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Insulin-like growth factor binding protein 1 predicts cardiovascular morbidity and mortality in patients with acute myocardial infarction and type 2 diabetes

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Running title: IGFBP-1 and CV risk in AMI patients with T2DM

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ABSTRACT

Objective: There are indications that the IGF system is related to both type 2 diabetes (T2DM) and cardiovascular (CV) disease. We tested the hypothesis that low IGF-I and high IGF binding protein-1 (IGFBP-1) predict future cardiovascular mortality and morbidity in patients with acute myocardial infarction (AMI) and T2DM.

Research design and methods: The DIGAMI 2 trial recruited 1253 patients with T2DM and AMI of which 575, the present material, were enrolled in a biochemical program with repeated blood sampling. Primary and secondary endpoints were adjudicated CV mortality and a composite of CV events (CV death, reinfarction or stroke). Multiple Cox proportional hazard regression was used to study the relationship between endpoints and the variables. Admission variables were used for the survival analysis and for blood glucose and HbA1c updated mean values during follow-up were also available.

Results: During a median follow-up period of 2.2 years, 131 (23%) patients died from all-cause and 102 (18%) from CV disease while 175 patients (30%) suffered from at least one CV event. The independent predictors for CV death in the Cox regression model were (HR, 95% CI): ln updated mean blood glucose (12.2 (5.8 - 25.7), age (+5 years; 1.5, 1.4-1.7), ln IGFBP-1 (1.4, 1.1-1.8) and ln S-Creatinine at admission (2.4, 1.3-4.2). The model predicting CV events contained the same variables (ln IGFBP-1 at admission; 1.2, 1.0-1.4).

Conclusions: High levels of IGFBP-1 at admission are associated with increased risk for CV mortality and morbidity in T2DM patients with AMI.

Patients with type 2 diabetes (T2DM) have an increased risk for cardiovascular disease (CVD) [1]. The prognosis after an acute myocardial infarction (AMI) does indeed deteriorate already at glucose levels in the upper normal range [2,3]. The importance is underlined by the high prevalence of AMI patients with previously undetected glucose abnormalities [4-6]. The increased CV risk can not be completely explained by traditional risk factors and mechanisms amplifying their impact are not fully understood [7]. Thus, the search for novel risk factors linking T2DM and CVD is important [8].

The Insulin like growth factor-I (IGF-I) system has been related to poor glucose control and low levels of IGF-I have been related to future T2DM [9]. In addition, the IGF-I system and especially low IGF-I, low IGF binding protein 1 (IGFBP-1) and high IGFBP-3 relate to increased CV risk [10,11]. Thus it is of interest to explore the IGF-I system as a potential novel risk factor and as a possible link between T2DM and subsequent CV complications [12]. The hepatic production of IGFBP-1 is down-regulated by insulin [13]. Accordingly, in the general population there is a correlation between low levels of IGFBP-1 and hyperinsulinemia and these findings may link to an increased CV risk [14,15]. However, the IGFBP-1 concentrations rise during the development of T2DM despite persisting hyperinsulinemia, indicating increased hepatic insulin resistance during disease progression [16,17]. These observations were supported by a report from van den Berghe et al. showing that patients admitted to the intensive care unit (ICU) with elevated levels of IGFBP-1 presented a poor prognosis regarding mortality, a finding that related to acute hepatic insulin resistance [18]. Furthermore, there is a correlation between high levels of IGFBP-1 and CV mortality in elderly men [19] findings in accordance with trends observed in a recently published study on AMI patients without previously known T2DM [20].

This study tests the hypothesis that low levels of IGF-I and high levels of IGFBP-1 predict future CV mortality and morbidity in patients with AMI and T2DM.

RESEARCH DESIGN AND METHODS

DIGAMI 2, a prospective, randomised trial compared three different management strategies in patients with type 2 diabetes and suspect AMI. An extensive description of the study design has been presented elsewhere [21]. In brief, 1 253 patients were randomized to one of three study arms receiving: a) a 24 hour insulin-glucose infusion followed by subcutaneous insulin-based long-term glucose control (Group 1, n= 474); b) the same initial treatment followed by glucose lowering treatment according to local practice (Group 2, n=473); or c) glucose lowering treatment according to local practice (Group 3, n=306). The objective was to compare total mortality and morbidity between these management strategies. There was no significant difference in the primary endpoint (total mortality) or secondary endpoint (mortality, non-fatal MI or stroke). Increasing mean plasma glucose during follow-up was, however, an independent predictor of fatal outcome, but could not explain all morbidity [21].

Present study population

A total of 575 of the DIGAMI 2 patients participated in a biochemistry program, with repeated blood sampling at admission before initiation of the glucose-insulin infusion, fasting at discharge and after 3 and 12 months. Blood glucose and HbA1c were collected at admission, (blood glucose also after 24 hours), after 3, 6, and thereafter every 6 months during the follow-up. The median study duration was 2.1 (Q1, Q3: 1.0, 3.0) years and no patient was lost to follow-up.

Laboratory analyses

Blood glucose was analyzed locally as whole blood glucose in mmol/L while HbA1c was analysed in a core laboratory (Department of Laboratory Medicine, Malmö Hospital, Sweden) by high-performance liquid chromatography on capillary blood applied on filter paper with an upper normal limit of 5.3% (Boehringer Mannheim Scandinavian AB, Bromma, Sweden). Concentrations of total serum IGF-I were determined by RIA in µg/L after separation of IGFs from IGFBPs by acid ethanol extraction and cryoprecipitation. To minimize interference of remaining IGFBPs, des (1-3) IGF-I was used as radioligand [22]. IGFBP-1 concentrations in serum were determined by RIA according to the method of Póvo et al [23]. The sensitivity of the RIA was 3 µg/l and the intra- and interassays CV were 3 % and 10 %, respectively.

Events during follow up

In this analysis CV mortality and a composite of CV events (CV death, reinfarction or stroke) served as primary and secondary endpoints respectively. An independent adjudication committee composed of experienced cardiologists adjudicated the events. They were provided with death certificates, autopsy reports, hospital and laboratory records and ECG:s but did not have any information on group belongings.

Statistical analyses and calculations

BMI was calculated as weight/height^2 (kg/m^2). Updated mean blood glucose was calculated as a simple mean value of all samples available (a maximum of nine occasions: admission, 24 hours, 3, 6, 12, 18, 24, 30 and 36 months) during the complete period of follow-up until an event occurred. As IGF-I decreases with age, a standardized IGF-I score (IGF-I SD) was also calculated as previously described [24]. Continuous variables are presented as median (quartile 1, quartile 3) and categorical variables as percentages. Differences between groups were compared by the Jonckheere-Terpstra's test or the χ^2-test. Multiple Cox proportional hazard regression was applied to investigate relations between candidate predictors from Table 2 with a *p*-value < 0.2 and the endpoints. All possible combinations of predictors were fitted into a best subset analysis and the models were compared using the Akaike information criterion. To limit the influence of extreme values, all continuous variables were log-transformed prior to analysis. A two-sided *p* value <0.05 was regarded as statistically significant. No adjustment for multiple testing have been performed. All analyses were done using SAS version 9.1.3 (SAS Institute) and Statistica 7.0 (StatSoft Inc.).

Ethical considerations

The study conformed to good clinical practice guidelines and followed the recommendations of the Helsinki Declaration. Written informed consent was obtained from all patients prior to enrolment.

RESULTS

Baseline characteristics

As outlined in Table 1, the present study population from DIGAMI 2 (n=575) was similar to the group where biochemistry was not available (n=678) apart from slightly lower blood glucose and lower creatinine at admission. In brief, 67% of the patients were men, the median age was 69.6 (Q1, Q3: 60.3, 76.7) years, 24% were smokers and the median BMI was 27.6 (Q1, Q3: 25.1, 30.7) kg/m^2. Thirty-three percent of the patients had a a history of previous AMI and the median duration of the T2DM was 5.7 (Q1, Q3: 1.1, 12.8) years.

Baseline characteristics of IGFBP-1 tertiles

Pertinent clinical and biochemical characteristics of the patients divided into tertiles of IGFBP-1 at admission are presented in Table 2. The number of patients in the different tertiles was equally distributed regarding randomized treatment groups. Patients in the highest tertile were older, more frequently females, had lower BMI and lower blood pressure. In addition they presented higher admission levels of blood glucose and creatinine but lower levels of triglycerides and IGF-I compared to those in the lowest tertiles. Furthermore, the patients in the highest IGFBP-1 tertile were less often treated with metformin prior to the study.

Prediction models for cardiovascular mortality and morbidity

During the follow-up period 131 (23%) patients died from all-cause and of these 102 (78%) from CVD. A total of 175 patients (30%) suffered from at least one CV event during the follow-up. There were no significant differences in CV death or the occurrence of CV events between the three randomised treatment groups.

Univariate survival analysis of the IGF-I system showed that ln IGF-I at admission, discharge (day 4-5), 3 or 12 months were not related to CV death (HR_{adm}:0.8, p=0.206; HR_{dis}:0.9, p=0.670; HR_{3M}:0.9, p=0.824; HR_{12M}:1.0, p=0.982) whereas levels of ln IGFBP-1 at admission, discharge, 3 months or 12 months were related to CV death (HR_{adm}:1.9, p<0.001; HR_{dis}: 1.8, p=0.002; HR_{3M} :1.5, p=0.059; HR_{12M}: 2.8, p=0.004) .

Analyses of the secondary endpoint showed that ln IGF-I at admission and 3 months were significantly related to future CV events but the discharge and 12 months levels were not (HR_{adm}: 0.7, p=0.026; HR_{dis}: 0.8, p=0.190; HR_{3M}: 0.5, p=0.012; HR_{12M}: 0.8, p=0.740). Ln IGFBP-1 was significantly related to CV events at all occasions (HR_{adm}: 1.5, p<0.001; HR_{dis}: 1.5, p=0.002; HR_{3M}: 1.5, p=0.012; HR_{12M}: 1.7, p=0.035).

In the best subset analysis of predictors at admission (Table 3) ln IGFBP-1 at admission remained significantly related to CV death (HR: 1.5, p<0.001) and to CV event (HR: 1.2, p=0.028). Age and ln creatinine at admission also remained significant. The predictive power of the models increased when updated mean blood glucose was entered into the models as an explanatory variable and ln updated mean blood glucose was correlated to both CV death and CV event (HR:12.2, p<0.001 and HR:10.2, p<0.001). Candidate predictors with p-values < 0.2, not included in the final models were admission blood glucose, IGF-I, BMI, gender, smoking status and previous coronary disease history.

Survival curves for tertiles of IGFBP-1 adjusted for updated mean blood glucose, age and creatinine are presented in Figure 1 A-B.

CONCLUSIONS

The main finding from the present study is that high levels of IGFBP-1 at admission, discharge, after 3 and 12 months are all related to subsequent CV morbidity and mortality in patients with AMI and T2DM. Many fatal and other events occurred relatively soon after hospital discharge, some already during hospitalization. From a clinical point of view this makes IGFBP-1 at admission an interesting prognostic predictor, which also remained after multiple adjustment.

IGFBP-1 has been described as the most likely acute regulator of IGF-I actions [25] and the hepatic production of IGFBP-1 is upregulated in response to pro-inflammatory cytokines [26] and physiological stresses [27] and downregulated by insulin [13]. There are several potential links between high levels of IGFBP-1 and increased CV risk. One is that the high affinity-binding of IGFBP-1 to IGF-I, that prevents the activation of receptor signalling, may attenuate known beneficial effects of IGF-I such as enhanced glucose uptake, improved insulin sensitivity and decreased hepatic glucose production [28]. Other mechanisms of IGF-I that could be disrupted by inhibition of IGFBP-1 are stabilization of atherosclerotic plaques [29] and beneficial effects on arterial blood flow and endothelial function [30]. However, IGF-I was not significantly related to the outcome after multivariate analysis. The univariate relationships between IGF-I and CV events at admission and three months could possibly indicate a correlation to age or the glucometabolic state since age and the updated mean value of blood glucose were by far, the strongest predictors in the multiple analyses.

Although several reports from cohort studies have presented relationships between low IGF-I and CVD it is only the Dan-MONICA study, based on a nationwide population registry in Denmark, that reported a significant relation between coronary heart disease events and IGF-I measured before the event. It was, however, only the combination of a low IGF-I and a high IGFBP-3 that predicted future events [11]. We recently investigated patients with AMI with newly detected abnormal glucose tolerance. They had low levels of IGF-I and high levels of IGFBP-3. The combination did, however, not relate to a worse prognosis [20]. In addition, although most studies measured total IGF-I and demonstrated relationships to T2DM [9] and to CVD [11] some studies indicated that the free fraction of IGF-I was the most valuable measure, which also related to an increased CV risk [31]. Free IGF-I was not measured in the present study due to the lack of a reliable commercial method. Anyhow, there is a strong inverse correlation between free IGF-I and IGFBP-1 [32]. Low levels of free IGF-I would therefore be expected in patients with high levels of IGFBP-1.

IGFBP-1 is a marker for hepatic insulin resistance [33]. This can be explained by the fact that when hepatic insulin resistance increases the hepatic suppression of IGFBP-1 decreases causing an increase in the IGFBP-1 concentrations. Critically ill patients with elevated levels of IGFBP-1 are unresponsive to administered insulin and have a worse long-term prognosis than patients with low levels of IGFBP-1 [34]. Furthermore, patients with severe hepatic cirrhosis have demonstrated high fasting levels of IGFBP-1 accompanied by elevated insulin levels. Interestingly, these patients had a less pronounced insulin-mediated suppression of IGFBP-1 during an oral glucose tolerance test (OGTT) [35]. A similar pattern has been described in patients with AMI and abnormal glucose tolerance among whom the suppression of IGFBP-1 was significantly decreased during an OGTT, a finding that has been interpreted as increased hepatic insulin resistance [20].

Patients with high levels of IGFBP-1 were less well gluco-metabolically controlled as indicated by their higher admission blood glucose and lower levels of IGF-I. In addition, these patients had lower BMI, blood pressure and triglycerides, correlations already reported on but interpreted as an inverse correlation between IGFBP-1 and cardiovascular risk factors [15].

An adaptation correlation to physiological stress is probably part of the explanation for the present findings. After a 30-min infusion of epinephrine in healthy men, levels of IGFBP-1 increased but returned to basal approximately 2 hours [36] It is unlikely that the present results may be completely explained by acute stress during the early phase of an AMI, since morbidity and mortality continued to relate to the levels of IGFBP-1 during follow-up.

The link between IGFBP-1 and pro-inflammatory cytokines has recently been demonstrated [26] and since both T2DM and CVD are conditions with increased inflammatory activity, this correlation must be considered as inevitable. We have not analyzed inflammation factors and could therefore not adjust for this in the analyses. However in a recently published study from our group, IGFBP-1 in the acute phase of an AMI did not correlate to C-reactive protein [20] and as mentioned previously, the risk continuous to be higher during follow-up, thus the acute inflammatory response from the AMI cannot explain the present findings.

Elevated levels of IGFBP-1 may theoretically be a result of chronic insulin deficiency. However, it is likely to assume that the patients suffer from various stages of insulin resistance and one third of the patients were treated with insulin prior to the study. Thus, any correlations between insulin levels and IGFBP-1 in the present population would be very difficult to interpret.

In conclusion, this study shows that high levels of IGFBP-1 in AMI-patients with T2DM are related to a substantially increased cardiovascular mortality and morbidity. We suggest that this may be due to hepatic insulin resistance and poor metabolic control and IGFBP-1 could be a novel marker for discovering high-risk patients in this particular group.

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Table 1. Clinical and biochemical characteristics of the DIGAMI 2 patients divided into availability of biochemistry or not. All biochemistry refers to admission values if not stated otherwise.

Variable	**Biochemistry available**	**Biochemistry Not available**
Number of patients	575	678
Age [years; median (Q1, Q3)]	69.6 (60.3-76.7)	69.7 (61.2, 77.0)
Male gender [n (%)]	385 (67)	451 (67)
BMI [kg/m2; median (Q1, Q3)]	27.6 (25.1, 30.7)	27.7 (25.3, 31.1)
Diabetes duration [yrs; med (Q1, Q3)]	5.3 (0.6, 12.3)	6.1 (1.1, 11.9)
Blood pressure [mm Hg; (Q1, Q3)]		
Systolic	130 (116, 150)	130 (120, 150)
Diastolic	73 (62, 84)	80 (70, 88)
Previous medical history [n (%)]		
Myocardial infarction	192 (33)	230 (34)
Angina pectoris	266 (46)	296 (44)
Heart failure	103 (18)	117 (17)
Hypertension	289 (50)	318 (47)
Hyperlipidemia	180 (31)	215 (32)
Current smoker [n (%)]	139 (24)	160 (24)
Medication prior to admission [n (%)]		
Insulin	182 (32)	208 (31)
Metformin	141 (25)	167 (25)
Glibenclamide	143 (25)	131 (19)
Beta-blockers	208 (36)	248 (37)
Aspirin 75 mg	159 (28)	170 (25)
ACE-inhibitor	178 (31)	211 (31)
Lipid Lowering	160 (28)	188 (28)
Randomized treatment group [n (%)]		
Group 1	211 (37)	263 (39)
Group 2	223 (39)	250 (37)
Group 3	141 (25)	164 (24)
Biochemistry at admission [median (Q1, Q3)]		
Blood-glucose (mmol/L)	11.9 (9.4, 15.1)	12.6 (10.0, 15.3)
HbA1c (%)	7.0 (6.1, 8.4)	7.0 (6.1, 8.3)
S-Creatinine (mmol/L)	91 (79, 112)	98 (83, 118)
S-Cholesterol (mmol/L)	5.0 (4.3, 5.9)	5.2 (4.3, 5.9)
S-Triglycerides (mmol/L)	1.7 (1.2, 2.6)	1.7 (1.2, 2,6)

Table 2. Clinical and biochemical characteristics of patients divided into tertiles of IGFBP-1 at admission. All biochemistry refers to admission values if not stated otherwise.

Variable	**IGFBP-1 tertiles**			*p*
	3.0 – 24.0 μ g/L	**25.0 – 42.0 μg/L**	**43.0 – 677 μg/L**	
Number of patients	165	169	167	
Age [years; median (Q1, Q3)]	65.3 (57.3, 73.3)	68.8 (60.5, 75.6)	73.0 (65.9, 79.6)	<0.001
Male gender [n (%)]	121 (73.3)	123 (72.8)	101 (60.5)	0.017
BMI [kg/m2; median (Q1, Q3)]	29.0 (26.7, 32.2)	27.5 (25.4, 30.2)	26.5 (23.7, 29.4)	<0.001
Diabetes duration [yrs; med (Q1, Q3)]	5.2 (1.2, 12.4)	5.4 (0.7, 12,5)	5.1 (0.4, 13.5)	N.S
Blood pressure [mm Hg; (Q1, Q3)]				
Systolic	134 (120, 150)	130 (116, 145)	125 (112, 145)	0.002
Diastolic	76 (67, 85)	72 (65, 82)	70 (60, 80)	<0.001
Current smoker [n (%)]	36 (21.8)	48 (28.4)	34 (20.4)	N.S
Medication prior to admission [n (%)]				
Insulin	53 (32.1)	54 (32.0)	53 (31.7)	N.S
Metformin	54 (32.7)	45 (26.6)	24 (14.4)	<0.001
Glibenclamide	46 (27.9)	41 (24.3)	44 (26.4)	N.S
Beta-blockers	63 (38.4)	69 (40.8)	54 (32.3)	N.S
Aspirin 75 mg	50 (30.3)	52 (30.8)	38 (23.4)	N.S
ACE-inhibitor	51 (30.9)	53 (31.4)	45 (27.0)	N.S
Lipid Lowering	53 (32.1)	48 (28.4)	41 (24.6)	N.S
Randomized treatment group [n (%)]				
Group 1	62 (37.6)	54 (32.0)	66 (39.5)	N.S
Group 2	65 (39.4)	70 (41.4)	59 (35.3)	N.S
Group 3	38 (23.0)	45 (26.6)	42 (25.2)	N.S
Biochemistry admission [median (Q1, Q3)]				
Blood-glucose (mmol/L)	11.4 (9.0, 14.0)	11.8 (9.3, 14.6)	12.8 (10.0, 16.8)	<0.001
HbA1c (%)	6.8 (6.1, 8.1)	7.0 (6.1, 8.3)	7.2 (6.0, 8.4)	N.S
S-Creatinine (mmol/L)	84 (75, 97)	94 (81, 109)	100 (84, 130)	<0.001
S-Cholesterol (mmol/L)	5.1 (4.2, 5.8)	5.0 (4.3, 6.0)	5.0 (4.3, 6.0)	N.S
S-Triglycerides (mmol/L)	2.1 (1.4, 3.0)	1.7 (1.1, 2.5)	1.6 (1.1, 2.3)	<0.001
S-IGF-I (μg/L)	141 (112, 169)	124 (93, 154)	106 (74, 131)	<0.001
S-IGF-I SD	0.0 (-1.1, 0.9)	-0,4 (-1.6, 0.5)	-1.0 (-2.1, 0.08)	<0.001

N.S = Not significant. *P* values are based on Jonckheere-Terpstra's test or χ-square test.

Table 3. Multiple prediction models for cardiovascular mortality and morbidity. All hazard ratios for biochemistry refer to the natural logarithm (ln) of the variable.

Variable	Chi^2	HR	95 % CI	*p*
CV death				
Model 1				
Age (+ 5 yrs)	44.5	1.6	1.4 – 1.8	<0.001
IGFBP-1 baseline (+ ln)	13.6	1.5	1.2 – 1.9	<0.001
Creatinine baseline (+ ln)	6.3	2.0	1.2 – 3.6	0.012
Model 2				
Age (+ 5 yrs)	44.5	1.5	1.4 – 1.7	<0.001
Updated mean blood glucose (+ ln)	43.9	12.2	5.8 – 25.7	<0.001
IGFBP-1 baseline (+ ln)	8.9	1.4	1.1 – 1.8	0.003
Creatinine baseline (+ ln)	8.4	2.4	1.3 – 4.2	0.004
CV event (cv death, reinfarction, stroke)				
Model 1				
Age (+ 5 yrs)	39.3	1.3	1.2 – 1.5	<0.001
Creatinine baseline (+ ln)	10.6	2.0	1.3 – 3.1	0.001
IGFBP-1 baseline (+ ln)	4.9	1.2	1.0 – 1.4	0.028
Model 2				
Updated mean blood glucose (+ ln)	60.6	10.2	5.7 – 18.2	<0.001
Age (+ 5 yrs)	39.9	1.3	1.2 – 1.4	<0.001
Creatinine baseline (+ ln)	16.2	2.4	1.6– 3.7	<0.001
IGFBP-1 baseline (+ ln)	2.8	1.2	1.0 – 1.4	0.096

Candidate predictor with *p*-values < 0.2 that were eliminated in the best subset analyses were admission blood glucose, HbA1c, IGF-I, S-cholesterol, S-triglycerides, BMI, gender, blood pressure, smoking status and previous coronary disease history.

Figure 1. Kaplan-Meier curves of tertiles of IGFBP-1 during follow-up related to A) Cardiovascular death and B) Cardiovascular event (CV death, reinfarction or stroke). All curves are adjusted for updated mean blood glucose, age and creatinine.