspect is if this relation is sustainable even after the acute phase of an AMI.

The aims of the present epidemiological study of patients participating in the DIGAMI 2 was 1) to characterize the AVP system measured as copeptin during and after an AMI 2) to expand previous knowledge on the relationship between copeptin and IGFBP-1 and 3) to study the impact of copeptin and IGFBP-1 on prognosis during and after an AMI and in patients with T2DM.

RESEARCH DESIGN AND METHODS

Study design

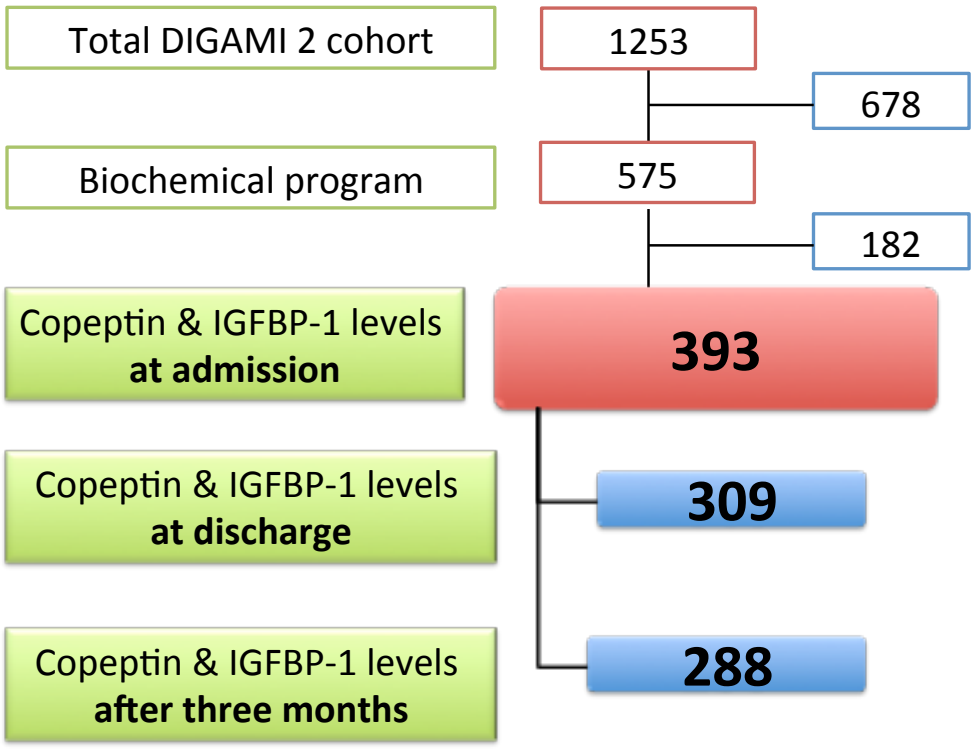
The DIGAMI 2 was a multicenter, prospective randomized, open trial with blinded evaluation comparing three different glucose lowering management strategies in patients with T2DM and suspected AMI [29]. In summary, 1253 patients (mean age 68 years; 67% males) were randomized, to receive 1) acute insulin-glucose infusion followed by insulin-based long-term glucose control (n=474), 2) insulin-glucose infusion followed by standard glucose control (n=473) and 3) routine metabolic management according to local practice (n=306). The median study duration was 2.1 (interquartile range 1.03 – 3.00) years and no patients were lost to follow up. The objective was to compare the mortality and morbidity difference between the groups. All events were adjudicated by an independent committee unaware of group allocation. There was no significant difference in mortality and morbidity among the three groups, which is the reason that the DIGAMI 2 database may be used for epidemiological studies like the present..

Patients

In a prespecified biochemistry substudy 575 of the DIGAMI 2 patients, regardless of treatment allocation, had blood samples stored and analyzed at hospital admission and discharge and 3 and 12 months thereafter. In the present epidemiological analyses copeptin and IGFBP-1 are studied at three different occasions: hospital admission; hospital discharge; and three months later.

Three subsets of patients are used [Figure 1] .The first subset includes 393 patients with copeptin and IGFBP-1 values at admission. The results from this cohort have been presented previously [28] but all results have been recalculated within the framework of the present project. With the first subset as a basis a second and third subset include patients, who apart from copeptin and IGFBP-1 at admission had copeptin available at discharge (n=309) and after three months (n=288).

Figure 1 Flow Chart showing division of different subgroups



Laboratory analyses

Copeptin concentration was measured with a sandwich immuno assay (LUMI test CT-proAVO, BRAHMS AG, Henningsdorf/Berlin, Germany). The lower detection limit was 0.4 pmol/l and the functional assay sensitivity (<20% interassay coefficient of variation) <1pmol/l [19, 28].

The serum IGFBP-1 concentrations were determined by radioimmunoassay according to the method of Póvoa et al [28, 30]. The sensitivity of RIA was 3 μg/l and intra- and interassays coefficient of variation 3 and 10% respectively. Blood glucose was analysed locally as whole blood glucose in mmol/L. HbA1c was analysed by high-performance liquid chromatography with an upper normal limit of 5.3%. Creatinine clearance (ml/min) was calculated using the Cockroft-Gault formula.

Statistical methods

Continuous variables are presented as median and interquartile ranges (IQRs), and categorical data as number and percentages unless otherwise stated. In Table 2 age, creatinine clearance, glucose at admission and HbA1c were stratified into dichotomous variables divided by biomarker value under or above median (see Table 1). Differences between groups of patients stratified by dichotomous variables were evaluated using Wilcoxon two sample test (Table 2). Associations between continuous variables were assessed using Spearman's Rank Correlation (Table 3).

The relationship between copeptin, IGFBP-1 and various events was assessed using Cox's proportional hazard regression and is presented as hazard ratio (HR) and 95% confidence intervals (CI). Because of skewed distributions, the copeptin and IGFBP-1 values were log transferred prior to analysis. The primary endpoint, was a composite of cardiovascular death and non-fatal myocardial infarction or stroke. The fatal and non-fatal components of the primary event served as secondary endpoints. Three different time-points (admission, discharge and after three months) were used as baseline depending on from which occasion the biomarkers were studied. Time to an event refers to the first event occurring after the given baseline regardless if it is the patients´ first, second, and so on, number of event.

Known predictors of outcome in the DIGAMI 2 trial (age, creatinine clearance, glucose at admission, and previous heart failure) were adjusted for in the mulitvariable analyses [28, 29]. Based on stepwise adjustments in the baseline cohort age and creatinine clearance were chosen to be included in the final model, together with copeptin. The same set of variables was used for all endpoints at all timepoints. A two tailed $p<0.05$ was considered significant. Kaplan-Meier curves were drawn to illustrate time trends for cardiovascular outcome by copeptin tertiles (p value=log-rank test for trend). All analyses were done using SAS version 9.3; SAS Institute).

RESULTS

Baseline characteristics

Clinical and biochemical characteristics for the three subsets of patients are outlined in Table 1. In brief about 70 % were men, the median age was 70 years, BMI was 28 kg/m^2, and the median duration of T2DM was 6-7 years. Thirty-eight percent of the patients had a history of previous AMI, about 20% previous congestive heart failure and 22% were current smokers. All three subsets had similar baseline characteristics apart from small differences as e.g. younger age, better renal function and higher use of metformin and lipid-lowering drugs in the last group.

Table 1

Baseline characteristics for patients in all three subsets. Data are n (%) or median (quartile 1–quartile 3). All variables refer to the time of hospital admission, unless otherwise stated.

	Admission	Discharge	Three months
n	393	309	288
Age (years)	70.0 (60.8-77.0)	69.7 (60.7-76.7)	67.7 (60.3-75.6)
Male sex	269 (68)	209 (68)	202 (70)
BMI (kg/m^2)	28 (25-31)	28 (25-31)	28 (26-31)
Diabetes duration (years)	6 (1.5-14)	7 (1-14)	6 (2-14)
Blood pressure (mmHg)			
Systolic	130 (120-150)	130 (120-150)	130 (120-150)
Diastolic	74 (63-85)	74 (64-85)	75 (65-85)
Previous medical history			
Myocardial infarction	148 (38)	117 (38)	109 (38)
Angina Pectoris	201 (51)	159 (51)	152 (53)
Heart failure	78 (20)	55 (18)	52 (18)
Hypertension	215 (55)	168 (55)	167 (58)
Hyperlipidemia	135 (34)	105 (34)	104 (36)
Current smoker	84 (22)	67 (22)	60 (21)
Medication prior to admission			
Insulin	135 (34)	104 (34)	103 (36)
Metformin	99 (25)	68 (22)	77 (27)
Sulphonylurea	153 (39)	121 (39)	111 (39)
Betablocker	175 (45)	133 (43)	133 (46)
Aspirin	206 (52)	157 (51)	149 (52)
ACE inhibitor	124 (32)	95 (31)	95 (33)
Lipid lowering	117 (30)	87 (28)	92 (32)
Randomized treatment group			
Group 1	147 (37)	119 (39)	109 (38)
Group 2	154 (39)	122 (39)	114 (40)
Group 3	92 (23)	68 (22)	65 (23)
Biochemistry at admission			
Blood glucose (mmol/l)	11.8 (9.2-14.9)	11.9 (9.4-14.9)	11.8 (9.2-14.9)
HbA1c (%)	7.1 (6.2-8.3)	7.2 (6.3-8.4)	7.1 (6.3-8.4)
Serum creatinine (μmol/l)	92 (78-110)	90 (77-107)	90 (77-107)

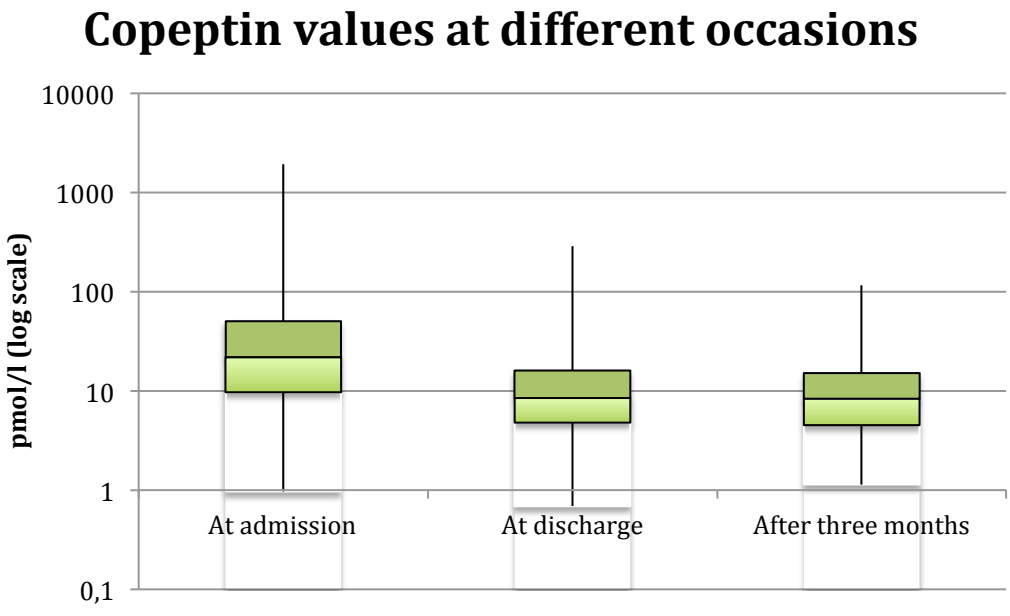
Creatinine Clearance (ml/min)	72 (50-93)	72 (51-94)	75 (53-96)
Serum cholesterol (mmol/l)	5.0 (4.2-5.8)	5.0 (4.2-5.9)	4.9 (4.1-5.8)
Serum triglycerides (mmol/l)	1.7 (1.2-2.6)	1.7 (1.2 -2.6)	1.8 (1.2-2.7)

Copeptin and IGFBP-1 levels

Copeptin

Figure 2 presents the copeptin levels at the different times of measurement. The levels at admission varied between 0.97 and 1936 pmol/l (median 21.8 pmol/l; mean 62.4 pmol/l; interquartile range 9.7-50.1 pmol/l) The levels at discharge were 0.63 and 286.0 pmol/l (median 8.5 pmol/l; mean 14.0 pmol/l; interquartile range 4.80-16.1 pmol/l) and after three months 1.14-116.0 pmol/l (median 8.40 pmol/l; mean 11.9 pmol/l; interquartile range 4.52-15.2 pmol/l).

Figure 2 Copeptin levels (pmol/l) at admission, discharge and after three months.



At all occasions patients with an age above and renal function below the median and those with heart failure had higher levels of copeptin (Table 2). The levels did not differ significantly between those with and without previous myocardial infarction, but patients with previous myocardial infarction had higher levels at discharge and after three months,. The levels did not differ in patients with glucose control (glucose or HbA1c) above or below median except at admission. The levels were independent of gender.

Copeptin consistently correlated with age and creatinine clearance (Table 3). There were significant correlations between copeptin at admission, discharge and after three months. Moreover there were significant correlations between copeptin and IGFBP-1 at all occasions.

Table 2

Median values (quartile 1–3) for copeptin (pmol/l) at admission, discharge and after three months by gender and some biochemical and clinical characteristics (p-value=Wilcoxon Two-Sample Test).

		Admission (n=393)	p	**Discharge (n=309)**	p	**Three months (n= 299)**	p
Males vs. females	Males	20.2 (9.6-43.5)	NS (p=0.20)	8.6 (5.2-15.9)	NS (p= 0.29)	8.2 (4.7-14.6)	NS (p=0.93)
	Females	24.7 (10.0-63.1)		8.2 (4.0-16.4)		8.5 (4.2-15.3)	
Age	Below median	14.7 (7.2-31.7)	<0.0001	6.5 (4.2-11.0)	<0.0001	6.6 (4.0-10.6)	<0.0001
	Above median	34.6 (13.5-72.6)		12.4 (6.0-21.6)		10.7 (5.5-18.3)	
Biochemistry at admission							
Creatinine Clearance	Below median	36.7 (13.6-69.6)	<0.0001	13.0 (5.9-21.7)	<0.0001	10.1 (5.4-19.1)	<0.0001
	Above median	13.8 (6.4-27.2)		6.8 (4.2-11.6)		6.8 (3.8-10.3)	

Glucose	Below median	18.3 (7.6-42.3)	p = 0.0058	8.5 (4.8-16.6)	NS (p = 0.87)	7.5 (4.2-14.7)	NS (p= 0.40)
	Above median	27.2 (11.9-58.1)		8.5 (4.8-16.1)		8.7 (5.0-15.2)	
HbA1c	Below median	22.8 (10.2-51.4)	NS (p=0.30)	8.9 (4.5-17.7)	NS (p =0.86)	8.5 (4.0-16.5)	NS (p=0.97)
	Above median	21.4 (8.2-49.2)		8.1 (5.2-14.2)		8.3 (5.0-13.9)	
Previously medical history							
Myocardial infarction	MI	23.15 (10.2-56.2)	NS (p=0.19)	11.5 (6.0-19.3)	p = 0.0003	10.3 (6.3-16.7)	p=0.0003
	No previous MI	21.2 (9.4-45.1)		7.2 (4.3-13.4)		7.0 (3.9-12.8)	
Heart failure	Heart failure	34.9 (16.7-66.4)	p= .0002	14.1 (9.5-23.5)	<0.0001	13.4 (8.9-25.8)	<0.0001
	No previous heart failure	19.4 (8.2-43.4)		7.3 (4.4-14.6)		7.3 (4.2-14.1)	
Hypertension	Hypertension	21.3 (9.6-43.8)	NS (p=0.66)	8.9 (4.8-16.8)	NS (p = 0.33)	8.5 (4.5-15.4)	NS (p=0.22)
	No previous hypertension	23.3 (9.7-56.4)		8.0 (4.6-15.1)		7.6 (4.5-13.8)	

Table 3

Associations between continuous variables (Spearman rank correlation) at admission, discharge and after three months.

	Admission (n=393)		**Discharge (n=309)**		**Three months (n= 299)**	
	Correlation coefficient	**p**	**Correlation coefficient**	**p**	**Correlation coefficient**	**P**
Age	0.41	<0.0001	0.42	<0.0001	0.35	<0.0001
BMI	-0.13	0.016	-0.029	NS (0.62)	0.11	NS (0.057)
Biochemistry at admission						
Creatinine Clearance	-0.45	<0.0001	-0.41	<0.0001	-0.30	<0.0001
Glucose	0.17	0.0008	0.028	NS (0.63)	0.063	NS (0.29)

HbA1c	-0.095	NS (0.074)	-0.0094	NS (0.88)	0.0096	NS (0.88)
Copeptin at admission	-	-	0.47	<0.0001	0.46	<0.0001
Copeptin at discharge	0.47	<0.0001	-	-	0.68165	<0.0001
Copeptin at three months	0.45	<0.0001	0.68	<0.0001	-	-
IGFBP-1 at admission	0.53	<.0.0001				
IGFBP-1 at discharge			0.31	<.0001		
IGFBP-1 at three months					0.14	0.015

IGFBP-1

The IGFBP-1 levels at admission varied between 3.0 and 677.0 (median 23.0; mean 42.0; interquartile range 12.0-51.0 (μg/l)). The levels at discharge were 3.0 - 308.0 (median 33.0; mean 40.2; interquartile range 18.0-52.0) (μg/l) and after three months 3.0-284.0 (median 36.0; mean 41.3; interquartile range 18.0-56.0(μg/l).

Male patients had lower values of IGFBP-1 compared to females. Patients with age above and renal function below the median had higher levels of IGFBP-1 (data not shown). In general, IGFBP-1 levels did not differ significantly between those with or without previous myocardial infarction, heart failure or hypertension except for slightly higher discharge levels of IGFBP-1 in patients with heart failure and lower IGFBP-1 in those with than without previous hypertension at three months. Patients with glucose levels above the median had significantly higher IGFBP-1 levels at admission and at discharge.

IGFBP-1 levels measured at admission, discharge and after three months correlated in between (Spearman correlation coefficients; admission-discharge 0.45; $p<0.001$, admission-three months 0.45; $p<0.001$, and discharge-three months 0.14; $p= 0.015$.).

Age, blood glucose and HbA1c correlated to IGFBP-1 at all studied time-points. IGFBP-1 levels were inversely correlated with creatinin clearance and BMI at admission, discharge and after three months (data).

Mortality and morbidity

During a median follow-up of 2.45 (1.05-3.00) years, 95 (24.2%) patients died, 77 (19.6%) from cardiovascular causes, while 59 (15.0%) had a non-fatal re-infarction and 25 (6.4%) a non-fatal stroke (6.4%).

In unadjusted analyses, copeptin predicted cardiovascular event, at all occasions: at admission HR 1.59 (95%CI 1.41-1.81; P<0.001), at discharge HR 1.66 (95%CI 1.35-2.04; P<0.001) and after three months HR 2.09 (95%CI 1.57-2.78 P<0.001) as well as non-fatal cardiovascular events and cardiovascular death (Table 4). Increasing levels of IGFBP-1 were predictors of cardiovascular events as well: at admission HR 1.49 (95%CI 1.26-1.77; P<0.001), at discharge 1.52 (1.17-1.97 P=0.0019) and after three months 1.40 (1.00-1.94; P=0.048 (Table 4). In a multiple model including both biomarkers copeptin was a predictor of all events apart from non-fatal reinfarction and stroke at discharge while IGFBP-1 did not remain as an independent predictor of cardiovascular event. In the final multiple model, including age and creatinine clearance, copeptin remained an independent predictor for all events at admission (cardiovascular event HR 1.35 (95%CI 1.16-1.57; P<0.001) and after three months (cardiovascular events HR 1.56 (95%CI 1.15-2.11; P=0.004) but not at discharge (cardiovascular events HR 1.26 (95%CI 0.99-1.59; P=0.057). In figure 3, Kaplan-Meier survival curves plot the impact of tertiles of copeptin levels on cardiovascular events at admission, discharge and after three months.

Table 4

Unadjusted and adjusted predictive ability of copeptin and IGFBP-1 assessed by Cox proportional hazard regression at admission, discharge and after three months-

		Cardiovascular event			**Cardiovascular death**			**Nonfatal reinfarction or stroke**		
		n	HR (95% CI)	p	n	HR (95% CI)	p	n	HR (95% CI)	p
Admission	**Univariable unadjusted** (n = 393)									
	log copeptin	138	1.59 (1.41-1.81)	<0.001	77	1.81 (1.54-2.14)	<0.001	77	1.35 (1.13-1.61)	0.008
	log IGFBP-1	138	1.49 (1.26-1.77)	<0.001	77	1.99 (1.57-2.51)	<0.001	77	1.11 (0.88-1.39)	NS (0.37)
	Multiple model including log copeptin and log IGFBP-1									
	log copeptin	138	1.53 (1.31-1.78)	<0.001	77	1.56 (1.27-1.92)	<0.001	77	1.43 (1.16-1.77)	0.009
	log IGFBP-1	138	1.10 (0.90-1.34)	NS (0.35)	77	1.41 (1.06-1.86)	0.017	77	0.87 (0.67-1.14)	NS (0.32)
	Multiple model adjusted									
	log copeptin (adjusted for age)	138	1.34 (1.22-1.60)	<0.001	77	1.49 (1.24-1.79)	<0.001	77	1.28 (1.06-1.55)	0.011
	log copeptin (adjusted for creatinine clearance)	129	1.37 (1.18-1.59)	<0.001	70	1.40 (1.15-1.72)	0.001	74	1.26 (1.03-1.55)	0.023
	Multiple model adjusted									
	log copeptin	129	1.351	<0.001	70	1.43 (1.16-	<0.001	74	1.26 (1.03-	0.028

	(adjusted for age and creatinine clearance)		(1.161-1.571)			1.76)			1.54)	
Discharge	**Univariable unadjusted**									
	log copeptin	100	1.66 (1.35-2.04)	<0.001	45	1.95 (1.46-2.60)	<0.001	59	1.35 (1.01-1.80)	0.040
	log IGFBP-1	100	1.52 (1.17-1.97)	0.0019	45	1.99 (1.30-3.03)	0.0014	59	1.42 (1.02-1.99)	0.039
	Multiple model including log copeptin and log IGFBP-1									
	log copeptin	100	1.55 (1.24-1.93)	<0.001	45	1.73 (1.26-2.37)	<0.001	59	1.24 (0.92-1.69)	NS (0.16)
	log IGFBP-1	100	1.27 (0.97-1.67)	NS (0.082)	45	1.55 (1.00-2.38)	NS (0.05)	59	1.31 (0.92-1.86)	NS (0.13)
	Multiple model adjusted									
	log copeptin (adjusted for age)	100	1.29 (1.03-1.63)	0.030	45	1.29 (0.92-1.81)	0.14	59	1.16 (0.85-1.59)	NS (0.36)
	log copeptin (adjusted for creatinine clearance)	96	1.34 (1.07-1.69)	0.012	43	1.42 (1.01-1.99)	0.044	58	1.21 (0.88-1.64)	NS (0.24)
	Multiple model adjusted									
	log copeptin (adjusted for age and creatinine clearance)	96	1.26 (0.99-1.59)	NS (0.057)	43	1.28(0.90-1.80)	NS (0.168)	58	1.15 (0.84-1.59)	NS (0.38)
Three months	**Univariable unadjusted**									

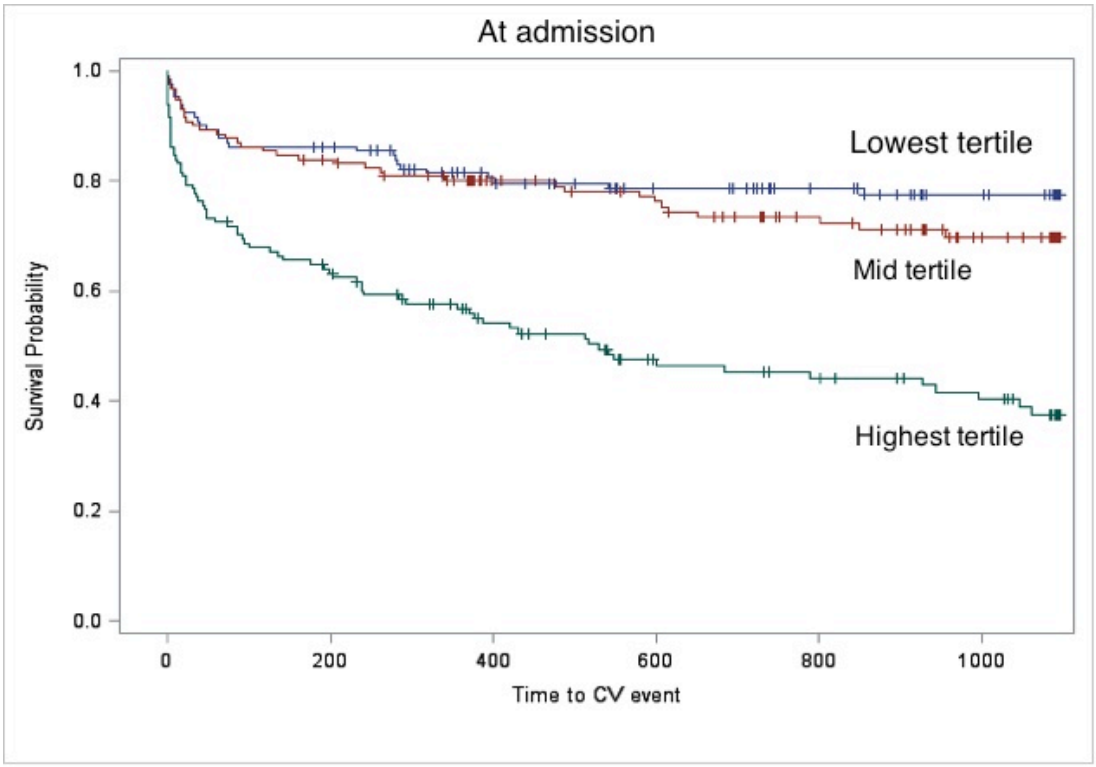
	log copeptin	70	2.09 (1.57-2.78)	<0.001	42	2.14 (1.48-3.10)	<0.001	38	2.03 (1.38-3.00)	<0.001
	log IGFBP-1	70	1.40 (1.00-1.94)	NS (0.05)	42	1.78 (1.13-2.79)	0.013	38	1.23 (0.80-1.89)	NS (0.35)
	Multiple model including log copeptin and log IGFBP-1									
	log copeptin	70	2.01 (1.49-2.73)	<0.001	35	1.85 (1.20-2.83)	0.0049	38	2.04 (1.35-3.08)	<0.001
	log IGFBP-1	70	1.11 (0.80-1.52)	NS (0.54)	35	1.95 (1.15-3.31)	0.013	38	1.00 (0.66-1.52)	NS (0.9)
	Multiple model adjusted									
	log copeptin (adjusted for age)	70	1.65 (1.22-2.22)	0.0012	42	1.54 (1.04-2.27)	0.031	38	1.73 (1.14-2.61)	0.0094
	log copeptin (adjusted for creatinine clearance)	67	1.65 (1.22-2.22)	0.0010	40	1.66 (1.12-2.44)	0.011	37	1.65 (1.10-2.50)	0.016
	Multiple model adjusted									
	log copeptin (adjusted for age and creatinine clearance)	67	1.56 (1.15-2.11)	0.004	40	1.52 (1.02-2.26)	0.040	37	1.61 (1.07-2.43)	0.022

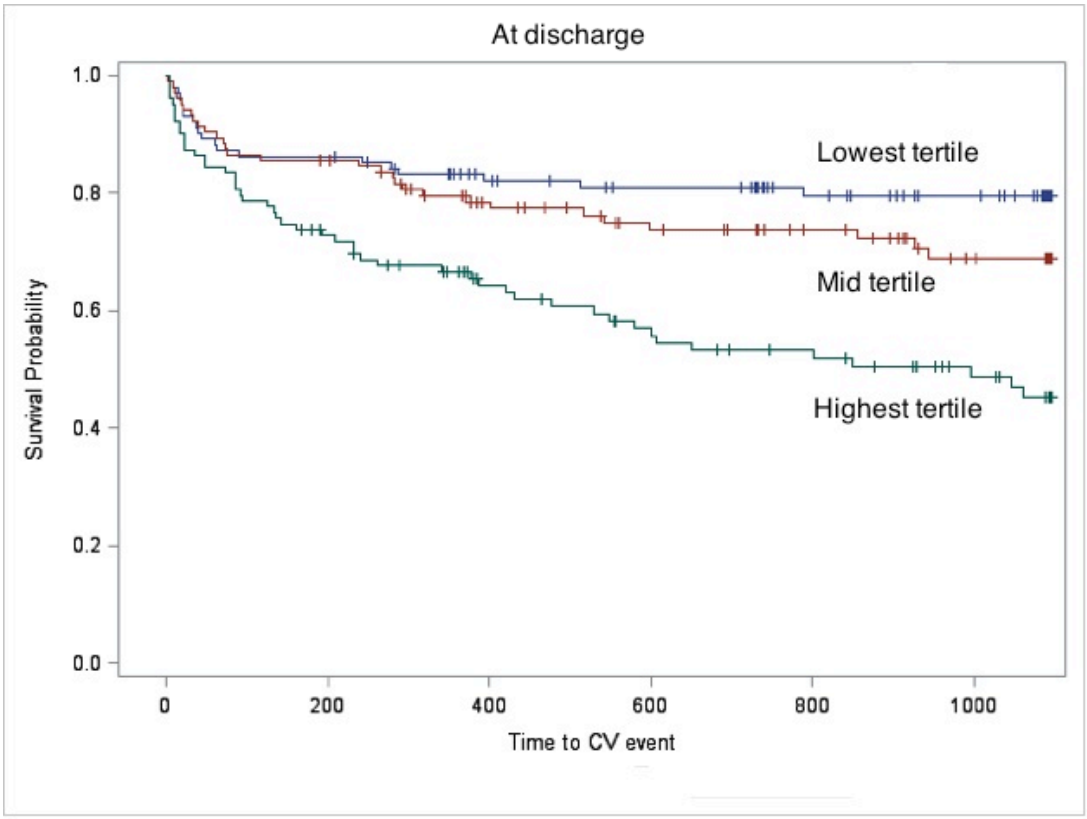
Figure 3 Kaplan-Meier curves for cardiovascular events by copeptin tertiles (min-max; median)

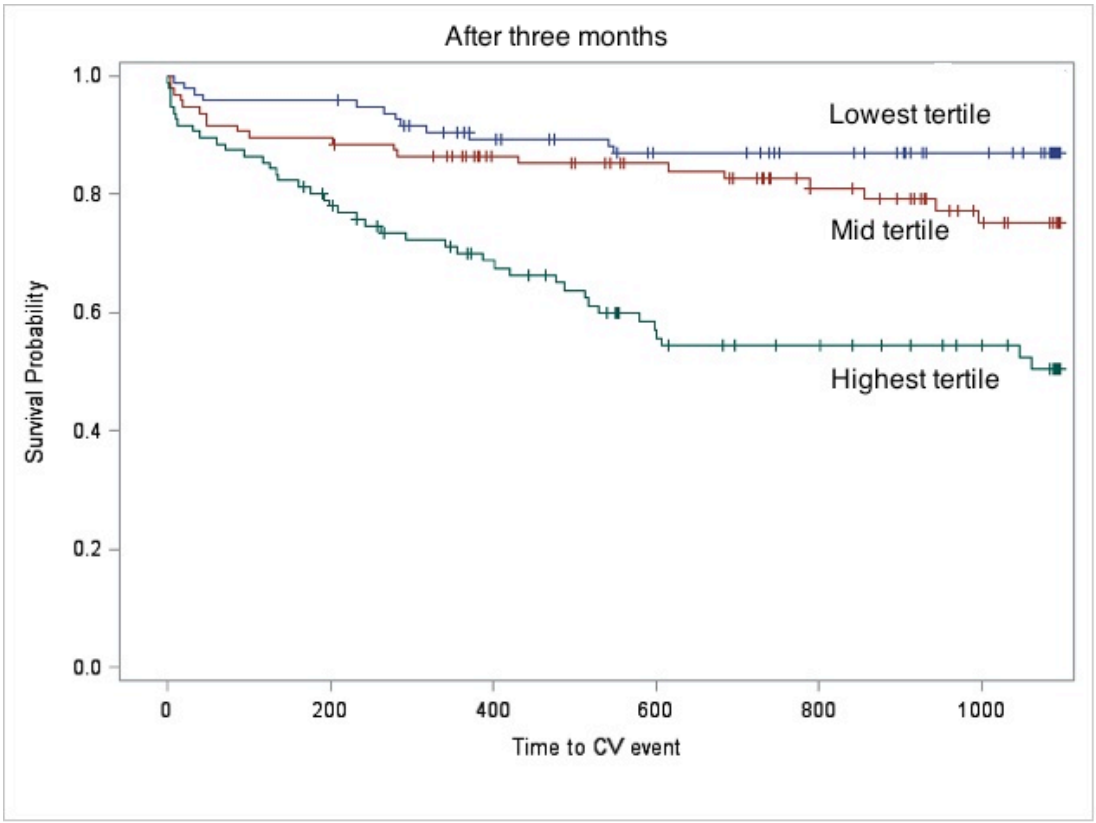
a) At admission Tertile 1: 0.97-12.5;6.3 pmol/l, Tertile 2: 12.7-37.1; 21.8 pmol/l, Tertile 3: 37.8-1936.0 ; 66.8 pmol/l. Log-rank test for trend < 0.0001.

b) At discharge Tertile 1: 0.69-5.62;4.0 pmol/l, Tertile 2: 5.7-13.2; 8.48 pmol/l, Tertile 3: 13.3-286.0 ; 20.1 pmol/l. Log-rank test for trend <0.0001.

c) At three months Tertile 1: 1.14-5.6; 3.72 pmol/l, Tertile 2: 5.62-12.0;8.40 pmol/l, Tertile 3: 12.4-116.0 18.4 pmol/l. Log-rank test for trend < 0.0001.


At admission
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
Time to CV event
Lowest tertile
Mid tertile
Highest tertile


At discharge
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
Time to CV event
Lowest tertile
Mid tertile
Highest tertile



CONCLUSIONS

The present report shows that copeptin levels were high at admission for AMI in patients with T2DM, decreased during hospitalization and remained relatively stable during the following three months. Copeptin levels at admission and three months after an AMI predicted cardiovascular events. Furthermore the previously reported relationship between copeptin and IGFBP-1 levels at hospital admission remained throughout the study period.

In this biochemical substudy of the DIGAMI 2 trial, comprising a well-defined patient cohort with T2DM, the copeptin levels were highest during the initial phase of AMI (median 21.8 pmol/l). Already at hospital discharge copeptin had decreased to a level (median 8.5 pmol/l) that remained stable during the three months of observation (median 8.4 pmol/l).

These levels are clearly elevated compared to those in healthy individuals [18]. For example in a healthy control study group (n=700; median age 61 years) with no cardiovascular diseases or treatment the median copeptin level was 3.8 pmol/l (0.44 to 44.3 pmol/l) [20].

The present results confirm previous findings that copeptin is elevated during hospitalization of an AMI, in particular in the early phase. Charpentier et al. recently showed that the median copeptin levels at admission for ACS was 14.0 pmol/l and with significantly higher median values in patients with NSTEMI (25% DM patients) (22.9 pmol/L) compared to those with unstable angina (11% DM patients) (median 9.1 pmol/l) [31]. This indicates that the levels of copeptin may relate to the extent of the ischemic myocardial damage. In a report by Khan et al. copeptin levels were highest on admission (first day versus second to fifth days $P<0.001$) and reached a plateau at days 3 to 5 [20]. This early rise in copeptin and a high sensitivity of ischemia are reasons why the biomarker is suggested to serve as a complement to troponin in the emergency room for rapid- rule out of AMI in patients with chest pain [17, 32].

Stress-related changes are the most plausible explanations for the rise in copeptin during the acute phase of an AMI. This does, however, not explain why the present cohort have higher copeptin levels than other post-AMI cohorts during the acute phase and higher than healthy controls after three months of follow-up. An explanation may be that, in addition to coronary artery disease, all patients in DIGAMI 2 had T2DM. In addition to be elevated in relation to stress, copeptin levels are higher among patients with than without diabetes. In a report from Enhöring et al. based on The Malmo Diet and Cancer study (MDC), patients with prevalent diabetes at baseline had higher values of copeptin compared to those with normal fasting glucose (6.9 vs 5.1 pmol/L; $p< 0.001$) [23]. Individuals in the top quartile of copeptin had a 2- to 3-fold excess risk of developing T2DM compared with those in the lowest quartile. This implicates that activation of the AVP system is involved in the pathogenesis of T2DM and not just elevated

as a result of stress. Furthermore these findings imply that the AVP system may not just be a promising biomarker for the prediction of T2DM but also a potential treatment target.

Increasing copeptin level during hospitalization for AMI [17, 20, 21] and in patients with stable angina referred for coronary angiography [33] is a negative prognostic predictor. The present study expands previous knowledge by including the findings from the DIGAMI 2 trial analyzing copeptin already at hospital admission [28] and by including following the values in T2DM patients with CAD into a stable condition. In the final Cox Hazard regression model, adjusted for age and creatinine cleareance, copeptin remained a significant predictor for the primary outcome as well as for cardiovascular death and nonfatal reinfarction and stroke, both at admission and after three months (Table 4). The explanation to that copeptin did not have predictive capacity at the time for hosital discharge is unknown. It may be related to different characteristics of the three subcohorts investigated but also a play of chance.

Age, gender and conditions such as congestive heart failure and, impaired renal function may, apart from being predictors of outcome per se, relate to AVP activation in different ways thereby influencing the prognostic impact of copeptin directly or indirectly. These confounders were taken into consideration in the present analyses.

Patient with above the median age had higher copeptin levels than those below (at admission; 34.6 pmol/l vs. 14.7 pmol/l; $p < 0.001$) (Table 2) and age correlated positively with copeptin at all time-points. In contrast to some previous studies the present report does not show any gender differences in copeptin levels. The relation between age and gender on AVP activation may however differ during different circumstances. In the previously mentioned healthy control study group, male individuals had higher median values of copeptin than females 4.3 (range 0.4-44.3) pmol/l versus 3.2 pmol/l (range 1.0-14.8) pmol/l $P<0.0005$ [20].

No correlation was found between copeptin and age. In the same study no significant differences were found between men (median 6.7; range 0.3 to 226 pmol/l) and women (median 7.6;range 0.6 to 441 pmol/l) in copeptin levels for AMI patients) but copeptin correlated with age ($r = 0.328$; $p<0.0005$) .

In the present report, increased copeptin had an inverse relation to renal function expressed as creatinine clearance (Table 3) This supports similar observations by for example by Khan et al [20]. In addition elevated copeptin have been associated with microalbumiuria [34]. One may speculate that this reflect physiological effects, in particular antidiuretic, of AVP and the distribution of V2 receptors in the kidneys [35].

Copeptin was higher in patients with than in those without heart failure (Table 2). AVP is part of the neurohormonal axis that is activated in heart failure and is associated with a poor outcome [35, 36]. Previous heart failure did, however, not remain significant in the final Cox regression analysis in the present study.

The other biomarker of interest in this study, IGFBP-1 predicted the primary endpoint during hospitalization but not after three months. There are several hypotheses to explain the relationship between high levels of IGFBP-1 and poor prognosis in patients with AMI and T2DM. The high attraction of IGFBP-1 to IGF-1 may diminish beneficial effects of IGF-1 such as improved insulin sensitivity, improved glucose uptake and diminished glucose production by the liver. The binding of IGFBP-1 could also interrupt with other favorable mechanisms of IGF-1 as the stabilization of atherosclerotic plaques and its positive effects on endothelial function and arterial blood flow[37].

When both biomarkers were included in a multiple model copeptin was the only remaining predictor of significance (Table 4). An interesting finding is that the correlation between copeptin and IGFBP-1, previously showed at admission [28], remained at discharge and after three months. It may be hypthesized that an IGFBP-1 increase is related to AVP-activation and not only caused by a stressinduced acute hepatic insulin resistance. If this is true it may be of importance for the pathophysiology of coronary artery disease and diabetes. Thus this hypothesis deserves further evaluation in mechanistic studies. If the results are confirmed it may have potential pharmacological implications since AVP receptor antagonists, so called vaptans, may be used to target this relation and hopefully improve prognosis for patients with T2DM and CAD[35].

Limitations

Although the present study reached the aims it had some unavoidable limitations. All patients did not have the biomarkers measured at all occasion resulting in slightly different patient subcohorts at admission, discharge and after three months, however, with fairly similar baseline characteristics. There are several explanations among them that some patients died during follow-up, a few did not show up for the follow up or biomarkers were not analyzed or stored correctly.

Another potential limitation is that this epidemiological study did not take the randomized treatment in the original DIGAMI study into account. Some patients were given insulin while others not. The use of exogenous insulin may have influenced the results, in particular the prognostic impact of IGFBP-1.

In summary elevated copeptin predicts cardiovascular events during and after hospitalization for AMI in patients with T2DM. Furthermore the previously shown relationship between copeptin and IGFBP-1 levels at hospital admission remained throughout three months of observation. The findings are interesting from a pathophysiological point of view, increasing the understanding of the association between T2DM and cardiovascular disease. These findings could be used for risk assessement by simplifying the discovery of high-risk patients but also to identify a potential pharmacological target to improve prognosis for T2DM patients with coronary artery disease.

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