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Growth Hormone for risk stratification and effects of therapy in acute myocardial infarction

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Dr. Bergmann holds ownership in Sphingotec AG which provided the GH assay, and is a member of the board of directors of Sphingotec GmbH.

Dr. Struck is employed by Sphingotec GmbH.

Dr. Ng has submitted patents on cardiovascular biomarkers on behalf of the University of Leicester.

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STRUCTURED ABSTRACT

Context

Excess growth hormone is associated with early mortality. In healthy populations, high GH levels was associated with increased mortality.

Objectives

We assessed the association of Growth Hormone (GH) with prognosis after acute myocardial infarction(AMI), and the effects of secondary prevention therapies on outcome stratified by GH levels.

Methods

GH was measured using a high-sensitivity assay in 953 (687 male, mean age 66.1 ± 12.8 years) AMI patients. The primary outcome was major adverse events (MACE, a composite of death, re-AMI, heart failure (HF) hospitalization).

Results

During 2 years follow-up, there were 281 major adverse event endpoints. Patients with MACE had higher levels of GH (median [range], 0.91 [0.04-26.28] µg/L) compared to event free survivors (0.59 [0.02-21.6], $p<0.0005$ using the Mann -Whitney test). In multivariate Cox survival analysis correcting for clinical variables, GH was a significant predictor of MACE (hazard ratio (95% confidence interval) 1.43(1.05-1.95),$p=0.026$ and 1.49 (1.10-2.02),$p=0.01$ respectively) with significant interactions with beta blocker therapy ($p=0.047$) and ACE/ARB therapy ($p=0.016$). Prescription of beta blocker therapy and ACE/ARB was most effective at reducing MACE in those patients in the top GH tertile ($p<0.0005$).

Conclusions

GH levels post-AMI are prognostic for MACE and may indicate those patients who benefit from beta blocker and ACE/ARB therapy.

ABBREVIATIONS AND ACRONYMS

AMI = Acute Myocardial Infarction

ACE/ARB = Angiotensin converting enzyme inhibitor or angiotensin receptor 1 blocker

eGFR = estimated glomerular filtration rate

MACE = Major Adverse Cardiac Events

Re-AMI = Recurrent Myocardial Infarction

GH = Growth hormone

HF = Heart Failure

hs-GH = high sensitivity growth hormone

NSTEMI = Non–ST-segment Elevation Myocardial Infarction

STEMI = ST-segment Elevation Myocardial Infarction

IHD = Ischemic heart disease

Introduction

Growth hormone (GH) is secreted from the anterior pituitary gland, in a pulsatile fashion (1), and has multiple physiological effects in addition to its anabolic effect on tissues. These include adipokinetic, diabetogenic and cardiovascular effects that include stimulation of left ventricular hypertrophy and elevation of blood pressure. In those patients with GH excess (acromegaly), premature death ensues from a cardiomyopathy with ventricular hypertrophy, arrhythmias, hypertension and heart failure (2). Indeed, administration of GH in acute settings (eg in intensive care units) is associated with an increased mortality (3).

Recently, there has been interest in the association of GH levels with outcomes in healthy populations. A prospective study of French policemen with long term follow-up demonstrated increased total and also cardiovascular mortality in those with elevated fasting GH levels (4). More recently, in a substudy of the Malmo Diet and Cancer study, Hallengren et al (5) demonstrated in a healthy population that higher fasting levels of GH were associated with increased total and cardiovascular mortality, as well as cardiovascular morbidity (incidence of ischemic heart disease (IHD), heart failure (HF), stroke).

These observations were documented despite the pulsatile nature of GH release. Moreover, previous assays of GH were relatively insensitive, although recently, a newly introduced high sensitivity assay (hs-GH) was able to quantify even the low normal range of GH levels (6) and enabled a more accurate assessment of the prognostic value of GH levels in the normal population (5).

It is also known that GH release is stimulated by hypoglycaemia, amino acid infusions, onset of slow wave sleep and also acute stress (1). There have been no studies on the relationship of GH with outcome in acute disease, such as myocardial infarction which constitutes a

stressful presentation that may affect GH secretion. In the present study, we sought to investigate whether GH, as determined by a high sensitivity assay, was associated with major adverse events after myocardial infarction. We were also interested to investigate whether the benefit of secondary prevention therapies prescribed after myocardial infarction varies with levels of GH.

Methods

Study Population. We studied 953 STEMI and NSTEMI patients admitted to University Hospitals of Leicester NHS trust between August 2004 and April 2007. This observational cohort study complied with the Declaration of Helsinki, was approved by the local ethics committee and all patients provided written informed consent. All patients with a diagnosis of acute myocardial infarction (AMI) had a cardiac troponin I level above the 99th centile with at least one of the following:- chest pain lasting >20 minutes or diagnostic serial electrocardiographic changes consisting of new pathological Q waves or ST-segment and T-wave changes (7). Patients with known malignancy, renal replacement therapy or surgery in the previous month were excluded. Estimated glomerular filtration rate (eGFR) was calculated from the simplified Modification of Diet in Renal Disease formula (8). All patients received standard medical treatment and revascularisation at the discretion of the attending physician. Medication on discharge from hospital was noted (aspirin, statin, beta blocker, angiotensin converting enzyme inhibitor or angiotensin receptor blocker (ACE/ARB), loop diuretics).

Plasma samples. Blood samples (anticoagulated with EDTA and aprotinin) were obtained immediately after diagnosis and within 36 h of symptom onset. Plasma was stored at -80°C until assayed in a single batch for blinded determination of plasma hs-GH.

Echocardiography. Transthoracic echocardiography was performed in 738 (77.4%) patients during the index admission, using either a Sonos 5500 or IE 33 instrument (Philips Medical Systems, Reigate, UK). A 16-segment left ventricular wall motion index (LVWMI) score was performed based on the American Society of Echocardiography method(9). In suitable patients left ventricular ejection fraction (LVEF) was calculated using the biplane method of discs formula. LV systolic dysfunction (LVSD) was defined as either an LVEF<40% or a LVWMI >1.8.

Biomarker assays. Troponin I was measured using the Centaur cTnI Ultra immunoassay (Siemens Healthcare Diagnostics), which has a CV (coefficient of variation) of 10% at 0.03 µg/L with a 99th percentile of 0.04 µg/L. Measurement of GH levels was performed with a high sensitivity 2-site chemiluminescence sandwich immunoassay similar to one previously described (6), using mouse monoclonal antibodies raised against human GH. The capture antibody (1.5 µg antibody/0.3 ml 100 mmol/l NaCl, 50 mmol/l Tris/HCl, pH 7.8,) was coated onto polystyrene tubes (Greiner Bio-One International AG, Austria) for 18 hours. Tubes were then blocked using 5 % bovine serum albumin. 50 µl of sample (or calibrator standards of human GH) were pipetted into antibody coated tubes, together with 200 µl methyl-acridinium ester labeled antibody and incubated at 22°C for 2 hours. Unbound tracer antibody was removed by washes (20 mmol/l PBS, pH 7.4, 0.1 % Triton X 100) and chemiluminescence determined on an AutoLumat LB 953 (Berthold Technologies GmbH).

The analytical assay sensitivity was 2ng/L GH and the functional assay sensitivity (<20% inter assay CV) was 10 ng/L.

End points. The primary composite endpoint was major adverse cardiac events (MACE) which included all-cause mortality, heart failure (HF) hospitalization or recurrent AMI (re-AMI), within 2 years of the index event. Hospitalization for HF was defined as a hospital readmission for which HF was the primary reason requiring treatment with high dose diuretics, inotropes or intravenous nitrate. Recurrent AMI was diagnosed using the universal definition (7). Endpoints were obtained by reviewing the local hospital databases and patients' records, the Office of National Statistics Registry and phone calls to patients.

Statistical analysis. Statistical analyses were performed on SPSS Version 20 (SPSS Inc, Chicago, Illinois). Biomarker levels were $\log_{10}$ transformed and hazard ratios for these were standardised to 1 SD increment of the $\log_{10}$ transformed biomarker. Non-parametric tests were employed for data analysis (Chi-squared, Mann-Whitney and Kruskal Wallis tests and Spearman (r_s) correlations). Cox survival analysis was used to assess the prognostic value of variables and biomarkers. Multivariate models were constructed using clinical variables, prescribed treatments, biomarkers (log troponin I and log hs-GH), including an interaction term between hs-GH and each treatment, in order to assess whether treatment responses differed according to GH levels. Kaplan-Meier survival analysis was used to visualise the treatment effects according to GH tertiles.

Results

Patient Characteristics

Following AMI, GH secretion was higher in females (median [range] 1.06 [0.03-26.28] μg/L) compared to males (0.58 [0.02-19.64] μg/L, p<0.0005). The characteristics of the study population are shown in Table 1, according to GH tertiles. Patients with higher GH levels were older, more often female, had higher levels of glucose and troponin, lower levels of eGFR. They also showed more signs of heart failure (Killip class greater than 1). However, there were no significant differences in prevalence of past histories of IHD, HF, diabetes or hypertension, and presence or absence of ST elevation on presenting ECGs. Patients with higher GH levels were less likely to receive aspirin, statins, beta blockers, ACE/ARB and more likely to receive diuretics on discharge (Table 1). Revascularisation rates were similar across GH tertiles.

Correlation analysis

Spearman correlation analysis (r_s) showed GH was significantly correlated to age (0.236), eGFR (-0.198) and troponin (0.161) (p value for all <0.0005), and weakly with wall motion score index (0.105, p<0.004) and peak creatine kinase (0.123, p<0.003). In multivariate analysis, age, troponin (p value for both <0.0005) and sex (p<0.001) remained independent predictors of GH.

Survival analysis

During follow-up over 2 years, there were 281 MACE, the primary composite endpoint (comprising 117 deaths, 71 HF hospitalisations and 93 re-AMIs). Patients with MACE had

higher levels of GH on presentation (median [range], 0.91 [0.04-26.28] μg/L) compared to event free survivors (0.59 [0.02-21.6], p<0.0005 using the Mann -Whitney test). Table 1 also illustrates the higher prevalence of MACE in patients with higher GH levels.

Table 2 reports the univariate hazard ratios of various factors, therapies and biomarkers that affected the outcome of MACE at 2 years. In multivariate analysis, individual therapies and their interaction with GH levels were examined. In all models age, Killip class>1, and eGFR were retained as independent predictors, together with GH levels. Beta blocker therapy was associated with lower MACE (p=0.03) and showed a statistically significant interaction with GH levels (p=0.047, Table 2). Therapy with ACE/ARB also showed interaction with GH levels (p=0.016, Table 2).

In analyses of interactions of aspirin, statins or diuretics with GH levels, no significant evidence of an interaction with GH levels was found (data not shown).

Kaplan-Meier survival analysis was used to visualise the interactions of therapies with beta blocker or ACE/ARB according to GH tertiles (Figure 1). For beta blocker therapy, MACE rates were lower for those prescribed compared to those who were not prescribed this treatment in the 2nd (p=0.009) and 3rd (highest) GH tertiles (p<0.0005). Similarly for ACE/ARB treatment, MACE rates were lower for those prescribed compared to those who were not prescribed this treatment in the 2nd (p=0.001) and 3rd GH tertiles (p<0.0005). In contrast, for both treatments, there was no difference in MACE rates between those prescribed or not prescribed these treatments in those patients in the lowest GH tertile.

Discussion

Previous studies in healthy populations have demonstrated a link between GH levels and total and cardiovascular mortality and morbidity (4,5). In the present study, we complement these findings by providing evidence of a link between higher GH levels and MACE following an acute cardiovascular presentation, namely myocardial infarction. GH secretion in such acute situations may represent an acute response to a stressful stimulus. However, there are no easily available high throughput methods for measuring the level of stress experienced by such patients presenting with acute illness. The GH secretion was higher in females following AMI, which resembles the findings using fasting GH levels in healthy subjects within the general population(5,6). We also demonstrated a weak correlation to troponin levels, which suggests some association with infarct size.

A number of secondary prevention therapies are now routinely prescribed following AMI, namely aspirin, statins, beta blockers and ACE/ARB based on evidence derived from large double blind therapeutic trials. In this study, we examined whether outcomes in patients prescribed or not prescribed these therapies differed according to the risk of poor outcome, as determined by the GH level. For some secondary prevention therapies eg beta blockers and ACE/ARB, there was evidence of a significant interaction between GH level and the treatment, suggesting that patients with the lowest GH levels may derive less benefit from these therapies compared to those with higher GH levels. Both of these treatments may have the most impact on patients with impairment of left ventricular function or adverse ventricular remodeling. In contrast, we found no significant interactions of GH levels with therapy using aspirin, statins or loop diuretics. Our findings are hypothesis generating for investigating the role of GH on stratifying risk post-AMI, and assessing the effects of

ACE/ARB or beta blockers on left ventricular function/remodeling. It also remains to be investigated whether these findings apply to healthy populations.

Limitations

Our findings are observational, and based on patients recruited in a single centre, with 2 admitting hospitals, and should be verified in other larger populations. The rate of early revascularisation in our NSTEMI population was low compared to more contemporary invasive approaches of revascularisation within 72 h of presentation. Prescription of the secondary prevention therapies was at the discretion of the prescribing physician, and was not randomised, so that unmeasured factors could have influenced the prescription rates and the adverse outcomes.

Conclusions

Following AMI, GH levels may provide independent prognostic information for poor outcomes, and could indicate the groups of patients who derive the most benefit from some secondary prevention therapies such as ACE/ARB and beta blockers. The potential of GH in stratified medicine should be examined in further larger randomised studies.

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Figure Legends

Figure 1: Kaplan-Meier Plots for the endpoint of MACE, according to GH tertiles. Event free survival for patients prescribed or not prescribed therapies on discharge are plotted. The upper panel refers to beta blocker therapy, and the lower panel to ACE/ARB therapy.

Table 1: Characteristics of the 953 AMI patients according to hs-GH tertiles on admission. Numerical data are presented as n (%). P values are quoted for the Kruskal Wallis or Chi squared tests for continuous or categorical variables respectively. Numbers (%) or Mean± SD are reported.

		hs-GH tertiles			
	All	1 <0.33 µg/L	2 0.33-1.37 µg/L	3 >1.37 µg/L	P Value
	n=953	n=315	n=323	n=315	
hs-GH µg/L	1.70 ± 2.85	0.14 ± 0.08	0.75 ± 0.31	4.24 ± 3.85	<0.0005
Demographics					
Age (years)	66.1 ± 12.8	61.9 ± 11.9	67.3 ± 12.6	69.0 ± 12.9	<0.0005
Male (%)	687 (72)	262 (83)	221 (68)	204 (65)	<0.0005
ST elevation AMI	459 (48)	139 (44)	154 (48)	166 (53)	NS
Previous History					
IHD	320 (34)	103 (33)	117 (36)	100 (32)	NS
Heart Failure	37 (4)	9 (3)	12 (4)	16 (5)	NS
Hypertension	493 (52)	149 (47)	166 (52)	178 (57)	NS
Diabetes Mellitus	227 (24)	74 (23)	76 (24)	77 (24)	NS
Killip Class>1	390 (41)	112 (36)	138 (43)	140 (45)	<0.05
Glucose (mmol/L)	8.8 ± 4.2	8.3 ± 3.4	8.8 ± 3.8	9.5 ± 4.9	<0.021
Troponin I (µg/L)	12.5 ± 24.6	9.2 ± 20.3	14.0 ± 25.6	14.4 ± 27.2	<0.0005
eGFR (ml/min/1.73m^2)	66.2 ± 19.9	71.1 ± 16.9	64.1 ± 19.0	63.5 ± 22.4	<0.0005

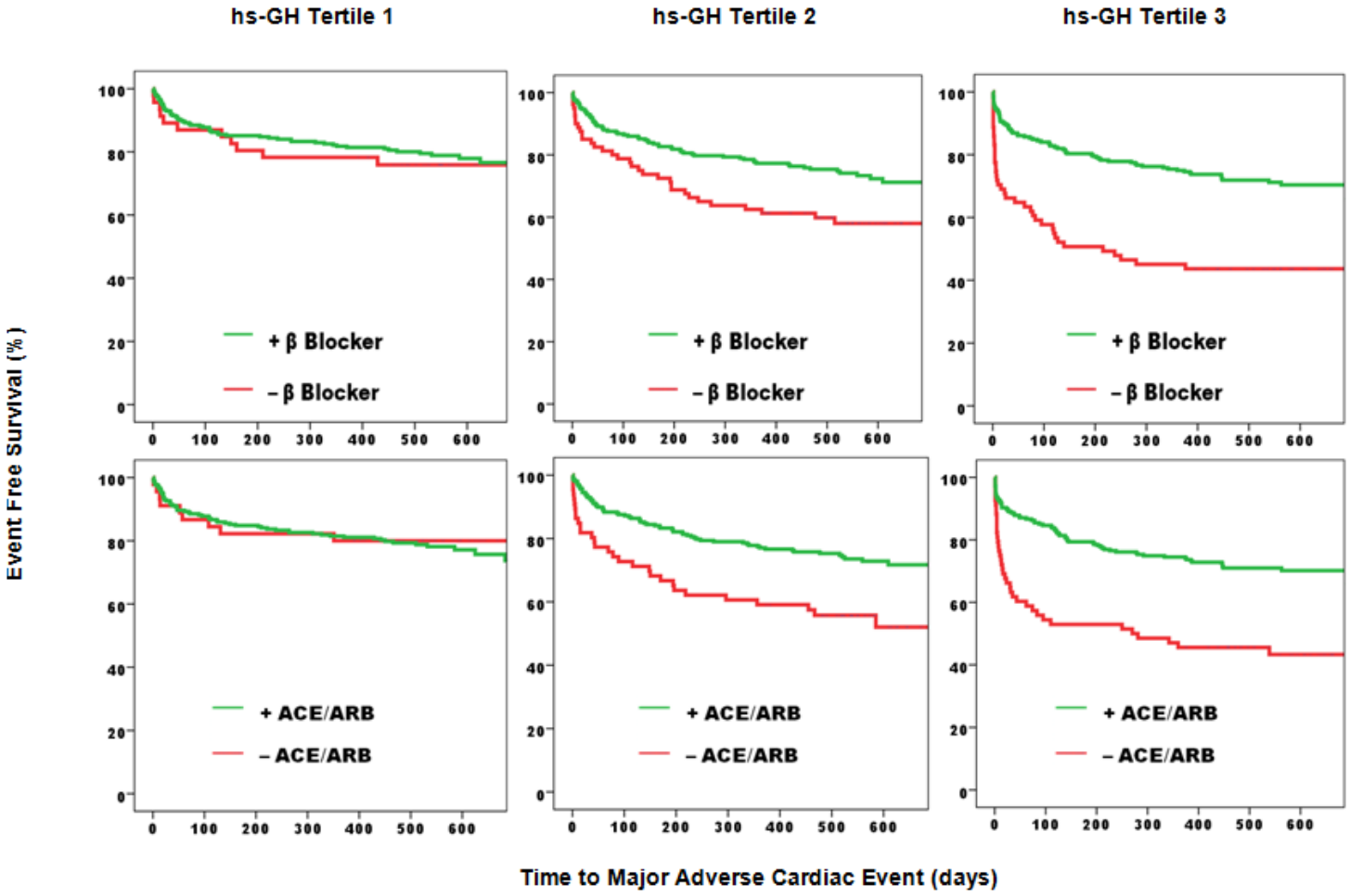
Treatment					
Aspirin	794 (83)	285 (90)	260(80)	249 (79)	<0.0005
Beta-blocker	755 (79)	269 (85)	242 (75)	244 (77)	0.004
ACE inhibitor or ARB*	774 (81)	270 (86)	257 (80)	247 (78)	0.041
Statin	824 (86)	287 (91)	278 (86)	259(82)	0.005
Loop Diuretic	242 (25)	57 (18)	92 (29)	93 (30)	0.001
Revascularisation	241 (25)	95 (30)	76 (24)	70 (22)	NS
End Points (2 years)					
Major Adverse Cardiac Events	281 (29)	70 (22)	99 (31)	112 (36)	0.001

*ARB = Angiotensin 2 receptor blocker

Table 2. Cox regression analysis for MACE at 2 years post-AMI. Multivariable analysis results are reported for model 1 and 2 which included clinical variables and hs-GH, with interaction terms hs-GH with beta blockers (model 1) or ACE/ARB (model 2).

	Univariable HR (95% CI)	**P**	**Multivariable Model 1 HR (95% CI)**	**P**	**Multivariable Model 2 HR (95% CI)**	**P**
Age (years)	1.05 (1.04-1.06)	0.001	1.03 (1.01-1.04)	0.001	1.03 (1.02-1.04)	0.001
Male Sex	0.59 (0.46-0.75)	0.001	1.13 (0.84-1.51)	NS	1.10 (0.83-1.47)	NS
ST elevation	0.97 (0.77-1.23)	NS	1.27 (0.92-1.76)	NS	1.31 (0.95-1.81)	NS
Killip class>1	2.62 (2.06-3.33)	0.001	1.66 (1.26-2.19)	0.001	1.67 (1.26-2.20)	0.001
eGFR (ml min^{-1} /1.73m^2)	0.97 (0.96-0.97)	0.001	0.99 (0.98-0.99)	0.006	0.98 (0.97-0.99)	0.001
Past history						
Ischemic heart disease	1.67 (1.32-2.11)	0.001	1.06 (0.80-1.42)	NS	1.03 (0.76-1.38)	NS
Hypertension	1.69 (1.32-2.15)	0.001	1.13 (0.85-1.50)	NS	1.18 (0.88-1.57)	NS
Diabetes	1.59 (1.23-2.04)	0.001	1.26 (0.94-1.69)	NS	1.27 (0.95-1.69)	NS
Treatment						
Aspirin	0.54 (0.41-0.72)	0.001	excluded		excluded	
Statin	0.38 (0.29-0.51)	0.001	excluded		excluded	
Loop Diuretic	2.30 (1.81-2.92)	0.001	excluded		excluded	
ACE/ARB	0.51 (0.39-0.66)	0.001	excluded		0.74 (0.53-1.04)	NS
β blocker	0.51 (0.39-0.65)	0.001	0.70 (0.52-0.97)	0.03	excluded	
Biomarkers						
Log Troponin (μg/L)	1.10 (0.97-1.26)	NS	1.13 (0.93-1.36)	NS	1.13 (0.93-1.37)	NS
Log hs-GH (μg/L)	1.76 (1.60-1.94)	0.001	1.43 (1.05-1.95)	0.026	1.49 (1.10-2.02)	0.01
β blocker * hs-GH			0.70 (0.49-0.99)	0.047		
ACE/ARB * hs-GH					0.65 (0.47-0.93)	0.016

Figure 1


hs-GH Tertile 1
hs-GH Tertile 2
hs-GH Tertile 3
Event Free Survival (%)
+ β Blocker
– β Blocker
+ ACE/ARB
– ACE/ARB
0
20
40
60
80
100
0
100
200
300
400
500
600
Time to Major Adverse Cardiac Event (days)