Means and methods for predicting renal failure in diabetes patients based on placental growth factor and soluble FLT1

Mittel und Verfahren zur Prognose von Nierenversagen bei Diabetikern auf Basis des plazentalen Wachstumsfaktors und löslichem Flt-1

Moyens et procédés pour prévoir l’insuffisance rénale chez des patients souffrant de diabète basés sur le facteur de croissance placentaire et le FLT1 solubles

Designated Contracting States:
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References cited:
• HEESCHEN C ET AL: "PLASMA LEVELS OF THE SOLUBLE FMS-LIKE TYROSINE KINASE 1 (VEGF RECEPTOR 1) MODULATE THE PROGNOSTIC IMPACT OF PLACENTAL GROWTH FACTOR IN PATIENTS POST MYOCARDIAL INFARCTION" CIRCULATION, AMERICAN HEART ASSOCIATION, DALLAS, TX, vol. 110, no. 17, SUPPL, 10 November 2004 (2004-11-10), page 411,ABSTR.NO.1942, XP009063846 ISSN: 0009-7322
The probability envisaged by the present invention allows that the prediction of an increased, normal or decreased risk, at least 95%, at least 97%, at least 98% or at least 99%. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, statistics for research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. These measures are highly desirable. Suitable therapeutic measures comprise the administration of blood lowering drugs, an altered lifestyle, and nutritional diets. A remaining issue in handling chronic renal failure, however, is its early diagnosis or an individual risk assessment for a given diabetes patient.

Renal failure is a major clinical complication in patients suffering from diabetes mellitus type I. Specifically, patients suffering from diabetes or being at least at risk of developing diabetes have a higher prevalence for developing renal failure than others (see, e.g., Villar 2007, Diabetes Care 30(12):3070-3076; Gilbert 2008, N Engl J Med 358:1628-1630). Renal failure may appear as chronic or acute renal failure. While the acute form of renal failure most often can be addressed only by kidney function replacement measures such as dialysis or transplantation, the chronic form is progressing in a manner which allows for other therapeutic interventions as well. At its end stage, chronic renal failure also requires kidney function replacement measures such as dialysis or kidney transplantation. It is to be understood that these measures in light of the high costs for the health care system and the severe, inconvenient and cumbersome side effects for the patient are to be avoided. Therefore, an early intervention with the progression of renal failure is highly desirable. Suitable therapeutic measures comprise the administration of blood lowering drugs, an altered lifestyle, and nutritional diets. A remaining issue in handling chronic renal failure, however, is its early diagnosis or an individual risk assessment for a given diabetes patient.

In its broadest sense, the present invention encompasses methods and uses as described in the appended claims.

Accordingly, the present invention relates to a method for predicting the risk of developing renal insufficiency for a subject suffering from diabetes mellitus comprising: a. determining the amounts of Placental Growth Factor and soluble Flt 1 (sFlt1) in a blood, plasma or serum sample of a subject suffering from diabetes mellitus; and b. comparing the amounts determined in step a) with reference amounts, whereby the risk of developing renal insufficiency is to be predicted, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 is indicative for an increased risk of developing renal insufficiency.

The method of the present invention is an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate to sample pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually or assisted by automation. Preferably, step (a) and/or (b) may in total or in part be assisted by automation, e.g., by a suitable robotic and sensory equipment for the determination in step (a) or a computer-implemented comparison in step (b).

The term “predicting the risk” as used herein refers to assessing the probability according to which a subject will develop renal failure within a certain time window, i.e. the predictive window. In accordance with the present invention, the predictive window, preferably, is at least 2 years, at least 4 years, at least 6 years, at least 8 years, at least 10 years or at least 12 years. The predictive window, also preferably, will be the entire life span of the subject. However, as will be understood by those skilled in the art, such an assessment is usually not intended to be correct for 100% of the subjects to be diagnosed. The term, however, requires that prediction can be made for a statistically significant portion of subjects in a proper and correct manner. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student’s t-test, Mann-Whitney test etc. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability envisaged by the present invention allows that the prediction of an increased, normal or decreased risk.
will be correct for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population. The term, preferably, relates to predicting an increased risk for renal failure compared to the average risk for developing renal failure in a population of subjects suffering from diabetes.  

[0010] The term "renal failure" as used herein refers to an impaired kidney function. Accordingly, during renal failure the kidney function is significantly decreased as determined by the glomerular filtration rate (GFR) or the protein content of the urine. Further symptoms of renal failure are well known in the art and described in standard text books of medicine. Preferably, a GFR of at least 60 ml/min for three or more months or detectable protein in the urine during said time period are indicative for a chronic renal failure. More preferably, renal failure as used herein is chronic renal failure. It will be understood that severe chronic renal failure requires dialysis or transplantation of the kidney exhibiting the said failure. Accordingly, the method of the present invention can be also applied to predict the need for dialysis or kidney transplantation of a patient. The term "diabetes mellitus" as used herein refers, preferably, to diabetes mellitus type I. The symptoms and clinical parameters associated with diabetes mellitus type I are well known in the art.  

[0011] The term "subject" as used herein relates to animals, preferably mammals, and, more preferably, humans. However, it is envisaged by the present invention that the subject shall be suffering from diabetes mellitus as specified elsewhere herein. Except for the diabetes, the subject shall be, more preferably, apparently healthy, in particular with respect to kidney function.  

[0012] The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, or urine, more preferably, samples of blood, plasma or serum. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, cell-, tissue- or organ samples are obtained from those cells, tissues or organs which express or produce the peptides referred to herein.  

[0013] The term "PIGF (Placental Growth Factor)" as used herein refers to a placenta derived growth factor which is a 149-amino-acid-long polypeptide and is highly homologous (53% identity) to the platelet-derived growth factor-like region of human vascular endothelial growth factor (VEGF). Like VEGF, PIGF has angiogenic activity in vitro and in vivo. For example, biochemical and functional characterization of PIGF derived from transfected COS-1 cells revealed that it is a glycosylated dimeric secreted protein able to stimulate endothelial cell growth in vitro (Maqlione1993, Oncogene 8 (4):925-31). Preferably, P1GF refers to human P1GF, more preferably, to human PIGF having an amino acid sequence as shown in Genebank accession number P49763, GI: 17380553 (Genebank is available from the NCBI, USA under www.ncbi.nlm.nih.gov/entrez) or a variant thereof. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific P1GF. Variants may be allelic variants, splice variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific P1GF or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Such fragments may be, e.g., degradation products of P1GF. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.  

[0014] The term "soluble (s)Flt-1" as used herein refers to polypeptide which is a soluble form of the VEGF receptor FLT1. It was identified in conditioned culture medium of human umbilical vein endothelial cells. The endogenous soluble FLT1 (sFLT1) receptor is chromatographically and immunologically similar to recombinant human sFLT1 and binds [125I] VEGF with a comparable high affinity. Human sFLT1 is shown to form a VEGF-stabilized complex with the extracellular domain of KDR/FK1-k in vitro. Preferably, sFLT1 refers to human sFLT1 or a variant thereof. More preferably, human sFLT1 can be deduced from the amino acid sequence of Flt-1 as shown in Genebank accession number P17948, GI: 125361. An amino acid sequence for mouse sFLT1 is shown in Genebank accession number BAA24499.1, GI: 2809071. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino acid sequence of the specific sFLT1. Variants may be allelic variants, splice variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific sFLT1 or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Such fragments may be, e.g., degradation products of sFLT1. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.  

[0015] Determining the amount of the polypeptides referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the polypeptide based on a signal which is obtained from the polypeptide itself and the intensity of which directly correlates with the number of molecules of the polypeptide present in the sample. Such a signal - sometimes referred to herein as intensity signal -may be obtained, e.g., by
measuring an intensity value of a specific physical or chemical property of the polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of a polypeptide can be achieved by all known means for determining the amount of a polypeptide in a sample. Said means comprise immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative for the presence or absence of the polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse-proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass-spectrometers, NMR- analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on Elecsys™ analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-Hitachi™ analyzers), and latex agglutination assays (available for example on Roche-Hitachi™ analyzers).

Preferably, determining the amount of a polypeptide comprises the steps of (a) contacting a cell capable of eliciting a cellular response the intensity of which is indicative of the amount of the polypeptide with the said polypeptide for an adequate period of time, (b) measuring the cellular response. For measuring cellular responses, the sample or processed sample is, preferably, added to a cell culture and an internal or external cellular response is measured. The cellular response may include the measurable expression of a reporter gene or the secretion of a substance, e.g. a peptide, polypeptide, or a small molecule. The expression or substance shall generate an intensity signal which correlates to the amount of the polypeptide.

Also preferably, determining the amount of a polypeptide comprises the step of measuring a specific intensity signal obtainable from the polypeptide in the sample. As described above, such a signal may be the signal intensity observed at an mass to charge (m/z) variable specific for the polypeptide observed in mass spectra or a NMR spectrum specific for the polypeptide.

Determining the amount of a polypeptide may, preferably, comprises the steps of (a) contacting the polypeptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display. Antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)2 fragments that are capable of binding antigen or hapten. The present invention also includes single chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semiquantitative or quantitative. Suitable methods are described in the following.

First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance.

Second, if the ligand also serves as a substrate of an enzymatic activity of the polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/ polypeptide" complex or the ligand which was bound by the polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the
reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for a detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myctag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels (e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3',5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemiluminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include 35S, 125I, 32p, 33P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling or other detection methods as described above.

[0020] The amount of a polypeptide may be, also preferably, determined as follows: (a) contacting a solid support comprising a ligand for the polypeptide as specified above with a sample comprising the polypeptide and (b) measuring the amount of polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracetes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

[0021] The term "amount" as used herein encompasses the absolute amount of a polypeptide, the relative amount or concentration of the said polypeptide as well as any value or parameter which correlates thereto or can be derived therefrom. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said polypeptides by direct measurements, e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., response levels determined from biological read out systems in response to the peptides or
intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

**[0022]** The term “comparing” as used herein encompasses comparing the amount of the polypeptide comprised by the sample to be analyzed with an amount of a suitable reference source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample. The comparison referred to in step (b) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the amount determined in step a) and the reference amount, it is possible to predict the risk for developing renal failure. Therefore, the reference amount is to be chosen so that either a difference or a similarity in the compared amounts allows identifying those subjects which have an increased, decreased or normal risk for developing renal failure.

**[0023]** Accordingly, the term “reference amounts” as used herein refers to amounts of the polypeptides which allow allocating the individual risk for developing renal failure into either the increased, decreased or normal risk group. Therefore, the reference may either be derived from (i) a subject known to have a decreased or normal risk of developing renal failure or (ii) a subject known to have an increased risk of developing renal failure. Moreover, the reference amounts, preferably, define thresholds. Suitable reference amounts or threshold amounts may be determined by the method of the present invention from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample. The reference amount serving as a threshold is derived from the upper limit of normal (ULN), i.e. the upper limit of the physiological amount to be found in a population of subjects (e.g. patients enrolled for a clinical trial). The ULN for a given population of subjects can be determined by various well known techniques. A suitable technique may be to determine the median of the population for the peptide or polypeptide amounts to be determined in the method of the present invention. The ULN for P1GF referred to herein, preferably, varies between 10 and 20 pg/ml. More preferably, the ULN is between 12 and 15 pg/ml, most preferably, it is 13 pg/ml. The ULN for sFLT1 referred to herein, preferably, varies between 80 and 150 pg/ml. More preferably, the ULN for the said sFLT1 is between 90 and 120 pg/ml, most preferably, it is 95 pg/ml.

**[0024]** In principle, it has been found that with respect to the ULN an increased amount of P1GF and sFLT1 are indicative for a subject suffering from diabetes and having an increased risk for developing renal failure. Thus, in the method of the present invention, with respect to the ULN an increased amount of PLGF and sFLT1 are indicative for said increased risk of developing renal failure.

**[0025]** It has been found in the study underlying the present invention that a combination of P1GF and sFLT1 as biomarkers is required for predicting the risk of developing renal failure in subjects suffering from diabetes in a reliable and efficient manner. Moreover, it has been found that each of said biomarkers is statistically independent from each other. Accordingly, the method of the present invention provides for highly reliable risk stratification. Based on the individual risk, suitable therapeutic measures can be started in order to avoid the severe consequences of progressing chronic renal failure such as dialysis or transplantation. Moreover, the method of the present invention can be implemented even in portable assays, such as test stripes. Therefore, the method is particularly well suited for prediction within large populations of diabetes patients even for ambulant patients. Thanks to the findings of the present invention, the severe consequences of chronic renal failure can be avoided. Moreover, it is possible to predict the need of dialysis or kidney transplantation.

**[0026]** The present invention also refers to a method of determining whether a subject suffering from diabetes mellitus is susceptible to a renal therapy comprising administration of blood pressure lowering drugs, aspirin, statins, ACE inhibitors or angiotensin II receptor blockers, said method comprising: a. determining the amounts of Placental Growth Factor and sFLT1 (soluble Flt1) in a blood, plasma or serum sample of a subject suffering from diabetes mellitus; and b. comparing the amounts determined in step a. with reference amounts, whereby a subject susceptible to a renal therapy is to be determined, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for a subject susceptible to said renal therapy.

**[0027]** The term “renal therapy” as used herein, preferably, relates to therapeutic measures which inhibit progression of renal failure. Said renal therapy comprises administration of blood pressure lowering drugs, most preferably, aspirin, statins, ACE inhibitors and angiotensin II receptor blockers (see Eddy 2005, Advances in Chronic Kidney Diseases 12 (4):353-365). In addition, life style recommendations can be given to a subject and/or nutritional diets, preferably, in combination with glucose level control.

**[0028]** The term “susceptible” as used herein means that a renal therapy applied to the subject will inhibit or at least ameliorate the progression of renal failure or its accompanying symptoms. It is to be understood that the assessment...
for susceptibility for the renal therapy will not be correct for all (100%) of the investigated subjects. However, it is envisaged
that at least a statistically significant portion can be determined for which the renal therapy can be successfully applied.
Whether a portion is statistically significant can be determined by techniques specified elsewhere herein.

[0029] In method, the reference amount is the ULN. Increased amounts of PLGF and sFLT1 with respect to the ULN are
indicated for a subject susceptible to a renal therapy.

[0030] The present disclosure also describes a method for predicting the risk of mortality for a subject suffering from
diabetes mellitus comprising:

a) determining the amounts of PLGF and sFLT1 in a sample of a subject suffering from diabetes mellitus; and
b) comparing the amounts determined in step a) with reference amounts, whereby the risk of mortality is to be
predicted.

[0031] The term "mortality" as used herein encompasses all causes of mortality. Preferably, the term relates to mortality
caued by cardiovascular events.

[0032] In a preferred embodiment of the aforementioned method, the said reference amount is the ULN. More preferably,
increased amounts of PLGF and sFLT1 with respect to the ULN are indicative for an increased risk of mortality.

[0033] The present invention also relates to an in vitro use of a device comprising: a. means for determining the amount
of Placental Growth Factor and sFLT1 (soluble Flt1); and b. means for comparing the amounts determined by the means
of a) with reference amounts, for predicting the risk of developing renal insufficiency for a subject suffering from diabetes
mellitus, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an
increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for an increased risk of developing
renal insufficiency.

[0034] The term "device" as used herein relates to a system of means comprising at least the aforementioned means
operatively linked to each other as to allow the prediction. Preferred means for determining the amount of one of the
aforementioned polypeptides as well as means for carrying out the comparison are disclosed above in connection with
the method of the invention. How to link the means in an operating manner will depend on the type of means included
into the device. For example, where means for automatically determining the amount of the peptides are applied, the
data obtained by said automatically operating means can be processed by, e.g., a computer program in order to obtain
the desired results. Preferably, the means are comprised by a single device in such a case. Said device may accordingly
include an analyzing unit for the measurement of the amount of the polypeptides in an applied sample and a computer
unit for processing the resulting data for the evaluation. The computer unit, preferably, comprises a database including
the stored reference amounts or values thereof recited elsewhere in this specification as well as a computer-implemented
algorithm for carrying out a comparison of the determined amounts for the polypeptides with the stored reference amounts
of the database. Computer-implemented as used herein refers to a computer-readable program code tangibly included
into the computer unit. Alternatively, where means such as test stripes are used for determining the amount of the
peptides or polypeptides, the means for comparison may comprise control stripes or tables allocating the determined
amount to a reference amount. The test stripes are, preferably, coupled to a ligand which specifically binds to the peptides
or polypeptides referred to herein. The strip or device, preferably, comprises means for detection of the binding of said
peptides or polypeptides to the said ligand. Preferred means for detection are disclosed in connection with embodiments
relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the
system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due
to the instructions and interpretations given in a manual. The means may appear as separate devices in such an
embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the
means without further ado. Preferred devices are those which can be applied without the particular knowledge of a
specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may
be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however,
processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices
comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing
the polypeptides referred to herein, Plasmon surface resonance devices, NMR spectrometers, mass- spectrometers etc.)
and/or evaluation units/devices referred to above in accordance with the method of the invention.

[0035] Furthermore, the present invention relates to an in vitro use of a device comprising: a. means for determining
the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and b. means for comparing the amounts determined
by the means of a) with reference amounts, whereby the device is adapted for determining whether the subject is
susceptible to a renal therapy, for determining whether a subject suffering from diabetes mellitus is susceptible to a renal
therapy comprising administration of blood pressure lowering drugs, aspirin, statins, ACE inhibitors or angiotensin II
receptor blockers, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the
ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for a subject susceptible
to renal therapy.
Even further, encompassed by this invention is an in vitro use of a kit comprising: a. means for determining the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and b. means for comparing the amounts determined by the means of a) with reference amounts, for predicting the risk of a subject with diabetes mellitus of developing renal insufficiency, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for an increased risk of developing renal insufficiency.

The present invention also contemplates an in vitro use of a kit comprising: a. means for determining the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and b. means for comparing the amounts determined by the means of a) with reference amounts, for determining whether a subject suffering from diabetes mellitus is susceptible for renal therapy comprising administration of blood pressure lowering drugs, aspirin, statins, ACE inhibitors or angiotensin II receptor blockers, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for a subject susceptible to said renal therapy.

The term "kit" as used herein refers to a collection of the aforementioned means, preferably, provided in separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention. Preferably, the kit comprises instructions for carrying out the said method of the present invention.

The term "kit" as used herein refers to a collection of the aforementioned means, preferably, provided in separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention. Preferably, the kit comprises instructions for carrying out the said method of the present invention.

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Example: PlGF and sFLT1 are predictors for an increased risk of developing renal failure in patients suffering diabetes mellitus type I.

A total of 891 patients suffering from diabetes type I were investigated for blood levels of sFLT1 and P1GF. Blood levels of sFLT1 and P1GF were determined using the commercially available Elecsys Immunoassays from Roche Diagnostics, DE.

Endpoints "all cause mortality" and "renal failure" were determined after 12 years in the present outcome study.

The results of the study are summarized in the following table. Moreover, PlGF, and sFLT1 are statistically independent from each other as shown by linear regression analysis (see Figure).

<table>
<thead>
<tr>
<th>Quartiles</th>
<th>all cause mortality (n=178)</th>
<th>ERSD (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 pg/ml</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>34</td>
<td>9</td>
</tr>
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<td>16</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>31</td>
<td>77</td>
<td>62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SFLT1</th>
<th>all cause mortality (n=178)</th>
<th>ERSD (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>69 pg/ml</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>79</td>
<td>34</td>
<td>18</td>
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<td>95</td>
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<td>15</td>
</tr>
<tr>
<td>169</td>
<td>75</td>
<td>43</td>
</tr>
</tbody>
</table>

Claims

1. A method for predicting the risk of developing renal insufficiency for a subject suffering from diabetes mellitus comprising:
a. determining the amounts of Placental Growth Factor and soluble Flt1 (sFlt1) in a blood, plasma or serum sample of a subject suffering from diabetes mellitus; and
b. comparing the amounts determined in step a) with reference amounts, whereby the risk of developing renal insufficiency is to be predicted, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 is indicative for an increased risk of developing renal insufficiency.

2. The method of claim 1, wherein the subject is apparently healthy with respect to kidney function.

3. A method of determining whether a subject suffering from diabetes mellitus is susceptible to a renal therapy comprising administration of blood pressure lowering drugs, aspirin, statins, ACE inhibitors or angiotensin II receptor blockers, said method comprising:
   a. determining the amounts of Placental Growth Factor and sFLT1 (soluble Flt1) in a blood, plasma or serum sample of a subject suffering from diabetes mellitus; and
   b. comparing the amounts determined in step a) with reference amounts, whereby a subject susceptible to a renal therapy is to be determined, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for a subject susceptible to said renal therapy.

4. The method of claim 3, wherein the subject is apparently healthy with respect to kidney function.

5. The method of any one of claims 1 to 4, wherein the ULN for Placental Growth Factor is 13 pg/ml.

6. The method of any one of claims 1 to 4, wherein the ULN for sFLT1 (soluble Flt1) is 95 pg/ml.

7. In vitro use of a device comprising:
   a. means for determining the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and
   b. means for comparing the amounts determined by the means of a) with reference amounts,
   for predicting the risk of developing renal insufficiency for a subject suffering from diabetes mellitus, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for an increased risk of developing renal insufficiency.

8. In vitro use of a device comprising:
   a. means for determining the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and
   b. means for comparing the amounts determined by the means of a) with reference amounts, whereby the device is adapted for determining whether the subject is susceptible to a renal therapy,
   for determining whether a subject suffering from diabetes mellitus is susceptible to a renal therapy comprising administration of blood pressure lowering drugs, aspirin, statins, ACE inhibitors or angiotensin II receptor blockers, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for a subject susceptible to renal therapy.

9. In vitro use of a kit comprising:
   a. means for determining the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and
   b. means for comparing the amounts determined by the means of a) with reference amounts,
   for predicting the risk of a subject with diabetes mellitus of developing renal insufficiency, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for an increased risk of developing renal insufficiency.

10. In vitro use of a kit comprising:
    a. means for determining the amount of Placental Growth Factor and sFLT1 (soluble Flt1); and
b. means for comparing the amounts determined by the means of a) with reference amounts,

for determining whether a subject suffering from diabetes mellitus is susceptible for renal therapy comprising administration of blood pressure lowering drugs, aspirin, statins, ACE inhibitors or angiotensin II receptor blockers, wherein the reference amount is the Upper Limit of Normal (ULN) and wherein with respect to the ULN an increased amount of Placental Growth Factor and sFLT1 (soluble Flt1) is indicative for a subject susceptible to said renal therapy.

**Patentansprüche**

1. Verfahren zur Vorhersage des Risikos einer an Diabetes mellitus leidenden Person, eine Niereninsuffizienz zu entwickeln, umfassend:
   a. Bestimmen der Mengen des Plazentalen Wachstumsfaktors und des löslichen Flt1 (sFlt1) in einer Blut-, Plasma- oder Serumprobe einer an Diabetes mellitus leidenden Person; und
   b. Vergleichen der in Schritt a) bestimmten Mengen mit Referenzmengen, wobei das Risiko eine Niereninsuffizienz zu entwickeln vorherzusagen ist, wobei die Referenzmenge die obere Grenze des Normalen (ULN) ist und wobei eine hinsichtlich der ULN erhöhte Menge an Plazentalem Wachstumsfaktor und sFlt1 indikativ für ein erhöhtes Risiko ist, eine Niereninsuffizienz zu entwickeln.

2. Verfahren nach Anspruch 1, wobei die Person in Bezug auf die Nierenfunktion scheinbar gesund ist.

3. Verfahren zur Bestimmung, ob eine an Diabetes mellitus leidende Person für eine Nierentherapie, umfassend die Verabreichung von blutdrucksenkenden Medikamenten, Aspirin, Statinen, ACE-Inhibitoren oder Angiotensin II-Rezeptor-Blockern, empfänglich ist, umfassend:
   a. Bestimmen der Mengen des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1) in einer Blut-, Plasma- oder Serumprobe einer an Diabetes mellitus leidenden Person; und
   b. Vergleichen der in Schritt a) bestimmten Mengen mit Referenzmengen, wobei eine Person, die empfänglich für eine Nierentherapie ist, zu bestimmen ist, wobei die Referenzmenge die obere Grenze des Normalen (ULN) ist, und wobei eine hinsichtlich der ULN erhöhte Menge an Plazentalem Wachstumsfaktor und sFLT1 (lösliches Flt1) indikativ für eine Person ist, die empfänglich für die Nierentherapie ist.

4. Verfahren nach Anspruch 3, wobei die Person in Bezug auf die Nierenfunktion scheinbar gesund ist.

5. Verfahren nach einem der Ansprüche 1 bis 4, wobei die ULN für den Plazentalen Wachstumsfaktor 13 pg/ml ist.

6. Verfahren nach einem der Ansprüche 1 bis 4, wobei die ULN für sFLT1 (lösliches Flt1) 95 pg/ml ist.

7. In vitro Verwendung einer Vorrichtung, umfassend:
   a. Mittel zur Bestimmung der Menge des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1); und
   b. Mittel für den Vergleich der durch die Mittel aus a) bestimmten Mengen mit Referenzmengen,
   zur Vorhersage des Risikos einer an Diabetes mellitus leidenden Person, eine Niereninsuffizienz zu entwickeln, wobei die Referenzmenge die obere Grenze des Normalen (ULN) ist, und wobei eine hinsichtlich der ULN erhöhte Menge an Plazentalem Wachstumsfaktor und von sFLT1 (lösliches Flt1) indikativ für ein erhöhtes Risiko ist, eine Niereninsuffizienz zu entwickeln.

8. In vitro Verwendung einer Vorrichtung, umfassend:
   a. Mittel zur Bestimmung der Menge des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1); und
   b. Mittel für den Vergleich der durch die Mittel aus a) bestimmten Mengen mit Referenzmengen, wobei die Vorhersage zur Bestimmung, ob die Person für eine Nierentherapie empfänglich ist, geeignet ist
   zur Bestimmung, ob eine an Diabetes mellitus leidende Person für eine Nierentherapie empfänglich ist, umfassend die Verabreichung von blutdrucksenkenden Medikamenten, Aspirin, Statinen, ACE-Inhibitoren oder Angiotensin II-Rezeptor-Blockern, wobei die Referenzmenge die obere Grenze des Normalen (ULN) ist, und wobei eine hinsichtlich
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...der ULN erhöhte Menge des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1) indikativ für eine Person ist, die empfänglich für eine Nierentherapie ist.

9. In vitro Verwendung eines Kits, umfassend:
   a. Mittel zur Bestimmung der Menge des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1); und
   b. Mittel für den Vergleich der durch die Mittel aus a) bestimmten Mengen mit Referenzmengen,

...für die Vorhersage des Risikos einer Person mit Diabetes mellitus, eine Niereninsuffizienz zu entwickeln, wobei die Referenzmenge die obere Grenze des Normalen (ULN) ist, und wobei eine hinsichtlich der ULN erhöhte Menge des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1) indikativ für ein erhöhtes Risiko ist, eine Niereninsuffizienz zu entwickeln.

10. In vitro Verwendung eines Kits, umfassend:
   a. Mittel zur Bestimmung der Menge des Plazentalen Wachstumsfaktors und von sFLT1 (lösliches Flt1); und
   b. Mittel für den Vergleich der durch die Mittel aus a) bestimmten Mengen mit Referenzmengen,

...zur Bestimmung, ob eine an Diabetes mellitus leidende Person für eine Nierentherapie empfänglich ist, umfassend die Verabreichung von blutdrucksenkenden Medikamenten, Aspirin, Statinen, ACE-Inhibitoren oder Angiotensin II-Rezeptor-Blockern, wobei die Referenzmenge die obere Grenze des Normalen (ULN) ist, und wobei eine hinsichtlich der ULN erhöhte Menge an Plazentalem Wachstumsfaktor und von sFLT1 (lösliches Flt1) indikativ für eine Person ist, die empfänglich für die besagte Nierentherapie ist.

Revendications

1. Procédé pour prédire le risque de développer une insuffisance rénale pour un sujet souffrant d’un diabète sucré comprenant :
   a. la détermination des quantités de facteur de croissance placentaire et de Flt1 soluble (sFlt1) dans un échantillon de sang, plasma ou sérum d’un sujet souffrant d’un diabète sucré ; et
   b. la comparaison des quantités déterminées à l’étape a) avec des quantités de référence, moyennant quoi le risque de développer une insuffisance rénale est à prévoir, dans lequel la quantité de référence est la limite supérieure de la normale (LSN) et dans lequel ce qui concerne la LSN une quantité accrue de facteur de croissance placentaire et de sFLT1 est indicatrice d’un risque accru de développer une insuffisance rénale.

2. Procédé selon la revendication 1, dans lequel le sujet est apparemment en bonne santé en ce qui concerne la fonction rénale.

3. Procédé de détermination si un sujet souffrant d’un diabète sucré est sensible à une thérapie rénale comprenant l’administration de médicaments antihypertenseurs, d’aspirine, de statines, d’inhibiteurs de l’ECA ou d’antagonistes des récepteurs de l’angiotensine II, ledit procédé comprenant :
   a. la détermination des quantités de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) dans le sang, le plasma ou le sérum d’un sujet souffrant de diabète sucré ; et
   b. la comparaison des quantités déterminées à l’étape a. avec des quantités de référence, moyennant quoi un sujet sensible à une thérapie rénale doit être déterminé, dans lequel la quantité de référence est la limite supérieure de la normale (LSN) et dans lequel ce qui concerne la LSN une quantité accrue de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) est indicatrice d’un sujet sensible à ladite thérapie rénale.

4. Procédé selon la revendication 3, dans lequel le sujet est apparemment en bonne santé en ce qui concerne la fonction rénale.

5. Procédé selon l’une quelconque des revendications 1 à 4, dans lequel la LSN pour le facteur de croissance placentaire est de 13 pg/ml.

6. Procédé selon l’une quelconque des revendications 1 à 4, dans lequel la LSN pour le sFLT1 (Flt1 soluble) est de...
7. Utilisation *in vitro* d'un dispositif comprenant :

a. un moyen pour déterminer la quantité de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) ; et  
b. un moyen pour comparer les quantités déterminées au moyen de a) avec des quantités de référence,

pour prédire le risque de développer une insuffisance rénale pour un sujet souffrant d'un diabète sucré, dans laquelle la quantité de référence est la limite supérieure de la normale (LSN) et dans laquelle en ce qui concerne la LSN une quantité accrue de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) est indicatrice d'un risque accru de développer une insuffisance rénale.

8. Utilisation *in vitro* d'un dispositif comprenant :

a. un moyen pour déterminer la quantité de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) ; et  
b. un moyen pour comparer les quantités déterminées au moyen de a) avec des quantités de référence, moyennant quoi le dispositif est adapté pour déterminer si le sujet est sensible à une thérapie rénale,

pour déterminer si un sujet souffrant d'un diabète sucré est sensible à une thérapie rénale comprenant l'administration de médicaments antihypertenseurs, d'aspirine, de statines, d'inhibiteurs de l'ECA ou d'antagonistes des récepteurs de l'angiotensine II, dans laquelle la quantité de référence est la limite supérieure de la normale (LSN) et dans laquelle en ce qui concerne la LSN une quantité accrue de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) est indicatrice d'un sujet sensible à la thérapie rénale.

9. Utilisation *in vitro* d'un kit comprenant :

a. un moyen pour déterminer la quantité de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) ; et  
b. un moyen pour comparer les quantités déterminées au moyen de a) avec des quantités de référence,

pour prédire le risque d'un sujet atteint d'un diabète sucré de développer une insuffisance rénale, dans laquelle la quantité de référence est la limite supérieure de la normale (LSN) et dans laquelle en ce qui concerne la LSN une quantité accrue de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) est indicatrice d'un risque accru de développer une insuffisance rénale.

10. Utilisation *in vitro* d'un kit comprenant :

a. un moyen pour déterminer la quantité de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) ; et  
b. un moyen pour comparer les quantités déterminées au moyen de a) avec des quantités de référence,

pour déterminer si un sujet souffrant d'un diabète sucré est sensible à une thérapie rénale comprenant l'administration de médicaments antihypertenseurs, d'aspirine, de statines, d'inhibiteurs de l'ECA ou d'antagonistes des récepteurs de l'angiotensine II, dans laquelle la quantité de référence est la limite supérieure de la normale (LSN) et dans laquelle en ce qui concerne la LSN une quantité accrue de facteur de croissance placentaire et de sFLT1 (Flt1 soluble) est indicatrice d'un sujet sensible à ladite thérapie rénale.
REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

• US 20060008829 A [0004]
• US 5744305 A [0020]

Non-patent literature cited in the description