Abstract:
The present invention relates to a method for diagnosing the cardiac state in a subject with coronary artery disease (CAD) undergoing or having undergone percutaneous coronary intervention (PCI). Due to the use of a specifically selected set of markers, in particular sPIT1, an ANP-type peptide and H-FABP, the invention surprisingly allows the early recognition of STENT complications in general and even the discrimination between different complications. The findings of the invention have implications in terms of patient supervision, monitoring, diagnostic procedures and therapeutic intervention.
Method for Diagnosing and Monitoring the Cardiac Pathophysiological State in a Subject Undergoing or Having Undergone Percutaneous Coronary Intervention (PCI)

The present invention relates to a method for diagnosing the cardiac state in a subject undergoing and/or having undergone percutaneous coronary intervention (PCI), to a method of monitoring a patient undergoing or having undergone PCI, to a method for recognizing and/or characterizing complications of PCI, to a method of recommending and/or deciding on diagnostic steps and/or therapy following PCI, to uses of one or a group of several peptide markers such as soluble fms-like tyrosine kinase-1 (sFlt1), hepatocyte growth factor (HGF), atrial natriuretic-type peptide (ANP-type peptide), and/or heart fatty acid binding protein (H-FABP), or variants of the aforementioned, as well as the use of antibodies directed against any one of these peptide markers in the methods of the invention. Also comprised by the present invention are kits and devices to carry out the methods of the present invention.

Coronary artery disease (CAD), also called atherosclerotic heart disease, is generally the consequence of the accumulation of atheromatous plaques within walls of the coronary arteries that supply the myocardium with oxygen and nutrients. CAD may progress over many years and may result in significant stenoses of coronary arteries, severely restricting the flow of oxygen-carrying blood to the myocardium.

Patients with CAD have frequently angina pectoris, in particular during or after physical stress, for example during or after exercise. Angina pectoris typically involves muffled, oppressive, constricting and often burning chest pain, localized behind the episternum. Patients typically describe the pain as a feeling of tightness in their chest. Typically, the pain may spread to the left, or, more rarely, to both arms and to the legs. However, pain may also spread to other regions, such as the neck, back, throat, or jaw, for example. Other symptoms of CAD are less specific and may include dyspnea, hypotension, tachycardia, increased sweating, paleness and anxiety.

In stable angina pectoris, the symptoms are generally of short duration and generally stop
some time after the stress has ended. However, besides physical stress, other events may cause stable angina pectoris, such as heavy meals, cold, psychological agitation, and sexual arousal.

In order to achieve a relief of symptoms mentioned above and of the signs of ischemic CAD in general, medication and interventions are available. Interventions include coronary bypass surgery and percutaneous coronary intervention (PCI), also referred to as coronary angioplasty. Bypass surgery is predominantly used in diffuse multivessel disease or CAD of small arteries.

PCI is chosen if localised stenoses of the main arteries have been identified. Most patients currently undergoing PCI have a localised stenosis of more than 70% of one or more vessels. PCI is specifically used to reduce or eliminate the symptoms of CAD. It is noted that, while PCI was demonstrated to improve the quality of life, it might not improve mortality when compared to medical therapy (Popma et al., Braunwald's Heart Disease, 8. Ed, p. 1419 ff).

PCI is performed in that a catheter is placed in a larger artery, such as the femoral artery and contrast agents are used to localize the coronary artery stenosis. In the past, a balloon has been inflated in the area of the stenosis, crushing the atherosclerotic plaque into the walls of the artery in order to restore blood flow. Therefore, the term balloon angioplasty is commonly used to describe this form of PCI. Currently, a STENT, that is a tube, for example a mesh tube, is placed on the balloon in order to prop the artery open and achieve a sustained blood flow.

Coronary angioplasty is not without risks. First, in the period of inflating the balloon, the artery is occluded for seconds to minutes, which may interrupt blood supply to the affected heart tissue completely, which may cause ischemic angina pectoris during the procedure. Furthermore, approximately 20 to 30% of patients have incomplete revascularization.

In addition, patients may suffer from complications of PCI, including myocardial infarction. Other complications may include arrhythmia after the procedure and stunned myocardium, which might progress into hibernating myocardium (Popma et al.).

For these reasons, the effects of PCI are monitored by angiography, in order to identify
restoration of blood flow. However, this is limited to the angioplasty procedure and does not allow the identification of complications occurring later on.

In addition, troponin peptide markers in the blood after PCI have been used to identify myocardial infarction, which may be the result from emboli releases during the intervention (Saadeddin et al., Med. Sci. Monit. 2000, 6: 708-712; Saleh et al., Am. J. Cardiol. 2006, 97: 830-834).

The present inventors have also shown that the peptide NT-proANP is rapidly released after PCI in case of necrosis (troponin T increase) and non-necrotic complications, such as temporary wall motion abnormalities and arrhythmia (WO2008/034750).

In WO 2009/150181, the inventors identified hibernation to be associated with continuous heart fatty acid binding protein elevation (H-FABP). However, significant elevation of this peptide marker has also been linked to the prediction of myocardial infarction (WO 2008/145689).

Recently, the present inventors have shown that patients presenting with chest pain or acute coronary syndrome show an increase of soluble fms-like tyrosine kinase-1 (sFITI) within minutes to hours after the event, although they did not develop myocardial infarction. Increase of sFITI was clearly associated with ischemia as indicated by its association with typical chest pain. In this group of patents, sFITI rapidly decreased after ischemia resolved indicating that sFITI represents a sensitive indicator of ischemia; similar data were obtained with hepatocyte growth factor (HGF) see EP 09177395.2.

In view of the above, it is an objective of the invention to identify or recognize complications of PCI. In particular, it is an objective to detect complications early, and to characterize them thoroughly.

It is a further objective to detect complications of PCI, to differentiate between various situations and complications, and to characterize the severity and/or acuteness of one or several complications so as to be able to take the appropriate additional diagnostic and therapeutic steps. In other words, there is a need for methods and tools that enable the skilled person to decide on diagnostic and therapeutic steps to be taken in a patient that undergoes and/or has undergone PCI.
For example, appropriate diagnostic steps would include additional diagnostic procedures such as echocardiography to identify wall motion abnormalities specifically new motion abnormalities, CT- or MRI scan, invasive or non-invasive angiography. If needed, therapeutic steps could include intervention by either PCI or cardiovascular surgery, or medication, for example.

Additional consequences from the post-procedural laboratory analysis should include the need for more or less intensive monitoring including continuous ECG monitoring. This includes also the site, where the patient needs to be supervised, such as the ICU, intermediate care or the general ward, for example.

In other words, there is a clear need for diagnostic and prognostic means. There is a need to obtain information that enables one to assess the outcome of PCI and/or to know which diagnostic therapeutic step(s), if any, will be the most adequate and promising following PCI.

Thus, the technical problem underlying the present invention can also be seen as the provision of means and methods for complying with the aforementioned needs.

The technical problem is solved by the invention as characterized and defined in the appended claims and elsewhere in this specification, in particular herein below.

In an aspect, the present invention relates to a method for diagnosing the cardiac state in a subject with coronary artery disease (CAD) undergoing and/or having undergone percutaneous coronary intervention (PCI). The cardiac state is preferably diagnosed based on the comparison of amounts of several markers determined in a sample of a subject with reference amounts of said markers.

In an aspect, the present invention relates to a method for diagnosing the cardiac state in a subject with CAD undergoing and/or having undergone PCI, based on the comparison of amounts of at least one marker selected from soluble fms-like tyrosine kinase-1 (sFLT1) and a variant of sFLT1, at least one marker selected from an atrial natriuretic-type peptide (ANP-type peptide), and a variant of an ANP-type peptide, and at least one marker selected from heart fatty acid binding protein (H-FABP) and a variant of H-FABP, with reference amounts.
In an aspect, the method of the invention comprises:

a) determining an amount of sFlT1 or of a variant of sFlT1 in a sample of the subject,
b) determining an amount of an ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject, and
c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject.

In the preferred embodiments, the method of the invention further comprises the step of d) diagnosing the cardiac state based on the comparison of the amounts of sFlT1 or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

In an embodiment, the present invention encompasses a method for diagnosing the cardiac state in a subject with coronary artery disease (CAD) undergoing and/or having undergone percutaneous coronary intervention (PCI), comprising

a) determining an amount of sFlT1 or of a variant of sFlT1 in a sample of the subject,
b) determining an amount of an ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject,
c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject, and
d) diagnosing the cardiac state based on a comparison of the amounts of sFlT1 or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

For the purpose of the present invention, the order of steps a) to c) is not relevant. These steps may be conducted simultaneously or successively, in any order, and may be conducted separately or in one single analysing unit or one kit simultaneously. Devices and kits suitable for working the method of the present invention will be described further below. The comparison and/or diagnosis made in step d) can generally only be conducted once the amount to be compared is determined in the respective step a), b) and c). Step d) may, for example, be split in separate steps, which separate steps may be conducted following the respective step a), b) or c), in which the respective amount is determined.
The samples mentioned in steps a), b) and c) in the methods of the invention may be different samples that are preferably taken within a short time interval, but at different points in time, that is, preferably all samples are taken within four hours, preferably two hours, more preferably within one hour from an individual. Alternatively, and preferably, samples mentioned in steps a) to c) in the methods of the invention may stem from one large sample, for example from one blood or serum sample, which is then subjected to the steps a), b) and c), possibly following preparation of subsamples for conducting each step a), b) and c) separately.

According to an embodiment, in step a), in addition to the amount of sFITI or of a variant thereof, an amount of hepatocyte growth factor (HGF) or of a variant thereof is determined.

In accordance with an embodiment of the present invention, in addition to sFITI or of a variant thereof, HGF or a variant thereof is determined in step a). Both markers, sFITI and HGF are in principle indicative for the same or a similar state, condition and situation, and substantially play a similar role for the diagnosis of the cardiac states and also in the goal and/or purposes of the other methods of the invention, such as monitoring, PCI assessment, risk prediction following PCI, detection, diagnosis, recognition or identification of complications following PCI, recommendations or decisions following PCI in terms of further diagnostic and/or therapeutic steps, and the like. However, HGF levels or amounts are raised for prolonged times, or even chronically with specific diseases, such as liver disease, for example. This is not the case with sFITI. SFITI has a more stable background and is thus preferred as a marker and preferably used in step a) for the purpose of the present invention.

In case that two markers are determined in one step, for example sFITI and HGF in step a), both amounts may be determined from one single sample or each of these two markers from two different samples, preferably taken within a short time interval as specified above.

According to the invention, amounts of at least three markers are determined, at least one in each step a), b) and c), respectively.
The samples mentioned in steps a) to c) are preferably taken before, during and/or following PCI.

According to a preferred embodiment, the samples of steps a) to c) in the methods of the invention are taken in the time interval starting with the start of the PCI and lasting up to about 24, about 12 hours following the end of PCI. More preferably, samples are taken in the interval starting at the end of PCI and lasting until about 8 hours, more preferably about 6 hours, and even more preferably about 4 hours and most preferably about 3 hours, or even within about 2 or about 1 hour following the end of the PCI.

The method of the invention is preferably repeated in case the amount of at least one marker determined in steps a) to c) is increased compared with the reference amount. In this way, samples taken in steps a) to c) may also be taken later than about 24 hours following PCI.

According to an embodiment, in step c) the amount of H-FABP or a variant thereof and of myoglobin or a variant thereof is determined. Accordingly, in step d), a metabolic state of the myocardium is diagnosed based on the determined amounts of H-FABP or a variant thereof and myoglobin or a variant thereof and the comparison of the amount of H-FABP to at least one reference amount for H-FABP and the comparison of the amount of myoglobin to at least one reference amount for myoglobin.

The methods and uses of the present invention are, independently, preferably in vitro methods. Moreover, they may comprise steps in addition to those explicitly mentioned above where appropriate. For example, further steps may relate to sample pre-treatments or evaluation of the results obtained by the method.

Accordingly, the amounts of the markers determined in the context of the present invention (sFlt1, HGF, ANP-type peptide, H-FABP, myoglobin, or of variants of each of the aforementioned) will preferably be determined from at least one sample of the respective subject. The sample is preferably taken independently and/or prior to the methods of the invention. The sample may be conserved, for example stored at temperatures below 0 °C before use in the methods, uses, devices and kits of the present invention. The sample may be selected from urine, blood, plasma or serum samples, for example, preferably from a serum or plasma sample, of the respective subject. The sample, once sampled, may be
stored, for example frozen, at conditions that substantially conserve the amount of the marker in the sample. It is noted that the term "sample" may refer to one single sample, to subsamples of one single sample and to different samples, a "different sample" being a separate sample taking at a different point in time from the subject.

The term "diagnosing", for the purpose of the present specification, means assessing, identifying, evaluating and/or classifying, for example the cardiac state in a subject, in particular of a subject with CAD.

The term "diagnosing" also refers to distinguishing, in particular between and/or among physiologically healthy subjects or stable patients having CAD, and between and/or among subjects suffering from or having one or more of the following complications following PCI: subjects having ischemia, incomplete revascularization (e.g. following PCI), an acute cardiac condition, arrhythmia, wall motion abnormalities, temporary and non-temporary wall stress, metabolic alterations such as stunned myocardium or hibernating myocardium, and other states associated with CAD in general or PCI complications, preferably during or after PCI. The complications cited beforehand - which in the context of the present invention are acute complications for following the event of PCI - are embodiments of the term "cardiac state", see hereinafter.

The term "cardiac state" as used in this specification preferably refers to the state, condition and/or situation of the myocardium and/or of the coronary vasculature, in particular coronary arteries in a subject. According to an embodiment, said cardiac state is a "cardiac pathophysiological state", which refers to the pathological situation or the physiological situation independently or both together. For example, the term may refer to the impact a pathological state in the coronary vasculature has on the physiological functioning of the heart. The term "cardiac state" may refer to the state of coronary arteries in terms of the extent of presence and/or absence of atherosclerotic plaques and/or stenosis.

Preferably, the term "cardiac state" refers to the state of the myocardium in terms of the absence or presence of certain specific pathologies, including but not limited to ischemia, arrhythmia, temporary and non-temporary wall stress, wall motion abnormalities, metabolic alterations such as stunning and/or hibernating myocardium.

According to a preferred embodiment, the term "cardiac state" refers to the presence or
absence of events or conditions, in particular acute events, that may occur during or following PCI and/or due to PCI, for example those mentioned above and/or cardiac syndrome, such as unstable angina pectoris and myocardial infarction. Preferably, in accordance with the invention, said cardiac state is diagnosed by aid of one or more markers present in one or more samples of a subject, which markers are indicative of specific physiological and/or pathological processes, such as in particular acute events in the body, in particular the heart. These markers preferably reflect the body's efforts to maintain, or the incapacity of maintaining, the (physiological) functioning of the heart.

According to an embodiment, the diagnosis of the cardiac state encompasses the diagnosis of at least one of the states or conditions selected from ischemia, temporary and non-temporary wall stress, wall motion abnormalities, and metabolic alteration, preferably stunning and hibernating myocardium. For example, the diagnosis allows resolving between at least two, at least three, at least four and most preferably between all of these clinical states in a subject.

According to an embodiment, the diagnosis of the cardiac state encompasses the diagnosis of the occurrence of an acute cardiac state during or following PCI and/or as a consequence of PCI. In particular, it encompasses the diagnosis of a cardiac pathology as defined above occurring acutely, for example during, following or due to PCI.

The term "subject", for the purpose of the present specification, encompasses humans and animals. Preferably, the term "subject" relates to warm blooded animals, more specifically mammals. The term "subject" thus encompasses livestock and mammal pets, such as dogs, cats, rabbits and the like. Most preferably, the term "subject" refers to humans, in particular humans having CAD. The "subject" preferably is a patient.

According to an embodiment, the "subject" refers to individuals undergoing and/or to a subject having undergone PCI, the term PCI being discussed further below. Preferably, the patient is a stable patient at least before PCI. "Stable" in the context of the present invention refers to patients representing unchanged symptoms and drug therapy for last four weeks at least before PCI, in addition the patients may not have symptoms of acute chest pain meeting the criteria of unstable angina. Furthermore, stable patients have normal kidney function as documented by creatinine values within the normal range. Preferably, the stable patient is an over 40 years, more preferably over 45, and most preferably over 50
years old male or female.

The term "percutaneous coronary intervention", generally abbreviated as "PCI" is well known to the person skilled in the art and generally refers to a surgical intervention. The term "percutaneous" means that the intervention involves access through the skin of a subject and/or operation through an opening in the skin, and the term "coronary" refers to the coronary arteries of the heart. Generally, PCI is done through the skin and through the lumen of the artery. Preferably, PCI refers to the treatment of stenotic coronary arteries as found, for example, in subjects suffering from CAD as defined elsewhere in this specification. Practically, a catheter with a deflated balloon at its tip is introduced into the coronary artery through a main artery in a limb, generally the femoral artery of the leg, or, less often, the radial or brachial artery in the arm. The catheter is then guided to the location of the stenosis. At the point of the stenosis the balloon is inflated thus compressing the atheromatous plaque and simultaneously stretching the flexible wall of the affected vessel. Therefore, PCI, also known as coronary angioplasty, is therefore often called balloon angioplasty. Sometimes, to prevent re-stenosis, a wire mesh tube (a "STENT") is introduced into the vessel to prop the vessel open at the position of the atherosclerotic plaque. The STENT may be a drug-eluting stent releasing pharmaceuticals that further reduce the risk of restenosis. Suitable pharmaceuticals are those that inhibit tissue growth. Other procedures that may be done during PCI include rotational or laser atherectomy and brachytherapy, for example.

According to a preferred embodiment, PCI involves STENT implantation and the subjects specified above are receiving or have received a STENT implantation.

The term "coronary artery disease", or "CAD", including atherosclerotic heart disease, is well known in the art and has its usual meaning. Briefly, CAD refers to the occurrence of atheromatous plaques within the walls of the coronary arteries that supply the myocardium with oxygen and nutrients. Generally, symptoms and signs of CAD are noted only in the advanced state of the disease. As the degree of the disease progresses, there may be substantial obstruction of the lumen of the coronary artery, restricting more or less the flow of oxygen to the myocardium. CAD may lead to myocardial ischemia and myocardial infarction, for example.
If an atherosclerotic lesion occludes at least 50% of the lumen of a major coronary artery, this condition is defined as 1-vessel disease. In the context of the present invention, the term "major coronary artery" comprises the right coronary artery, the left anterior descending artery and the circumflex artery. The occlusion of two or three major coronary arteries by at least 50% each is defined as 2- or 3-vessel disease. More preferably, the patient suffers from 1-vessel disease, 2-vessel disease or 3-vessel disease. The number of stenoses in a specific vessel does not affect the definition of vessel disease.

The patient may also suffer from diffuse multi-vessel disease. In this condition no occlusion of the major coronary arteries meets the criteria set forth above for 1-, 2- or 3-vessel disease. The occlusions rather affect minor arteries, nevertheless resulting in ischemia in at least parts of the myocardium.

According to an embodiment, CAD as referred to herein is stable CAD, as opposed to acute cardiovascular events. In stable CAD there are generally stable symptoms and signs, such as stable angina pectoris, for example, generally characterised by the absence of a life-threatening condition. In acute cardiovascular events, on the contrary, there is an acute, life-threatening situation, such as acute angina pectoris, acute coronary syndrome, including acute myocardial ischemia and/or myocardial infarction.

In an embodiment, the invention is suitable to diagnose the cardiac state in a subject with CAD, the subject having undergone and/or undergoing PCI. The steps by which the cardiac state can be diagnosed are disclosed in further detail below. The method of the invention preferably allows the recognition, identification and/or determination of specific conditions, symptoms, signs and/or indications for conditions related to CAD, such as the amelioration and/or deterioration of a condition, the presence of an acute event and/or of the change to an acute condition and the like, as far as the arteries of the heart and/or the heart in general are concerned, in particular following PCI.

The method of the invention encompasses the determination of one or more markers present, for example at different amounts, or absent, in at least one body liquid, such as serum, blood or urine of a subject.

A "marker" for the purpose of the present invention, is a molecule present in the body, in particular in a body fluid of a subject. A marker may be and frequently is a hormone, and
may thus but need not be associated with biochemical processes at specific situations, orga

n, tissues in the body. A marker, or the amount thereof, may be indicative of a condition occurring in the body or in a part of the body, for example in an organ or tissue of a subject, for example a physiological and/or pathological condition. Furthermore, a combination of at least one, two, three or more markers, or their amount, may be indicative for such a condition in accordance with the invention.

Markers specifically encompassed by the present specification are sFlt1, variants thereof, HGF, variants thereof, an ANP-type peptide and variants thereof, in particular ANP and NT-proANP, variants of ANP-type peptides, H-FABP and variants of H-FABP.

The markers used for the purpose of the present invention are preferably markers indicating acute events and/or acute complications, which markers may be referred to herein as acute markers. An acute marker is a marker the amount of which in a body fluid of a subject changes and in particular increases when there is an acute event, for example an acute change of a specific condition or state in the subject. In general, the amount of the marker in the body fluid goes back to the original amounts, or stabilises and persists at the increased or another amount. An "acute marker" is thus a marker that is suitable to indicate a change in a condition, in particular which indicates an acute condition, such as an acute complication of PCI, for example, such as the cardiac states and conditions indicated in this specification, for example. The invention also encompasses the use of other markers. For example in case there is a complication indicated by at least one acute marker, it may be necessary to use further markers in order to assess the impact of the acute event on a longer term and in order to monitor the patient closely.

According to an embodiment, a marker is a peptide. The term "peptide" generally has its usual meaning in the art. Preferably, in accordance with this specification, it refers to a molecule comprising a peptide bond. More preferably, it refers to a molecule comprising a plurality of amino acid moieties linked together by peptide bonds. Amino acids in peptides may be selected from the twenty two standard (proteinogenic) amino acids or, less preferably but not excluded, from non-standard amino acids, for example as formed by post translational modification. Peptides include di-, tri-, oligo- and polypeptides, comprising, respectively, two, three, 4 to 10 and more than 10 amino acid moieties. Preferably, peptides used as markers in the context of this specification are oligo- and/or polypeptides. The peptides may be characterised by post-translational modifications, such
as, for example, attachment to other functional groups, such as acetate, phosphate, lipids, and/or carbohydrates, changing the chemical nature of an amino acid moiety or of the entire peptide.

In an embodiment, the methods of the invention comprise the step of determining an amount of at least one marker in a sample of a subject.

The term "determining" or "determine", in particular in the context of determining the amount of a marker in a sample, generally relates to the generation or provision of a value or parameter, including but not limited to a numerical value, which at least approximately corresponds to, or is a function of the actual, real amount, or which simply correlates with the actual, real value or parameter of the amount of the marker in the sample. The value or parameter may be generated (obtained) as disclosed elsewhere in this specification, for example using commercially available tests or other methodology, for example as disclosed herein with respect to any specific marker. In general, the term "determining" involves the reading and/or measuring of a signal, for example a light signal, wherein the signal is in any logical way related to the amount of a marker in the sample, and preferably to the amount in the body fluid of the subject. The term "determining" may also involve producing the signal, and/or generating and/or estimating said value or parameter from said signal and/or measurement.

Determining the amount of a peptide or polypeptide marker may, preferably, comprise the steps of (a) contacting the peptide with a specific ligand, (optionally) removing non-bound ligand, (b) 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 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 marker 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.

In accordance with the present invention, determining the amount of a marker, such as a peptide or polypeptide can be achieved by all known means for determining the amount of a marker in a sample, for example. 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), for example.

General methods for determining the amount of a marker in a sample include but are not limited to measurement methods such as precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), western blotting, and mass spectrometry), can be used alone or in combination with other detection methods.

The term "amount" as used herein encompasses the absolute amount (e.g., of markers as specified herein, e.g. sFIT1 or HGF), the relative amount or concentration, 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 markers by direct measurements, e.g., intensity values in mass spectra or NMR spectra and extinction values, for example. 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 nature of the "amount" thus generally depends on the methodology used for determining the amount. Very often, signals obtained when determining an amount are transformed or translated, for example mathematically and/or electronically, or optically, for example by direct comparison with a reference signal, to a numerical value, which is an approximation of the actual amount.

According to an embodiment, if expressed as a numerical value rather than a parameter, the amount may be expressed, for example, in a weight unit, for example grams (g), milligrams (mg), micrograms (µg), nanograms (ng), or picograms (pg) for example, or as a weight per volume unit (g/l), (mg/l, µg/ml, µg/µl, pg/ml, for example), as a weight
per weight unit (g/g, mg/g, µg/g, mg/mg, µg/mg, for example), or as a molar concentration mol/l, mmol/l, µmol/l, µmol/ml, for example). Of course, the aforementioned units are merely produced for the purpose of illustration. It is also possible that a parameter of an amount is transformed, for example by way of a mathematical method, to a numerical value, such as those mentioned above.

It is noted that, for the purpose of the method of the invention, the amount of one or more markers in a sample may be determined immediately after the taking of a sample, or the sample may be stored under conditions that substantially conserve, that is, do not substantially affect or change the amount of the marker in the sample, or under conditions that cause a change in the amount of marker that can be corrected by mathematical methods taking the time spent between taking the sample and determining the amount of a marker into account. According to an embodiment, the amount of any one, several or all markers is determined within a time period of 0-48, 0-36, 0-24, 0-12, 0-6, 0-4, most preferably 0-2 hours following the taking of the sample.

The present invention encompasses determining an amount of a marker indicative for ischemia. More particularly, the methods of the present invention comprise the step of determining an amount of sFIT1 and/or of a variant thereof.

In vivo, sFIT1 is believed to bind to and to reduce free circulating levels of proangiogenic factors VEGF (vascular endothelial growth factor) and thus disables or inhibits proteins and hormones that cause blood vessel growth. For the purpose of the present invention, sFIT1 is used as an indicator for the presence of proangiogenic factors in serum of a subject. Since such factors are released in case of ischemia, sFIT1 is used as an indicator of the presence of an ischemic state or ischemia.

The term "soluble fms-like tyrosine kinase-1", more shortly also referred to as "sFIT1" herein, refers to a polypeptide, which is a soluble form of the VEGF receptor FIT1. It was identified in conditioned culture medium of human umbilical vein endothelial cells. The endogenous soluble FIT1 (sFIT1) receptor is chromatographic ally and immunologically similar to recombinant human sFIT1 and binds VEGF with a comparable high affinity. Human sFIT1 is shown to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro. Preferably, sFIT1 refers to human sFIT1. More preferably, human sFIT1 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 sFlt1 is shown in Genebank accession number BAA24499.1, GI: 2809071.

Moreover, it is to be understood that a variant of sFlt1 as referred to in accordance with the present invention may have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific sFlt1. Variants may be allelic variants, splice variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific sFlt1 or the aforementioned types of variants, preferably as long as these fragments have the essential immunological and biological properties as referred to herein. Preferably, the sFlt1 variants have immunological properties (i.e. epitope composition) and/or biological properties comparable to those of human sFlt1. Thus, the variants are generally recognizable by the aforementioned means or ligands used for determination of the amount of sFlt1. Such fragments may be, e.g., degradation products of sFLT1. Further included are variants which differ due to posttranslational modifications such as glycosylation, phosphorylation or myristylation. Preferably the biological property of sFlt1 is the ability to bind to VEGF with a high affinity and/or to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1. The biological property of sFlt-1 is, preferably, its ability to form a VEGF-stabilized complex with KDR/Flk-1 in vitro.

Variants of sFlt1 also encompass degradation products, byproducts or precursors occurring in the course of the biosynthesis or anabolism of sFlt1, in as far as the determination of any one of the former can in any way be related to the actual amount of sFlt1 in the sample, and/or if the diagnostic value as disclosed in this specification with respect to the amount of the degradation product, byproduct or precursor of sFlt1 is similar or substantially the same as for sFlt1 itself.

Preferably, a degree of identity as indicated in this specification, in particular with respect to peptide markers specified herein, is to be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of amino acid sequence
in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Add. APL. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman Proc. Natl. Acad Sci. (USA) 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment and, thus, the degree of identity. Preferably, the default values of 5.00 for gap weight and 0.30 for gap weight length are used.

For example, an amount of sFlt1 can be determined using the Quantikine Human Soluble VEGF RI/Flt-1 Immunoassay (catalogue no.: DVR100B or PCVR100B) of R&D Systems, Inc., Minneapolis, MN, USA. This test allows for quantitative determination of the sFlt1 marker in a sample.

According to an embodiment, in addition to determining an amount of sFlt1 or a variant thereof, the present invention encompasses determining amount of another marker indicative for ischemia. In particular, HGF is indicative for ischemia.

"Hepatocyte growth factor", more shortly also referred to as "HGF" in this specification, was first identified in 1984 and 1985 and purified as a potent mitogen of primary cultured hepatocytes. An inactive precursor of HGF is secreted as a single-chain polypeptide, which is cleaved by serine proteases into two chains, an alpha and a beta chain. A disulfide bond between the two chains produces the active, heterodimeric form. 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, tumorogenesis, and tissue regeneration. It is generally secreted as a single inactive polypeptide and is cleaved by serine proteases into a 69-kDa alpha-chain and 34-kDa beta-chain. The protein belongs to the plasminogen subfamily of SI peptidases but has no detectable protease activity. Alternative splicing of this gene produces multiple transcript variants encoding different isoforms. 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 GL33859835. Preferably, "HGF" refers to human HGF.

Moreover, it is to be understood that a variant of HGF as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific HGF. Variants may be allelic variants, splice variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific HGF or the aforementioned types of variants as long as these fragments have the essential immunological and/or biological properties as referred to herein. Preferably, the HGF variants have immunological properties (i.e. epitope composition) and/or biological properties comparable to those of human HGF. Thus, the variants are preferably recognizable by the means or ligands used for determination of the amount of HGF disclosed elsewhere in this specification. Such fragments may be, e.g., degradation products of HGF. Further included are variants which differ due to posttranslational modifications such as glycosylation, phosphorylation or myristylation. Preferably the biological property of HGF is the ability to bind to the proto-oncogenic c-Met receptor.
Variants of HGF also encompass degradation products, byproducts or precursors occurring in the course of the biosynthesis or anabolism of HGF, in as far as the determination of the any one of the former can in any way be related to the actual amount of HGF in the sample, and/or if the diagnostic value as disclosed in this specification with respect to the amount of the variant HGF, in particular the amount of the degradation product, byproduct or precursor of HGF is similar or substantially the same as for HGF itself.

An amount of HGF can be determined using the Quantikine Human HGF Immunoassay (catalogue no.: DHG00, SHG00, and PDHG00) from the same company. This test allows for quantitative determination of HGF in a sample.

The method of the present invention comprises a step of determining an amount of at least one marker indicative for temporary and non-temporary wall stress, wall motion abnormalities and arrhythmia. Preferably, this marker is selected from an ANP-type peptide and a variant of ANP-type peptide in a sample of a subject.

The term "atrial natriuretic-type peptide", more shortly also referred to as "ANP-type peptide" in this specification, encompasses, amongst other pre-proANP, proANP, and NT-proANP, "preprocardiodilatin", ANF (atrial natriuretic factor), and ANP (see e.g. Bonow, 1996, Circulation 93: 1946-1950). The pre-pro peptide comprises a short signal peptide, which is enzymatically cleaved off to release the pro peptide. The pro peptide is further cleaved into an N-terminal pro peptide (NT-proANP) and the active hormone ANP with 28 amino acids. ANP has a vasodilatory effect and causes excretion of water and sodium via the urinary tract. The amino acid sequence of the human pre-proANP, comprising 151 amino acids is disclosed under accession number AAA35529 and NP_006163. ANP generally encompasses the 28 C-terminal amino acids of aal24 to 151 of the pre-proANP.

ANP has a shorter half-life than its generally inactive counterpart NT-proANP. ANP is produced and released exclusively from the atrium. The amount of ANP may therefore predominantly reflect atrial function.

It is understood that a "variant of an ANP-type peptide" encompasses also variants of the specific ANP-type peptides mentioned in this specification. Such variants may have at least the same essential biological and immunological properties as the specific cardiac natriuretic-type peptides. 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 any one of said cardiac natriuretic-type peptides. Moreover, it is to
be understood that a variant of an ANP-type peptide as referred to in accordance with the
present invention preferably has 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 the specific ANP-type peptide, preferably over the entire length of the specific
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 specific ANP-type
peptides or the aforementioned types of variants as long as these fragments have the
essential immunological and/or biological properties of the respective ANP-type peptide,
for example as referred to above. Preferably, the NT-proANP or ANP variants have
immunological properties (i.e. epitope composition) comparable to those of NT-proANP or
ANP, respectively. Thus, the variants are preferably recognizable by the means or ligands
used for determination of the amount of the ANP-type peptides as specified elsewhere in
this specification. Such fragments may be, e.g., degradation products of the peptides of the
present invention. Further included are variants which differ due to posttranslational
modifications such as phosphorylation or myristylation.

Variants of ANP-type peptides also encompass degradation products, byproducts or
precursors occurring in the course of the biosynthesis or anabolism of ANP-type peptides,
in as far as the determination of any one of the former can in any way be related to the
actual amount of an ANP-type peptide in the sample, and/or if the diagnostic value as
disclosed in this specification with respect to the amount of the degradation product,
byproduct or precursor of the ANP-type peptide is the similar or substantially the same as
for the respective ANP-type peptide itself.

Preferably the biological property of ANP and its variant is the vasodilatory ability and/or
the ability of sodium and water excretion. As mentioned, other ANP-type peptides such as
pre-proANP, proANP, NT-proANP generally may lack this activity, but are encompassed
as ANP-type peptides. Their variants may thus also lack this activity.

Specifically, an amount of an ANP-type peptide (in particular NT-proANP) may be determined using the proANP(1-98) immunoassay (catalogue no.: BI-20892) by Biomedica Medizinprodukte, Vienna, Austria. This test allows for quantitative determination of the proANP marker in a sample.

According to a preferred embodiment, in step b) the amount of NT-proANP, of ANP or of a variant of any of the two aforementioned is determined, more preferably of NT-proANP.

The present invention encompasses determining an amount of a marker indicative for myocardial metabolic alterations, such as, in particular, stunning and/or hibernating myocardium. More particularly, the method of the present invention comprises the step c) of determining an amount of at least one marker selected from heart fatty acid binding protein (H-FABP) or a variant of H-FABP.

"Heart-type fatty acid binding protein" is also referred to as "heart fatty acid binding protein", or more shortly, "H-FABP" in this specification. H-FABP is also known as FABP3. H-FABP as used herein, preferably, relates to human H-FABP. The cDNA sequence as well the protein sequence of human H-FABP is well known in the art and was first described by Peeters et al. (Biochem. J. 276 (Pt 1), 203-207 (1991)). Moreover, the sequence of human H-FABP can be found, preferably, in Genebank entry U57623.1 (cDNA sequence) and AAB02555.1 (protein sequence), or also under accession number NP_004093.1. The major physiological function of H-FABP is thought to be the transport of free fatty acids, see e.g. Storch et al., Biochem. Biophys. Acta. 1486 (2000), 28-44.

A variant of H-FABP as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino acid sequence of H-FABP. Variants may be allelic variants, splice variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific H-FABP or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above, in particular the transport of free fatty acids. Such fragments may be, e.g., degradation
products of H-FABP. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation. Preferably the biological property of H-FABP is the transport of long-chain fatty acids from the cell membrane to their intracellular sites of metabolism in the mitochondria, where they enter the citric acid cycle.

Variants of H-FABP also encompass degradation products, byproducts or precursors occurring in the course of the biosynthesis or anabolism of H-FABP, in as far as the determination of any one of the former can in any way be related to the actual amount of H-FABP in the sample, and/or if the diagnostic value as disclosed in this specification of the amount of the degradation product, byproduct or precursor of H-FABP is similar or substantially the same as for H-FABP itself.

The amount of H-FABP in a sample can be determined by using the human H-FABP ELISA Kit for human heart type fatty acid binding protein (catalogue no.: HK402 or HK401), of HyCult Biotechnology, Uden, The Netherlands. This test allows for quantitative determination of H-FABP in a sample.

As has been mentioned above, tests for determining the amounts of markers and variants thereof in accordance with steps a) to c) of the methods of the invention are commercially available.

The method of the invention generally comprises a comparison of the amounts of the marker, as determined in steps of the method of the invention, to respective reference amounts.

The term "comparing" or "comparison" as used herein encompasses comparing the amount of the marker, for example the peptide, polypeptide, or protein comprised in the sample to be analyzed or determined with a reference amount. The reference amount is disclosed elsewhere in this specification. It is to be understood that comparing as used herein preferably refers to a comparison of corresponding parameters or values, for example, 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. In other words, the amount determined in steps a), b) and/or c) is compared with a reference amount that is actually comparable, or comparison with which makes statistically, physically and/or
scientifically sense.

The term "comparison" compasses, for example, the comparison of numerical values, of parameters, or of signals directly, such as signal strengths, for example without a numerical value being produced, area under the curve and the like. In particular, the term "comparison" refers to a comparison of "amounts" as defined elsewhere in this specification. The comparison comprises and encompasses all kinds of comparative operations, such as mathematical operations, such as subtraction and/or division in order to produce a difference and/or a ratio, for example.

In this context, the term "as determined in steps a) to c)" is used, for example, in step d), encompasses the comparison of comparable, preferably substantially the same marker as determined specifically and respectively in the respective step. Although this should be clear, it is shortly illustrated by an example with respect to step b): If, in step b) an amount of NT-proANP is determined, step d), preferably encompasses the comparison with a reference amount of NT-proANP, and not with any one of a variant of NT-proANP, ANP and a variant of ANP, for example. This principle preferably applies to all steps, a), b) and c) of the method of the invention equally.

The comparison referred to in step d) of the method of the present invention may be carried out manually or may be computer assisted. For a computer assisted comparison, the value of the determined amount may be compared by a data processing unit, for example using a computer program, to values corresponding to references which are stored in a database.

The data processing unit or the computer program may further evaluate the result of the comparison, that is, automatically provide the desired assessment, for example diagnosis, in a suitable output format. In other words, the diagnosis in step d) of the methods of the present invention may be conducted by the same data processing unit that is used to conduct the comparison mentioned in step d).

Based on the comparison of the amount(s) determined in step a), b) and/or c) to reference amount(s), it is possible to diagnose the cardiac state, to recognize and/or identify complications of PCI and/or the occurrence of complications due to or following PCI, and/or to recommend and/or to decide on diagnostic steps and/or therapy.
A "reference amount" with which amounts determined in steps a), b) and c) are compared in step d) in the various aspects and embodiments of the invention, may be a predetermined amount or may need to be or can be determined, for example as discussed below.

The "reference amount" is in particular such that a diagnosis or assessment as specified in step d) of the various aspects and embodiments of the invention can be conducted. In particular, the reference amounts are such that it is possible to diagnose the cardiac state, to recognize and/or identify complications of or due to PCI and/or the occurrence of complications due to or following PCI to recommend and/or to decide on diagnostic steps and/or therapy. These diagnosis and assessments generally depend on the amounts of several, preferably all markers as determined in steps a), b) and c), and reference amounts are thus preferably established for all markers together and/or in combination.

With respect to the markers used in the methods of the invention, the term "reference amount", preferably, refers to an amount of a sFII, a variant thereof, HGF, a variant thereof, an ANP-type peptide, a variant thereof, and/or of H-FABP or a variant thereof, respectively. The amount is such that a differentiation can be made between a patient, in whom there is any kind of complication and/or acute event during an/or due to PCI and in a patient in whom there was no complication, in particular no complication deserving further attention or measures. Preferably, in patients having complications due to or following PCI, also a differentiation between different cardiac, in particular coronary or myocardial clinical states or different acute events can be made. According to an embodiment, the reference amounts are such that the various relevant clinical states, complications and/or acute events of PCI can be resolved. In particular, a distinguished diagnosis can preferably be made in step d) on the basis of the amounts of markers measured in steps a) to c) of the method of the invention.

The differentiation between clinical states or acute events then allows the taking and/or deciding on appropriate diagnostic and/or therapeutic steps, such as specified elsewhere in this specification. In this way, the present invention has clinical implications in terms of patient supervision, monitoring, diagnostic procedures and therapeutic interventions.

As mentioned above, in order to make the diagnosis, monitoring and assessments, and so forth, in steps d) of the various aspects and embodiments of the invention, preferably at least one marker should be determined in each of steps a) through c), so that there are at
least three amounts to be compared with the corresponding at least three reference amounts.

Of course, reference amounts are generally specific for each marker and may be also be specific to a specific variant of a marker.

According to an embodiment, the reference amount of any one, several or all markers is specific for each individual subject, and is thus determined for each individual subject separately, in a personalized manner, in particular from a sample taken before PCI. In accordance with this embodiment, the reference amount is a specific, personalised and/or individual reference amount.

Patients having CAD and in particular stable CAD may have levels or amounts of certain markers as specified in this specification that may be above the levels in a healthy subject not suffering from CAD, as is shown in Tables 1 and 2 further below. Furthermore, the amounts of markers as specified herein in a subject may depend on various factors such as age, gender, condition, disease state and the like.

In particular, levels of markers in the blood of a subject, in particular the level of an ANP-type peptide or F-FABP may fluctuate substantially between subjects, although resting generally constant within a specific subject. This also and in particular applies to subjects being in or suffering from a stable condition, such as stable CAD.

As mentioned in more detail elsewhere in this specification, the markers as specified herein, in particular sFIT1, HGF, ANP-type peptides and H-FABP, are preferably considered as acute markers.

According to an embodiment, the reference amount of any one, several or all markers as specified herein are determined on the basis of an amount of the respective marker or its variant, in another sample of a specific subject, said other sample being taken before said PCI. This preferably applies to the reference amount of the ANP-type peptide, the variant thereof, H-FABP or the variant thereof, as determined in steps b) and c) of the method of the invention. Preferably, however, it applies to all markers.

In this regard, the expression "determined on the basis of" as used in the larger expression
"determined on the basis of an amount of the respective marker" means that the reference amount may in any way, for example by mathematical, computer-assisted, statistical and/or empiric means, be derived from the amount of the respective marker in the sample taken before PCI. Preferably, the expression "determined on the basis of" means "corresponds substantially to" or "is equal to".

According to a preferred embodiment, the reference amount of the ANP-type peptide, the variant thereof, H-FABP, or the variant thereof, independently, corresponds to an amount of the respective marker or its variant, determined in another sample of said subject, said other sample taken before said PCI.

According to a preferred embodiment, the reference amount of any one, several or all of sFIT1 or the variant thereof, HGF or the variant thereof, the ANP-type peptide, the variant thereof, H-FABP, or the variant thereof, independently, corresponds to an amount of the respective marker or its variant, in another sample of said subject, said other sample taken before said PCI.

"Before PCI" preferably refers to a period of about ≤7 days, ≤6 days, ≤5 days, ≤4 days, preferably ≤ about 96 hours, preferably ≤ about 48 hours, more preferably ≤ about 24 hours, most preferably ≤ about 12 hours before the start of the PCI. These time periods apply in particular to the moment of the taking of the sample from which the reference amount is determined, and not necessarily to the moment of determining the reference amount, for example by conducting the specific commercially available test, which is generally some time after the taking of the sample. Accordingly, these time periods express the moment in which the amount of the marker in the blood of the subject is expected to correspond to an amount that is suitable to determine the reference amount.

The respective reference amount may be determined on the basis of a plurality of separate measurements of the amount of the respective marker in the subject, all separate measurements being made from one or several samples taken before PCI. This may give more statistical weight to the actual amount of the specific marker in the subject. On the other hand, the reference amount of any specific marker may be determined only once from a sample of the subject taken before PCI. This is possible, for example, thanks to highly precise and reliable tests and procedures, including automated procedures, for determining amounts of the markers specified herein. The reference amount determined
before PCI can then be used to recognize the occurrence of a complication during or due to PCI.

As becomes clear from the above, the method of the present invention is preferably suitable or adapted to detect a change and in particular an increase of an amount of a marker in a body fluid, in particular in the blood of a specific, individual subject. Accordingly, the reference amount is preferably an amount determined from an individual subject itself and thus applies specifically to the respective individual subject only.

In accordance with an embodiment, an amount of any marker as specified herein, as determined in accordance with steps a) to c) of the invention, which amount being above the reference amount of the respective marker, generally reflects an increase of the amount of said marker in said subject.

According to an embodiment, an amount of a marker in a subject is found to be "increased" or there is an "increase" of a marker with respect to and/or compared with a reference amount if an amount of a marker, in particular as determined in any one of steps a) to c) is > about 15%, preferably > about 20%, more preferably > about 30%, > about 40%, > about 50%, > about 60%, > about 70%, > about 80%, > about 90%, > about 100% above the reference amount, in particular as determined in the subject before PCI.

For the purpose of illustration, typical amounts of markers encompassed by the present invention as determined in healthy patients and of stable patients having CAD are given in Tables 1 and 2 below. These amounts can give an idea about the value of reference amounts, and reference amounts may, for example, be located within the range between the 25th and the 75th percentile in Tables 1 and/or 2.

**Table 1**: Amounts of markers in patients having stable CAD

<table>
<thead>
<tr>
<th>Marker</th>
<th>Amount (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50th percentile</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>233.8</td>
</tr>
<tr>
<td>Troponin T</td>
<td>11.1</td>
</tr>
<tr>
<td>sFlT1</td>
<td>80.7</td>
</tr>
<tr>
<td>HGF</td>
<td>1385</td>
</tr>
</tbody>
</table>
The data shown in Table 1 stem from a total of 79 patients (49 males, 30 females, mean age 59 years) with established or suspected coronary artery disease and scheduled for angiography. All patients were clinically stable. Blood samples were taken before intervention and tested for the markers listed in Table 1. All patients had normal kidney function as documented by creatinine values within the normal range.

Table 2: Amounts of markers in apparently healthy subjects

<table>
<thead>
<tr>
<th>Marker</th>
<th>Amount (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50th percentile</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>37.25</td>
</tr>
<tr>
<td>Troponin T</td>
<td>0.0</td>
</tr>
<tr>
<td>sFIT1</td>
<td>71</td>
</tr>
<tr>
<td>HGF</td>
<td>1320</td>
</tr>
<tr>
<td>H-FABP</td>
<td>1257</td>
</tr>
<tr>
<td>NT-proANP</td>
<td>860</td>
</tr>
</tbody>
</table>

The data shown in Table 2 stem from a group of 149 clinically healthy individuals (51 males, mean age 40 years, range 20 - 52 years and 97 females, mean age 41 years, range 18-56 years) who can be considered as an apparently healthy control population. All subjects have normal blood pressure, a normal electrocardiogram, no diabetes mellitus and no history of cardiac disease of any type and also no cardiovascular risk factors. In addition they have normal kidney function.

As can be seen from Tables 1 and 2, the markers sFIT1 and HGF are similar between healthy patients and patients having stable CAD. Furthermore, the range of amounts in these markers is comparatively narrow. From the data used to prepare Tables 1 and 2, it is possible to deduce specific reference amounts for the purpose of the invention.

As can also be seen from Tables 1 and 2, the amounts of the markers H-FABP and NT-proANP are different between healthy subjects and patients with stable CAD. In particular, the ranges of amounts covered by these markers are substantially larger in the stable CAD.
patients than in healthy subjects. The dependence of the cardiac function is thus more clearly shown with H-FABP and the ANP-type marker, and less with sFITI and HGF. Due to the fluctuations of NT-proANP and H-FABP, it is more difficult to provide reliable predetermined, non-personalized reference amounts that have general validity for all patients with respect to H-FABP and the ANP-type peptide.

Nevertheless, the skilled person may specify a specific, pre-determined and non-individualized reference amounts from values in Table 1 and/or 2, for example.

For the purpose of the present specification, and in particular in the context of reference amounts, the term "allows diagnosing" can also be understood as "is indicative for".

In accordance with the above data, and in the context of reference amounts, the following amounts of the respective markers are indicative for a healthy subject, which means that amounts equal to or higher than the following reference amounts indicate that a subject is not healthy and suffers from an acute complication of its cardiac state as used and defined in the context of the present invention, during/following PCI or due to/as a consequence of PCI. The higher the amount of the respective marker, the higher is the probability that the individual suffers from an acute complication of its cardiac state as defined in the context of the present invention.

In general, the reference amount for sFITI for a healthy individual is about 80 pg/ml, more preferably about 75 pg/ml, even more preferably about 70 pg/ml.

According to an embodiment, the reference amount for HGF is about 1650 pg/ml, more preferably about 1500 pg/ml, even more preferably about 1300 pg/ml.

According to an embodiment, the reference amount for an ANP-type peptide, such as ANP or NT-proANP, for example, is about 1000 pg/ml, more preferably about 900 pg/ml, even more preferably about 850 pg/ml.

According to an embodiment, the reference amount of H-FABP is about 1500 pg/ml, more preferably about 1350 pg/ml, even more preferably about 1250 pg/ml.

In all cases of predetermined, fixed and/or non-personalized reference amounts indicated
above, these reference amounts have less diagnostic value and are thus less preferred than personalized reference amounts determined from the concerned, individual patient before PCI, as described in more detail elsewhere in this specification.

With respect to the diagnosis of a specific cardiac condition or state on the basis of these reference amounts, the same as specified elsewhere in this specification applies.

In general, the reference amounts of sFlt1 and/or HGF is preferably such that an amount of sFlt1 and/or HGF, respectively, in a sample of a subject that is increased compared with the reference amount is indicative for an ischemic state, a severe ischemic state, and a very severe ischemic state in a subject, in particular for an acute ischemic state.

Furthermore, the reference amount of sFlt1 and/or HGF is/are such that, when combined with reference amounts of other markers, the further, different and preferably more specific clinical interpretations can be made.

The reference amounts of an ANP-type peptide is preferably such that an amount of an ANP-type peptide in a sample of a subject that is increased compared with the reference amount is indicative for arrhythmias and/or wall stress and/or wall motion abnormalities in a subject, in particular acute arrhythmias and/or wall stress and/or wall motion abnormalities.

The reference amount of H-FABP is preferably such that an amount of H-FABP peptide in a sample of a subject that is increased compared with the reference amount is indicative for at least one selected from myocardial stunning and myocardial hibernation in a subject, in particular acute stunning.

According to a preferred embodiment, the following diagnostic algorithm can be applied:

Table 3: Peptide marker levels and a diagnostic algorithm

<table>
<thead>
<tr>
<th>Peptide marker level</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>sFlt-1</td>
<td>HGF</td>
</tr>
<tr>
<td></td>
<td>H-FABP</td>
</tr>
<tr>
<td></td>
<td>ANP-type, preferably NT-proANP</td>
</tr>
<tr>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Not increased</td>
<td>Not increased</td>
</tr>
<tr>
<td>Not increased</td>
<td>Not increased</td>
</tr>
<tr>
<td>Increased</td>
<td>Increased, preferably more than 100%</td>
</tr>
<tr>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Increase of the peptide marker level indicated in Table 3, above is meant to refer to an increase in the level of the respective marker determined based on a pre-PCI sample, or an increase compared to the corresponding reference amount.

The reference amounts mentioned above represent preferred embodiments of the present invention. Reference amounts may be experimentally and/or clinically determined, and may, as a consequence, be changed, adapted or re-determined, for example from samples taken within the same time intervals as specified above for the taking of the samples used in steps a) to c).

According to an embodiment, the reference amount of any markers used herein (sFITI, HGF, ANP-type peptide, H-FABP, and variants of the aforementioned) is determined by methods known to the person skilled in the art, in particular by statistical means.

In general, for determining the respective amount(s)/amount(s) or amount ratios allowing to establish the desired diagnosis in accordance with the respective embodiment of the present invention, ("threshold", "reference amount"), the amount(s)/amount(s) or amount ratios of the respective marker, in particular peptide or peptides are determined in appropriate patient groups. According to the diagnosis to be established, the patient group may, for example, comprise only healthy individuals, or may comprise healthy individuals and individuals suffering from the pathophysiological state which is to be determined, or may comprise only individuals suffering from the pathophysiological state which is to be determined, or may comprise individuals suffering from the various pathophysiological
states to be distinguished, by the respective marker(s) using validated analytical methods. The results which are obtained are collected and analyzed by statistical methods known to the person skilled in the art. The obtained threshold or reference values are then established in accordance with the desired probability of suffering from the disease (the term "disease" referring also to one or more specific pathological or clinical state(s) or conditions(s) as detailed elsewhere in this specification) and linked to the particular threshold value or reference amount. For example, it may be useful to choose the median value, the 60th, 70th, 80th, 90th, 95th or even the 99th percentile of the healthy and/or non-healthy patient collective, in order to establish the threshold value(s), reference amount(s) or amount ratios.

A reference amount of a diagnostic marker can be established, and the amount of the marker in a patient sample can simply be compared to the reference amount. The sensitivity and specificity of a diagnostic and/or prognostic test depends on more than 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 (or pathological or clinical state as specified herein) will likely overlap. Under such conditions, a test, for example in a test kit, or method 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 recognition and/or identification of a condition. ROC curves can be used even when test results do not 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, reference amounts of 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.

While exemplary panels are described herein, one or more markers may be replaced,
added, or subtracted from these exemplary panels while still providing clinically useful
results. Panels may comprise both specific markers of a disease (e.g., markers that are
increased or decreased in arrhythmia but not in other disease states) and/or non-specific
markers (e.g., markers that are increased or decreased due to inflammation, regardless
of the cause; markers that are increased or decreased due to changes in hemostasis, regardless
of the cause, etc.). While certain markers may not individually be definitive in the methods
described herein, a particular "fingerprint" pattern of changes may, in effect, act as a
specific indicator of disease state. As discussed above, pattern of changes may be obtained
from a single sample, or may optionally consider temporal changes in one or more
members of the panel (or temporal changes in a panel response value).

In order to test if a chosen reference value yields a sufficiently safe diagnosis of patients
suffering from the disease of interest, one may for example determine the efficiency (E) of
the methods of the invention for a given reference amount using the following formula:

\[ E = \frac{TP}{TO} \times 100; \]

wherein TP = true positives and TO = total number of tests = TP + FP + FN + TN, wherein
FP = false positives; FN = false negatives and TN = true negatives. E has the following
range of values: 0 < E < 100). Preferably, a tested reference value yields a sufficiently safe
diagnosis provided the value of E is at least about 50, more preferably at least about 60,
more preferably at least about 70, more preferably at least about 80, more preferably at least about 90, more preferably at least about 95, more preferably at least about 98.

The diagnosis if individuals are healthy or suffer from a certain pathophysiological state is made by established methods known to the person skilled in the art. The methods differ in respect to the individual pathophysiological state.

The algorithms to establish the desired diagnosis are laid out in the present application, in the passages referring to the respective embodiment, to which reference is made.

Accordingly, the present invention also comprises a method of determining the reference amount indicative for a physiological and/or a pathological state and/or a certain pathological state, comprising the steps of determining in appropriate patient groups the amounts of the appropriate marker(s), collecting the data and analyzing the data by statistical methods and establishing the threshold values.

The term "about" as used in this specification refers to +/- 20%, preferably +/-10%, preferably, +/- 5% of a given measurement, value or amount, for example of a given reference amount.

As the above indications with respect to the determination of reference values demonstrate, the methods and uses of the invention do rarely have the purpose of having 100% sensitivity and specificity, that is, the diagnosis will not in all cases be completely correct. However, the methods and uses of the invention are such that they allow the prediction of the making of a diagnosis with reasonable, preferably high probability and thus have important predictive, diagnostic and/or clinical value.

As is apparent from the above, the cardiac state is preferably diagnosed on the basis of the comparison as conducted in step d). More specifically, a deviation of an amount of a marker as determined in steps a) to c) from the reference amount of the respective marker provides an information that can be used in diagnosing said pathophysiological state.

According to an embodiment, in addition to the definition of the term "comparison" elsewhere in this specification, the "comparison" is preferably resulting in the diagnosis or detection of a presence or absence of a difference between an amount as determined in any
one of steps a) to c) and to the reference amount of the respective marker. In particular, the comparison determines the occurrence or not of an increase of any marker with respect to the reference amount. Furthermore, the "comparison" preferably yields information with respect to the extent of such a difference, in particular increase.

5 The cardiac state may be diagnosed on the basis of the comparison of the amount of markers with reference amounts as discussed elsewhere in this specification, but in particular as discussed herein below.

10 In particular, an increase of an amount of at least one marker determined in step a) of the method of the invention with respect to the respective reference amount is indicative for the presence of ischemia, in particular for an ischemic state, a severe ischemic state, and a very severe ischemic state in a subject.

15 An increase of an amount of at least one marker determined in step b) of the method of the invention with respect to the respective reference amount is indicative for at least one selected from arrhythmia, wall motion abnormalities and wall stress, including temporary and non-temporary wall stress.

20 An increase of an amount of at least one marker determined in step c) of the method of the invention with respect to the reference amount is indicative for at least one selected from stunning and hibernating myocardium.

The extent by which an amount of a marker determined in any one of steps a) to c) is increased is generally indicative for the severity of the respective cardiac state, acute event and/or diagnosis. This generally applies to all markers. For example, in case of sFIT1, the extent by which the reference amount is trespassed by an amount determined in step a) is indicative for the severity of the ischemic state.

30 The term "ischemia" or "ischemic state", as used herein, is well known to the skilled person. In general, it relates to the state of an impaired blood supply that is not sufficient for metabolic needs, in particular for oxygen supply to the affected tissue. In the context of the present specification, "ischemia" is preferably cardiac ischemia and thus not related to bowel, brain and cutaneous ischemia. Ischemia may be associated with, lead to or cause an altered myocardial function depending on the extent of ischemic myocardium and duration.
of ischemia. Myocardial function normalizes rapidly after a single episode of ischemia lasting less than 2 minutes. As ischemia increases in duration and/or severity, there is a temporal delay in recovery of function that occurs, despite the fact that blood flow has been restored. A 15 minute occlusion of a vessel results, e.g., in a 6 h altered myocardial function although the blood flow is restored. This reversible event is not associated with myocardial necrosis. Preferably, the term "ischemia" encompasses a reversible cardiac dysfunction and a non reversible cardiac injury, as well as a process leading to, being associated with or causing a reversible cardiac dysfunction or a non reversible cardiac injury. Ischemia or the ischemic state also encompasses a cardiac injury, the latter being associated with, leading to and/or causing a myocardial infarct.

The cardiac pathophysiological state of ischemia is preferably diagnosed, in accordance with the invention, if the amount of sFIT1 and/or HGF, or of variants of any one of the aforementioned, preferably as determined in step a), is increased compared with the respective reference amount.

The term "arrhythmia", also known as cardiac dysrhythmia, as used in this specification, is well known to the skilled person. It is a term for any one of a large and heterogeneous group of conditions in which there is an abnormal electrical activity in the heart. The heart beat may be too fast or too slow, and may be regular or irregular. Some arrhythmias are life-threatening medical emergencies that can result in cardiac arrest and sudden death. Others cause symptoms such as an abnormal awareness of heart beat (palpitations), and may be merely annoying. Extra-systoles occur as single additional contractions of the myocardium or as salvos of repeated additional contractions. They may affect the complete myocardium or only the atria or only the ventricles. Most preferably, an arrhythmia as referred to in the present application has detrimental effects on the circulation.

The cardiac, and in particular myocardial pathophysiological state of arrhythmia is preferably diagnosed, in accordance with the invention, if the amount of an ANP-type peptide or of a variant thereof, is increased with respect to the respective reference amount, and if amounts of sFIT1 and/or HGF or variants of any one or more thereof, preferably as determined in steps b) and a), are not increased compared with their respective reference amounts.

The term "wall stress" and "wall motion abnormalities", as used in this specification, is
well known to the skilled person. Stress on the wall of the heart is generally caused by substantial and acute increase of blood volume in the heart or if part of the contracting musculature of the heart fails, or when blood transport is arrhythmic. The aforementioned situations result in or are associated with fluctuations of the transported blood volume which exerts fluctuating pressures and stresses to the heart, generally causing wall stress. Wall stress may be temporary and non-temporary. Wall stress is generally associated with wall motion abnormalities, which may be diagnosed by echocardiography, for example. In general, wall stress causes immediately increase of ANP-type peptides, and, if it extends for longer time periods, for example > 4 hours, increase of BNP-type peptide, such as NT-proBNP. Wall stress may be temporary or non-temporary. Generally, patients with cardiac insufficiency have non-temporary wall stress. Acute events are generally associated with fluctuations of transported blood volume in the left ventricle and thus generally may cause wall stress. The wall motion abnormalities are, preferably, caused by an impaired contractility of the affected areas of the myocardium. Stunned or hibernating myocardium as described below frequently displays wall motion abnormalities.

The cardiac pathophysiological state of wall stress, in particular temporary wall stress is preferably diagnosed, in accordance with the invention, if the amount of sFlt1 and/or HGF, and of the ANP-type peptide, or variants of any one or more of the aforementioned, preferably as determined in steps a) and b), are increased compared with their respective reference amounts.

The terms "stunning", also described as "stunned myocardium" is well known to the skilled person. This term refers to a temporal delay in the recovery of the myocardial function following ischemia, despite the fact that blood flow has been restored. A defining feature of isolated myocardial stunning is that myocardial function remains depressed while resting myocardial perfusion is normal. Thus, there is a dissociation of the usual close relationship between the subendocardial flow and function. Stunned myocardium may also occur after demand-induced ischemia. This may occur, for example, in a patient suffering from stable CAD when exercising, so that oxygen demand in myocytes exceeds supply due to a stenotic atherosclerotic plaque, limiting flow rate in a coronary artery, for example. Ischemic events leading to stunning are, for example, short term (generally < 20 min) total occlusions of a coronary artery, which may occur, for example during balloon inflation during the STENT implantation procedure. Stunning also occurs due to medium-term (0.3-5 hours) partial occlusions of a coronary artery. In stunning, the myocardial function may
be regionally depressed for some time after perfusion is restored, as can be experimentally shown by measuring left ventricular wall thickening during myocardial contraction. The delay for functional restoration following restoration of blood flow generally depends on the length and severity of the ischemic event. Normally, stunning lasts from 1 hour to up to a week. If, however, repetitive reversible ischemia develops before function normalizes, stunning can lead to a state of persistent dysfunction or chronic stunning, in which the complete myocardial function is achieved only weeks after perfusion is restored. Chronic stunning may, in turn, lead to hibernating myocardium, discussed further below, in case of reversible repetitive ischemia and depressed resting flow.

The cardiac states of stunning, along with severe ischemia and wall stress are preferably diagnosed, in accordance with the invention, if the amount of sFlt1, and/or HGF, ANP-type peptide, and/or H-FABP, or of variants of any one or more of the aforementioned, preferably as determined in steps a) to c), are increased compared with their respective reference amounts.

The term "hibernating myocardium", also referred to as "myocardial hibernation" as used in this specification, is well known to the skilled person. In general, a hibernating myocardium is a condition in which myocardial contractility, metabolism and ventricular function are reduced in order to cope with a reduced oxygen supply. It is a chronic, but in many cases reversible cardiac dysfunction that is caused by prolonged myocardial hypoperfusion and that persists generally for some time that may last up to 12 months, even if normal blood flow is restored. Thus, hibernating myocardial cells are temporarily asleep, but still viable, and can "wake up" to normal function when the blood supply is fully restored by revascularization.

A "hibernating myocardium", for the purpose of the present invention, preferably is a persistent myocardial dysfunction that occurs when myocardial perfusion is chronically reduced but sufficient to maintain the viability of the myocardial tissue (see e.g. Braunwald's Heart Disease 8th Ed. 2005 Elsevier Publishers, Chapters 48 and 54).

Thus, the cardiac state or condition of a hibernating myocardium is preferably a state, in which one or a plurality of myocardial regions show a chronically depressed contractile ability, but are still viable. It is known in the art that hibernating myocardium can cause abnormal systolic or diastolic ventricular function or both. Moreover, the skilled person
knows that myocardial hibernation is generally a state of persistent ventricular dysfunction that can be reversed by revascularization. Preferably, the term "hibernating myocardium" does not include myocardial stunning, which is a transient posts ischemic dysfunction defined elsewhere in this specification. However, it is known the art that stunning myocardium and hibernating myocardium can coexist and that stunning myocardium may turn into hibernating myocardium, particularly in cases of repetitively stunned tissues. Reviews on myocardial hibernation are, e.g., given by Heusch et al. (Am J Physiol Heart Circ Physiol 288:984-999, 2005) and Kalra et al. (Kalra DK, Zoghbi WA: Myocardial hibernation in coronary artery disease. Curr Atheroscler Rep 2002, 4:149-155). The existence of a hibernating myocardium was first shown in subjects after bypass surgery. Angiographic studies in subjects which underwent coronary angioplasty, e.g., revealed immediate recovery of global and regional systolic, as well as diastolic, function after revascularization. It has been proposed that the formation of hibernating myocardial regions is due to chronic ischemia and is a mechanism to prevent myocardial necrosis and other ischemic symptoms. The exact molecular mechanisms underlying hibernation are still not completely understood. However, they may include impaired calcium metabolism by the sarcoplasmic reticulum and reduced sensitivity of myofibrils to calcium. Metabolically, in the hibernating myocardium maximum the rate of glucose uptake during insulin stimulation and creatine phosphate and ATP levels are not regionally altered, contrasting the depressed ATP levels in stunning.

It is noted that a "hibernating myocardium", for the purpose of the present specification, preferably relates to a chronic hibernating myocardium, and preferably does not refer to short term hibernation, which refers to reduced regional oxygen consumption and energy uptake during persistent hypoperfusion. The ability of short-term hibernation to prevent necrosis is limited by the severity and duration of ischemia, with irreversible injury developing generally after periods of more than 24 hours.

The cardiac state of a hibernating myocardium is preferably diagnosed, in accordance with the invention, if the amount of H-FABP or a variant thereof is increased compared with the respective reference amount, and if amounts of sFlt1, HGF, ANP-type peptide or variants of the aforementioned, preferably as determined in steps a) to c), are not increased compared with their respective reference amounts.

The methods of the present invention are suitable to diagnose "hibernating myocardium"
and thus to detect the occurrence of viable, but dysfunctional myocardial tissue.

In particular, according to an embodiment of a method of the invention, in step d), said cardiac state of said subject is diagnosed, as the case may be as detailed further below under no. 1 to 6. As used in no. 1 to 6, the peptide determined in step a) refers to sFIT1 or to a variant of sFIT1; the peptide determined in step b) refers to an ANP-type peptide or to a variant of an ANP-type peptide; the peptide determined in step c) refers to H-FABP or to a variant of H-FABP.

1) An amount of the peptide determined in step a) that is increased compared with the respective reference amount is indicative for ischemia. In particular, it is indicative for an ischemic complication following PCI. If the increase is >100%, this indicates severe ischemia.

2) An amount of the peptide determined in step a) not increased compared with the respective reference amount, and an amount of the peptide determined in step b) increased compared with the respective reference amount is indicative for arrhythmia.

3) An amount of the peptide determined in step a) increased compared with the respective reference amount, and an amount of the peptide determined in step b) increased compared with the respective reference amount is indicative for temporary wall stress.

4) An amount of the peptide determined in step a) not increased compared with the respective reference amount, an amount of the peptide determined in step b) not increased compared with the respective reference amount, and an amount of the peptide determined in step c) increased compared with the respective reference amount, are indicative for hibernating myocardial regions.

5) An amount of the peptide determined in step a) increased compared with the respective reference amount, an amount of the peptide determined in step b) increased compared with the respective reference amount, and an amount of the peptide determined in step c) increased compared with the respective reference amount is indicative for severe ischemia, wall stress, and stunning, in particular due to prolonged ischemia with metabolic alterations.
In particular, if the H-FABP is increased over 100%, this is generally associated with an increase of sFlt1, (HGF, if determined) and the ANP-type peptide, or variants thereof, of >100% and is indicative for new wall motion abnormality.

6) An amount of the peptide determined in step a) not increased compared with the respective reference amount, an amount of the peptide determined in step b) not increased compared with the respective reference amount, and an amount of the peptide determined in step c) not increased compared with the respective reference amount is indicative for an absence of complications of PCI.

According to an aspect, the present invention provides a method for monitoring a subject with CAD before, during and/or after PCI, the method comprising:

i) conducting steps a) to c) of the method of diagnosing the cardiac state in a subject with CAD in accordance with the invention;

ii) after a period of time, repeating steps a) to c) of the method of diagnosing the cardiac state in a subject with CAD in accordance with the invention;

Undiagnosing a change of the cardiac state of the subject on the basis of a comparison of the amounts of sFlt1, the variant thereof, the ANP-type peptide, the variant thereof, H-FABP, the variant thereof, as determined in step ii) with the amounts of the respective marker as determined in a previous step, and thereby monitoring PCI in the subject.

According to an embodiment, steps ii) and/or iii) may be repeated several times, in dependence of the cardiac condition of a patient. For example, steps ii) and iii) may be repeated as long as necessary, for example until a subject is in a stable cardiac state. In this manner, monitoring of the subject is achieved. Monitoring may thus be continued over prolonged time until the absence of any acute cardiac condition is diagnosed.

The expression "in a previous step" may thus preferably refer to step i), but in case step ii) is repeated it may refer to step i) but also to a step that is previous, preferably directly preceding the repeated step ii) and/or iii).

Step ii) is conducted after a period of time following step i) the length of period of time may be made dependent of the cardiac state of the subject, with shorter periods of time being chosen in case of severe acute conditions and longer periods of time in case if a
subject is in a stable condition. In general time periods may lie in the range of 0.5 hours to 48 hours, preferably 1 hour to 24 hours, more preferably 1.5 hours to 12 hours, most preferably 2 hours to 8 hours.

According to an embodiment, at least step i) is conducted before PCI. Amounts of markers determined before PCI may be used as reference amounts as specified elsewhere in this specification. In this case, the monitoring encompasses an overall period starting before PCI and extending until after PCI, preferably until the cardiac state of a subject is stable. "Before PCI" refers to time periods before the start of PCI as defined elsewhere in this specification, preferably to a time period that is ≤ about 48 hours, ≤ about 24 hours, more preferably ≤ about 12 hours and most preferably ≤ about 8 hours before the PCI.

It is noted that time periods indicated with respect to the present invention, in particular time periods as specified with respect to "before PCI" or "after PCI", or the expression "after a period of time" in particular between conducting step i) and step ii) refer to the time periods that have passed between the taking of samples or to the moment of the taking of a sample and not necessarily to the moment of the determination of an amount of a specific marker in the sample. With respect to the time periods between the moment of the taking of a sample and the moment of determination of an amount of the marker using, for example, the appropriate test, the same as specified elsewhere in this specification applies.

Accordingly, the expression "at least step i) is conducted before PCI" preferably means "conducting step i) by using at least one sample taken before PCI for conducting steps a) to c)". In other words, the sample referred to in steps a) to c) is taken before PCI in accordance with this embodiment.

With respect to the time passed between the taking of a sample and the actual determination of an amount of the marker in the sample, the same as specified elsewhere in this specification applies.

If step i) is conducted before PCI, step ii) is preferably conducted during or shortly after PCI. "After PCI", for the purpose of the present specification, is not limited to a specific time after the end of the intervention.

It is important and thus preferred that at least one of steps i) and ii) is conducted at least
once shortly after the end of PCI, in particular within a time period of \( \leq \) about 8 hours, preferably \( \leq \) about 6 hours more preferably \( \leq \) about 4 hours. Since the markers that are determined for the purpose of the present invention are acute markers, it is necessary to determine the amounts of the markers in a sample taken towards the end of the PCI procedure or shortly after termination of PCI. After the indicated periods, the amount of the marker in the blood of a subject may change again, for example may drop again to previous amounts, and no reliable diagnosis can be made with respect to acute events in this case.

In accordance with the aforementioned, the expression "step ii) is conducted during or shortly after PCI" preferably means "conducting step ii) by using at least one sample taken during or shortly after PCI for conducting steps a) to c)".

According to a preferred embodiment, step i) is conducted before and step ii) is conducted after PCI. If there is a change, and in particular an increase of an amount of a marker found on the basis of the comparison of the amounts of a markers determined in steps i) and ii), it is possible to diagnose an acute condition and/or a cardiac state as specified elsewhere in this specification.

According to another embodiment, steps i) and steps ii) are both conducted after PCI. In this case, a subject is monitored after PCI, for example in case there has been any kind of complication or any change in the cardiac state due to or following PCI and monitoring of the subject is thus recommended.

According to an embodiment, any one or both of steps i) and/or ii) are conducted during PCI. The amounts of the markers determined in the methods of the invention generally increase very quickly in case of an acute event and/or a complication and by conducting steps i) and/or ii) during PCI such a acute event and/or complication may be diagnosed very early.

According to a preferred embodiment, step i) is conducted before PCI, steps ii) and/or iii) are conducted during PCT, and steps ii) and iii) are repeated after PCI. In this case, the subject is monitored during and after PCI. Furthermore, in this embodiment the amounts determined in step i) may be used as reference amounts.
It is noted that the subject may be monitored during PCI but is preferably monitored after PCI. Monitoring after PCI does not exclude that step i) be conducted before PCI, for example in order to determine a reference amount for the purpose of comparison and diagnosis as disclosed elsewhere in this specification.

Generally, during the PCI, the subject undergoing PCI and the effects of PCI on a subject are monitored, for example by angiography, to identify restoration of blood flow. As this is generally limited to the duration of the procedure, the steps of the embodiments of the methods of the invention directed to monitoring are preferably conducted and/or continued after PCI, in particular, said step i) and/or step ii) is preferably conducted on the basis of a sample taken towards the end of the PCI procedure or very shortly after termination of PCI.

If there is an increase in at least one marker, this is generally indicative for a change of the cardiac state in the subject. Specific acute states or pathophysiological conditions can be diagnosed on the basis of the presence or absence of an increase of one, several or all markers as determined in steps a) to c) as set forth elsewhere in this specification.

The determination of amounts of a respective marker over time, in particular before, during and after PCI can be used, for example to assess if there are complications due to PCI, if PCI did not bring any improvement or deterioration, and/or the presence or absence of any acute event such as specified elsewhere in this specification. For example, the occurrence of a life threatening condition is a complication of PCI. In this way, it is possible to monitor PCI in a subject with CAD. It is thus also possible to detect changes in the cardiac state in a subject over time.

In an aspect, the present invention provides a method for recognizing and/or characterizing complications of PCI in a subject. The embodiments of the method of diagnosing a cardiac state in a subject preferably also apply to other methods, in particular to the method of recognizing and/or characterizing complications of PCI in a subject.

In an embodiment, the present invention provides a method for recognizing and/or characterizing complications of PCI in a subject, comprising

a) determining an amount of sFIT1 or of a variant of sFIT1 in a sample of the subject,
b) determining an amount of an ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject,
c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject,
d) recognizing and/or characterizing complications of PCI based on a comparison of the amounts of sFlt1 or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

The subject of the method of the invention is preferably a subject undergoing and/or having undergone PCI.

In accordance with the invention, a complication of PCI can be recognized and/or identified if the amount of one or more, two or more, or three or more markers determined in any one of steps a) to c) is increased if compared to the reference amount of the respective marker.

"Complications of PCI" encompass, in particular, any one or more selected from ischemia, wall stress, wall motion abnormalities, cardiac metabolic alterations, such as stunned myocardium and hibernating myocardium, which are all discussed in further detail elsewhere in this specification.

It is noted that the invention preferably concerns the recognition and/or identification of an acute event such as those mentioned above, occurring during, shortly (up to 4 hours) after PCI and/or due to the PCI.

Based on the comparison conducted in step d), the complications of PCI can be characterized. The characterization preferably encompasses the differentiation between the various or different complications as specified elsewhere herein, for example under no. 1 to 5 discussed hereinabove.

Furthermore, the characterization preferably encompasses an assessment of the severity and/or acuteness of a complication. The latter may be characterized on the basis of the extent or amount by which the amount of a marker determined in any one of steps a) to c) is increased compared with the respective reference amount. For example, an increase of
sFITI of about 20% determined in step d) indicates ischemia, whereas an increase of about 100% or more indicates the presence of a severe ischemia. The same principle preferably applies to the other markers, such as those determined in steps b) and c).

In an aspect, the invention provides a method for recommending or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD. The embodiments of the method of diagnosing a cardiac state in a subject preferably also apply to other methods, in particular to the method of recommending or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD.

According to an embodiment the invention provides a method for recommending or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD, comprising:

a) determining an amount of sFITI or of a variant of sFITI in a sample of the subject,

b) determining an amount of an ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject,

c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject,

d) recommending or deciding on diagnostic steps and/or therapy following PCI based on a comparison of the amounts of sFITI or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

On the basis of the comparison in step d) further diagnostic steps and/or therapy may be recommended or decided, in particular if there were complications due to or during PCI, for example if the amount of at least one marker determined in steps a) to c) is increased with respect to a reference amount.

Similarly, following the diagnosis of the cardiac state in a subject, the recognition and/or characterization of complications following PCI, and risk prediction as disclosed elsewhere in the specification, further diagnostic steps and/or therapy may be recommended or decided.

Further diagnostic steps that can be recommended or decided include diagnostic
procedures such as echocardiography (ECG) to identify wall motion abnormalities, in
particular new motion abnormalities (for example, in the situation of no. 3) and 5) above),
CT- or MRI scans, invasive and non-invasive angiography.

Therapy that can be recommended or decided may include medication and, if needed,
intervention by either further PCI or cardiovascular surgery.

Further recommendations and/or decisions from the comparison in step d) include the
intensity of monitoring. For example, continuous ECG monitoring may be recommended
and/or decided.

Still further recommendations and/or decisions from the comparison in step d) include the
determination of the site where the subject is supervised or located. For example, it can be
recommended or decided to locate a patient in the intensive care unit (ICU), in the
intermediate care unit or in the general ward.

Only for illustration purposes, in case the amounts of all markers determined in steps a) to
c) are above their respective reference amounts, there are severe complications following
PCI, and in step d) of an embodiment of an invention further diagnostic steps such as
angiography, continuous ECG monitoring are preferably recommended or decided.
Furthermore, intervention by PCI or ECG needs to be assessed and the subject is
supervised in the ICU.

In particular, when the amount of any marker is increased by >100% if compared to the
reference amount, or, in the case of monitoring, if an amount determined in step ii) is
increased by >100% if compared to an amount of the respective marker determined in a
previous step, the respective subject deserves further clinical attention and work up. Such
work up includes the repeated measurement of these markers after 2 to 4 hours, for
example by repeating steps ii) and iii) of the method of monitoring of the present invention
and in addition the determination of Troponin T and/or Troponin I as an indicator of
myocardial necrosis and NT-proBNP as an indicator of sustained cardiac function.
Moreover, these individuals deserve continuous ECG monitoring and echocardiography for
comparison with the pre-treatment ECHO results. If appropriate further diagnostic
procedures might be warranted, such as CT and MRI scan including under stress
conditions as well as angiography without or with intervention (as needed).
In an embodiment, the present invention provides a method of diagnosing CAD in a subject, comprising

a) determining an amount of sFlt1 or of a variant of sFlt1 in a sample of the subject,
b) determining an amount of ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject,
c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject,
d) diagnosing the coronary artery disease based on comparing the amounts of sFlt1 or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

In an aspect, the present invention provides the use of one or more markers selected from sFlt1, a variant thereof, HGF, a variant thereof, an ANP-type peptide, a variant thereof, and H-FABP and/or a variant thereof, for diagnosing the cardiac state in a subject having undergone PCI.

In another aspect, the present invention provides the use of one or more markers selected from sFlt1, a variant thereof, HGF, a variant thereof, an ANP-type peptide, a variant thereof, H-FABP, and a variant thereof, for monitoring a subject with CAD undergoing and/or having undergone PCI.

According to an embodiment of the above uses of the invention, at least three different markers, in particular at least sFlt1 or a variant thereof, an ANP-type peptide or a variant thereof, and H-FABP or a variant thereof are used.

According to another embodiment, at least three or at least four markers are used, in particular sFlt1 and optionally also HGF, besides H-FABP and the ANP-type peptide, and/or, independently, variants of the aforementioned markers.

In an aspect, the present invention provides the use of one or more antibodies selected from an anti-sFlt1 antibody, an anti-HGF antibody, an anti-ANP-type peptide antibody, and an anti-H-FABP antibody, or from one or more antibodies directed against a variant of the respective antigen, or a fragment of the antibody, for diagnosing the cardiac state in an
subject undergoing and/or having undergone PCI.

In general, an antibody directed against any specific marker as specified herein (for example, an anti-sFlT1 antibody) may be replaced, independently of the antibodies against the other markers, by an antibody against a variant of the respective marker (in accordance with the above example: an anti-variant-of-sFlT1 antibody).

In another aspect, the present invention provides the use of one or more antibodies selected from an anti-sFlT1 antibody, an anti-HGF antibody, an anti-ANP-type peptide antibody, and an anti-H-FABP antibody, or from an antibody directed against a variant of the respective antigen, or a fragment of the antibody, for monitoring a subject with CAD undergoing or having undergone PCI.

According to an embodiment, at least three or at least four antibodies are used, in particular at least one or both of the group of anti-sFlT1 and anti-HGF, besides anti-ANP-type peptide and anti-H-FABP antibodies.

According to an embodiment of the above uses of antibodies, at least an anti-sFlT1 antibody, an anti-ANP peptide antibody, and an anti-H-FABP antibody are used, and/or antibodies directed against one or more variants, independently, of the aforementioned markers.

Anti-ANP-type antibodies are preferably selected from anti-NT-proANP and anti-ANP antibodies.

Antibodies, for the purpose of the present specification, are preferably specific antibodies. "Specific" means, for example, that said antibodies specifically bind to the respective marker. The antibodies are specific, in particular, if they do not bind to another marker and/or to other epitopes potentially present in a sample of a subject, which other epitopes are not related to the present invention.

Furthermore, "specific" means that an antibody does bind only one of the markers of the present invention, and/or the variant thereof, but not other markers used for the purpose of the present invention.
Antibodies may be monoclonal or polyclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(\(ab\))\(_2\) fragments that are capable of binding antigen or hapten. Antibodies and fragments are discussed in more detail elsewhere in this specification. Monoclonal antibodies are preferred.

Further embodiments of the various methods of the invention described elsewhere in this specification and in particular further above also apply to the uses of markers and of anti-marker antibodies of the invention. In particular, the invention encompasses the use of the markers for other purposes, in particular diagnostic purposes, as specified elsewhere in this specification.

The invention encompasses the use of markers or anti-marker antibodies as specified herein for one or more selected independently from: monitoring a subject undergoing and/or having undergone PCI; recognizing and/or characterizing complications of PCI in a subject, in particular a subject undergoing and/or having undergone PCI; and recommending and/or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD.

For example, the invention encompasses the use of one, two or all three of sFITI, an ANP-type peptide and H-FABP, or variants of the respective marker, for detecting acute events occurring due to or during PCI or shortly (for example, up to 4 hours) thereafter.

According to further embodiments, the present invention provides the use of one, two, preferably three or more markers selected from the group of sFITI or a variant thereof, an ANP-type peptide or a variant thereof, and H-FABP or a variant thereof for manufacturing a diagnostic composition. The diagnostic composition may be used for one or more selected from diagnosing the cardiac state of a subject undergoing and/or having undergone PCI, monitoring a subject undergoing and/or having undergone PCI; recognizing and/or characterizing complications of PCI in a subject, in particular a subject undergoing and/or having undergone PCI; and recommending and/or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD.

According to further embodiments, the present invention provides the use of one, two, preferably three or more selected from the group of an anti-sFITI antibody or an antibody directed against a variant of sFITI, an anti-ANP-type peptide antibody or an antibody directed against a variant of an ANP-type peptide, and an anti-H-FABP antibody or an
antibody directed against a variant of H-FABP for manufacturing a diagnostic composition. The diagnostic composition may be used for one or more selected from diagnosing the cardiac state of a subject undergoing and/or having undergone PCI, monitoring a subject undergoing and/or having undergone PCI; recognizing and/or characterizing complications of PCI in a subject, in particular a subject undergoing and/or having undergone PCI; and recommending and/or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD.

The present invention also encompasses a kit and/or a device, preferably suitable to conduct the methods of the present invention.

In an aspect, the present invention provides a device for diagnosing the cardiac state in a subject with CAD undergoing and/or having undergone PCI, comprising:

1) at least one analysing unit for determining the amounts of a) at least one marker selected from sFlt1, a variant thereof, HGF and a variant thereof, b) at least one marker selected from an ANP-type peptide and a variant thereof, and c) at least one marker selected from H-FABP, and a variant thereof, in one or more samples of the subject, preferably one or more blood or serum samples;

2) at least one evaluation unit for making at least one comparison of the amounts determined by unit 1) with reference amounts, and for assisting and/or conducting the diagnosis of said cardiac state on the basis of said comparison.

The term "device" as used herein preferably relates to a system of means, units or elements (hereinafter simply "means") comprising 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 in the device. For example, where an analysis unit for automatically determining the amount of the markers 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 one or more marker in an applied sample and an evaluation unit for processing the
resulting data.

According to an embodiment, the device comprises an analysing unit capable of measuring an amount of sFIT1 or a variant thereof, an ANP-type peptide or a variant thereof, and H-FABP or a variant thereof, in one or more applied samples of a subject.

According to an embodiment, in addition or instead of the capacity of measuring an amount of sFIT1 or a variant thereof, the analysing unit is capable of measuring an amount of HGF or a variant thereof.

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 for determining the amount of the respective markers, 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 any one of the respective markers, in particular, sFIT1, ANP-type peptide, H-FABP, or variants thereof, and/or HGF in addition to sFIT1. The strip or device, preferably, comprises means for detection of the binding of any one of said respective markers 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, for example on a display unit and/or as a print-out, 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 an aspect, the present invention provides a kit for diagnosing the cardiac state in a subject with CAD, the subject undergoing and/or having undergone PCI comprising:

1) at least one analysing unit for determining the amounts of a) at least one marker selected from sFITI and a variant thereof, b) at least one marker selected from an anti-ANP-type peptide and a variant thereof, and c) at least one marker selected from H-FABP, and a variant thereof, in one or more samples of the subject, preferably one or more blood or serum samples;

2) at least one evaluation unit for making at least one comparison of the amounts determined by unit 1) with reference amounts, and for assisting and/or conducting the diagnosis of said cardiac state on the basis of said comparison.

The term "kit" as used herein refers to a collection of the components mentioned elsewhere in this specification, in particular at least one analysing unit and at least one evaluation unit, which may or may not be packaged together. The components of the kit may be comprised in 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 aforementioned methods using the kit of the present invention.

The kit preferably comprises one or more analyzing agents, preferably at least one analysing agent per marker of which an amount is to be determined in the methods of the invention.

According to an embodiment, the kit comprises at least agents capable of specifically
recognizing sFlT1 or a variant thereof, an ANP-type peptide or a variant thereof, H-FABP or a variant thereof, in one or more samples of a subject.

According to an embodiment, the kit comprises, in addition to the agent capable of specifically recognizing sFlT1 or a variant thereof, an agent specifically recognizing HGF or a variant thereof.

The said agent shall upon binding to sFlT1, HGF, an ANP-type peptide, H-FABP and/or variants of the aforementioned, respectively and as the case may be, preferably, be capable of generating a detectable signal, the intensity of which correlates to the amount of the respective marker or the variant thereof present in the sample. Dependent on the type of signal which is generated, methods for detection of the signal can be applied which are well known in the art. Analyzing agents which are preferably used for the kit of the present invention include antibodies or aptamers. The analyzing agent may be present on a test stripe as described elsewhere herein. The amounts of the sFlT1, HGF, an ANP-type peptide, H-FABP, or variants of the aforementioned, thus detected can then be further evaluated in the evaluation unit.

Preferred evaluation units to be used for the kit of the present invention include those referred to elsewhere herein.

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

**Examples**

In the following examples, the following tests were used for the determination of the amounts of the respective peptides:

Soluble FlT-1 (sFlT1) was determined with a sFlT1 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 sFlT1 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 sample. A sandwich complex comprising sFlT1 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.

HGF (hepatocyte growth factor) was tested by an enzyme linked immunoassay (ELISA) (RD Systems, Minneapolis, Catalogue Nr. DHG00) using a monoclonal antibody specific for HGF and a precoated microplate. Standards and samples are pipetted into the wells and any HGF present is bound by the immobilized antibody. After washing away unbound substances, an enzyme-linked polyclonal antibody specific for HGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of HGF bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

NT-proANP was determined with the proANP ELISA assay (B1-20892) obtained from Biomedica, Vienna, Austria. This sandwich assay comprises a polyclonal sheep NT-proANP specific antibody bound to a microtiterstrip. The sample is added to the microtiterstrip so that the proANP can bind to the antibody. After binding of the proANP to the first antibody a second proANP specific antibody is added to the vessel. This second antibody is conjugated with horseradish peroxidase (HRP). After incubation the unbound enzyme-conjugated antibody is removed by washing the microtiterstrip. Finally, tetramethylbenzidine (TMB) is added as a substrate for the HRP. The more proANP the sample contains, the more conjugated antibody binds. Thus, the total activity of HRP present in the vessel depends on the amount of proANP in the sample and the initial rate of TMB converted is a measure for the amount of NT-proANP in the sample.

The amount of H-FABP is determined by an ELISA, using the HBT ELISA Test Kit for human heart type fatty acid binding protein (HyCult Biotechnology, Uden, The Netherlands).

The named tests are also preferably employed in the general context of the present invention for the determination of the respective peptide markers.
Example 1: Monitoring of peptide markers in patients undergoing PCI

30 patients with stable CAD who received a STENT implantation were included into this study. Patients were excluded from the study if they had symptoms of stable or unstable angina within the last 2 weeks. For inclusion into the study, creatinine had to be in the normal range, indicating normal kidney function. Patients had a mean age of 61 years (49 to 71), 18 were males and 12 females.

Serum samples were taken 6 to 24 hours before intervention, and 4 hours thereafter. Samples were centrifuged within 30 minutes and the supernatant was frozen and kept at minus 20 C until used for analysis, analysis was done in a single run.

A change, in particular an increase, in concentration was assumed if concentrations differed by at least 20 % if compared to concentrations before intervention.

During the intervention, patients were monitored by electrocardiogram (ECG), and in some cases by echocardiography. Further information on the progress and potential occurrence of acute events was obtained by angiography.

Stable patients could leave the hospital the day following PCI.

Results

Table 4 below summarises the changes of the value of each peptide marker separately compared to the value determined before PCI.

As shown in Table 4, among the 30 patients with stable CAD who underwent angiography and STENT implantation, an increase of sFITI (more than 20%) was noted in 23 patients, this increase in sFITI exceeded 100% in 13 patients. HGF increased in 22/30 patients (more than 20%) and in 13/30 more than 100 % from baseline. Seven patients had no increase in sFITI and 8 patients no increase in HGF. Patients who had no increase in sFITI also had no increase in HGF.

An increase of NT-proANP (more than 20%) was noted in 17 patients, this increase exceeded 100% in 6 patients.
H-FABP increased in 14 patients (more than 20%), this increase exceeded 100% in 6 patients.

**Table 4**: Increases in monitored peptide markers in blood following PCI

<table>
<thead>
<tr>
<th>Concentration change</th>
<th>Peptide marker</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase over 20%</td>
<td>sFlT1</td>
<td>23/30</td>
</tr>
<tr>
<td>Increase over 100%</td>
<td>HGF</td>
<td>14/30</td>
</tr>
</tbody>
</table>

As evident from the increase in level of sFlt-1, the study indicated that ischemia was noted in 77% (23) of the patients due to the stent procedure and severe ischemia was found in 47% (sFlT1 exceeding 100 % increase).

Patients without sFlT1 increase had no chest pain. However, only 17 patients with a sFlT1 increase (20%) had temporary chest pain, 12 of them in the severe ischemia group. This distribution was identical in the HGF group.

An NT-proANP increase was noted in 17 patients. This increase exceeded 100% in 6 patients. An NT-proANP increase without increase in sFlT1 and HGF was seen in 4 patients, 3 of them had arrhythmia of at least 20 seconds during the procedure. Among the patients who had an NT-proANP increase of more than 100% (6), 4 had severe ischemia and 3 of these 6 had temporary wall motion abnormalities on the angiography film.

Table 5 below compares the outcome of changes of NT-proANP with the one of sFlT1 and HGF.

**Table 5**: Peptide markers sFlT1 and HGF in PCI patients having increased NT-proANP values.

<table>
<thead>
<tr>
<th>Peptide marker</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proANP</td>
<td>sFlT1 / HGF</td>
</tr>
<tr>
<td>increase 20-100%</td>
<td>no change</td>
</tr>
<tr>
<td>increase &gt;100%</td>
<td>no change</td>
</tr>
</tbody>
</table>
As shown in Table 4, increases of H-FABP were found in 14 patients, and in 6 of them this increase exceeded 100%. In 4 of the 14 patients with H-FABP increase, there was no increase of HGF or NT-proANP. This finding was consistent with an increase in wall motion abnormality 2 weeks after the procedure in 3 patients and required late intervention because of suspected extended hibernation.

In case of an H-FABP increase of over 100% (6 patients), this was associated with sFlT1, HGF and NT-proANP increase of more than 100%, and in 4 patients with detectable new wall motion abnormality. This finding can be interpreted as ischemia associated stunning. Table 6 below summarises these findings, showing the data obtained for sFlT1, HGF, and NT-proANP compared to H-FABP.

**Table 6**: Soluble FIT-1, HGF and NT-proANP peptide marker values in PCI patients having increased H-FABP values.

<table>
<thead>
<tr>
<th>Peptide marker</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-FABP</td>
<td>sFIT1 / HGF</td>
</tr>
<tr>
<td>increase &gt;20%</td>
<td>no increase</td>
</tr>
<tr>
<td>increase &gt;20%</td>
<td>increase &gt;20%</td>
</tr>
<tr>
<td>increase &gt;20%</td>
<td>increase &gt;20%</td>
</tr>
</tbody>
</table>

The results described above are surprising, because previous experiments of stress testing did not reveal that sFIT1 could be useful as biochemical marker. On the other hand, more recent findings show that increased sFIT1 is indicative for ischemia in patients with acute coronary syndrome without progression to myocardial infarction.

The present inventors demonstrate in particular that certain markers, in particular sFIT1 and HGF, allow the detection of ischemic complications after STENT implantation. Even more surprisingly, the use of sFIT-1 allows a more definite interpretation of markers already shown to be useful after PCI.

As shown, NT-proANP increases without increases of sFIT1 or HGF point to arrhythmia
which is consistent with the clinical findings. A H-FABP increase without increase of sFlT1, HGF and NT-proANP is consistent with extension of hibernating myocardial regions after intervention, a complication which can be treated by intervention. Increases of sFlT1, HGF and NT-proANP indicate temporary wall stress and increase in all marker sFlT1, HGF, NT-proANP and H-FABP are indicators of severe ischemia, wall stress and even more importantly detection of stunning effect due to prolonged ischemia with metabolic alterations.

In summary the detection of ischemia by sFlT1 allows surprisingly the early recognition of STENT complications and even more surprisingly the resolution of clinical states previously identified using NT-proANP and H-FABP.

The findings have clinical implications in terms of patient supervision, monitoring, diagnostic procedures and therapeutic interventions, in particular as mentioned elsewhere in this specification.
Claims

1. A method for diagnosing the cardiac state in a subject with coronary artery disease (CAD) undergoing and/or having undergone percutaneous coronary intervention (PCI), comprising
   a) determining an amount of soluble fms-like tyrosine kinase-1 (sFlT1) or of a variant of sFlT1 in a sample of the subject,
   b) determining an amount of an atrial natriuretic-type peptide (ANP-type peptide) or of a variant of an ANP-type peptide in a sample of the subject,
   c) determining an amount of heart-type fatty acid binding protein (H-FABP) or of a variant of H-FABP in a sample the subject, and
   d) diagnosing the cardiac state based on the comparison of the amounts of sFlT1 or the variant thereof, the ANP-type peptide or the variant thereof, and H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

2. The method of any one of claim 1, wherein the diagnosis of said cardiac state encompasses the diagnosis of at least one of the states or conditions selected from ischemia, temporary and non-temporary wall stress, wall motion abnormalities, and metabolic alteration, preferably stunning and hibernating myocardium.

3. The method of any one of the preceding claims, wherein said the diagnosis of said cardiac state encompasses the diagnosis of the occurrence of an acute cardiac state.

4. The method of any one or the preceding claims, wherein, in step a), in addition to the amount of sFlT1 or of a variant thereof, an amount of hepatocyte growth factor (HGF) or of a variant thereof is determined.

5. The method of any one of the preceding claims, wherein the cardiac state as diagnosed in step d) is used to identify the presence or absence of complications of the PCI.

6. The method of any one of the preceding claims, wherein the reference amount of any one of sFlT1 or the variant thereof, if applicable HGF or the variant thereof, the
ANP-type peptide, the variant thereof, H-FABP, or the variant thereof, independently, corresponds to an amount of the respective marker or its variant, in another sample of said subject, said other sample taken before said PCI.

7. A method for monitoring a subject with CAD before, during and/or after PCI, the method comprising:
   i) conducting steps a) to c) of the method of claim 1;
   ii) after a period of time, repeating said steps a) to c) of the method of claim 1;
   iii) diagnosing a change of the cardiac state of the subject on the basis of a comparison of the amounts of sFlT1, the variant thereof, the ANP-type peptide, the variant thereof, H-FABP, the variant thereof, as determined in step ii) with the amounts of the respective marker as determined in a previous step, and thereby monitoring PCI in the subject.

8. A method for recognizing and/or characterizing complications of PCI in a subject, comprising
   a) determining an amount of sFlT1 or of a variant of sFlT1 in a sample of the subject,
   b) determining an amount of an ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject,
   c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject,
   d) recognizing and/or characterizing complications of PCI based on a comparison of the amounts of sFlT1 or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

9. A method for recommending or deciding on diagnostic steps and/or therapy following PCI in a subject with CAD, comprising:
   a) determining an amount of sFlT1 or of a variant of sFlT1 in a sample of the subject,
   b) determining an amount of an ANP-type peptide or of a variant of an ANP-type peptide in a sample of the subject,
   c) determining an amount of H-FABP or of a variant of H-FABP in a sample the subject,
d) recommending or deciding on diagnostic steps and/or therapy following PCI based on a comparison of the amounts of sFIT1 or the variant thereof, the ANP-type peptide or the variant thereof, H-FABP or the variant thereof, as determined in steps a) to c), with respective reference amounts.

10. Use of one or more markers selected from sFIT1, a variant thereof, HGF, a variant thereof, an ANP-type peptide, a variant thereof, and H-FABP a variant thereof, for diagnosing the cardiac state in a subject undergoing and/or having undergone PCI.

11. Use of one or more antibodies selected from an anti-sFIT1 antibody, an anti-HGF antibody, an anti-ANP-type peptide antibody, and an anti-H-FABP antibody, or from an antibody directed against a variant of the respective antigen, or a fragment of the antibody, for diagnosing the cardiac state in a subject undergoing and/or having undergone PCI.

12. Use of one or more markers selected from sFIT1, a variant thereof, HGF, a variant thereof, an ANP-type peptide, a variant thereof, H-FABP and a variant thereof, for monitoring a subject with CAD undergoing and/or having undergone PCI.

13. Use of one or more antibodies selected from an anti-sFIT1 antibody, an anti-HGF antibody, an anti-ANP-type peptide antibody, and an anti-H-FABP antibody, or from an antibody directed against a variant of the respective antigen, or a fragment of the antibody, for monitoring a subject with CAD undergoing or having undergone PCI.

14. A kit for diagnosing the cardiac state in a subject with CAD, the subject undergoing and/or having undergone PCI comprising:
   1) at least one analysing unit for determining the amounts of a) at least one marker selected from sFIT1 and a variant thereof, b) at least one marker selected from an anti-ANP-type peptide and a variant thereof, and c) at least one marker selected from H-FABP and a variant thereof, in one or more samples of the subject, preferably one or more blood or serum samples;
   2) at least one evaluation unit for making at least one comparison of the amounts determined by unit 1) with reference amounts, and for assisting and/or conducting the diagnosis of said cardiac state on the basis of said comparison.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/68

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

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category</th>
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<th>Relevant to claim No.</th>
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<td>X</td>
<td>wo 2008/034750 AI (HOFFMANN LA ROCHE; ROCHE DIAGNOSTICS GMBH; HESS G) 27 March 2008 (2008-03-27) cited in the application on the whole document, in particular paragraph spanning pages 17 and 18; sentence spanning pages 26 and 27; claims 1, 15, 16</td>
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<td>A</td>
<td>wo 2009/150181 A2 (ROCHE DIAGNOSTICS GMBH; HOFFMANN LA ROCHE; HESS G) 17 December 2009 (2009-12-17) cited in the application on the whole document, in particular paragraph spanning pages 13 and 14; page 38, line 31 to page 39, line 8; page 47, lines 26-30; example 3</td>
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Further documents are listed in the continuation of Box C. 

* Special categories of cited documents:

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

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"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

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"A" document member of the same patent family

Date of the actual completion of the international search

8 November 2011

Date of mailing of the international search report

15/11/2011

Name and mailing address of the ISA/Authorized officer

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

Weber, Peter
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