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(54) **Title**: METHOD FOR STAGING MYOCARDIAL INFARCTION AND FOR MONITORING EFFICACY OF INTERVENTION

(57) **Abstract**: The present invention relates to a method for the stage of myocardial infarction in a subject showing a ST segment elevated myocardial infarction (STEMI). Moreover, the present invention relates to a method for identifying a subject being susceptible to cardiac intervention, wherein the subject suffers from a ST segment elevated myocardial infarction (STEMI). The methods of the present invention are based on the determination of soluble fms-like tyrosine kinase-1 (sFLT-1), heart fatty acid binding protein (H-FABP) and a cardiac troponin or a variant thereof in a sample of said subject, and comparing the amounts of sFLT-1, H-FABP, and the cardiac troponin to at least one reference amount. Also comprised by the present invention are kits or devices to carry out the methods of the present invention.

Method for staging myocardial infarction and for monitoring efficacy of intervention

The present invention relates to a method for the evaluation of the stages (staging) of myocardial infarction including related stages, i.e. stages preceding myocardial infarction and/or leading to or being associated with myocardial infarction and/or co-existing with myocardial infarction, in a subject showing signs and symptoms of acute chest pain or acute coronary syndrome and being diagnosed with a ST-segment elevation myocardial infarction (STEMI). Moreover, the present invention relates to a method for identifying a subject, being diagnosed with a ST-segment elevation myocardial infarction (STEMI), which is susceptible to cardiac intervention/treatment, and to a method of deciding on the intervention/treatment. The present invention also includes a method of monitoring the efficacy of the intervention/treatment of the ST-segment elevation myocardial infarction. Also comprised are kits and devices for carrying out the methods of the present invention. The methods of the present invention are based on the determination of each of the peptides: soluble fms-like tyrosine kinase-1 (sFLT-1), heart fatty acid binding protein (H-FABP) and a cardiac troponin from the group troponin T and troponin I in a sample of said subject and comparing the amount of the above-cited peptides to at least one reference amount. Also comprised by the present invention are kits or devices to carry out the methods of the present invention.

An aim of modern medicine is to provide personalized or individualized treatment regimens. Those are treatment regimens which take into account a patient's individual needs or risks. A particularly important risk is the presence of a cardiovascular complication, especially of an acute cardiovascular event. Cardiovascular complications belong to the leading causes of morbidity and mortality in the Western hemisphere. For individual treatment of a person who suffers from a cardiovascular complication, a reliable diagnosis has a significant impact on the success of the treatment of said person. This is particularly important for patients showing signs and symptoms of acute coronary syndrome (ACS).

Patients with signs of acute coronary syndrome have a significantly increased risk of experiencing non reversible cardiac injury or even cardiac death and, therefore, need to be identified among the patients with nontraumatic chest symptoms (Morrow et al., National academy of clinical biochemistry guidelines: Clinical characteristics and utilization of biochemical markers in acute coronary syndrome, 2007, Circulation;115;356-375). An acute coronary syndrome is caused by a sudden blockage in a coronary artery, significantly reducing or cutting off the blood supply to connected areas of the myocard (heart muscle) and resulting in ischemia (lack of blood supply).

Clinical symptoms of acute coronary syndrome are believed to be caused by acute myocardial ischemia. Patients with chest pain or signs and symptoms of instable angina or acute coronary syndrome (ACS) frequently present to their doctor as an emergency or to the emergency room. Clinical evaluation of these patients includes a medical history specifically directed to evidence of existing cardiovascular disease or their risk factors, analysis of the type of symptoms as described, as well as clinical signs associated with acute coronary syndrome such as evidence of pulmonary edema, hypotension and/or. tachy- or bradycardia (Circulation 106: 1893, 2002 / Task force on Practice guidelines of the American Heart Association). In addition an ECG and laboratory tests are performed. From the evidence obtained, a diagnosis is established which includes confirmation of the suspected ACS and a differential diagnosis which subform of ACS the individual suffers from (unstable angina pectoris UAP, ST-segment elevation myocardial infarction STEMI or non-ST-segment elevation myocardial infarction NSTEMI).

Heart tissue becomes necrotic in case of significant and/or persisting ischemia. Myocardial infarction (MI), also termed heart attack, is known as cell necrosis in the myocard (heart tissue) resulting from ischemia, as described by The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction (The Joint European Society of Cardiology/American College of Cardiology Committee: Universal definition of myocardial infarction, European Heart Journal (2007), 28, 2525 - 2538). Myocardial infarction is caused by the sudden occlusion or significant narrowing of a coronary artery. The sudden narrowing or occlusion of a coronary artery is frequently caused by the formation of a thrombus after plaque disruption. In case of insufficient collaterals (which is frequently the case) blood flow is obstructed and the affected myocardium becomes ischemic.

The electrocardiogram (ECG) provides important information for the diagnosis. This information and the interpretation of it are known to the person skilled in the art. Particularly, if the ECG shows elevated ST segments, a ST segment elevated myocardial infarction (STEMI) is diagnosed. If the ECG does not show elevated ST segments, a non ST elevated MI (NSTEMI) is diagnosed when a cardiac troponin level characteristic for myocardial infarction is detected in a sample of the respective patient.

In case the ECG (electrocardiogram) shows ST-segment elevation, a ST-segment elevation myocardial infarction is diagnosed, i.e. the clinical diagnosis of a myocardial infarction is established. The diagnosis is a final clinical diagnosis which may be confirmed by the determination of troponin T or I which often does not give the necessary information as in general, patients present within the first 4 – 6 hours after onset of symptoms, before the cardiac specific necrosis marker starts to be released from the myocardium and circulates in increased amounts in the serum/plasma. Subsequent to the diagnosis of myocardial infarction, the patient is considered for evaluation of an appropriate therapy aiming at limiting the damage to the myocard caused by the ischemia/occlusion, in general by reperfusion therapy (i.e. totally or partly reestablishing blood flow in the occluded artery).

In the non-prepublished application "Method for diagnosing and monitoring cardiac ischemia in patients with acute chest pain and without myocardial infarction" filed on 27. November 2009 and carrying the application number EP 09 177 395.2, a method for diagnosing and/or monitoring an ischemic state in a subject showing signs and symptoms of acute coronary syndrome but not fulfilling the diagnostic criteria for a myocardial infarction is disclosed, which comprises the steps of a) determining the amount of soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof in a sample of said subject, b) comparing the amount of sFLT-1 or a variant thereof determined in step a) to at least one reference amount, and c) diagnosing the ischemic state based on the information obtained in step b) and preferably based on the information obtained in a) and b). In embodiments of this invention, further markers like heart fatty acid binding protein (H-FABP) and/or a cardiac troponin can be determined, and/or and ECG is recorded, in order to rule in/rule out myocardial infarction in the subject. This invention does not refer to patients having STEMI.

Because of the frequent difficulty to assess the beginning of symptoms and to identify the correct time of onset of myocardial infarction, staging the myocardial infarction (also called evaluation of the stage of the myocardial infarction) even in ST-segment elevation

myocardial infarction where the diagnosis of myocardial infarction can be made at presentation remains a significant challenge. The staging of myocardial infarction is of significant importance for the following reasons:

It is well established and has been discussed extensively that the duration of coronary artery occlusion is of significance in terms of nature and extent of myocardial damage and determines also possible benefits of reperfusion therapy. The first stage of damage occurring after artery occlusion is ischemia (ischemic myocard) which is fully reversible if sufficient blood flow of the occluded artery (reperfusion) is reestablished within a short period in time. A reperfusion of the ischemic myocardium within 20 minutes after occlusion-induced ischemia results in viable myocardium with reversible cardiac dysfunction (postischemic dysfunction), avoiding necrosis with sequential scarring. If a reperfusion is achieved within 2 – 4 hours after occlusion-induced ischemia, non reversible cardiac injury (in general necrosis) will develop and in addition postischemic dysfunction in other parts of the myocardium. In case of permanent occlusion the affected myocardium becomes completely necrotic (i.e. suffers from non reversible cardiac injury) with neighboring regions being also affected, in general by postischemic dysfuction. Thus early intervention is needed to save myocardium by protecting the affected myocardium from necrosis and to prevent late sequelae of necrosis such as heart failure and, after all, reduce long-term and short-term mortality.

Even in ST-segment elevation myocardial infarction, a successful reperfusion of the myocard (i.e. partially or totally reopening the occluded artery or arteries), when performed at the right point in time, will prevent the myocard from non reversible injury which would have occurred in case no reperfusion intervention had been carried out. This is shown by the time development of the ST-segment elevation which may persist over several hours, during the period of time when ischemia develops into reversible cardiac dysfunction/non reversible cardiac injury. Even though ST-segment elevation dissolves in case of a succesful reperfusion intervention, the ECG recording does not permit to monitor the various stages of myocard infarction laid out beforehand.

ST elevation myocardial infarction represents an emergency condition. It is well appreciated that benefit from therapy is highest, if the patient presents early and undergoes early intervention (Gersh et al, JAMA 293:979, 2005). For example if the interventions occurs later than about six to eight hours after occlusion-induced ischemia , there is no proven benefit from intervention.(Gersh et al). In principle two types of intervention are

available: percutaneous coronary intervention (PCI) with the application of a STENT or balloon, or thrombolysis. A combination of both methods is also known. Fibrinolysis is considered to be only effective if the patient presents within 3 hours after the acute event.

Each of these interventions are associated with complication. Fibrinolysis can e.g. cause bleeding. Therefore this approach has absolute and relative contraindications. Absolute contraindications include, inter alia, prior cerebral hemorrage, ischemic stroke within the past 3 months, active bleeding disorders etc. Relative contraindications include active peptic ulcer, pregnancy, recent internal bleeding etc.. Indication for PCI needs to consider the proportion of viable myocardium subtended by the treated coronary artery and assessing the risk of the PCI procedure (Smith S.C, et al J Am Coll Cardiol 47 (1) e 1 – 121,2006). Other elements to consider include baseline lesion morphology, underlying cardiac function, renal insufficiency and associated medical conditions such as cancer.

PCI represents an invasive medical intervention carrying risks, such risks include mortality in the order of 1 % in general but higher in STEMI (Smith et al), additional myocardial infarction or its extension through embolisation of thrombus material, vessel rupture and bleeding. In addition PCI is associated with an increased risk of arrhythmia including ventricular fibrillation.

Morphological changes induced by STEMI are heterogenous e.g. because of incomplete thrombus closure of the vessel, spontaneous lysis or atherosclerosis in other parts of the vessel that might affect downstream processes. This variability in pathophysiological course of STEMI is not captured by time from acute event.

Infarction size is an important determinant of prognosis in patients with STEMI, including both short-term prognosis and long-term prognosis. Individuals suffering from large STEMIs often die within hours or days after infarction, frequently from cardiac arrest or arrythmia. Survivors with large infarcts frequently exhibit late impairment of ventricular function, and the long-term mortality rate is higher than for survivors with small infarcts, who tend not to develop cardiac decompensation. Cardiac decompensation frequently results in heart failure being the cause behind long-term mortality.

Limiting infarction size and, thus, improving mortality rate has attracted a great deal of attention. Efforts to limit the size of the infarct have centered early reperfusion (i.e. restoration of blood flow in the affected myocardium). Despite the many advances in

reperfusion therapy for STEMI, practical clinical decision-making in the case of individual patients is complex being a cause for the underuse of reperfusion therapy. Lack of knowledge in particular about the point in time of arrival after the onset of symptoms (early or late) and of the various states of ischemia, reversible cardiac dysfunction (postischemic dysfunction) and non reversible cardiac injury (in general necrosis) appears to be the major cause underlying the underuse of reperfusion of STEMI in routine practice. A further major factor appears to be the balance of risk (bleeding) versus benefit in particular in fibrinolysis reperfusion treatment patients.

Thus, there is an urgent need for methods to improve the assessment of the point in time and to monitor the damage in particular in STEMI, in order to select the appropriate treatment and to improve assessment of benefit to risk. Such method should also provide information related to the potential success of reperfusion programs. A method should be found allowing to diagnose, identify, evaluate or assess, in a patient suffering from a myocardial infarction, in particular suffering from STEMI, the cardiac pathophysiological state, including cardiac ischemia, reversible cardiac dysfunction (postischemic dysfunction) and states of non reversible cardiac injury (necrosis) and the degree (extent) to which these pathophysiological states have already progressed. Based on the results of the diagnosis/identification/evaluation/assessment, a decision or recommendation for therapy should be based. The method should also allow the exclusion/inclusion of ischemia, reversible cardiac dysfunction and states of non reversible cardiac injury and further classification of patients presenting with STEMI so as to provide an improved diagnostic and therapeutic work up. The method should permit to decide on therapeutic measures to limit the infarct size or even, as the case may be, totally avoid infarcted (i.e. necrotic) myocard areas. The method and means should avoid at least some of the drawbacks of the current techniques as laid out above.

Thus, the technical problem underlying the present invention must be seen as the provision of means and methods for complying with at least one of the aforementioned needs.

The technical problem is solved by the embodiments characterized in the claims and herein below.

Accordingly, the present invention relates to a method for diagnosing or determining the stage of myocardial infarction in a subject showing a ST segment elevated myocardial infarction (STEMI), based on the comparison of the amounts of each of the following

markers, determined in a sample of said subject, to at least one reference amount: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof.

This method of the present invention may comprise at least one of the following steps: a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof; in a sample of said subject; b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, preferably the amount of each marker is compared to its respective reference amount.

The diagnosis or determination of the stage of myocardial infarction may be established based on the information obtained in step b) and preferably based on the information obtained in a) and b).

The present invention also provides for a method for diagnosing the stage of myocardial infarction in a subject showing a ST segment elevation myocardial infarction (STEMI), comprising the steps of

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, of H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b).

The present invention also relates to a method for monitoring the stage of myocardial infarction in a subject showing a ST segment elevated myocardial infarction (STEMI), based on the comparison of the amounts of each of the following markers, determined in a

sample of said subject at least at two different points in time, to at least one reference amount: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof.

This method of the present invention may comprise at least one of the following steps: a) determining, at least at two different points in time, the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof; b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount.

The present invention also provides for a method for monitoring the stage of myocardial infarction in a subject showing a ST segment elevation myocardial infarction (STEMI), comprising the steps of

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, of H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b).

Based on the monitoring a diagnosis may be established using the information obtained in step b) and preferably based on the information obtained in a) and b), at each point in time when the amounts of the markers were determined

In a preferred embodiment of the present invention, the method (i) further comprises a step of collecting a sample from the patient by a minimal-invasive step or (ii) excludes a step of collecting a sample from the patient by a minimal-invasive step or (iii) excludes a surgical step or (iv) excludes a step of collecting a sample or (v) is an in vitro method.

The state of the art in respect to diagnosis of myocardial infarction including the criteria applied for its diagnosis are described by The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction (The Joint European Society of Cardiology/American College of Cardiology Committee: Universal definition of myocardial infarction, l.c.), which are the latest criteria at the time of the filing of the present application. Evaluation of medical history (history of coronary artery disease CAD) is one criterion for the diagnosis of patients exhibiting symptoms of ACS (e.g. chest pain for more than 20 min). These patients are furthermore diagnosed for fulfilling further criteria, namely using electrocardiogram (ECG), on the one hand, and troponin testing, on the other hand. In case of a diagnostic ECG (i.e. a ECG showing ST-elevation), a myocardial infarction (STEMI) is diagnosed. The ECG, however, does not provide profound information on the pathological state of the individual under examination, i.e. the person skilled in the art is not aware of the degree of ischemia which has already occurred, and if reversible cardiac dysfunction and/or non reversible cardiac injury have already taken place. In cases of an ECG not meeting the diagnostic criteria of non ST-elevation myocardial infarction at presentation and a Troponin test result not meeting the diagnostic criteria of a myocardial infarction, this procedure is repeated after 4 – 8 hours. In case ECG and troponin determination continue not to meet the diagnostic criteria of myocardial infarction, the patient is discharged with the diagnosis of exclusion of myocardial infarction.

The term "acute coronary syndrome" (ACS) and the criteria for diagnosing ACS are understood and known to the person skilled in the art. The term relates to a constellation of clinical symptoms caused by acute myocardial ischemia. The ischemia itself results from the formation of a thrombus on an unstable atherosclerotic plaque in a coronary artery. It is known in the art that ACS may be accompanied by symptoms such as epigastric, arm, wrist or jaw discomfort or pain, in particular chest pain, whereby in particular, the chest pain lasts for longer than 20 minutes and may radiate to the arm, back or shoulder. Further symptoms of an acute cardiovascular event may be unexplained nausea or vomiting, shortness of breath, weakness, dizziness, lightheadedness, sweating or syncope as well as any combinations thereof. Generally, these clinical symptoms, especially chest pain, occur suddenly; they may appear at rest or after minimal exertion. ACS patients can show unstable angina pectoris (UAP) or these individuals can suffer from a myocardial infarction (MI). MI can be a ST-segment elevation MI (STEMI) or a non-ST-segment elevation MI (NSTEMI). MI is classified as belonging to coronary heart diseases (CHD)

and is preceded by other events also classified as belonging to CHD, like unstable angina pectoris (UAP). Symptomatic for UAP is chest pain which is relieved by sublingual administration of nitroglycerine. UAP is caused by a partial occlusion of the coronary vessels leading to hypoxemia and myocardial ischemia..

Myocardial necrosis is the pathological state underlying myocardial infarction, i.e. by pathological criteria myocardial infarction is defined by myocardial cell death (necrosis) due to prolonged periods of ischemia, see The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction (The Joint European Society of Cardiology/American College of Cardiology Committee: Universal definition of myocardial infarction, l.c.). The clinical definition for myocardial infarction, therefore, is based on evidence for necrosis in a clinical setting consistent with myocardial ischemia. Evidence for ischemia and/or necrosis can be taken from the electrocardiogram, frequently from the form of the ST-segment. Generally, STEMI is diagnosed by electrocardiography, in case the electrocardiogram (ECG) shows ST-segment elevation (indicating ischemic myocard). The determination of a cardiac troponin amount at least six hours after the onset of symptoms of ACS allows confirming the diagnosis of MI (here: STEMI) and, in cases where a MI could not be diagnosed due to the absence of characteristic waves or segments in the ECG, differentiating UAP and NSTEMI. If the Troponin amount is elevated (indicating myocardial damage or death) a NSTEMI is assumed. MI may occur without obvious symptoms, i.e. the subject does not show any discomfort, and the MI is not preceded by stable or unstable angina pectoris.

From the foregoing, it is clear that the clinical diagnosis of MI differs from the pathological diagnosis (established by the examination of tissue samples). The clinical diagnosis of necrosis is not based on unambigous proof, but on signs of ischemia (like ST-segment elevation). The present invention is drawn to individuals to which the clinical diagnosis of MI, in particular STEMI, applies which, in accordance with the foregoing, includes ischemic myocard, reversible cardiac dysfunction and non reversible cardiac injury. Therefore, the term "evaluating the stages of myocardial infarction" refers to determining or assessing the degree or extent of the stage of ischemia and/or reversible cardiac dysfunction and/or non reversible cardiac injury of the individual under examination, as all these stages may occur subsequently or simultaneously after an occlusion of a coronary artery in an individual. The term "evaluating the stages of myocardial infarction" does not refer to merely diagnosing necrosis or the extent to which necrosis has occurred (which would be the pathological diagnosis).

In a preferred embodiment of the present invention, an ECG of the respective subject is determined simultaneously or around the time of the determination of sFLT-1, H-FABP and a cardiac troponin; measuring the ECG may also precede or follow the determination of sFLT-1, H-FABP and a cardiac troponin, preferably provided that the interval between the ECG and the marker determination and/or sampling is not more than about 30 minutes. The stage of the myocardial infarction, therefore, is preferably determined simultaneously with or after measuring an ECG, by determining the amounts of sFLT-1, H-FABP and a cardiac troponin. Even in case the ECG, by clinical criteriae, unambigously shows that a myocardial infarction has occurred (e.g. by a ST-segment elevation which is recognized in the art as showing a myocardial infarction), the information gathered by determining the amounts of the peptides determined in the context of the present invention allows to diagnose, identify, assess or evaluate and, furthermore, to monitor the stage of the myocardial infarction and, in consequence, a rapid decision on the appropriate treatment to be carried out on the individual.

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

The term "diagnosing" as used herein means assessing, identifying, evaluating or classifying the stage of the myocardial infarction in a subject showing a ST segment elevated myocardial infarction (STEMI), in particular if the subject suffers from an ischemic state (leading to or being associated with a reversible cardiac dysfunction or to a non reversible cardiac injury), and/or from a reversible cardiac dysfunction and/or from non reversible cardiac injury. The term "diagnosing" also refers to distinguishing, in subjects showing a ST segment elevated myocardial infarction (STEMI), between ischemia (which, as the case may be, will lead to a reversible cardiac dysfunction or to non reversible cardiac injury), a reversible cardiac dysfunction and non reversible cardiac injury. It has to be born in mind, however, that all above-cited pathological states can be present in a subject, as the case may be.

The term “ischemia" or "ischemic state”, as used herein, relates to the state of an impaired blood supply not sufficient for metabolic needs, in particular not sufficient for oxygen supply, to the affected tissue. Ischemia may be associated with, lead to or cause a reversible cardiac dysfunction (postischemic dysfunction) 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 period of about 15 minute occlusion of a vessel results, e.g., in a roughly 6 h reversible cardiac dysfunction, although the blood flow is restored. This reversible event is not associated with or does not lead to myocardial necrosis. Preferably, the term “ischemia" or "ischemic state” encompasses a process leading to, being associated with or causing a reversible cardiac dysfunction or a non reversible cardiac injury.

In accordance with the present invention, ischemia is diagnosed by determining the amount of sFLT-1 in a sample of the individual. The amount of sFLT-1 is also indicative of the relative degree of ischemia, as the amount of sFLT-1 correlates with the degree of ischemia.

The term "reversible cardiac dysfunction", also termed “postischemic dysfunction” in the context of the present invention, relates to an impaired pumping capacity or activity of the heart, which is fully reversible and preferably occurs without leaving any significant structural deterioration to the heart (including necrosis) of a significant number of cardiomyocytes. Preferably, the impaired pumping capacity or activity does not cause any significant injury to the subject's body. A reversible cardiac dysfunction may be immediately reversible, i.e. within a few seconds or minutes like in cases of very short ischemic periods. Examples of reversible cardiac dysfunctions include myocardial stunning and myocardial hibernation.

Both terms are known to the person skilled in the art. In both cases, reversibility may be delayed for hours or days.

Myocardial stunning is observed already in acute events, in particular MI and also other states of severe ischemia. Myocardial stunning is fully reversible after reperfusion. In a stunned myocardium, myocardial function is depressed at rest but myocytes remain viable.

Left ventricular dysfunction may be reversible in stunning. Stunned myocardium is most commonly observed after a transient period of ischemia followed by reperfusion (depressed function at rest but preserved perfusion). The ischemic episodes can be single or multiple, brief or prolonged, but never severe enough to result in non reversible cardiac injury. Resting blood flow is normal (i.e. blood flow is normal after reperfusion); uptake of normal contractility is delayed after successful reperfusion. In case stenosis persists, acute stunning may transform into chronic stunning, which takes a longer time to resolve after reperfusion (less than about 24h in acute stunning (also termed post-ischemic stunning) versus days to weeks in chronic stunning).

Myocardial hibernation refers to a chronic state and occurs after prolonged periods of ischemia and increased stenosis severity. "Hibernation" refers to chronically dysfunctional myocardium with the potential to recover following revascularisation/reperfusion. Hibernation is a response of the myocardium to prolonged phases of myocardial hypoperfusion (lack of blood supply), resulting in reduced blood flow initiated by the myocardium itself. Hibernating myocard is often found in patients having coronary artery disease. Hibernating myocard is also often found in patients having a prior experience of myocardial infarction, and where no sufficient reperfusion has been achieved when MI was treated. In hibernation a chronically stenosed vessel subtends the affected myocardium.

A preferred definition of stunning and hibernation of myocardium, is provided by Braunwald's Heart Disease, Eigth Edition, Saunders Elsevier, pages 367/368 and 1184 - 1191.

In a preferred embodiment of the present invention, subjects having a reversible cardiac dysfunction, in particular stunned myocard and/or myocardial hibernation, can be diagnosed by determining the amount of H-FABP.

The term "non reversible cardiac injury" is generally known in the field and preferably relates to cardiac injury which is associated with cell death, preferably necrosis of cardiomyocytes, for example by necrotic process. In the context of the present invention, non reversible cardiac injury is diagnosed by determining the amount of a cardiac troponin, in general troponin T or troponin I, in particular troponin T. "Non reversible cardiac injury" includes both the cases that

a) necrosis occurs in a localized, connected myocardium area ("connected myocardium area necrosis"), leading to or being associated with continous, macroscopic scarred tissue

subsequent to the artery occlusion causing ischemia, which is the case in myocardial infarction;

b) necrosis occurs in non-localized, non-connected myocardium regions ("non-connected myocardium regions necrosis") whose size each is inferior to that resulting in macroscopic scarred tissue, which is supposed to be the case in ischemic processes e.g in coronary artery disease, where the initial process leading to or being associated with ischemia is not the occlusion of a defined artery.

The present invention allows to determine the stage of the myocardial infarction. Before the present invention, the stage of the MI (which can also be referred to as the relative period in time having elapsed after the artery occlusion) could only be assessed based on statements by the subjects when discomfort and chest pain had started (onset of symptoms), which leaves lots of incertainty. The individual may not recall when discomfort started, or symptoms were not significant enough in the early stage.

The clinical course of STEMI varies significantly. This includes the extent and duration of ischemia (or even recurrent ischemia), as well as metabolic abnormalities with respect to extent and duration. Variables effecting ischemia include preexisting conditions, in particular the extent of atherosclerosis and dynamics of thrombus formation, level of the arterial occlusion, spontaneous thrombus lysis, embolism from the thrombus in distal smaller arteries, and further variables known to the person skilled in the art. All these variables have an impact on treatment decisions and are not captured by ECG findings, contrary to the method of the present invention. Thus the method of the present invention provides further important insight into pathologic processes associated with STEMI and provides information useful for clinical decision making beyond ECG and clinical information.

The term "subject" as used herein relates to animals, preferably mammals, and, more preferably, humans.

The term "simultaneously" as used herein relates to carrying out an activity, preferably the determination of a marker used in the context of the present invention, at the same point in time. This preferably includes measurements wherein the determination of one marker is slightly deferred over the determination of another marker, e.g. for seconds or a few minutes, e.g. 1 minute, 2, 3, 4, 5, 6, 7, 8, 9 or 10 minutes.. It is essential that the determination of the later marker is not deferred in a way that its amounts may change to

an extent such that a different diagnosis will result. "Determining a marker" relates to the taking of the body fluid sample in which the amount of the marker is determined, or the determination and perception of the marker amount, or both.

The term "determining (the amount of a marker) at least at two different points in time" as used herein is meant to encompass the determination of the marker amount in intervals, wherein the second and each further sample will be taken in an interval which ensures an effective monitoring of the ischemic state. In general, the interval between each sample is about 15 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 90 minutes, about 2 hours, about 3, 4, 5, or 6 hours. For example, the initial sample is taken about 1 hour after the onset of symptoms of acute coronary syndrome or immediately after the presentation of the subject to the physician, and each further sample is taken about 1 hour after the initial samples. The number of samples taken will depend on the evaluation of the ischemia.

In one further embodiment of the present invention, the amount of sFLT-1 measured in an individual is used to diagnose myocardial infarction. This is in particular advantageous in cases the individual can be observed for prolonged periods of time, preferably more than 6 hours, more preferably more than 8 hours, even more preferably more than 12 hours, in particular more than 24 hours.

Accordingly, the present invention also relates to a method of diagnosing myocardial infarction, in particular non ST elevated myocardial infarction, in a subject showing the signs and symptoms of acute coronary syndrome ACS but not fulfilling the diagnostic criteria for a myocardial infarction, comprising the steps of

a) determining the amount of soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof in a sample of said subject,

b) comparing the amount of sFLT-1 or a variant thereof as determined in step a) to at least one reference amount, and

c) diagnosing myocardial infarction, in particular non ST elevated myocardial infarction based on the information obtained in step b), preferably based on the information obtained in a) and b).

It is also provided a method of diagnosing myocardial infarction, comprising the steps of

a) determining the amount of troponin T in a sample of said subject,

b) comparing the amount of troponin T or a variant thereof as determined in step a) to at least one reference amount, and

c) diagnosing myocardial infarction, based on the information obtained in step b), preferably based on the information obtained in a) and b).

The term "cardiac Troponin", refers to all Troponin isoforms expressed in cells of the heart and, preferably, the subendocardial cells. These isoforms are well characterized in the art as described, e.g., in Anderson 1995, Circulation Research, vol. 76, no. 4: 681-686 and Ferrieres 1998, Clinical Chemistry, 44: 487-493. Preferably, cardiac Troponin refers to Troponin T and/or Troponin I, and, most preferably, to Troponin T. It is to be understood that isoforms of Troponins may be determined in the method of the present invention together, i.e. simultaneously or sequentially, or individually, i.e. without determining the other isoform at all. Amino acid sequences for human Troponin T and human Troponin I are disclosed in Anderson, loc cit and Ferrieres 1998, Clinical Chemistry, 44: 487-493.

The term "cardiac Troponin" encompasses also variants of the aforementioned specific Troponins, i.e., preferably, of Troponin I, and more preferably, of Troponin T. Such variants have at least the same essential biological and immunological properties as the specific cardiac Troponins. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing the said cardiac Troponins. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about92%, 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 Troponin, preferably over the entire length of the specific troponin. 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 cardiac Troponins or the aforementioned types of variants

as long as these fragments have the essential immunological and biological properties as referred to above. Preferably, the cardiac troponin variants have immunological properties (i.e. epitope composition) comparable to those of human troponin T or troponin I. Thus, the variants shall be recognizable by the aforementioned means or ligands used for determination of the amount of the cardiac troponins. Such fragments may be, e.g., degradation products of the Troponins. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation. Preferably the biological property of troponin I and its variant is the ability to inhibit actomyosin ATPase or to inhibit angiogenesis in vivo and in vitro, which may e.g. be detected based on the assay described by Moses et al. 1999 PNAS USA 96 (6): 2645-2650). Preferably the biological property of troponin T and its variant is the ability to form a complex with troponin C and I, to bind calcium ions or to bind to tropomyosin, preferably if present as a complex of troponin C, I and T or a complex formed by troponin C, troponin I and a variant of troponin T.

In a preferred embodiment of the present invention, non reversible cardiac injury, preferably necrosis, in the individual presenting with STEMI is diagnosed using a cardiac troponin, preferably troponin T or troponin I, in particular troponin T.

Troponin T was tested with the high sensitive Troponin T Test using the ELECSYS 2010 Analyser (Roche Diagnostics, Mannheim, Germany). The test was carried out according to the instructions of the manufacturer. The test has a measuring range from 0.003 – 10 ng/ml (or 3 – 10000 pg/ml). The precision of the test was found to be between 0.8 to 2.6 percent, depending on the troponin concentration in the sample. Preferably, the Troponin amount which is detectable may relate to any concentration that is equal or larger than 0.001 ng/ml, 0.002 ng/ml, 0.005 ng/ml, 0.0075 ng/ml, or 0.01 ng/ml. More preferably, the cardiac Troponin amount which is detectable relates to any concentration that is equal or larger than 0.002 ng/ml. The term "cardiac Troponin amount" as used herein relates to the concentration of a cardiac Troponin, preferably of TnT. Preferably, the term relates to the concentration of a cardiac Troponin in a plasma or serum sample of a subject. Preferably, in the methods of the present invention troponin is determined using high sensitive Troponin T Tests. Troponin T tests with improved sensitivity (high sensitive troponin tests) exhibit frequently detectable troponin levels in patients with stable coronary artery disease which are unrelated to an acute event and thus nondiagnostic for myocardial infarction. In single cases sensitive troponin tests may aid in the early recognition of myocardial infarction in case of significant increase from low troponin levels.

The term "soluble (s)FLT-1" as used herein refers to polypeptide which is a soluble form of the VEGF receptor FLT1. It was identified in conditioned culture medium of human umbilical vein endothelial cells. The endogenous soluble FLT1 (sFLT1) receptor is chromatographically and immunologically similar to recombinant human sFLT1 and binds [125I] VEGF with a comparable high affinity. Human sFLT1 is shown to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro. Preferably, sFLT1 refers to human sFLT1. More preferably, human sFLT1 can be deduced from the amino acid sequence of Flt-1 as shown in Genebank accession number P17948, GI: 125361. An amino acid sequence for mouse sFLT1 is shown in Genebank accession number BAA24499.1, GI: 2809071. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific sFLT1. The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity 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. Variants may be allelic variants, splice variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein

include fragments of the specific sFLT1 or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Preferably, the sFLT-1 variants have immunological properties (i.e. epitope composition) and/or biological properties comparable to those of human sFLT-1. Thus, the variants shall be recognizable by the aforementioned means or ligands used for determination of the amount of sFLT-1. 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 sFLT-1 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.

Heart-type fatty acid binding protein, herein also referred to as H-FABP, is a small cytosolic protein that functions as the principal transporter of long-chain fatty acids in the cardiomyocyte, from the cell membrane to their intracellular sites of metabolism in the mitochondria, where they enter the citric acid cycle. H-FABP is present in the myocardium and it is generally thought to be released rapidly into the circulation in response to reversible cardiac dysfunction/myocardial injury. Several studies show that H-FABP is a early biochemical marker of myocardial infarction e.g. Okamoto et al., Clin Chem Lab Med 38(3):231-8 (2000) Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction. Clinical evaluation of H-FABP in comparison with myoglobin and creatine kinase isoenzyme MB; O'Donoghue et al., Circulation, 114;550-557 (2006) Prognostic Utility of Heart-Type Fatty Acid Binding Protein in patients with acute coronary syndrome or Ruzgar et al., Heart Vessels, 21;209-314 (2006) The use of human heart-type fatty acid-binding protein as an early diagnostic marker of myocardial necrosis in patients with acute coronary syndrome, and its comparison with troponinT and its creatine kinase-myocardial band).

H-FABP as used herein encompasses also variants of H-FABP polypeptides, respectively. Such variants have at least the same essential biological and immunological properties as the specific H-FABP polypeptide. 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 the said H-FABP polypeptide, respectively. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least

50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific H-FABP polypeptide. The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity 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. Variants referred to above may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific H-FABP polypeptide or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Such fragments may be, e.g., degradation products of the H-FABP polypeptide. 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.

In the context of the present invention it is to be expected that at least one of the three markers sFLT-1, H-FABP and a cardiac troponin will be elevated in the person having STEMI. However, the amount of each of the markers, their relative ratios and, in a preferred embodiment, the evaluation of the marker amounts over time give important information of the severeness of the individual pathological states (i.e. ischemia, reversible

cardiac dysfunction and non reversible cardiac injury) and, in the preferred embodiment, the evaluation of the states over time.

The individual amount of each marker gives guidance on the stage of the myocardial infarction and the risk for a deterioration of the pathological state in the individual. It is to be understood that ischemia is the least severe pathological state in an individual suffering from STEMI. Ischemia may resolve on its own, not causing reversible cardiac dysfunction and/or non reversible cardiac injury if resolution occurs early after the acute event and/or if ischemia is not severe enough to cause cardiac dysfunction/injury even in the short time frame until resolution occurs (as indicated by the amount of sFLT-1).

The stage following ischemia and (and being more severe) is reversible cardiac dysfunction, in general myocardial stunning which can be diagnosed/assessed by determining an amount of H-FABP. Preferably, relative to an increased amount of sFLT-1, an increased amount of H-FABP indicates an even higher risk of suffering from non reversible cardiac injury.

The next stage – following ischemia and reversible cardiac dysfunction - is non reversible cardiac injury, in general necrosis which is diagnosed by a cardiac troponin (troponin T and troponin I). An elevated amount of cardiac troponin shows that at least a part of the myocard cells has died. In the context of the present invention, an increasing amount of a cardiac troponin over a certain period of time, or an almost constant level over a certain period of time, is indicative of an ongoing necrotic process, wherein the severity of the necrosis is indicated by the cardiac troponin amounts. It is contemplated that in case of connected myocardium area necrosis (as in the case in myocardial infarction), cardiac troponin amounts are higher than in non-connected myocardium regions necrosis, as is the case in stable chronic CAD patients.

In the context of the present invention, amounts of a cardiac troponin indicative of necrotic processes as a consequence of myocardial infarction, in particular STEMI, are amounts higher than those found in stable individuals suffering from coronary artery disease CAD.

It has to be kept in mind that in the context of carrying out the teachings of the present invention, the amount of a cardiac troponin determined in an individual suffering from STEMI and developing or suffering from connected myocardium area necrosis may be inferior to those typically regarded as indicating myocardial infarction (but higher than

those found in stable chronic CAD patients having non-connected myocardium regions necrosis), if the cardiac troponin is determined at an early stage of the infarction, in general prior to about 6 to 8 hours after the acute event (i.e. artery occlusion by a thrombus). In the context of the present invention, this case is considered as indicating myocardial infarction and connected myocardium area necrosis. It is supposed, however, that necrotic processes are still ongoing and have not reached the final state, pointing to the fact that myocardium can still be saved by appropriate therapeutic measure taken immediately.

If the subject presents to the emergency department and the markers according to the invention are recorded at a very early stage after the acute event (showing a STEMI), the amount of sFLT-1 may be increased, but not that of the other markers H-FABP and/or a cardiac troponin. In such a case, a very high amount of sFLT-1 indicating a high degree of ischemia (i.e. a large number of myocard cells is ischemic) will point to the fact that the myocardial infarction is still at its early stage, and that the individual is at an increased risk of suffering from reversible cardiac dysfunction and/or non reversible cardiac injury. This marker amount constellation, however, also points to the fact that the entire myocard or at least most of the myocard may be saved by timely appropriate treatment.

As the pathologic effects of the acute event progress to another stage, not only the amount of sFLT-1 may be elevated, but also the amounts of H-FABP and/or a cardiac troponin. The information relative to the stage of the myocardial infarction will then be taken form the combination of the amounts of each of the markers which can be determined, and the intervention will be based thereon. For example, in case only the sFLT-1 and the H-FABP levels are elevated, the individual, in any case, has not (yet) suffered from substantial myocard necrosis, but the vessel occlusion is severe and has already lead to reversible cardiac dysfunction, besides ischemic myocard. Under these circumstances, a large part of the myocard may be saved by timely, appropriate treatment. However, probability is high that not the entire myocard may be saved and mortality risk is also still high.

At very late points in from the onset of the symptoms associated with the acute event, the amount of sFLT-1 may be low (i.e. has declined), and the same holds true for H-FABP (its amount may be so low that reversible cardiac dysfunction cannot be diagnosed any longer). In such a case, the amount of a cardiac troponin may be elevated, indicating progression of the pathological effects of the acute event, e.g. resulting in large areas of non reversible cardiac injury, in general necrosis. The information relative to the stage of the myocardial infarction will then be taken form the combination of the amounts of each of the markers

which can be determined. In this case, the marker amounts show that only minor or even, as the case may be, that no portions of the myocard may be saved anymore by timely, appropriate treatment. In acute events duration of the increase of sFlT-1 (and H-FABP) depends on the clinical course of the event (protracted course yes or no) however sFlT1 might return to the normal range within 4 hours in case of time limited ischemia, this might also be the case for H-FABP.

Thanks to the present invention, it is possible to diagnose, identify, evaluate or assess, in a patient suffering from a myocardial infarction, in particular suffering from STEMI, the cardiac pathophysiological state, including cardiac ischemia, reversible cardiac dysfunction (postischemic dysfunction) and states of non reversible cardiac injury (necrosis) and the degree (extent) to which these pathophysiological states have already progressed. The method allows the exclusion/inclusion of ischemia, reversible cardiac dysfunction and states of non reversible cardiac injury and further classification of patients presenting with STEMI so as to provide an improved diagnostic and therapeutic work up.

While the above conclusions apply to the patient groups as a whole, it is to be understood that the diagnostic information taken by the person skilled in the art obtained from the determined marker amounts leaves some room for interpretation and depends, inter alia, on the individual patient's condition and medical history presenting to the physician, the absolute amount of each of the markers, their relative development over time and the ratio of two or even all three of the markers. The interpretation of the marker amount information will be carried out by the person skilled in the art for each individual case.

The term "variant" of the markers of the invention, i.e. of H-FABP, sFlt-1 and troponin, also relates to splicing variants, mutants and to a post-translationally modified peptide such as glycosylated peptide. A "variant" is also a peptide which has been modified after collection of the sample, for example by covalent or non-covalent attachment of a label, particularly a radioactive or fluorescent label, to the peptide. Measuring the amount of a peptide modified after collection of the sample is understood as measuring the amount of the originally non-modified peptide.

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, or urine, more preferably, samples of blood, plasma or serum. Tissue or organ samples are also

encompassed by the term "sample" and may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, cell-, tissue- or organ samples are obtained from those cells, tissues or organs which express or produce the peptides referred to herein. Preferably, the term "sample" refers to a plasma or serum sample, more preferably to a serum sample.

The methods of the present invention may encompass a step of collecting a sample, which optionally may be an invasive step. The sample may be collected by way of an invasive step, preferably minimal invasively, such as by venopuncture. The minimal invasive collection also encompasses the case where the sample is collected by use of a needle (lancette) which when applied to the skin, preferably the skin of a finger, elicits outflow of a small volume of blood which may then be collected for determining the amount of the markers in the sample. The sample is preferably collected by way of a safe routine procedure, preferably by persons that do not need to have a strong medical training and the sample collection preferably poses no significant health risk for the person subjected to the sample collection.

In another embodiment of the methods of the present invention the methods of the present invention exclude a surgical step or excludes a step of collecting a sample invasively, preferably, the methods exclude a step of collecting a sample. More preferably, the methods of the present invention are in vitro methods.

In the present invention, the sample is obtained at an appropriate point in time which is known to the skilled person. It has to be taken into consideration that the subject under examination suffers from an acute pathophysiological state (STEMI), requiring rapid diagnosis and a rapid decision on an appropriate treatment. Preferably, the sample is obtained from a subject according the present invention shortly, e.g. after about 1 h, not more than about 2 hours, not more than about 3 hours, not more than about 4 hours, not more than about 5 hours, or not more than about 6 hours after the onset of symptoms of acute coronary syndrome. Preferably, the sample will be taken immediately (i.e. within a few minutes, i.e. within about 5, about 10, about 15, about 30, about 45, or about 1h) after presentation of the subject to the physician or after arrival of the patient in the hospital, emergency unit, intensive care unit or ambulance.

In case the amounts of sFLT-1 and H-FABP and a cardiac troponin are determined repeatedly, preferably at least twice, to monitor the severity of the ischemia, the second and each further sample will be taken in an interval which ensures an effective monitoring of the ischemic state. In general, the interval between two samples is about 15 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 90 minutes, about 2 hours, about 3, about 4, about 5, or about 6 hours. For example, the initial sample is taken about 1 hour after the onset of signs and/or symptoms of acute coronary syndrome or immediately after the presentation of the subject to the physician, and each further sample is taken about 1 hour after the initial sample. The number of samples taken will depend on the outcome of the diagnosis and the obtained evaluation of the ischemia.

Determining the amount of a cardiac troponin, preferably troponin T, or the amount of sFLT-1, or the amount of H-FABP, or any other peptide or polypeptide or protein referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. The terms polypeptide and protein are used interchangeably throughout this application. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide present in the sample. Such a signal – sometimes referred to herein as intensity signal -may be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of the peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of a peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Said means comprise immunoassay devices and methods which may utilize labelled 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 Elecsys™ analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-Hitachi™ analyzers), and latex agglutination assays (available for example on Roche-Hitachi™ analyzers).

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

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

Determining the amount of a peptide or polypeptide may, preferably, comprise the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example,

random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display. Antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and $F(ab)_2$ fragments that are capable of binding antigen or hapten. The present invention also includes single chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

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

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

a detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labelling may be done by direct or indirect methods. Direct labelling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labelling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a coloured reaction product, fluorescence or chemoluminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include ^{35}S, ^{125}I, ^{32}P, ^{33}P and the like. A radioactive label can be detected by any method

known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labelling or other detection methods as described above.

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

Preferably, the amount of sFLT-1 and the amount of H-FABP and the amount of a cardiac troponin and, as the case may be, the amounts of other peptides measured in the context of the present invention are determined in a blood sample, e.g., a serum or plasma sample, obtained from a subject as defined in the present invention. Preferably, such a determination is done by ELISA. Such a determination by ELISA can be done, e.g., by using the Quantikine Human Soluble VEGF R1/Flt-1 Immunoassay (for sFLT-1), R&D Systems, Inc., Minneapolis, MN, USA. The amounts of H-FABP as specified elsewhere in this application can be determined by using the HBT ELISA Test Kit for human heart type fatty acid binding protein (HyCult Biotechnology, Uden, The Netherlands) for the determination of the amount of H-FABP.

The term "amount" as used herein encompasses the absolute amount (e.g., of sFLT-1, H-FAB, a cardiac troponin), the relative amount or concentration (e.g, of sFLT-1, H-FAB, a cardiac troponin) as well as any value or parameter which correlates thereto. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said peptides by direct measurements, e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., expression amounts determined from biological read out systems in response to the peptides or intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

The term "comparing" as used herein encompasses comparing the amount of the peptide, polypeptide, protein comprised by the sample to be analyzed with an amount of a suitable reference source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample. The comparison referred to in step (b) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the amount(s) determined in step a) to

suitable reference amount(s), it is possible to diagnose ischemia, reversible cardiac dysfunction and/or non reversible cardiac injury in said subject. It is to be understood that amounts of sFLT-1, H-FABP, a cardiac troponin as determined in step (a) of the methods of the presents invention are compared in step (b) to reference amounts for sFLT-1, H-FABP, a cardiac troponin as specified elsewhere in this application.

The term "reference amounts" as used herein in this embodiment of the invention refers to amounts of the polypeptides which allow diagnosing if an individual is a physiologically healthy subject, or a subject having an ischemic state leading to or being associated with a reversible cardiac dysfunction or to non reversible cardiac injury, and/or a subject having reversible cardiac dysfunction and/or non reversible cardiac injury.

Therefore, the reference amounts will in general be derived from subjects known to be a physiologically healthy, or subjects having an ischemic state leading to or being associated with a reversible cardiac dysfunction or to non reversible cardiac injury, or subjects having reversible cardiac dysfunction and/or non reversible cardiac injury.

Accordingly, the term "reference amount" as used herein either refers to an amount which allows determining the ischemic state (which may also be referred to as "degree of ischemia" in the context of the present invention) and/or reversible cardiac dysfunction (in general stunning, but also hibernation, as the case may be) and/or non reversible cardiac injury (in general necrosis) in a subject having STEMI, i.e. in a subject as defined in the present invention. The comparison with reference amounts permits to diagnose if the ischemia will lead to a reversible cardiac dysfunction (in general stunning) and/or non reversible cardiac injury (in general necrosis). Preferably, the onset of symptoms of ACS has occurred recently before the sample was obtained or collected from said subject, preferably, within a 6 hour period, more preferably within a 4 hour period, and most preferably within a 2 hour period.

Reference amounts for sFLT-1, H-FABP, a cardiac troponin for the severity of the degree of ischemia and/or reversible cardiac dysfunction (in general stunning) and/or non reversible cardiac injury (in general necrosis) may be derived from subjects as defined in the present invention diagnosed as having STEMI and presenting at various points in time after the occurrence of acute symptoms, preferably symptoms of ACS, and where the subject's outcome was determined, namely occurrence of MI, in particular STEMI, myocardial stunning, myocardial hibernation.

In the context of the present invention, reference amounts for sFLT-1, H-FABP, a cardiac troponin are preferably determined from individuals having stable chronic CAD without signs of ACS. It is known to the person skilled in the art that in stable chronic CAD patients, cardiac troponin levels are raised, being indicative of necrotic processes occuring as a consequence of ischemia during the coronary artery disease. Amounts for sFLT-1, H-FABP, a cardiac troponin higher than those found in stable chronic CAD patients are indicative of ischemic, postischemic dysfunction and necrotic processes which are more pronounced than in chronic patients.

In the context of the present invention a preferred reference amount is the 50^{th} or the 75^{th} percentile of sFLT-1, H-FABP or cardiac troponin determined in a group of subjects having stable CAD without signs of ACS. Preferably, the group comprises at least 100 or at least 150 subjects.

In an alternative embodiment, reference amounts for sFLT-1, H-FABP, a cardiac troponin are preferably determined from a collective of healthy individuals without signs of CAD and/or ACS. Reference amounts for sFLT-1, H-FABP, a cardiac troponin indicative of ischemic, postischemic dysfunction and necrotic processes can be established in accordance with the desired probability of suffering from the disease and linked to the particular threshold value. The person skilled in the art will know percentile to chose, for example, 80^{th}, 90^{th}, 95^{th} or even the 99^{th} percentile of the healthy patient collective. In the context of the present invention, it is preferred to choose the 95^{th} or, alternatively, the 99^{th} percentile, in order to establish the reference amounts. Further guidance on the determination of the relevant reference amounts is generally known to the skilled artisan and is also provided in the examples. Preferably, the patient collective comprises at least 100 or at least 200 individuals.

In all embodiments of the present invention, the amounts/amounts of the respective markers used therein (a cardiac troponin, in particular troponin T; sFLT-1, H-FABP) are determined by methods known to the person skilled in the art.

In general, for determining the respective amounts/amounts 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 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 pathopysiological state which is to be determined, or may comprise only individuals suffering from the pathopysiological 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 values are then established in accordance with the desired probability of suffering from the disease and linked to the particular threshold value. For example, it may be useful to choose the median value, the 60^{th}, 70^{th}, 80^{th}, 90^{th}, 95^{th} or even the 99^{th} percentile of the healthy and/or non-healthy patient collective, in order to establish the threshold value(s), reference value(s) or amount ratios.

Alternatively, the levels may be determined as "normal ranges" as known in the state of the art. The levels may also be determined or further refined by studies on individuals suffering from ST-elevation myocardial infarction and correlating any physiological findings in these patients with the levels observed in the individuals. Such studies may also allow to tailor the levels according to certain patient subgroups, e.g. elderly patients or patients with pre-existing coronary artery disease. Guidance on how such studies may be carried out can also be obtained from the examples included in this specification

A reference value of a diagnostic marker can be established, and the amount of the marker in a patient sample can simply be compared to the reference value. 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 will likely overlap. Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap indicates where the test cannot distinguish normal from disease. A threshold is selected, above which (or below which, depending on how a marker changes with the disease) the test is considered to be abnormal and below which the test is considered to be normal. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification of a condition. ROC curves can be used even when test results don't necessarily give an accurate number.

As long as one can rank results, one can create an ROC curve. For example, results of a test on "disease" samples might be ranked according to degree (say l=low, 2=normal, and 3=high). This ranking can be correlated to results in the "normal" population, and a ROC curve created. These methods are well known in the art. See, e.g., Hanley et al, Radiology 143: 29-36 (1982).

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

In other embodiments, a positive likelihood ratio, negative likelihood ratio, odds ratio, or hazard ratio is used as a measure of a test's ability to predict risk or diagnose a disease. In the case of a positive likelihood ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In the case of a negative likelihood ratio, a value of 1 indicates that a negative result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a negative result is more likely in the test group; and a value less than 1 indicates that a negative result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a positive or negative likelihood ratio of at least about 1.5 or more or about 0.67 or less, more preferably at least about 2 or more or about 0.5 or less, still more preferably at least about 5 or more or about 0.2 or less, even more preferably at least about 10 or more or about 0.1 or less, and most preferably at least about 20 or more or about 0.05 or less. The term "about" in this context refers to +/- 5% of a given measurement.

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

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

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 bacterial infection, 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, that 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 value using the following formula:

$$E = (TP / TO) \times 100;$$

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 threshold 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.

Unless stated differently, the term "about" as used herein refers to +/- 20%, preferably +/- 10%, preferably, +/- 5% of a given measurement or value.

Preferably, the reference amounts for sFLT-1 indicating ischemic states as defined in the present invention (threshold amounts) are the following: about 92 pg/ml, more preferably about 95 pg/ml, even more preferably about 109 pg/ml, in particular about 124 pg/ml. Amounts of sFLT-1 below the above-cited values are indicative for a non ischemic state not associated with or not leading to reversible cardiac dysfunction or non reversible cardiac injury. Amounts of sFLT-1 equal to or larger than the above-quoted reference

amounts are indicative for an ischemic state associated with or leading to reversible cardiac dysfunction or non reversible cardiac injury.

Further preferred reference amounts of sFlT-1 indicating ischemic states are the 95th or 99th percentiles determined in a group of healthy subjects or the 50th or 75th percentiles determined in a group of subjects suffering from stable CAD. Each of said groups, preferably, comprises at least 100, 150, 200, 250 or 300 subjects.

Preferably, the reference amounts for H-FABP for reversible cardiac dysfunction, as defined in the present invention (threshold amounts) are the following: about 820 pg/ml, preferably about 1015 pg/ml, more preferably about 2236 pg/ml, in particular about 3380 pg/ml. Amounts of H-FABP equal to or larger than the above-quoted reference amounts are indicative for a reversible cardiac dysfunction. Amounts of H-FABP below the above-cited values are indicative for a reversible cardiac dysfunction. Reversible cardiac dysfunction, preferably, is myocardial stunning or myocardial hibernation.

It is evident, that the levels given below can serve only as a first classification of the status of a subject. For example, the diagnosis may also be dependent on pre-existing cardiac disorders, the age or the general health status of the subject.

Further preferred reference amounts of H-FABP indicating reversible cardiac dysfunction are the 95th or 99th percentiles determined in a group of healthy subjects or the 50th or 75th percentiles determined in a group of subjects suffering from stable CAD. Each of said groups, preferably, comprises at least 100, 150, 200, 250 or 300 subjects.

Preferred Troponin T amounts indicative for non reversible cardiac dysfunction (necrosis) in the context of the present invention may be, but are not limited to, an amount of at least about 0,003 ng/ml, preferably about 0,005 ng/ml, more preferably about 0,007 ng/ml, in particular about 0,023 ng/ml. It is contemplated that these values indicate necrotic processes where necrotic tissue is still developing, resulting in connected myocardium area necrosis.

Further preferred reference amounts of troponin T indicating non reversible cardiac dysfunction (necrosis) are the 95th or 99th percentiles determined in a group of healthy subjects or the 50th or 75th percentiles determined in a group of subjects suffering from

stable CAD. Each of said groups, preferably, comprises at least 100, 150, 200, 250 or 300 subjects.

In a further embodiment of the present invention, troponin T amounts of at least about 0.1 ng/ml, of at least about 0.2 ng/ml, and of at least about 0.3 ng/ml are indicative of myocardium necrosis associated with myocardial infarction (connected myocardium area necrosis). These amounts are based on the requirements of The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction, lc., and are considered to be indicative for MI. In the context of the present invention, the above-cited amounts may be taken into account for confirmation of MI in a subject and/or for diagnosing advanced necrosis. They are, in general, not taken as reference amout for necrotic processes, for being present only at later stages of a myocardial infarction (due to delayed release of troponins), whereas the present invention is drawn to early diagnosis of the stages of myocardial infarction.

In case an ischemic state and/or a reversible cardiac injury is diagnosed, the respective individual may require further examination in respect to cardiac or cardiovascular diseases. In a preferred embodiment, accordingly, the present invention comprises steps of further diagnosing the individual in whom the amounts of sFLT-1 and/or H-FABP have been determined. Appropriate further diagnostic methods in the context of the present invention include stress testing of various kinds, e.g. stress exercise ECG, stress exercise echocardiography, stress exercise computer tomography, stress exercise thallium scan; and angiography (invasive or virtual, e.g. by spiral computer tomography).

The term "Troponin amount which is indicative for myocardial infarction" relates to a commonly accepted Troponin concentration that indicates non-reversible cardiac injury to an extent chracteristic for a myocardial infarction. Preferably, the amount of the biomarker determined in the sample, preferably the amount of cardiac Troponin, considered as being indicative for myocardial infarction relates to a concentration that is above the 95th, preferably above the 99th percentile concentration of a suitable reference population (cut-off score). This amount is based upon a recommendation that was made by The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction (The Joint European Society of Cardiology/American College of Cardiology Committee: Universal definition of myocardial infarction, l.c.) The person skilled in the art knows how to select a suitable reference population and how to determine the 95th and 99th percentile concentration. It is to be understood that this concentration may differ based on the used

assay for determining the cardiac Troponin concentration and based on the selected reference population.

It is to be noted that the values for NT-proANP may change due to the occurrence of chronic diseases in an individual, e.g. heart failure, renal impairment or failure. In case of a chronic disease, the values cited above may be significantly higher (e.g. 2 5, 7, 8 or 10 times higher) than the values cited beforehand which are applicable for individuals not having a chronic disease, in particular not a chronic heart disease, further to the acute coronary syndrome.
The term "at least one reference amount" means one or more than one reference amount, e.g. two reference amounts.

In a preferred embodiment of the present invention, the determination of the above-cited markers is carried out in intervals, in order to determine the evaluation of the marker amount. This may be helpful in the assessment if an acute event occurs/has occurred or not.

An acute event is assumed if he determined amount of at least one of the markers sFLT-1, H-FABP, a cardiac troponin and/or NT-proANP is larger than the reference amounts cited beforehand. Preferably, the deviation is, at least about 20%, or at least about 30 %, or at least about 50 %, or at least about 100 %, more preferably at least about 200 %, even more preferably at least about 500 %, in particular at least about 1000 %.

The time interval in between two determinations of a given marker (or the markers) is at least about 15 minutes, at least about 30 minutes, at least about 45 minutes, at least about 1 hour, at least about 90 minutes, at least about 2 hours, at least about 3, at least about 4, at least about 5, or at least about 6 hours. The person skilled in the art is aware that the time interval and the deviation may vary, in accordance with the state of the individual, the trend of the amount of the respective marker to change (i.e. the development of ischemia and/or circulatory impairment), etc.

It is to be understood that according to the method of the present invention described herein above and below, sFLT-1 H-FABP and a cardiac troponin or means for the determination thereof can be used for the manufacture of a diagnostic composition for diagnosing the stage of myocardial infarction in a subject showing a ST segment elevated myocardial infarction (STEMI).

The present invention also relates to a method for stratifying or assessing the risk of the deterioration of the pathological state in a subject having a ST segment elevated myocardial infarction (STEMI), based on the comparison of the amounts of each of the following markers, determined in a sample of said subject, to at least one reference amount: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof.

This method of the present invention may comprise at least one of the following steps ~~of~~: a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof; in a sample of said subject; b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, preferably the amount of each marker is compared to its respective reference amount.

The assessment of the risk of the deterioration of the pathological state in a subject may be established based on the information obtained in step b) and preferably based on the information obtained in a) and b).

Accordingly, the present invention provides a method for stratifying or assessing the risk of the deterioration of the pathological state in a subject having a ST segment elevated myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b)

d) assessing the risk of the deterioration of the pathological state, based on the information obtained in c).

The present invention further relates to a method for identifying a subject susceptible to cardiac intervention, whereby the subject has a ST segment elevated myocardial infarction (STEMI), based on the comparison of the amounts of each of the following markers, determined in a sample of said subject, to at least one reference amount: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof.

This method of the present invention may comprise at least one of the following steps ~~of~~: a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof; in a sample of said subject; b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, preferably the amount of each marker is compared to its respective reference amount.

The identification of a subject susceptible to cardiac intervention, whereby the subject has a ST segment elevated myocardial infarction (STEMI), may be established based on the information obtained in step b) and preferably based on the information obtained in a) and b).

Accordingly, the present invention provides a method for identifying a subject susceptible to cardiac intervention, whereby the subject has a ST segment elevated myocardial infarction (STEMI), comprising the steps of

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b)

d) identifying the subject, based on the information obtained in c).

In general, an individual is susceptible to a cardiac intervention when the amount of at least one of the markers sFLT-1, H-FABP or cardiac troponin is higher than the reference amounts specified below, indicating that the individual suffers from cardiac ischemia, reversible cardiac dysfunction and/or non reversible cardiac injury.

Moreover, the present invention relates to a method of recommending or deciding on a possible cardiac intervention or initiating a possible cardiac intervention in a subject having a ST segment elevated myocardial infarction (STEMI), based on the comparison of the amounts of each of the following markers, determined in a sample of said subject, to at least one reference amount: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof.

This method of the present invention may comprise at least one of the following steps ~~of~~: a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof; in a sample of said subject; b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, preferably the amount of each marker is compared to its respective reference amount.

Therecommendation of a possible cardiac intervention may be established based on the information obtained in step b) and preferably based on the information obtained in a) and b).

Accordingly, the present invention provides a method of recommending or deciding on a possible cardiac intervention or initiating a possible cardiac intervention in a subject having a ST segment elevated myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b)

d) recommending or deciding the initiation of a cardiac intervention, initiating the cardiac intervention or refraining from the cardiac intervention, based on the information obtained in step c).

In general, a physician will recommend a or decide on a or initiate a cardiac intervention in an individual when the amount of at least one of the markers sFLT-1, H-FABP or cardiac troponin is higher than the reference amounts specified below, indicating that the individual suffers from cardiac ischemia, reversible cardiac dysfunction and/or non reversible cardiac injury. The kind of intervention will be determined depending on the stage of the myocardial infarction, i.e. the relative amount of the markers. Further to the marker amounts, the considerations specified below preferably will be taken into account in the therapy decision/recommendation, i.e. recommendations and guidance from the prior art in respect to treatment of ischemic and/or stunning and/or necrotic states in myocardial infarction based on medical experience.

Still, the present invention relates to a method of monitoring a cardiac intervention in a subject having a ST segment elevated myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof; in a sample of said subject,

b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b)

d) monitoring the cardiac intervention, based on the information obtained in step c).

Monitoring is based on the amount of at least one of the markers sFLT-1, H-FABP or cardiac troponin, preferably determined at different points in time. When the amount(s) of a marker which prior to the cardiac intervention was higher than the reference amounts specified below (indicating that the individual suffers from cardiac ischemia, reversible cardiac dysfunction and/or non reversible cardiac injury) goes down in the course of the intervention, this shows that the intervention is successful. The intervention may be adapted, depending on the development of the respective marker amounts over time. Further to the marker amounts, the considerations specified below preferably will be taken into account in the therapy decision/recommendation, i.e. recommendations and guidance from the prior art in respect to treatment of ischemic and/or stunning and/or necrotic states in myocardial infarction based on medical experience.

In all embodiments cited beforehand, i.e. a method for stratifying or assessing the risk of the deterioration of the pathological state, a method for identifying a subject susceptible to cardiac intervention, a method of recommending or deciding on a possible cardiac intervention or initiating a possible cardiac intervention, and a method of monitoring a cardiac intervention in a subject having a ST segment elevated myocardial infarction, the determination of each of the cited markers is preferably carried out at least at two different points in time

In the context of the present invention it is to be expected that at least one of the three markers sFLT-1, H-FABP and a cardiac troponin will be elevated in the person having

STEMI. However, the amount of each of the markers, their relative ratio and, in a preferred embodiment, the evaluation of the marker amounts over time give important information of the severeness of the individual pathological states (i.e. ischemia, reversible cardiac dysfunction and non reversible cardiac injury) and, in the preferred embodiment, the evaluation of the states over time.

The individual amount of each marker gives guidance on the stage of the myocardial infarction and the risk for a deterioration of the pathological state in the individual. It is to be understood that ischemia is the less severe pathological state in an individual suffering form STEMI (as ST-elevation is indicative of ischemia). Ischemia may resolve on its own, not causing reversible cardiac dysfunction and/or non reversible cardiac injury if resolution occurs early after the acute event and/or if ischemia is not severe enough to cause cardiac dysfunction/injury even in the short time frame until resolution occurs (as indicated by the amount of sFLT-1).

The stage following ischemia (and being more severe) is reversible cardiac dysfunction, in general myocardial stunning, which can be diagnosed/assessed by determining an amount of H-FABP. Relative to an increased amount of sFLT-1, an increased amount of H-FABP indicates an even higher risk of suffering from non reversible cardiac injury and the patient should be treated within a short delay.

The pathophysiological stage following ischemia and reversible cardiac dysfunction is non reversible cardiac injury, in general necrosis which is diagnosed by a cardiac troponin (troponin T and troponin I). An elevated amount of cardiac troponin shows that at least a part of the myocard cells has died. An increasing amount of a cardiac troponin, or an almost constant level over time, is indicative of an ongoing necrotic process. Ongoing necrotic processes should preferrably be treated within a minimum delay.

Preferably, the reference amounts for sFLT-1 indicating ischemic states as defined in the present invention (threshold amounts) are the following: about 92 pg/ml, more preferably about 95 pg/ml, even more preferably about 109 pg/ml, in particular about 124 pg/ml. Amounts of sFLT-1 below the above-cited values are indicative for a non ischemic state not associated with or not leading to reversible cardiac dysfunction or non reversible cardiac injury. Amounts of sFLT-1 equal to or larger than the above-quoted reference amounts are indicative for an ischemic state associated with or leading to reversible cardiac dysfunction or non reversible cardiac injury.

Further preferred reference amounts of sFlT-1 indicating ischemic states are the 95th or 99th percentiles determined in a group of healthy subjects or the 50th or 75th percentiles determined in a group of subjects suffering from stable CAD. Each of said groups, preferably, comprises at least 100, 150, 200, 250 or 300 subjects.

In case an ischemic disease is diagnosed by the amounts of sFLT-1, the respective individual, in a preferred embodiment of the present invention, receives a treatment regimen as specified below, also referred to as "cardiac intervention".

Preferably, in case the determined sFLT-1 values do not indicate an ischemic state, the individual does not require any further examination in respect to cardiac diseases. In general, such individual can be released home.

Preferably, the reference amounts for H-FABP for reversible cardiac dysfunction, as defined in the present invention (threshold amounts) are the following: about 820 pg/ml, preferably about 1015 pg/ml, more preferably about 2236 pg/ml, in particular about 3380 pg/ml. Amounts of H-FABP equal to or larger than the above-quoted reference amounts are indicative for a reversible cardiac dysfunction. Amounts of H-FABP below the above-cited values are indicative for a reversible cardiac dysfunction. Reversible cardiac dysfunction, preferably, is myocardial stunning or myocardial hibernation.

Further preferred reference amounts of H-FABP indicating a reversible cardiac dysfunction are the 95th or 99th percentiles determined in a group of healthy subjects or the 50th or 75th percentiles determined in a group of subjects suffering from stable CAD. Each of said groups, preferably, comprises at least 100, 150, 200, 250 or 300 subjects.

In case an ischemic state and/or a reversible cardiac injury is diagnosed, the respective individual may require further examination in respect to cardiac or cardiovascular diseases. In a preferred embodiment, accordingly, the present invention comprises steps of further diagnosing the individual in whom the amounts of sFLT-1 and/or H-FABP have been determined. Appropriate further diagnostic methods in the context of the present invention include stress testing of various kinds, e.g. stress exercise ECG, stress exercise echocardiography, stress exercise computer tomography, stress exercise thallium scan; and angiography (invasive or virtual, e.g. by spiral computer tomography).

In case an ischemic disease is diagnosed by the amounts of sFLT-1, the respective individual may require further examination in respect to cardiac or cardiovascular diseases. In a preferred embodiment, these further diagnostic methods in the context of the present invention include stress testing of various kinds, e.g. stress exercise e.g., stress exercise echocardiography, stress exercise computer tomography, stress exercise thallium scan; and angiography (invasive or virtual, e.g. by spiral computer tomography). Depending on the results of the further examination, the respective individual will receive a treatment regimen as specified below, also referred to as "cardiac intervention".

Preferred Troponin T amounts indicative for non reversible cardiac dysfunction (necrosis) in the context of the present invention may be, but are not limited to, an amount of at least about 0,003 ng/ml, preferably about 0,005 ng/ml, more preferably about 0,007 ng/ml, in particular about 0,023 ng/ml. It is contemplated that these values indicate necrotic processes where necrotic tissue is still developping, resulting in connected myocardium area necrosis.

Further preferred reference amounts of troponin T indicating a non reversible cardiac dysfunction (necrosis) are the 95^{th} or 99^{th} percentiles determined in a group of healthy subjects or the 50^{th} or 75^{th} percentiles determined in a group of subjects suffering from stable CAD. Each of said groups, preferably, comprises at least 100, 150, 200, 250 or 300 subjects.

In a further embodiment of the present invention, troponin T amounts of at least about 0.1 ng/ml, of at least about 0.2 ng/ml, and of at least about 0.3 ng/ml are indicative of myocardium necrosis associated with myocardial infarction (connected myocardium area necrosis). These amounts are based on the requirements of The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction, lc., and are considered to be indicative for MI. In the context of the present invention, the above-cited amounts may be taken into account for confirmation of MI in a subject and/or for diagnosing advanced necrosis. They are, in general, not taken as reference amout for necrotic processes, for being present only at later stages of a myocardial infarction (due to delayed release of troponins), whereas the present invention is drawn to early diagnosis of the stages of myocardial infarction.

The clinical course of STEMI varies significantly (as already laid out beforehand), including the extent and duration of ischemia (or even recurrent ischemia), as well as

metabolic abnormalities with respect to extent and duration. Variables effecting ischemia include preexisting conditions, in particular the extent of atherosclerosis and dynamics of thrombus formation, level of the arterial occlusion, spontaneous thrombus lysis, embolism from the thrombus in distal smaller arteries, and further variables known to the person skilled in the art. All these variables have an impact on treatment decisions and are not captured by ECG findings, contrary to the method of the present invention.

The same holds true for interventions, for fibrinolysis as well as for PCI. Effects may vary from no effect, partial thrombolysis or complete reperfusion. Likewise, these effects can not be captured by merely ECG recording. Similarly embolism associated with intervention may result in ischemia, metabolic changes or even necrosis, the same is true for other complications specifically from PCI such as rethrombosis, dissection or bleeding at the intervention site. Again, effects of these complications are not fully recognized by ECG.

Although late spontaneous reperfusion occurs in some patients, thrombotic occlusion persists in the majority of patients with STEMI while the myocardium is undergoing necrosis. Timely reperfusion of jeopardized myocardium by PCI and/or fibrinolysis is the best way of restoring the balance between myocardial oxygen supply and demand. When fibrinolysis is administered, the extent of protection appears to be related directly to the rapidity with which reperfusion is carried out after the onset of coronary occlusion. When reperfusion is achieved with PCI (including stent deployment), the extent of myocardial salvage is less time-dependent than that for fibrinolysis. The causes underlying this phenomenon are believed to lie in restoration of full antegrade flow in the infarct artery with PCI whereas efficacy of fibrinolytic agents should decrease as coronary thrombi mature with the passage of time. Analyses adjusting for baseline risk, however, demonstrate a statistically significant increase in mortality with progressive delays between the onset of symptoms and PCI. Each 30-minute delay from symptom onset to PCI increases by 8 percent the relative risk of 1-year mortality.

PCI is in general supposed to be superior to fibrinolysis. However, the absolute risk difference between the two reperfusion strategies varies. A wider range of patients who need to be treated (or who are harmed) use PCI as compared with fibrinolysis as the reperfusion strategy in patients with STEMI.

Several issues should be considered in selecting the type of reperfusion therapy:

the stage of STEMI in the indicidual patient before reperfusion therapy can be initiated; for patients treated by fibrinolysis or PCI, the stage of the STEMI (time from the onset of symptoms) is an important predictor of mortality, underscoring the need for prompt reperfusion whichever strategy is selected.

Risk of STEMI: Patients at highest risk of mortality from STEMI account for the majority of deaths from STEMI. Accordingly, the mortality benefit associated with PCI is largest in patients who are at highest risk of mortality; the mortality benefit of PCI decreases progressively as the patient's risk of mortality from STEMI decreases, such that the mortality advantage of PCI is no longer evident among patients whose 30-day mortality rate is estimated to be between 2 and 3 percent if treated with fibrinolytic therapy.

Risk of bleeding: In patients with an increased risk of bleeding, particularly intracranial hemorrhage, therapeutic decision-making strongly favors a PCI-based reperfusion strategy. If PCI is unavailable, the benefit of pharmacological reperfusion should be balanced against the risk of bleeding. A decision analysis suggests that when no PCI is available, fibrinolytic therapy should still be favored over no reperfusion treatment in case of very severe, advanced STEMI. For patients who are not candidates for acute reperfusion because of lack of availability of PCI and contraindications to fibrinolysis, aspirin and anti-thrombin therapy can be prescribed.

Thus every effort should be made to provide reperfusion therapy even in clinical circumstances in which there is a perceived increase in the risk of bleeding. Arrangements for urgent primary PCI should be made for patients with a constellation of advanced age, low body weight, and hypertension on presentation because of the substantially increased risk of intracranial hemorrhage with fibrinolytic therapy. When the estimated delay to implementation of primary PCI is substantial (>90 minutes), fibrinolysis (with a fibrin-specific agent) may be preferable to no reperfusion therapy in such patients when the risk from the STEMI is high (e.g., anterior infarction with hemodynamic compromise). In the setting of absolute contraindications to fibrinolysis (see Table 51-3) and lack of access to PCI facilities, antithrombin therapy and antiplatelet therapy should be prescribed because of the small but finite chance.

Time required for transportation to a skilled PCI center: The greatest operational impediment to routine implementation of a PCI reperfusion strategy is the delay required for transportation to a skilled PCI center. Although several trials reported that referral to a

PCI center was superior to fibrinolysis administered in a local hospital, such studies were conducted in dedicated health care systems with extremely short transportation and door-to-balloon times at the PCI centers. Evidence exists to suggest that if the delay to implementation of primary PCI is greater than 1 hour, the mortality advantage compared with administration of a fibrin-specific agent is lost.

For example, if the subject presents to the emergency department and the markers according to the invention are recorded at a very early stage after the acute event (showing a STEMI), the amount of sFLT-1 may be increased, but not that of the other markers H-FABP and/or a cardiac troponin. In such a case, a very high amount of sFLT-1 indicating a high degree of ischemia (i.e. a large number of myocard cells is ischemic) will point to the fact that the myocardial infarction is still at its early stage, and that the individual is at an increased risk of suffering from reversible cardiac dysfunction and/or non reversible cardiac injury. This marker amount constellation, however, also points to the fact that the entire myocard or at least most of the myocard can be saved by timely appropriate treatment. It also shows that fibrinolysis is applicable for being in a stage shortly after onset artery occlusion, where fibrinolysis is still highly effective and PCI should not give advantageous results (which may be important in case of a contra-indication for PCI, e.g. long transportation to a PCI center).

At a later stage after the acute event, not only the amount of sFLT-1 may be elevated, but also the amounts of H-FABP and/or a cardiac troponin. The information relative to the stage of the myocardial infarction will then be taken form the combination of the amounts of each of the markers which can be determined, and the intervention will be based thereon. In case only the sFLT-1 and the H-FABP levels are elevated, the individual, in any case, has not suffered from substantial myocard necrosis, but the vessel occlusion is severe and has already lead to reversible cardiac dysfunction, besides ischemic myocard. Here, a large part of the myocard may be saved by timely, appropriate treatment, but it cannot be expected that the entire myocard can be saved, and mortality risk is still high. PCI should be preferred to fibrinolysis.

At very late points in time after the acute event, the amount of sFLT-1 may be low (i.e. has declined), and the same holds true for H-FABP (its amount may be so low that reversible cardiac dysfunction cannot be diagnosed any longer); however, the amount of a cardiac troponin may be elevated, indicating large areas of non reversible cardiac injury, in general necrosis. The information relative to the stage of the myocardial infarction will then be

taken form the combination of the amounts of each of the markers which can be determined. In the present case, the marker amounts show that only minor or even, as the case may be, no portions of the myocard can be saved by timely, appropriate treatment. Treatment of the patient by PCI has to be considered, however, depending on the patient's state.

The skilled person is aware that in view of the information as laid out above on the decision-making on reperfusion treatment, many variations to the treatments as proposed are conceivable, and the person skilled in the art is capable of individually establishing treatment decisions (or treatment recommendations) in respect to an individual suffering from STEMI in which the stage of the infarction has been diagnosed following the method of the present invention.

Thanks to the aforementioned method, a risk/success stratification can be easily performed before subjecting a patient to a cardiac intervention. In case the patient turns out to be not susceptible for a cardiac intervention, said dangerous, time and/or cost intensive therapy can be avoided. Thus, besides preventing a subject from the adverse and severe side effects accompanying a cardiac intervention, the method of the present invention will be beneficial for the health system in that resources will be saved.

It is to be understood in the context of the aforementioned method of the present invention that a subject suffering from STEMI and having an ischemic state or a development of its ischemic state which is supposed to result in reversible cardiac dysfunction or non reversible cardiac injury, is susceptible to cardiac intervention.

The term "identifying" as used herein means assessing whether a subject will be susceptible for a cardiac intervention or not. As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for all (i.e. 100%) of the subjects to be identified. The term, however, requires that a statistically significant portion of subjects can be identified (e.g. a cohort in a cohort study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student´s t-test, Mann-Whitney test etc.. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. More

preferably, at least 60%, at least 70%, at least 80% or at least 90% of the subjects of a population can be properly identified by the method of the present invention.

The term "cardiac intervention" encompasses those treatment regimens considered appropriate by the person skilled in the art. The term comprises interventions by surgery, microsurgery, other invasive therapies affecting the cardiovascular system and, preferably, the heart, as well as conservative (non-surgery) methods of treatment. Conservative methods are known in the art and include non-pharmacological methods and pharmacological methods. Pharmacological methods relate to the administration of pharmaceuticals. Appropriate pharmaceuticals include ACE inhibitors, in particular Enalapril, Captopril, Ramipril, Trandolapril; angiotensin receptor antagonists and aldosterone antagonists, in particular Losartan, Valsartan, Irbesartan, Candesartan, Telmisartan, Eprosartan, Spironolactone; statines, in particular Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, Rosuvastatin, Simvastatin; beta blockers like proprenolol, metoprolol, bisoprolol, carvedilol, bucindolol, nebivolol; nitrates; adrenergic agonists, like dobutamine, dopamine, epinephrine, isoprotenerol, norepinephrine, phenylephrine; antiplatelet agents, in particular aspirin and clopidrogel; anticoagulants, in particular warfarin, heparin, thrombin inhibitors, thrombinolytic drugs. Preferably, cardiac interventions as used herein are treatment regimens which aim to restore the proper oxygen supply of the heart. This is, preferably, achieved by restoring the blood flow throughout the blood vessels supporting the heart, i.e. the coronary blood vessels. Those blood vessels may be impaired due to, e.g., thrombotic or atherosclerotic plaques. Accordingly, cardiac interventions shall, preferably, comprise a destruction and/or removal of such plaques and a restoration of the vessel, if necessary. Preferred cardiac interventions in accordance with the present invention are selected from the group consisting of percutaneous coronary angioplasty, percutaneous transluminal coronary balloon angioplasty, laser angioplasty, coronary stent implantation, bypass implantation or intraluminal techniques aiming to restore blood flow, vessel patency, stabilize plaque, and/or reduce intracoronary thrombus load.

Moreover, encompassed by the present invention is a kit or device for carrying out the methods of the present invention comprising means for determining the amount of sFLT-1, H-FABP and a cardiac troponin, in a sample of a subject and means for comparing said amount to at least one reference amount.

The term "kit" as used herein refers to a collection of the aforementioned means, preferably, provided separately or within a single container. Optionally, the kit may additionally comprise a user's manual for interpreting the results of any measurement(s) with respect to diagnosing and/or monitoring, in a subject, the stage of myocardial infarction, and/or stratifying or assessing the risk of the deterioration of the pathological state, and/or identifying a subject susceptible to cardiac intervention, and/or recommending or deciding on a possible cardiac intervention, and/or monitoring a cardiac intervention in a subject, as defined in the present invention. Particularly, such manual may include information about what determined amounts corresponds to what kind of diagnosis. This is outlined in detail elsewhere in this specification. Additionally, such user's manual may provide instructions about correctly using the components of the kit for determining the amount of the respective biomarkers.

The term "device" as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to allow diagnosing and/or monitoring, in a subject, the stage of myocardial infarction, and/or stratifying or assessing the risk of the deterioration of the pathological state, and/or identifying a subject susceptible to cardiac intervention, and/or recommending or deciding on a possible cardiac intervention, and/or monitoring a cardiac intervention in a subject. Preferred means for determining the amount of sFLT-1, H-FABP and a cardiac troponin and means for carrying out the comparison are disclosed above in connection with the method of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the amount of the peptides are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the peptides or polypeptides in an applied sample and a computer unit for processing the resulting data for the evaluation. Alternatively, where means such as test stripes are used for determining the amount of the peptides or polypeptides, the means for comparison may comprise control stripes or tables allocating the determined amount to a reference amount. The test stripes are, preferably, coupled to a ligand which specifically binds to the peptides or polypeptides referred to herein. The strip or device, preferably, comprises means for detection of the binding of said peptides or polypeptides to the said ligand. Preferred means for detection are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the system brings

together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the sFLT-1, H-FABP and a cardiac troponin. Plasmon surface resonance devices, NMR spectrometers, mass- spectrometers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

The present invention also relates to the use of a kit or device for determining the amount of sFLT-1, H-FABP and a cardiac troponin in a sample of a subject and/or means for determining the amount of sFLT-1, H-FABP and a cardiac troponin and/or means for comparing the amount of sFLT-1, H-FABP and a cardiac troponin to at least one reference amount for: diagnosing and/or monitoring, in a subject, the stage of myocardial infarction, and/or stratifying or assessing the risk of the deterioration of the pathological state, and/or identifying a subject susceptible to cardiac intervention, and/or recommending or deciding on a possible cardiac intervention, and/or monitoring a cardiac intervention in a subject.

The present invention also relates to the use of sFLT-1, H-FABP and a cardiac troponin and/or means for determining the amount of sFLT-1, H-FABP and a cardiac troponin and/or means for comparing the amount of sFLT-1, H-FABP and a cardiac troponin to at least one reference amount for the manufacture of a diagnostic composition for: diagnosing and/or monitoring, in a subject, the stage of myocardial infarction, and/or stratifying or assessing the risk of the deterioration of the pathological state, and/or identifying a subject susceptible to cardiac intervention, and/or recommending or deciding on a possible cardiac intervention, and/or monitoring a cardiac intervention in a subject.

The present invention also relates to the use of antibodies against sFLT-1, H-FABP and a cardiac troponin for diagnosing and/or monitoring, in a subject, the stage of myocardial infarction, and/or stratifying or assessing the risk of the deterioration of the pathological state, and/or identifying a subject susceptible to cardiac intervention, and/or recommending

or deciding on a possible cardiac intervention, and/or monitoring a cardiac intervention in a subject.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

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

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

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

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

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

Finally, a further embodiment of the present invention relates to a method for diagnosing acute kidney disease in a subject comprising the steps of

a) determining the amount of neutrophil gelatinase associated lipocalin (NGAL) or a variant thereof in a sample of the subject;
b) comparing the measured amount of NGAL or the variant thereof to a reference amount;

whereby the results obtained in step b) indicate whether the subject suffers from acute kidney disease.

Preferably, the method of the present invention comprises the steps of a) determining the amount of neutrophil gelatinase associated lipocalin (NGAL)or a variant thereof in a

sample of the subject; b) comparing the measured amount of NGAL or the variant thereof to a reference amount; and c) diagnosing whether the subject suffers from acute kidney disease.

NGAL is known to the person skilled in the art.

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:

Troponin T was determined with the high sensitive hs Troponin T immunoassay test by Roche Diagnostics, using the ELECSYS 2010 Analyser. 1st incubation: 50 µL of a human serum sample, a biotinylated monoclonal anti-cardiac troponin T-specific antibody, and a monoclonal anti-cardiac troponin T-specific antibody labeled with a ruthenium complex ($Ru(bpy)^{2+}_3$) react to form a sandwich complex. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Chemiluminescent emission is induced by application of a voltage to the electrode and is measured by a photomultiplier. Results are determined via a calibration curve. This troponin T assay has a lower detection limit of 1 pg/ml.

sFlt-1 was determined with a sFlt-1 immunoassay to be used with the Elecsys and COBAS analyzers from Roche Diagnostics, Mannheim, Germany. The assay is based on the sandwich principle and comprises two monoclonal sFlt-1 specific antibodies. The first of these is biotinylated and the second one is labeled with a Tris(2,2'-hipyridyl)ruthenium(TT)-complex. In a first incubation step both antibodies are incubated with the human serum sample. A sandwich complex comprising sFlt-1 and the two different antibodies is formed. In a next incubation step streptavidin-coated beads are added to this complex. The beads bind to the sandwich complexes. The reaction mixture is then aspirated into a measuring cell where the beads are magnetically captured on the

surface of an electrode. The application of a voltage then induces a chemiluminescent emission from the ruthenium complex which is measured by a photomultiplier. The amount of light is dependent on the amount of sandwich complexes on the electrode. This sFlT-1 assay has a lower detection limit of 10 pg/ml.

H-FABP was determined by using the HBT ELISA Test Kit for human heart type fatty acid binding protein (HyCult Biotechnology, Uden, The Netherlands). The test is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. The microwells have been pre-coated with monoclonal antibody recognizing human H-FABP. Diluted human serum samples or standards are incubated together with a peroxidase conjugated second antibody (tracer) in the microwells for the incubation period, during which human H-FABP is bound to the solid phase. the tracer antibody is bound to the solid phase. Color develops proportionally to the amount of H-FABP in the sample. The enzyme reation is stopped by the addition of citric acid, the absorbance at 450 nm is measured spectrophotometrically. A standard curve is obtained, from which unknown H-FABP concentrations can be determined. The H-FABP-assay has a lower detection limit of 100 pg/ml.

The named tests are also preferably employed in the general context of the present invention for the determination of the respective peptides.

Example 1. Patients and Determination of Reference Values

149 Persons, mean age 42,8 years were tested for the presence of sFlT1, H-FABP and troponin T, at the time of testing and within the previous 2 weeks they were asymptomatic, specifically they had no chest pain, there was also no evidence of cardiac dysfunction as indicated by NT-pro BNP testing. The patients did not display CAD.
Results (see Table 1):

Table 1:

	sFlT1	**H-FABP**	**Troponin T**
Median	52 pg/ml	420 pg/ml	below detection limit
25 %	42 pg/ml	210 pg/ml	below detection limit
75 %	71 pg/ml	580 pg/ml	below detection limit
90%	81 pg/ml	790 pg/ml	2,0 pg/ml

95%	92 pg/ml	820 pg/ml	2,95 pg/ml
99%	109 pg/ml	1015 pg/ml	4,50 pg/ml

In the context of the present invention, preferably the 95th percentile is regarded as an appropriate reference value: Individuals showing marker values above those are considered to suffer from an ischemic state. More preferably, the 99th percentile defines the reference value indicative for an ischemic state. In respect to NT-proANP, preferably the 95th and more preferably the 99th percentile are likewise considered to be the reference value which defines which values are indicative of a circulatory impairment. Preferably the 99 Percent percentile of the reference population was chosen, as these individuals did not have evidence of coronary artery disease or were at risk of coronary artery disease.

Example 2. Patients and Determination of Reference Values

A total of 234 patients (122 males, 112 females (mean age 52.1 years) suffering from coronary artery disease were included into the study. All patients were clinically stable for at least 3 weeks prior to the study and they did not show signs and symptoms of chest pain or even acute coronary syndrome. 82 patients had one-vessel disease, 60 had two-vessel disease and 92 had three-vessel disease (determined by angiography) which followed clinical examination, at which also blood was taken by venipuncture. Angiography did not show evidence of acute thrombus formation in any of the patients. All patients had normal kidney function as assessed by creatinine levels within the normal range.

Blood taken by venipuncture was centrifuged within 30 minutes and serum samples were stored at – 20 Celsius until analysed.

Results (see Table 2):

Table 2:

	sFlT1	**H-FABP**	**Troponin T**
Median	95 pg/ml	2236 pg/ml	6,60 pg/ml
25 %	75 pg/ml	1548 pg/ml	1,24 pg/ml
75%	124 pg/ml	3380 pg/ml	23,00 pg/ml
95%	184 pg/ml	4836 pg/ml	82,00 pg/ml

In the context of the present invention, preferably the 50th percentile is regarded as an appropriate reference value: Individuals showing marker values above those are considered to suffer from an ischemic state and/or reversible cardiac dysfunction and/or myocardial necrosis. More preferably, the 75th percentile defines the reference value indicative for an ischemic state and/or reversible cardiac dysfunction and/or myocardial necrosis.

The patients had stable coronary artery disease. However one, two or three vessel disease which may be related to chronic ischemia, in addition minor ischemic events (as they may be asymptomatic) cannot be fully excluded in this population, thus for determination of the reference value the 75 th percentile was chosen. A patient was defined as suffering from 1-vessel disease if an atherosclerotic lesion occluded at least 50 % of the lumen of one coronary artery. The occlusion of two or three major coronary arteries by at least 50 % was defined as 2- or 3-vessel disease. The number of stenoses in a specific vessel did not affect the definition of vessel disease.

Example 3:

A total of 25 patients (14 men und 11 women) of mean age 62.1 years with unstable angina were included into the study. All patients had normal kidney function as assessed by creatinine levels within the normal range of the test. Twenty-two patients had ST elevation myocardial infarction, 3 patients had a nondiagnostic electrocardiogram (no ST elevation).

Serum samples were taken at presentation, then every 15 minutes within the first 90 min, thereafter at 24, 48, 72 and 96 hours.

Samples were tested for the presence of Troponin T using a high sensitive Test, sFlT1 and H-FABP.

Interventions (coronary angiography with restoration of the flow of the obstructed coronary) vessels was carried out in some, but not all of the patients. The following patients were treated at the indicated points in time:

patient 16 at 60 min; patient 35 at 120 min; patient 6 at 45 min; patient 14 at 30 min; patient 30 at 30 min; patient 31 at 30 min; patient 32 at 15 min; patient 12 at 30 min; patient 24 at 15 min; patient 26 at 45 min; patient 37 at 60 min.

Patients were split into 4 groups as follows:
group 1: patients with Troponin T levels exceeding 1000 pg/ml at presentation (9 patients),
group 2: patients with Troponin T levels between 100 and 1000 pg/ml at presentation (8 patients)
group 3: patients with Troponin T levels below 100 pg/ml at presentation (5 patients)
group 4 : patients without ST elevation (3 patients).

The individual results are shown in table 2.

group 1	Pat-No.	Time	TnT hs pg/ml	SFLT-1 pg/ml	H-FABP pg/ml
	10	baseline	1228.64	139.05	93700.97
	10	15 min	1398.82	1098.86	95988.30
	10	30 min	2859.39	1113.47	112846.54
	10	45 min	1219.27	142.83	86354.08
	10	60 min	14350.00	1295.15	137315.62
	10	24 h	3076.77	182.76	15282.98
	16	baseline			
	16	15 min	1255.18	2269.53	544193.69
	16	45 min	2872.90	1497.61	296258.81
	16	24 h	2638.43	117.17	10063.54
	16	48 h	1218.92	84.25	3259.78
	16	72 h	1273.81	39.25	2591.82
	16	96 h	1300.38	44.47	3899.56
	20	baseline			
	20	15 min	7704.50	1554.49	395499.83
	20	30 min	2003.75	2564.58	435370.46
	20	45 min	5427.34	1964.59	390676.67

20	60 min	2068.69	2570.04	501107.09
20	90 min	9629.04	1138.73	343894.63
20	48 h	3327.47	194.69	6983.19
20	72 h	2954.48	96.82	4055.94
23	baseline	1382.27	1245.13	5013.54
23	30 min	1368.62	1685.84	7400.64
23	45 min	1328.91	1666.66	6579.58
23	60 min	1390.01	1368.08	6680.34
23	90 min	1324.03	587.85	7298.92
23	24 h	leer	108.15	3931.26
34	baseline	1037.31	298.67	4493.51
34	15 min	1209.09	1005.92	5036.49
34	45 min	2739.74	1270.47	7765.38
34	60 min	3578.00	1099.19	9166.57
34	90 min	3524.40	547.13	9742.16
34	24 h	1846.85	86.54	2047.14
35	baseline			
35	15 min	4112.82	3483.18	211424.30
35	30 min	6269.31	2915.86	243904.58
35	45 min	8034.60	2363.96	240983.22
35	60 min	8507.35	2180.02	253952.59
35	90 min	9351.36	1572.43	225254.11
35	24 h	5385.87	111.75	42599.62
35	96 h	2869.76	132.04	7633.23
36	baseline	4331.69	1297.47	452655.48
36	15 min	4369.35	935.80	388182.56
36	30 min	15650.00	1821.11	478177.88
36	45 min	18270.00	1767.07	535661.34
36	60 min	15800.00	1872.34	531627.23
36	90 min	22680.00	1492.52	516717.89
36	24 h	13270.00	175.21	218439.22
38	baseline	1035.18	632.59	236477.25
38	15 min	2346.25	895.61	581695.66

	38	30 min	2464.90	879.38	50000.00
	38	45 min	8967.83	1037.99	50000.00
	38	60 min	14030.00	671.24	50000.00
	38	90 min	12290.00	850.12	50000.00
	40	baseline			
	40	15 min	2254.00	1965.76	525791.13
	40	30 min	9979.00	2464.49	610202.69
	40	45 min		2408.10	642321.88
	40	60 min	18030.00	2260.30	614200.69
	40	90 min	27190.00	1132.72	618598.12
	40	24 h	9255.00	105.22	166628.11
group 2	Pat-No.	Time	TnT hs pg/ml	SFLT-1 pg/ml	H-FABP pg/ml
	6	baseline	174.09	279.83	164110.02
	6	15 min	468.13	821.29	288930.17
	6	30 min	3210.13	874.91	503334.12
	6	60 min	5690.49	831.04	520596.73
	6	90 min	8836.83	597.39	421875.98
	6	24 h	3187.00	113.33	19167.56
	6	48 h	2635.56	59.19	5433.79
	6	96 h	1404.98	61.01	3087.54
	14	baseline	484.20	275.45	146370.30
	14	15 min	829.30	853.04	276457.85
	14	45 min	2796.00	1086.63	355265.31
	14	60 min	3168.00	983.00	296629.23
	14	90 min	3606.00	656.18	274885.63
	14	24 h	3457.00	196.85	22818.45
	14	48 h	3812.91	130.29	5699.75
	14	72 h	4169.13	108.45	2975.77
	14	96 h	1948.50	50.38	2321.62
	22	baseline	219.58	80.66	58241.32
	22	15 min	251.69	1087.84	38975.97
	22	30 min	386.53	1322.83	37465.36

22	45 min	398.23	1223.07	33948.49
22	60 min	436.95	1153.29	34404.50
22	90 min	leer	1064.54	29654.29
22	48 h	698.12	141.64	5866.24
29	baseline	633.86	926.95	52800.11
29	15 min	759.43	872.21	133513.11
29	30 min	2958.67	581.53	126243.10
29	45 min	1875.75	885.05	139950.56
29	60 min	3594.90	553.50	138048.47
29	90 min	5159.57	310.00	125326.48
29	24 h	1930.93	54.12	6526.16
30	baseline	426.15	992.40	537847.54
30	15 min	673.60	1893.94	398964.80
30	45 min	1117.69	2734.38	422073.07
30	60 min	1297.32	2514.33	363497.31
30	90 min	1939.07	2443.36	285032.71
30	24 h	2856.91	116.84	25766.10
31	baseline	254.25	244.81	167590.91
31	15 min	527.38	789.57	157641.68
31	45 min	1236.84	546.56	47186.05
31	60 min	982.82	891.14	51746.68
31	90 min	leer	1541.85	10000.00
32	baseline	193.90	1441.58	131844.70
32	30 min	374.90	1761.42	121651.85
32	45 min	leer	1985.41	10000.00
32	60 min	496.75	2030.86	110010.75
32	90 min	556.28	1380.81	70961.53
32	24 h	1084.66	292.72	9914.02
44	baseline	564.05	1085.01	11959.67
44	15 min	12730.00	2020.07	353526.20
44	30 min	2550.00	1722.52	18587.90
44	45 min	2854.00	1407.96	18704.03
44	60 min	1661.00	800.00	10322.02

44	90 min	2814.00	1182.49	17654.27

group 3	Pat-No.	Time	TnT hs pg/ml	SFLT-1 pg/ml	H-FABP pg/ml
	12	baseline	18.47	1947.73	5966.00
	12	15 min	694.71	1529.25	305446.58
	12	45 min	2678.05	802.78	121157.69
	12	60 min	2031.60	1374.19	176989.52
	12	24 h	1757.17	134.16	7491.19
	12	48 h	1057.02	127.22	4505.62
	12	72 h	1239.18	139.83	2469.31
	12	96 h	114.11	287.74	1838.40
	24	baseline	60.58	795.99	73153.99
	24	30 min	152.96	729.44	123115.48
	24	45 min	175.32	389.82	94897.95
	24	90 min	271.28	167.11	71254.66
	26	baseline	26.45	2497.98	17537.01
	26	15 min	62.51	2090.20	91422.63
	26	30 min	492.47	2979.53	368124.71
	26	60 min	733.82	3038.67	437530.38
	26	90 min	1316.79	2578.16	330619.55
	26	24 h	5138.53	127.31	23605.32
	37	baseline	73.59	654.19	13436.94
	37	15 min	157.67	1889.30	18660.51
	37	30 min	291.38	2483.42	26863.82
	37	45 min	270.16	2482.11	27488.78
	37	90 min	519.84	2065.09	32580.89
	37	24 h		303.71	10000.00
	37	96 h	2335.00	89.55	16840.97
	41	baseline	45.68	737.27	13525.00
	41	15 min	1720.33	1254.84	50000.00
	41	30 min	21230.00	827.62	50000.00

41	45 min	23000.00	806.33	50000.00
41	60 min	17760.00	911.73	50000.00
41	90 min	40650.00	354.75	50000.00

group 4	Pat-No.	Time	TnT hs pg/ml	SFLT-1 pg/ml	H-FABP pg/ml
	27	baseline	11.47	325.09	6278.00
	27	15 min	8.47	2618.06	5611.04
	27	30 min	leer	2027.07	5212.50
	27	60 min	10.19	1155.05	4947.26
	27	90 min	leer	1210.07	5179.33
	39	baseline	49.77	2081.88	9926.09
	39	15 min	46.40	2553.21	9256.77
	39	30 min	52.97	3497.74	13345.24
	39	45 min	50.99	3163.95	11276.59
	39	60 min		3464.07	11512.86
	39	90 min	50.60	3059.84	8679.78
	42	baseline		2167.95	9106.57
	42	15 min	49.24	2463.13	12210.65
	42	30 min	49.94	2618.74	10505.23
	42	45 min	55.17	2642.33	10018.46
	42	60 min		2659.46	9905.60
	42	90 min	77.17	2384.83	10380.23
	42	24 h	89.25	2049.54	12376.51
	42	48 h	119.08	827.71	13563.48

As can be seen from the individual cases, results are highly variable with regard to evidence and extent of ischemia, level of metabolic alteration and their duration. Intervention did not result in resolution of ischemia in all patients. Intervention resulted in the improvement of metabolic abnormalities in the majority of patients, but with some delay as is consistent with stunning effects.

As can be seen from the individual cases the determination of sFlT1 and H-FABP add significant information beyond current information in the early diagnosis of ST elevation myocardial infarction and its follow up.

Benefits from intervention are related to low Troponin T at presentation associated with highy elevated sFlT1 and specifically H-FABP.

Example 4

5 patients with diagnosed ST-segment elevation myocardial infarction (STEMI) were analysed at entry into the emergency room and approximately 3 hours thereafter. They were 5 males and three females, mean age 62,3 years (range 52 – 71 years), all patients were considered to have suffered from myocardial infarction within the last two hours before entry into the emergency room, they had no diagnostic troponin at entry into the study and their kidney function was within the normal range based on the upper limit of normal of the creatinine test. During the observation period none of the patients had a PCI that would affect the markers measured.

At presentation patients 1,2,5,6 and 9 had evidence of ischemia and metabolic abnormalities however the troponin T value was compatible with chronic artery disease in all cases and thus would reflect a timepoint for intervention to rescue the ischemic myocardium, 3 hours later all patients has increased Troponin T levels to a variable extent indicating myocardial necrosis, in general indicators of ischemia declined whereas in most patients metabolic abnormalities still increased indicating that percutaneous intervention would still be beneficial but not to the extent when compared to timepoint 0.

The results are presented in Table 2

Table 2:.

		Time Interval after entry	**sFlt-1**	**hs-TnT**	**H-FABP**
Patient no		hours	pg/ml	pg/ml	pg/ml

1		0	2160.48	1.81	4998.19
		4	274.03	20.33	11689.25
2		0	7382.76	22.03	10442.23
		3	6673.91	481.41	131250.00
5		0	5041.05	27.91	8241.65
		3	445.19	70.89	17864.14
6		0	7397.13	28.83	8930.32
		2	6492.55	1227.00	58960.77
12		0	4048.29	10.10	4740.93
		3	1682.39	168.91	120116.69

Claims

1. A method for diagnosing the stage of myocardial infarction in a subject showing a ST segment elevation myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, of H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b).

2. The method according to claim 1, wherein an amount of sFLT-1 or a variant thereof below or equal to the reference amount is indicative for a non ischemic state not associated with or not leading to a reversible cardiac dysfunction or non reversible cardiac injury, and an amount of sFLT-1 or a variant thereof larger than the reference amount is indicative for an ischemic state associated with or leading to a reversible cardiac dysfunction or a non reversible cardiac injury.

3. The method according to claim 2, wherein the reference amount for sFLT-1 or a variant thereof indicative for an ischemic state is about 92 pg/ml, preferably about 95 pg/ml, more preferably about 109 pg/ml, in particular about 124 pg/ml.,

4. The method according to any of claims 1 to 3, wherein an amount of H-FABP or a variant thereof larger than the reference amount is indicative for a reversible cardiac dysfunction, in particular myocardial stunning and/or myocardial hibernation.

5. The method according to claim 4, wherein a reference amount for H-FABP or a variant thereof is about 820 pg/ml, preferably about 1015 pg/ml, more preferably about 2236 pg/ml, in particular about 3380 pg/ml.

6. The method according to any of claims 1 to 5, wherein the cardiac troponin is troponin T and an amount of troponin T or a variant thereof larger than the reference amount is indicative for non reversible cardiac injury, in particular necrosis.

7. The method according to claim 6, wherein a reference amount for troponin T or a variant thereof is about about 0,003 ng/ml, preferably about 0,005 ng/ml, more preferably about 0,007 ng/ml, in particular about 0,023 ng/ml.

8. A method according to any of claims 1 to 7, wherein in step a) only the amount of troponin T or a variant thereof is determined, in step b) only the amount of troponin T or a variant thereof as determined in step a) is compared to at least one reference amount, and in step d) myocardial infarction is diagnosed based on the information obtained in step b), preferably based on the information obtained in a) and b).

9. The method according to any one of claims 1 to 8, wherein the method
   i) further comprises a step of collecting a sample from the patient by a minimal-invasive step,
   ii) excludes a step of collecting a sample from the patient by a minimal-invasive step,
   iii) excludes a surgical step,
   iv) excludes a step of collecting a sample, or
   is an in vitro method.

10. A method for monitoring the stage of myocardial infarction in a subject showing a ST segment elevation myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; and a cardiac troponin or a variant thereof,

b) comparing the amounts of sFLT-1 or a variant thereof, of H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b).

11. A method of deciding on a possible cardiac intervention or initiating a possible cardiac intervention in a subject having a ST segment elevated myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof; in a sample of said subject,

b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b)

d) deciding the initiation of a cardiac intervention, initiating the cardiac intervention or refraining from the cardiac intervention, based on the information obtained in step c).

12. A method of monitoring a cardiac intervention in a subject having a ST segment elevated myocardial infarction (STEMI), comprising

a) determining the amount of each of the following markers in a sample of said subject: soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof; in a sample of said subject,

b) comparing the amounts of sFLT-1 or a variant thereof, H-FABP or a variant thereof and of the cardiac troponin or a variant thereof, determined in step a), to at least one reference amount, and

c) diagnosing the stage of the myocardial infarction based on the information obtained in step b) and preferably based on the information obtained in a) and b)

d) monitoring the cardiac intervention, based on the information obtained in step c).

13. The method according to claim 12, wherein the cardiac intervention is selected from the group of: administration of pharmaceuticals; percutaneous coronary angioplasty; percutaneous transluminal coronary balloon angioplasty; laser angioplasty; coronary stent implantation; bypass implantation or intraluminal techniques aiming to restore blood flow, vessel patency, stabilize plaque, and/or reduction of intracoronary thrombus load.

14. A kit or device for carrying out the methods as laid out in any one of claims 1 to 14 comprising means for determining the amount of soluble fms-like tyrosine kinase-1 (sFLT-1) or a variant thereof; heart fatty acid binding protein (H-FABP) or a variant thereof; a cardiac troponin or a variant thereof in a sample of a subject, and means for comparing the amount of sFLT-1, H-FABP, and the cardiac troponin to at least one reference amount.

15. Use of antibodies against sFlT-1, H-FABP and a cardiac troponin for diagnosing the stage of myocardial infarction in a subject showing a ST segment elevation myocardial infarction (STEMI).

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/054073

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/74
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/218498 A1 (BUECHLER KENNETH F [US] ET AL) 20 September 2007 (2007-09-20) examples 3, 4 tables 1, 2 claims 1-44	1-15
Y	WO 2009/033831 A1 (HOFFMANN LA ROCHE [CH]; ROCHE DIAGNOSTICS GMBH [DE]; HESS GEORG [DE];) 19 March 2009 (2009-03-19) examples 1,2 claims 1-22	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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"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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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 2011	03/05/2011

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1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/054073

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ISHII JUNNICHI ET AL: "Prognostic value of serum concentration of heart-type fatty acid-binding protein relative to cardiac troponin T on admission in the early hours of acute coronary syndrome", CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY, WASHINGTON, DC LNKD- DOI:10.1373/CLINCHEM.2004.047662, vol. 51, no. 8, 1 August 2005 (2005-08-01) , pages 1397-1404, XP002454289, ISSN: 0009-9147 the whole document -----	1-15

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/054073

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007218498 A1	20-09-2007	NONE	
WO 2009033831 A1	19-03-2009	EP 2198307 A1	23-06-2010
		JP 2010539460 T	16-12-2010
		US 2010285595 A1	11-11-2010