



(43) International Publication Date
30 August 2012 (30.08.2012)

(10) International Publication Number
WO 2012/113773 A1

- (51) International Patent Classification:
C12Q 1/48 (2006.01) *G01N 33/74* (2006.01)
- (21) International Application Number:
PCT/EP2012/052905
- (22) International Filing Date:
21 February 2012 (21.02.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
11155457.2 22 February 2011 (22.02.2011) EP
- (71) Applicant (for DE only): **ROCHE DIAGNOSTICS GMBH** [DE/DE]; Sandhofer Strasse 116, 68305 Mannheim (DE).
- (71) Applicant (for all designated States except DE, US): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacher Strasse 124, CH-4070 Basel (CH).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **HESS, Georg** [DE/DE]; Oppenheimer Strasse 82, 55130 Mainz (DE). **HORSCH, Andrea** [DE/DE]; Christian-Morgenstern-Strasse 11, 68259 Mannheim (DE). **ZDUNEK, Dietmar** [DE/DE]; Elly-Ney-Strasse 3, 82327 Tutzing (DE).
- (74) Agent: **DR. HUHN, Michael**; N 4, 1, 68161 Mannheim (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(54) Title: DIAGNOSIS OF ISCHEMIA USING SFLT-1 AND HGF AFTER INTERVENTION AS AN EARLY INDICATOR OF COMPLICATION

(57) Abstract: The present invention relates to the field of laboratory diagnostics. Specifically means and methods for diagnosing complications of procedures such as angiography or angioplasty in a subject are described. Moreover, the subclassification of the type of complication is disclosed. The invention describes the use of hepatocyte growth factor, soluble fms-like tyrosine kinase 1, cardiac troponins, urinary liver type fatty acid binding protein, adiponectin, neutrophil gelatinase associated lipocalin (NGAL), albumin and kidney injury molecule 1 for the aforementioned purposes.



WO 2012/113773 A1

5

Diagnosis of ischemia using sFIT-1 and HGF after intervention as an early indicator of complication

10 The present invention relates to the field of laboratory diagnostics. Specifically means and methods for diagnosing complications of procedures such as angiography or angioplasty in a subject are described. Moreover, the subclassification of the type of complication is disclosed. The invention describes the use of soluble fms-like tyrosine kinase 1, hepatocyte growth factor, cardiac troponins, urinary liver type fatty acid binding protein, neutrophil
15 gelatinase associated lipocalin (NGAL), adiponectin, albumin and kidney injury molecule 1 for the aforementioned purposes.

Workup in clinical practice comprises taking a medical history followed by a physical examination and ordering laboratory tests and assessing their results. In general this leads
20 to a final diagnosis or a working hypothesis which is frequently associated with additional diagnostic procedures including imaging. Imaging might be noninvasive such as in abdominal ultrasound or echocardiography, computed tomography or magnetic resonance imaging although these diagnostic procures might require the application of contrast agents. If contrast agents are applied with high pressure or in high concentrations they
25 might (partially) replace blood and exert toxicity. This is typically the case in angiography where a needle is placed into an artery or a vein and a catheter is guided under x-ray observation to the desired place and then the contrast agent is injected under high pressure in order to obtain the desired information. The contrast agent is then diluted in the blood, which perfuses organs of the body. Typical examples for angiography are coronary
30 angiography in search for chronic artery disease, angiography of the *arteria carotis*, the renal arteries or the peripheral arteries. Once a diagnosis is established, angiography may be followed by angioplasty, e.g. percutaneous coronary intervention (PCI). In case of PCI a balloon is placed at the location of the stenosis covered by a mesh tube. Then the balloon is inflated and the mesh tube (STENT) is placed into the artery wall and the blood flow is
35 restored in case the procedure is successful. When the balloon is inflated, the artery is occluded for several seconds or even minutes. In case the STENT is applied to the carotis artery blood flow is stopped in one carotid artery while the blood flow is unaffected in the

second carotid artery and the *arteriae vertebrales* which are linked through the *circulus arteriosus willisii*. Therefore, the blood flow to the brain is not significantly reduced as long as these arteries are not occluded or subject to significant stenosis. In the renal artery or in a peripheral artery STENT implantation almost always results in stop or significant reduction in blood flow to the organ in question because parallel vessels that could serve as collaterals are not available.

WO 2010/144553 discloses a method for diagnosing if, after a reperfusion of a blood vessel in a subject has occurred, generally after percutaneous coronary intervention (PCI), atherectomy, laser angioplasty, or arterial bypass graft surgery, the said blood vessel reoccludes again. Reocclusion is diagnosed measuring the level of sFLT-1 present in a biological sample of the respective subject, and wherein an increase in the level of sFLT-1 in said subject compared to the level of sFLT-1 in a subject not suffering from reocclusion of a blood vessel indicates reocclusion of a blood vessel in said subject.

In case of intervention an additional risk exists: thrombotic material may be released from the local site and may be transported to a smaller artery where it blocks blood flow and creates damage if left untreated. An early common phenomenon of contrast agent application or possible result of intervention is ischemia. Ischemia however is not easily recognised. In acute kidney injury, for example, ischemia is only recognised by an increase of serum creatinine after several days. Ischemia during cardiac intervention might be recognized by ECG or clinically (chest pain). However, in many cases it may also be asymptomatic.

Thus there is an urgent need to identify complications of angiography with or without intervention and of the administration of contrast agents. In the case of ischemic complications this is particularly important because ischemia may lead to cell death.

Therefore, the present invention provides a method for diagnosing or predicting the complications of a procedure that temporarily impairs blood supply in at least one artery based on the comparison of the amounts of soluble fms-like tyrosine kinase 1 (sFLT-1) or a variant thereof and/or hepatocyte growth factor (HGF) or a variant thereof determined beforehand in a sample of a subject, to at least one reference amount.

The method comprises at least one of the following steps: a) determining the amount of soluble fms-like tyrosine kinase 1 (sFLT-1) or a variant thereof and/or hepatocyte growth factor (HGF) or a variant thereof in a sample of the subject; b) comparing the amount of

the at least one marker determined in step a) to at least one reference amount; and c) diagnosing/predicting the complication of a procedure that temporarily impairs blood supply in at least one artery based on the comparison carried out in step b).

5 It is also provided a method for diagnosing or predicting a complication of a procedure that temporarily impairs blood supply in at least one artery comprising the steps of

a) determining the amount of at least one marker from the group soluble fms-like tyrosine kinase 1 (sFlt-1) or a variant thereof and hepatocyte growth factor (HGF) or a variant
10 thereof, in a sample of the subject;

b) comparing the amount of the at least one marker determined in step a) to a reference amount; and

15 c) diagnosing/predicting the complication of a procedure that temporarily impairs blood supply in at least one artery based on the comparison carried out in step b).

According to the present invention, it is preferred, in one embodiment, to determine the
20 amounts of both sFlt-1 or a variant thereof and of HGF or a variant thereof.

In a further embodiment of the present invention, it is preferred to determine the amount of only one marker from the group sFlt-1 or a variant thereof and of HGF or a variant thereof. If only one of the markers is determined, it is preferred to determine the amount of sFlt-1.

25

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

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a
35 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. The amounts of HGF, sFIT-1 and cardiac troponins are, preferably, determined in blood, serum or plasma samples. The amount of a marker for kidney damage, i.e. U-LFABP, NGAL, KIM-1, adiponectin or albumin, is, preferably, determined in a urine sample.

Preferably, the sample for the determination of HGF and/or sFIT-1 is taken about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours or about 6 hours after the procedure that temporarily impairs blood supply in at least one artery. More preferably the sample is taken about 4 hours after the procedure. Preferably, the sample used for determining the reference amount is taken before, e.g. within about 24 h, preferably within about 12 h, preferably within about 8 h, preferably within about 4 h, preferably within about 1 h before the procedure that temporarily impairs blood supply in at least one artery is initiated or while the procedure is performed.

The subject is, preferably, a human, preferably a woman or a man, who undergoes a procedure that temporarily impairs blood supply in at least one artery. Preferably, prior to carrying out the procedure that temporarily impairs blood flow, the subject suffers from a stenosis, preferably a stenosis of at least one of the following vessels: renal artery, *Arteria mesenterica*, *Arteria hepatica* or *Arteria lienalis*, and in a coronary artery (coronary vessel). The stenosis may have been partly or entirely removed by the procedure that temporarily impairs blood flow, i.e. the subject may still suffer from the stenosis specified beforehand, as the case may be, to the same or a lesser extent, or the stenosis which was the reason for applying the procedure that temporarily impairs blood flow has been entirely removed by the procedure. The coronary vessel may be a small vessel, a medium vessel or a large vessel, e.g. the right coronary artery or the left coronary artery or large branches thereof known to the person skilled in the art. Also preferably, prior to carrying out the procedure that temporarily impairs blood flow, the subject suffers from at least one of the following conditions: peripheral artery disease, *Angina abdominalis*, stable coronary artery disease or stable *Angina pectoris*. More preferably, the procedure that temporarily impairs blood supply in at least one artery is performed in the subject to treat one of the aforementioned conditions. These conditions may have been partly or entirely disappeared after the procedure that temporarily impairs blood flow, i.e. the subject may still suffer from the conditions specified beforehand, as the case may be, to the same or a lesser

extent, or the condition which was the reason for applying the procedure that temporarily impairs blood flow has been entirely removed by the procedure.

The subject is, preferably, stable before the procedure temporarily impairing blood supply in at least one artery in accordance with the invention is performed, meaning that the condition of a stable subject did not deteriorate suddenly before or during the procedure. Preferably, the subject shall not suffer or have suffered from acute, subacute or recent myocardial infarction, unstable angina pectoris or stroke. Moreover, the subject shall preferably not show signs or symptoms of a contrast agent induced anaphylactoid reaction to the contrast agent. The effects of an anaphylactoid reaction are similar to those of an anaphylactic reaction, the main difference being the stimulation of mast cell degranulation. In an anaphylactoid reaction mast cell degranulation is triggered directly without the mediation of further mediators of the immune system.

Preferably, a stable subject is defined by the kinetics of sFIT-1 and/or HGF before the procedure temporarily impairing blood supply in at least one artery is performed. In a stable subject the amount of sFIT-1 or HGF is, preferably, stable until the point in time when the aforementioned procedure is performed or initiated, during a period of time of at least about 4 hours, of at least about 3 hours, of at least about 2 hours, of at least about 1 hour, of at least about 30 minutes or of at least 15 minutes.

An amount of HGF and/or of sFIT-1 indicating a stable subject is an amount that does not increase or decrease in the aforementioned time span by more than about 5 %, by more than about 10 %, by more than about 15 %, by more than about 20 %, by more than about 25 % or by more than about 30 %.

The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

Furthermore, the onset of certain acute diseases or disorders may increase the amounts of the markers of the present invention. When the disease or disorder subsides, the amount decreases again. If the methods of the present invention are performed during a period of increase or decrease of the markers used in the method of the present invention, due to an acute disease or disorder (and not due to the method of the invention temporarily impairing blood supply), the results may be misleading. An increase of the markers due to a preexisting disease may falsely indicate the presence of a complication following the procedure temporarily impairing blood supply in at least one artery in accordance with the

invention. Even when the preexisting disease or disorder subsides, the resulting decreasing levels of the markers may superimpose an increase that is attributable to a complication. This might result in a false negative diagnosis. The kinetic of the increase and the decrease of the markers of the present invention differ. Generally, upon the onset of a disease or disorder they increase more rapidly than they decrease after the disease or disorder subsides.

Accordingly, the subject, preferably, when starting the method temporarily impairing blood supply in accordance with the present invention, is not about to recover from an acute disease or disorder having caused increased amounts of said markers, wherein the acute disease or disorder has occurred within a certain period of time prior starting a procedure temporarily impairing blood supply in accordance with the invention. Said period of time, preferably, is at least about 24 hours, or at least about 48 hours. Acute diseases or disorders that lead to increasing amounts of HGF are, preferably, hepatic diseases, more preferably acute or chronic hepatitis, acute or chronic liver disease and liver cirrhosis. Diseases or disorders that lead to increasing amounts of sFIT-1 are, preferably, acute cardiovascular events and acute infections.

Furthermore, the subject did, preferably, not undergo surgical interventions within about 24 hours, about 48 hours or about 72 hours prior to the performance of the procedure that temporarily impairs blood supply in at least one artery.

If the subject suffers from a disease or disorder that causes permanently increased or decreased amounts of the markers of the present invention, he/she may still be eligible for the method of the present invention. As the diagnosis as well as the identification of a complication of a procedure that temporarily impairs blood supply in at least one artery is preferably based on changes of the amounts of the markers of the present invention, preferably changes of amounts of the markers over time, rather than based on absolute reference amounts, permanent increases or decreases may not mask the changes induced by a complication. However, if a preexisting disease or disorder causes permanently increased amounts of a marker of the present invention, the reference amounts given below, may have to be adjusted because a given absolute increase results in different relative increases dependent on the base amount. Such adjustments can be readily determined by the skilled person.

A disease or disorder that causes a permanent increase or decrease of the markers of the present invention is, preferably, a chronic condition, i.e. it remains stable for an extended

period of time. A “permanent increase or decrease” is, preferably, an increase or decrease that remains unchanged for at least about 1 month, at least about 2 months, at least about 4 months or at least about 6 months.

- 5 In a preferred embodiment of the present invention, subjects suffering from a disease or disorder that causes a permanent increase or decrease of the markers of the present invention, preferably a chronic condition, are excluded from the methods of the present invention.
- 10 In another preferred embodiment of the present invention, subjects suffering from impaired renal function, preferably subjects suffering from renal failure, in particular subjects suffering from chronic and end stage renal failure are excluded from the methods of the present invention. In another preferred embodiment, subjects with renal hypertension are excluded from the methods of the present invention. Preferably, the “subject” as used
- 15 herein excludes subjects suffering from impaired renal function, preferably subjects suffering from renal failure, in particular subjects suffering from chronic and end stage renal failure, more preferably subjects with renal hypertension, most preferably all of the subjects suffering from one of the diseases and conditions mentioned in this sentence. In this context, “renal failure” is regarded as an impaired glomerular filtration rate (GFR)
- 20 lying below the usual ranges of 60 to 120 ml/min, preferably below 60 ml/min. Chronic renal failure is a long-standing, progressive deterioration of renal function which often results in end stage renal failure. End stage renal failure is diagnosed when the GFR reaches a rate of up to about 30 ml/min. GFR is determined by the creatinine clearance, which is known to the person skilled in the art. Subjects with impaired renal function show
- 25 higher levels of troponin I and troponin T than those cited above, due to an impaired clearance of the peptide. The levels vary with the severity of the renal impairment. This may also apply in respect to the further markers used in the context of the present invention; as the case may be, this may occur to a lesser extent than for troponin T and troponin I.

30

The severity of renal impairment is divided into various grades, as displayed below.

- 0: ≥ 90 ml/min
- 1: ≥ 90 ml/min with microalbuminuria
- 2: $\geq 60 - < 90$ ml/min
- 35 3: $\geq 30 - < 60$ ml/min
- 4: $\geq 15 - < 30$ ml/min
- 5: < 15 ml/min

(see National Kidney Foundation, as published in: Am J. Kidney Dis 39 suppl 1, 2002; Clinical Practice Guidelines for chronic kidney disease).

5 The term “procedure that temporarily impairs blood supply in at least one artery” refers to any medical treatment for diagnostic and/or for therapeutic purposes which reduces the blood supply in an artery of a subject. Preferably, the impairment is associated with, preferably caused by or follows, complete or partial filling of the lumen of the artery with a medical device or instrument such as an endoscope or a catheter, by temporarily replacing or diluting the blood in said artery with a fluid or by ligating the artery. All the
10 aforementioned procedures lead to a decreased blood and oxygen supply to those parts (e.g. cells, tissues or organs) of the body that depend on this artery, preferably the parts are located downstream of the site of complete or partial filling of the lumen of the artery.

15 More preferably, procedures that temporarily impair blood supply in at least one artery are intravascular ultrasound, angioplasty, angiography and the peripheral administration of a contrast agent.

The term intravascular ultrasound, as used herein refers to a procedure involving the insertion of a catheter with a miniaturized ultrasound probe into the vessel of a subject.
20 This method of ultrasound is mainly used to determine the amount of atheromatous plaque in an artery. In a clinical setting intravascular ultrasound is mainly used for the examination of vessels which cannot be appropriately visualized by angiography, e.g. due to the presence of several overlapping vessels at one place. In addition to that, intravascular ultrasound has been used in research to verify the results of angiography. Since this
25 procedure involves the introduction of a catheter into an artery, it might lead to a temporary reduction of the amount of blood flowing through the artery.

The term “angioplasty” refers to percutaneous transluminal angioplasty, a procedure for mechanically widening a narrowed blood vessel (stenosis of a blood vessel). A typical
30 cause for the stenosis of a blood vessel is atherosclerosis. A collapsed balloon is passed with the aid of a guide wire to the location of the stenosis. Typically, this happens under X-ray control. At the site of the stenosis the balloon is inflated under high pressure (6 to 20 atmospheres), thus widening the lumen of vessel by pressing the atherosclerotic plaque into the flexible walls of the vessel. This procedure decreases the blood flow through the
35 vessel and, in consequence, decreases the blood supply, as long as the balloon is inflated because the balloon blocks the lumen of the vessel. This may cause ischemia in those tissues that depend on this artery for their oxygen supply.

In order to prevent restenosis of the vessel STENTS are frequently implanted during angioplasty. A STENT is a wire mesh tube which is attached in its collapsed form at the outside of the balloon. Inflation of the balloon causes the inflation of the STENT. After deflation of the balloon the STENT remains inflated in the artery, thus keeping it open. A
5 STENT may be a drug-eluting stent releasing pharmaceuticals that further reduce the risk of restenosis. Suitable pharmaceuticals are those that inhibit tissue growth.

Angioplasty as referred to in the present application may be performed with STENT-implantation or without STENT-implantation.

10 Angioplasty is, preferably, performed in a coronary artery (known as percutaneous coronary intervention (PCI)), in a renal artery or in a peripheral artery.

The term "angiography" refers to a medical imaging technique for visualizing the lumen of
15 a blood vessel, preferably an artery. Access to the subject's blood vessels is typically gained through the femoral artery or the femoral or jugular vein. Under X-ray control a catheter is guided into the blood vessel of interest. Once the catheter is in place, a contrast agent is applied to the vessel, typically under high pressure. Hence, the contrast agent replaces the blood in the artery completely or partially or it dilutes the blood. Due to the
20 different absorption of X-rays by the contrast agent as compared to blood, the lumen of the vessel can then be visualized. Because the contrast agent does not carry oxygen, the tissues supplied by the artery experience a decreased oxygen supply as long as significant amounts of contrast agent are present in the artery. This may cause ischemia of said tissues.

25 Angiography as referred to in the present application is, preferably, performed in a coronary artery, in a renal artery or in a peripheral artery.

The administration of a contrast agent as a part of angiography may also lead to ischemia of the kidney independent of the location of angiography. After the application to the
30 vessel that is to be visualized by angiography, the contrast agent is gradually diluted by the blood. It is excreted by the kidneys into the urine. As a part of the renal clearance of contrast agents their concentration increases in the renal arteries. As a consequence of this concentration process the blood is diluted by contrast agent in the renal arteries. This may result in ischemia of the kidneys.

35 Furthermore, it is also preferred if the subject to which the methods according to the invention is applied is not a female pregnant individual. These individuals are preferably excluded from the methods of the present invention, as it is known to the person skilled in

the art that sFlt-1 amounts/levels can be increased under certain physiological or pathophysiological conditions occurring in pregnancy. For example, it is known that sFlt-1 levels are increased before and at delivery, and also when the female subject suffers from preeclampsia, i.e. in all cases where the placenta is released from the body. Accordingly, in preferred embodiments the subject according to the present invention is not a pregnant woman suffering from preeclampsia or which is close to delivery.

The term “resulting in” or “resulting from” as used herein encompasses a temporal, an association (coincidence) or a causal relationship between two events. E.g. in the case of a blockage of a lumen of a vessel which results in a kidney damage, the term is meant to convey that (i) the blockage is followed by a kidney damage (temporal relationship), or (ii) the blockage is associated in the sense of coinciding with a kidney damage or (iii) blockage causes a kidney damage.

As set forth above, a renal complication of the administration of a contrast agent does not require the direct application of the contrast agent to the kidneys or its direct application to any other organ. It is sufficient that diluted contrast agent is present in the blood of the subject. Therefore, in a preferred embodiment of the present invention the procedure that temporarily impairs blood supply in at least one artery is the peripheral administration of a contrast agent.

The term “peripheral administration of a contrast agent” refers to the administration of a contrast agent to any blood vessel of the subject. Preferably, the contrast agent is administered under low pressure so that it does not dilute or displace the blood at the site of administration sufficiently to result in a complication at this site. The site of administration is, preferably, a small vein of the subject. However, as described above, the renal clearance of the contrast agent may result in a renal complication even though the concentration of the contrast agent in the general circulation of the subject is low. A contrast agent is typically administered peripherally if the blood vessels of the subject are to be visualized by computed tomography (CT). This procedure is known in the art as computed tomography angiography (CTA). It is used to detect atherosclerosis in various vessels of the subject, to examine the pulmonary arteries to rule out pulmonary embolism, to identify aneurisms of the aorta or other major vessels, to detect a dissection of the aorta or to detect thrombosis in the veins. CTA can replace angiography for many diagnostic indications. It is cheaper than angiography and results in less discomfort to the subject.

The term "contrast agent" refers to x-ray absorbing substances that are administered to the blood vessels of a subject to make the vessels visible by x-ray examination or computed tomography. Preferably, a contrast agent as referred to in the present application is an iodinated compound. The x-ray absorbing effect and, thus, the visibility of the blood vessels increase with the iodine content of the contrast agent. The term "contrast agent" encompasses ionic as well as anionic contrast agents. Ionic contrast agents are, preferably, diatrizoic acid, metrizoic acid and loglicic acid. Non-ionic contrast agents are, preferably, Iopamidol, Iohexol, Ioxilan, Iopromide and Iodixanol.

The term "impaired arterial blood supply" refers to a decreased volume of blood that passes the artery in question in a given unit of time and hence, an impaired (in general reduced) supply of blood and of compounds mandatory to maintain metabolism. As compared to blood supply under normal conditions, i.e. without the procedure that temporarily impairs blood supply in at least one artery, blood supply is, preferably, reduced by at least about 50 %, by at least about 65 %, by at least about 80 %, by at least about 90 % or by at least about 95 %. In an embodiment of the present invention, blood supply and/or blood flow is completely inhibited.

In order that the method according to the present invention can be carried out, preferably, the temporary impairment of blood supply in at least one artery lasts up to about 30 s., up to about 1 min., up to about 2 min., up to about 3 min., up to about 4 min., up to about 5 min., up to about 7.5 min. or up to about 10 min. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

The mechanism which results in the temporary impairment of blood supply in at least one artery depends on the procedure applied. In the case of angioplasty with or without STENT-implantation the available lumen of the artery is at least partially obstructed by a solid body, preferably the balloon of the catheter. In the case of angiography, the injected contrast agent displaces or dilutes the blood flowing through the artery. Thus, in the case of angiography the passage of fluid through the vessel is not impaired, but blood is replaced at least partially by contrast agent. Because the contrast agent does not carry oxygen, the injection of contrast agent into an artery has a similar effect as an obstruction of the artery.

A complication resulting from a procedure that temporarily impairs blood supply in at least one artery may be a direct or an indirect complication.

A direct complication is a complication that is caused by the impaired blood supply through the artery where said procedure is performed. Thus, the complication is typically located in the organ that was targeted by the procedure. A direct complication of a procedure that temporarily impairs blood supply in at least one renal artery is, preferably, a renal complication. In the case of PCI with or without STENT-implantation or in the case of coronary angiography, the complication is, preferably, a coronary complication. In the case of a procedure that temporarily impairs blood supply in a peripheral artery, the complication is, preferably, located in the muscle that is supplied by the peripheral artery in question.

An indirect complication is not caused by the impaired blood supply through the artery where the procedure that temporarily impairs blood supply in at least one artery is performed. Any invasive procedure in a blood vessel may cause thrombosis or may dislocate already existing thrombotic material or may release atherosclerotic plaque. However, the release of thrombotic material is typically caused by angioplasty. Thrombotic material may be transported via the bloodstream. As an artery branches in the organ that it supplies, the individual branches of the artery have a smaller diameter than the supplying artery itself. A piece of thrombotic material that is transported in the artery gets stuck once it reaches a vessel of sufficiently small diameter. Thus, the procedure that temporarily impairs blood supply in at least one artery causes an indirect complication in the organ that is targeted by said procedure.

In the case of the administration of a contrast agent during angiography or the peripheral administration of a contrast agent a renal complication may arise independently of the location of the administration of the contrast agent because diluted contrast agent is concentrated in the renal artery. In the present application renal complications of the administration of a contrast agent are considered a subgroup of indirect complications of a procedure that temporarily impairs blood supply in at least one artery of a subject.

The term “complication of a procedure that temporarily impairs blood supply in at least one artery” or, briefly, “complication” refers to any effect of a procedure that temporarily impairs blood supply in at least one artery that is detrimental to the health or well-being of the subject.

The complication is, preferably, an ischemic complication. An ischemic complication is a complication that is characterized by an imbalance of the oxygen supply and the oxygen requirement of a tissue. If the oxygen requirement exceeds the oxygen supply, ischemia is

the consequence. Depending on its severity and duration ischemia may lead to cell death and, thus, tissue damage.

Moreover, the complication is, preferably, classified according to the affected organ. Consequently, a complication as referred to in the present application is, preferably, a cardiac complication, a renal complication or a complication of the skeletal muscle.

A cardiac complication is, preferably caused by coronary angiography or PCI. In the context of the present invention, "cardiac complication" particularly relates to coronary artery disease (CAD), stable angina pectoris SAP, acute coronary syndrome ACS, unstable angina pectoris UAP, myocardial infarction MI (including ST-elevated MI and non-ST-elevated MI), left ventricular dysfunction LVD, heart failure including congestive heart failure CHF, diastolic heart failure, systolic heart failure, or cardiovascular death,

A cardiac complication according to the invention may also be arrhythmia, bradycardia and tachycardia.

A cardiac complication can be systolic or diastolic (i.e. primarily affecting the ejection phase (systole) or the filling phase (diastole) of the affected ventricle).

More particularly, "cardiac complication" relates to ACS, UAP, MI (including ST-elevated MI and non-ST-elevated MI). The aforementioned complications, in an embodiment of the invention, may lead to LVD, heart failure including congestive heart failure CHF, diastolic heart failure, systolic heart failure, or cardiovascular death. Preferably, the cardiac complication is characterized by structural damage to the myocardium due the death of cardiomyocytes. As lost myocardial cells are generally replaced by scar tissue rather than functional cardiomyocytes, cardiac complications may have a lasting effect on the subject. In the case of a cardiac complication the localization of the affected coronary artery by angiography and its reopening by angioplasty may be necessary.

A renal complication is, preferably, caused by angiography or angioplasty of a renal artery or by any administration of a contrast agent. The consequence of a renal complication is, preferably, characterized by an increase of serum creatinine about 12 to 24 hours after the administration of the contrast agent. Typically, a renal complication only has a transient effect. In severe cases hemodialysis as renal replacement therapy may become necessary for a certain duration.

In the context of the present invention, the term “renal complication” relates to any damage or disease, reversible and non-reversible, of the kidney or parts of the kidney, e.g. reversible and non-reversible glomerular and tubular damage. Tubular damage may be associated with tubular repair. The kidney damage or repair may occur without acute kidney injury AKI, or kidney damage may be associated with AKI. In AKI serum creatinine levels rise slowly and it may take 2 – 3 days before kidney injury becomes apparent, in general indicated by an increase in creatinine of at least 0.3 mg/dl or an increase of more than 50 % from baseline (Devarajan, Expert Opinion Med Diagn 2008, 2: 387 – 398). Finally, AKI may lead to chronic renal failure (i.e. the renal complication according to the invention then is kidney damage with chronic renal failure). A definition for renal failure is found elsewhere in the specification.

A complication of the skeletal muscle is, preferably, caused by angiography or angioplasty of a peripheral artery. Preferably, it is associated with the death of myocytes in the affected muscle.

WO 2010/144553 discloses a method for diagnosing if, after a reperfusion of a blood vessel in a subject has occurred, generally after thrombolytic therapy (i.e administration of a thrombolytic agent known to the person skilled in the art, e.g. heparin, streptokinase, urokinase), percutaneous coronary intervention (PCI), atherectomy, laser angioplasty, or arterial bypass graft surgery, the said blood vessel reoccludes again. This a well-known phenomenon (which is also referred to as “reinfarction”) occurring after therapeutic measures, preferably the above-cited measures, have been undertaken to reperfuse a vessel, is well known to the person skilled in the art, and is limited to the heart. Vessel reperfusion is in general carried out after a cardiovascular conditions causing an occlusion of the vessel has occurred. In case a stent has been inserted to reperfuse a vessel, reocclusion (reinfarction or “stent thrombosis”) may occur with 24 h (acute). from 24 h – 30 days (subacute), from 30 days – 1 year (late) and after 1 year (very late). It is furthermore known that with drug eluting stents, frequently very late stent thrombosis is observed, about 0.2 – 0.5 % per year. In case of death of the respective individual, the suspected underlying cause of reinfarction is regarded as definite if the subject suffered from ACS symptoms and the cause was confirmed pathologically and by angiography. Reinfarction is regarded as the probable cause if unexplained death occurs within 30 days, and as a possible cause if unexplained death occurs after 30 days.

In WO 2010/144553, reocclusion is diagnosed by carrying out the steps of: (a) obtaining a biological sample from said subject, wherein said biological sample is obtained after

reperfusion of a blood vessel; and (b) measuring the level of sFLT-1 present in said biological sample, wherein an increase in the level of sFLT-1 in said subject compared to the level of sFLT-1 in a subject not suffering from reocclusion of a blood vessel indicates reocclusion of a blood vessel in said subject. In an embodiment, a further marker from
5 annexin V, f3-enolase, cardiac troponin I, cardiac troponin T, 20 creatine kinase-MB, glycogen phosphorylase-BB, heart-type fatty acid binding protein, C-reactive protein, growth differentiation factor 15, phosphoglyceric acid mutase-MB, S-100ao, myoglobin, actin, myosin, and lactate dehydrogenase The said cardiovascular condition include acute coronary syndrome, atherosclerosis, transient ischemic attack, systolic dysfunction,
10 diastolic dysfunction, aneurysm, aortic dissection, myocardial ischemia, acute myocardial infarction (AMI), acute ST-segment elevation myocardial infarction (STEMI), acute non-ST-segment elevation myocardial infarction (NSTEMI), angina pectoris, unstable angina (UA), and stable angina (SA), myocardial infarction, congestive heart failure, dilated congestive cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, cor
15 pulmonale, arrhythmia, valvular heart disease, endocarditis, pulmonary embolism, venous thrombosis, peripheral vascular disease, and peripheral artery disease. By "reocclusion" or "restenosis" is meant the reoccurrence of stenosis (i.e., narrowing) of a blood vessel, leading to restricted blood flow. For example, reocclusion may pertain to a blocked or narrowed artery that has been treated to clear the blockage or occlusion and that has
20 subsequently become reoccluded. Reocclusion is defined as a reduction in the circumference of the lumen of the blood vessel by, 25 e.g., 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100%. Alternatively, reocclusion may refer to stenosis that results in reduced organ perfusion. Reocclusion may occur in a subject with, e.g., a cardiovascular condition. The disclosure of WO 2010/144553 is restricted to the
25 said conditions and the diagnosis of reocclusion.

WO 2010/144553 diagnoses a complication occurring after reperfusion is attained in a subject, i.e. at a later point in time, and wherein the complication is not related to the reperfusion. The complication is restricted to reocclusion of a cardiac vessel (reinfarction),
30 preferably occurring after a therapeutic method, in particular thrombolytic therapy (i.e. administration of a thrombolytic agent known to the person skilled in the art, e.g. heparin, streptokinase, urokinase), percutaneous coronary intervention (PCI), atherectomy, laser angioplasty, or arterial bypass graft surgery. In contrast to WO 2010/144553, the present invention is directed towards the diagnosis of a complication occurring as a consequence of
35 reperfusion, i.e. following the reperfusion and being related to it.

The present invention does not include the teachings of WO 2010/144553 as laid out beforehand. In particular, the method of the present invention does not include diagnosing reocclusion of a blood vessel after reperfusion of said blood vessel in a subject by determining the amounts of sFlt-1 in said subject after reperfusion of the said vessel, and
5 wherein reperfusion is attained by at least one of the methods of thrombolytic therapy, percutaneous coronary intervention (PCI), atherectomy, laser angioplasty, and arterial bypass graft surgery. In a further embodiment, the present invention does not comprise diagnosing reocclusion of a blood vessel after reperfusion of said blood vessel occurs following an intervention, preferably a therapeutic intervention, on the respective subject.

10 The term “diagnosing” refers to the process of assessing whether a subject suffers from a complication of a procedure that temporarily impairs blood supply in at least one artery. The term “diagnosing” may also refer to classifying or assessing of a complication according to the organ that is affected and mechanism underlying the complication (direct
15 or indirect complication). The process of classifying/assessing a complication is also referred to as “identifying the complication of the procedure that temporarily impairs blood supply in at least one artery”.

On the other hand, it is to be understood that increased amounts of the markers of the
20 present invention may precede the onset of actual damage caused by the procedure. Thus, in a further embodiment the present invention provides a method for predicting a complication of a procedure that temporarily impairs blood supply in at least one artery. The term “predicting” or “prediction” as used in the present application refers to assessing or identifying the risk of the future onset of a complication. The term “predicting” also
25 includes those cases in which the increase of the markers of the present invention occurs prior to the onset of organ damage caused by the procedure that temporarily impair blood supply in at least one artery.

It is to be understood that the diagnosis/prognosis according to the present invention may
30 not be correct for all subjects. However, the diagnosis shall be correct for a statistically significant proportion of subjects. Whether an proportion 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.. 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 treatment shall be effective 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 "soluble fms-like tyrosine kinase 1", briefly "sFIT-1", as used herein refers to polypeptide which is a soluble form of the VEGF receptor FIT-1. It was identified in conditioned culture medium of human umbilical vein endothelial cells. The endogenous soluble FIT-1 (sFIT-1) receptor is chromatographically and immunologically similar to recombinant human sFIT-1 and binds VEGF with a comparable high affinity. Human sFIT-1 has been shown to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro. Preferably, sFIT-1 refers to human sFIT-1. More preferably, human sFIT-1 can be deduced from the amino acid sequence of FIT-1 as shown in Genebank accession number P17948, GI: 125361. An amino acid sequence for mouse sFIT-1 is shown in Genebank accession number BAA24499.1, GI: 2809071. Due to its binding to VEGF sFIT-1 inhibits VEGF-mediated angiogenesis. sFIT-1 has been shown to be a marker for chronic ischemia in subjects suffering from coronary artery disease (EP 10156911.9). In subjects with acute coronary syndromes the increase of sFIT-1 precedes the onset of cardiomyocyte death as indicated by an increase of sFIT-1 followed by an increase of troponins in case of substantial myocyte death (EP 10156911.9).

The biological property of sFIT-1 is, preferably, its ability to form a VEGF-stabilized complex with KDR/Flk-1 in vitro.

The term "sFIT-1" also refers to variants of the above described sFIT-1 peptide. The term "variant" encompasses variants of the specific sFIT-1 peptide of the present application having at least the same essential biological and immunological properties as sFIT-1. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing sFIT-1. 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of sFlt-1, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of sFlt-1 or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

Hepatocyte growth factor (HGF) was first identified in 1984 and 1985 and purified as a potent mitogen of primary cultured hepatocytes. HGF is single-chain precursor form, and further processing by serine proteases into the two-chain form is coupled to its activation. Serine proteases responsible for the activation of HGF include HGF activator or HGF converting enzyme and urokinase-type plasminogen activator (uPA). The receptor for HGF was identified as a c-met proto-oncogene product. The c-Met receptor is composed of a 50-kDa a-chain and 145-kDa h-chain. Binding of HGF to the c-Met receptor induces activation of tyrosine kinase, resulting in subsequent phosphorylation of C-terminally clustered tyrosine residues. HGF has an organotrophic role in the regeneration and protection of various organs, including the liver, lung, stomach, pancreas, heart, brain, and kidney. Hepatocyte growth factor regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signalling cascade after binding to the proto-oncogenic c-Met receptor. Hepatocyte growth factor is secreted by mesenchymal cells and acts as a multi-functional cytokine on cells of mainly epithelial origin. Its ability to stimulate mitogenesis, cell motility, and matrix invasion gives it a central role in angiogenesis (e.g. following ischemia), tumorigenesis, and tissue regeneration. It is secreted as a single inactive polypeptide and is cleaved by serine proteases into a 69-kDa alpha-chain and 34-kDa beta-chain. A disulfide bond between the alpha and beta chains produces the active, heterodimeric molecule. The protein belongs to the plasminogen subfamily of S1 peptidases but has no detectable protease activity. Alternative splicing of this gene produces multiple transcript variants encoding different isoforms that are also

encompassed by the term “HGF”. An amino acid sequence for mouse HGF is shown in Genebank accession number NP034557.2, GI: 46048249. An amino acid sequence for human HGF is shown in Genebank accession number NP000592.3 GI:33859835. Preferably, HGF refers to human HGF.

5

Preferably, the biological property of HGF is the ability to bind to the proto-oncogenic c-Met receptor.

The term “HGF” also refers to variants of the above described HGF peptide. The term “variant” encompasses variants of the specific HGF peptide of the present application having at least the same essential biological and immunological properties as HGF. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing HGF. 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of HGF, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of HGF or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

25

The term “cardiac Troponin” refers to all Troponin isoforms expressed in cells of the heart and, preferably, the subendocardial cells. These isoforms are well characterized in the art as described, e.g., in Anderson 1995, Circulation Research, vol. 76, no. 4: 681-686 and Ferrieres 1998, Clinical Chemistry, 44: 487-493. Preferably, cardiac Troponin refers to Troponin T and/or Troponin I, and, most preferably, to Troponin T. It is to be understood that isoforms of Troponins may be determined in the method of the present invention

30

together, i.e. simultaneously or sequentially, or individually, i.e. without determining the other isoform at all. Amino acid sequences for human Troponin T and human Troponin I are disclosed in Anderson, loc cit and Ferrieres 1998, Clinical Chemistry, 44: 487-493.

- 5 Preferably the biological property of troponin I and its variant is the ability to inhibit actomyosin ATPase or to inhibit angiogenesis in vivo and in vitro, which may e.g. be detected based on the assay described by Moses et al. 1999 PNAS USA 96 (6): 2645-2650). Preferably the biological property of troponin T and its variant is the ability to form a complex with troponin C and I, to bind calcium ions or to bind to tropomyosin, 10 preferably if present as a complex of troponin C, I and T or a complex formed by troponin C, troponin I and a variant of troponin T.

The term “cardiac troponin” also refers to variants of the above described troponins. The term “variant” encompasses variants of the specific troponins of the present application 15 having at least the same essential biological and immunological properties as the troponins. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing cardiac troponins. Moreover, it is to be understood that a variant as referred to in accordance with 20 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino 25 sequence of the cardiac troponin, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the cardiac troponin or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

The term “kidney injury molecule-1” (KIM-1) relates to a type 1 membrane protein containing a unique six-cysteine Ig domain and a mucin domain in its extracellular portion. KIM-1 which is the sequence of rat 3-2 cDNA contains an open reading frame of 307 amino acids.

5 The protein sequence of human cDNA clone 85 also contains one Ig, mucin, transmembrane, and cytoplasmic domain each as rat KIM-1. All six cysteines within the Ig domains of both proteins are conserved. Within the Ig domain, the rat KIM-1 and human cDNA clone 85 exhibit 68.3% similarity in the protein level. The mucin domain is longer, and the cytoplasmic domain is shorter in clone 85 than rat KIM-1, with similarity of 49.3
10 and 34.8% respectively. Clone 85 is referred to as human KIM-1 (for the structure of KIM-1 proteins see e.g. Ichimura et al., J Biol Chem, 273 (7), 4135-4142 (1998), in particular Fig. 1). Recombinant human KIM-1 exhibits no cross-reactivity or interference to recombinant rat- or mouse-KIM-1.

15 KIM-1 mRNA and protein are expressed in high levels in regenerating proximal tubule epithelial cells which cells are known to repair and regenerate the damaged region in the postischemic kidney. KIM-1 is an epithelial cell adhesion molecule (CAM) up-regulated in the cells, which are dedifferentiated and undergoing replication after renal epithelial injury.
20 A proteolytically processed domain of KIM-1 is easily detected in the urine soon after acute kidney injury (AKI) so that KIM-1 performs as an acute kidney injury urinary biomarker (Expert Opin. Med. Diagn. (2008) 2 (4): 387-398).

25 Preferably, the biological property of KIM-1 is its ability to induce cellular dedifferentiation and replication after renal epithelial injury.

The term “KIM-1” also refers to variants of the above described KIM-1 peptide. The term “variant” encompasses variants of the specific KIM-1 peptide of the present application having at least the same essential biological and immunological properties as KIM-1. In particular, they share the same essential biological and immunological properties if they
30 are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing KIM-1. 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
35 substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least

about 97%, at least about 98%, or at least about 99% identical with the amino sequence of KIM-1, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of KIM-1 or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

The term „albumin“ refers to a to a globular protein mainly found in blood. They reach a concentration of 3.5 g/dl to 4.5 g/dl and represent approximately 60 % of the plasma proteins. Albumin is, preferably, human albumin. Mature human albumin comprises 585 amino acids and has a molecular weight of approximately 66,470 Da. The preproprotein has, preferably, an amino acid sequence as defined by the NCBI reference sequence NP_000468.1.

Albumin plays an important role in maintaining the colloid osmotic pressure of the blood, transports free fatty acids, thyroid hormones, unconjugated bilirubin and many drugs. Moreover, it buffers the pH of the blood.

Preferably, the biological activity of albumin is the ability to bind fatty acids.

The term “albumin” also refers to variants of the above described albumin peptide. The term “variant” encompasses variants of the specific albumin peptide of the present application having at least the same essential biological and immunological properties as albumin. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing albumin. 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of albumin, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of albumin or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

The term “neutrophil gelatinase-associated Protein” (NGAL) refers to a protein having a molecular mass of 25 kDa in its glycosylated form and approximately 21 kDa in its deglycosylated form. It comprises 178 amino acids and has an amino acid sequence as described by Kjeldsen et al. in 1993 (Journal of Biological Chemistry, 268: 10425-10432). It is sometimes found as a heterodimer with human neutrophil gelatinase (matrix metalloproteinase 9). Some evidence indicates that binding of NGAL prevents the degradation of matrix metalloproteinase 9 (Yan et al., 2001, Journal of Biological Chemistry, 276: 37258-37265). The expression of NGAL is known to be up-regulated in subjects with acute renal dysfunction, especially after renal ischemic injury (Wagener et al., 2006, Anesthesiology, 105: 485-491).

Preferably, the biological property of NGAL is the prevention the degradation of matrix metalloproteinase 9 bund to NGAL (Yan et al., 2001, loc. cit.).

The term “NGAL” also refers to variants of the above described NGAL peptide. The term “variant” encompasses variants of the specific NGAL peptide of the present application having at least the same essential biological and immunological properties as NGAL. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing NGAL. 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of NGAL, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of NGAL or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

Adiponectin is a polypeptide (one of several known adipocytokines) secreted by the adipocyte. In the art, adiponectin is frequently also referred to as Acrp30 and apM1. Adiponectin has recently been shown to have various activities such as anti-inflammatory, antiatherogenic, preventive for metabolic syndrome, and insulin sensitizing activities.

Adiponectin is encoded by a single gene, and has 244 amino acids, its molecular weight is approximately 30 kilodaltons. The mature human adiponectin protein encompasses amino acids 19 to 244 of full-length adiponectin. A globular domain is thought to encompass amino acids 107 – 244 of full-length adiponectin. The sequence of the adiponectin polypeptide is well known in the art, and, e.g., disclosed in WO/2008/084003.

Preferably, the biological property of adiponectin is the sensitization of cells for the effects of insulin.

The term “adiponectin” also refers to variants of the above described adiponectin peptide. The term “variant” encompasses variants of the specific adiponectin peptide of the present application having at least the same essential biological and immunological properties as adiponectin. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing adiponectin. 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of adiponectin, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of adiponectin or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

The term “liver-type fatty acid binding protein” (L-FABP, frequently also referred to as FABP1 herein also referred to as liver fatty acid binding protein) relates to a polypeptide being a liver type fatty acid binding protein and to a variant thereof. Liver-type fatty acid binding protein is an intracellular carrier protein of free fatty acids that is expressed in the proximal tubules of the human kidney. For a sequence of human L-FABP, see e.g. Chan et al.: Human liver fatty acid binding protein cDNA and amino acid sequence, Functional and evolutionary implications, J. Biol. Chem. 260 (5), 2629-2632 (1985) or GenBank Acc. Number M10617.1.

As L-FABP is preferably determined in a urine sample of the respective subject, it may also be referred to, in the context of the present invention, as “urinary liver-type fatty acid binding protein” or “U-LFABP”.

- 5 The term “L-FABP”, preferably, does not include heart FABP, brain FABP and intestine FABP.

Preferably, the biological activity of LFABP is the binding of fatty acids.

- 10 The term “L-FABP” also refers to variants of the above described L-FABP peptide. The term “variant” encompasses variants of the specific L-FABP peptide of the present application having at least the same essential biological and immunological properties as L-FABP. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification,
15 e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing L-FABP. 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 about 50%, at least about 60%, at least about 70%, at
20 least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of L-FABP, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of L-FABP or the aforementioned types of
25 variants as long as these fragments have the essential immunological and biological properties as referred to above.

- The term “creatine kinase” (CK) refers to an enzyme that converts creatine to phosphocreatine. Phosphocreatine serves as a buffer for ATP in cells with a high turnover
30 of ATP such as neurons in the brain, skeletal muscle cells, photoreceptor cells of the retina or spermatozoa. The cytosolic CK consists of a dimer. These dimers may be homodimers made up from creatine kinase, brain (CKB) or creatine kinase, muscle (CKM). It may also be a heterodimer consisting of one molecule CKB and one molecule CKM. In the context of the present invention, creatine kinase is, preferably, the homodimer consisting of two
35 molecules CKM. Preferably, CKM has an amino acid sequence as defined by NCBI sequence NP_001815.

Preferably, the biological activity of creatine kinase is the transfer of a phosphate residue from ATP to creatine.

The term "CK" also refers to variants of the above described CK peptide. The term "variant" encompasses variants of the specific CK peptide of the present application having at least the same essential biological and immunological properties as CK. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing CK. 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 about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of CK, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of CK or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above.

Determining the amount of sF1T-1, or a cardiac troponin, preferably troponin T, of HGF, creatine kinase, U-LFABP, KIM-1, adiponectin or albumin or any other peptide or polypeptide or protein referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. The terms polypeptide and protein are used interchangeable throughout this application. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide 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 peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or 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 peptide or polypeptide can be achieved by all known means for determining the amount of a peptide 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 peptide or 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 peptide or 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 ElecsysTM analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-HitachiTM analyzers), and latex agglutination assays (available for example on Roche-HitachiTM analyzers).

Preferably, determining the amount of a peptide or 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 peptide or polypeptide with the said peptide or 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 peptide or polypeptide.

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

Determining the amount of a peptide or polypeptide may, preferably, comprise the steps of (a) contacting the peptide 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 peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or 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 term "antibody" refers to an antibody binding to a peptide selected from the group consisting of sFlt-1, HGF, a cardiac troponin, U-LFABP, KIM-1, NGAL, adiponectin, albumin or creatine kinase. Preferably, the ligand or agent binds specifically to the peptide or 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 peptide or 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 semi-quantitative 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 peptide or 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/peptide or polypeptide” complex or the ligand which was bound by the peptide or 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 labelled 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. Labelling may be done by direct or indirect methods. Direct labelling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labelling 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, digoxigenin, His-Tag, Glutathion-S-
5 Transferase, FLAG, GFP, myc-tag, 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
10 ("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), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate,
15 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 coloured reaction product, fluorescence or chemoluminescence, 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
20 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
25 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
30 immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFA), 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 labelling or other detection methods as described above.

5 The amount of a peptide or polypeptide may be, also preferably, determined as follows: (a) contacting a solid support comprising a ligand for the peptide or polypeptide as specified above with a sample comprising the peptide or polypeptide and (b) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is
10 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, duracytes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to
15 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
20 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 labelled, carrying different ligands. Methods of producing such
25 arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

Preferably, the amounts of a cardiac troponin, sFIT1, HGF, creatine kinase and, as the case may be, the amounts of other peptides measured in the context of the present invention are
30 determined in a blood sample, e.g., a serum or plasma sample, obtained from a subject as defined in the present invention. In the case of U-LFABP, KIM-1, adiponectin, albumin and NGAL the amount is, preferably in the urine of the subject. Preferably, such a determination is done by ELISA. Such a determination of sFIT-1 by ELISA can be done,

e.g., by using the ELECSYS sFIT-1 test by Roche Diagnostics, Mannheim, Germany. The amount of troponin T can be determined by the COBAS assay, Roche Diagnostics Mannheim, Germany. The amount of HGF can be determined with the Quantikine human HGF assay by R&D systems, Minneapolis, USA. The amount of U-LFABP can be determined with the L-FABP ELISA-Kit from CMIC Co., Ltd, Japan. The amount of KIM-1 can be determined with the the Human KIM-1 (catalogue number DY 1750) ELISA Development kit from R&D-Systems. The amount of (multimeric) adiponectin can be determined by using the test EIA from ALPCO diagnostics® (USA).

The term “amount” as used herein encompasses the absolute amount (e.g., of sFIT-1, a cardiac troponin, HGF, creatine kinase, KIM-1, albumin, NGAL, adiponectin or U-LFABP), the relative amount or concentration (e.g., of sFIT-1, a cardiac troponin, HGF, creatine kinase, KIM-1, albumin, NGAL, adiponectin or U-LFABP) as well as any value or parameter which correlates thereto. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said peptides 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., expression amounts 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.

The term “comparing” as used herein encompasses comparing the amount of the peptide, polypeptide, protein comprised by the sample to be analyzed with an amount of a 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 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(s) determined in step a) to reference amount(s), it is possible to diagnose a complication of a procedure that temporarily impairs blood supply

in at least one artery. It is to be understood that amounts of sFIT-1 or HGF as determined in step (a) of the methods of the presents invention are compared in step (b) to reference amounts for sFIT-1 or HGF as specified elsewhere in this application. Similarly, the amounts of the cardiac troponin, creatine kinase, KIM-1, adiponectin, albumin, NGAL or U-LFABP are compared to reference amounts of the cardiac troponin, creatine kinase or U-LFABP.

According to the method of the present invention a measured amount of one of the markers of the present invention indicates a complication of the procedure that temporarily impairs blood supply in at least one artery if it is increased with respect to a reference amount. The term "increased amount" is known to the person skilled in the art. The person skilled in the art is able to determine actual values/amounts for the relevant biochemical markers which correspond to increased amounts/values.

In a preferred embodiment of the present invention, the reference amount of a marker of the present invention is the amount of the marker that is determined in a sample of the subject that is taken before the procedure is performed. Said amount is also referred to as "baseline amount". The sample for the determination of the baseline amount is, preferably, taken immediately before the procedure that temporarily impairs blood supply in at least one artery is initiated. More preferably, it is taken not more than 60 minutes, not more than about 30 minutes or not more than about 15 before said procedure is initiated.

In a further embodiment, the actual amount for an "increased amount" can be defined according to percentiles of the amounts observed in sample of a representative cohort of individuals who did not suffer from a complication of the procedure that temporarily impairs blood supply in at least one artery (preferably, the cohort comprises at least 100, more preferably at least 500, most preferably at least 1000 individuals). A non-increased level may correspond to the maximum level observed in e.g. the 90%, the 95%, the 97.5%, the 98% or even the 99%percentile of said group of individuals. Conversely, an increased amount corresponds to amounts above said maximum levels.

Alternatively, a "non increased amount" may be determined by referring to amounts known in the state of the art as "normal ranges". The increased amount may also be determined or further refined by studies performed on individuals undergoing a procedure that temporarily impairs blood supply in at least one artery and correlating any complications of said procedure with the amounts observed in the individuals. Such studies may also allow tailoring the reference amounts according to certain subject sub-groups,

e.g. subjects with known coronary artery disease, elderly subjects, subjects with renal disease or apparently healthy individuals. Guidance on how such studies may be carried out can also be obtained from the Examples included in this specification.

- 5 The value of the amounts considered as "increased" may also be chosen according to the desired sensitivity or specificity (stringency) of exclusion. The higher the desired sensitivity, the lower is the specificity of exclusion and vice versa. In the above example, the higher the percentile chosen to determine an "increased amount", the more stringent is the exclusion criterion, i.e. less individuals would be considered as having suffered from a
- 10 complication of the procedure that temporarily impairs blood supply in at least one artery.

Further below, examples for actual reference amounts are provided for sFlt-1 and HGF. It is evident, that the levels reference amounts below can serve only as a first classification of the risk of an individual. For example, the risk may also dependent on the general health

15 status of the individual.

With respect to sFlt-1 and HGF, the term "reference amount" refers to an amount of sFlt-1 or HGF that allows the identification of a subject having suffered from a complication of the procedure that temporarily impairs blood supply in at least one artery.

20

With respect to a cardiac troponin, creatine kinase, U-LFABP, NGAL, KIM-1, adiponectin and albumin, the term "reference amount", preferably, refers to an amount of creatine kinase, U-LAFBP, NGAL, KIM-1, albumin or adiponectin, respectively, that allows the identification of a complication as a renal complication, an amount of creatine kinase that

25 allows the identification of a complication as a complication of the skeletal muscle or an amount of the cardiac troponin that allows the identification of the complication as a cardiac complication.

The sensitivity and specificity of a diagnostic and/or prognostic test depends on more than

30 just the analytical "quality" of the test – they also depend on the definition of what constitutes an abnormal result. In practice, Receiver Operating Characteristic curves, or "ROC" curves, are typically calculated by plotting the value of a variable versus its relative frequency in "normal" and "disease" populations. For any particular marker of the invention, a distribution of marker amounts for subjects with and without a disease will

35 likely overlap. Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap indicates where the test cannot distinguish normal from disease. A threshold is selected, above which (or below which,

depending on how a marker changes with the disease) the test is considered to be abnormal and below which the test is considered to be normal. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification of a condition. ROC curves can be used even when test results don't necessarily give an accurate number. As long as one can rank results, one can create an ROC curve. For example, results of a test on "disease" samples might be ranked according to degree (say 1=low, 2=normal, and 3=high). This ranking can be correlated to results in the "normal" population, and a ROC curve created. These methods are well known in the art. See, e.g., Hanley et al, Radiology 143: 29-36 (1982).

In certain embodiments, markers and/or marker panels are selected to exhibit at least about 70% sensitivity, more preferably at least about 80% sensitivity, even more preferably at least about 85% sensitivity, still more preferably at least about 90% sensitivity, and most preferably at least about 95% sensitivity, combined with at least about 70% specificity, more preferably at least about 80% specificity, even more preferably at least about 85% specificity, still more preferably at least about 90% specificity, and most preferably at least about 95% specificity. In particularly preferred embodiments, both the sensitivity and specificity are at least about 75%, more preferably at least about 80%, even more preferably at least about 85%, still more preferably at least about 90%, and most preferably at least about 95%. The term "about" in this context refers to +/- 5% of a given measurement.

In other embodiments, a positive likelihood ratio, negative likelihood ratio, odds ratio, or hazard ratio is used as a measure of a test's ability to predict risk or diagnose a disease. In the case of a positive likelihood ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In the case of a negative likelihood ratio, a value of 1 indicates that a negative result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a negative result is more likely in the test group; and a value less than 1 indicates that a negative result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a positive or negative likelihood ratio of at least about 1.5 or more or about 0.67 or less, more preferably at least about 2 or more or about 0.5 or less, still more preferably at least about 5 or more or about 0.2 or less, even more preferably at least about 10 or more or about 0.1 or less, and most

preferably at least about 20 or more or about 0.05 or less. The term "about" in this context refers to +/- 5% of a given measurement.

5 In the case of an odds ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit an odds ratio of at least about 2 or more or about 0.5 or less, more preferably at least about 3 or more or
10 about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less. The term "about" in this context refers to +/- 5% of a given measurement.

15 In the case of a hazard ratio, a value of 1 indicates that the relative risk of an endpoint (e.g., death) is equal in both the "diseased" and "control" groups; a value greater than 1 indicates that the risk is greater in the diseased group; and a value less than 1 indicates that the risk is greater in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a hazard ratio of at least about 1.1 or more or
20 about 0.91 or less, more preferably at least about 1.25 or more or about 0.8 or less, still more preferably at least about 1.5 or more or about 0.67 or less, even more preferably at least about 2 or more or about 0.5 or less, and most preferably at least about 2.5 or more or about 0.4 or less. The term "about" in this context refers to +/- 5% of a given measurement.

25

Amounts and amount ratios of sFIT-1 and/or HGF higher than or equal to the reference amount (i.e. increased amounts), preferably, indicate that the subject suffers from a complication of a procedure that temporarily impairs blood supply in at least one artery. The reference amount (increased amount) for HGF and/or sFIT-1 in the sample taken after
30 the procedure is, preferably, about 120 %, about 130 %, about 140 %, about 160 %, about 180 %, about 200 %, about 220 % or about 250 % of the baseline amount. Most preferably, the reference amount (increased amount) is about 130 % or about 200 % of the baseline amount. In connection with the reference amount (increased amount), the amounts of sFIT-1 and/or HGF determined in the sample taken before the procedure is initiated (i.e. the
35 baseline amount) are defined as 100 %. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

If no complication of the procedure that temporarily impairs blood supply in at least one artery is diagnosed according to the method of the present invention, the subject is eligible for early discharge if he does not show other signs or symptoms of a complication of the procedure that temporarily impairs blood supply in at least one artery.

If the method of the present invention indicates the presence of a complication of the procedure that temporarily impairs blood supply in at least one artery, closer monitoring of the subject may be required. As the type of complication depends on the kind and the location of the procedure, monitoring may focus on renal function (in the case of any administration of a contrast agent, angiography of the renal artery or angioplasty of the renal artery), or it may focus on cardiac function (in the case of PCI or coronary angiography), or it may focus on the skeletal muscle (in the case of angiography or angioplasty of a peripheral artery).

If the procedure that temporarily impairs blood supply in at least one artery is performed on an outpatient (i.e. a subject not admitted to a hospital) and the method of the present invention indicates the presence of a complication, the admission of the subject into a hospital should be considered.

If the procedure that temporarily impairs blood supply in at least one artery potentially causes a renal complication and the method of the present invention indicates the presence of a complication, protective measures are recommended. These include, preferably, maintenance of blood pressure, careful fluid balance (e.g. the administration of sodium bicarbonate), the administration of Acetylcysteine and the avoidance of drugs that are potentially toxic to the kidney. Moreover, renal function should be closely monitored, e.g. by the determination of serum creatinine.

In the case of a cardiac complication the localization of the affected coronary artery by angiography and its reopening by angioplasty may become necessary.

The determination of markers for specific damage to the aforementioned organs is preferred for the monitoring of a subject suffering from a complication of the procedure that temporarily impairs blood supply in at least one artery. Suitable markers are disclosed further below in the present application.

Advantageously, the present invention allows the diagnosis and/or prediction of a complication of a procedure that temporarily impairs blood supply in at least one artery

independently of clinical signs and symptoms of the subject. Thus, it detects and/or predicts complications in their early stages before significant organ damage occurs and it detects small complications that may cause permanent damage even though the subject never shows clinical signs or symptoms. Hence, early intervention to limit the consequences of the complication is possible. In some subjects the presence of complications can be excluded. These subjects can be saved a prolonged hospitalization or further diagnostic procedures if no other signs of complications are present. Thus, the individual subject can be treated according to his/her needs and the resources of the health system as a whole can be applied to those subjects in greatest need thereof. For the first diagnosis of a complication it is preferable to use the general ischemia markers sFIT-1 and/or HGF rather than the specific markers for organ damage because increases of sFIT-1 and HGF can be detected within about 4 hours after the procedure while cardiac troponins and U-LFABP often take about 24 hours to increase to their maximum (see examples 2 and 3). Thus, sFIT-1 and HGF allow an earlier decision about further treatment of the subject.

Because the diagnosis of the mere presence of a complication of a procedure that temporarily impairs arterial blood supply in at least one artery does not offer much guidance for the selection of a specific therapy, it is desirable to supplement the aforementioned diagnosis by the identification of the type and location of the complication.

Therefore, a preferred embodiment of the present invention relates to a method for classifying the kind of complication resulting from a procedure that temporarily impairs arterial blood supply in at least one artery based on the comparison of the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for damage to the skeletal muscle determined beforehand in a sample of the subject to at least one reference amount.

The method comprises at least one of the following steps: a) determining the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for damage to the skeletal muscle in a first sample of the subject; b)

It is also provided a method for classifying the kind of complication resulting from the procedure that temporarily impairs arterial blood supply in at least one artery, the method comprising the steps of

- a) determining the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for damage to the skeletal muscle in a first sample of the subject;
- b) comparing the amount of the at least one marker determined in step a) to a reference amount; and
- c) classifying the kind of complication resulting from the procedure that temporarily impairs arterial blood supply in at least one artery based on the comparison carried out in step b).

Preferably, the above described method is performed after the occurrence of a complication of a procedure that temporarily impairs blood supply in at least one artery is diagnosed or even predicted, as set forth at the very beginning of the present application and in accordance with the present invention. However, because the method of the present invention requires the acquisition of a sample prior to the initiation of the procedure that temporarily impairs blood supply in at least one artery (wherein the sample can be obtained by the same person and/or at the same place or facility by which/where the comparison and/or the diagnosis are carried out), all necessary samples for the identification of the presence of the complication, are preferably, taken together with the samples necessary for diagnosing the complication. The determination of the amounts of the markers for identifying the complication may then be delayed until the presence of the complication is diagnosed based on the determination of HGF and/or sFIT-1.

The amounts of the markers that allow the classification of the complication of the procedure that temporarily impairs blood supply in at least one artery are, preferably determined in a sample that is taken about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours or about 6 hours after the procedure that temporarily impairs blood supply in at least one artery. More preferably the sample is taken about 4 hours after the procedure.

The term “classifying” refers to the process of exactly identifying the kind of complication and may also be termed “assessing” or “identifying”. Preferably, the complication is classified/assessed according to the affected organ.

Coronary complications

Preferred markers for coronary damage are cardiac troponins or variants thereof.

An amount of a cardiac troponin that is equal to or higher than the reference amount indicates the presence of a coronary complication of the procedure that temporarily impairs blood supply in at least one artery.

- 5 Preferably, the reference amount for troponin T is about 120 %, about 130 %, about 140 %, about 160 %, about 180 %, about 200 %, about 220 % or about 250 % of the baseline amount, i.e. the amount of the troponin determined in the sample that was taken before the procedure that temporarily impairs blood supply in at least one artery was initiated. Most preferably, the reference amount is about 130 % or about 200 % of the baseline amount.
- 10 The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

As the amounts of cardiac troponin increase not as fast as the amounts of the other markers of the present invention, in another preferred embodiment of the present invention the

15 baseline amount for the cardiac troponin is the amount determined in a sample that is taken 4 hours after the procedure that temporarily impairs blood supply in at least one artery is performed.

Moreover, independent of the choice of the baseline amount it is also preferred to take the

20 sample for the determination of the cardiac troponin about 24 hours after the procedure that temporarily impairs blood supply in at least one artery is performed.

In case the subject's renal function is impaired (independently of a "renal complication" which may be diagnosed/predicted in an embodiment of the present invention) it is to be

25 understood that the amount of the cardiac troponin at baseline may be increased. Hence, in a subject with a pre-existing renal disease the relative increases of troponin indicating a coronary complication are lower than those described above.

Renal complications

30

Preferred markers for kidney damage are urinary liver-type fatty acid binding protein (U-LFABP) or a variant thereof, neutrophil gelatinase associated lipocalin (NGAL) or a variant thereof, adiponectin or a variant thereof, KIM-1 or a variant thereof and albumin or a variant thereof. Of these markers U-LFABP or a variant thereof and NGAL are

35 especially preferred.

An amount or of U-LFABP equal to or higher than the reference amount indicates the presence of a renal complication of the procedure that temporarily impairs blood supply in at least one artery.

- 5 Preferably, the reference amount for U-LFABP is about 120 %, about 130 %, about 140 %, about 160 %, about 180 %, about 200 %, about 220 % or about 250 of the amount determined in the sample that was taken before the procedure that temporarily impairs blood supply in at least one artery was initiated (the "baseline amount"). Most preferably, the reference amount is about 130 % or about 200 %. The term "about" as used herein
10 refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

- Preferably, the reference amount for NGAL is about 150 %, about 200 %, about 150 %, about 300 %, about 350 % or about 400 % of the amount determined in the sample that
15 was taken before the procedure that temporarily impairs blood supply in at least one artery was initiated. Most preferably, the reference amount is about 300 % or about 400 %. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

- 20 An amount of KIM-1, adiponectin or albumin larger than the reference amount indicates the presence of a renal complication of the procedure that temporarily impairs blood supply in at least one artery.

Damage to the skeletal muscle

25

A preferred marker for damage to the skeletal muscle is creatine kinase.

- An amount or of creatine kinase equal to or higher than the reference amount indicates the presence of a complication of a skeletal muscle of the procedure that temporarily impairs
30 blood supply in at least one artery.

- For the identification of a complication of the procedure that temporarily impairs blood supply in at least one artery a stable subject is a subject as defined above. In addition to
35 that, the amount of the at least one marker selected from the group consisting of markers for coronary complications, markers for renal complications and markers for damage to the skeletal muscle in the subject did, preferably, not increase at least about 2 hours, at least

about 4 hours or at least about 6 hours before the procedure that temporarily impairs blood supply in at least one artery was performed. If the marker is a cardiac troponin or U-LFABP, the amount of said marker does not increase by more than about 5 % or by more than about 10 %.

5

Moreover, if the subject recovered recently from an acute disease or disorder that causes an increase of one of the markers used for the identification of a complication, he/she is not eligible for a diagnosis according to the method of the present invention. Preferably, the term “recently” refers to an interval of about 1 day, about 2 days, about 3 days, about 4
10 days, about 6, days, about 8 days, about 10 days, about 12 days or about 14 days. If the marker is U-LFABP, the interval is, more preferably, about 1 day.

15

Advantageously, the method for identifying the complication of the procedure that temporarily impairs blood supply in at least one artery allows the subclassification of subjects suffering from such a complication according to the affected organ. Thus, the method offers guidance for further diagnostic and/or therapeutic procedures. This decreases the time required for a diagnosis and the initiation of a suitable therapy. The timely initiation of the appropriate therapy limits the consequences of the complication. In
20 cases where a longer duration of the complication leads to irreversible damage this is especially important. A cardiac complication, for example, may have non reversible consequences because ischemic parts of the myocardium die after a certain duration of ischemia and dead cardiomyocytes are replaced by scar tissue rather than functional myocardium.

25

In an especially preferred embodiment of the present invention the method for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery is performed first. If said method indicates that no such complication is present, an early discharge of the subject may be recommended unless there are other indicators of
30 complications. If the method for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery indicates the presence of such a complication, the method for identifying the complication of the procedure that temporarily impairs arterial blood supply in at least one artery is, preferably, performed. Thus, the physician receives information about the organ affected by the complication. This allows the timely
35 ignition of further diagnostic and/or therapeutic measures.

Moreover, the present invention relates to a device for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject comprising

- a) an analyzing unit for determining the amount of a marker selected from the group consisting of sFIT-1 or a variant thereof and HGF or a variant thereof in a sample of a subject;
- b) an evaluation unit for comparing the determined amounts to reference amounts.

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 practise the method of the present invention. Preferred means for determining the amounts of the markers of the present invention, and 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 an analysis unit for automatically determining the amount of the gene products of the present invention is applied, the data obtained by said automatically operating analysis unit can be processed by, e.g., a computer as evaluation unit in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case.

Said device, preferably, includes an analyzing unit for the measurement of the amount of sFIT-1 and/or HGF in an applied sample and an evaluation unit for processing the resulting data. Preferably, the evaluation unit comprises a database with the stored reference amounts and a computer program code which when tangibly embedded on a computer carries out the comparison of the determined amounts and the reference amounts stored in the database. More preferably, the evaluation unit comprises a further computer program code which allocates the result of the comparison to a risk prediction. In such a case, it is, also preferably, envisaged that the evaluation unit comprises a further database wherein the reference amounts are allocated to the risks.

Alternatively, where means such as test stripes are used in the analyzing unit for determining the amount of sFIT-1 and/or the HGF, the evaluation unit 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 sFIT-1 or HGF. The strip or device, preferably, comprises means for detection of the binding of said sFIT-1 or HGF 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 analysis unit and the evaluation unit 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 analysis unit and the evaluation unit 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 gene product, Plasmon surface resonance devices, NMR spectrometers, mass-spectrometers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

In a preferred embodiment of the present invention the analyzing unit of the device further comprises means for determining the amounts of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage, markers for brain damage and markers for skeletal muscle damage in a sample of a subject and the evaluation unit comprises means for comparing said amounts to reference amounts.

Moreover, the present invention relates to a kit for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject comprising

- a) a specific ligand for determining the amount of a marker selected from the group consisting of sFLT-1 or a variant thereof and/ HGF or a variant thereof in a sample of a subject;
- b) an evaluation unit for comparing the determined amounts to reference amounts.

The term "kit" as used herein refers to a collection of the aforementioned components of which may or may not be packaged together. The components of the kit may be comprised by separate vials (i.e. as a kit of separate parts) or provided in a single vial. Moreover, it is to be understood that the kit of the present invention is to be used for practising the methods referred to herein above. It is, preferably, envisaged that all components are

provided in a ready-to-use manner for practising the methods referred to above. Further, the kit preferably contains instructions for carrying out the said methods. The instructions can be provided by a user's manual in paper- or electronic form. For example, the manual may comprise instructions for interpreting the results obtained when carrying out the
5 aforementioned methods using the kit of the present invention. The kit shall comprise a specific ligand. This specific ligand is capable of specifically recognizing HGF and/or sFIt-1 in a sample of the subject. Moreover, said specific ligand shall upon binding to the HGF and/or sFIt-1, preferably, be capable of generating a detectable signal, the intensity of which correlates to the amount of HGF or sFIt-1 present in the sample. Dependent on
10 the type of signal which is generated, methods for detection of the signal can be applied which are well known in the art. Specific ligands which are preferably used for the kit of the present invention include antibodies or aptamers. The specific ligand may be present on a test stripe as described elsewhere herein. The amounts of HGF and/or sFIt-1 thus detected can then be further evaluated in the evaluation unit. Preferred evaluation units to
15 be used for the kit of the present invention include those referred to elsewhere herein.

In a preferred embodiment of the present invention the kit further comprises a specific ligand/specific ligands for determining is the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and
20 markers for skeletal muscle damage in a sample of a subject and an evaluation unit for comparing said amount ratios to reference amounts.

The present invention also relates to the use of a kit or device for determining the amount of sFIt-1 or a variant thereof and/or HGF or a variant thereof in a sample of a subject, comprising means for determining the amount of sFIt-1 or the variant thereof and/or HGF
25 or the variant thereof and/or means for comparing the amount or the amount ratio of sFIt-1 or the variant thereof and/or HGF or the variant thereof to at least one reference value for: diagnosing and/or predicting a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject.

30

The present invention also relates to the use of: an antibody against sFIt-1 or a variant thereof and/or an antibody against HGF or a variant thereof and/or of means for determining the amount of sFIt-1 or a variant thereof and/or HGF or a variant thereof and/or of means for comparing the amount or the amount ratio of sFIt-1 or the variant
35 thereof and/or HGF or the variant thereof to at least one reference value for the manufacture of a diagnostic composition for: diagnosing and/or predicting a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject.

The present invention also relates to the use of: sFIT-1 or a variant thereof and/or HGF or a variant thereof and/or of means for determining the amount of sFIT-1 or a variant thereof and/or HGF or a variant thereof, and/or of means for comparing the amount or the amount ratio of sFIT-1 or the variant thereof and/or of HGF or the variant thereof to at least one reference value for: diagnosing and/or predicting a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject.

The present invention also relates to the use of: an antibody against at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for skeletal muscle damage and/or of means for determining the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for skeletal muscle damage and/or of means for comparing the amount or the amount ratio of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for skeletal muscle damage to at least one reference value for the manufacture of a diagnostic composition for: the identification of the complication of the procedure that temporarily impairs arterial blood supply in at least one artery.

The present invention also relates to the use of: at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage, markers and markers for skeletal muscle damage and/or of means for determining the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for skeletal muscle damage, and/or of means for comparing the amount or the amount ratio of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for skeletal muscle damage to at least one reference value for: the identification of the complication of the procedure that temporarily impairs arterial blood supply in at least one artery.

It is also provided for a method for diagnosing a myocardial infarction in a subject comprising the steps of

- a) determining the amount of a cardiac troponin or a variant thereof in a sample of the subject;
- b) comparing the measured amount of the cardiac troponin or the variant thereof to a reference amount;

whereby the results obtained in step b) indicate whether the subject suffers from a myocardial infarction.

Preferably, the method of the present invention comprises the steps of a) determining the amount of a cardiac troponin or a variant thereof in a sample of the subject; b) comparing the measured amount of the cardiac troponin or the variant thereof to a reference amount; and c) diagnosing whether the subject suffers from a myocardial infarction.

Cardiac troponins are known to the person skilled in the art. Preferably, a cardiac troponin is troponin T or troponin I.

A further embodiment of the present invention relates to a method for diagnosing heart failure in a subject comprising the steps of

a) determining the amount of a natriuretic peptide or a variant thereof in a sample of the subject;

b) comparing the measured amount of the natriuretic peptide or the variant thereof to a reference amount;

whereby the results obtained in step b) indicate whether the subject suffers from heart failure.

Preferably, the method of the present invention comprises the steps of a) determining the amount of a natriuretic peptide or a variant thereof in a sample of the subject; b) comparing the measured amount of the natriuretic peptide or the variant thereof to a reference amount; and c) diagnosing whether the subject suffers from a heart failure.

Natriuretic peptides are known to the person skilled in the art. Preferably, a natriuretic peptide is BNP or NT-proBNP.

The following examples are merely intended to illustrate the present invention. They shall not limit the scope of the claims in any way.

Example 1: Analytical Methods

sFlt-1 was determined with a sFlt-1 immunoassay to be used with the Elecsys and COBAS analyzers from Roche Diagnostics, Mannheim, Germany. The assay is based on the sandwich principle and comprises two monoclonal sFlt-1 specific antibodies. The first of these is biotinylated and the second one is labeled with a Tris(2,2'-

bipyridyl)ruthenium(TT)-complex. In a first incubation step both antibodies are incubated with the human serum sample. A sandwich complex comprising sFlt-1 and the two different antibodies is formed. In a next incubation step streptavidin-coated beads are added to this complex. The beads bind to the sandwich complexes. The reaction mixture is then aspirated into a measuring cell where the beads are magnetically captured on the surface of an electrode. The application of a voltage then induces a chemiluminescent emission from the ruthenium complex which is measured by a photomultiplier. The amount of light is dependent on the amount of sandwich complexes on the electrode. The test is capable of measuring sFlt1-concentrations from 10 to 85000 pg/ml.

Troponin T was measured with an immunoassay (hs troponin T, product number 05092744, from December 2008) by Roche Diagnostics to be used with the above described systems. The test is capable of measuring troponin T-concentrations from 1 to 10000 pg/ml.

HGF was determined with the Quantikine human HGF assay by R&D systems, Minneapolis, USA. This test is based on the sandwich-principle as well. However, compared to the abovementioned tests by Roche Diagnostics the principle is modified. The sample is added to a microtiter well which is coated with an antibody to the respective biomarker. The marker in the sample is, thus, immobilized at the wall of the vessel. Unbound substances are then removed by washing. After that, a second antibody against the marker is added to the well. This antibody is linked to an enzyme. After a further wash to remove any unbound enzyme-conjugated antibody, a substrate solution is added to the well. The enzyme forms a colored reaction product from the substrate. The color development is stopped after a predetermined time and the intensity of the color is measured with a photometer. The concentration of the marker in the sample is calculated by comparing the measured color intensity with a calibration curve. The limit of detection of the HGF assay is below 40 pg/ml.

U-L-FABP was determined by using the L-FABP ELISA-Kit from CMIC Co., Ltd, Japan. The test is based on an ELISA 2-step assay. L-FABP standard or urine samples are firstly treated with pretreatment solution, and transferred into an anti-L-FABP antibody coated microplate containing assay buffer and incubated. During this incubation, L-FABP in the reaction solution binds to the immobilized antibody. After washing, the 2nd Antibody-peroxidase conjugate is added as the secondary antibody and incubated, thereby forming a complex of the L-FABP antigen sandwiched between the immobilized antibody and the conjugate antibody. After incubation, the plate is washed and substrate for enzyme reaction

is added, color develops according to the L-FABP antigen quantity. The L-FABP concentration is determined based on the optical density.

Example 2: Subjects with angiography and subsequent STENT implantation

Subjects:

A total of 31 subjects (18 males, 13 females) were included into the study, mean age 61 years (range 49 – 71 years). All subjects suffered from stable coronary artery disease. Subjects were excluded from the study if they had symptoms of stable or unstable angina pectoris within the past 2 weeks. All subjects had normal kidney function as indicated by creatinine levels within the normal range of the test (below 1.3 mg/dl). The subjects had an angiography through a line placed into the femoral artery in order to localise the stenosis or multiple stenoses for STENT implantation. Thereafter a STENT was placed onto a balloon to be localised into the area of the stenosis, the STENT was placed by inflating the balloon. Success of STENT implantation was monitored by angiography after the procedure was completed, in all cases the stenosis was successfully opened. During the intervention contrast media was applied, e.g. to monitor the success of the STENT implantation.

Samples were taken before the procedure (baseline sample), as well as 4 and 24 h after the procedure. Samples were centrifuged within 30 minutes and the serum was kept at – 20 degrees Celsius until tested. In addition to blood samples urine samples were taken at the same time and were also kept at – 20 degrees Celsius until tested.

The amounts of sFIT-1 and HGF in the baseline sample were compared to the amounts of said markers in samples from the same subject taken 4 h and 24 h after the procedure. An increase of sFIT-1 and HGF respectively was regarded as an increase of at least 30 % from baseline of each marker (i.e. before intervention) to 4 h after the intervention. In case of no increase or an increase of less than 30 % in the time period defined above this was regarded as no increase. The same definition applied to U-LFABP (increase by at least 30% from baseline to 4 h). In case of troponin T an increase by at least 30 % from the timepoint 4 hours after baseline to the timepoint 24 h after baseline was regarded to be an increase.. If the marker did not increase or increased by less than 30 % this was not regarded as an increase. A large increase of any of the markers was assumed if the increase exceeded 100 % with respect to the time intervals described above for each marker.

According to the definition above the following data were obtained for sFIT-1 and HGF at 4 h after STENT implantation. This is depicted in Table 1.

5

Table 1: Increases of sFIT-1 and HGF from baseline to the 4 hours after intervention (given in percent of the amount at baseline) are stratified into groups of no increase (below 30%), increase 30 to below 100 %, increase between 100 % and 500 % and increase above 500 %.		
	sFIT-1	HGF
No increase (below 30%)	8/31	9/31
Increase 30 to below 100 %	8/31	7/31
100 % to 500 % increase	6/31	2/31
More than 500 % increase	9/31	13/31

As can be derived from Table 1, large increases of the amounts of ischemia markers were observed in approximately 50 % of the subjects after STENT implantation, while the rest did not have large increases of the amounts of markers of ischemia as compared to the baseline sample. Subjects with an increase of the amount of HGF of less than 100 % as compared to the baseline sample also had no increase of the amount of sFIT-1 of more than 100 % and vice versa. Subjects who had no increase or an increase below 100 % for sFIT-1 were identical with subjects who displayed no increase or an increase below 100 % for HGF. Moreover, subjects who had an increase of more than 100 % for sFIT-1 were identical with subjects who displayed an increase of more than 100 % for HGF. This indicates that sFIT-1 and HGF can be used interchangeably.

When subjects were divided into subjects who had an increase of the amount of HGF and sFIT-1 of more or less than 100 percent as compared to the baseline sample the following data were obtained:

25

Table 2: Increases of U-LFABP (from baseline to 4 hours after the intervention) and troponin T (from 4 hours after the intervention to 24 hours after the intervention) are shown for subjects of less than 100 % and with more than 100 % increase of HGF/sFLT-1.

	(increase of U-LFABP)	(increase of Troponin T)
Increase of HGF and sFIT-1 less than 100 %	5/16	2/16
Increase of HGF and sFIT-1 more than 100 %	11/15	10/15

5 The data shown in Table 2 illustrate that angiography followed by STENT implantation was followed in the majority of subjects by the induction of ischemia which varied with respect to its extent (different levels of increase of sFIT-1 and HGF). Table 2 shows that subjects with higher increases of sFIT-1 and/or HGF (i.e. more than 100 % of the baseline amount) had a higher probability of organ damage. As shown in Table 1 sFIT1 and HGF
10 identified the same subjects if an increase below or above 100 % of the respective marker was used. Hence, sFIT-1 and HGF, respectively, can be used to obtain the desired information with respect to already existing or future organ damage. These data show that the extent of ischemia as indicated by the increase of sFIT-1 and/or HGF predicts the risk of organ damage. Cardiac damage is detected by an increase of troponin T and kidney
15 damage is detected by an increase of U-LFABP. Thus, the data illustrate that sFIT-1 and/or HGF can be used to identify subjects at increased risk of procedure related organ damage (as identified by cardiac troponin T and kidney damage associated U-LFABP). The data have implications on the subject's discharge. In case of no increase of sFIT-1 or HGF is detectable, the subject may be eligible for an early discharge. If an increase of sFIT-1 or
20 HGF is detected, the subject requires closer monitoring for organ damage and – as the case may be – specific therapy.

The following examples of individual subjects shall illustrate the finding:

Subject 1:

	Timepoint: [h]	0	4	24
5	sFIT-1 [pg/ml]	76	152	186
	HGF [pg/ml]	2432	5782	3483
	Troponin T [pg/ml]	40	155	1375
	U-LFABP [pg/ml]	26	25	29

10 This subject had a procedure related increase of sFIT-1 and HGF. He developed a myocardial infarction as demonstrated by increased troponin amounts after 24 hours. However, he failed to develop kidney damage associated with the procedure, although U-LFABP before the procedure was elevated indicating pre-existing tubular damage. However U-LFABP did not increase after the procedure indicating that the procedure was
 15 not associated with additional kidney damage. In this case the increase of sFIT-1 clearly indicated cardiac ischemia associated with cardiac necrosis.

Subject 2:

20	sFIT-1 [pg/ml]	63	1883	156
	HGF [pg/ml]	1054	19385	2375
	Troponin T [pg/ml]	13	9	14
	U-LFABP [pg/ml]	13	56	32

25 In subject 2 shown above large increases in sFIT-1 and HGF indicated the presence of ischemia. Said increases were associated an increase of U-LFABP (indicating kidney damage) but not with cardiac necrosis as demonstrated by the constant amounts of troponin T.

30 Subject 3:

	sFIT-1 [pg/ml]	106	374	161
	HGF [pg/ml]	1936	11456	3910
	Troponin T [pg/ml]	64	102	247
	U-LFABP [pg/ml]	30	60	86

35

Subject 3 suffered from a complication of STENT implantation as shown by the increases of the amounts of sFIT-1 and HGF. Since the amounts of both troponin T and U-LFAB increased the presence of a renal as well as a cardiac complication are likely

5 Subject 4:

sFIT-1 [pg/ml]	82	113	227
HGF [pg/ml]	1623	1528	6081
Troponin T [pg/ml]	9	6	8
U-LFABP [pg/ml]	4	10	16

10

In subject 4 increased amounts of HGF and sFIT-1 indicate the presence of a complication of STENT implantation. As shown by the stable amount of troponin T and the increased amount of U-LFABP the complication was a renal complication while the heart remained unaffected.

15

Subject 5:

sFIT-1 [pg/ml]	68	81	87
HGF [pg/ml]	987	896	1018
Troponin T [pg/ml]	11	13	12
U-LFABP [pg/ml]	7	9	8

20

No increase of sFIT-1 or HGF was detectable in subject 5. Consequently, the stable amounts of troponin T and U-LFABP indicate that there was no cardiac or renal complication.

25

The data of the five individual subjects presented above clearly illustrate and support the usefulness of sFIT-1 and or HGF for the identification of ischemia associated with angiography and subsequent STENT implantation. As can be derived from subject 5, if there is no increase of sFIT-1 no cardiac or renal complication is to be expected and the subject can be safely discharged. In contrast, increases of sFIT-1 and/or HGF represent an early sign of organ complication. In coronary STENT implantation this complication can be of cardiac or renal origin and can be detected by specific organ damage markers, this case U-LFABP for kidney damage and cardiac troponin T for cardiac damage. Such early information about possible or ongoing (but still not detectable) target organ damage

30

provides the physician with information that can be translated into improved monitoring of a subject with or without application of specific interventions.

Example 3: Subjects with angiography but without STENT implantation

5

In order to assess whether the increases in ischemia markers were only related to STENT implantation, we tested in addition 5 subjects who only underwent coronary angiography and no STENT implantation. This is outlined in the following cases:

10

Case 1:

	Time [h]	0	4	24
15	sFIT-1 [pg/ml]	49	75	43
	HGF [pg/ml]	1180	1940	1130
	Troponin T [pg/ml]	15	11	14
	U-LFABP [pg/ml]	12	10	26

20 The increases of HGF and sFIT-1 in case 1 demonstrate that the subject suffered from a complication of angiography. The stable amount of troponin T and the increased amount of U-LFABP indicate that the complication was a renal complication.

25 Case 2:

	Time [h]	0	4	24
	sFIT-1 [pg/ml]	70	567	79
	HGF [pg/ml]	1100	12800	1078
	Troponin T [pg/ml]	2	4	4
30	U-LFABP [pg/ml]	4	4	28

Large increases of both HGF and sFIT-1 indicate significant ischemia in case 2. Increased U-LFABP and stable troponin T indicate the complication was a renal complication.

Case 3

	Time [h]	0	4	24
	sFIT-1 [pg/ml]	46	125	39
	HGF [pg/ml]	480	2890	430
5	Troponin T [pg/ml]	17	38	39
	U-LFABP [pg/ml]	20	19	57

In case 3 the increases of HGF and sFIT-1 indicate the presence of a complication of angiography in the subject. Both troponin T and U-LFABP increased. Thus, the subject
 10 suffered from a renal complication in addition to a cardiac complication.

Case 4

	Time [h]	0	4	24
15	sFIT-1 [pg/ml]	63	63	417
	HGF [pg/ml]	1064	950	6200
	Troponin T [pg/ml]	11	7	8
	U-LFABP [pg/ml]	11	5	21

20 Subject 4 suffered from an anaphylactoid reaction to the contrast agent with a resulting decrease of blood pressure. Therefore, the amounts of sFIT-1 and HGF took 24 hours to increase rather than the usual 4 hours. The increased amounts of the markers were caused by the anaphylactoid reaction rather than an ischemic complication as defined in the present application.

25

Case 5

	Time [h]	0	4	24
	sFIT-1 [pg/ml]	59	232	66
	HGF [pg/ml]	710	5870	648
30	Troponin T [pg/ml]	0	2	4
	U-LFABP [pg/ml]	5	6	33

The increased amounts of sFIT-1 and HGF indicate the presence of a complication of angiography in case 5. The stable amount of troponin T and the increased amount of U-LFABP indicate a renal complication.

5 The data of the subjects shown in example 3 clearly show that the method of the invention is not restricted to individuals undergoing a STENT implantation, but can also be used for subjects undergoing other procedures temporarily impairing blood supply like coronary angiography. In these latter cases the ischemia is typically related to renal but not to cardiac damage. (except for Subject 3). Moreover, ischemia is frequently of shorter
10 duration. This is illustrated by a faster decrease of sFIT-1 (to normal or near normal values at 24 h) as compared to subjects undergoing angiography with subsequent intervention (such as those shown in example 2). This is consistent with the fact that less contrast medium is used and that there is no or only little manipulation in the coronary arteries that might give rise to complications such as thromboembolism or vascular dissection.

15

It should be noted that, typically, the dynamics of sFIT-1 and HGF are short lived (a rapid increase is followed by a rapid decrease as shown in Subject 2 in example 2). However, in case of organ damage ischemia may last (as is the case in Subject 1 in example 2) who developed myocardial infarction. While the duration of ischemia might provide important
20 information (as is the case in Subject 1 in example 2), the prediction of an organ complication relies on the early (intervention associated) change of the amounts of sFIT-1 and/or HGF in general preceding the increase of organ damage markers.

Conclusion

25

The data obtained in the above Examples 1 to 3 illustrates the diagnostic and prognostic value of the ischemia markers sFIT-1 and HGF in the diagnosis of complications of interventions. Angiography alone often primarily results in kidney damage and requires follow up specifically in those subjects who are discharged early. In addition to follow up
30 examination sufficient fluid consumption and avoidance of nephrotoxic drugs are recommended.

In case of STENT implantation both cardiac and kidney damage require consideration and monitoring. In case of cardiac damage further diagnostic procedures and additional intervention should be performed as needed. In case of intervention of other target organs
5 appropriate monitoring of organ damage is required.

Lack of increased levels of ischemia markers, i.e. an increase in the amount of sFlt-1 of below 100% renders target organ complications unlikely and makes monitoring unnecessary or less intensive.

10

The identification of ischemia based on sFlt-1 or HGF independent of its localization is a valuable tool for monitoring interventions specifically in cases where ischemia is not associated with symptoms (as in the kidney) or in cases where symptoms are ameliorated by anaesthetics (such as in cardiac intervention). The information provided has clear
15 clinical consequences as outlined above.

Claims

5

1. A method for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject, comprising the steps of

10

- a) determining the amount of at least one marker from the group soluble fms-like tyrosine kinase 1 (sFIT-1) or a variant thereof and hepatocyte growth factor (HGF) or a variant thereof, in a sample of the subject;

- b) comparing the amount of the at least one marker determined in step a) to a reference amount; and

15

- c) diagnosing the complication of a procedure that temporarily impairs blood supply in at least one artery based on the results of the comparison carried out in step b).

2. The method of claim 1, wherein the complication is an ischemic complication.

20

3. The method of claim 1 or 2, wherein the complication is a coronary complication, a renal complication or a complication of a skeletal muscle.

25

4. The method of any of claims 1 to 3, wherein the procedure that temporarily impairs arterial blood supply is selected from the group consisting of intravascular ultrasound, angiography, angioplasty and the peripheral administration of a contrast agent.

30

5. The method of any of claims 1 to 4, wherein the complication is a renal complication that results from (i) the administration of a contrast agent in connection with angiography or (ii) the peripheral administration of a contrast agent.

35

6. A method for classifying a complication resulting from a procedure that temporarily impairs arterial blood supply in at least one artery, the method comprising the steps of

a) determining the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage and markers for skeletal muscle damage in sample of the subject;

b) comparing the amount of the at least one marker determined in step a) to a reference amount; and

c) classifying the complication of the procedure that temporarily impairs blood flow in at least one artery based on the results of the comparison carried out in step b).

7. The method of claim 6, wherein

a. the marker for coronary damage is a cardiac troponin or a variant thereof; and/or

b. the marker for kidney damage is selected from the group consisting of urinary liver-type fatty acid binding protein (U-LFABP) or a variant thereof, neutrophil gelatinase associated lipocalin (NGAL) or a variant thereof, adiponectin or a variant thereof, KIM-1 or a variant thereof and albumin or a variant thereof; and/or

c. the marker for damage to the skeletal muscle is creatine kinase or a variant thereof

8. The method of claim 7, wherein

a. an increased amount of the at least one marker for kidney damage indicates the presence of a renal complication; and/or

b. an increased amount of the marker for coronary damage indicates a coronary complication; and/or

c. an increased amount of the marker for damage to the skeletal muscle indicates a complication of a skeletal muscle.

9. The method of any of claims 6 to 8, wherein the presence of a complication of a procedure that temporarily impairs blood supply in at least one artery is diagnosed according to the method of claim 1 before said complication is classified according to the method of any of claims 6 to 8.

10. The method according to any of claims 1 to 9, wherein the reference amount is the amount of the respective marker determined in a sample of the subject that was taken before the sample of step (a), preferably before or while the procedure that temporarily impairs blood supply in at least one artery is performed.

11. Use of a specific ligand for determining sFIT-1 or a variant thereof and/or a specific ligand for determining HGF or a variant thereof for diagnosing and/or predicting a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject.

12. Device for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject comprising

- a. an analyzing unit for determining the amount of a marker selected from the group consisting of sFIT-1 or a variant thereof and HGF or a variant thereof in a sample of a subject; and
- b. an evaluation unit for comparing the determined amounts to reference amounts.

13. A kit for diagnosing a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject comprising

- a. specific ligand for determining the amount of a marker selected from the group consisting of sFIT-1 or a variant thereof and/or HGF or a variant thereof in a sample of a subject; and
- b. an evaluation unit for comparing the determined amounts to reference amounts.

14. The device or kit of claim 12 or 13, further comprising an analyzing unit/a specific ligand for determining the amount of at least one marker selected from the group consisting of markers for coronary damage, markers for kidney damage, markers for brain damage and markers for skeletal muscle damage in

sample of the subject, optionally means for calculating the ratio of said amount, and means for comparing the determined amount or the amount to a reference amount.

5 15. A method for predicting a complication of a procedure that temporarily impairs blood supply in at least one artery in a subject , comprising the steps of

- 10 a) determining the amount of at least one marker from the group soluble fms-like tyrosine kinase 1 (sFIT-1) or a variant thereof and hepatocyte growth factor (HGF) or a variant thereof, in a sample of the subject;
- b) comparing the amounts of the markers determined in step a) to a reference amount; and
- c) predicting the complication based on the results of the comparison in step b),

15

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/052905

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/48 G01N33/74
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/144553 A2 (TUFTS MEDICAL CT INC [US]; KAPUR NAVIN K [US]; KARAS RICHARD [US]; MEN) 16 December 2010 (2010-12-16) claims page 1, line 7 - line 20 page 4, line 28 - line 32 -----	1-15
X	US 2007/218498 A1 (BUECHLER KENNETH F [US] ET AL) 20 September 2007 (2007-09-20) claims -----	1-15
X	US 5 827 673 A (MATSUMORI AKIRA [JP]) 27 October 1998 (1998-10-27) claims -----	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

19 March 2012

Date of mailing of the international search report

30/03/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Routledge, Brian

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/052905

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010144553 A2	16-12-2010	US 2010323380 A1 WO 2010144553 A2	23-12-2010 16-12-2010
US 2007218498 A1	20-09-2007	NONE	
US 5827673 A	27-10-1998	NONE	