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(54) **Title:** SFLT-1 AND TROPONIN T AS BIOMARKERS OF PULMONARY EMBOLISM

(57) **Abstract:** The present invention relates to the field of laboratory diagnostics. Specifically means and methods for diagnosing a complication of pulmonary embolism in a patient, means and methods for recommending an appropriate treatment and the monitoring of said patient are disclosed. The invention describes the use of soluble fms-like tyrosine kinase 1 and cardiac troponins for the aforementioned purposes.



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sFIT-1 and troponin T as biomarkers of pulmonary embolism

10 The present invention relates to the field of laboratory diagnostics. Specifically means and methods for diagnosing a complication of pulmonary embolism in a patient, means and methods for recommending an appropriate treatment and the monitoring of said patient are disclosed. The invention describes the use of soluble fms-like tyrosine kinase 1 and cardiac troponins for the aforementioned purposes.

15 Pulmonary embolism (PE) is the result of a thrombus which is released further downstream from large veins, frequently the V. iliaca or the V. femoralis. Typically, PE is associated with a hypercoagulative state, i.e. a condition characterized by excessive coagulation. This condition may have extrinsic causes such as surgery, immobility or cancer or intrinsic causes, typically genetic disorders. Risk factors for PE comprise advanced age, obesity,
20 malignancy, chronic obstructive pulmonary disease, acute infections and long-haul air travel. However, even the immobility during a bus ride may be sufficient to cause the formation of a thrombus which may finally lead to PE. In addition to the aforementioned factors, a genetic predisposition may increase the risk of PE in a patient. For example, the factor V Leiden mutation is present in approximately 5 % of the Caucasian population and
25 is linked to pulmonary embolism and thrombosis in early adulthood (Goldhaber, SZ Chapter 72, p. 1863 in "Heart Disease" Ed.: E. Braunwald, 8th Edition). The mortality of patients suffering from PE is high and may reach 15 %. The diagnosis of PE is difficult because it may be confused with other diseases. The symptoms of PE may, for example, include chest pain, dyspnea or syncope, which are also signs of myocardial infarction or
30 heart failure.

The pathophysiology of PE is complex. In general, embolism results in anatomical obstruction of a pulmonary artery, resulting in increased pulmonary arterial (PA) pressure and right ventricular dilation as well as increased right ventricular wall tension. These
35 effects in turn lead to increased right ventricular oxygen demand and right ventricular ischemia. Because the increased volume of a dilated right ventricle exerts pressure on the left ventricle, the preload of the left ventricle is reduced, thus causing left ventricular heart

failure, hypotension and reduced systemic and myocardial perfusion, which may possibly result in systematic and/or myocardial ischemia.

5 D-dimer has been shown to have diagnostic value for excluding deep vein thrombosis and pulmonary embolism. If the amounts of D-dimer are below a threshold amount, the presence of PE in the patient in question is highly unlikely. However, the usefulness of D-dimer is compromised by the fact that D-dimer is frequently increased in diseases other than PE. Particularly, increased amounts of D-dimer are frequently found in hospitalized persons. Thus, D-dimer has only a limited value in this group of patients. Moreover, NT-proBNP as a marker of cardiac function, troponin T as a marker of cardiac necrosis and GDF-15 as a further cardiac marker have been described as useful for the prediction of the outcome of pulmonary embolism (Lankeit et al., 2008, Am. J. Crit. Care, 177: 1018-1025; Vuilleumier et al., Thrombosis Research, 121: 617-624; 2008; Vuilleumier et al., 2008, Thrombosis and Hemostasis, 391-398). It has been shown previously that right ventricular
10 dilation was only associated with increased amounts of natriuretic peptides but not with increased amounts of cardiac troponins (Vuilleumier et al., 2008, Thrombosis Research, 121: 617-624).
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20 Recently, the present inventors have shown that patients presenting with an acute coronary syndrome show an increase of soluble fms-like tyrosine kinase-1 (sFlt-1) within minutes to hours after the event, although they did not develop myocardial infarction. Increase of sFlt-1 was clearly associated with myocardial ischemia as indicated by its association with typical chest pain. In this group of patents, sFlt-1 rapidly decreased after ischemia resolved indicating that sFlt-1 represents a sensitive indicator of myocardial ischemia, see
25 EP 09177395.2.

The application WO 2011/054829, having a priority date of 3 November 2009 and published on 12 May 2011 relates to a method for rapidly diagnosing if an acute medical event in an emergency patient is associated with a circulatory and/or an ischemic
30 complication, comprising the steps of

a) determining the amount of an ANP-type peptide in a sample of a patient;

b) determining the amount of sFlt-1 in a sample from a patient;

35 c) comparing the amounts measured in steps a) and b) to reference amounts; and

d) establishing a diagnosis based on the results of c).

The term "acute medical event" refers to a condition of a patient which induces or causes the patient to seek medical assistance. Said condition may be a serious, potentially life threatening condition such as a failure of vital body functions. The term also refers to the sudden deterioration of a previously stable condition. Organs whose functions are essential for survival are e.g. the lung, the heart, the kidneys and the liver. The term "circulatory complication" refers to a sudden deterioration of the function of the heart. Such a deterioration is e.g. caused by cardiac arrhythmia, transient cardiac arrest or pulmonary embolism. Accordingly, this application teaches that sFlt-1 may indicate the occurrence of pulmonary embolism.

As shown above, the complications of pulmonary embolism result from a complex interplay of different factors such as right ventricular heart failure, hypotension and ischemia. In order to select the optimal treatment for a patient it is essential to recognize these elements, in particular ischemia which generally leads to a deterioration of the affected organ. As pulmonary embolism is a medical emergency, the assessment of a patient should be quick and simple. Ideally, it should be possible to assess the patients' condition at the point of care. In principle, biomarkers are means that comply with this requirement. However, the biomarkers known in the art for the assessment of pulmonary embolism (natriuretic peptides, cardiac troponins and D-dimer) are not specific for pulmonary embolism and its consequences. As described above, D-dimer is often increased in hospitalized patients for other reasons than pulmonary embolism. Increased amounts of natriuretic peptides and/or cardiac troponins may be caused by pre-existing chronic cardiac diseases such as coronary artery disease or chronic heart failure rather than by PE.

Consequently, the problem underlying the present invention can be seen as the provision of means and methods for an improved clinical work up of patients suffering from pulmonary embolism. It is especially desirable to provide a method which allows fast and early diagnosis while not requiring specialized skills, preferably of ischemia, in particular of myocardial and/or systemic ischemia. Preferably, the results of the method should not be influenced by pre-existing diseases or disorders of the patient. The present invention solves this technical problem by the embodiments described in the claims and herein below.

Therefore, the present invention relates to a method for diagnosing a complication of pulmonary embolism, preferably systemic ischemia, in a patient, based on the comparison

of soluble fms-like tyrosine kinase 1 (sF1T-1) or a variant thereof determined beforehand in a sample of the patient to at least one reference amount.

- The method of the present invention comprises at least one of the following steps: a) determining the amount of sF1T-1 or a variant thereof in a sample of the patient; b) comparing the amount of sF1T-1 or the variant thereof determined in step a) to a reference amount; and c) determining the patient's risk of suffering from complications of pulmonary embolism based on the comparison of step b).
- It is also provided for a method for diagnosing a complication of pulmonary embolism, preferably systemic ischemia, in a patient comprising the steps of
- a) determining the amount of sF1T-1 or a variant thereof in a sample of the patient;
 - b) comparing the amount of sF1T-1 or the variant thereof determined in step a) to a reference amount; and wherein a complication of pulmonary embolism in the patient is diagnosed based on the comparison of step b).

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

The term "pulmonary embolism" (PE) refers to a disorder that is caused by a complete or partial obstruction of at least one pulmonary artery. Most frequently, PE is caused by a blood clot (embolus) which originates from a vein of the patient and is transported through the venous system towards the *vena cava*. A thrombus which is transported by the circulation is also referred to as an embolus. After passing the right atrium and the right ventricle, the embolus reaches the main pulmonary artery. At the bifurcation, the branch of the left and right pulmonary artery, the embolus is then transported into the left or right lung. Depending on its size, the embolus lodges in a larger or smaller pulmonary vessel. Large emboli may even be located at the bifurcation of the pulmonary artery, thus blocking blood flow both through the left and right pulmonary artery.

Nevertheless, PE may also be of nonthrombotic origin. Sources of nonthrombotic pulmonary embolism are, preferably, fat, tumor mass and air. Fat embolism can most frequently be observed after blunt trauma complicated by long-bone fractures. Air embolism may be a complication of the placement or removal of a large venous catheter.

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The term “complication of pulmonary embolism” refers to a deterioration of the patient’s health resulting from pulmonary embolism. In principle, a complication of PE may be the impairment of any physiological function of the patient. In the context of the present invention, a complication of PE is, preferably, characterized by an impairment of the normal blood circulation or an impairment of the normal pulmonary function in the patient in question. An impairment of blood circulation is any condition that impairs the transport of oxygenated blood to any organ or tissue of the body. An impairment of pulmonary function is any condition that impairs the exchange of oxygen and/or carbon dioxide between blood and air in at least a part of a lung. Preferably, the impairment of blood circulation and/or the impairment pulmonary function results in ischemia in at least one organ or tissue.

In PE, ischemia may be systemic or localized to certain organs or tissues. Systemic ischemia is typically the result of left ventricular heart failure. However, some of the complications of PE may be accompanied by localized ischemia. Right ventricular heart failure increases the workload and, concomitantly, the oxygen consumption of the right ventricle. In addition to this, the right ventricular walls stiffen, thus impairing the blood supply of the myocardium. Right ventricular failure may cause myocardial ischemia.

Preferably, the complication of PE is an ischemic complication. Most preferably, the complication of pulmonary embolism is myocardial ischemia or systemic ischemia. “Myocardial ischemia”, as used herein, is ischemia of at least a part of the myocardium. The term “presence of myocardial ischemia” does not exclude the presence of ischemia in another organ or tissue besides the myocardium. “systemic ischemia”, as used herein, is ischemia of at least one organ, tissue or part of the body. In one embodiment of the invention, “systemic ischemia” refers to ischemia of at least one organ, tissue or part of the body excluding the myocardium, e.g. the liver, kidney, feet or hands. In another, preferred embodiment of the invention, “systemic ischemia” refers to ischemia of at least one organ, tissue or part of the body including the myocardium, e.g. the myocardium, liver, kidney, feet or hands. Typically systemic ischemia in a patient suffering from pulmonary embolism is caused by the decreased systemic blood pressure of the patient. A decreased systolic blood pressure decreases systemic perfusion and, concomitantly, oxygen supply. It

especially affects those organs with a high metabolic oxygen requirement such as the kidney or liver or those body parts with the greatest distance to the heart such as feet and hands. In patients suffering from PE systemic ischemia typically results from left ventricular heart failure. As set forth in example 3, the blood pressure of patients with
5 initially high amounts of sFIT-1 was at the lower end of the normal range (about 90 mm Hg). Thus, increased amounts of sFIT-1 which indicate insufficient systemic perfusion are frequently associated with a decreased blood pressure.

The term “myocardial ischemia within systemic ischemia” refers to the phenomenon that
10 myocardial ischemia is indicated by an increased amount of a cardiac troponin and that, furthermore, it cannot be excluded that the respective patient suffers from systemic ischemia of an organ other than the heart, further to myocardial ischemia. As sFIt-1 indicates systemic ischemia irrespective of the affected organ (including the heart), tissue or body part and is, hence, not specific, an increased amount of a cardiac troponin together
15 with an increased amount of sFIt-1 indicates myocardial ischemia, without, however, indicating if the increase in sFIt-1 is exclusively due to myocardial ischemia, or if the increase is caused by organs further to the heart.

Preferably, the term “resulting from” as used in the present application encompasses a
20 temporal relationship, an association (coincidence), or a causal relationship between pulmonary embolism and the deterioration of the patient’s health. Here, i.e. in the case of pulmonary embolism, the term is meant to convey that (i) pulmonary embolism is followed by a deterioration of the patient’s health, (temporal relationship), or (ii) pulmonary embolism is associated in the sense of coinciding with a deterioration of the patients
25 health, or (iii) pulmonary embolism causes the deterioration of the patient’s health.

Patients suffering from pulmonary embolism frequently suffer from right ventricular heart failure as a complication of PE. This condition is caused by the increased resistance of the pulmonary arteries due to occlusion by the embolus and/or due to the release of
30 vasoconstricting agents. These effects increase the afterload of the right ventricle. If the increase of the afterload is sufficiently high, right ventricular heart failure develops.

Left ventricular heart failure may typically develop in patients suffering from pulmonary embolism as a consequence of right ventricular heart failure. Right ventricular heart failure
35 causes a dilation of the right ventricle. The left and right ventricles are both contained by the pericardium, the outer envelope of the heart, which is a rigid structure and does not respond immediately to increasing pressure. Therefore, the dilation of the right ventricle

necessarily decreases the available space for the left one. Thus, the filling of the left ventricle during diastole is impaired and the amount of blood pumped by each contraction of the left ventricle decreases. If left ventricular heart failure is severe enough, the remaining output of the heart is insufficient to maintain normal systemic blood pressure.

5 Hence, depending on the severity of left ventricular heart failure, the patient's blood pressure decreases. This condition leads to systemic underperfusion and, thus, systemic ischemia. A severe drop of systemic blood pressure below a systolic pressure of 90 mm Hg is known in the art as shock. The systemic underperfusion resulting from shock may result in the death of the patient.

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An impairment of pulmonary function may result from the occlusion of a pulmonary artery. In such a situation, deoxygenated blood cannot be transported to those parts of the lung which are supplied by this artery. This decreases the area of the lungs which is available for gas exchange between blood and air. If the affected area of the lung is sufficiently large, the oxygen requirement of the body cannot be met because the oxygenation of blood in the lung is insufficient to meet the systemic requirement, resulting in systemic ischemia. In the context of the present invention, the term "artery" refers to all vessels which lead away from the heart. Systemic arteries transport oxygenated blood from the left ventricle to the rest of the body except for the lungs. Pulmonary arteries transport deoxygenated blood from the right ventricle to the lungs where it is oxygenated.

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The term "diagnosing" refers to the process of assessing whether or not the patient in question suffers from a complication of pulmonary embolism. The person skilled in the art knows that such an assessment is usually not correct for all patients. However, the method of the present invention shall allow the correct assessment for a statistically significant portion of the patients. 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.

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

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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.

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Preferably, the sample is taken at any time after and as long as the patient suffers from pulmonary embolism. More preferably, the sample is taken as soon as the suspicion arises that the patient might be suffering from pulmonary embolism, preferably at presentation of the patient at the clinic, hospital, emergency unit, ambulance or emergency doctor or
10 resident doctor. Most preferably, the sample is taken when the diagnosis of PE is confirmed.

The term “patient”, preferably, refers to an animal, more preferably to a mammal and, most preferably, to a human. Preferably, the patient does not suffer from advanced
15 atherosclerosis. Also preferably, the patient does not suffer from acute coronary syndrome (ACS). More preferably, the patient does not suffer from instable angina pectoris or myocardial infarction (MI).

Preferably, the patient does not suffer from class III or class IV heart failure according to
20 the classification of the New York Heart Association (NYHA). NYHA class II heart failure is characterized by slight limitation of physical activity while the patient is comfortable at rest. Ordinary physical activity results in fatigue, dyspnea, palpitation and angina pain. Patients with more severe heart (NYHA classes II and IV) failure show these symptoms during less than ordinary physical activity or even at rest. More preferably, the patient has
25 no acute heart failure and no history of heart failure. Also preferably, the patient is not pregnant.

Moreover, the patient has, preferably, normal kidney function as defined by a glomerular filtration rate of more than 60 ml per minute and 1.73 m² body surface or by a serum
30 creatinine value lower than 1.3 mg/dl.

The term “acute coronary syndrome” (ACS) is known to the person skilled in the art and refers to an acute coronary disorder characterized by ischemia of the myocardium. It is subdivided into unstable angina pectoris and myocardial infarction.

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The term “unstable angina pectoris” UAP is known to the person skilled in the art and refers to an acute cardiac disorder resulting from the sudden occlusion of a coronary vessel

by atherosclerotic plaque. It is characterized by at least one of the following features: (i) occurrence of chest pain at rest and usually lasting at least 20 minutes, (ii) occurrence of chest pain for the first time and (iii) increasing frequency and/or severity of the bouts of chest pain. The ischemia caused by unstable angina pectoris leads to myocardial ischemia.

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The term "myocardial infarction" (MI) is known to the person skilled in the art. Pathologically, myocardial infarction is characterized by areas of necrotic myocardium in the patient. The clinical definition is based on the symptoms of the patient, the electrocardiogram and laboratory diagnostics. Typically, though not universally, the patient experiences chest pain. In the electrocardiogram, ST-element elevation or a new bundle branch block are considered as indicative for MI. Most frequently, creatine kinase and cardiac troponins are used for the laboratory diagnostics of MI. The aforementioned markers are released from the cardiomyocytes upon cellular injury. sFIT-1 is a further marker for MI which was recently introduced. sFIT-1 is released during periods of ischemia. Hence the amounts of the aforementioned markers in the blood may be increased in a patient suffering from myocardial infarction.

Because pulmonary embolism as well as the acute coronary syndrome may result in myocardial ischemia and the release of sFIT-1, increased amounts of sFIT-1 relative to the reference amount in a patient suffering from the acute coronary syndrome in addition to suspected or confirmed pulmonary embolism cannot be attributed to PE with certainty. Hence, in these patients sFIT-1 may not be reliable marker for the diagnosis of complications of PE.

In a preferred embodiment of the present invention, the patient is a patient at increased risk of suffering from PE and/or an increased risk of suffering from a severe form of PE. Preferably, a patient at increased risk of suffering from PE and/or an increased risk of suffering from a severe form of PE is selected from the group consisting of patients with major thrombophilias, hospitalized patients with medical illnesses (preferably pneumonia or heart failure), patients with an acute infection, patients suffering from chronic obstructive pulmonary disease, women using hormonal contraception, postmenopausal women receiving estrogen replacement therapy, patients undertaking long-haul air travel, patients suffering from cancer, obese patients, patients having undergone surgery or trauma, smokers, patients suffering from arterial diseases and patients having a pacemaker, an implantable coronary defibrillator or an indwelling central venous catheter. Major thrombophilias are either acquired or inherited. Typical acquired major thrombophilias are antiphospholipid antibody syndrome and hyperhomocysteinemia. Typical inherited major

thrombophilias are factor V Leiden, prothrombin gene mutation 20210, antithrombin III deficiency, protein C deficiency and protein S deficiency.

5 More preferably, the patient is suspected to suffer from pulmonary embolism. Because the symptoms of the acute coronary syndrome and pulmonary embolism are similar, ACS is, preferably, excluded in a patient suspected to suffer from pulmonary embolism. A patient is suspected to suffer from pulmonary embolism rather than ACS if the electrocardiogram does not show changes indicative of ACS and a clinical model shows a high probability of PE. Preferred clinical models for determining the probability of PE are described by Wells
10 PS et al., 1998, Annals of Internal Medicine, 129: 997-1005 and in Braunwald's Heart Disease, 8th Edition, Table 72-4. Moreover, a patient with suspected PE does not suffer from chest pain which radiates into the left arm, back, belly or neck, especially after physical activity (the typical symptoms of ACS). Finally, the patient suspected to suffer from PE does not suffer from symptomatic heart failure (NHYA class II or higher).

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Even more preferably, pulmonary embolism has already been diagnosed in the patient. PE is, preferably, diagnosed by angiography and/or computed tomography.

Contrary to the teachings of WO 2011/054829 (see introductory part) that sFIt-1 may
20 indicate the occurrence of pulmonary embolism, the present invention teaches that in a patient suffering from or suspected to suffer from PE, an elevated amount of sFIt-1 indicates a complication of PE, and wherein the complication (preferably myocardial or systemic ischemia) will not mandatorily occur.

25 An increased amount of sFIT-1 relative to the reference amount indicates that the patient suffers from a complication of pulmonary embolism, preferably systemic ischemia. The diagnosis of a complication of PE has therapeutic implications. If the patient does not suffer from a complication, the major concern is the prevention of repeated PE in the future. Hence, anticoagulation therapy is sufficient for said patient. In the absence of
30 evidence of complications of PE obtained by methods other than the method of the present invention the patient is eligible for an early discharge from the hospital.

Preferably, the presence of a complication of PE, preferably of systemic ischemia, or myocardial ischemia within systemic ischemia, indicates that further treatment in addition
35 to anticoagulation is required. Preferred additional treatments are the administration of thrombolytics, the insertion of a vena cava filter and embolectomy.

Moreover, circulatory support should be considered in a patient suffering from a complication of PE, preferably of systemic ischemia, or myocardial ischemia within systemic ischemia. The term "circulatory support" refers to any therapy that aims at increasing or stabilizing the systemic blood pressure in a patient. Preferably, circulatory support aims at maintaining a systolic blood pressure of at least 90 mm Hg. More preferably, vasopressors are administered for circulatory support. Preferred vasopressors are epinephrine, norepinephrine and dopamine.

The term "anticoagulation therapy" refers to the treatment of a patient with pharmaceuticals that prevent blood from clotting. As PE is typically caused by a blood clot, a reduced clotting of the patient's blood decreases the probability of future PE in the patient. Preferred pharmaceuticals for anticoagulation therapy are unfractionated heparin, low-molecular-weight heparin, warfarin, rivaroxaban, apixaban and dabigatran.

The term "inferior cava filter" refers to a medical device that is to be inserted into the inferior vena. These filters prevent the transport of an embolus from the veins of the patient to the right atrium. Thus, future occlusions of the pulmonary arteries by emboli are prevented because emboli do not reach the lungs anymore.

The term "embolectomy" refers to an intervention that aims at the removal of the embolus from the pulmonary artery where it lodges. Embolectomy is either surgical embolectomy or catheter embolectomy.

In surgical embolectomy, the chest of the patient is opened, a cardiopulmonary bypass is performed and the embolus is then removed. This technique is suitable as an emergency measure in patients with a high risk of complications of PE or with already apparent complications of PE.

In catheter embolectomy, a catheter is inserted into the vena cava of the patient and moved through the right heart to the pulmonary artery where the embolus lodges. The thrombus is then destroyed mechanically. Catheter embolectomy may be assisted by pharmacological thrombolysis. Moreover, a stent may be temporarily placed in the affected pulmonary artery to keep it open. A stent is a wire mesh tube which is attached in its collapsed form at the outside of a balloon catheter. Once, the catheter is guided to the desired artery, inflation of the balloon causes the inflation of the STENT. After deflation of the balloon the STENT remains inflated in the artery, thus keeping it open.

The term "thrombolytics" refers to pharmaceuticals that are able to dissolve clotted blood. These pharmaceuticals have two beneficial effects in pulmonary embolism: (i) They dissolve the embolus in the pulmonary artery, thus restoring pulmonary circulation. (ii) They dissolve thrombi in the deep veins, thus preventing their release into the circulation and future PE. Preferred thrombolytics are streptokinase, urokinase and recombinant tissue plasminogen activators, in particular alteplase, reteplase and tenecteplase. The most preferred thrombolytic is alteplase.

In a further preferred embodiment of the invention, the amount of a cardiac troponin or a variant thereof is determined further to the amount of sFIT-1 or a variant thereof. Based on the determination of sFIT-1 or a variant thereof and a cardiac troponin or a variant thereof it is e.g. possible to diagnose whether the patient suffers from myocardial ischemia within systemic ischemia.

Hence, a further preferred embodiment of the method of the present invention relates to a method for diagnosing if a complication of pulmonary embolism in a patient is myocardial ischemia within systemic ischemia, based on the determination of the amounts of sFIT-1 or a variant thereof and a cardiac troponin or a variant thereof and the comparison of the determined amounts to reference amounts.

Preferably, the method comprises at least one of the following steps: a) determining the amount of sFIT-1 or a variant thereof and the amount of a cardiac troponin or a variant thereof in the sample of the patient; b) comparing the amounts of sFIT-1 or the variant thereof and of the cardiac troponin or the variant thereof determined in step a) to reference amounts; and c) diagnosing whether the patient suffers from myocardial ischemia within systemic ischemia, based on the comparison of step b).

Hence, it is also provided for a method for diagnosing if a complication of pulmonary embolism in a patient is myocardial ischemia within systemic ischemia, comprising the steps of

- a) determining the amount of sFIT-1 or a variant thereof and the amount of a cardiac troponin or a variant thereof in the sample of the patient;
- b) comparing the amounts of sFIT-1 or the variant thereof and of the cardiac troponin or the variant thereof determined in step a) to reference amounts; and

- c) wherein it is diagnosed whether the patient suffers from myocardial ischemia within systemic ischemia, based on the comparison of step b).

In the context of this embodiment of the present invention the term "diagnosing" not only refers to the assessment whether a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, is present or absent in the patient in question. A diagnosis based on the combined determination of the amounts of sFIT-1 or a variant thereof and a cardiac troponin or a variant thereof allows the assessment whether the complication of PE is myocardial ischemia within systemic ischemia. It is to be understood that the differentiation between no ischemia, systemic ischemia and myocardial ischemia within systemic ischemia may not be correct for each patient diagnosed according to the method of the present invention. However, it is required that a statistically significant proportion of patients is diagnosed correctly. Whether a proportion of patients is significant, can be determined without further ado by the skilled person using the statistical methods disclosed above in this application.

An increased amount of sFIT-1 relative to the reference amount and a non-increased amount of the cardiac troponin relative to the reference amount, preferably, indicate the presence of systemic ischemia. Increased amounts of both sFIT-1 relative to the reference amount and the cardiac troponin relative to the reference amount, preferably, indicate the presence of myocardial ischemia within systemic ischemia. Myocardial ischemia may be associated with low level cardiomyocyte death and/or cardiomyocyte necrosis. Non-increased amounts of both sFIT-1 and the cardiac troponin, each relative to the reference amount indicate the absence of myocardial as well as systemic ischemia.

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In the case of myocardial ischemia, a more aggressive treatment with thrombolytics or embolectomy should be considered in order to prevent or limit non-reversible damage to the myocardium. In the case of systemic ischemia circulatory support should be considered.

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In an embodiment of the present invention the amount of sFIT-1 or a variant thereof determined in the patient is compared to the reference amount prior to the determination of the amount of the cardiac troponin. If the comparison of sFIT-1 to the reference amount indicates the absence of an ischemic complication, the steps of determining the amount of a cardiac troponin and comparing the determined amount to a reference amount can be omitted. It is to be understood that the above-described method increased amounts of sFIT-1 relative to the reference amount are present in myocardial as well as systemic ischemia

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while non-increased amounts of sFIT-1 relative to the reference amount indicate the absence of any kind of ischemia. Therefore, in the absence of ischemia, the determination of the amount of a cardiac troponin is not necessary.

- 5 The present invention, furthermore, also relates to a method of ruling in/ruling out a complication of pulmonary embolism, preferably systemic ischemia, or myocardial ischemia within systemic ischemia,.

10 The method for ruling in/ruling out a complication of pulmonary embolism, preferably systemic ischemia, preferably comprises at least one of the following steps: a) determining the amount of sFIT-1 or a variant thereof in a sample of the patient; b) comparing the amount of sFIT-1 or the variant thereof determined in step a) to a reference amount; and wherein ruling in/ruling out of the complication of pulmonary embolism, preferably of systemic ischemia, in the patient is decided based on the comparison of step b).

15

It is also provided for a method for ruling in/ruling out a complication of pulmonary embolism, preferably of systemic ischemia, in a patient suffering from or suspected to suffer from pulmonary embolism comprising the steps of

- 20 a) determining the amount of sFIT-1 or a variant thereof in a sample of the patient;
- b) comparing the amount of sFIT-1 or the variant thereof determined in step a) to a reference amount; and
- 25 c) wherein ruling in/ruling out of the complication of pulmonary embolism, preferably of systemic ischemia, is carried out based on the comparison of step b).

In a preferred embodiment of the method ruling in/ruling out a complication of pulmonary embolism, preferably of myocardial ischemia within systemic ischemia, in a patient
30 suffering from or suspected to suffer from pulmonary embolism, the amount of a cardiac troponin or a variant thereof is additionally determined in step a) and compared in step b) to a reference amount, whereby ruling in/ruling out a complication of pulmonary embolism, preferably of myocardial ischemia within systemic ischemia, is carried out based on the comparison of step b).

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Preferably, non-increased amounts of sFIT-1 and the cardiac troponin (as compared to the respective reference amounts) indicate that a complication of pulmonary embolism can be ruled out.

- 5 Preferably an increased amount of sFIT-1 and a non-increased amount of the cardiac troponin (as compared to the respective reference amounts) indicate that systemic ischemia is to be ruled in.

10 Increased amounts of both sFIT-1 and the cardiac troponin (as compared to the respective reference amounts) that in addition to systemic ischemia, myocardial ischemia is to be ruled in (myocardial ischemia within systemic ischemia.)

15 As the diagnosis of a complication of pulmonary embolism, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, has therapeutic implications, the present invention further relates to a method for recommending a therapy of PE based on the determination of sFIT-1 or a variant thereof.

20 The method for recommending a treatment of PE, preferably comprises at least one of the following steps: a) determining the amount of sFIT-1 or a variant thereof in a sample of the patient; b) comparing the amount of sFIT-1 or the variant thereof determined in step a) to a reference amount; and wherein a therapy of the complication of pulmonary embolism in the patient is recommended based on the comparison of step b).

25 It is also provided for a method for recommending a therapy of a complication of pulmonary embolism, preferably of systemic ischemia, in a patient suffering from or suspected to suffer from pulmonary embolism comprising the steps of

- a) determining the amount of sFIT-1 or a variant thereof in a sample of the patient;
- 30 b) comparing the amount of sFIT-1 or the variant thereof determined in step a) to a reference amount; and
- c) wherein the therapy of the complication of pulmonary embolism, preferably of systemic ischemia, in the patient is recommended based on the comparison of step
35 b).

In a preferred embodiment of the method for recommending a therapy of a complication of pulmonary embolism, preferably of myocardial ischemia within systemic ischemia, in a patient, the amount of a cardiac troponin or a variant thereof is additionally determined in step a) and compared in step b) to a reference amount, whereby a therapy of a complication of pulmonary embolism, preferably myocardial ischemia within systemic ischemia, is recommended in step c).

Preferably, non-increased amounts of sFIT-1 and the cardiac troponin (as compared to the respective reference amounts) indicate that the patient is eligible for an early discharge.

Preferably an increased amount of sFIT-1 and a non-increased amount of the cardiac troponin (as compared to the respective reference amounts) indicate that the blood pressure of patient should be monitored to detect a hemodynamic instability early. Circulatory support should also be considered.

Increased amounts of both sFIT-1 and the cardiac troponin (as compared to the respective reference amounts) indicate that the patient requires close monitoring in an intensive care unit and circulatory support if the blood pressure falls below 90 mm Hg. Embolectomy or thrombolysis should be considered.

Preferably, a non-increased amount of sFIT-1 and an increased amount of the cardiac troponin (as compared to the respective reference amounts) indicate that myocardial ischemia in this patient results from a disease different from PE. Hence, the recommended therapy for this patient depends on the disease underlying the increased amount of the cardiac troponin.

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The term "soluble fms-like tyrosine kinase 1", briefly "sFIT-1", as used herein refers to polypeptide which is a soluble form of the VEGF receptor Flt-1. It was identified in conditioned culture medium of human umbilical vein endothelial cells. The endogenous soluble Flt-1 (sFIT-1) receptor is chromatographically and immunologically similar to recombinant human sFIT-1 and binds VEGF with a comparable high affinity. Human sFIT-1 has been shown to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro. Preferably, sFIT-1 refers to human sFIT-1. More preferably, human sFIT-1 can be deduced from the amino acid sequence of Flt-1 as shown in Genebank accession number P17948, GI: 125361. An amino acid sequence for mouse sFIT-1 is shown in Genebank accession number BAA24499.1, GI: 2809071. Due to its binding to VEGF sFIT-1 inhibits VEGF-mediated angiogenesis. sFIT-1 has been shown to be a marker for chronic ischemia in patients suffering from coronary artery disease (EP

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10156911.9). In patients with acute coronary syndromes the increase of sFIT-1 precedes the onset of cardiomyocyte death as indicated by an increase of sFIT-1 followed by an increase of troponins in case of substantial myocyte death (EP 10156911.9).

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

The term “sFIT-1” also refers to variants of the above described sFIT-1 peptide. The term “variant” encompasses variants of the specific sFIT-1 peptide of the present application
10 having at least the same essential biological and immunological properties as sFIT-1. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing sFIT-1. Moreover, it is to be understood that a variant as referred to in accordance with the present
15 invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of
20 sFIT-1, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of sFIT-1 or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. The term "about" as used herein refers to +/- 20%, more preferably +/-
25 10%, most preferably, +/- 5% of a given measurement or value.

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
30 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
35 are disclosed in Anderson, loc cit and Ferrieres 1998, Clinical Chemistry, 44: 487-493.

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
5 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.

The term "cardiac troponin" also refers to variants of the above described troponins. The
10 term "variant" encompasses variants of the specific troponins of the present application having at least the same essential biological and immunological properties as the troponins. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing cardiac
15 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 about 92%, at least about
20 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of the cardiac troponin, preferably over the entire length of the peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the cardiac troponin or the aforementioned types of variants as long as these fragments have the essential
25 immunological and biological properties as referred to above. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

Determining the amount of sFIT-1, or a cardiac troponin, preferably troponin T, or any
30 other peptide or polypeptide or protein referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. The terms polypeptide and protein are used interchangeable throughout this application. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the
35 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.

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

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

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

Determining the amount of a peptide or polypeptide may, preferably, comprise the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display. Antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)₂ fragments that are capable of binding antigen or hapten. The present invention also includes single chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the term "antibody" refers to an antibody binding to a peptide selected from the group consisting of sFlt-1 and a cardiac troponin. 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.

5 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
10 polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for
15 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
20 and measurement of the ligand. Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of
25 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
30 higher order ligands. Suitable tags include biotin, digoxigenin, 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
35 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 ³⁵S, ¹²⁵I, ³²P, ³³P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according to 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 (DELFA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling 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 amounts of sFIT1, 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 patient as defined in the present invention. Such a determination of sFIT-1 by ELISA can be done, e.g., by using the ELECSYS sFIT-1 test by Roche Diagnostics, Mannheim, Germany. The amount of troponin T can be determined by the COBAS assay, Roche Diagnostics Mannheim, Germany. The amount of NT-proBNP can be determined by the COBAS assay, Roche Diagnostics Mannheim, Germany.

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The term “amount” as used herein encompasses the absolute amount (e.g., of sFIT-1, a cardiac troponin, or a natriuretic peptide), the relative amount or concentration (e.g. of sFIT-1 or 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.

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The term “comparing” as used herein encompasses comparing the amount of the peptide, polypeptide, protein comprised by the sample to be analyzed with an amount of a reference source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to

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a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample. The comparison referred to in step (b) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the amount(s) determined in step a) to reference amount(s), it is possible to diagnose a complication of pulmonary embolism. It is to be understood that amount of sFIT-1 as determined in step (a) of the methods of the presents invention are compared in step (b) to a reference amount for sFIT-1 as specified elsewhere in this application. Similarly, the amounts of the cardiac troponin determined in step a) shall be compared to reference amounts for said markers.

According to the present invention, the measured amount of sFIT-1 indicates whether a patient suffers from a complication of PE. If the amounts of sFIT-1 and a cardiac troponin are determined in combination, it can be additionally diagnosed whether the complication of PE is myocardial or systemic ischemia. The terms used in this context, i.e. "non-increased amount" "increased amount" are known to the person skilled in the art. The person skilled in the art is able to determine actual values for the relevant biochemical markers which correspond to these levels.

For example, the reference amounts may be assigned according to percentiles of the levels observed in a representative sample of apparently healthy individuals below an age of 50 years (preferably, the sample comprises at least 100, more preferably at least 500, most preferably at least 1000 individuals). E.g., a non-increased level may correspond to the maximum level observed in the 95th percentile.

Alternatively, the reference amounts 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 performed on individuals undergoing stress testing and correlating any adverse events with the levels observed in the individuals. Such studies may also allow tailoring the levels according to certain patient sub-groups, e.g. patients with known coronary artery disease, elderly patients, or apparently healthy individuals. Guidance on how such studies may be carried out can also be obtained from the Examples included in this specification.

The value of the amount considered as "increased", i.e. above the reference amount may also be chosen according to the desired sensitivity or specificity (stringency) of exclusion. The higher the desired sensitivity, the lower is the specificity of exclusion and vice versa. In the above example, the higher the percentile chosen to determine each amount, the more
5 stringent is the exclusion criterion, i.e. less individuals would be considered as suffering from a complication of PE.

Below, examples for actual reference amounts are provided for sFfT-1 and troponin T. It is evident, that the amounts given below can serve only as a first indication of the
10 presence/absence of PE in a patient. For example, the value of a normal or increased amount relative to the reference amount may also depend on the on the general health status of the individual. It is generally known the artisan how to take these conditions into account.

15 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
20 invention, a distribution of marker amounts for patients 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
25 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
30 1=low, 2=normal, and 3=high). This ranking can be correlated to results in the "normal" population, and a ROC curve created. These methods are well known in the art. See, e.g., Hanley et al, Radiology 143: 29-36 (1982).

In certain embodiments, markers and/or marker panels are selected to exhibit at least about
35 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" as used herein refers to +/- 20%, more preferably +/- 10%, most preferably, +/- 5% of a given measurement or value.

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

In the case of an odds ratio, a value of 1 indicates that a positive result is equally likely among patients 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" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

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

Preferably, the reference amount for determining if the sFIT-1 amount is indicative of a complication of pulmonary embolism is derived from the 75th percentile, 90th percentile or 95th percentile of a collective of healthy patients. More preferably, the reference amount is derived from the 95th percentile. The collective comprises, preferably, more than 100 or more than 150 patients. More preferably, a reference amount of sFIT-1 is an amount higher than about 90 pg/ml, higher than about 150 pg/ml, higher than about 200 pg/ml or higher than about 250 pg/ml. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

For troponin T, the reference amount of sFIT-1 indicating myocardial ischemia is derived from the 75th percentile, 90th percentile or 95th percentile of a collective of patients suffering from stable coronary artery disease. More preferably, the reference amount is derived from the 75th percentile. The collective comprises, preferably, more than 40 or more than 100 patients. More preferably, a reference amount of troponin is about 14 pg/ml, about 17 pg/ml, about 20 pg/ml or about 25 pg/ml. More preferably, the reference amount of troponin T is about 20 pg/ml. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

As set forth above, sFIT-1 is a specific marker for right ventricular stress caused by pulmonary embolism. Successful treatment of PE decreases the resistance of the pulmonary circulation and, concomitantly, right ventricular stress. If treatment of PE is unsuccessful, the resistance of the pulmonary circulation remains elevated or may even increase further. This is reflected by non-decreasing or increasing amounts of sFIT-1. Therefore, sFIT-1 can also be used to monitor the course of pulmonary embolism.

Hence, another preferred embodiment of the present invention relates to a method for monitoring a patient suffering from pulmonary embolism preferably from a complication of pulmonary embolism, preferably of systemic ischemia, based on the determination of the amounts of sFIT-1 or a variant thereof determined beforehand in a first and in a second sample of the patient.

The method of the present invention comprises at least one of the following steps: a) determining the amount of sFIT-1 or a variant thereof in a first sample and at least a second sample of the patient; b) comparing the amount of sFIT-1 or the variant thereof determined in the second sample to the amount of sFIT-1 or the variant thereof determined in the first sample; and c) Assessing whether the complication of PE in the patient improves or deteriorates based on the comparison of step b).

It is also provided for a method for monitoring a patient suffering from pulmonary embolism preferably from a complication of pulmonary embolism, preferably of systemic ischemia, comprising the steps of

- a) determining the amount of sFIT-1 or a variant thereof in a first sample and at least a second sample of the patient;
- b) comparing the amount of sFIT-1 or the variant thereof determined in the second sample to the amount of sFIT-1 or the variant thereof determined in the first sample; and
- c) wherein it is assessed whether the complication of PE, preferably of systemic ischemia, in the patient ameliorates or deteriorates based on the comparison of step b).

The term “monitoring a patient suffering from PE, preferably from a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia,” refers to the process of assessing whether the condition of the patient improves or deteriorates. Preferably, an improving condition of the patient is characterized by decreasing ischemia and a deteriorating condition of the patient is characterized by increasing ischemia.

Preferably, a higher amount of sFIT-1 in the second sample as compared to the first sample indicates that the condition of the patient is deteriorating. A decreased amount of sFIT-1 in the second sample as compared to the first sample, preferably, indicates that the patient's

condition is improving. A constant amount of sFlt-1 in the first and second sample indicates that the patient's condition is stable. Preferably, the increase or decrease of the amount of sFlt-1 is statistically significant. Whether an increase/decrease is statistically significant can be determined by the person skilled in the art without further ado by
5 applying the statistical methods laid out elsewhere in this application.

More preferably, the decrease of sFlt-1 is at least about 25 %, at least about 50 %, at least about 75 % or at least about 90 %. Most preferably, the amount of sFlt-1 decreases by at least about 25 %. The term "about" as used herein refers to +/- 20%, more preferably +/-
10 10%, most preferably, +/- 5% of a given measurement or value.

Also more preferably, the increase of sFlt-1 is at least about 50 %, at least about 100 %, at least about 150 % or at least about 200 %. Most preferably, the amount of sFlt-1 increases by at least about 100 %. The term "about" as used herein refers to +/- 20%, more
15 preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

The first sample is, preferably, taken at any time as long as the patient suffers from PE or is suspected to suffer from PE. More preferably, it is taken at presentation of the patient.

20 Preferably, the second sample is taken at any time after the first sample. More preferably, the interval between taking the two samples is about 3 hours about 6 hours, about 12 hours or about 24 hours. Most preferably, the interval is about 6 hours. If the condition of the patient is improving and/or if the risk of complications of PE is low, the interval is, preferably, about 12 hours, about 24 hours or about 48 hours. More preferably, the interval
25 for the aforementioned patient is about 24 hours. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

In a preferred embodiment of the present invention, the patient to be monitored according
30 to the above-described method receives treatment against PE. This treatment is, preferably, anticoagulation therapy. Thus, the method of the present invention can be applied to monitor, whether the treatment of a patient suffering from PE is successful or not.

If the condition of the patient improves, this, preferably, indicates that the patient receives
35 the appropriate therapy and no changes are required.

If the condition of the patient does not improve or even deteriorates, this, preferably, indicates that the patient receives insufficient therapy. Hence, a switch to more a more aggressive therapy should be considered and the patient should be monitored closely for hemodynamic instability. More aggressive therapies are, preferably, the insertion of a vena cava filter, thrombolysis, surgical embolectomy and catheter embolectomy. If the condition of the patient worsens even though thrombolysis is initiated, this indicates, preferably, that surgical or catheter embolectomy should be performed. Circulatory support may also be considered.

In order to monitor the patient's condition continuously it is preferred to take samples regularly in the above defined intervals as long as the patient suffers from PE. If this is done, the amounts of sFIT-1 or the variant thereof in any two samples can be compared, provided that the sample which serves as first sample is taken earlier than the sample serving as second sample.

As set forth above, cardiac troponins and their variants serve as indicators of myocardial ischemia. Thus, the combined determination of the amounts of sFIT-1 and a cardiac troponin allow a closer monitoring of the patient's condition.

Therefore, in a another preferred embodiment, the method for monitoring a patient suffering from PE, preferably from a complication of PE, preferably myocardial ischemia within systemic ischemia, comprises the steps of

- a) determining the amounts of sFIT-1 or a variant thereof and of a cardiac troponin or a variant thereof in a first sample and at least a second sample of the patient;
- b) comparing the amounts of sFIT-1 and of the cardiac troponin or the variant thereof determined in the second sample to the amounts of sFIT-1 or the variant thereof and of the cardiac troponin or the variant thereof determined in the first sample; and
- c) wherein it is assessed whether the complication of PE, preferably myocardial ischemia within systemic ischemia, in the patient ameliorates or deteriorates based on the comparison of step b).

The combined combination of sFIT-1 and a cardiac troponin allows the monitoring of myocardial ischemia as well as systemic ischemia. Consequently, a decreased amount of a cardiac troponin in the second sample as compared to the first sample indicates that

myocardial ischemia and concomitant necrosis in the patient are subsiding. Said decrease of the amount of the cardiac troponin is, preferably, statistically significant. Whether a decrease is statistically significant can easily be determined with the statistical method described elsewhere in the present application.

5 More preferably, the decrease of the cardiac troponin is at least about 25 %, at least about 50 %, at least about 75 % or at least about 90 %. Most preferably, the amount of sFIT-1 decreases by at least about 25 %. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

10 Also more preferably, the increase of the cardiac troponin is at least about 50 %, at least about 100 %, at least about 150 % or at least about 200 %. Most preferably, the amount of sFIT-1 increases by at least about 100 %. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

15 In a preferred embodiment of the present invention, each of the samples taken for monitoring a patient suffering from PE is additionally compared to the reference values disclosed for the embodiments of the invention relating to the diagnosis of a complication of PE. If increasing amounts of sFIT-1 in the patient remain below the reference amounts indicating a complication of PE, the treatment regimen does not need to be changed despite
20 increasing amounts of sFIT-1. On the other hand, if increasing amounts of sFIT-1 reach or surpass one of these reference amounts, a more aggressive therapy should be strongly considered.

Surprisingly, it has been found in the study underlying the present invention that a strong
25 decrease of the amount of sFIT-1 in the sample taken 12 hours after presentation of the patient as compared to the sample taken at presentation indicates that the amount of sFIT-1 in the samples taken after said sample will continue to decrease. In most cases the amount of sFIT-1 will reach normal or almost normal values after less than 48 hours. Thus, a strong initial decrease of the amount of sFIT-1 predicts that the complication of pulmonary
30 embolism in the patient, preferably systemic ischemia, will not worsen or may even resolve within about 2 days. Hence, in a patient showing such a decrease aggressive therapies are not necessary.

Hence, in another embodiment, the present invention relates to a method for predicting the
35 course of pulmonary embolism in a patient based on the determination of the amounts of sFIT-1 or a variant thereof determined beforehand in a first and in a second sample of the patient.

The method of the present invention comprises at least one of the following steps: a) determining the amount of sFIT-1 or a variant thereof in a first and second sample of the patient; b) comparing the amounts of sFIT-1 or the variant thereof determined in the first and second sample; and c) predicting the course of pulmonary embolism in the patient based on the comparison of step b).

It is also provided for a method for predicting the course of pulmonary embolism, preferably the course of a complication of pulmonary embolism, preferably systemic ischemia, in a patient comprising the steps of

- a) determining the amounts of sFIT-1 or a variant thereof in a first and second sample of the patient;
- b) comparing the amount of sFIT-1 or the variant thereof determined in the second sample to the amount of sFIT-1 or the variant thereof determined in the first sample; and
- c) wherein the course of pulmonary embolism preferably the course of a complication of PE, preferably systemic ischemia, in the patient is predicted based on the comparison of step b).

The term "predicting the course of pulmonary embolism" refers to the process of assessing whether the complications of pulmonary embolism will subside or endure. Preferably, subsiding complications of PE are defined by decrease of ischemia over time. Decrease of ischemia (and, thus, a subsiding complication of PE) is, preferably, indicated by a normalization of the amount of sFIT-1 within about 24 hours, about 36 hours, about 48 hours or about 60 hours after taking the first sample. More preferably, the interval is about 48 hours. The term "normalization of the amount of sFIT-1", preferably, refers to a decrease of the amount of sFIT-1 to less than about 250 pg/ml, less than about 200 pg/ml, less than about 150 pg/ml or less than about 100 pg/ml. In the context of the present application, enduring ischemia is defined by amounts of sFIT-1 which do not decrease as set forth above. In a patient suffering from enduring ischemia the amounts of sFIT-1 determined in the intervals given beforehand may even increase. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

The first sample for the prediction of the course of PE is, preferably, taken at presentation of the patient. The second sample is, preferably, taken about 4 hours, about 8 hours, about 12 hours, about 16 hours or about 20 hours after the first sample. More preferably, the interval is about 12 hours. The term "presentation of the patient", preferably, refers to the time when the patient first seeks medical assistance in a hospital, in an emergency room or by a general practitioner. Preferably, PE is suspected in the patient when the first sample is taken. However, taking the first sample may be delayed until the diagnosis of PE is confirmed. Preferably, the first sample is not taken more than about 30 minutes, more than about 60 minutes or more than about 90 minutes after the diagnosis of PE is confirmed for the patient. More preferably, the first sample is taken less than about 30 minutes after the presentation of the patient. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

Preferably, a decrease of the amount of sFIT-1 in the second sample as compared to the first sample by at least about 50 %, at least about 75 % or at least about 90 %, indicates that ischemia will subside within the interval given above. More preferably, the decrease of sFIT-1 which predicts a subsiding complication of PE is about 50 %. Preferably, a decrease of sFIT-1 below about 50 % or even an increase of sFIT-1 predicts that the complication of PE will endure. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

Moreover, a decrease of sFIT-1 between the first and second sample as described above indicates that significant systemic and/or myocardial ischemia will not occur or that it will subside (if present at presentation) as indicated by the troponin amounts. Preferably significant myocardial ischemia within systemic ischemia, is defined by the amount of a cardiac troponin higher than about 25 pg/ml, higher than about 30 pg/ml or higher than about 35 pg/ml. More preferably, significant ischemia (myocardial ischemia within systemic ischemia) is defined by a troponin amount of more than about 30 pg/ml. The term "about" as used herein refers to +/- 20%, more preferably +/-10%, most preferably, +/- 5% of a given measurement or value.

Therefore, the amount of sFIT-1 does not decrease as described above, this indicates that said patient is in need of more aggressive therapy, preferably embolectomy and/or the administration of thrombolytics.

Consequently another embodiment of the present invention relates to a method for recommending the appropriate therapy of a patient suffering from pulmonary embolism

based on the determination of the amounts of sFIT-1 or a variant thereof determined beforehand in a first and in a second sample of the patient.

- The method of the present invention comprises at least one of the following steps: a) determining the amount of sFIT-1 or a variant thereof in a first and at least a second sample of the patient; b) comparing the amounts of sFIT-1 or the variant thereof determined in the first and second sample; and c) recommending the appropriate therapy of the patient based on the comparison of step b).
- It is also provided for a method for recommending the appropriate therapy of a patient suffering from pulmonary embolism, preferably from a complication of PE, preferably of systemic ischemia, comprising the steps of
- a) determining the amount of sFIT-1 or a variant thereof in a first and at least a second sample of the patient;
 - b) comparing the amounts of sFIT-1 or the variant thereof determined in the first and second sample; and
 - c) wherein the appropriate therapy of the patient for a complication of PE, preferably of systemic ischemia, is recommended based on the comparison of step b).

If the amount of sFIT-1 in the second sample does not decrease as set forth above, a more aggressive therapy of PE is, preferably, recommended. Because of the side effects of more aggressive therapies of PE, these should only be recommended if a clear benefit for the patient in question can be expected. Typically, this will be the case if the patient is likely to suffer from enduring complications of PE.

As set forth above, cardiac troponins and their variants serve as indicators of myocardial ischemia.

Therefore, in a another preferred embodiment, the method for recommending the appropriate therapy of a patient suffering from pulmonary embolism, preferably from a complication of PE, preferably of myocardial ischemia within systemic ischemia, comprises the steps of

- a) determining the amounts of sFIT-1 or a variant thereof and of a cardiac troponin or a variant thereof in a first sample and at least a second sample of the patient;
- b) comparing the amounts of sFIT-1 and of the cardiac troponin or the variant thereof
5 determined in the second sample to the amounts of sFIT-1 or the variant thereof and of the cardiac troponin or the variant thereof determined in the first sample; and
- c) wherein the appropriate therapy of the patient, for a complication of PE, preferably of myocardial ischemia within systemic ischemia, is recommended based on the
10 comparison of step b).

The combined combination of sFIT-1 and a cardiac troponin allows determining myocardial ischemia as well as systemic ischemia. Consequently, a decreased amount of a cardiac troponin in the second sample as compared to the first sample indicates that
15 myocardial ischemia and concomitant necrosis in the patient are subsiding. Said decrease of the amount of the cardiac troponin is, preferably, statistically significant. Whether a decrease is statistically significant can easily be determined with the statistical method described elsewhere in the present application.

20 Preferably, the term “more aggressive therapy of PE” refers to thrombolysis, surgical embolectomy, catheter embolectomy and the insertion of a vena cava filter.

Side effects of surgical embolectomy are those that are known to the person skilled in the art to be normally associated with major surgery. In particular, said side effects include
25 pulmonary aspiration of gastric contents, hemorrhage, infection and hypostatic pneumonia. In the case of catheter embolectomy, side effects are, typically, associated with the administration of a contrast agent. In particular, side effects of catheter embolectomy include acute kidney failure.

30 Thombolysis moves the normal equilibrium of hemostasis towards fibrinolysis. Thus, the administration of thrombolytics increases the risk of hemorrhage. The most severe form of hemorrhage is intracerebral hemorrhage, i.e. hemorrhage in the brain because his condition may cause non-reversible neurological damage.

35 The person skilled in the art knows that a recommendation according to the method of the present invention is usually not correct for each patient. However, the method of the present invention shall allow the correct recommendation for a statistically significant

portion of the patients. 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 described elsewhere in this application.

- 5 The person skilled in the art knows that factors other than the severity or predicted duration of PE, preferably systemic ischemia or myocardial ischemia within systemic ischemia, may additionally influence the recommendation of a treatment. Preferably, intracranial bleeding within the last three months, ischemic stroke within the last three months, bleeding diathesis, uncontrolled or controlled hypertension, prolonged anticoagulant use
10 within the last two weeks, pregnancy, active peptic ulcers and hemorrhagic or diabetic retinopathies speak against the treatment of a patient with thrombolytics. If any of the aforementioned conditions is present in the patient, higher reference amounts of sFIT-1 than those cited above may be required for the recommendation of thrombolysis. Depending on the condition of the patient a treatment with thrombolytics may even be
15 absolutely contraindicated.

Advantageously, the present invention provides novel methods for the diagnosis and monitoring of complications of pulmonary embolism, preferably of systemic ischemia and/or myocardial ischemia within systemic ischemia, and for predicting their further
20 course. The known markers for complications of PE such as cardiac troponins, natriuretic peptides and GDF-15 may be increased due to pre-existing diseases or disorders of the patient. In contrast to this, sFIT-1 is more specific for acute ischemia. Hence, the determination of sFIT-1 alone or in combination with one of the previously described biomarkers increases the reliability of the diagnosis of a complication of PE, preferably of
25 systemic ischemia. Particularly the combined determination of sFIT-1 and a cardiac troponin allows not only for the diagnosis of a complication of PE, preferably of myocardial ischemia within systemic ischemia, but furthermore for its classification. Increased amounts of a cardiac troponin in addition to increased amounts of sFIT-1 relative to the respective reference amounts indicate ongoing myocardial necrosis in the patient
30 while an increased amount of sFIT-1 alone indicates that no myocardial necrosis is present.

The surprising discovery that a marked decrease of sFIT-1 within the first 12 hours after presentation of the patient predicts the rapid improvement of ischemia further assists in clinical decision making.

35

Thus, the diagnostic and prognostic methods of the present invention help identifying of those patients which are in greatest need of more aggressive treatments. Because

aggressive treatments such as embolectomy and thrombolysis carry a high risk of undesired side effects, they should be reserved for those patients who can expect a significant benefit from them. Patients without complications of PE or patients with a good prognosis can be spared the risks and inconveniences of unnecessary therapies. Moreover the targeting of aggressive treatments to the patients with the greatest need allows a more economical use of the resources of the health system. Hence the individual patient as well as the health system as a whole benefit from the diagnosis and prediction according to the methods of the present invention.

Moreover, the present invention relates to a device for carrying out the methods of the present invention comprising

a) an analyzing unit for determining the amount of sF1T-1 or a variant thereof in a sample of the patient; and

b) an evaluation unit for processing the resulting data.

The term “device” as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to practise the method of the present invention. Preferred means for determining the amounts of the markers of the present invention, and means for carrying out the comparison are disclosed above in connection with the method of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where an analyzing unit for automatically determining the amount of the gene products of the present invention is applied, the data obtained by said automatically operating analyzing unit can be processed by, e.g., a computer as evaluation unit in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case.

In particular, the present invention relates a device comprising an analyzing unit for the measurement of the amount of sF1T-1 in an applied sample and an evaluation unit for processing the resulting data.

Preferably, the analyzing unit comprises a combination of a reading device and a specific ligand. The reading device is adapted to detect a signal generated or suppressed (e.g. in a competitive ELISA) by the binding of the specific ligand to the target molecule.

In one preferred embodiment of the present invention, the specific ligand is dissolved in a liquid carrier, e.g. a buffer solution. In this embodiment, the analyzing unit may be easily adapted to detect different target molecules because the specificity of the reading device only depends on the specificity of the specific ligand. In order to be compatible with the reading device, a specific ligand only has to generate the kind of signal that can be recognized by the reading device. Thus, using ligands with different specificities but the same signal-generating mechanism allows the easy adaptation of the analyzing unit to different diagnostic methods. The analyzing unit just has to comprise reservoirs for ligands with different specificities. The loading of the reading device with the sample as well as the addition of the specific ligand to the sample can be performed by automated liquid handling systems known to the person skilled in the art. Therefore, this type of analyzing unit is especially well suited for the use in the clinical laboratory because it allows a high degree of automation and, thus, a high throughput of samples.

In another preferred embodiment of the present invention, the specific ligand is bound to a solid carrier, preferably a test stripe. In this case the reading device is adapted to detect a signal generated or suppressed by the binding of the target molecule to the specific ligand immobilized on the solid carrier. The handling of solid carriers is less amenable to automation. However, a sample can easily be transferred to the solid carrier at the point of care. Hence, this type of analyzing unit lends itself to applications at the bedside. Therefore, this embodiment is especially preferred for situations where a rapid assessment of the patient's condition is essential, e.g. for the diagnosis, risk stratification or selection of treatment of an emergency patient. In addition to this, the combination of a portable reading device and a specific ligand bound to a solid carrier is advantageous for the use by an individual patient for monitoring his/her condition.

If test stripes are used which generate a colour signal that can be recognized by the eye, a reading device can be omitted from the analyzing unit of the present invention. In this case the analyzing unit does not comprise a reading device.

In a further preferred embodiment of the present invention, the analyzing unit comprises biosensors and/or arrays and/or Plasmon surface resonance products and/or NMR spectrometers and/or mass-spectrometers referred to above in accordance with the method of the invention.

Preferably, the analyzing unit comprises means for automatically transmitting the measured data, preferably as intensity values of the generated signal, to the evaluation unit

so that they can be further processed without the need for human intervention. If the components of the device, i.e. the analyzing unit and evaluation unit, are physically linked, the measured data can be transmitted by electrical circuits. However, it is also envisaged by the present invention that the analyzing unit and the evaluation unit may be physically
5 separated. In this case, means for wireless transmittal of the data from the analyzing unit to the evaluation unit are preferred. Such means are well known to the person skilled in art. In such an embodiment a multiplicity of analyzing units may be connected to single central evaluation unit.

10 Preferably, the evaluation unit is adapted for processing the values transmitted by the analyzing unit.

In the simplest embodiment of the present invention, "processing of the resulting data" by the evaluation unit merely refers to calculating amounts, concentrations or amount ratios of
15 the markers of the present invention. For this purpose, the evaluation unit, preferably, comprises a database comprising values of a calibration curve. In this case, the evaluation unit generates an output of raw data in the form of amounts, concentrations or amount ratios that have to be interpreted by the clinician. However, in a more preferred embodiment of the present invention, the evaluation unit further comprises a database with
20 the specific reference amounts disclosed elsewhere in the present application and a computer program which when tangibly embedded on a computer carries out the comparison of the determined amounts and the reference amounts. In an even more preferred embodiment, the evaluation unit furthermore comprises computer program where the comparison is transformed into the desired result according to the method of the
25 present invention. The "desired result" is a diagnosis, prediction, risk stratification or treatment recommendation. Thus, "processing the resulting data" by an evaluation unit comprising such a database and a computer program allows the automatic processing of the raw data generated by the analyzing unit until the point where a diagnosis, prediction, risk stratification or treatment recommendation according to the method of the present
30 invention is possible.

In a preferred embodiment of the present invention, the device further comprises a control unit. Preferably, said control unit comprises a storage device comprising the measuring parameters for the method of the present invention in computer readable form. Said
35 measuring parameters, preferably, comprise, the specific ligand to be used, incubation times and temperatures and the dilution of the sample. Said control unit is linked to the analyzing unit so that the activity of the analyzing unit can be actuated by the output of the

control unit. In addition to this, the control unit is, preferably, linked to the evaluation unit so that the reference amounts for processing the data according to the method of the present invention can be chosen according to the method of the present invention. Because the evaluation unit as well as the control unit, preferably, comprise a microprocessor as well as a computer readable storage device, both units can be merged into a single unit. Therefore, in a preferred embodiment of the present invention, the control unit is integrated into the evaluation unit.

In another preferred embodiment of the present invention, the device comprises a manual, further to or in place of the control unit. The manual may comprise the following: the measuring parameters for the method of the present invention, comprising the specific ligand to be used, incubation times and temperatures and the dilution of the sample, the reference amounts according to the method of the present invention.

Moreover, the present invention relates to a kit of reagents suitable for use in the analyzing unit of the present invention. Preferably, said kit of reagents comprises a specific ligand for the markers of the present invention. In a preferred embodiment of the present invention the kit of reagents further comprises at least one calibration sample containing a known amount of the marker(s) of the present invention. In another preferred embodiment of the present invention, the kit additionally comprises calibration curves, reference amounts and algorithms for practicing the method of the present invention. Preferably, said information is provided in computer readable form so that it can be uploaded to the evaluation unit and/or control unit. The information may also be provided as a user manual as described above.

The present invention also relates to the use of a device for determining the amount of sFIT-1 or a variant thereof in a sample of a patient, comprising an analyzing unit for determining the amount of sFIT-1 or a variant thereof in a sample of the patient and an evaluation unit for processing the resulting data for: diagnosing a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, in a patient, monitoring the course of a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, in a patient or recommending a therapy for a patient suffering from a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia.

In a preferred embodiment, the invention relates to the use of a device further comprising an analyzing unit for determining the amount of a cardiac troponin or a variant thereof in a

sample of the patient for diagnosing if a complication of pulmonary embolism in a patient is or myocardial ischemia within systemic ischemia.

5 The present invention also relates to the use of: an antibody against sFlt-1 or a variant thereof for: diagnosing a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, in a patient, monitoring the course of a complication of PE, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, in a patient or recommending a therapy for a patient suffering from a complication of PE, preferably of systemic ischemia or myocardial ischemia within
10 systemic ischemia.

In a preferred embodiment of the invention an antibody against a cardiac troponin or a variant thereof is additionally used for diagnosing if a complication of pulmonary embolism in a patient is systemic ischemia or myocardial ischemia within systemic
15 ischemia, wherein

- a) an increased amount of sFlt-1 and a non-increased amount of the cardiac troponin relative to the respective reference amounts indicate the presence of systemic ischemia; and
20
- b) increased amounts of both sFlt-1 and the cardiac troponin relative to the respective reference amounts indicate the presence of or myocardial ischemia within systemic ischemia.

25 The present invention also relates to the use of: sFlt-1 or a variant thereof for: diagnosing a complication of PE, preferably systemic ischemia, in a patient, monitoring the course of a complication of PE, preferably of systemic ischemia, in a patient or recommending a therapy for a patient suffering from a complication of PE, preferably of systemic ischemia.

30 In a preferred embodiment of the invention a cardiac troponin or a variant thereof is additionally used for diagnosing if a complication of pulmonary embolism in a patient is or myocardial ischemia within systemic ischemia.

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

- 35
- a) determining the amount of a cardiac troponin or a variant thereof in a sample of the patient;

b) comparing the measured amount of the cardiac troponin or the variant thereof to a reference amount;

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

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

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

15

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

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

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

25 whereby the results obtained in step b) indicate whether the patient 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 patient; b) comparing
30 the measured amount of the natriuretic peptide or the variant thereof to a reference amount; and c) diagnosing whether the patient 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.

35

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

5 **Example 1:**

Measuring methods

sFlt-1 was determined with a sFlt-1 immunoassay to be used with the Elecsys and COBAS analyzers from Roche Diagnostics, Mannheim, Germany. The assay is based on the sandwich principle and comprises two monoclonal sFlt-1 specific antibodies. The first of
10 these is biotinylated and the second one is labeled with a Tris(2,2'-bipyridyl)ruthenium(TT)-complex. In a first incubation step both antibodies are incubated with the human serum sample. A sandwich complex comprising sFlt-1 and the two different antibodies is formed. In a next incubation step streptavidin-coated beads are
15 added to this complex. The beads bind to the sandwich complexes. The reaction mixture is then aspirated into a measuring cell where the beads are magnetically captured on the surface of an electrode. The application of a voltage then induces a chemiluminescent emission from the ruthenium complex which is measured by a photomultiplier. The amount of light is dependent on the amount of sandwich complexes on the electrode. The
20 test is capable of measuring sFlt-1-concentrations from 10 to 85000 pg/ml.

Troponin T was measured with an immunoassay (hs troponin T, product number 05092744, from December 2008) by Roche Diagnostics to be used with the above described systems. The test is capable of measuring troponin T-concentrations from 1 to
25 10000 pg/ml.

Samples for the determination of the above described biomarkers were taken at presentation of the patient (1st sample, 0 hours), 12 hours after presentation (2nd sample), 24 hours after presentation (3rd sample) and 48 hours after presentation (4th sample).
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Example 2: Reference amounts

2.1 Healthy subjects

sFlt-1 and troponin T were determined in 149 clinically healthy subjects (52 males, mean age 40 years, range 20 to 52 years; 97 females, mean age 41 years, range 18 to 56 years). The subjects had a normal systemic blood pressure in repeated measurements, a normal
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electrocardiogram, no diabetes mellitus and no history of cardiac disease or other diseases that would have put them at increased risk of cardiac disorders. The data of these subjects are shown in table 1.

Table 1: Overview over the healthy individuals		
Percentile	sFIT-1 [pg/ml]	Troponin T [pg/ml]
25 th	65	0
50 th	71	0
75 th	78	0
95 th	88	3

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As can be seen, the amount of sFIT-1 in healthy subjects does not exceed 100 pg/ml. Taken together with the results of example 2, this shows 100 pg/ml to be a suitable reference amount (see table 2A) for ruling out a complication of pulmonary embolism. If a complication of pulmonary embolism can be ruled out, the patient is not need of a close monitoring for hemodynamic instability and/or aggressive treatments such as embolectomy or thrombolysis.

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2.2 Patients suffering from stable coronary artery disease associated with heart failure

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sFIT-1 and troponin T were determined in 42 patients suffering from coronary artery disease associated with stable heart failure, i.e. without acute cardiac events in the last 3 months as established by clinical signs and symptoms. For the last month this finding was confirmed by laboratory evidence. All patients had a left ventricular ejection fraction below 40 %. In order to exclude acute cardiac events, the patients were monitored for 4 weeks prior to study entry with respect to ECG, echocardiography, clinical signs and symptoms, drug therapy (including compliance of drug therapy, changes of drug and dose were not allowed). In addition to this, the patients did not change their body weight by more than 2 kg during the observation period. Samples were obtained before follow-up, and during follow up at 2 weeks, months and 3 months. The medians of sFIT-1 and troponin T are shown in tables 2A and 2B. The amounts of sFIT-1 and troponin T were stable during the observation period.

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Table 2A: sFIT-1 [pg/ml] in patients suffering from stable heart failure			
Percentile	25 th	50 th	75 th
Baseline	68	77	87
2 weeks	65	76	81
1 months	61	74	87
3 months	66	77	88

Table 2B: Troponin T [pg/ml] in patients suffering from stable heart failure			
Percentile	25 th	50 th	75 th
Baseline	7	14	20
2 weeks	6	11	18
1 months	6	12	18
3 months	6	11	19

As can be seen from table 2A patients with stable coronary artery disease have sFIT-1 levels below 100 pg/ml. Hence, this amount of sFIT-1 is a suitable reference amount for ruling out a complication of pulmonary embolism in a patient suffering from pulmonary embolism.

The 75th percentile of troponin T did not exceed 20 pg/ml at any time (see tab. B). Thus, this amount of troponin T is a suitable reference amount for ruling in myocardial ischemia in a patient.

Increased amounts of sFIT-1 as compared to the reference amount indicate that the patient suffers from ischemia, i.e. that the oxygen supply of the patient's body is insufficient. Myocardial necrosis as indicated by a measured troponin amount higher than the reference amount is the consequence of myocardial ischemia. In order to be certain that said myocardial ischemia is a complication of pulmonary embolism the presence of the acute coronary syndrome, myocardial infarction and unstable angina pectoris have to be excluded.

If lower reference amounts are chosen, the proportion of false-positive diagnoses will increase because amounts lower than 20 pg/ml troponin T are frequently found in patients suffering from stable coronary artery disease (see also example 2.1 for troponin T in healthy individuals).

Example 3: Patients suffering from pulmonary embolism.

A total of 19 patients with pulmonary embolism were included into the study. Pulmonary embolism was confirmed by multi slice computed tomography. Blood samples were obtained at presentation (sample 1), at 12 h (sample 2), 24 h (sample 3) and 48 h (sample 4) after presentation.

Systolic pulmonary pressure was assessed in the majority of patients and considered abnormal when it exceeded 25 mm Hg. Increased systolic pulmonary pressure indicates that the blood flow through the lung is impaired or obstructed. Acutely impaired blood flow through the lungs associated with increased systolic pulmonary pressure may result in right ventricular dilation. Additionally, the majority of patients received an echocardiography to assess right ventricular function which was graded as normal (0) or abnormal (1). Patients with a basal right ventricular (RV) diameter of 2.0 cm to 2.8 cm were considered as having normal RV function. Patients with a RV diameter exceeding 2.8 cm were considered as having impaired RV function. Impaired RV function typically results from acute increases of systolic PA pressure.

Systolic PA pressure and right ventricular function were determined once 3 to 6 hours after presentation.

Patients were placed according to their sFIT-1 levels into 4 groups: not increased sFIT-1 at any time (Table 3A), initially increased sFIT-1 levels which decreased thereafter (Table 3B), sustained increases of sFIT-1 levels (Table 3C) and intermittently increased sFIT-1 levels (Table 3D).

Table 3A: Patients with PE and without ischemia						
Patient	Sample	Troponin T [pg/ml]	sFIT-1 [pg/ml]	NT- proBNP [pg/ml]	Right ventricular function	Pulmonary artery pressure [mm Hg]
193	1	0	65	26	1	39
216	1	80	70	964	1	35
216	2	24	50	900		
223	1	0	72	68	1	nd*

223	2	0	65	42		
223	3	0	63	42		

* not determined

As depicted in Table 3 A, all patients had sFIT1 levels within the normal range throughout the observation period. They had normal NT-proBNP and troponin T levels (except patient 216) although echocardiography indicated abnormal right ventricular function in all patients. Patient 216 had elevated NT-proBNP (964 pg/ml) and troponin T levels due to concomitant coronary artery disease (i.e. the increased troponin T did not result from a complication of PE). All patients with normal sFIT1 levels were clinically stable. In the absence of coronary artery disease normal amounts of troponin T, sFIT-1 and NT-pro BNP levels indicate an uncomplicated pulmonary embolism in spite of impaired right ventricular function and increased systolic pulmonary pressure. Patients with these characteristics are candidates for early discharge. If NT-proBNP and troponin T are elevated, but the amount of sFIT1 is below 100 pg/ml, pre-existing coronary artery disease requires consideration as the cause of troponin T and NT-proBNP elevation instead of pulmonary embolism.

Table 3B: Patients with PE and with initial ischemia							
Patient	Sample	Troponin T [pg/ml]	sFIT-1 [pg/ml]	Decrease of sFIT-1 [%]	NT-proBNP [pg/ml]	Right ventricular function	Pulmonary artery pressure [mm Hg]
31	1	0	2253		67	0	nd*
31	2	0	267	88	53		
31	3	12	151		98		
176	1	110	446		61	1	45
176	2	41	240	46	1005		
176	3	19	44		392		
176	4	26	47		227		
187	1	20	1166		1786	0	37
187	2	17	454	61	1196		
187	3	6	292		442		

224	1	0	2170		412	1	39
224	2	0	290	87	262		
224	3	0	54		282		
230	1	28	4028		2287	1	45
230	2	16	339	92	2812		
230	3	9	355		1212		
268	1	55	3228		216	1	49
268	2	14	60	98	2090		

* not determined

Patients with initially high sFIT-1 levels as shown in table 3B were marginally stable (systolic blood pressure above 90 mmHg but below 110 mmHg). All but 2 patients (patients 31 and 187) had impaired right ventricular function. All but one patient (patient 224) had increased troponin T levels, which generally decreased over time.

In all patients the sFIT-1 levels fell by more than 40 % between presentation and 12 hours after presentation. In all patients except for patient 230 sFIT-1 continued to decrease in the samples taken after that point in time. In patient 268 sFIT-1 was not determined at 24 hours or 48 hours.

None of patients showed a troponin T amount higher than 26 pg/ml in the last sample taken indicating that at this point in time no significant myocardial ischemia was present.

The results show that patients with diagnosed PE and ischemia indicated by increased sFIT-1 levels may or may not have increased troponin T levels. This means that ischemia was present, as indicated by elevated sFIT-1 levels. The troponin T levels provide additional diagnostic information allowing to differentiate systemic ischemia (low troponin levels) from myocardial ischemia (increased troponin levels).

Table 3C: Patients with PE and with sustained ischemia

Patient	Sample	Troponin T [pg/ml]	sFIT-1 [pg/ml]	NT- proBNP [pg/ml]	Right ventricular function	Pulmonary artery pressure [mm Hg]
66	1	53	119	1020	0	30

66	2	10	374	1262		
66	3	19	308	306		
68	1	58	393	902	1	40
68	2	305	339	6101		
68	3	82	782	16571		
68	4	45	283	8109		
202	1	42	199	11634	1	55
202	2	54	864	15127		
235	1	5	288	133	0	23
235	2	6	273	126		
235	3	4	134	119		

* not determined

The patients shown in table 3C had sustained elevations of sFIT-1 levels, i.e. they had sFIT-1 levels higher than 100 pg/ml at presentation and the sFit-1 levels did not fall by at least 40% in the period between presentation and 12 hours after presentation. Thus, this group of patients is characterized by enduring systemic ischemia.. In some cases the sFit1 levels even rose over time. They had a systolic blood pressure of about 100 mg Hg, i.e. at the lower end of the normal range. Hence the observed systemic ischemia most likely results from underperfusion due to low systemic blood pressure. sFIT-1 as an indicator of ischemia did not correlate to troponin T levels as indicators of myocardial ischemia. The data of tables 3A to 3C show that the absence of ischemia as indicated by low amounts of sFIT-1 correlates with the absence of myocardial ischemia as indicated by low troponin T levels. Increased amounts of sFIT-1 may or may not correlate with increased troponin T levels. Hence, sFIT-1 and troponin T provide independent and different diagnostic information for patients suffering from pulmonary embolism. However, the patients with initial ischemia and a strong decrease of sFIT-1 within the first 12 hours after presentation showed stable or further decreasing amounts of sFIT-1 in the samples taken 24 h and 48 h after presentation (see table 3B). Thus, a strong initial decrease of sFIT-1 predicts that systemic ischemia in the patient will not worsen subsequently or may even resolve. Similarly, troponin T levels in most patients with a strong initial decrease of sFIT-1 were normal (20 pg/ml) or almost normal (26 pg/ml in patient 176) 24 h and 48 h after

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presentation. Thus, a strong initial decrease of sFIT-1 predicts that future onset of substantial myocardial necrosis unlikely in the patient.

Table 3D: Patients with PE and with intermittent ischemia						
Patient	Sample	Troponin T [pg/ml]	sFIT-1 [pg/ml]	NT- proBNP [pg/ml]	Right ventricular function	Pulmonary artery pressure [mm Hg]
29	1	63	91	2203	nd*	nd
29	2	70	846	2456		
29	3	59	246	965		
29	4	33	383	301		
30	1	20	60	145	0	nd
30	2	19	309	113		
30	3	23	284	48		
52	1	7	94	57	0	20
52	2	0	182	60		
67	1	0	46	679	0	45
67	2	1	422	1747		
67	3	6	261	1061		
69	1	2	57	820	1	40
69	2	6	279	288		
69	3	4	45	92		
186	1	4678	5093	3408	nd*	nd

* not determined

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The patients shown in table 3D suffered from intermittent ischemia characterized by normal levels of sFIT-1 (i.e. below 100 pg/ml) at presentation followed by an increase to levels above 100 pg/ml indicating development of ischemia. While troponin T levels increased parallel to sFIT-1 in some patients (patients 29 and 30), this trend could not be observed in the other patients belonging to this group.. This indicates that increases of

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sFIT-1 in the patients without increases of troponin T reflect systemic ischemia rather than myocardial ischemia. One patient had an impaired right ventricular function (patient 69). Patients 67 and 69 had a temporary decrease in systolic blood pressure at the time of increase of sFIT1 levels. It appears likely that these patients suffered from a recurrent pulmonary embolism during the course of the study.

5 Patient 186 suffered from a massive pulmonary embolism associated with massive ischemia and cardiac necrosis. This patient died.

Conclusion:

10 In most patients a PA pressure above 39 mm Hg was associated with impaired right ventricular function. Given the relationship between acute PA hypertension and right ventricular heart failure, this finding is not surprising. The lacking correlation between the former two parameters and the amount of sFIT-1 demonstrate that sFIT-1 offers diagnostic information which is independent of and different from right ventricular function.

15 Similarly, sFIT-1 levels do not correlate with NT-proBNP levels. Hence, both markers cover different clinical aspects of pulmonary embolism.

The combined determination of sFIT-1 and troponin T can be used for refining the assessment of a complication of pulmonary embolism. sFIT-1 shows the presence/absence of systemic ischemia while troponin T indicates whether myocardial ischemia is present in addition to systemic ischemia.

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Patients with normal sFIT-1 and troponin T levels (see table 3A) are clinically stable. They do not require close monitoring and specific treatment and are thus candidates for early discharge.

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Patients with elevated sFIT-1 levels and normal troponin T at presentation are considered clinically unstable, they require intense monitoring for blood pressure and circulatory support, especially if systolic blood pressure falls below 90 mm Hg at more than one occasion. A patient with increased troponin T in addition to increased sFIT-1 at presentation suffers from systemic ischemia and myocardial damage, such a patient requires intense monitoring at the intensive care unit and circulatory support if systolic blood pressure falls below 90 mmHg at more than one occasion. He is a candidate for embolectomy.

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In patients in whom sFIT-1 decreases on follow up show improvement in their cardiac function even if troponin T and NT-pro BNP remain unchanged, the decision of embolectomy can be delayed if they remain clinically stable. If the decrease of sFIT-1 between presentation and 12 h after presentation is strong ($> 40\%$), there is a high probability that the condition of the patient will not worsen in the next 36 hours (see table 3B).

Patients with continuously elevated sFIT1 (table 3C) or increasing sFIT-1 (table 3D) as a sign for persistent or even newly beginning systemic ischemia and hemodynamic instability are candidates for circulatory support and embolectomy, especially if troponin T (and NT-proBNP as additional marker) remain persistently elevated or if these markers are increasing.

Because of its rapid response to ischemia and its short half-life (in contrast to troponin T and NT-pro BNP and imaging methods) sFIT-1 provides up timely information on the clinical state of the patient. As an indicator of complications of PE it is superior to blood pressure because decreasing blood pressure as such does not indicate whether the patient already suffers from ischemia (being the actual danger associated with low blood pressure). In contrast to this, sFIT-1 measures the consequences of hemodynamic stability or instability, i.e. sufficient oxygen supply of the body or ischemia. Moreover, blood pressure frequently changes within short periods. Hence, a single measurement of blood pressure gives no information about the oxygen supply because a low blood pressure at one measurement may be an outlier. sFIT-1 integrates the consequences of the hemodynamic state of the patient over time. Thus sFIT-1 provides important additional information to conventional markers and imaging methods currently in practice or under consideration.

Claims

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1. A method for diagnosing systemic ischemia, in a patient suffering from or suspected to suffer from pulmonary embolism comprising the steps of

- 10 a) determining the amount of sFIT-1 or a variant thereof in a sample of the patient;
b) comparing the amount of sFIT-1 or the variant thereof determined in step a) to a reference amount; and
c) wherein an increased amount of sFIT-1 relative to the reference amount indicates the presence of systemic ischemia in the patient.

15 2. The method of claim 1 comprising the further steps of

- a1) determining the amount of a cardiac troponin or a variant thereof in a sample of the patient;
b1) comparing the amount of the cardiac troponin or the variant thereof determined in
20 step a1) to a reference amount; and
c1) wherein it is diagnosed whether the patients suffers from myocardial ischemia within systemic ischemia, based on the comparison of step b1).

3. The method of claim 2, wherein

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- i. the absence of myocardial ischemia and systemic ischemia indicates that the patient is eligible for an early discharge;
ii. the presence of systemic ischemia and the absence of myocardial ischemia indicates that the blood pressure of the patient should be monitored closely;
30 iii. the presence of both myocardial and systemic ischemia indicates that the patient requires close monitoring in an intensive care unit and that embolectomy or thrombolysis should be considered;
iv. the presence of myocardial ischemia without systemic ischemia indicates that myocardial ischemia is caused by a disease different from pulmonary
35 embolism.

4. A method for monitoring a patient suffering from a complication of pulmonary embolism, preferably systemic ischemia or myocardial ischemia within systemic ischemia, comprising the steps of
 - 5 a) determining the amount of sFIT-1 or a variant thereof and optionally of a cardiac troponin or a variant thereof in a first sample and at least a second sample of the patient;
 - b) comparing the amount of sFIT-1 or the variant thereof and optionally of a cardiac troponin or a variant thereof determined in the second sample to the amount of
10 sFIT-1 or the variant thereof and optionally of a cardiac troponin or a variant thereof determined in the first sample; and
 - c) wherein it is assessed whether the complication of pulmonary embolism, preferably systemic ischemia, optionally myocardial ischemia within systemic ischemia, in the patient ameliorates or deteriorates based on the comparison of step b).
- 15 5. The method of claim 4, wherein a decrease of the amount of sFIT-1 or the variant thereof relative to the reference amount is indicative of an amelioration of the complication of pulmonary embolism and wherein an increase of the amount of sFIT-1 or the variant thereof relative to the reference amount is indicative of an deterioration
20 of the complication of pulmonary embolism.
6. The method of claim 5, wherein the patient receives anticoagulation therapy.
7. The method of claim 5 or 6, wherein a higher amount of sFIT-1 or the variant thereof
25 in the second sample as compared to the first sample indicates the need for insertion of a vena cava filter, thrombolysis or embolectomy.
8. A method for predicting the course of pulmonary embolism, preferably the course of a complication of pulmonary embolism, preferably systemic ischemia, in a patient
30 suffering from or suspected to suffer from pulmonary embolism comprising the steps of
 - a) determining the amount of sFIT-1 or a variant thereof and optionally of a cardiac troponin or a variant thereof in a first and second sample of a patient;
 - 35 b) comparing the amount of sFIT-1 or the variant thereof and optionally of the cardiac troponin or a variant thereof determined in the second sample to the amount of

sFIT-1 or the variant thereof and optionally of the cardiac troponin or a variant thereof determined in the first sample; and

- 5 c) wherein the course of pulmonary embolism preferably the course of a complication of pulmonary embolism, preferably systemic ischemia, optionally myocardial ischemia within systemic ischemia, in the patient is predicted based on the comparison of step b).

9. The method of claim 8, wherein the interval between isolation of the first and second sample is at least 4h.

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10. The method of claim 8 or 9, wherein a decrease of the amount sFIT-1 or the variant thereof of at least 40 % indicates that the complication of pulmonary embolism, preferably myocardial and/or systemic ischemia, will not deteriorate in the future.

15 11. A method for recommending a therapy of a complication of pulmonary embolism, preferably of systemic ischemia, in a patient suffering from or suspected to suffer from pulmonary embolism comprising the steps of

- 20 a) determining the amount of sFIT-1 or a variant thereof and, optionally, of a cardiac troponin or a variant thereof, in a sample of the patient;
- b) comparing the amount of sFIT-1 or the variant thereof and, optionally, of the cardiac troponin or a variant thereof, determined in step a) to a reference amount; and
- 25 c) wherein the therapy of the complication of pulmonary embolism, preferably of systemic ischemia or myocardial ischemia within systemic ischemia, in the patient is recommended based on the comparison of step b).

30 12. A method for ruling in/ruling out a complication of pulmonary embolism, preferably of systemic ischemia, in a patient suffering from or suspected to suffer from pulmonary embolism comprising the steps of

- a) determining the amount of sFIT-1 or a variant thereof and, optionally, of a cardiac troponin or a variant thereof, in a sample of the patient;
- 35 b) comparing the amount of sFIT-1 or the variant thereof and, optionally, of the cardiac troponin or a variant thereof, determined in step a) to a reference amount; and

- c) wherein ruling in/ruling out of the complication of pulmonary embolism, preferably of systemic ischemia, is carried out based on the comparison of step b).

13. A device for carrying out the methods of the present invention comprising

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- a) an analyzing unit for determining the amount of sFlt-1 or a variant thereof in a sample of the patient; and
- b) an evaluation unit for processing the resulting data.

- 10 14. Use of sFlt-1 or a variant thereof or of an anti-sFlt-1 antibody or an anti-sFlt1-variant antibody or a fragment of the aforementioned antibodies for diagnosing a complication of pulmonary embolism, preferably myocardial and/or systemic ischemia, in a patient.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/057783

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/48 G01N33/68
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q 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 practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/144553 A2 (TUFTS MEDICAL CT INC [US]; KAPUR NAVIN K [US]; KARAS RICHARD [US]; MEN) 16 December 2010 (2010-12-16) claims 1-2, 7 page 1, lines 5-6 page 1, lines 19-20 page 2, lines 7-33 page 3, lines 11-20 page 5, lines 1-4 page 6, lines 21-30 page 7, line 1 - page 8, line 7 page 8, lines 27-29 page 10, lines 17-20 ----- -/-	1-4, 7-14

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/057783

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/057783

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