hGH Determination for Use to Guide Prevention of a Major Adverse Cardiac Event or a Cardiovascular Disease in a Subject

Subject matter of the present invention is a method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker)) or HMG-CoA reductase inhibitors (statins) comprising the steps:

- determining the level of hGH , and/or its isoforms in a blood sample of said subject and
- comparing the determined level of hGH, and/or its isoforms in said blood sample with a pre-determined threshold and
- wherein in case the determined level of hGH, and/or its isoforms is above said pre-determined threshold then the subject is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein in case the determined level of hGH, and/or its isoforms is below said pre-determined threshold then the subject is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins).

The use of biomarkers for stratification of patients suffering from or being at risk to develop cardiovascular disease for a successful pharmacologic intervention is principally known. However, this has been restricted so far to biomarkers associated with vasoactive or inflammatory activities, e.g in WO/2010/128071, in Richards AM, et al. Australia-New Zealand Heart Failure Group. Journal of the American College of Cardiology 2001, 37(7):1781-1787 and in Ridker et al: The New England journal of medicine 2008, 359(21):2195-2207.

Human growth hormone (hGH) has never been described in this context. It is only known that relatively elevated levels of plasma hGH in the normal population (especially in males) are associated with an increased risk for the development of incident adverse cardiac events in WO/2014/108396.

Here we describe that elevated levels of plasma hGH, and/or its isoforms in subjects are associated with the success of therapy with hypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins), whereas such therapy is not efficacious in patients with relatively low levels of plasma hGH, and/or its isoforms. Thus, measurement of hGH, and/or its isoforms in subjects is a suitable to stratify patients for pharmacological therapy.

The present disclosure relates to a method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject and
- comparing the determined level of hGH, and/or its isoforms in said blood sample with a pre-determined threshold and
- wherein in case the determined level of hGH, and/or its isoforms is above said pre-determined threshold then the subject is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein in case the determined level of hGH, and/or its isoforms is below said pre-determined threshold then the subject is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins).

In one aspect, the present disclosure relates to a method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject and
- comparing the determined level of hGH, and/or its isoforms in said blood sample with a pre-determined threshold and
- wherein in case the determined level of hGH, and/or its isoforms is above said pre-determined threshold then the subject is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein in case the determined level of hGH, and/or its isoforms is below said pre-determined threshold then the subject is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein said isoforms of hGH is selected from the group consisting of: hGH isoform 1, hGH isoform 2, hGH isoform 3 and hGH isoform 4.

In another aspect, the present disclosure relates to the use of an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) in the manufacture of a medicament, for preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods as disclosed herein as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs.

In a further aspect, the present disclosure relates to the use of an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) in the manufacture of a

medicament, for preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods as disclosed herein as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs wherein said subject does not have hypertension.

In yet another aspect, the present disclosure relates to a method for monitoring the therapy response of a subject at vascular risk that is treated with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject taken at a time point X1 with antihypertensive drugs and
- determining the level of hGH, and/or its isoforms in a blood sample of said subject taken at a time point X2 with antihypertensive drugs, and
- wherein time point X1 is either before the beginning of therapy or after starting the therapy but in any event earlier than time point X2, and
- wherein time point X2 is after starting the therapy and in any event later than time point X1

- comparing the determined level of hGH, and/or its isoforms in said blood sample taken at point X1 with the determined level of hGH, and/or its isoforms in said blood sample taken at time point X2
- wherein a lower level of hGH, and/or its isoforms in said blood sample taken at time point X2 compared to the level of hGH, and/or its isoforms in said blood sample taken at time point X1 indicates a therapy response and
- wherein said isoforms of hGH is selected from the group consisting of: hGH isoform 1, hGH isoform 2, hGH isoform 3 and hGH isoform 4.

In one specific embodiment said subject does not have hypertension (at least >140mmHG (systolic) to 90 mmHG (diastolic)).

In one specific embodiment said subject has not yet been treated with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins).

In one embodiment reducing the vascular risk by lowering blood pressure in a subject means preventing a major cardiovascular event and/or a cardiovascular disease in said subject.

In one specific embodiment of the method said subject has suffered an acute myocardial infarction or acute heart failure within the last 2 weeks, preferably within the last one week, preferably within the last 36 hours.

In one specific embodiment of the method according to the invention said subject has never had a major cardiovascular event and has never had any cardiovascular disease. In another specific embodiment of the method according to the invention said subject has carotid plaque but no symptoms of carotid artery disease. An established method to detect the presence of an atherosclerotic disease and to monitor its regression, arrest or progression is the measurement of the intima-media thickness (IMT) (de Groot et al., Nature Reviews Cardiology 5, 280-288 (May 2008) | doi:10.1038/ncpcardio1163). This is a measurement of the thickness of tunica intima and tunica media, the innermost two layers of the wall of an artery. The measurement is usually made by external ultrasound and occasionally by internal, invasive ultrasound catheters. The FDA has approved IMT as a surrogate marker of atherosclerotic disease for application in clinical trials. The extent of IMT has been associated with cardiovascular outcome and its change of over time (statistically significant of IMT per year) with efficacy of drugs (de Groot et al., Nature Reviews Cardiology 5, 280-288 (May 2008) | doi:10.1038/ncpcardio1163; Hedblad et al., Circulation. 2001;103:1721-1726.)

Said major cardiovascular event or cardiovascular disease may be selected from the group comprising heart failure, artherosclerosis, hypertension, cardiomyopathy, myocardial infarction and stroke.

In one embodiment of the invention said subject is male.

In one embodiment of the method according to the invention said method is used for prevention of a major cardiovascular event or prevention of a cardiovascular disease.

Said major cardiovascular event may be selected from the group comprising myocardial infarction, acute heart failure, stroke and cardiovascular death.

The blood sample may be selected from the group comprising whole blood, serum, and plasma.

In one embodiment of the invention the blood sample is a fasting sample.

Fasting sample means a sample, which has been drawn from a subject, which has not consumed any food or solubilized nutrients for at least 8 hours prior to blood sampling. In a specific embodiment, such fasting sample is taken from a subject between 7:00 and 11:30 a,m., after the subject has fastened at least 8 hours.
In another embodiment fasting levels of growth hormone (hGH) means the level of growth hormone (hGH) determined in blood, serum or plasma of fasted subjects that did not have food uptake 12 h prior sample taking. In said embodiment fastened subject means a subject that had 12 h prior sample taking no food up-take.

The term "subject" as used herein refers to a living human or non-human organism. Preferably herein the subject is a human subject. The subject may be healthy or diseased if not stated otherwise. The term “elevated level” means a level above a certain threshold level.

In one specific embodiment of the invention the measurement method or assay that is used to determine the level of hGH, and/or its isoforms has an analytical sensitivity, which is sufficient to quantify the lower 95th percentile of the normal range from fasting healthy, preferably at least 40 years old males with an interassay CV, which does not exceed 20%. An example for such assay is described in WO/2014/108396

Assay sensitivity may be defined by the “analytical” assay sensitivity that is defined as hGH concentration (standard curve readout) at a median signal of e.g. 20 determinations of hGH depleted sample + 2 standard deviations (SD).

Alternatively, the assay sensitivity may be defined by the "functional" assay sensitivity, which reflects more the limit of detection in a clinical routine. The functional assay sensitivity is defined as determination of the inter assay coefficient of variation (CV) of plasma (patient samples) in a variety of individual samples with different hGH concentrations. The functional assay sensitivity is the lowest hGH concentration with an inter assay CV of less than 20%.

Assay sensitivities may depend upon the used method of calibration and the used hGH assay. Different hGH assays may detect different sets of hGH isoforms, leading to different quantitative results meaning leading to different thresholds and sensitivity needs depending on the assay and the calibration method used,

To overcome this possible heterogeneity of different hGH assays, assay calibrations may be corrected by correlating plasma hGH values of individual subjects. If the slope of the correlation curve is different from 1, the hGH values (the calibration) can be corrected by the differential factor leading to a comparable calibration, comparable sensitivity analysis and comparable thresholds.

The above correction may be performed in order to compare the results obtained by different assays.

The assay described in example 1 predominantly recognizes hGH isoform 1 and is calibrated by the recombinant Human Growth Hormone (NIBSC code 98/574, National Institute for Biological Standards and Control, Herfordshire, UK).

In a specific method according to the invention said fasting level of growth hormone (hGH), and/or its isoforms in a bodily fluid is determined by an ultrasensitive assay having an analytical assay sensitivity (that is defined as hGH concentration at a median signal of 20 determinations of hGH depleted sample + 2 standard deviations (SD)) of less than 100 pg/ml, preferred less than 50 pg/ml, preferred less than 30 pg/ml, preferred less than 20 pg/ml, preferred less than 10 pg/ml, preferred less than 5 pg/ml, preferred 2 pg/ml. Measurement of hGH is expressed in ng/ml (1ng/ml=2.6 mU/L).

Alternatively, the ultrasensitive assay having a functional assay sensitivity (see above) of less than 400 pg/ml, preferred less than 200 pg/ml, preferred less than 120 pg/ml, preferred less than 80 pg/ml, preferred less than 40 pg/ml, preferred less than 20 pg/ml, preferred 10 pg/ml most preferred less than 8,5 pg/ml. Measurement of hGH is expressed in ng/ml.

The assay can be an immunoassay or another detection method, for instance mass spectrometry.

For immunization and for calibration we used recombinant Human Growth Hormone (NIBSC code 98/574, National Institute for Biological Standards and Control, Herfordshire, UK)

In one embodiment of the invention said assay is conducted as described in Example 1.

Said assay may be selective for one specific hGH isoform or maybe specific for and determines more than one or all secreted isomers of hGH selected from the group comprising hGH Isoform 1, Isoform 2, Isoform 3, and Isoform 4 (see SEQ ID NO.1-4) in said bodily fluid.

Isoforms of growth hormone (hGH) may be selected from the group comprising hGH isoform 1 (22KD), hGH isoform 2, hGH isoform 3, and hGH isoform 4.

Sequences of the isoforms:

SEQ ID NO.1: hGH Isoform 1 (22KD)
MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE
EA
YIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLR
SVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSH
NDD
ALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF

SEQ ID NO.2: hGH Isoform 2
MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEF
NPQ
TSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSN
VYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCF
RKD
MDKVETFLRIVQCRSVEGSCGF

SEQ ID NO.3: hGH Isoform 3
MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE
EA
YIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQTLMGRLEDGS
PRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSC
GF

SEQ ID NO.4: hGH Isoform 4
MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE
EA
YIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQIFK
QTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF

In one specific embodiment of the invention the measurement method or that is used to determine the level of hGH, and/or its isoforms is an assay having an assay sensitivity of at least 50 pg/ml.

In one specific embodiment of the invention the measurement method that is used to determine the level of hGH, and/or its isoforms is an immuno assay having an assay sensitivity of at least 50 pg/ml.

In one embodiment of the invention said subject the pre-determined threshold is from above 330 pg/ml, preferably above 1370 pg/ml. In one embodiment of the invention the pre-determined threshold is from above 330 pg/ml, preferably above 1370 pg/ml in a subject that has suffered an acute myocardial infarction within the last 2 weeks, preferably within the last one week, preferably within the last 36 hours.

In one embodiment the threshold may be pre-determined as follows:

- Comparison of concentration of hGH, and/or its isoforms in a bodily fluid obtained from said subject with the median of the levels of hGH, and/or its isoforms in a bodily fluid obtained from an ensemble of pre-determined samples in a randomly selected population of subjects having comparable baseline conditions as said subject,

- Comparison of concentration of hGH, and/or its isoforms in a bodily fluid obtained from said subject with a quantile of the levels of hGH, and/or its isoforms in a bodily fluid obtained from an ensemble of pre-determined samples in a population of subjects having comparable baseline conditions as said subject,

- Calculation based on Cox Proportional Hazards analysis or by using Risk index calculations such as the NRI (Net Reclassification Index) or the IDI (Integrated Discrimination Index).

The person skilled in the art understands that a pre-determined threshold has to be seen in the context of assay used, calibration methods used and other factors.

Subject matter according to the present invention is a method wherein the level, preferably fasting level, of growth hormone (hGH), and/or its isoforms is determined by using a binder to growth hormone (hGH), and/or its isoforms.

In one embodiment of the invention said binder is selected from the group comprising an antibody, an antibody fragment or a non-Ig-Scaffold binding to growth hormone (hGH), and/or its isoforms.

In one specific embodiment of the invention an immunoassay is used that comprises at least two antibodies that bind to hGH, and/or its isoforms.

According to the invention the diagnostic binder is selected from the group consisting of antibodies *e.g.* IgG, a typical full-length immunoglobulin, or antibody fragments containing at least the F-variable domain of heavy and/or light chain as *e.g.* chemically coupled antibodies (fragment antigen binding) including but not limited to Fab-fragments including Fab minibodies, single chain Fab antibody, monovalent Fab antibody with epitope tags, *e.g.* Fab-V5Sx2; bivalent Fab (mini-antibody) dimerized with the CH3 domain; bivalent Fab or multivalent Fab, *e.g.* formed via multimerization with the aid of a heterologous domain, *e.g.* via dimerization of dHLX domains, *e.g.* Fab-dHLX-FSx2; F(ab')2-fragments, scFv-fragments, multimerized multivalent or/and multispecific scFv-fragments, bivalent and/or bispecific diabodies, BITE® (bispecific T-cell engager), trifunctional antibodies, polyvalent antibodies, *e.g.* from a different class than G; single-domain antibodies, *e.g.* nanobodies derived from camelid or fish immunoglobulines.

In a preferred embodiment each of the at least two antibodies that bind to hGH, and/or its isoforms have an affinity towards hGH, and/or its isoforms of at least 10^7 M^{-1}, preferred 10^8 M^{-1}, preferred affinity constant is greater than 10^9 M^{-1}, most preferred greater than 10^{10} M^{-1}. A person skilled in the art knows that it may be considered to compensate lower affinity by applying a higher dose of compounds and this measure would not lead out-of-the-scope of the invention. Binding affinity may be determined using the Biacore method, offered as service analysis *e.g.* at Biaffin, Kassel, Germany (http://www.biaffin.com/de/).

To determine the affinity of the antibodies, the kinetics of binding of hGH to immobilized antibody was determined by means of label-free surface plasmon resonance using a Biacore 2000 system (GE Healthcare Europe GmbH, Freiburg, Germany). Reversible immobilization of the antibodies was performed using an anti-mouse Fc antibody covalently coupled in high density to a CM5 sensor surface according to the manufacturer's instructions (mouse antibody capture kit; GE Healthcare) {Lorenz et al.," Functional Antibodies Targeting IsaA of Staphylococcus aureus Augment Host Immune Response and Open New Perspectives for Antibacterial Therapy"; Antimicrob Agents Chemother. 2011 January; 55(1): 165–173}.

In addition to antibodies other biopolymer scaffolds are well known in the art to complex a target molecule and have been used for the generation of highly target specific biopolymers. Examples are aptamers, spiegelmers, anticalins and conotoxins. Non-Ig scaffolds may be protein scaffolds and may be used as antibody mimics as they are capable to bind to ligands or antigenes. Non-Ig scaffolds may be selected from the group comprising tetranectin-based non-Ig scaffolds (*e.g.* described in US 2010/0028995), fibronectin scaffolds (*e.g.* described in EP 1266 025; lipocalin-based scaffolds (*e.g.* described in WO 2011/154420); ubiquitin scaffolds (*e.g.* described in WO 2011/073214), transferring scaffolds (*e.g.* described in US 2004/0023334), protein A scaffolds (*e.g.* described in EP 2231860), ankyrin repeat based scaffolds (*e.g.* described in WO 2010/060748), microproteins preferably microproteins forming a cystine knot) scaffolds (*e.g.* described in EP 2314308), Fyn SH3 domain based scaffolds (*e.g.* described in WO 2011/023685) EGFR-A-domain based scaffolds (*e.g.* described in WO 2005/040229) and Kunitz domain based scaffolds (*e.g.* described in EP 1941867).

In one embodiment of the invention it may be a so-called POC-test (point-of-care), that is a test technology that allows performing the test within less than 1 hour near the patient without the requirement of a fully automated assay system. One example for this technology is the immunochromatographic test technology.

In one embodiment of the invention such an assay is a sandwich immunoassay using any kind of detection technology including but not restricted to enzyme label, chemiluminescence label, electrochemiluminescence label, preferably a fully automated assay. In one embodiment of the invention such an assay is an enzyme labeled sandwich assay. Examples

of automated or fully automated assay comprise assays that may be used for one of the following systems: Roche Elecsys®, Abbott Architect®, Siemens Centauer®, Brahms Kryptor®, Biomerieux Vidas®, Alere Triage®.

A variety of immunoassays are known and may be used for the assays and methods of the present invention, these include: radioimmunoassays ("RIA"), homogeneous enzyme-multiplied immunoassays ("EMIT"), enzyme linked immunoadsorbent assays ("ELISA"), apoenzyme reactivation immunoassay ("ARIS"), dipstick immunoassays and immuno-chromotography assays.

In one embodiment of the invention at least one of said two binders is labeled in order to be detected.

The preferred detection methods comprise immunoassays in various formats such as for instance radioimmunoassay (RIA), chemiluminescence- and fluorescence-immunoassays, Enzyme-linked immunoassays (ELISA), Luminex-based bead arrays, protein microarray assays, and rapid test formats such as for instance immunochromatographic strip tests.

In a preferred embodiment said label is selected from the group comprising chemiluminescent label, enzyme label, fluorescence label, radioiodine label.

The assays can be homogenous or heterogeneous assays, competitive and non-competitive assays. In one embodiment, the assay is in the form of a sandwich assay, which is a non-competitive immunoassay, wherein the molecule to be detected and/or quantified is bound to a first antibody and to a second antibody. The first antibody may be bound to a solid phase, *e.g.* a bead, a surface of a well or other container, a chip or a strip, and the second antibody is an antibody which is labeled, *e.g.* with a dye, with a radioisotope, or a reactive or catalytically active moiety. The amount of labeled antibody bound to the analyte is then measured by an appropriate method. The general composition and procedures involved with "sandwich assays" are well-established and known to the skilled person {Ludvigsson J, Andersson E, Ekbom A, Feychting M, Kim J-L, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11(1):450}.

In another embodiment the assay comprises two capture molecules, preferably antibodies which are both present as dispersions in a liquid reaction mixture, wherein a first labelling

component is attached to the first capture molecule, wherein said first labelling component is part of a labelling system based on fluorescence- or chemiluminescence-quenching or amplification, and a second labelling component of said marking system is attached to the second capture molecule, so that upon binding of both capture molecules to the analyte a measurable signal is generated that allows for the detection of the formed sandwich complexes in the solution comprising the sample.

In another embodiment, said labeling system comprises rare earth cryptates or rare earth chelates in combination with fluorescence dye or chemiluminescence dye, in particular a dye of the cyanine type.

In the context of the present invention, fluorescence based assays comprise the use of dyes, which may for instance be selected from the group comprising FAM (5-or 6-carboxyfluorescein), VIC, NED, Fluorescein, Fluoresceinisothiocyanate (FITC), IRD-700/800, Cyanine dyes, such as CY3, CY5, CY3.5, CY5.5, Cy7, Xanthen, 6-Carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), TET, 6-Carboxy-4',5'-dichloro-2',7'-dimethodyfluorescein (JOE), N,N,N',N'-Tetramethyl-6-carboxyrhodamine (TAMRA), 6-Carboxy-X-rhodamine (ROX), 5-Carboxyrhodamine-6G (R6G5), 6-carboxyrhodamine-6G (RG6), Rhodamine, Rhodamine Green, Rhodamine Red, Rhodamine 110, BODIPY dyes, such as BODIPY TMR, Oregon Green, Coumarines such as Umbelliferone, Benzimides, such as Hoechst 33258; Phenanthridines, such as Texas Red, Yakima Yellow, Alexa Fluor, PET, Ethidiumbromide, Acridinium dyes, Carbazol dyes, Phenoxazine dyes, Porphyrine dyes, Polymethin dyes, and the like.

In the context of the present invention, chemiluminescence based assays comprise the use of dyes, based on the physical principles described for chemiluminescent materials in {D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743-53. Epub 2008/01/24}. Preferred chemiluminescent dyes are acridiniumesters.

As mentioned herein, an "assay" or "diagnostic assay" can be of any type applied in the field of diagnostics. Such an assay may be based on the binding of an analyte to be detected to one or more capture probes with a certain affinity. Concerning the interaction between capture

molecules and target molecules or molecules of interest, the affinity constant is preferably more than 10^8 M^{-1}.

In the context of the present invention, "binder molecules" are molecules which may be used to bind target molecules or molecules of interest, *i.e.* analytes (*i.e.* in the context of the present invention hGH and/or isomers thereof), from a sample. Binder molecules must thus be shaped adequately, both spatially and in terms of surface features, such as surface charge, hydrophobicity, hydrophilicity, presence or absence of lewis donors and/or acceptors, to specifically bind the target molecules or molecules of interest. Hereby, the binding may for instance be mediated by ionic, van-der-Waals, pi-pi, sigma-pi, hydrophobic or hydrogen bond interactions or a combination of two or more of the aforementioned interactions between the capture molecules and the target molecules or molecules of interest. In the context of the present invention, binder molecules may for instance be selected from the group comprising a nucleic acid molecule, a carbohydrate molecule, a PNA molecule, a protein, an antibody, a peptide or a glycoprotein. Preferably, the binder molecules are antibodies, including fragments thereof with sufficient affinity to a target or molecule of interest, and including recombinant antibodies or recombinant antibody fragments, as well as chemically and/or biochemically modified derivatives of said antibodies or fragments derived from the variant chain with a length of at least 12 amino acids thereof.

Chemiluminescent label may be acridinium ester label, steroid labels involving isoluminol labels and the like.

Enzyme labels may be lactate dehydrogenase (LDH), creatinekinase (CPK), alkaline phosphatase, aspartate aminotransferace (AST), alanine aminotransferace (ALT), acid phosphatase, glucose-6-phosphate dehydrogenase and so on.

In one embodiment of the invention at least one of said two binders is bound to a solid phase as magnetic particles, and polystyrene surfaces.

In one embodiment of the assays for determining the level of growth hormone (hGH), and/or its isoforms in a sample according to the present invention such assay is a sandwich assay, preferably a fully automated assay. It may be an ELISA fully automated or manual. It may be a so-called POC-test (point-of-care). Examples of automated or fully automated assay comprise assays that may be used for one of the following systems: Roche Elecsys®, Abbott

Architect®, Siemens Centauer®, Brahms Kryptor®, Biomerieux Vidas®, Alere Triage®. Examples of test formats are provided above.

In one embodiment of the assays for determining the level of growth hormone (hGH), and/or its isoforms in a sample according to the present invention at least one of said two binders is labeled in order to be detected. Examples of labels are provided above.

In one embodiment of the assays for determining the level of growth hormone (hGH), and/or its isoforms in a sample according to the present invention at least one of said two binders is bound to a solid phase. Examples of solid phases are provided above.

In one embodiment of the assays for determining the level of growth hormone (hGH), and/or its isoforms in a sample according to the present invention said label is selected from the group comprising chemiluminescent label, enzyme label, fluorescence label, radioiodine label.

A further subject of the present invention is a kit comprising an assay according to the present invention wherein the components of said assay may be comprised in one or more container.

In one embodiment of the invention the level of hGH, and/or its isoforms is determined by mass spectrometry.

In one specific embodiment said methods according to the present invention is used for monitoring the vascular risk of said subject. Thus, subject matter of the present invention is also a method of determining at least twice, preferably annually, whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject and
- comparing the determined level of hGH, and/or its isoforms in said blood sample with a pre-determined threshold and
- wherein in case the determined level of hGH, and/or its isoforms is above said pre-determined threshold then the subject is identified as having a vascular risk that can be

reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and

- wherein in case the determined level of hGH, and/or its isoforms is below said pre-determined threshold then the subject is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins)

In said monitoring method the above mentioned method steps are conducted at least once a year.

The method according to the present invention may in one embodiment of the invention further comprise:

- recommendation of administration of antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms, is above said pre-determined threshold and who is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs or
- recommendation of withholding with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is below said pre-determined threshold and who is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs

The method according to present invention may in one embodiment of the invention further comprise:

- administration of antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is above said pre-determined

threshold and who is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs or

- withholding with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is below said pre-determined threshold and who is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs.

In one embodiment of the invention the hypertensive drug is selected from the group comprising Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), and Beta-Adrenoreceptor Blocker (Beta-blocker), statins and a combination thereof as well as a combination with other drugs, such as for instance diuretics.

In one embodiment of the invention the Angiotensin converting enzyme inhibitor (ACE inhibitor) is selected from the group comprising Benazepril, Captopril, Cilazapril, Enalapril, Fosinopril, Imidapril, Lisinopril, Moexipril, Perindopril, Quinapril, Ramipril, Spirapril, Trandolapril, and Zofenopril.

In one embodiment of the invention the Angiotensin Receptor Blocker (ARB) is selected from the group comprising Azilsartan, Azilsartanmedoxomil, Candesartan, Candesartancilexetil, Eprosartan, Fimasartan, Irbesartan, Losartan, Milfasartan, Olmesartan, Pomisartan, Pratosartan, Ripisartan, Saprisartan, Tasosartan, Telmisartan, and Valsartan.

In one embodiment of the invention the Beta-Adrenoreceptor Blocker (Beta-blocker) is selected from the group comprising Atenolol, Bisoprolol, Metoprolol, Nebivolol, Esmolol, Betaxolol, Acebutolol, Celiprolol, Propranolol, Bupranolol, Timolol, Carvedilol, Sotalol, Nadolol, Pindolol, Oxprenolol, Alprenolol and Carteolol

In one embodiment of the invention the statin is selected from the group comprising Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin, Simvastatin, Cerivastatin and Mervastatin (Compactin) and a combination thereof as well as a combination with other drugs.

Subject matter of the present invention is an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins for use in preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of the present invention as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs .

Subject matter of the present invention is an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of the present invention as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs and wherein said subject does not have hypertension.

Subject matter of the present invention is an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherin said subject is identified according to the methods of the present invention as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs and wherein said subject does not have hypertension and wherein said Angiotensin converting enzyme inhibitor (ACE inhibitor) is selected from the group comprising Benazepril, Captopril, Cilazapril, Enalapril, Fosinopril, Imidapril, Lisinopril, Moexipril, Perindopril, Quinapril, Ramipril, Spirapril, Trandolapril, and Zofenopril.

Subject matter of the present invention is an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherin said subject is

identified according to the methods of the present invention as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs and wherein said subject does not have hypertension and wherein said Angiotensin Receptor Blocker (ARB) is selected from the group comprising Azilsartan, Azilsartanmedoxomil, Candesartan, Candesartancilexetil, Eprosartan, Fimasartan, Irbesartan, Losartan, Milfasartan, Olmesartan, Pomisartan, Pratosartan, Ripisartan, Saprisartan, Tasosartan, Telmisartan, and Valsartan.

Subject matter of the present invention is an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of the present invention as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs and wherein said subject does not have hypertension and wherein said statin is selected from the group comprising: Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin, Simvastatin, Cerivastatin and Mervastatin (Compactin).

Subject matter of the present invention is an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of the present invention as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs and wherein said subject does not have hypertension and wherein said Beta-Adrenoreceptor Blocker (Beta-blocker) is selected from the group comprising Atenolol, Bisoprolol, Metoprolol, Nebivolol, Esmolol, Betaxolol, Acebutolol, Celiprolol, Propranolol, Bupranolol, Timolol, Carvedilol, Sotalol, Nadolol, Pindolol, Oxprenolol, Alprenolol and Carteolol.

Most of the above active compounds may be formulated in different salt forms. Pharmaceutically acceptable salts may be obtained while using the common procedures. Thus, subject matter are also all pharmaceutically acceptable salts of the above mentioned active compounds.

Such salt forms may be common alkaline and acid addition salts obtained from conversion with respective organic or inorganic acids. The acids which form salts of the active compounds include sulfuric acid, sulfonic acid, phosphoric acid, nitrous acid, nitric acid, perchloric acid, hydrobromic acid, hydrochloric acid, formic acid, acetic acid, propionic acid, succinic acid, oxalic acid, glucuronic acid (in the left and dextrorotatory form), lactic acid, malic acid, tartaric acid, (hydroxymalonic, hydroxypropanoicdicarbonic acid), fumaric acid, citric acid, ascorbic acid, maleic acid, malonic acid, hydroxymaleic acid, pyruvic acid, phenylacetic acid (o-, m-, p-) toluic acid, benzoic acid, p-aminobenzoic acid, salicylic, p-amino-salicylic acid, methylsulfonic-, ethylsulfonic-, hydroxy methylsulfonic acid, ethylenesulfonic acid, p-toluenesulfonic, naphthalenesulfonic, naphthylaminosulfonic acid, sulfanilic acid, camphorsulfonic acid, quinic acid, o-methyl mandelic acid, picric acid (2,4,6-trinitrophenol), adipic acid, amino acids such as methionine, tryptophane, arginine, and especially acidic amino acids such as glutamine or aspartic acid.

In case acid substituents are present in said active compounds also basic addition salts may be formed, particularly with alkali metals, as well as with amino acids. Therefore, alkali metal salts such as the sodium, potassium, lithium salt or the magnesium, calcium salt, alkylamino salts or salts formed with amino acids, for example with amino acids such as lysine alkaline.

The preferred specific salts in accordance with the invention are selected from a group comprising sulphate, bisulphate, nitrate, phosphate, hydrogen phosphate, hydrochloride, hydrobromide, hydroiodide, acetate, tartrate, lactate, citrate, gluconate, fumarate, maleate, hydroxyl maleate, succinate, pamoate, benzoate, propionate, pyruvate, oxalate, malonate, cinnamate, salicylate, alkyl sulphonate, aryl sulphonate, and aralkyl sulphonate.

Suitable salts of the active compounds are all acid addition salts or all salts with bases. Particular mention may be made of the pharmacologically tolerable inorganic and organic acids and bases customarily used in pharmacy.

Those suitable are, on the one hand, water insoluble and, particularly, watersoluble acid addition salts with acids such as, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, nitric acid, sulphuric acid, acetic acid, citric acid, D-gluconic acid, benzoic acid, 2-(4-hydroxybenzoyl)benzoic acid, butyric acid, sulphosalicylic acid, maleic acid, lauric acid, malic acid such as (-)-L-malic acid or (+)-D-malic acid, fumaric acid, succinic acid, oxalic acid, tartaric acid such as (+)-L-tartaric acid or (-)-D-tartaric acid or meso-tartaric acid, embonic acid, stearic acid, toluenesulphonic acid, methanesulphonic acid or 3-hydroxy-2-naphthoicacid, the acids being employed in salt preparation – depending on whether a mono- or polybasic acid is concerned and depending on which salt is desired – in an equimolar

quantitative ratio or one differing therefrom. Further, glutamate and aspartate are suitable salts of the above mentioned active compounds.

Pharmacologically intolerable salts, which can be obtained, for example, as process products or by-products during the preparation of the compounds according to the present invention on an industrial scale, are converted into pharmacologically tolerable salts by processes known to the person skilled in the art.

The term "pharmaceutical acceptable" in the context of the invention means that the relevant derivatives, functional equivalents, salts, solvates, hydrates, excipients, carrier, diluents, and solvents according to the invention are safe and effective for the comprised use in mammals and that possess the desired biological activity and/or function, respectively.

Some selected embodiments of the inventions are listed below:

1) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject and
- comparing the determined level of hGH, and/or its isoforms in said blood sample with a pre-determined threshold and
- wherein in case the determined level of hGH, and/or its isoforms is above said pre-determined threshold then the subject is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein in case the determined level of hGH, and/or its isoforms is below said pre-determined threshold then the subject is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins).

2) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 1,

wherein said subject does not have hypertension (at least >140mmHG (systolic) to 90 mmHG (diastolic)).

3) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 1 or 2 wherein the blood sample is taken from a subject that has not yet treated with antihypertensive drugs.

4) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 1, 2 or 3 wherein reducing the vascular risk by lowering blood pressure in a subject means preventing a major cardiovascular event and/or a cardiovascular disease in said subject.

5) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of claims 1 to 4 wherein said subject has never had a major cardiovascular event and has never had any cardiovascular disease.

6) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the claims 1 to 4 wherein said subject has suffered an acute myocardial infarction or acute heart failure within the last 2 weeks, preferably within the last one week, preferably within the last 36 hours.

7) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the claims 1 to 6 wherein said antihypertensive drug is selected from the group comprising Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), and Beta-Adrenoreceptor Blocker (Beta-blocker), statins or combinations thereof.

8) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the

preceding claims wherein the blood sample is selected from the group comprising whole blood, serum, and plasma.

9) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the blood sample is a fasting sample.

10) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the measurement method that is used to determine the level of hGH, and/or its isoforms has a sensitivity of at least 50 pg/ml.

11) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the measurement method that is used to determine the level of hGH, and/or its isoforms is an immuno assay having an assay sensitivity of at least 50 pg/ml.

12) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the pre-determined threshold is 1370 pg/ml, preferably 330 pg/ml.

13) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according any of the claims 1-12 wherein the level of hGH, and/or its isoforms is determined by an immunoassay that comprises at least two antibodies that bind to hGH, and/or its isoforms.

14) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 11 wherein each of the at least two antibodies that bind to hGH, and/or its isoforms have an affinity towards hGH, and/or its isoforms that is at least $10^8 M^{-1}$.

15) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein said method is used for prevention of a major cardiovascular event and/or a cardiovascular disease and/or a pre-stage of cardiovascular event and/or a cardiovascular disease in said subject selected from the group comprising heart failure, artherosclerosis, hypertension, cardiomyopathy, myocardial infarction and stroke.

16) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the method further comprises:

- recommendation of administration of antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and who is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs or
- recommendation of withholding with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is below said pre-determined threshold and who is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs

17) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the method further comprises:

- administration of antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and who is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs or

- withholding of antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is below said pre-determined threshold and who is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs.

18) An antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in a method of preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of any of claims 1-17 as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs.

19) An antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) for use in a method of preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of any of claims 1-17 as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs wherein said subject does not have hypertension.

Examples

Example 1, us-hGH Assay

Plasma samples were measured using a hGH assay as described previously in WO/2014/108396. The measurement was made with an ultra-sensitivity chemiluminescence immunoassay (see example 1 us-hGH assay). The detection limit was 2 pg/ml, functional assay sensitivity was 8.5 pg/ml. Measurement of hGH is expressed in ng/ml (1ng/ml=2.6 mU/L).

Chemicals

If not stated otherwise, chemicals were obtained at p.a. grade from Merck (Darmstadt, Germany).

Antigen

For immunization and for calibration we used recombinant Human Growth Hormone (NIBSC code 98/574, National Institute for Biological Standards and Control, Herfordshire, UK).

Development of Antibodies

Mouse monoclonal antibodies against hGH were developed by UNICUS (Karlsburg, Germany).

The antibodies were generated according to the following method:

A BALB/c mouse were immunized with 100 µg hGH at day 0 and 14 (emulsified in 100 µl complete Freund's adjuvant) and 50 µg at day 21 and 28 (in 100 µl incomplete Freund's adjuvant). Three days before the fusion experiment was performed, the animal received 50 µg of the conjugate dissolved in 100 µl saline, given as one intraperitonal and one intra venous injection.

Splenocytes from the immunized mouse and cells of the myeloma cell line SP2/0 were fused with 1 ml 50 % polyethylene glycol for 30 s at 37 °C. After washing, the cells were seeded in 96-well cell culture plates. Hybrid clones were selected by growing in HAT medium [RPMI 1640 culture medium supplemented with 20 % fetal calf serum and HAT-supplement]. After

two weeks the HAT medium is replaced with HT Medium for three passages followed by returning to the normal cell culture medium.

The cell culture supernatants were primary screened for antigen specific IgG antibodies three weeks after fusion. The positive tested microcultures were transferred into 24-well plates for propagation. After retesting the selected cultures were cloned and recloned using the limiting-dilution technique and the isotypes were determined. (Lane, R.D. "A short-duration polyethylene glycol fusiontechnique for increasing production of monoclonal antibody-secreting hybridomas", J. Immunol. Meth. 81: 223-228; (1985) - Ziegler, B. *et al.* "Glutamate decarboxylase (GAD) is not detectable on the surface of rat islet cells examined by cytofluorometry and complement-dependent antibody-mediated cytotoxicity of monoclonal GAD antibodies", Horm. Metab. Res. 28: 11-15, (1996))

Monoclonal antibody production

We selected 5 antibodies for further investigations.

Antibodies were produced via standard antibody production methods (Marx *et al.*, Monoclonal Antibody Production (1997), ATLA 25, 121) and purified via Protein A-chromatography. The antibody purities were > 95 % based on SDS gel electrophoresis analysis.

Labelling and coating of antibodies.

All antibodies were labelled with acridinium ester according the following procedure:

Labelled compound (tracer): 100 µg (100 µl) antibody (1 mg/ml in PBS, pH 7.4), was mixed with 10 µl Acridinium NHS-ester (1 mg/ml in acetonitrile, InVent GmbH, Germany) (EP 0353971) and incubated for 20 min at room temperature. Labelled antibody was purified by gel-filtration HPLC on Bio-Sil SEC 400-5 (Bio-Rad Laboratories, Inc., USA) The purified labelled antibody was diluted in (300 mmol/l potassiumphosphate, 100 mmol/l NaCl, 10 mmol/l Na-EDTA, 5 g/l bovine serum albumin, pH 7.0). The final concentration was approx. 800.000 relative light units (RLU) of labelled compound (approx. 20 ng labeled

antibody) per 200 µl. Acridiniumester chemiluminescence was measured by using an AutoLumat LB 953 (Berthold Technologies GmbH & Co. KG).

Solid phase antibody (coated antibody):

Solid phase: Polystyrene tubes (Greiner Bio-One International AG, Austria) were coated (18 h at room temperature) with antibody (1.5 µg antibody/0.3 ml 100 mmol/l NaCl, 50 mmol/l Tris/HCl, pH 7.8). After blocking with 5 % bovine serum albumin, the tubes were washed with PBS, pH 7.4 and vacuum dried.

hGH Immunoassay:

50 µl of sample (or calibrator) was pipetted into coated tubes, after adding labeled antibody (200 ul), the tubes were incubated for 2 h at 18-25 °C. Unbound tracer was removed by washing 5 times (each 1 ml) with washing solution (20 mmol/l PBS, pH 7.4, 0.1 % Triton X-100). Tube-bound labelled antibody was measured by using the LB 953. Using a fixed concentration 1ng/ml of hGH. The signal (RLU at 1ng hGH /ml) to noise (RLU without us-hGH) ratio of different antibody combinations is given in table 1. All antibodies were able to generate a sandwich complex with any other antibody. The antibody pair with strongest signal to noise ratio (best sensitivity) was subsequently used to perform the us-hGH-immunoassay: hGH G12 antibody was used as coated tube antibody and hGH H4 antibody was used as labelled antibody.

Table 1: Results of noise to ratio determinations between different pairs of hGH antibodies.

hGH antibody	Solid phase antibody	H2	H8	G12	H4	D7
Labelled antibody						
H2		/	9.665	11.005	9.259	10.102
H8		8.512	/	7.833	8.446	6.384
G12		10.112	9.846	/	10.905	7.751
H4		11.213	8.675	12.225	/	6.843
D7		2.488	2.761	3.954	2.713	/

Calibration:

The assay was calibrated, using dilutions of recombinant hGH (WHO International Standard, NIBSC code 98/574), diluted in 20 mM K2PO4, 6 mM EDTA, 0,5% BSA, 50uM Amastatin, 100uM Leupeptin, pH 8.0. (Fig. 1)

Assay specifications

The analytical assay sensitivity (mean relative light units of 20 determinations of hGH free sample plus 2 S.D.) was 2pg/ml of hGH and the functional assay sensitivity (see above) was 8,5 pg/ml. Recovery and dilution was > 85% intra measurement range of 5-10.000 pg/ml hGH. The coefficient of correlation of N=997 samples between the us-hGH assay and a hGH assay specific for recombinant hGH (22KD) was r=0.98 and a r of 0.95 was found for an assay recognizing preferentially hGH isoforms naturally produced by the pituitary (Bidlingmaier M, Suhr J, Ernst A, Wu Z, Keller A, Strasburger CJ, et al. High-sensitivity chemiluminescence immunoassays for detection of growth hormone doping in sports. Clinical chemistry. 2009;55(3):445-53. Epub 2009/01/27). These dataindicating the suitability of all hGH isoform measurements within the present invention.

Example 2, 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 and was approved by the local ethics committee; written informed consent was obtained from patients. AMI was diagnosed if a patient 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. 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 (Smilde TD, van Veldhuisen DJ, Navis G, Voors AA, Hillege HL. Drawbacks and prognostic value of formulas estimating renal function in patients with chronic heart failure and systolic dysfunction. Circulation 2006;114:1572-80). All patients received standard medical treatment and revascularisation was at the discretion of the attending physician.

Plasma samples: Blood samples (anticoagulated with EDTA and aprotinin) were drawn after 15 minutes bed rest, 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 hGH.

Exampie 3, Analysis

The efficacy of treatment of patients with acute myocardial infarction with various drugs was analysed depending on the patients' hGH concentration, which was determined within a timeframe of maximally 36 hours after symptom onset. The drugs analysed were a) Angiotensin converting enzyme inhibitor (ACE) or Angiotensin Receptor Blocker (ARB), and b) beta-blocker. The endpoint for determining efficacy was occurrence of major adverse cardiac events (MACE) within one year after symptom onset. MACE was defined as a combination of the following endpoints: all-cause mortality, hospitalization due to heart failure, or re-acute myocardial infarction (re-AMI).

Angiotensin converting enzyme inhibitor (ACE) or Angiotensin Receptor Blocker (ARB):

The patient cohort consisted of 953 cases with 245 MACE endpoints within 1 year. Various clinical variables were associated with prediction of MACE within one year, as determined by Cox regression analysis (table 1). When hGH concentrations were added to the statistical model (table 2), hGH was an independent predictor of MACE, and, surprisingly, also the interaction of hGH and treatment with ACE/ARB. Kaplan-Meier analysis for the occurrence of MACE depending on treatment with ACE/ARB was performed for the patient population after separation into hGH tertiles, that is patient with low (<330 pg/mL), medium (330 – 1370 pg/mL) and high concentrations (> 1370 pg/mL) of hGH. The number of MACE for the subgroups are shown in table 3. As shown in table 4, the ACE/ARB treatment effect on MACE was dependent on hGH tertiles and significant only for hGH tertiles 2 and 3, that is for medium and high hGH concentrations. The effect was more pronounced in hGH tertile 3 than in hGH tertile 2. Fig. 2-4 show respective Kaplan-Meier-Plots for the three hGH tertiles.

Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)
age	.032	.006	29.831	1	.000	1.032
sex	.138	.119	1.360	1	.244	1.148
PMHmi_angina	.257	.115	5.024	1	.025	1.293
pmhbp	.284	.114	6.162	1	.013	1.329
PMHdiabetes	.265	.119	4.957	1	.026	1.304
KIIIIpAbove1	.493	.116	17.922	1	.000	1.637
eGFR	-.015	.003	20.395	1	.000	.985
ClassSTEMI	.307	.115	7.077	1	.008	1.359
DischACE_ARB	-.369	.116	10.142	1	.001	.692

Table 1: Multivariate Cox regression analysis for the prediction of MACE within 1 year.

Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)
age	.033	.007	21.337	1	.000	1.033
sex	.046	.144	.102	1	.749	1.047
PMHmi_angina	.038	.143	.071	1	.789	1.039
pmhbp	.123	.140	.768	1	.381	1.131
PMHdiabetes	.267	.145	3.375	1	.066	1.305
KIIIIpAbove1	.553	.138	16.070	1	.000	1.739
eGFR	-.019	.004	19.937	1	.000	.981
ClassSTEMI	.636	.145	19.167	1	.000	1.889
DischACE_ARB	-.591	.162	13.243	1	.000	.554
ZLoghGH	.375	.145	6.658	1	.010	1.455
DischACE_ARB*ZLoghGH	-.359	.166	4.657	1	.031	.698

Table 2: Multivariate Cox regression analysis for the prediction of MACE within 1 year including hGH and interaction with ACE/ARB therapy.

Case Processing Summary

				Censored	
hGHaTertiles	DischACE_ARB	Total N	N of Events	N	Percent
1.00	0	45	9	36	80.0%
	1	270	50	220	81.5%
	Overall	315	59	256	81.3%
2.00	0	66	27	39	59.1%
	1	257	59	198	77.0%
	Overall	323	86	237	73.4%
3.00	0	68	37	31	45.6%
	1	247	63	184	74.5%
	Overall	315	100	215	68.3%
Overall	Overall	953	245	708	74.3%

Table 3: Case Processing Summary for MACE depending on ACE/ARB treatment in patients with low (tertile 1), medium (tertile 2) and high (tertile 3) concentartions of hGH.

Pairwise Comparisons

			0		1	
	hGHaTertiles	DischACE_ARB	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	1.00	0			.070	.791
		1	.070	.791		
	2.00	0			10.492	.001
		1	10.492	.001		
	3.00	0			25.970	.000
		1	25.970	.000		

Table 4: Statistical comparison of ACE/ARB treatment effect on MACE depending on hGH tertiles.

Beta-blockers

In the same patient cohort, multivariate Cox regression analysis revealed also a significant interaction of hGH and treatment with Beta-blockers for prediction of 1 year MACE (table 5). Kaplan-Meier analysis for the occurrence of MACE depending on treatment with Beta-blockers was performed for the patient population after separation into hGH tertiles, that is patient with low, medium and high concentrations of hGH. The number of MACE for the subgroups are shown in table 6. As shown in table 7, the Beta-blockers treatment effect on MACE was dependent on hGH tertiles and significant only for hGH tertiles 2 and 3, that is for medium and high hGH concentrations. The effect was more pronounced in hGH tertile 3 than in hGH tertile 2. Fig.5-7 show respective Kaplan-Meier-Plots for the three hGH tertiles.

Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)
age	.031	.007	17.475	1	.000	1.031
sex	.086	.145	.356	1	.551	1.090
PMHmi_angina	.082	.141	.337	1	.562	1.085
pmhbp	.076	.140	.291	1	.590	1.079
PMHdiabetes	.282	.147	3.671	1	.055	1.326
KillipAbove1	.528	.139	14.540	1	.000	1.696
eGFR	-.021	.004	23.658	1	.000	.979
ClassSTEMI	.573	.144	15.936	1	.000	1.774
DischBetaBlocker	-.383	.157	5.933	1	.015	.682
ZLoghGH	.415	.153	7.348	1	.007	1.514
DischBetaBlocker*ZLoghGH	-.399	.172	5.365	1	.021	.671

Table 5: Multivariate Cox regression analysis for the prediction of MACE within 1 year including hGH and interaction with Beta-blocker therapy.

Case Processing Summary

hGHtertiles	DischBetaBlocker	Total N	N of Events	Censored	
				N	Percent
1.00	0	46	10	36	78.3%
	1	269	49	220	81.8%
	Overall	315	59	256	81.3%
2.00	0	80	30	50	62.5%
	1	242	55	187	77.3%
	Overall	322	85	237	73.6%
3.00	0	71	39	32	45.1%
	1	244	61	183	75.0%
	Overall	315	100	215	68.3%
Overall	Overall	952	244	708	74.4%

Table 6: Case Processing Summary for MACE depending on Beta-blocker treatment in patients with low (tertile 1), medium (tertile 2) and high (tertile 3) concentartions of hGH.

Pairwise Comparisons

			0		1	
	hGHtertiles	DischBetaBlocker	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	1.00	0			.359	.549
		1	.359	.549		
	2.00	0			7.355	.007
		1	7.355	.007		
	3.00	0			29.334	.000
		1	29.334	.000		

Table 7: Statistical comparison of Beta-blocker treatment effect on MACE depending on hGH tertiles.

Characteristics of the 953 AMI patients according to hGH tertiles on admission are given in table 8.

		hGH tertiles (pg/mL)			
	All	1 <330	2 330-1370	3 >1370	P Value
	n=953	n=315	n=323	n=315	
hGH pg/mL	1700 ± 2850	140 ± 80	750 ± 0.310	4240 ± 3850	<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

Table 8: 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.

*ARB = Angiotensin 2 receptor blocker

	Univariable HR (95% CI)	P	Multivariable Model 1 HR (95% CI)	P	Multivariable Model 2 HR (95% CI)	P

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], 910 [40-26 280] pg/mL) compared to event free survivors (590 [20-21 600], p<0.0005 using the Mann -Whitney test). Table 8 also illustrates the higher prevalence of MACE in patients with higher GH levels.

Table 9 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. Age, Killip class>1, eGFR were retained in all models as independent predictors, together with GH levels. Beta blocker therapy was associated with lower MACE (p=0.03) and showed a significant interaction with GH levels (p=0.047, Table 9). Therapy with ACE/ARB also showed a significant interaction with GH levels (p=0.016, Table 9).

Kaplan-Meier survival analysis was used to visualise the interactions of therapies with beta blocker or ACE/ARB according to GH tertiles (Figure 8). For beta blocker therapy, there were significant differences in MACE rates between those prescribed compared to those who were not prescribed this treatment in the 2nd (p=0.009) and 3rd GH tertiles (p<0.0005). For ACE/ARB treatment, there were significant differences in MACE rates between 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.

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 hGH	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 * hGH			0.70 (0.49-0.99)	0.047		
ACE/ARB * hGH					0.65 (0.47-0.93)	0.016

Table 9.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 hGH, with interaction terms hGH with beta blockers (model 1) or ACE/ARB (model 2).

Example 4, Malmö Diet and Cancer study

The Malmö Diet and Cancer study - cardiovascular cohort (MDC-CC) is a prospective cohort examined in the early 90's. Further details about this study can be found in earlier publications (Berglund et al. 1993. Journal of Internal Medicine 233: 45-51). We performed a cross-sectional analysis of the relationship between hs-GH and intima media thickness (IMT) in MDC-CC. In multiple regression models with IMT as the dependent variable and the standardized value of the natural logarithm of hs-GH as independent we analyzed 4425 individuals from this cohort with values on both hs-GH and the mean IMT in the common carotid artery (IMT_{cca}) and 3397 participants with values on the maximum IMT at the bifurcation (IMT_{bulb}). Separate models were performed for IMT_{cca} and IMT_{bulb} and they were adjusted for either sex and age or a set of traditional cardiovascular risk factors: sex, age, current smoking, systolic blood pressure, anti-hypertensive medication, BMI, LDL-C, HDL-C and diabetes mellitus.

Carotid ultrasound was performed at baseline. In brief, a predefined extent of the right carotid bifurcation was scanned for presence of plaques and the maximum IMT at the bifurcation (IMT_{bulb}) and the mean IMT in the common carotid artery (IMT_{cca}) was measured.In the initial cross-sectional regression model of 3397 (1957 females, 58%) individuals in the MDC-CC hs-GH exhibited a significant positive correlation with IMT_{bulb} in the whole cohort (P=0.003) and in males (P=0.003) (Table 10). This association was essentially unaffected when adjusting for traditional cardiovascular risk factors (P=0.002 and P=0.005 respectively). The results among females were not significant. Fasting levels of hs-GH were not associated with the IMT_{cca} in the 4425 individuals (2658 females, 60%) that were available in this analysis.

Higher fasting levels of hs-GH were associated with an increased IMT in the carotid bulb in males. The relationship between hs-GH and IMT at the carotid bulb in males is in accordance with previous results of higher fasting values of hs-GH being associated to cardiovascular disease, which was also preferentially seen in males (Hallgren et al. 2014. JACC 64: 1452-1460; Maison et al. 1998. BMJ [Clinical Research ed] 316: 1132-1133). IMT_{bulb} is a good measure of atherosclerosis (Naqvi and Lee. 2014. JACC Cardiovasc Imaging 7: 1025-1038), and this finding thus supports a hypothesis of the connection between hs-GH and cardiovascular diseases being mediated by atherosclerosis.

Table 10: Linear regression with IMT at baseline in the MDC-CC study as dependent variable and hs-GH as independent. One crude model adjusted for sex and age and one model in addition adjusted for traditional cardiovascular risk factors.

Dependent	Gender	Model	Beta	95%CI	P
IMT_{bulb}	ALL	Crude	0.060	0.020 to 0.099	0.003
		Adjusted	0.066	0.025 to 0.106	0.002
	MALE	Crude	0.075	0.025 to 0.125	0.003
		Adjusted	0.074	0.022 to 0.125	0.005
	FEMALE	Crude	0.029	-0.014 to 0.072	0.184
		Adjusted	0.038	-0.006 to 0.083	0.089
IMT_{cca}	ALL	Crude	-0.012	-0.046 to 0.021	0.477
		Adjusted	0.017	-0.017 to 0.052	0.319
	MALE	Crude	0.011	-0.033 to 0.054	0.634
		Adjusted	0.024	-0.020 to 0.068	0.280
	FEMALE	Crude	-0.027	-0.063 to 0.009	0.145
		Adjusted	0.001	-0.036 to 0.038	0.967

Crude models adjusted for sex and age. Adjusted model adjusted for: sex, age, systolic blood pressure, antihypertensive medication, diabetes mellitus, current smoking, BMI, LDL-C and HDL-C. The β coefficients are expressed as the increment of standardized values of the natural logarithm of IMT per 1 increment of standardized values of the natural logarithm of hs-GH. Abbreviations: IMT_{cca}, mean value of intima media thickness in the common carotid artery. IMT_{bulb}, max value of the intima media thickness at the carotid bifurcation.

Example 5, BCAPS

Study description

The β-Blocker Cholesterol-Lowering Asymptomatic Plaque Study (BCAPS) was a randomized, double blind, placebo-controlled, single-center clinical trial that took place between 1994 and 1999. A detailed description of BCAPS can be found in the original paper (Hedblad et al. 2001. Circulation 103: 1721-1726), following here is a brief summary. The original study population consisted of 361 men and 432 women (total n=793) 49 to 70 years of age recruited from MDC-CC. Subjects included in BCAPS had plaque in the right carotid artery but no symptoms of carotid artery disease. Exclusion criteria were: myocardial infarction, angina pectoris or stroke within the previous 3 months; previous surgery in the right carotid artery; regular use of β-blockers or statins; blood pressure >160 (systolic) or >95 (diastolic) mm Hg; total cholesterol > 8.0 mmol/l;

hyperglycemia suspected to require insulin treatment; and conditions that in the view of the investigator made the participant unsuitable for the trial.

All participants provided written consent and the study was approved by The Ethics Committee of Lund University.

The study consisted of 4 different treatment groups and the participants were randomized to 1 of the following: placebo/placebo, metoprolol CR/XL (25 mg once daily)/placebo, fluvastatin (40 mg once daily)/placebo or metoprolol CR/XL (25 mg once daily)/fluvastatin (40 mg once daily).

The treatment period was 36-months. Fasting blood samples were drawn at baseline and at 12, 24 and 36 months. HDL-C, total cholesterol and TG were measured according to standard procedures at the Department of Clinical Chemistry, University Hospital of Malmö. LDL-C levels were calculated according to the Friedewald formula. GH levels were measured in stored fasting plasma samples, which were frozen immediately to -80°C at the different examinations. A number of the samples stored in the freezer were missing or had insufficient plasma for the analysis of hs-GH. Subjects in which either the baseline-value of GH or the 12-month value of GH was missing were excluded from further analysis. After this adjustment the study consisted of 472 individuals. All samples were measured using the hs-hGH immunoassay as described above.

Ultrasound

Carotid ultrasound was performed at baseline, 18 and 36 months (Hedblad et al. 2001. Circulation 103: 1721-1726). In brief, a predefined extent of the right carotid bifurcation was scanned for presence of plaques and the maximum IMT at the bifurcation (IMT_{bulb}) and the mean IMT in the common carotid artery (IMT_{cca}) was measured.

Statistical analyses

In the analyses where treatment groups are compared, the groups are either the 4 randomization groups or they are divided in to 2 groups which are fluvastatin vs non-fluvastatin (i.e placebo) or metoprolol vs non-metoprolol. Since fasting hs-GH is known to differ between men and women all models were performed separate for men and women as well as combined. All models were adjusted for age, sex (if not already gender separated) and the natural logarithm of the fasting level of hs-GH at baseline.

To investigate if fasting hs-GH was affected by treatment with fluvastatin or metoprolol we performed multiple regression analysis with ΔGH (baseline value subtracted from 12

month value) as the dependent variable and the treatment groups as independent variables.

We then analyzed if hs-GH affects the treatment effect of the different medicines on the IMT in a series of multiple regression models with IMT at 36 months as the dependent variable. In these analyses the larger randomization groups were used to gain more power. An interaction term between the treatment groups multiplied with ΔGH or the natural logarithm of hs-GH, depending on model, was created to evaluate this interaction. A small number of individuals were lost during follow-up and could not complete the ultrasound examination of IMT at 36 months. When analyzing the IMT, the latest IMT-values (i.e. at 18 months) are used for these individuals and the analysis is adjusted for time between IMT-measurements. The models were in addition adjusted for the natural logarithm of IMT at baseline.

All analyses were performed in SPSS (version 22.0.0, SPSS Inc., Chicago, Ill). A 2-sided P-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics of the 472 individuals in the BCAPS study population are shown in table 11. The females in all groups had higher values of hs-GH at baseline compared to males. The subjects in which hs-GH could not be measured due to missing samples at either baseline or 12 months did not differ in their baseline characteristics. The missing samples were evenly distributed over the different treatment groups, but generally more men than women were in the group of missing samples. This made the male/female ratio marginally different in two of the four treatment groups (table 11).

In linear regression models the change in levels of hs-GH at 12 months compared to baseline (ΔGH) were related against the different treatment groups (Table 12). When analyzing the whole cohort none of the analyses were significant. In males treatment with fluvastatin/metoprolol had a significant negative correlation with ΔGH compared against placebo (P=0.015). There was also a significant negative correlation between ΔGH and fluvastatin treatment compared to placebo (P=0.046). Among females the correlations were positive and significant for fluvastatin/metoprolol (P=0.010) and borderline significant for fluvastatin (P=0.051).

Male subjects treated with fluvastatin had a greater reduction of hs-GH over 12 months as compared to subjects not treated with fluvastatin. In males, treatment with metoprolol/fluvastatin compared to placebo/placebo was significantly associated with a

greater reduction of hs-GH over 12 months of treatment independently of baseline level of hs-GH. Moreover, when all male subjects receiving fluvastatin (metoprolol/fluvastatin and placebo/fluvastatin) were compared with all subjects with no fluvastatin treatment, fluvastatin treatment was also associated with a greater 12 months reduction of hs-GH, suggesting that the fasting value of hs-GH is lowered by treatment with fluvastatin in males. However in this context it should be noted that the change of hs-GH over 12 months in the fluvastatin/placebo-group was not significantly different when compared to the placebo/placebo group.

Table 11: Clinical characteristics of the study population in BCAPS.

Variable	Placebo/Placebo	Metoprolol/Placebo	Fluvastatin/Metoprolol	Fluvastatin/Placebo
Number of participants	117	118	117	120
Female (%)	78 (66.7)	78 (66.1)	70 (59.8)	72 (60.0)
Age, mean (SD), years	61.5 (5.7)	60.3 (5.6)	62.3 (5.0)	61.8 (5.4)
Height, mean (SD), cm	168 (10)	167 (8)	169 (8)	169 (9)
Baseline				
Body Mass Index, Mean (SD), kg/m2	25.5 (3.6)	25.5 (3.6)	25.1 (2.7)	25.7 (3.7)
LDL-C, mean (SD), mmol/L	4.07 (0.86)	4.18 (0.92)	4.15 (0.88)	4.16 (0.82)
HDL-C, mean (SD), mmol/L	1.48 (0.40)	1.39 (0.37)	1.41 (0.35)	1.37 (0.35)
IMT_{cca}, median (IQR)	0.88 (0.78-0.96)	0.88 (0.77-0.99)	0.86 (0.79-0.98)	0.86 (0.78-0.97)
IMT_{bulb}, median (IQR)	1.74 (1.46-2.09)	1.77 (1.49-2.24)	1.84 (1.52-2.29)	1.81 (1.55-2.17)
Growth Hormone - males, geometric mean, 95%CI, pg/mL	240 (150-390)	170 (110-270)	230 (140-380)	190 (130-260)
Growth Hormone - females, geometric mean, 95%CI, pg/mL	1260 (970-1660)	1070 (780-1440)	1080 (840-1390)	1210 (940-1570)
12 months				
LDL-C, mean (SD), mmol/L	4.01 (0.82)	4.17 (0.87)	3.21 (0.85)	3.27 (0.74)
Growth Hormone - males, geometric mean, 95%CI, pg/mL	250 (160-410)	170 (110-280)	220 (150-340)	160 (110-230)
Growth Hormone - females, geometric mean, 95%CI, pg/mL	830 (640-1090)	860 (660-1130)	1090 (820-1460)	810 (610-1070)
36 months				
IMT_{cca}, median (IQR)	0.91 (0.82-1.04)	0.90 (0.83-1.02)	0.87 (0.78-0.98)	0.87 (0.77-0.99)
IMT_{bulb}, median (IQR)	1.98 (1.71-2.35)	1.89 (1.60-2.43)	1.99 (1.60-2.44)	2.02 (1.68-2.30)

Abbreviations: LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; IMT, intima media thickness.
Missing values in IMTbulb: placebo/placebo, n=3; metoprolol/placebo n=3; Fluvastatin/Metoprolol, n=5; fluvastatin/placebo, n=8.
Missing values in HDL-C and LDL-C: placebo/placebo, n=1; metoprolol/fluvastatin n=1.

Table 12: Multiple linear regression models of ΔGH (12 months - baseline) vs different treatment groups.

Gender	Model	Treatment group	B	95%CI	P
ALL	All groups	Metoprolol-Placebo	-0.11	-0.53 to 0.31	0.600
		Fluvastatin-Metoprolol	0.25	-0.17 to 0.67	0.241
		Fluvastatin-Placebo	-0.11	-0.53 to 0.31	0.595
	All Fluvastatin	Fluvastatin	0.12	-0.18 to 0.42	0.423
	All Metoprolol	Metoprolol	0.13	-0.17 to 0.43	0.399
MALE	All groups	Metoprolol-Placebo	-0.26	-0.79 to 0.28	0.342
		Fluvastatin-Metoprolol	-0.64	-1.16 to -0.13	0.015
		Fluvastatin-Placebo	-0.36	-0.88 to 0.15	0.165
	All Fluvastatin	Fluvastatin	-0.37	-0.73 to -0.01	0.046
	All Metoprolol	Metoprolol	-0.27	-0.63 to 0.10	0.150
FEMALE	All groups	Metoprolol-Placebo	-0.06	-0.63 to 0.51	0.842
		Fluvastatin-Metoprolol	0.77	0.185 to 1.35	0.010
		Fluvastatin-Placebo	0.02	-0.56 to 0.59	0.956
	All Fluvastatin	Fluvastatin	0.41	0.00 to 0.83	0.051
	All Metoprolol	Metoprolol	0.33	-0.09 to 0.74	0.119

Models adjusted for: age and standardized values of natural logarithm of hs-GH at baseline. In addition adjusted for sex in the subgroup all.

Three different models are executed: in "all groups" the different treatment groups are each one compared against placebo. In "all fluvastatin" the individuals receiving fluvastatin are compared against individuals not receiving fluvastatin and vice versa in "all metoprolol". B coefficients are expressed as the increment of ΔGH with treatment of the medicine in question as compared to placebo.

Figure description

Fig. 1: shows a typical us-hGH assay dose/ signal curve.

Fig. 2: Kaplan-Meier Plot for efficacy of ACE/ARB treatment on MACE in patients with hGH concentrations in tertile 1.

Fig. 3: Kaplan-Meier Plot for efficacy of ACE/ARB treatment on MACE in patients with hGH concentrations in tertile 2.

Fig. 4: Kaplan-Meier Plot for efficacy of ACE/ARB treatment on MACE in patients with hGH concentrations in tertile 3.

Fig. 5: Kaplan-Meier Plot for efficacy of Beta-blocker treatment on MACE in patients with hGH concentrations in tertile 1.

Fig. 6: Kaplan-Meier Plot for efficacy of Beta-blocker treatment on MACE in patients with hGH concentrations in tertile 2.

Fig. 7: Kaplan-Meier Plot for efficacy of Beta-blocker treatment on MACE in patients with hGH concentrations in tertile 3.

Fig. 8: Kaplan-Meier Plot for efficacy of Beta-blocker or ACE/ARB treatment on MACE in patients with hGH concentrations in tertile 1, 2 and 3, respectively.

Claims

1) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject and
- comparing the determined level of hGH, and/or its isoforms in said blood sample with a pre-determined threshold and
- wherein in case the determined level of hGH, and/or its isoforms is above said pre-determined threshold then the subject is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein in case the determined level of hGH, and/or its isoforms is below said pre-determined threshold then the subject is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) and
- wherein said isoforms of hGH is selected from the group consisting of: hGH isoform 1, hGH isoform 2, hGH isoform 3 and hGH isoform 4.

2) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 1, wherein said subject does not have hypertension (at least >140mmHG (systolic) to 90 mmHG (diastolic)).

3) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 1 or 2 wherein the blood sample is taken from a subject that has not yet treated with antihypertensive drugs.

4) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 1, 2 or 3 wherein reducing the vascular risk by lowering blood pressure in a subject means preventing a major cardiovascular event and/or a cardiovascular disease in said subject.

5) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of claims 1 to 4 wherein said subject has never had a major cardiovascular event and has never had any cardiovascular disease.

6) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the claims 1 to 4 wherein said subject has suffered an acute myocardial infarction or acute heart failure within the last 2 weeks, preferably within the last one week, preferably within the last 36 hours.

7) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the claims 1 to 6 wherein said antihypertensive drug is selected from the group comprising Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), and Beta-Adrenoreceptor Blocker (Beta-blocker), statin or combinations thereof.

8) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the blood sample is selected from the group comprising whole blood, serum, and plasma.

9) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the blood sample is a fasting sample.

10) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the measurement method that is used to determine the level of hGH, and/or its isoforms has a sensitivity of at least 50 pg/ml.

11) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the measurement method that is used to determine the level of hGH, and/or its isoforms is an immuno assay having an assay sensitivity of at least 50 pg/ml.

12) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the pre-determined threshold is 330 pg/ml or above, preferably 1370 pg/ml or above.

13) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according any of the claims 1-12 wherein the level of hGH, and/or its isoforms is determined by an immunoassay that comprises at least two antibodies that bind to hGH, and/or its isoforms.

14) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to claim 11 wherein each of the at least two antibodies that bind to hGH and/or its isoforms have an affinity towards hGH, and/or its isoforms that is at least $10^8 M^{-1}$.

15) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein said method is used for prevention of a major cardiovascular event and/or a cardiovascular disease in said subject selected from the group

comprising heart failure, artherosclerosis, hypertension, cardiomyopathy, myocardial infarction and stroke.

16) Method of determining whether a subject has a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs according to any of the preceding claims wherein the method further comprises:

- recommendation of administration of antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and who is identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs or
- recommendation of withholding with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) to a subject whose determined level of hGH, and/or its isoforms is below said pre-determined threshold and who is identified as not having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs.

17) Use of an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) in the manufacture of a medicament, for preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of any of claims 1-16 as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs.

18) Use of an antihypertensive drug (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) in the manufacture of a medicament, for preventing a major cardiovascular event and/or a cardiovascular disease in a subject whose determined level of hGH, and/or its isoforms is above said pre-determined threshold and wherein said subject is identified according to the methods of any of claims 1-16 as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs wherein said subject does not have hypertension.

19) A method for monitoring the therapy response of a subject at vascular risk that is treated with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) comprising the steps:

- determining the level of hGH, and/or its isoforms in a blood sample of said subject taken at a time point X1 with antihypertensive drugs and
- determining the level of hGH, and/or its isoforms in a blood sample of said subject taken at a time point X2 with antihypertensive drugs, and
- wherein time point X1 is either before the beginning of therapy or after starting the therapy but in any event earlier than time point X2, and
- wherein time point X2 is after starting the therapy and in any event later than time point X1

- comparing the determined level of hGH, and/or its isoforms in said blood sample taken at point X1 with the determined level of hGH, and/or its isoforms in said blood sample taken at time point X2
- wherein a lower level of hGH, and/or its isoforms in said blood sample taken at time point X2 compared to the level of hGH, and/or its isoforms in said blood sample taken at time point X1 indicates a therapy response and
- wherein said isoforms of hGH is selected from the group consisting of: hGH isoform 1, hGH isoform 2, hGH isoform 3 and hGH isoform 4.

20) A method for monitoring the therapy response of a subject at vascular risk that is treated with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) according to claim 19 wherein the determined level of hGH, and/or its isoforms in said blood sample taken at point X1 is above a pre-determined threshold and the subject has been identified as having a vascular risk that can be reduced by blood pressure lowering therapy with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins).

21) A method for monitoring the therapy response of a subject at vascular risk that is treated with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) according to claim 19 or 20 wherein the time point X1 is before starting the therapy.

22) A method for monitoring the therapy response of a subject at vascular risk that is treated with antihypertensive drugs (e.g. Angiotensin converting enzyme inhibitor (ACE inhibitor), Angiotensin Receptor Blocker (ARB), Beta-Adrenoreceptor Blocker (Beta-blocker) and/or statins) according to any of claims 19 to 21 wherein time point X2 is 12 months, 24 Months, or 36 months after time point X1.