Title: LTBP2 AS A BIOMARKER FOR LUNG INJURY

Abstract: The application discloses LTBP2 as a new biomarker for pulmonary injury; methods for the diagnosis, prediction, prognosis and/or monitoring of said pulmonary injury based on measuring said biomarker; and kits and devices for measuring said biomarker and/or performing said methods.
FIELD OF THE INVENTION

The invention relates to protein- and/or peptide-based biomarkers useful for the diagnosis, prediction, prognosis and/or monitoring of diseases and conditions in subjects, in particular pulmonary injury and mortality, in particular due to inflammation; and to related methods, kits and devices.

BACKGROUND OF THE INVENTION

In many diseases and conditions, a favourable outcome of prophylactic and/or therapeutic treatments is strongly correlated with early and/or accurate diagnosis, prediction, prognosis and/or monitoring of a disease or condition. Therefore, there exists a continuous need for additional and preferably improved manners for early and/or accurate diagnosis, prediction, prognosis and/or monitoring of diseases and conditions to guide the treatment choices.

A major cause of human death is represented by pulmonary diseases or complications such as for instance chronic obstructive pulmonary disease (COPD) or pneumonia, sometimes leading to irreversible pulmonary injury and death. Patients with pulmonary inflammation often present themselves in emergency departments (ED) with symptoms such as one or more of cough, shortness of breath or increased respiratory rate. Unfortunately, these symptoms are neither sensitive nor specific and are related to a whole array of possible underlying pathologies ranging from anxiety and hyperventilation to life-threatening pulmonary, cardiac or metabolic causes thereby preventing rapid and accurate triage and risk-stratification.

Natriuretic peptides (NP) have been recognized as quantitative biomarkers of cardiac hemodynamic stress in the early diagnosis and risk-stratification of dyspneic patients by allowing accurately identifying patients experiencing increased cardiac stress. However, biomarkers identifying pulmonary stress and accurately detecting patients at highest risk of pulmonary complications resulting ultimately in increased mortality risk are currently still missing.

The present invention addresses the above needs in the art by identifying biomarkers for pulmonary inflammation and providing uses thereof.

SUMMARY OF THE INVENTION

Having conducted extensive experiments and tests, the inventors have found that levels of latent transforming growth factor beta binding protein 2 (LTBP2) are closely indicative of
mortality in subjects presenting themselves with dyspnea. Especially mortality due to lung injury is highly correlated to LTBP2 levels in the blood of the subject. In particular, in clinical samples from 299 patients LTBP2 showed a significant association with several tested clinical parameters related to pulmonary injury.

Further, the median area under the ROC curve (AUC) value of LTBP2 ("ROC" stands for receiver operating characteristic) for discriminating subjects with increased mortality due to pulmonary dysfunction, is 0.95 which is highly relevant. The AUC value is a combined measure of sensitivity and specificity and a higher AUC value (i.e., approaching 1) in general indicates an improved performance of the test.

Accordingly, the inventors have identified LTBP2 as a new biomarker advantageous for evaluating pulmonary dysfunction, especially of predicting unfavourable lung related complications and/or mortality due to lung injury, in particular pulmonary inflammation and pulmonary death.

Further provided is a method for determining or predicting pulmonary dysfunction in a subject comprising measuring the quantity of LTBP2 in a sample from said subject. Particularly provided is a method for the diagnosis, prediction, prognosis and/or monitoring of lung dysfunction in a subject comprising measuring LTBP2 levels in a sample from said subject. As used throughout this specification, measuring the levels of LTBP2 and/or other biomarker(s) in a sample from a subject may particularly denote that the examination phase of a method comprises measuring the quantity of LTBP2 and/or other biomarker(s) in the sample from the subject. One understands that methods of diagnosis, prediction, prognosis and/or monitoring of diseases and conditions generally comprise an examination phase in which data is collected from and/or about the subject.

In an embodiment, a method for the diagnosis, prediction and/or prognosis of pulmonary dysfunction, preferably due to inflammation, comprises the steps of: (i) measuring the quantity of LTBP2 in a sample from the subject; (ii) comparing the quantity of LTBP2 measured in (i) with a reference value of the quantity of LTBP2, said reference value representing a known diagnosis, prediction and/or prognosis of pulmonary dysfunction or normal lung function; (iii) finding a deviation or no deviation of the quantity of LTBP2 measured in (i) from the reference value; and (iv) attributing said finding of deviation or no deviation to a particular diagnosis, prediction and/or prognosis of pulmonary dysfunction or normal lung function in the subject.

The method for the diagnosis, prediction and/or prognosis of pulmonary dysfunction, preferably due to inflammation, and in particular such method comprising steps (i) to (iv) as set forth in the previous paragraph, may be performed for a subject at two or more successive time points and the respective outcomes at said successive time points may be compared, whereby the presence or absence of a change between the diagnosis, prediction and/or
prognosis of pulmonary dysfunction at said successive time points is determined. The method thus allows monitoring a change in the diagnosis, prediction and/or prognosis of pulmonary dysfunction in a subject over time.

In an embodiment, a method for monitoring pulmonary dysfunction comprises the steps of: (i) measuring the quantity of LTBP2 in samples from a subject from two or more successive time points; (ii) comparing the quantity of LTBP2 between the samples as measured in (i); (iii) finding a deviation or no deviation of the quantity of LTBP2 between the samples as compared in (ii); and (iv) attributing said finding of deviation or no deviation to a change in pulmonary function or pulmonary dysfunction in the subject between the two or more successive time points. The method thus allows monitoring pulmonary dysfunction or pulmonary function in a subject over time.

Throughout the present disclosure, methods suitable for monitoring any one condition or disease as taught herein can *inter alia* allow to predict the occurrence of the condition or disease, or to monitor the progression, aggravation, alleviation or recurrence of the condition or disease, or response to treatment or to other external or internal factors, situations or stressors, *etc.* Advantageously, monitoring methods as taught herein may be applied in the course of a medical treatment of the subject, preferably medical treatment aimed at alleviating the so-monitored condition or disease. Such monitoring may be comprised, *e.g.*, in decision making whether a patient may be discharged, needs a change in treatment or needs further hospitalisation or treatment.

Similarly, throughout the present disclosure, methods suitable for prognosticating any one condition or disease as taught herein can *inter alia* allow to prognosticate the occurrence of the condition or disease, or to prognosticate the progression, aggravation, alleviation or recurrence of the condition or disease, or response to treatment or to other external or internal factors, situations or stressors, *etc.* may allow to prognosticate.

As shown in the experimental section, clinical parameters typifying pulmonary dysfunction, *etc.* associate with elevated levels of LTBP2. In particular said pulmonary dysfunction can be caused by inflammation, either due to local lung inflammation or due to inflammatory factors or agents originating from other tissues such as *e.g.* the kidney. Consequently, prediction or diagnosis of pulmonary dysfunction or a poor prognosis of pulmonary dysfunction can in particular be associated with an elevated level of LTBP2.

For example but without limitation, an elevated quantity (i.e., a deviation) of LTBP2 in a sample from a subject compared to a reference value representing the prediction or diagnosis of no pulmonary dysfunction (i.e., normal pulmonary function) or representing a good prognosis for pulmonary dysfunction respectively indicates that the subject has or is at risk of developing pulmonary dysfunction or indicates a poor prognosis for pulmonary dysfunction in
the subject (such as, e.g., a prognosis that the pulmonary dysfunction patient will progress towards permanent or irreversible lung fibrosis, or lung injury, eventually leading to pulmonary death).

In an aspect, the present invention thus provides for the use of latent transforming growth factor beta binding protein 2 (LTBP2) or a fragment thereof as a blood biomarker for the diagnosis, prediction, prognosis and/or monitoring of pulmonary dysfunction in a subject, particularly pulmonary injury leading to increased mortality in said subject.

The degree of pulmonary dysfunction or pulmonary injury can be assessed as being:

(i) no injury,

(ii) pulmonary injury with reversible damage which can lead to complications when left untreated, or

(iii) pulmonary injury with potential irreversible or irreparable physiological damage, morbidity or mortality.

In a further aspect, the present invention provides the use of LTBP2 or a fragment thereof as a blood biomarker for assessing the risk of developing severe pulmonary complications such as severe chronic obstructive pulmonary disease (COPD), pneumonia, or pulmonary death.

In a preferred embodiment, the LTBP2 biomarker is used in combination with one or more kidney derived markers selected from the group comprising: creatinine, cystatin C, NGAL, beta-trace protein, kidney injury molecule 1, and interleukin-18 (IL-18), and/or one or more other biomarkers selected from the group comprising: proinflammatory cytokines, interferon gamma, interleukine-2 (IL-2), interleukine-10 (IL-10), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor-beta (TGF-beta), interleukine-8 (IL-8), interleukine-6 (IL-6), interleukine-18 (IL-18), macrophage inflammatory protein (MIP)-2, monocyte chemoattractant protein (MCP)-1, interleukine-1 beta (IL-1beta), interleukine-1 alpha (IL-1 alfa), tumor necrosis factor-alpha (TNF-alfa), serum amyloid A (SAA), fractalkine (CX3CL1), C-reactive protein (CRP), procalcitonin (PCT), and white bloodcell count.

Alternatively the LTBP2 biomarker may be used in combination with one or more natriuretic peptides selected from BNP, proBNP and NTproBNP as a biomarker, or with markers indicative of sepsis such as procalcitonin, lactate, or CRP.

Furthermore, the LTBP2 biomarker can be used in combination with determining clinical history, physical examination, electrocardiogram, pulse oximetry, blood tests, chest X-ray, echocardiography, pulmonary function tests, computer tomography (CT)-angiography, and/or thoracic impedance.

Further markers indicating a reduction of pulmonary inflammation in a subject can be used in combination with the LTBP2 biomarker.
As taught herein, the level of LTBP2, such as for example the LTBP2 concentration in blood, serum, plasma and/or urine, correlates with the degree of lung injury, particularly due to inflammation of the lung.

Also disclosed is a method to determine whether a subject is or is not (such as, for example, still is, or is no longer) in need of a therapy to treat pulmonary dysfunction, comprising: (i) measuring the quantity of LTBP2 in the sample from the subject; (ii) comparing the quantity of LTBP2 measured in (i) with a reference value of the quantity of LTBP2, said reference value representing a known diagnosis, prediction and/or prognosis of pulmonary dysfunction or normal lung function; (iii) finding a deviation or no deviation of the quantity of LTBP2 measured in (i) from said reference value; (iv) inferring from said finding the presence or absence of a need for a therapy to treat pulmonary dysfunction. A therapy may be particularly indicated where steps (i) to (iii) allow for a conclusion that the subject has or is at risk of having pulmonary dysfunction or has a poor prognosis for pulmonary dysfunction, such as for example but without limitation, where the quantity of LTBP2 in the sample from the subject is elevated (i.e., a deviation) compared to a reference value representing the prediction or diagnosis of no pulmonary dysfunction (i.e., normal lung function). Without limitation, a patient having pulmonary dysfunction upon admission to or during stay in a medical care centre may be tested as taught herein for the necessity of initiating or continuing a treatment of said pulmonary dysfunction, and may be discharged when such treatment is no longer needed or is needed only to a given limited extent.

Exemplary therapies for pulmonary dysfunction encompass without limitation mechanical ventilation, diuresis or fluid restriction, treatment with corticosteroids or nitric oxide (NO) as a pulmonary vasodilator, as demonstrated in the examples, LTBP2 can identify subjects at risk of developing pulmonary complications in a subject population presenting themselves with (acute) dyspnea. Dyspnea (dyspnœa or shortness of breath) is a common and distressing symptom which may be connected to a range of underlying pathologies, such as, e.g., lung inflammation, pneumonia, sepsis, lung cancer, chronic obstructive pulmonary disease (COPD), congestive or acute heart failure, and renal dysfunction. To treat a patient manifesting with dyspnea adequately, the underlying problem needs to be established.

Accordingly, in methods for the diagnosis, prediction, prognosis and/or monitoring of pulmonary dysfunction as taught herein, the subject may present himself with (be manifest with) dyspnea. Preferably, the dyspnea may be acute dyspnea. Said methods may particularly allow to discriminate between (subjects having) dyspnea associated with or caused by pulmonary dysfunction and (subjects having) dyspnea associated with or caused by other conditions.
As also shown in the examples, the inventors have found that LTBP2 levels upon admission in subjects manifesting with acute dyspnea were significantly higher in those subjects who will have died within one year post-admission compared to those subjects who will have remained alive at one year. This distinction was even greater when the patient population was divided based on the cause of death being linked to pulmonary dysfunction or not. Consequently, the inventors have realised LTBP2 as a new biomarker advantageous for predicting or prognosticating mortality in patients with dyspnea, particularly acute dyspnea, in particular due to lung injury or dysfunction, preferably caused by pulmonary inflammation. Said inflammation can be directly in the lung or can be caused by inflammatory factors produced by other organs, such as the kidney or in case of reperfusion injury of the brain or heart.

Hence, provided is also a method for the prediction of mortality in a subject due to lung injury, particularly due to lung inflammation in a subject having dyspnea and/or acute heart failure and/or renal dysfunction, comprising measuring the quantity of LTBP2 in a sample from said subject. Also provided is a method for the prognosis that the pulmonary dysfunction, particularly due to lung inflammation in a subject having dyspnea and/or acute heart failure and/or renal dysfunction, will result in death of the subject, comprising measuring the quantity of LTBP2 in a sample from said subject. Preferably, the dyspnea may be acute dyspnea. Preferably, the renal dysfunction may be chronic renal dysfunction, particularly chronic kidney disease. Without limitation, the dyspnea may be associated with or caused by AHF and/or by renal dysfunction; or the dyspnea may be associated with our caused by conditions other than AHF and renal dysfunction; or the subject may have AHF and/or renal dysfunction without dyspnea symptoms.

In an embodiment, the method for the prediction of mortality in a subject or for the prognosis that the pulmonary dysfunction will result in death of the subject comprises the steps of: (i) measuring the quantity of LTBP2 in a sample from the subject; (ii) comparing the quantity of LTBP2 measured in (i) with a reference value of the quantity of LTBP2, said reference value representing a known prediction or prognosis of mortality; (iii) finding a deviation or no deviation of the quantity of LTBP2 measured in (i) from the reference value; and (iv) attributing said finding of deviation or no deviation to a particular prediction of mortality or prognosis of the pulmonary dysfunction in the subject.

The present methods for the prediction of mortality in a subject or for the prognosis that the pulmonary dysfunction will result in death of the subject may be preferably performed for a subject once the subject presents with or is diagnosed with dyspnea, such as acute dyspnea or dyspnea associated with acute heart failure or renal dysfunction, more preferably upon the initial (first) presentation or diagnosis of said diseases and conditions.
As shown in the experimental section, increased mortality rate due to pulmonary dysfunction, more particularly due to inflammatory events, in populations of dyspneic subjects, subjects with AHF and/or subjects with renal failure is associated with elevated levels of LTBP2. Consequently, prediction of increased mortality in a subject (increased risk or chance of death within a predetermined time interval) due to lung injury or dysfunction or poor prognosis of the pulmonary dysfunction in a subject can in particular be associated with an elevated level of LTBP2.

For example but without limitation, an elevated quantity (i.e., a deviation) of LTBP2 in a sample from a subject compared to a reference value representing the prediction of a given mortality or given prognosis of the pulmonary dysfunction (i.e., a given, such as a normal, risk or chance of death within a predetermined time interval) indicates that the subject has a comparably greater risk of deceasing within said time interval.

Without limitation, mortality may be suitably expressed as the chance of a subject to decease within an interval of for example several months or several years from the time of performing a prediction or prognostication method, e.g., within about 30 days, 2 months, 3 months, 6 months or within about 1 year or within about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9 or about 10 years from the time of performing the prediction or prognosis method.

In an exemplary but non-limiting experiment LTBP2 levels provided satisfactory discrimination between normal and increased mortality in patients presenting themselves with dyspnea, AHF, or renal dysfunction when the time interval for considering the alive vs. dead status was set at 1 year from the time of performing the prediction or prognosis method. Hence, in embodiments mortality may be suitably expressed as the chance of a subject to decease within an interval of between 6 months and 2 years and preferably within 1 year from performing the prediction or prognosis method.

It shall be appreciated that finding of increased mortality risk in a subject can guide therapeutic decisions to treat the subject’s diseases or conditions. This will enable the practitioner to initiate a treatment potentially reducing the mortality risk of the subject due to lung injury or pulmonary death drastically.

Hence, provided are methods for the diagnosis, prediction, prognosis and/or monitoring of any one of: pulmonary dysfunction, particularly by pulmonary inflammation, dyspnea associated with or caused by pulmonary dysfunction, pulmonary inflammation, renal dysfunction or failure, acute heart failure, left ventricular hypertrophy, or cardiac fibrosis and/or increased mortality due to pulmonary dysfunction, particularly by pulmonary inflammation, in a subject comprising measuring LTBP2 levels in a sample from said subject.
In an embodiment, a method for the diagnosis, prediction and/or prognosis of lung injury, particularly due to inflammation comprises the steps of: (i) measuring the quantity of LTBP2 in a sample from the subject; (ii) comparing the quantity of LTBP2 measured in (i) with a reference value of the quantity of LTBP2, said reference value representing a known diagnosis, prediction and/or prognosis of lung injury, particularly due to inflammation; (iii) finding a deviation or no deviation of the quantity of LTBP2 measured in (i) from the reference value; and (iv) attributing said finding of deviation or no deviation to a particular diagnosis, prediction and/or prognosis of lung injury, particularly due to inflammation in the subject.

The method for the diagnosis, prediction and/or prognosis of lung injury, particularly due to inflammation, and in particular such method comprising steps (i) to (iv) as set forth in the previous paragraph, may be performed for a subject at two or more successive time points and the respective outcomes at said successive time points may be compared, whereby the presence or absence of a change between the diagnosis, prediction and/or prognosis of lung injury, at said successive time points is determined. The method thus allows monitoring a change in the diagnosis, prediction and/or prognosis of lung injury, particularly due to inflammation in a subject over time.

In an embodiment, a method for monitoring lung injury, particularly due to inflammation, comprises the steps of: (i) measuring the quantity of LTBP2 in samples from a subject from two or more successive time points; (ii) comparing the quantity of LTBP2 between the samples as measured in (i); (iii) finding a deviation or no deviation of the quantity of LTBP2 between the samples as compared in (ii); and (iv) attributing said finding of deviation or no deviation to a change in lung injury in the subject between the two or more successive time points. The method thus allows assessing the degree of lung injury, particularly due to inflammation and monitoring the disease progression a subject over time.

Prediction or diagnosis of any one of lung injury, particularly due to inflammation or a poor prognosis of lung injury, particularly due to inflammation, can in particular be associated with an elevated level of LTBP2.

For example but without limitation, an elevated quantity (i.e., a deviation) of LTBP2 in a sample from a subject compared to a reference value representing the prediction or diagnosis of no lung injury (i.e., healthy state) or representing a good prognosis for possible alleviation or reversible lung injury, respectively indicates that the subject has or is at risk of having lung injury, particularly due to inflammation or indicates a poor prognosis for lung injury, particularly increased mortality due to pulmonary dysfunction in the subject.

Also provided is a method for assessing or predicting the risk of developing severe pulmonary complications such as severe COPD, pneumonia, or pulmonary death in a subject, wherein
the examination phase of the method comprises measuring the quantity of LTBP2 or a fragment thereof in a blood sample from the subject.

Also disclosed is a method to determine whether a subject is or is not (such as, for example, still is, or is no longer) in need of a therapy to treat lung injury, particularly due to inflammation, comprising: (i) measuring the quantity of LTBP2 in the sample from the subject; (ii) comparing the quantity of LTBP2 measured in (i) with a reference value of the quantity of LTBP2, said reference value representing a known diagnosis, prediction and/or prognosis; (iii) finding a deviation or no deviation of the quantity of LTBP2 measured in (i) from said reference value; (iv) inferring from said finding the presence or absence of a need for a therapy to treat lung injury, particularly due to inflammation.

A therapy may be particularly indicated where steps (i) to (iii) allow for a conclusion that the subject has or is at risk of obtaining serious lung injury, particularly due to inflammation, or has an increased risk of acquiring irreversible damage to the lung, possibly leading to pulmonary death, where the quantity of LTBP2 in the sample from the subject is elevated (i.e., a deviation) compared to a reference value representing the prediction or diagnosis of no lung injury (i.e., healthy state). Without limitation, a patient having impaired pulmonary function, dyspnea, or other lung-related syndromes and disorders upon admission to or during stay in a medical care centre may be tested as taught herein for the necessity of starting or continuing a treatment of said lung injury, and may be discharged when such treatment is no longer needed or is needed only to a given limited extent.

Any one diagnosis, prediction, prognosis and/or monitoring method as taught herein may preferably allow for sensitivity and/or specificity (preferably, sensitivity and specificity) of at least 50%, at least 60%, at least 70% or at least 80%, e.g., ≥ 85% or ≥ 90% or >95%, e.g., between about 80% and 100% or between about 85% and 95%.

Reference throughout this specification to "diseases and/or conditions" encompasses any such diseases and conditions as disclosed herein insofar consistent with the context of such a recitation, in particular but without limitation including diseases or disorders due to pulmonary dysfunction, particularly by pulmonary inflammation; dyspnea associated with or caused by pulmonary dysfunction, pulmonary inflammation, renal dysfunction or failure, acute heart failure, left ventricular hypertrophy, or cardiac fibrosis; and/or increased mortality due to pulmonary dysfunction or failure.

The present methods for the diagnosis, prediction, prognosis and/or monitoring of the diseases or conditions may be used in individuals who have not yet been diagnosed as having such (for example, preventative screening), or who have been diagnosed as having such, or who are suspected of having such (for example, display one or more characteristic symptoms), or who are at risk of developing such (for example, genetic predisposition;
presence of one or more developmental, environmental or behavioural risk factors). The methods may also be used to detect various stages of progression or severity of the diseases or conditions. The methods may also be used to detect response of the diseases or conditions to prophylactic or therapeutic treatments or other interventions. The methods can furthermore be used to help the medical practitioner in deciding upon worsening, status-quo, partial recovery, or complete recovery of the patient from the diseases or conditions, resulting in either further treatment or observation or in discharge of the patient from medical care centre.

The present methods enable the medical practitioner to monitor the disease state or condition of a critically ill patient e.g. presenting himself with dyspnea, by measuring the level of LTBP2 in a sample of the patient. For example, a decrease in LTBP2 level as compared to a prior LTBP2 level (e.g., at the time of the admission to ED) indicates the disease or condition in the subject is improving or has improved, while an increase of the LTBP2 level as compared to a prior LTBP2 level (e.g., at the time of the admission to ED) indicates the disease or condition in the subject has worsened or is worsening. Such worsening could possibly result in the death of the subject.

In view of the present disclosure, also provided are:

- the use of LTBP2 as a marker (biomarker);
- the use of LTBP2 as a marker (biomarker) for any one disease or condition as taught herein;
- the use of LTBP2 for diagnosis, prediction, prognosis and/or monitoring;
- the use of LTBP2 for diagnosis, prediction, prognosis and/or monitoring of any one disease or condition as taught herein;

particularly wherein said condition or disease may be chosen from pulmonary dysfunction, particularly by pulmonary inflammation, dyspnea associated with or caused by pulmonary dysfunction, pulmonary inflammation, renal dysfunction or failure, acute heart failure, left ventricular hypertrophy, or cardiac fibrosis and/or increased mortality due to pulmonary dysfunction, particularly by pulmonary inflammation.

In the present diagnosis, prediction, prognosis and/or monitoring methods the measurement of LTBP2 may also be combined with the assessment of one or more further biomarkers or clinical parameters relevant for the respective diseases and conditions.

Consequently, also disclosed herein are methods, wherein the examination phase of the methods further comprises measuring the presence or absence and/or quantity of one or more such other markers in the sample from the subject. In this respect, any known or yet unknown suitable marker could be used.
A reference throughout this specification to biomarkers "other than LTBP2" or "other biomarkers" generally encompasses such other biomarkers which are useful for the diagnosis, prediction, prognosis and/or monitoring of the diseases and conditions as disclosed herein. By means of example and not limitation, biomarkers useful in evaluating renal dysfunction include creatinine (i.e., serum creatinine clearance), Cystatin C and neutrophil gelatinase-associated lipocalin (NGAL), beta-trace protein, kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18). Further biomarkers useful in the present disclosure include inter alia B-type natriuretic peptide (BNP), pro-B-type natriuretic peptide (proBNP), amino terminal pro-B-type natriuretic peptide (NTproBNP) and C-reactive peptide, and fragments or precursors of any one thereof.

Hence, disclosed is a method for the diagnosis, prediction and/or prognosis of the diseases or conditions as taught herein in a subject comprising the steps: (i) measuring the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers in the sample from the subject; (ii) using the measurements of (i) to establish a subject profile of the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers; (iii) comparing said subject profile of (ii) to a reference profile of the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers, said reference profile representing a known diagnosis, prediction and/or prognosis of the conditions, symptoms and/or parameter values according to the invention; (iv) finding a deviation or no deviation of the subject profile of (ii) from the reference profile; (v) attributing said finding of deviation or no deviation to a particular diagnosis, prediction and/or prognosis of the respective diseases or conditions in the subject.

Applying said method at two or more successive time points allows for monitoring the desired diseases or conditions.

The present methods may employ reference values for the quantity of LTBP2, which may be established according to known procedures previously employed for other biomarkers. Such reference values may be established either within (i.e., constituting a step of) or external to (i.e., not constituting a step of) the methods of the present invention as defined herein. Accordingly, any one of the methods taught herein may comprise a step of establishing a reference value for the quantity of LTBP2, said reference value representing either (a) a prediction or diagnosis of the absence of the diseases or as taught herein or a good prognosis thereof, or (b) a prediction or diagnosis of the diseases or conditions as taught herein or a poor prognosis thereof.

A further aspect provides a method for establishing a reference value for the quantity of LTBP2, said reference value representing:

(a) a prediction or diagnosis of the absence of the diseases or conditions as taught herein, or
(b) a prediction or diagnosis of the diseases or conditions as taught herein or of the risk of obtaining said disease or disorder, comprising:

(i) measuring the quantity of LTBP2 in:

(i a) one or more samples from one or more subjects not having the respective diseases or conditions or not being at risk of having such, or

(ii a) as measured in (i a) as the reference value representing the prediction or diagnosis of the absence of the respective diseases or conditions, or

(ii b) as measured in (i b) as the reference value representing the prediction or diagnosis of the respective diseases or conditions.

The present methods may otherwise employ reference profiles for the quantity of LTBP2 and the presence or absence and/or quantity of one or more other biomarkers, which may be established according to known procedures previously employed for other biomarkers. Such reference profiles may be established either within (i.e., constituting a step of) or external to (i.e., not constituting a step of) the present methods. Accordingly, the methods taught herein may comprise a step of establishing a reference profile for the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers, said reference profile representing either (a) a prediction or diagnosis of the absence of the diseases or conditions as taught herein, or (b) a prediction or diagnosis of the diseases or conditions as taught herein.

A further aspect provides a method for establishing a reference profile for the quantity of LTBP2 and the presence or absence and/or quantity of one or more other biomarkers useful for the diagnosis, prediction, prognosis and/or monitoring of the diseases or conditions as taught herein, said reference profile representing:

(a) a prediction or diagnosis of the absence of the respective diseases or conditions, or

(b) a prediction or diagnosis of the respective diseases or conditions or of the risk of having said respective diseases or conditions, comprising:

(i) measuring the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers in:
(i a) one or more samples from one or more subjects not having the respective diseases or conditions or not being at risk of having such; or

(i b) one or more samples from one or more subjects having the respective diseases or conditions or being at risk of having such;

(ii)

(ii a) using the measurements of (i a) to create a profile of the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers; or

(ii b) using the measurements of (i b) to create a profile of the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers;

(iii)

(iii a) storing the profile of (ii a) as the reference profile representing the prediction or diagnosis of the absence of the respective diseases or conditions; or

(iii b) storing the profile of (ii b) as the reference profile representing the prediction or diagnosis of the respective diseases conditions.

Further provided is a method for establishing a LTBP2 base-line or reference value in a subject or population of subjects, comprising: (i) measuring the quantity of LTBP2 in the sample(s) from the subject(s) at different time points wherein the subject(s) is (are) not suffering from the diseases or conditions as taught herein, and (ii) calculating the range or mean value of the subject(s), which is the LTBP2 base-line or reference value for subject(s) not suffering from the diseases or conditions as taught herein.

Preferably, the subject as intended in any one of the present methods is human.

The quantity of LTBP2 and/or the presence or absence and/or quantity of the one or more other biomarkers may be measured by any suitable technique such as may be known in the art. For example, the quantity of LTBP2 and/or the presence or absence and/or quantity of the one or more other biomarkers may be measured using, respectively, a binding agent capable of specifically binding to LTBP2 and/or to fragments thereof, and a binding agent capable of specifically binding to said one or more other biomarkers. For example, the binding agent may be an antibody, aptamer, spiegelmer, photoaptamer, protein, peptide, peptidomimetic or a small molecule. For example, the quantity of LTBP2 and/or the presence or absence and/or quantity of the one or more other biomarkers may be measured using an immunoassay technology or a mass spectrometry analysis method or a chromatography method, or a combination of said methods.

Further disclosed is a kit for the diagnosis, prediction, prognosis and/or monitoring of the diseases or conditions as taught herein in a subject, the kit comprising (i) means for measuring
the quantity of LTBP2 in a sample from the subject, and optionally and preferably (ii) a
reference value of the quantity of LTBP2 or means for establishing said reference value,
wherein said reference value represents a known diagnosis, prediction and/or prognosis of the
respective diseases or conditions. The kit thus allows one to: measure the quantity of LTBP2
in the sample from the subject by means (i); compare the quantity of LTBP2 measured by
means (i) with the reference value of (ii) or established by means (ii); find a deviation or no
deviation of the quantity of LTBP2 measured by means (i) from the reference value of (ii); and
consequently attribute said finding of deviation or no deviation to a particular diagnosis,
prediction and/or prognosis of the respective diseases or conditions in the subject.

A further embodiment provides a kit for the diagnosis, prediction, prognosis and/or monitoring
of the diseases or conditions as taught herein in a subject, the kit comprising (i) means for
measuring the quantity of LTBP2 in a sample from the subject and (ii) means for measuring
the presence or absence and/or quantity of one or more other biomarkers in the sample from
the subject, and optionally and preferably (iii) means for establishing a subject profile of the
quantity of LTBP2 and the presence or absence and/or quantity of said one or more other
biomarkers, and optionally and preferably (iv) a reference profile of the quantity of LTBP2 and
the presence or absence and/or quantity of said one or more other biomarkers, or means for
establishing said reference profile, said reference profile representing a known diagnosis,
prediction and/or prognosis of the conditions, symptoms and/or parameter values according to
the invention. Such kit thus allows one to: measure the quantity of LTBP2 and the presence or
absence and/or quantity of said one or more other biomarkers in the sample from the subject
by respectively means (i) and (ii); establish (e.g., using means included in the kit or using
suitable external means) a subject profile of the quantity of LTBP2 and the presence or
absence and/or quantity of said one or more other biomarkers based on said measurements;
compare the subject profile with the reference profile of (iv) or established by means (iv); find a
deviation or no deviation of said subject profile from said reference profile; and consequently
attribute said finding of deviation or no deviation to a particular diagnosis, prediction and/or
prognosis of the respective diseases or conditions in the subject.

The means for measuring the quantity of LTBP2 and/or the presence or absence and/or
quantity of the one or more other biomarkers in the present kits may comprise, respectively,
one or more binding agents capable of specifically binding to LTBP2 and/or to fragments
thereof, and one or more binding agents capable of specifically binding to said one or more
other biomarkers. For example, any one of said one or more binding agents may be an
antibody, aptamer, spiegelmer, photoaptamer, protein, peptide, peptidomimetic or a small
molecule. For example, any one of said one or more binding agents may be advantageously
immobilised on a solid phase or support. The means for measuring the quantity of LTBP2
and/or the presence or absence and/or quantity of the one or more other biomarkers in the
present kits may employ an immunoassay technology or mass spectrometry analysis technology or chromatography technology, or a combination of said technologies.

Disclosed is thus also a kit for the diagnosis, prediction, prognosis and/or monitoring of the diseases or conditions as taught herein comprising: (a) one or more binding agents capable of specifically binding to LTBP2 and/or to fragments thereof; (b) preferably, a known quantity or concentration of LTBP2 and/or a fragment thereof (e.g., for use as controls, standards and/or calibrators); (c) preferably, a reference value of the quantity of LTBP2, or means for establishing said reference value. Said components under (a) and/or (c) may be suitably labelled as taught elsewhere in this specification.

Also disclosed is a kit for the diagnosis, prediction and/or prognosis of the diseases or conditions as taught herein comprising: (a) one or more binding agents capable of specifically binding to LTBP2 and/or to fragments thereof; (b) one or more binding agents capable of specifically binding to one or more other biomarkers; (c) preferably, a known quantity or concentration of LTBP2 and/or a fragment thereof and a known quantity or concentration of said one or more other biomarkers (e.g., for use as controls, standards and/or calibrators); (d) preferably, a reference profile of the quantity of LTBP2 and the presence or absence and/or quantity of said one or more other biomarkers, or means for establishing said reference profiles. Said components under (a), (b) and/or (c) may be suitably labelled as taught elsewhere in this specification.

Further disclosed is the use of the kit as described herein for the diagnosis, prediction, prognosis and/or monitoring of the diseases or conditions as taught herein.

Also disclosed are reagents and tools useful for measuring LTBP2 and optionally the one or more other biomarkers concerned herein.

Hence, disclosed is a protein, polypeptide or peptide array or microarray comprising (a) LTBP2 and/or a fragment thereof, preferably a known quantity or concentration of said LTBP2 and/or fragment thereof; and (b) optionally and preferably, one or more other biomarkers, preferably a known quantity or concentration of said one or more other biomarkers.

Also disclosed is a binding agent array or microarray comprising: (a) one or more binding agents capable of specifically binding to LTBP2 and/or to fragments thereof, preferably a known quantity or concentration of said binding agents; and (b) optionally and preferably, one or more binding agents capable of specifically binding to one or more other biomarkers, preferably a known quantity or concentration of said binding agents.

Also disclosed are kits as taught here above configured as portable devices, such as, for example, bed-side devices, for use at home or in clinical settings.
A related aspect thus provides a portable testing device capable of measuring the quantity of LTBP2 in a sample from a subject comprising: (i) means for obtaining a sample from the subject, (ii) means for measuring the quantity of LTBP2 in said sample, and (iii) means for visualising the quantity of LTBP2 measured in the sample.

In an embodiment, the means of parts (ii) and (iii) may be the same, thus providing a portable testing device capable of measuring the quantity of LTBP2 in a sample from a subject comprising (i) means for obtaining a sample from the subject; and (ii) means for measuring the quantity of LTBP2 in said sample and visualising the quantity of LTBP2 measured in the sample.

In an embodiment, said visualising means is capable of indicating whether the quantity of LTBP2 in the sample is above or below a certain threshold level and/or whether the quantity of LTBP2 in the sample deviates or not from a reference value of the quantity of LTBP2, said reference value representing a known diagnosis, prediction and/or prognosis of the diseases or conditions as taught herein. Hence, the portable testing device may suitably also comprise said reference value or means for establishing the reference value.

In an embodiment, the threshold level is chosen such that the quantity of LTBP2 in the sample above said threshold level indicates that the subject has or is at risk of having the respective disease or condition or indicates a poor prognosis for such in the subject, and the quantity of LTBP2 in the sample equal to or below said threshold level indicates that the subject does not have or is not at risk of having the diseases or conditions as taught herein or indicates a good prognosis for such in the subject.

In an embodiment, the portable testing device comprises a reference value representing the prediction or diagnosis of the absence of the diseases or conditions as taught herein or representing a good prognosis for such, or comprises means for establishing said reference value, and an elevated quantity of LTBP2 in the sample from the subject compared to said reference value indicates that the subject has or is at risk of having the respective disease or condition or indicates a poor prognosis for such in the subject. In another embodiment, the portable testing device comprises a reference value representing the prediction or diagnosis of the diseases or conditions as taught herein or representing a poor prognosis for such, or comprises means for establishing said reference value, and a comparable quantity of LTBP2 in the sample from the subject compared to said reference value indicates that the subject has or is at risk of having the respective disease or condition or indicates a poor prognosis for such in the subject.

These and further aspects and preferred embodiments are described in the following sections and in the appended claims.
BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates sequences of full length LTBP2 (SEQ ID NO.1). The signal peptide is indicated in small caps. Also indicated is the selected MASSterclass quantified peptide (bold, italic, underlined / SEQ ID NO.2).

Figures 2A and 2B represent box plot graphs illustrating LTBP2 normalized levels (Fig. 2A) and NTpro-BNP levels (pg/ml) (Fig. 2B) respectively in (A) 30 day survivors, (B) 30 day cardiac non-survivors and (C) 30 day pulmonary non-survivors. The p-value for survivors versus non-survivors because of pulmonary causes is <0.001.

Figures 3A and 3B represent box plot graphs illustrating LTBP2 normalized levels (Fig. 3A) and NTpro-BNP levels (pg/ml) (Fig. 3B) respectively in (A) one year survivors, (B) one year cardiac non-survivors and (C) one year pulmonary non-survivors. The p-value for survivors versus non-survivors because of pulmonary causes is <0.08.

Figure 4 represents a bar chart illustrating the relationship between LTBP2 deciles and one-year all-cause mortality.

DETAILED DESCRIPTION

As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps.

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

The term "about" as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of and from the specified value, in particular variations of +/-10% or less, preferably +/-5% or less, more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" refers is itself also specifically, and preferably, disclosed.

All documents cited in the present specification are hereby incorporated by reference in their entirety.
Unless otherwise specified, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions may be included to better appreciate the teaching of the present invention.

The term "biomarker" is widespread in the art and may broadly denote a biological molecule and/or a detectable portion thereof whose qualitative and/or quantitative evaluation in a subject is predictive or informative (e.g., predictive, diagnostic and/or prognostic) with respect to one or more aspects of the subject’s phenotype and/or genotype, such as, for example, with respect to the status of the subject as to a given disease or condition.

Reference herein to "disease(s) and/or condition(s) as taught herein" or a similar reference encompasses any such diseases and conditions as disclosed herein insofar consistent with the context of such a recitation, in particular pulmonary inflammation.

The term "pulmonary dysfunction" encompasses any disease or disorder that results in an impaired lung functioning, i.e. wherein the functioning of the lung or lung tissue is inadequate. Non-limiting examples are pulmonary inflammation, pneumonia, bronchitis, dyspnea, COPD, emphysema, etc. Some non-limiting examples are described below.

The terms "pulmonary inflammation" or "inflammation of the lung" may be used interchangeably herein and generally encompasses states, diseases and conditions in which the functioning of the lung or lung tissue is inadequate due to inflammation.

The pulmonary inflammation may be caused by a septic event or an aseptic event or may be caused by inflammatory substances generated in another organ such as by inflammatory substances generated upon acute kidney injury or reperfusion injury of the heart.

Signs and symptoms of pulmonary inflammation may include without limitation any one or more of cough; chest pain; fever; difficult breathing such as dyspnea; cyanosis or bluish skin; sharp chest pain; chest tightness; chills; sputum or mucus production; wheezing; weight loss; poor appetite and tiredness.

Dyspnea (dyspnoea or shortness of breath) is known per se and may particularly refer to a common and distressing symptom experienced by subjects as unpleasant or uncomfortable respiratory sensations that may be more particularly defined as a "subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity". Dyspnea may be connected to a range of underlying pathologies.

The pulmonary inflammation caused by a septic event may be selected from one or more of pneumonia, bronchitis or chronic obstructive pulmonary disease (COPD).
The terms "pneumonia", "bronchitis" and "chronic obstructive pulmonary disease" (COPD), as used herein, carry their respective art-established meanings. By means of further guidance, the term "pneumonia" generally refers to an inflammatory condition of the lung in particular affecting the microscopic air sacs or alveoli. Pneumonia may be caused by an infection by bacteria, viruses, fungi or parasites, or may be caused otherwise such as by autoimmune disease, chemicals or drugs. Pneumonia includes infectious pneumonia and noninfectious pneumonia or idiopathic interstitial pneumonia such as diffuse alveolar damage, organizing pneumonia, nonspecific interstitial pneumonia, lymphocytic interstitial pneumonia, desquamative interstitial pneumonia, respiratory bronchiolitis interstitial lung disease and usual interstitial pneumonia.

The term "bronchitis" generally refers to inflammation of the mucous membranes of the bronchi or airways that carry airflow from the trachea into the lungs. Bronchitis encompasses acute and chronic bronchitis. Acute bronchitis is characterized by the development of a cough, with or without the production of sputum or mucus that is expectorated (coughed up) from the respiratory tract. Acute bronchitis often occurs during the course of an acute viral illness such as the common cold or influenza. Chronic bronchitis, a type of chronic obstructive pulmonary disease, is characterized by the presence of a productive cough that lasts for three months or more per year for at least two years. Chronic bronchitis most often develops due to recurrent injury to the airways caused by inhaled irritants such as cigarette smoke or air pollution.

The term "chronic obstructive pulmonary disease" (COPD), also known as "chronic obstructive lung disease" (COLD), "chronic obstructive airway disease" (COAD), "chronic airflow limitation" (CAL) or "chronic obstructive respiratory disease" (CORD), is the co-occurrence of chronic bronchitis and emphysema.

Emphysema is known per se and may particularly refer to an enlargement of the air spaces distal to the terminal bronchioles, with destruction of their walls. The destruction of the air space walls reduces the surface area available for the exchange of oxygen and carbon dioxide during breathing and reduces the elasticity of the lung itself, which results in a loss of support for the airways that are embedded in the lung. These airways are more likely to collapse causing further limitation to airflow.

The pulmonary inflammation caused by an aseptic event may be selected from one or more of silicosis, ischemia, anaphylactic episode or lupus.

The term "silicosis", also known as Potter's rot, is a form of occupational lung disease caused by inhalation of crystalline silica dust. Silicosis is typically marked by inflammation and scarring in forms of nodular lesions in the upper lobes of the lungs.
The terms "ischemia", "ischaemia" or "ischemic stress" generally refer to a disease or condition characterized by a restriction in blood supply, i.e. a shortage of oxygen, glucose and other blood-borne nutrients, with resultant damage or dysfunction of tissue. Ischemia can be renal ischemia, myocardial ischemia, brain ischemia, mesenteric ischemia, ischemic colitis, ischemic stroke, limb ischemia or cutaneous ischemia. Ischemia can be chronic or acute.

The terms "anaphylactic episode" or "anaphylaxis" generally refer to a serious allergic reaction that is rapid in onset and may cause death. Anaphylaxis can result in a number of symptoms including throat swelling, an itchy rash, and low blood pressure.

The term "lupus", also known as "systemic lupus erythematosus" (SLE), is a systemic autoimmune disease (or autoimmune connective tissue disease) that can affect any part of the body. Lupus may refer to a Type III hypersensitivity reaction caused by antibody-immune complex formation. There is no one specific cause of SLE, however, SLE may be caused by a number of environmental triggers and by genetic susceptibility.

The pulmonary inflammation may be caused by inflammatory substances generated in another organ such as by inflammatory substances generated upon acute kidney injury or reperfusion injury of the heart or brain.

The inflammatory substances may be Proinflammatory cytokines, interferon gamma, IL-2, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), TGF-beta, IL 8 (CXCL1), IL-6, IL-18, macrophage inflammatory protein (MIP)-2, monocyte chemoattractant protein (MCP)-1 are increased in kidney ischemia but also: IL-1beta, IL-1 alfa, TNF-alfa are increased in cisplatin-induced AKI. Other markers include: Fractalkine (CX3CL1).

The terms "acute kidney injury" (AKI), "acute kidney failure" or "acute renal failure" may be used interchangeably. AKI may be staged (classified, graded) into 5 distinct stages using the "RIFLE" (Risk, Injury, Failure, Loss, end-stage renal disease) staging system as set out here below (based on Lameire et al. 2005, Lancet 365: 417-430):

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR (based on serum creatinine) criteria</th>
<th>Urine output criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Risk&quot;</td>
<td>Serum creatinine increased 1.5 times &lt; 0.5 imL / kg / h for 6 h</td>
<td>GFR=glomerular filtration rate</td>
</tr>
<tr>
<td>&quot;Injury&quot;</td>
<td>Serum creatinine increased 2.0 times &lt; 0.5 imL / kg / h for 12 h</td>
<td></td>
</tr>
<tr>
<td>&quot;Failure&quot;</td>
<td>Serum creatinine increased 3.0 times, &lt; 0.3 imL / kg / h for 24 h or creatinine &gt;355 mM/L when there was an acute rise of &gt; 44 mM/L or anuria for 12 h</td>
<td></td>
</tr>
<tr>
<td>&quot;Loss&quot;</td>
<td>Persistent acute renal failure &gt; 4 weeks</td>
<td>----</td>
</tr>
</tbody>
</table>
"End-stage" End-stage renal disease > 3 months

Acute kidney injury may also be staged using the "AKIN" (Acute Kidney Injury Network) criteria as set out here below (based on Bagshaw et al. 2008, Nephrol. Dial. Transplant., 23(5): 1569-1574):

<table>
<thead>
<tr>
<th>Stage</th>
<th>Serum creatinine criteria</th>
<th>Urine output criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Increase in serum creatinine &gt;26.2 µmol/l</td>
<td>&lt;0.5 ml/kg/h for ≥6 h or increase to ≥150-1 99% (1.5- to 1.9-fold) from baseline</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Increase in serum creatinine to 200-299%</td>
<td>&lt;0.5 ml/kg/h for &gt;12 h</td>
</tr>
<tr>
<td>(≥2.9 fold) from baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>Increase in serum creatinine to ≥300%</td>
<td>&lt;0.3 ml/kg/h &gt;24 h or anuria ≥12 h</td>
</tr>
<tr>
<td>(≥3-fold) from baseline or serum creatinine</td>
<td>≥354 µmol/l with an acute rise of</td>
<td></td>
</tr>
<tr>
<td>at least 44 µmol/l or initiation of RRT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other staging methods for renal failure resulting in similar or comparable classifications of different stages of renal failure may be used herein.

The term "reperfusion injury" generally refers to tissue damage caused when blood supply returns to the tissue after a period of ischemia or lack of oxygen.

The inventors realised the use of LTBP2 or a fragment thereof as a blood biomarker for the diagnosis, prediction, prognosis and/or monitoring of pulmonary inflammation in a subject, wherein said diagnosis, prediction, prognosis and/or monitoring pulmonary inflammation comprises assessing the degree of the pulmonary inflammation in the subject.

The complications related to pulmonary injury may encompass lung infarction, loss of functional lung tissue, emphysema, lung fibrosis, atelectasis, pleuritis, pulmonary hypertension.

The degree of pulmonary injury may be assessed as being: (i) no injury, (ii) pulmonary inflammation with reversible or repairable damage which can lead to complications when left untreated, or (iii) pulmonary inflammation with potential irreversible or irreparable physiological damage, morbidity or mortality.

The term "morbidity" generally refers to a diseased state, disability, or poor health due to any cause. The term may be used to refer to the existence of any form of disease, or to the degree that the condition affects the patient. Among critically ill patients, the level of morbidity is often measured by ICU scoring systems such as APACHE II, SAPS II and III, Glasgow Coma scale, PIM2, and SOFA.
The term "mortality" generally refers to the state or condition of being mortal or susceptible to death. An increased mortality is in the light of the present invention especially directed to having a high risk of dying due to pulmonary complications, more specifically due to pulmonary death.

The kidney derived biomarker may be one or more of creatinine (i.e., serum creatinine clearance), Cystatin C and neutrophil gelatinase-associated lipocalin (NGAL), beta-trace protein, kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18).

The inflammatory biomarker may be one or more of Proinflammatory cytokines, interferon gamma, IL-2, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), TGF-beta, IL 8 (CXCL1), IL-6, IL-18, macrophage inflammatory protein (MIP-)2, monocyte chemoattractant protein (MCP)-1 are increased in kidney ischemia but also: IL-1beta, IL-1alpha, TNF-alpha are increased in cisplatin-induced AKI. Other markers include: Fractalkine (CX3CL1), CRP, procalcitonin, white bloodcell count.

The term "natriuretic peptides" generally refers to one or more of pro-B-type natriuretic peptide, amino terminal pro-B-type natriuretic peptide and B-type natriuretic peptide. As used herein, the terms "pro-B-type natriuretic peptide" (also abbreviated as "proBNP") and "amino terminal pro-B-type natriuretic peptide" (also abbreviated as "NTproBNP") and "B-type natriuretic peptide" (also abbreviated as "BNP") refer to peptides commonly known under these designations in the art. As further explanation and without limitation, in vivo proBNP, NTproBNP and BNP derive from natriuretic peptide precursor B preproprotein (preproBNP). In particular, proBNP peptide corresponds to the portion of preproBNP after removal of the N-terminal secretion signal (leader) sequence from preproBNP. NTproBNP corresponds to the N-terminal portion and BNP corresponds to the C-terminal portion of the proBNP peptide subsequent to cleavage of the latter C-terminally adjacent to amino acid 76 of proBNP.

The term "lung fibrosis" or "pulmonary fibrosis", also described as "scarring of the lung", generally refers to the formation or development of excess fibrous connective tissue in the lungs.

The terms "predicting" or "prediction", "diagnosing" or "diagnosis" and "prognosticating" or "prognosis" are commonplace and well-understood in medical and clinical practice. It shall be understood that the phrase "a method for diagnosing, predicting and/or prognosticating" a given disease or condition may also be interchanged with phrases such as "a method for the diagnosis, prediction and/or prognosis" of said disease or condition or "a method for making (or determining or establishing) a diagnosis, prediction and/or prognosis" of said disease or condition, or the like.

By means of further explanation and without limitation, "predicting" or "prediction" generally refer to an advance declaration, indication or foretelling of a disease or condition in a subject.
not (yet) having said disease or condition. For example, a prediction of a disease or condition in a subject may indicate a probability, chance or risk that the subject will develop said disease or condition, for example within a certain time period or by a certain age. Said probability, chance or risk may be indicated inter alia as an absolute value, range or statistics, or may be indicated relative to a suitable control subject or subject population (such as, e.g., relative to a general, normal or healthy subject or subject population). Hence, the probability, chance or risk that a subject will develop a disease or condition may be advantageously indicated as increased or decreased, or as fold-increased or fold-decreased relative to a suitable control subject or subject population. As used herein, the term "prediction" of the conditions or diseases as taught herein in a subject may also particularly mean that the subject has a 'positive' prediction of such, i.e., that the subject is at risk of having such (e.g., the risk is significantly increased vis-a-vis a control subject or subject population). The term "prediction of no" diseases or conditions as taught herein as described herein in a subject may particularly mean that the subject has a 'negative' prediction of such, i.e., that the subject's risk of having such is not significantly increased vis-a-vis a control subject or subject population.

The terms "diagnosing" or "diagnosis" generally refer to the process or act of recognising, deciding on or concluding on a disease or condition in a subject on the basis of symptoms and signs and/or from results of various diagnostic procedures (such as, for example, from knowing the presence, absence and/or quantity of one or more biomarkers characteristic of the diagnosed disease or condition). As used herein, "diagnosis of the diseases or conditions as taught herein in a subject may particularly mean that the subject has such, hence, is diagnosed as having such. "Diagnosis of no" diseases or conditions as taught herein in a subject may particularly mean that the subject does not have such, hence, is diagnosed as not having such. A subject may be diagnosed as not having such despite displaying one or more conventional symptoms or signs reminiscent of such.

The terms "prognosticating" or "prognosis" generally refer to an anticipation on the progression of a disease or condition and the prospect (e.g., the probability, duration, and/or extent) of recovery.

A good prognosis of the diseases or conditions taught herein may generally encompass anticipation of a satisfactory partial or complete recovery from the diseases or conditions, preferably within an acceptable time period. A good prognosis of such may more commonly encompass anticipation of not further worsening or aggravating of such, preferably within a given time period.

A poor prognosis of the diseases or conditions as taught herein may generally encompass anticipation of a substandard recovery and/or unsatisfactorily slow recovery, or to substantially
no recovery or even further worsening of such and more particularly resulting in death of the diseased subject.

The term "subject" or "patient" as used herein typically denotes humans, but may also encompass reference to non-human animals, preferably warm-blooded animals, more preferably mammals, such as, e.g., non-human primates, rodents, canines, felines, equines, ovines, porcines, and the like.

The terms "sample" or "biological sample" as used herein include any biological specimen obtained from a subject. Samples may include, without limitation, whole blood, plasma, serum, red blood cells, white blood cells (e.g., peripheral blood mononuclear cells), saliva, urine, stool (i.e., faeces), tears, sweat, sebum, nipple aspirate, ductal lavage, tumour exudates, synovial fluid, cerebrospinal fluid, lymph, fine needle aspirate, amniotic fluid, any other bodily fluid, cell lysates, cellular secretion products, inflammation fluid, semen and vaginal secretions. Preferred samples may include ones comprising LTBP2 protein in detectable quantities. In preferred embodiments, the sample may be whole blood or a fractional component thereof such as, e.g., plasma, serum, or a cell pellet. Preferably the sample is readily obtainable by minimally invasive methods allowing removal or isolation of said sample from the subject. Samples may also include tissue samples and biopsies, tissue homogenates and the like. Preferably, the sample used to detect LTBP2 levels is blood plasma. Also preferably, the sample used to detect LTBP2 levels is urine.

The term "plasma" defines the colourless watery fluid of the blood that contains no cells, but in which the blood cells (erythrocytes, leukocytes, thrombocytes, etc.) are suspended, containing nutrients, sugars, proteins, minerals, enzymes, etc.

A molecule or analyte such as a protein, polypeptide or peptide, or a group of two or more molecules or analytes such as two or more proteins, polypeptides or peptides, is "measured" in a sample when the presence or absence and/or quantity of said molecule or analyte or of said group of molecules or analytes is detected or determined in the sample, preferably substantially to the exclusion of other molecules and analytes.

The terms "quantity", "amount" and "level" are synonymous and generally well-understood in the art. The terms as used herein may particularly refer to an absolute quantification of a molecule or an analyte in a sample, or to a relative quantification of a molecule or analyte in a sample, i.e., relative to another value such as relative to a reference value as taught herein, or to a range of values indicating a base-line expression of the biomarker. These values or ranges can be obtained from a single patient or from a group of patients.
An absolute quantity of a molecule or analyte in a sample may be advantageously expressed as weight or as molar amount, or more commonly as a concentration, e.g., weight per volume or mol per volume.

A relative quantity of a molecule or analyte in a sample may be advantageously expressed as an increase or decrease or as a fold-increase or fold-decrease relative to said another value, such as relative to a reference value as taught herein. Performing a relative comparison between first and second parameters (e.g., first and second quantities) may but need not require first to determine the absolute values of said first and second parameters. For example, a measurement method can produce quantifiable readouts (such as, e.g., signal intensities) for said first and second parameters, wherein said readouts are a function of the value of said parameters, and wherein said readouts can be directly compared to produce a relative value for the first parameter vs. the second parameter, without the actual need first to convert the readouts to absolute values of the respective parameters.

As used herein, the term "LTBP2" corresponds to the protein commonly known as latent transforming growth factor beta binding protein 2 (LTBP2), also known as GLC3D, LTBP3, MSTP031, C14orf141, i.e. the proteins and polypeptides commonly known under these designations in the art. The terms encompass such proteins and polypeptides of any organism where found, and particularly of animals, preferably vertebrates, more preferably mammals, including humans and non-human mammals, even more preferably of humans. The terms particularly encompass such proteins and polypeptides with a native sequence, i.e., ones of which the primary sequence is the same as that of LTBP2 found in or derived from nature. A skilled person understands that native sequences of LTBP2 may differ between different species due to genetic divergence between such species. Moreover, the native sequences of LTBP2 may differ between or within different individuals of the same species due to normal genetic diversity (variation) within a given species. Also, the native sequences of LTBP2 may differ between or even within different individuals of the same species due to post-transcriptional or post-translational modifications. Accordingly, all LTBP2 sequences found in or derived from nature are considered "native". The terms encompass LTBP2 proteins and polypeptides when forming a part of a living organism, organ, tissue or cell, when forming a part of a biological sample, as well as when at least partly isolated from such sources. The terms also encompass proteins and polypeptides when produced by recombinant or synthetic means.

Exemplary LTBP2 includes, without limitation, human LTBP2 having primary amino acid sequence as annotated under NCBI Genbank (http://www.ncbi.nlm.nih.gov/) accession number NP_000419 (sequence version 1) as reproduced in Fig. 1 (SEQ ID NO: 1). A skilled person can also appreciate that said sequences are of precursor of LTBP2 and may include
parts which are processed away from mature LTBP2. For example, in Figure 1, an LTBP2 signal peptide is indicated in small caps in the amino acid sequence.

In an embodiment the circulating LTBP2, e.g., secreted form circulating in the blood plasma, may be detected, as opposed to the cell-bound or cell-confined LTBP2 protein.

The reference herein to LTBP2 may also encompass fragments of LTBP2. Hence, the reference herein to measuring LTBP2, or to measuring the quantity of LTBP2, may encompass measuring the LTBP2 protein or polypeptide, such as, e.g., measuring the mature, active and/or the processed soluble/secreted form (e.g. plasma circulating form) of LTBP2 and/or measuring one or more fragments thereof. For example, LTBP2 and/or one or more fragments thereof may be measured collectively, such that the measured quantity corresponds to the sum amounts of the collectively measured species. In another example, LTBP2 and/or one or more fragments thereof may be measured each individually. Preferably, said fragment of LTBP2 is a plasma circulating form of LTBP2. The expression "plasma circulating form of LTBP2" or shortly "circulating form" encompasses all LTBP2 proteins or fragments thereof that circulate in the plasma, i.e., are not cell- or membrane-bound. Without wanting to be bound by any theory, such circulating forms can be derived from the full-length LTBP2 protein through natural processing, or can be resulting from known degradation processes occurring in said sample. In certain situations, the circulating form can also be the full-length LTBP2 protein, which is found to be circulating in the plasma. Said "circulating form" can thus be any LTBP2 protein or any processed soluble form of LTBP2 or fragments of either one, that is circulating in the sample, i.e. which is not bound to a cell- or membrane fraction of said sample.

Unless otherwise apparent from the context, reference herein to any protein, polypeptide or peptide encompasses such from any organism where found, and particularly preferably from animals, preferably vertebrates, more preferably mammals, including humans and non-human mammals, even more preferably from humans.

Further, unless otherwise apparent from the context, reference herein to any protein, polypeptide or peptide and fragments thereof may generally also encompass modified forms of said protein, polypeptide or peptide and fragments such as bearing post-expression modifications including, for example, phosphorylation, glycosylation, lipidation, methylation, cysteinylation, sulphonation, glutathionylation, acetylation, oxidation of methionine to methionine sulfoxide or methionine sulphone, and the like.

In an embodiment, LTBP2 and fragments thereof, or other biomarkers as employed herein and fragments thereof, may be human, i.e., their primary sequence may be the same as a corresponding primary sequence of or present in a naturally occurring human peptides, polypeptides or proteins. Hence, the qualifier "human" in this connection relates to the primary sequence of the respective proteins, polypeptides, peptides or fragments, rather than to their
origin or source. For example, such proteins, polypeptides, peptides or fragments may be present in or isolated from samples of human subjects or may be obtained by other means (e.g., by recombinant expression, cell-free translation or non-biological peptide synthesis).

The term "fragment" of a protein, polypeptide or peptide generally refers to N-terminally and/or C-terminally deleted or truncated forms of said protein, polypeptide or peptide. The term encompasses fragments arising by any mechanism, such as, without limitation, by alternative translation, exo- and/or endo-proteolysis and/or degradation of said protein or polypeptide, such as, for example, in vivo or in vitro, such as, for example, by physical, chemical and/or enzymatic proteolysis. Without limitation, a fragment of a protein, polypeptide or peptide may represent at least about 5%, or at least about 10%, e.g., ≥ 20%, ≥ 30% or ≥ 40%, such as ≥ 50%, e.g., ≥ 60%, ≥ 70% or ≥ 80%, or even ≥ 90% or ≥ 95% of the amino acid sequence of said protein, polypeptide or peptide.

For example, a fragment may include a sequence of ≥ 5 consecutive amino acids, or ≥ 10 consecutive amino acids, or ≥ 20 consecutive amino acids, or ≥ 30 consecutive amino acids, e.g., >40 consecutive amino acids, such as for example ≥ 50 consecutive amino acids, e.g., ≥ 60, ≥ 70, ≥ 80, ≥ 90, ≥ 100, ≥ 200, ≥ 300, ≥ 400, ≥ 500 or ≥ 600 consecutive amino acids of the corresponding full length protein.

In an embodiment, a fragment may be N-terminally and/or C-terminally truncated by between 1 and about 20 amino acids, such as, e.g., by between 1 and about 15 amino acids, or by between 1 and about 10 amino acids, or by between 1 and about 5 amino acids, compared to the corresponding mature, full-length protein or its soluble or plasma circulating form.

In an embodiment, fragments of a given protein, polypeptide or peptide may be achieved by in vitro proteolysis of said protein, polypeptide or peptide to obtain advantageously detectable peptide(s) from a sample. For example, such proteolysis may be effected by suitable physical, chemical and/or enzymatic agents, e.g., proteinases, preferably endoproteinases, i.e., protease cleaving internally within a protein, polypeptide or peptide chain. A non-limiting list of suitable endoproteinases includes serine proteinases (EC 3.4.21), threonine proteinases (EC 3.4.25), cysteine proteinases (EC 3.4.22), aspartic acid proteinases (EC 3.4.23), metalloproteinases (EC 3.4.24) and glutamic acid proteinases. Exemplary non-limiting endoproteinases include trypsin, chymotrypsin, elastase, Lyso bacter enzymogenes endoproteinase Lys-C, Staphylococcus aureus endoproteinase Glu-C (endopeptidase V8) or Clostridium histolyticum endoproteinase Arg-C (clostripain). Further known or yet to be identified enzymes may be used; a skilled person can choose suitable protease(s) on the basis of their cleavage specificity and frequency to achieve desired peptide forms. Preferably, the proteolysis may be effected by endopeptidases of the trypsin type (EC 3.4.21.4), preferably trypsin, such as, without limitation, preparations of trypsin from bovine pancreas,
human pancreas, porcine pancreas, recombinant trypsin, Lys-acetylated trypsin, trypsin in solution, trypsin immobilised to a solid support, etc. Trypsin is particularly useful, *inter alia* due to high specificity and efficiency of cleavage. The invention also contemplates the use of any trypsin-like protease, *i.e.*, with a similar specificity to that of trypsin. Otherwise, chemical reagents may be used for proteolysis. For example, CNBr can cleave at Met; BNPS-skatole can cleave at Trp. The conditions for treatment, *e.g.*, protein concentration, enzyme or chemical reagent concentration, pH, buffer, temperature, time, can be determined by the skilled person depending on the enzyme or chemical reagent employed.

Also provided is thus an isolated fragment of LTBP2 as defined here above. Such fragments may give useful information about the presence and quantity of LTBP2 in biological samples, whereby the detection of said fragments is of interest. Hence, the herein disclosed fragments of LTBP2 are useful biomarkers. A preferred LTBP2 fragment may comprise, consist essentially of or consist of the sequence as set forth in SEQ ID NO: 2.

The term "isolated" with reference to a particular component (such as for instance, a protein, polypeptide, peptide or fragment thereof) generally denotes that such component exists in separation from - for example, has been separated from or prepared in separation from - one or more other components of its natural environment. For instance, an isolated human or animal protein, polypeptide, peptide or fragment exists in separation from a human or animal body where it occurs naturally.

The term "isolated" as used herein may preferably also encompass the qualifier "purified". As used herein, the term "purified" with reference to protein(s), polypeptide(s), peptide(s) and/or fragment(s) thereof does not require absolute purity. Instead, it denotes that such protein(s), polypeptide(s), peptide(s) and/or fragment(s) is (are) in a discrete environment in which their abundance (conveniently expressed in terms of mass or weight or concentration) relative to other proteins is greater than in a biological sample. A discrete environment denotes a single medium, such as for example a single solution, gel, precipitate, lyophilisate, *etc*. Purified peptides, polypeptides or fragments may be obtained by known methods including, for example, laboratory or recombinant synthesis, chromatography, preparative electrophoresis, centrifugation, precipitation, affinity purification, *etc*.

Purified protein(s), polypeptide(s), peptide(s) and/or fragment(s) may preferably constitute by weight ≥ 10%, more preferably ≥ 50%, such as ≥ 60%, yet more preferably ≥ 70%, such as ≥ 80%, and still more preferably ≥ 90%, such as ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98%, ≥ 99% or even 100%, of the protein content of the discrete environment. Protein content may be determined, *e.g.*, by the Lowry method (Lowry et al. 1951. *J Biol Chem* 193: 265), optionally as described by Hartree 1972 (Anal Biochem 48: 422-427). Also, purity of peptides or polypeptides may be
determined by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain.

Further disclosed are isolated LTBP2 or fragments thereof as taught herein comprising a detectable label. This facilitates ready detection of such fragments. The term "label" as used throughout this specification refers to any atom, molecule, moiety or biomolecule that can be used to provide a detectable and preferably quantifiable read-out or property, and that can be attached to or made part of an entity of interest, such as a peptide or polypeptide or a specific-binding agent. Labels may be suitably detectable by mass spectrometric, spectroscopic, optical, colorimetric, magnetic, photochemical, biochemical, immunochemical or chemical means. Labels include without limitation dyes; radiolabels such as $^{32}$P, $^{33}$P, $^{35}$S, $^{125}$I, $^{131}$I; electron-dense reagents; enzymes (e.g., horse-radish phosphatase or alkaline phosphatase as commonly used in immunoassays); binding moieties such as biotin-streptavidin; haptens such as digoxigenin; luminogenic, phosphorescent or fluorogenic moieties; mass tags; and fluorescent dyes alone or in combination with moieties that can suppress or shift emission spectra by fluorescence resonance energy transfer (FRET).

For example, the label may be a mass-altering label. Preferably, a mass-altering label may involve the presence of a distinct stable isotope in one or more amino acids of the peptide vis-à-vis its corresponding non-labelled peptide. Mass-labelled peptides are particularly useful as positive controls, standards and calibrators in mass spectrometry applications. In particular, peptides including one or more distinct isotopes are chemically alike, separate chromatographically and electrophoretically in the same manner and also ionise and fragment in the same way. However, in a suitable mass analyser such peptides and optionally select fragmentation ions thereof will display distinguishable m/z ratios and can thus be discriminated. Examples of pairs of distinguishable stable isotopes include H and D, $^{13}$C and $^{15}$C, $^{14}$N and $^{15}$N or $^{16}$O and $^{18}$O. Usually, peptides and proteins of biological samples analysed in the present invention may substantially only contain common isotopes having high prevalence in nature, such as for example H, $^{12}$C, $^{14}$N and $^{16}$O. In such case, the mass-labelled peptide may be labelled with one or more uncommon isotopes having low prevalence in nature, such as for instance D, $^{13}$C, $^{15}$N and/or $^{18}$O. It is also conceivable that in cases where the peptides or proteins of a biological sample would include one or more uncommon isotopes, the mass-labelled peptide may comprise the respective common isotope(s).

Isotopically-labelled synthetic peptides may be obtained inter alia by synthesising or recombinantly producing such peptides using one or more isotopically-labelled amino acid substrates, or by chemically or enzymatically modifying unlabelled peptides to introduce thereto one or more distinct isotopes. By means of example and not limitation, D-labelled peptides may be synthesised or recombinantly produced in the presence of commercially
available deuterated L-methionine \( \text{CH}_2\text{-S-CD}_2\text{CD}_2\text{-CH(NH}_2\text{)-COOH} \) or deuterated arginine \( \text{H}_2\text{NC(=NH)-NH-(CD}_2\text{)_3-CD(NH}_2\text{)-COOH} \). It shall be appreciated that any amino acid of which deuterated or \(^{15}\text{N}\)- or \(^{13}\text{C}\)-containing forms exist may be considered for synthesis or recombinant production of labelled peptides. In another non-limiting example, a peptide may be treated with trypsin in \( \text{H}_2^{16}\text{O} \) or \( \text{H}_2^{18}\text{O} \), leading to incorporation of two oxygens (\(^{16}\text{O} \) or \(^{18}\text{O} \), respectively) at the COOH-termini of said peptide (e.g., US 2006/105415).

Accordingly, also contemplated is the use of LTBP2 and isolated fragments thereof as taught herein, optionally comprising a detectable label, as (positive) controls, standards or calibrators in qualitative or quantitative detection assays (measurement methods) of LTBP2, and particularly in such methods for the diagnosis, prediction, prognosis and/or monitoring of pulmonary dysfunction, in particular pulmonary injury in subjects. The proteins, polypeptides or peptides may be supplied in any form, *inter alia* as precipitate, vacuum-dried, lyophilisate, in solution as liquid or frozen, or covalently or non-covalently immobilised on solid phase, such as for example, on solid chromatographic matrix or on glass or plastic or other suitable surfaces (e.g., as a part of peptide arrays and microarrays). The peptides may be readily prepared, for example, isolated from natural sources, or prepared recombinantly or synthetically.

Further disclosed are binding agents capable of specifically binding to any one or more of the isolated fragments of LTBP2 as taught herein. Also disclosed are binding agents capable of specifically binding to only one of isolated fragments of LTBP2 as taught herein. Binding agents as intended throughout this specification may include *inter alia* an antibody, aptamer, spiegelmer, photoaptamer, protein, peptide, peptidomimetic or a small molecule.

A binding agent may be capable of binding both the plasma circulating form and the cell-bound or retained from of LTBP2. Preferably, a binding agent may be capable of specifically binding or detecting the plasma circulating form of LTBP2.

The term "specifically bind" as used throughout this specification means that an agent (denoted herein also as "specific-binding agent") binds to one or more desired molecules or analytes, such as to one or more proteins, polypeptides or peptides of interest or fragments thereof substantially to the exclusion of other molecules which are random or unrelated, and optionally substantially to the exclusion of other molecules that are structurally related. The term "specifically bind" does not necessarily require that an agent binds exclusively to its intended target(s). For example, an agent may be said to specifically bind to protein(s) polypeptide(s), peptide(s) and/or fragment(s) thereof of interest if its affinity for such intended target(s) under the conditions of binding is at least about 2-fold greater, preferably at least about 5-fold greater, more preferably at least about 10-fold greater, yet more preferably at
least about 25-fold greater, still more preferably at least about 50-fold greater, and even more preferably at least about 100-fold or more greater, than its affinity for a non-target molecule.

Preferably, the agent may bind to its intended target(s) with affinity constant \( K_A \) of such binding \( K_A \geq 1 \times 10^8 \text{ M}^{-1} \), more preferably \( K_A \geq 1 \times 10^7 \text{ M}^{-1} \), yet more preferably \( K_A \geq 1 \times 10^6 \text{ M}^{-1} \), even more preferably \( K_A \geq 1 \times 10^6 \text{ M}^{-1} \), and still more preferably \( K_A \geq 1 \times 10^5 \text{ M}^{-1} \) or \( K_A \geq 1 \times 10^4 \text{ M}^{-1} \), wherein \( K_A = [\text{SBA}_T]/[\text{SBA}][\text{T}] \), SBA denotes the specific-binding agent, T denotes the intended target. Determination of \( K_A \) can be carried out by methods known in the art, such as for example, using equilibrium dialysis and Scatchard plot analysis.

Specific binding agents as used throughout this specification may include \textit{inter alia} an antibody, aptamer, spiegelmer, photoaptamer, protein, peptide, peptidomimetic or a small molecule.

As used herein, the term "antibody" is used in its broadest sense and generally refers to any immunologic binding agent. The term specifically encompasses intact monoclonal antibodies, polyclonal antibodies, multivalent (e.g., 2-, 3- or more-valent) and/or multi-specific antibodies (e.g., bi- or more-specific antibodies) formed from at least two intact antibodies, and antibody fragments insofar they exhibit the desired biological activity (particularly, ability to specifically bind an antigen of interest), as well as multivalent and/or multi-specific composites of such fragments. The term "antibody" is not only inclusive of antibodies generated by methods comprising immunisation, but also includes any polypeptide, e.g., a recombinantly expressed polypeptide, which is made to encompass at least one complementarity-determining region (CDR) capable of specifically binding to an epitope on an antigen of interest. Hence, the term applies to such molecules regardless whether they are produced in vitro or in vivo.

An antibody may be any of IgA, IgD, IgE, IgG and IgM classes, and preferably IgG class antibody. An antibody may be a polyclonal antibody, e.g., an antiserum or immunoglobulins purified there from (e.g., affinity-purified). An antibody may be a monoclonal antibody or a mixture of monoclonal antibodies. Monoclonal antibodies can target a particular antigen or a particular epitope within an antigen with greater selectivity and reproducibility. By means of example and not limitation, monoclonal antibodies may be made by the hybridoma method first described by Kohler et al. 1975 (Nature 256: 495), or may be made by recombinant DNA methods (e.g., as in US 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using techniques as described by Clackson et al. 1991 (Nature 352: 624-628) and Marks et al. 1991 (J Mol Biol 222: 581-597), for example.

Antibody binding agents may be antibody fragments. "Antibody fragments" comprise a portion of an intact antibody, comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, Fv and scFv fragments; diabodies; linear...
antibodies; single-chain antibody molecules; and multivalent and/or multispecific antibodies
formed from antibody fragment(s), e.g., dibodies, tribodies, and multibodies. The above
designations Fab, Fab', F(ab')2, Fv, scFv etc. are intended to have their art-established
meaning.

The term antibody includes antibodies originating from or comprising one or more portions
derived from any animal species, preferably vertebrate species, including, e.g., birds and
mammals. Without limitation, the antibodies may be chicken, turkey, goose, duck, guinea fowl,
quail or pheasant. Also without limitation, the antibodies may be human, murine (e.g., mouse,
rat, etc.), donkey, rabbit, goat, sheep, guinea pig, camel (e.g., Camelus bactrianus and
Camelus dromaderius), llama (e.g., Lama paccos, Lama glama or Lama vicugna) or horse.

A skilled person will understand that an antibody can include one or more amino acid
deletions, additions and/or substitutions (e.g., conservative substitutions), insofar such
alterations preserve its binding of the respective antigen. An antibody may also include one or
more native or artificial modifications of its constituent amino acid residues (e.g., glycosylation,
etc.).

Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are
well known in the art, as are methods to produce recombinant antibodies or fragments thereof
(see for example, Harlow and Lane, "Antibodies: A Laboratory Manual", Cold Spring Harbour
Laboratory, New York, 1988; Harlow and Lane, "Using Antibodies: A Laboratory Manual", Cold
Spring Harbour Laboratory, New York, 1999, ISBN 0879695447; "Monoclonal Antibodies: A
Antibodies: A Practical Approach", by Dean & Shepherd, eds., Oxford University Press 2000,

The term "aptamer" refers to single-stranded or double-stranded oligo-DNA, oligo-RNA or
oligo-DNA/RNA or any analogue thereof, that can specifically bind to a target molecule such
as a peptide. Advantageously, aptamers can display fairly high specificity and affinity (e.g., K_A
in the order 1x10^9 M^-1) for their targets. Aptamer production is described *inter alia* in US
5,270,163; Ellington & Szostak 1990 (Nature 346: 818-822); Tuerk & Gold 1990 (Science 249:
505-510); or "The Aptamer Handbook: Functional Oligonucleotides and Their Applications", by
"photoaptamer" refers to an aptamer that contains one or more photoreactive functional
groups that can covalently bind to or crosslink with a target molecule. The term
"peptidomimetic" refers to a non-peptide agent that is a topological analogue of a
responding peptide. Methods of rationally designing peptidomimetics of peptides are known
in the art. For example, the rational design of three peptidomimetics based on the sulphated 8-
mer peptide CCK26-33, and of two peptidomimetics based on the 11-mer peptide Substance P, and related peptidomimetic design principles, are described in Horwell 1995 (Trends Biotechnol 13: 132-134). Speigelmers are aptamers constituted out of L-nucleotides in stead of D-nucleotides. Speigelmers are more stable since the mammalian body does not comprise the necessary machinery to destroy L-oligonucleotides.

The term "small molecule" refers to compounds, preferably organic compounds, with a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e.g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, e.g., up to about 4000, preferably up to 3000 Da, more preferably up to 2000 Da, even more preferably up to about 1000 Da, e.g., up to about 900, 800, 700, 600 or up to about 500 Da.

Hence, also disclosed are methods for immunising animals, e.g., non-human animals such as laboratory or farm, animals using (i.e., using as the immunising antigen) the herein taught fragments of LTBP2, optionally attached to a presenting carrier. Immunisation and preparation of antibody reagents from immune sera is well-known per se and described in documents referred to elsewhere in this specification. The animals to be immunised may include any animal species, preferably warm-blooded species, more preferably vertebrate species, including, e.g., birds and mammals. Without limitation, the antibodies may be chicken, turkey, goose, duck, guinea fowl, quail or pheasant. Also without limitation, the antibodies may be human, murine (e.g., mouse, rat, etc.), donkey, rabbit, goat, sheep, guinea pig, camel, llama or horse. The term "presenting carrier" or "carrier" generally denotes an immunogenic molecule which, when bound to a second molecule, augments immune responses to the latter, usually through the provision of additional T cell epitopes. The presenting carrier may be a (poly)peptidic structure or a non-peptidic structure, such as inter alia glycans, polyethylene glycols, peptide mimetics, synthetic polymers, etc. Exemplary non-limiting carriers include human Hepatitis B virus core protein, multiple C3d domains, tetanus toxin fragment C or yeast Ty particles.

Immune sera obtained or obtainable by immunisation as taught herein may be particularly useful for generating antibody reagents that specifically bind to one or more of the herein disclosed fragments of LTBP2.

Further disclosed are methods for selecting specific-binding agents which bind (a) one or more of the LTBP2 fragments taught herein, substantially to the exclusion of (b) LTBP2 and/or other fragments thereof. Conveniently, such methods may be based on subtracting or removing binding agents which cross-react or cross-bind the non-desired LTBP2 molecules under (b). Such subtraction may be readily performed as known in the art by a variety of affinity
separation methods, such as affinity chromatography, affinity solid phase extraction, affinity magnetic extraction, etc.

Any existing, available or conventional separation, detection and quantification methods can be used herein to measure the presence or absence (e.g., readout being present vs. absent; or detectable amount vs. undetectable amount) and/or quantity (e.g., readout being an absolute or relative quantity, such as, for example, absolute or relative concentration) of LTBP2 and/or fragments thereof and optionally of the one or more other biomarkers or fragments thereof in samples (any molecules or analytes of interest to be so-measured in samples, including LTBP2 and fragments thereof, may be herein below referred to collectively as biomarkers).

For example, such methods may include immunoassay methods, mass spectrometry analysis methods, or chromatography methods, or combinations thereof.

The term "immunoassay" generally refers to methods known as such for detecting one or more molecules or analytes of interest in a sample, wherein specificity of an immunoassay for the molecule(s) or analyte(s) of interest is conferred by specific binding between a specific-binding agent, commonly an antibody, and the molecule(s) or analyte(s) of interest. Immunoassay technologies include without limitation direct ELISA (enzyme-linked immunosorbent assay), indirect ELISA, sandwich ELISA, competitive ELISA, multiplex ELISA, radioimmunoassay (RIA), ELISPOT technologies, and other similar techniques known in the art. Principles of these immunoassay methods are known in the art, for example John R. Crowther, "The ELISA Guidebook", 1st ed., Humana Press 2000, ISBN 0896037282.

By means of further explanation and not limitation, direct ELISA employs a labelled primary antibody to bind to and thereby quantify target antigen in a sample immobilised on a solid support such as a microwell plate. Indirect ELISA uses a non-labelled primary antibody which binds to the target antigen and a secondary labelled antibody that recognises and allows to quantify the antigen-bound primary antibody. In sandwich ELISA the target antigen is captured from a sample using an immobilised ‘capture’ antibody which binds to one antigenic site within the antigen, and subsequent to removal of non-bound analytes the so-captured antigen is detected using a ‘detection’ antibody which binds to another antigenic site within said antigen, where the detection antibody may be directly labelled or indirectly detectable as above. Competitive ELISA uses a labelled ‘competitor’ that may either be the primary antibody or the target antigen. In an example, non-labelled immobilised primary antibody is incubated with a sample, this reaction is allowed to reach equilibrium, and then labelled target antigen is added. The latter will bind to the primary antibody wherever its binding sites are not yet occupied by non-labelled target antigen from the sample. Thus, the detected amount of bound labelled antigen inversely correlates with the amount of non-labelled antigen in the sample. Multiplex
ELISA allows simultaneous detection of two or more analytes within a single compartment (e.g., microplate well) usually at a plurality of array addresses (see, for example, Nielsen & Geierstanger 2004. J Immunol Methods 290: 107-20 and Ling et al. 2007. Expert Rev Mol Diagn 7: 87-98 for further guidance). As appreciated, labelling in ELISA technologies is usually by enzyme (such as, e.g., horse-radish peroxidase) conjugation and the end-point is typically colorimetric, chemiluminescent or fluorescent, magnetic, piezo electric, pyroelectric and other.

Radioimmunoassay (RIA) is a competition-based technique and involves mixing known quantities of radioactively-labelled (e.g., $^{125}$I- or $^{131}$I-labelled) target antigen with antibody to said antigen, then adding non-labelled or 'cold' antigen from a sample and measuring the amount of labelled antigen displaced (see, e.g., "An Introduction to Radioimmunoassay and Related Techniques", by Chard T, ed., Elsevier Science 1995, ISBN 0444821198 for guidance).

Generally, any mass spectrometric (MS) techniques that can obtain precise information on the mass of peptides, and preferably also on fragmentation and/or (partial) amino acid sequence of selected peptides (e.g., in tandem mass spectrometry, MS/MS; or in post source decay, TOF MS), are useful herein. Suitable peptide MS and MS/MS techniques and systems are well-known per se (see, e.g., Methods in Molecular Biology, vol. 146: "Mass Spectrometry of Proteins and Peptides", by Chapman, ed., Humana Press 2000, ISBN 089603609x; Biemann 1990. Methods Enzymol 193: 455-79; or Methods in Enzymology, vol. 402: "Biological Mass Spectrometry", by Burlingame, ed., Academic Press 2005, ISBN 9780121828073) and may be used herein. MS arrangements, instruments and systems suitable for biomarker peptide analysis may include, without limitation, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS; MALDI-TOF post-source-decay (PSD); MALDI-TOF/TOF; surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) MS; electrospray ionization mass spectrometry (ESI-MS); ESI-MS/MS; ESI-MS/(MS)$^n$ (n is an integer greater than zero); ESI 3D or linear (2D) ion trap MS; ESI triple quadrupole MS; ESI quadrupole orthogonal TOF (Q-TOF); ESI Fourier transform MS systems; desorption/ionization on silicon (DIOS); secondary ion mass spectrometry (SIMS); atmospheric pressure chemical ionization mass spectrometry (APCI-MS); APCI-MS/MS; APCI- (MS)$^n$; atmospheric pressure photoionization mass spectrometry (APPI-MS); APPI-MS/MS; and APPI- (MS)$^n$. Peptide ion fragmentation in tandem MS (MS/MS) arrangements may be achieved using manners established in the art, such as, e.g., collision induced dissociation (CID). Detection and quantification of biomarkers by mass spectrometry may involve multiple reaction monitoring (MRM), such as described among others by Kuhn et al. 2004 (Proteomics 4: 1175-86). MS peptide analysis methods may be advantageously combined with upstream peptide or protein separation or fractionation methods, such as for example with the chromatographic and other methods described herein below.
Chromatography can also be used for measuring biomarkers. As used herein, the term "chromatography" encompasses methods for separating chemical substances, referred to as such and vastly available in the art. In a preferred approach, chromatography refers to a process in which a mixture of chemical substances (analytes) carried by a moving stream of liquid or gas ("mobile phase") is separated into components as a result of differential distribution of the analytes, as they flow around or over a stationary liquid or solid phase ("stationary phase"), between said mobile phase and said stationary phase. The stationary phase may be usually a finely divided solid, a sheet of filter material, or a thin film of a liquid on the surface of a solid, or the like. Chromatography is also widely applicable for the separation of chemical compounds of biological origin, such as, e.g., amino acids, proteins, fragments of proteins or peptides, etc.

Chromatography as used herein may be preferably columnar (i.e., wherein the stationary phase is deposited or packed in a column), preferably liquid chromatography, and yet more preferably HPLC. While particulars of chromatography are well known in the art, for further guidance see, e.g., Meyer M., 1998, ISBN: 047198373X, and "Practical HPLC Methodology and Applications", Bidlingmeyer, B. A., John Wiley & Sons Inc., 1993. Exemplary types of chromatography include, without limitation, high-performance liquid chromatography (HPLC), normal phase HPLC (NP-HPLC), reversed phase HPLC (RP-HPLC), ion exchange chromatography (IEC), such as cation or anion exchange chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), size exclusion chromatography (SEC) including gel filtration chromatography or gel permeation chromatography, chromatofocusing, affinity chromatography such as immuno-affinity, immobilised metal affinity chromatography, and the like.

Chromatography, including single-, two- or more-dimensional chromatography, may be used as a peptide fractionation method in conjunction with a further peptide analysis method, such as for example, with a downstream mass spectrometry analysis as described elsewhere in this specification.

Further peptide or polypeptide separation, identification or quantification methods may be used, optionally in conjunction with any of the above described analysis methods, for measuring biomarkers in the present disclosure. Such methods include, without limitation, chemical extraction partitioning, isoelectric focusing (IEF) including capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITP), capillary electrochromatography (CEC), and the like, one-dimensional polyacrylamide gel electrophoresis (PAGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), capillary gel electrophoresis (CGE), capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), free flow electrophoresis (FFE), etc.
The various aspects and embodiments taught herein may further rely on comparing the quantity of LTBP2 measured in samples with reference values of the quantity of LTBP2, wherein said reference values represent known predictions, diagnoses and/or prognoses of diseases or conditions as taught herein.

For example, distinct reference values may represent the prediction of a risk (e.g., an abnormally elevated risk) of having a given disease or condition as taught herein vs. the prediction of no or normal risk of having said disease or condition. In another example, distinct reference values may represent predictions of differing degrees of risk of having such disease or condition.

In a further example, distinct reference values may represent the diagnosis of a given disease or condition as taught herein vs. the diagnosis of no such disease or condition (such as, e.g., the diagnosis of healthy, or recovered from said disease or condition, etc.). In another example, distinct reference values may represent the diagnosis of such disease or condition of varying severity.

In yet another example, distinct reference values may represent a good prognosis for a given disease or condition as taught herein vs. a poor prognosis for said disease or condition. In a further example, distinct reference values may represent varyingly favourable or unfavourable prognoses for such disease or condition.

Such comparison may generally include any means to determine the presence or absence of at least one difference and optionally of the size of such different between values or profiles being compared. A comparison may include a visual inspection, an arithmetical or statistical comparison of measurements. Such statistical comparisons include, but are not limited to, applying a rule. If the values or biomarker profiles comprise at least one standard, the comparison to determine a difference in said values or biomarker profiles may also include measurements of these standards, such that measurements of the biomarker are correlated to measurements of the internal standards.

Reference values for the quantity of LTBP2 may be established according to known procedures previously employed for other biomarkers.

For example, a reference value of the quantity of LTBP2 for a particular diagnosis, prediction and/or prognosis of given disease or condition as taught herein may be established by determining the quantity of LTBP2 in sample(s) from one individual or from a population of individuals characterised by said particular diagnosis, prediction and/or prognosis of said disease or condition (i.e., for whom said diagnosis, prediction and/or prognosis of pulmonary inflammation holds true). Such population may comprise without limitation \( \geq 2, \geq 10, \geq 100, \) or even several hundreds or more individuals.
Hence, by means of an illustrative example, reference values of the quantity of LTBP2 for the
diagnoses of a given disease or condition as taught herein vs. no such disease or condition
may be established by determining the quantity of LTBP2 in sample(s) from one individual or
from a population of individuals diagnosed (e.g., based on other adequately conclusive means,
such as, for example, clinical signs and symptoms, imaging, ECG, etc.) as, respectively,
having or not having said disease or condition.

In an embodiment, reference value(s) as intended herein may convey absolute quantities of
LTBP2. In another embodiment, the quantity of LTBP2 in a sample from a tested subject may
be determined directly relative to the reference value (e.g., in terms of increase or decrease, or
fold-increase or fold-decrease). Advantageously, this may allow the comparison of the quantity
of LTBP2 in the sample from the subject with the reference value (in other words to measure
the relative quantity of LTBP2 in the sample from the subject vis-a-vis the reference value) without
the need first to determine the respective absolute quantities of LTBP2.

The expression level or presence of a biomarker in a sample of a patient may sometimes
fluctuate, i.e. increase or decrease significantly without change (appearance of, worsening or
improving of) symptoms. In such an event, the marker change precedes the change in
symptoms and becomes a more sensitive measure than symptom change. Therapeutic
intervention can be initiated earlier and be more effective than waiting for deteriorating
symptoms. Early intervention at a more benign status may be carried out safely at home,
which is a major improvement from treating seriously deteriorated patients in the emergency
room.

Measuring the LTBP2 level of the same patient at different time points may in such a case
thus enable the continuous monitoring of the status of the patient and may lead to prediction of
worsening or improvement of the patient’s condition with regard to a given disease or condition
as taught herein. A home or clinical test kit or device as indicated herein can be used for this
continuous monitoring. One or more reference values or ranges of LTBP2 levels linked to a
certain disease state (e.g. pulmonary inflammation or no pulmonary inflammation) for such a
test can e.g. be determined beforehand or during the monitoring process over a certain period
of time in said subject. Alternatively, these reference values or ranges can be established
through data sets of several patients with highly similar disease phenotypes, e.g. from healthy
subjects or subjects not having the disease or condition of interest. A sudden deviation of the
LTBP2 levels from said reference value or range can predict the worsening of the condition of
the patient (e.g. at home or in the clinic) before the (often severe) symptoms actually can be
felt or observed.

Also disclosed is thus a method or algorithm for determining a significant change in the level of
the LTBP2 marker in a certain patient, which is indicative for change (worsening or improving)
In clinical status. In addition, the invention allows establishing the diagnosis that the subject is recovering or has recovered from a given disease or condition as taught herein.

In an embodiment the present methods may include a step of establishing such reference value(s). In an embodiment, the present kits and devices may include means for establishing a reference value of the quantity of LTBP2 for a particular diagnosis, prediction and/or prognosis of a given disease or condition as taught herein. Such means may for example comprise one or more samples (e.g., separate or pooled samples) from one or more individuals characterised by said particular diagnosis, prediction and/or prognosis of said disease or condition.

The various aspects and embodiments taught herein may further entail finding a deviation or no deviation between the quantity of LTBP2 measured in a sample from a subject and a given reference value.

A "deviation" of a first value from a second value may generally encompass any direction (e.g., increase: first value > second value; or decrease: first value < second value) and any extent of alteration.

For example, a deviation may encompass a decrease in a first value by, without limitation, at least about 10% (about 0.9-fold or less), or by at least about 20% (about 0.8-fold or less), or by at least about 30% (about 0.7-fold or less), or by at least about 40% (about 0.6-fold or less), or by at least about 50% (about 0.5-fold or less), or by at least about 60% (about 0.4-fold or less), or by at least about 70% (about 0.3-fold or less), or by at least about 80% (about 0.2-fold or less), or by at least about 90% (about 0.1-fold or less), relative to a second value with which a comparison is being made.

For example, a deviation may encompass an increase of a first value by, without limitation, at least about 10% (about 1.1-fold or more), or by at least about 20% (about 1.2-fold or more), or by at least about 30% (about 1.3-fold or more), or by at least about 40% (about 1.4-fold or more), or by at least about 50% (about 1.5-fold or more), or by at least about 60% (about 1.6-fold or more), or by at least about 70% (about 1.7-fold or more), or by at least about 80% (about 1.8-fold or more), or by at least about 90% (about 1.9-fold or more), or by at least about 100% (about 2-fold or more), or by at least about 150% (about 2.5-fold or more), or by at least about 200% (about 3-fold or more), or by at least about 500% (about 6-fold or more), or by at least about 700% (about 8-fold or more), or like, relative to a second value with which a comparison is being made. The examples section shows that in the experiments done, the increase in LTBP2 levels between 30-day survivors and pulmonary non-survivals is about 6 fold, i.e. lies within the range of 4.5 - 7 fold, preferably 5.5 - 6.5 fold.
The one-year survival score indicates that the LTBP2 levels show an increase in pulmonary non-survivors versus survivors, of about 5.2-fold, i.e. by about 4 to 6-fold. In essence, the invention thus shows that LTBP2 values are generally elevated by at least 2-fold, preferably at least 3-fold, more preferably at least 4-fold in pulmonary non-survivors vs. survivors.

Preferably, a deviation may refer to a statistically significant observed alteration. For example, a deviation may refer to an observed alteration which falls outside of error margins of reference values in a given population (as expressed, for example, by standard deviation or standard error, or by a predetermined multiple thereof, e.g., ±1xSD or ±2xSD, or ±1xSE or ±2xSE). Deviation may also refer to a value falling outside of a reference range defined by values in a given population (for example, outside of a range which comprises >40%, >50%, >60%, >70%, >75% or >80% or >85% or >90% or >95% or even >100% of values in said population).

In a further embodiment, a deviation may be concluded if an observed alteration is beyond a given threshold or cut-off. Such threshold or cut-off may be selected as generally known in the art to provide for a chosen sensitivity and/or specificity of the diagnosis, prediction and/or prognosis methods, e.g., sensitivity and/or specificity of at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 85%, or at least 90%, or at least 95%.

For example, in an embodiment, an elevated quantity of LTBP2 in the sample from the subject - preferably at least about 1.1-fold elevated, or at least about 1.2-fold elevated, more preferably at least about 1.3-fold elevated, even more preferably at least about 1.4-fold elevated, yet more preferably at least about 1.5-fold elevated, such as between about 1.1-fold and 3-fold elevated or between about 1.5-fold and 2-fold elevated - compared to a reference value representing the prediction or diagnosis of no given disease or condition as taught herein or representing a good prognosis for said disease or condition indicates that the subject has or is at risk of having said disease or condition or indicates a poor prognosis for the disease or condition in the subject. Said elevated quantity of LTBP2 in the sample of the subject is typically indicative of an increased risk of obtaining or developing a pulmonary condition, possibly leading to irreversible pulmonary injury or dysfunction and in the worst case to pulmonary death.

When a deviation is found between the quantity of LTBP2 in a sample from a subject and a reference value representing a certain diagnosis, prediction and/or prognosis of a given disease or condition as taught herein, said deviation is indicative of or may be attributed to the conclusion that the diagnosis, prediction and/or prognosis of said disease or condition in said subject is different from that represented by the reference value.
When no deviation is found between the quantity of LTBP2 in a sample from a subject and a reference value representing a certain diagnosis, prediction and/or prognosis of a given disease or condition as taught herein, the absence of such deviation is indicative of or may be attributed to the conclusion that the diagnosis, prediction and/or prognosis of said disease or condition in said subject is substantially the same as that represented by the reference value.

The above considerations apply analogously to biomarker profiles.

When two or more different biomarkers are determined in a subject, their respective presence, absence and/or quantity may be together represented as a biomarker profile, the values for each measured biomarker making a part of said profile. As used herein, the term "profile" includes any set of data that represents the distinctive features or characteristics associated with a condition of interest, such as with a particular diagnosis, prediction and/or prognosis of a given disease or condition as taught herein. The term generally encompasses *inter alia* nucleic acid profiles, such as for example genotypic profiles (sets of genotypic data that represents the genotype of one or more genes associated with a condition of interest), gene copy number profiles (sets of gene copy number data that represents the amplification or deletion of one or more genes associated with a condition of interest), gene expression profiles (sets of gene expression data that represents the mRNA levels of one or more genes associated with a condition of interest), DNA methylation profiles (sets of methylation data that represents the DNA methylation levels of one or more genes associated with a condition of interest), as well as protein, polypeptide or peptide profiles, such as for example protein expression profiles (sets of protein expression data that represents the levels of one or more proteins associated with a condition of interest), protein activation profiles (sets of data that represents the activation or inactivation of one or more proteins associated with a condition of interest), protein modification profiles (sets of data that represents the modification of one or more proteins associated with a condition of interest), protein cleavage profiles (sets of data that represent the proteolytic cleavage of one or more proteins associated with a condition of interest), as well as any combinations thereof.

Biomarker profiles may be created in a number of ways and may be the combination of measurable biomarkers or aspects of biomarkers using methods such as ratios, or other more complex association methods or algorithms (e.g., rule-based methods). A biomarker profile comprises at least two measurements, where the measurements can correspond to the same or different biomarkers. A biomarker profile may also comprise at least three, four, five, 10, 20, 30 or more measurements. In one embodiment, a biomarker profile comprises hundreds, or even thousands, of measurements.

Hence, for example, distinct reference profiles may represent the prediction of a risk (e.g., an abnormally elevated risk) of having a given disease or condition vs. the prediction of no or
normal risk of having said disease or condition. In another example, distinct reference profiles may represent predictions of differing degrees of risk of having said disease or condition.

In a further example, distinct reference profiles can represent the diagnosis of a given disease or condition as taught herein vs. the diagnosis no such disease or condition (such as, e.g., the diagnosis of healthy, recovered from said disease or condition, etc.). In another example, distinct reference profiles may represent the diagnosis of said disease or condition of varying severity.

In a yet another example, distinct reference profiles may represent a good prognosis for a disease or condition as taught herein vs. a poor prognosis for said disease or condition. In a further example, distinct reference profiles may represent varyingly favourable or unfavourable prognoses for such disease or condition.

Reference profiles used herein may be established according to known procedures previously employed for other biomarkers.

For example, a reference profile of the quantity of LTBP2 and the presence or absence and/or quantity of one or more other biomarkers for a particular diagnosis, prediction and/or prognosis of a given disease or condition as taught herein may be established by determining the profile in sample(s) from one individual or from a population of individuals characterised by said particular diagnosis, prediction and/or prognosis of said disease or condition (i.e., for whom said diagnosis, prediction and/or prognosis of said disease or condition holds true). Such population may comprise without limitation ≥ 2, ≥ 10, ≥ 100, or even several hundreds or more individuals. Said additional biomarkers have been defined elsewhere in the text as being indicative for pulmonary inflammation or other pulmonary injury of dysfunction conditions, or can alternatively be kidney or heart related.

Hence, by means of an illustrative example, reference profiles for the diagnoses of a given disease or condition as taught herein vs. no such disease or condition may be established by determining the biomarker profiles in sample(s) from one individual or from a population of individuals diagnosed as, respectively, having or not having said disease or condition.

In an embodiment the present methods may include a step of establishing such reference profile(s). In an embodiment, the present kits and devices may include means for establishing a reference profile for a particular diagnosis, prediction and/or prognosis of a given disease or condition as taught herein. Such means may for example comprise one or more samples (e.g., separate or pooled samples) from one or more individuals characterised by said particular diagnosis, prediction and/or prognosis of said disease or condition.
Further, art-known multi-parameter analyses may be employed mutatis mutandis to determine deviations between groups of values and profiles generated there from (e.g., between sample and reference biomarker profiles).

When a deviation is found between the sample profile and a reference profile representing a certain diagnosis, prediction and/or prognosis of a given disease or condition as taught herein, said deviation is indicative of or may be attributed to the conclusion that the diagnosis, prediction and/or prognosis of said disease or condition in said subject is different from that represented by the reference profile.

When no deviation is found between the sample profile and a reference profile representing a certain diagnosis, prediction and/or prognosis of a given disease or condition as taught herein, the absence of such deviation is indicative of or may be attributed to the conclusion that the diagnosis, prediction and/or prognosis of said disease or condition in said subject is substantially the same as that represented by the reference profile.

The present invention further provides kits or devices for the diagnosis, prediction, prognosis and/or monitoring of any one disease or condition as taught herein comprising means for detecting the level of the LTBP2 marker in a sample of the patient. In a more preferred embodiment, such a kit or kits of the invention can be used in clinical settings or at home. The kit according to the invention may be used for diagnosing said disease or condition, for monitoring the effectiveness of treatment of a subject suffering from said disease or condition with an agent, or for preventive screening of subjects for the occurrence of said disease or condition in said subject.

In a clinical setting, the kit or device may be in the form of a bed-side device or in an emergency team setting, e.g. as part of the equipment of an ambulance or other moving emergency vehicle or team equipment or as part of a first-aid kit. The diagnostic kit or device may assist a medical practitioner, a first aid helper, or nurse to decide whether the patient under observation is developing an acute heart failure, after which appropriate action or treatment can be performed.

A home-test kit gives the patient a readout which he can communicate to a medicinal practitioner, a first aid helper or to the emergency department of a hospital, after which appropriate action can be taken. Such a home-test device is of particular interest for people having either a history of, or are at risk of suffering from any one disease or condition as taught herein or have a history or are at risk of suffering from dyspnea. Such subjects with a high risk for a disease or condition as taught herein or having a history of dyspnea could certainly benefit from having a home test device or kit according to the invention at home, inter alia because they can then easily distinguish between a pulmonary inflammation event and
another event causing the dyspnea, resulting in an easier way of determining the actions to be taken to resolve the problem.

Typical kits or devices according to the invention comprise the following elements:

a) a means for obtaining a sample from the subject

5  b) a means or device for measuring the amount of the LTBP2 marker in said sample and visualizing whether the amount of the LTBP2 marker in said sample is below or above a certain threshold level or value, indicating whether the subject is suffering from a given disease or condition as taught herein or not.

In any of the embodiments of the invention, the kits or devices may additionally comprise c)

means for communicating directly with a medical practitioner, an emergency department of the hospital or a first aid post, indicating that a person is suffering from said disease or condition or not.

The term "threshold level or value" or "reference value" is used interchangeably as a synonym and is as defined herein. It may also be a range of base-line (e.g. "dry weight") values determined in an individual patient or in a group of patients with highly similar disease conditions.

In any of the embodiments of the invention, the device or kit or kits of the invention can additionally comprise means for detecting the level of an additional marker in the sample of said patient. Additional markers could for example be creatinine (i.e., serum creatinine clearance), Cystatin C and neutrophil gelatinase-associated lipocalin (NGAL), beta-trace protein, kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18) or proinflammatory cytokines, interferon gamma, IL-2, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), TGF-beta, IL 8 (CXCL1), IL-6, IL-18, macrophage inflammatory protein (MIP)-2, monocyte chemoattractant protein (MCP)-1, IL-1beta, IL-1 alpha, TNF-alpha, and fragments or precursors of any one thereof. Other markers include: Fractalkine (CX3CL1), CRP, procalcitonin, white bloodcell count, one or more natriuretic peptides, and fragments or precursors of any one thereof.

Any of kits as defined herein may be used as a bed-side device for use by the subject himself or by a clinical practitioner.

Non-limiting examples are: systems comprising specific binding molecules for said one or more markers attached to a solid phase, e.g. lateral flow strips or dipstick devices and the like well known in the art. One non-limiting example to perform a biochemical assay is to use a test-strip and labelled antibodies which combination does not require any washing of the membrane. The test strip is well known, for example, in the field of pregnancy testing kits where an anti-hCG antibody is present on the support, and is carried complexed with hCG by
the flow of urine onto an immobilised second antibody that permits visualisation. Other non-limiting examples of such home test devices, systems or kits can be found for example in the following U.S. patents: 6,107,045, 6,974,706, 5,108,889, 6,027,944, 6,482,156, 6,511,814, 5,824,268, 5,726,010, 6,001,658 or U.S. patent applications: 2008/0090305 or 2003/0109067.

In a preferred embodiment, the invention provides a lateral flow device or dipstick. Such dipstick comprises a test strip allowing migration of a sample by capillary flow from one end of the strip where the sample is applied to the other end of such strip where presence of an analyte in said sample is measured. In another embodiment, the invention provides a device comprising a reagent strip. Such reagent strip comprises one or more test pads which when wetted with the sample, provide a colour change in the presence of an analyte and/or indicate the concentration of the protein in said sample.

In order to obtain a semi-quantitative test strip in which only a signal is formed once the level of any one or more markers in the sample is higher than a certain predetermined threshold level or value, a predetermined amount of fixed capture antibodies for LTBP2 or a fragment thereof can be present on the test strip. This enables the capture of a certain amount of LTBP2 or a fragment thereof present in the sample, corresponding to the threshold level or value as predetermined. The remaining amount of LTBP or a fragment thereof (if any) bound by e.g. a conjugated or labelled binding molecules can then be allowed to migrate to a detection zone which subsequently only produces a signal if the level of said one or more biomarkers in the sample is higher than the predetermined threshold level or value.

Another possibility to determine whether the amount of the LTBP2 protein in the sample is below or above a certain threshold level or value, is to use a primary capturing antibody capturing all LTBP2 protein present in the sample, in combination with a labeled secondary antibody, developing a certain signal or colour when bound to the solid phase. The intensity of the colour or signal can then either be compared to a reference colour or signal chart indicating that when the intensity of the signal is above a certain threshold signal, the test is positive (i.e. pulmonary inflammation is imminent). Alternatively, the amount or intensity of the colour or signal can be measured with an electronic device comprising e.g. a light absorbance sensor or light emission meter, resulting in a numerical value of signal intensity or color absorbance formed, which can then be displayed to the subject in the form of a negative result if said numerical value is below the threshold value or a positive result if said numerical value is above the threshold value. This embodiment is of particular relevance in monitoring the LTBP2 level in a patient over a period of time.

The reference value or range can e.g. be determined using the home device in a period wherein the subject is free of a given disease or condition, giving the patient an indication of his base-line LTBP2 level. Regularly using the home test device will thus enable the subject to
notice a sudden change in LTBP2 levels as compared to the base-line level, which can enable him to contact a medical practitioner.

Alternatively, the reference value can be determined in the subject suffering from a given disease or condition as taught herein, which then indicates his personal LTBP2 "risk level", i.e. the level of LTBP2 which indicates he is or will soon be exposed to said disease or condition. This risk level is interesting for monitoring the disease progression or for evaluating the effect of the treatment. Reduction of the LTBP2 level as compared to the risk level indicates that the condition of the patient is improving.

Furthermore, the reference value or level can be established through combined measurement results in subjects with highly similar disease states or phenotypes (e.g. all having no disease or condition as taught herein or having said disease or condition).

Non-limiting examples of such semi-quantitative tests known in the art, the principle of which could be used for the home test device according to the present invention are the HIV/AIDS test or Prostate Cancer tests sold by Sanitoets. The home prostate test is a rapid test intended as an initial semi-quantitative test to detect PSA blood levels higher than 4 ng/ml in whole blood. The typical home self-test kit comprises the following components: a test device to which the blood sample is to be administered and which results in a signal when the protein level is above a certain threshold level, an amount of diluent e.g. in dropper pipette to help the transfer of the analytes (i.e. the protein of interest) from the sample application zone to the signal detection zone, optionally an empty pipette for blood specimen collection, a finger pricking device, optionally a sterile swab to clean the area of pricking and instructions of use of the kit.

Similar tests are also known for e.g. breast cancer detection and CRP-protein level detection in view of cardiac risk home tests. The latter test encompasses the sending of the test result to a laboratory, where the result is interpreted by a technical or medical expert. Such telephone or internet based diagnosis of the patient's condition is of course possible and advisable with most of the kits, since interpretation of the test result is often more important than conducting the test. When using an electronic device as mentioned above which gives a numerical value of the level of protein present in the sample, this value can of course easily be communicated through telephone, mobile telephone, satellite phone, E-mail, internet or other communication means, warning a hospital, a medicinal practitioner or a first aid team that a person is, or may be at risk of, suffering from pulmonary failure. A non-limiting example of such a system is disclosed in U.S. patent 6,482,156.

The presence and/or concentration of LTBP2 in a sample can be measured by surface plasmon resonance (SPR) using a chip having LTBP2 binding molecule immobilized thereon, fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer
(BRET), fluorescence quenching, fluorescence polarization measurement or other means known in the art. Any of the binding assays described can be used to determine the presence and/or concentration of LTBP2 in a sample. To do so, LTBP2 binding molecule is reacted with a sample, and the concentration of LTBP2 is measured as appropriate for the binding assay being used. To validate and calibrate an assay, control reactions using different concentrations of standard LTBP2 and/or LTBP2 binding molecule can be performed. Where solid phase assays are employed, after incubation, a washing step is performed to remove unbound LTBP2. Bound, LTBP2 is measured as appropriate for the given label (e.g., scintillation counting, fluorescence, antibody-dye etc.). If a qualitative result is desired, controls and different concentrations may not be necessary. Of course, the roles of LTBP2 and LTBP2 binding molecule may be switched; the skilled person may adapt the method so LTBP2 binding molecule is applied to sample, at various concentrations of sample.

A LTBP2 binding molecule according to the invention is any substance that binds specifically to LTBP2. Examples of a LTBP2 binding molecule useful according to the present invention, includes, but is not limited to an antibody, a polypeptide, a peptide, a lipid, a carbohydrate, a nucleic acid, peptide-nucleic acid, small molecule, small organic molecule, or other drug candidate. A LTBP2 binding molecule can be natural or synthetic compound, including, for example, synthetic small molecule, compound contained in extracts of animal, plant, bacterial or fungal cells, as well as conditioned medium from such cells. Alternatively, LTBP2 binding molecule can be an engineered protein having binding sites for LTBP2. According to an aspect of the invention, a LTBP2 binding molecule binds specifically to LTBP2 with an affinity better than $10^{-9}$ M. A suitable LTBP2 binding molecule can be determined from its binding with a standard sample of LTBP2. Methods for determining the binding between LTBP2 binding molecule and LTBP2 are known in the art. As used herein, the term antibody includes, but is not limited to, polyclonal antibodies, monoclonal antibodies, humanised or chimeric antibodies, engineered antibodies, and biologically functional antibody fragments (e.g. scFv, nanobodies, Fv, etc) sufficient for binding of the antibody fragment to the protein. Such antibody may be commercially available antibody against LTBP2, such as, for example, a mouse, rat, human or humanised monoclonal antibody.

In a preferred embodiment, the binding molecule or agent is capable of binding both the mature membrane- or cell-bound LTBP2 protein or fragment. In a more preferred embodiment, the binding agent or molecule is specifically binding or detecting the soluble form, preferably the plasma circulating form of LTBP2, as defined herein.

According to one aspect of the invention, the LTBP2 binding molecule is labelled with a tag that permits detection with another agent (e.g. with a probe binding partner). Such tags can be, for example, biotin, streptavidin, his-tag, myc tag, maltose, maltose binding protein or any...
other kind of tag known in the art that has a binding partner. Example of associations which can be utilised in the probe:binding partner arrangement may be any, and includes, for example biotin:strepavidin, his-tag:metal ion (e.g. N^{2+}), maltose:maltose binding protein.

The specific-binding agents, peptides, polypeptides, proteins, biomarkers etc. in the present kits may be in various forms, e.g., lyophilised, free in solution or immobilised on a solid phase. They may be, e.g., provided in a multi-well plate or as an array or microarray, or they may be packaged separately and/or individually. The may be suitably labelled as taught herein. Said kits may be particularly suitable for performing the assay methods of the invention, such as, e.g., immunoassays, ELISA assays, mass spectrometry assays, and the like.

The term "modulate" generally denotes a qualitative or quantitative alteration, change or variation specifically encompassing both increase (e.g., activation) or decrease (e.g., inhibition), of that which is being modulated. The term encompasses any extent of such modulation.

For example, where modulation effects a determinable or measurable variable, then modulation may encompass an increase in the value of said variable by at least about 10%, e.g., by at least about 20%, preferably by at least about 30%, e.g., by at least about 40%, more preferably by at least about 50%, e.g., by at least about 75%, even more preferably by at least about 100%, e.g., by at least about 150%, 200%, 250%, 300%, 400% or by at least about 500%, compared to a reference situation without said modulation; or modulation may encompass a decrease or reduction in the value of said variable by at least about 10%, e.g., by at least about 20%, by at least about 30%, e.g., by at least about 40%, by at least about 50%, e.g., by at least about 60%, by at least about 70%, e.g., by at least about 80%, by at least about 90%, e.g., by at least about 95%, such as by at least about 96%, 97%, 98%, 99% or even by 100%, compared to a reference situation without said modulation.

Preferably, modulation of the activity and/or level of intended target(s) (e.g., LTBP2 gene or protein) may be specific or selective, i.e., the activity and/or level of intended target(s) may be modulated without substantially altering the activity and/or level of random, unrelated (unintended, undesired) targets.

Reference to the "activity" of a target such as LTBP2 protein may generally encompass any one or more aspects of the biological activity of the target, such as without limitation any one or more aspects of its biochemical activity, enzymatic activity, signalling activity and/or structural activity, e.g., within a cell, tissue, organ or an organism.

In the context of therapeutic or prophylactic targeting of a target, the reference to the "level" of a target such LTBP2 gene or protein may preferably encompass the quantity and/or the
availability (e.g., availability for performing its biological activity) of the target, e.g., within a cell, tissue, organ or an organism.

For example, the level of a target may be modulated by modulating the target’s expression and/or modulating the expressed target. Modulation of the target’s expression may be achieved or observed, e.g., at the level of heterogeneous nuclear RNA (hnRNA), precursor mRNA (pre-mRNA), mRNA or cDNA encoding the target. By means of example and not limitation, decreasing the expression of a target may be achieved by methods known in the art, such as, e.g., by transfecting (e.g., by electroporation, lipofection, etc.) or transducing (e.g., using a viral vector) a cell, tissue, organ or organism with an antisense agent, such as, e.g., antisense DNA or RNA oligonucleotide, a construct encoding the antisense agent, or an RNA interference agent, such as siRNA or shRNA, or a ribozyme or vectors encoding such, etc. By means of example and not limitation, increasing the expression of a target may be achieved by methods known in the art, such as, e.g., by transfecting (e.g., by electroporation, lipofection, etc.) or transducing (e.g., using a viral vector) a cell, tissue, organ or organism with a recombinant nucleic acid which encodes said target under the control of regulatory sequences effecting suitable expression level in said cell, tissue, organ or organism. By means of example and not limitation, the level of the target may be modulated via alteration of the formation of the target (such as, e.g., unfolding, or interactions leading to formation of a complex), and/or the stability (e.g., the propensity of complex constituents to associate to a complex or disassociate from a complex), degradation or cellular localisation, etc. of the target.

The term "antisense" generally refers to a molecule designed to interfere with gene expression and capable of specifically binding to an intended target nucleic acid sequence. Antisense agents typically encompass an oligonucleotide or oligonucleotide analogue capable of specifically hybridising to the target sequence, and may typically comprise, consist essentially of or consist of a nucleic acid sequence that is complementary or substantially complementary to a sequence within genomic DNA, hnRNA, mRNA or cDNA, preferably mRNA or cDNA corresponding to the target nucleic acid. Antisense agents suitable herein may typically be capable of hybridising to their respective target at high stringency conditions, and may hybridise specifically to the target under physiological conditions.

The term "ribozyme" generally refers to a nucleic acid molecule, preferably an oligonucleotide or oligonucleotide analogue, capable of catalytically cleaving a polynucleotide. Preferably, a "ribozyme" may be capable of cleaving mRNA of a given target protein, thereby reducing translation thereof. Exemplary ribozymes contemplated herein include, without limitation, hammer head type ribozymes, ribozymes of the hairpin type, delta type ribozymes, etc. For teaching on ribozymes and design thereof, see, e.g., US 5,354,855, US 5,591,610, Pierce et

The term "pharmaceutically acceptable" as used herein is consistent with the art and means compatible with the other ingredients of a pharmaceutical composition and not deleterious to the recipient thereof.

As used herein, "carrier" or "excipient" includes any and all solvents, diluents, buffers (such as, \textit{e.g.}, neutral buffered saline or phosphate buffered saline), solubilisers, colloids, dispersion media, vehicles, fillers, chelating agents (such as, \textit{e.g.}, EDTA or glutathione), amino acids (such as, \textit{e.g.}, glycine), proteins, disintegrants, binders, lubricants, wetting agents, emulsifiers, sweeteners, colorants, flavourings, aromatisers, thickeners, agents for achieving a depot effect, coatings, antifungal agents, preservatives, antioxidants, tonicity controlling agents, absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active substance, its use in the therapeutic compositions may be contemplated.

The present active substances (agents) may be used alone or in combination with any therapies known in the art for the disease and conditions as taught herein ("combination therapy"). Combination therapies as contemplated herein may comprise the administration of at least one active substance of the present invention and at least one other pharmaceutically or biologically active ingredient. Said present active substance(s) and said pharmaceutically or biologically active ingredient(s) may be administered in either the same or different pharmaceutical formulation(s), simultaneously or sequentially in any order.

The dosage or amount of the present active substances (agents) used, optionally in combination with one or more other active compound to be administered, depends on the
individual case and is, as is customary, to be adapted to the individual circumstances to achieve an optimum effect. Thus, it depends on the nature and the severity of the disorder to be treated, and also on the sex, age, body weight, general health, diet, mode and time of administration, and individual responsiveness of the human or animal to be treated, on the route of administration, efficacy, metabolic stability and duration of action of the compounds used, on whether the therapy is acute or chronic or prophylactic, or on whether other active compounds are administered in addition to the agent(s) of the invention.

Without limitation, depending on the type and severity of the disease, a typical daily dosage might range from about 1 μg/kg to 100 mg/kg of body weight or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. A preferred dosage of the active substance of the invention may be in the range from about 0.05 mg/kg to about 10 mg/kg of body weight. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g., every week or every two or three weeks.

As used herein, a phrase such as "a subject in need of treatment" includes subjects that would benefit from treatment of a given disease or condition as taught herein. Such subjects may include, without limitation, those that have been diagnosed with said condition, those prone to contract or develop said condition and/or those in whom said condition is to be prevented.

The terms "treat" or "treatment" encompass both the therapeutic treatment of an already developed disease or condition, as well as prophylactic or preventative measures, wherein the aim is to prevent or lessen the chances of incidence of an undesired affliction, such as to prevent the chances of contraction and progression of a disease or condition as taught herein. Beneficial or desired clinical results may include, without limitation, alleviation of one or more symptoms or one or more biological markers, diminishment of extent of disease, stabilised (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and the like. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "prophylactically effective amount" refers to an amount of an active compound or pharmaceutical agent that inhibits or delays in a subject the onset of a disorder as being sought by a researcher, veterinarian, medical doctor or other clinician. The term "therapeutically effective amount" as used herein, refers to an amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a subject that is being sought by a researcher, veterinarian, medical doctor or other clinician, which may include *inter alia* alleviation of the symptoms of the disease or condition being treated. Methods are known...
in the art for determining therapeutically and prophylactically effective doses for the present compounds.

The above aspects and embodiments are further supported by the following non-limiting examples.

EXAMPLES

Example 1: MASTerclass targeted protein quantitation for LTBP2

**MASSTERCLASS experimental setup**

MASTerclass assays use targeted tandem mass spectrometry with stable isotope dilution as an end-stage peptide quantitation system (also called Multiple Reaction Monitoring (MRM) and Single Reaction Monitoring (SRM). The targeted peptide is specific (*i.e.*, proteotypic) for the specific protein of interest. *i.e.*, the amount of peptide measured is directly related to the amount of protein in the original sample. To reach the specificity and sensitivity needed for biomarker quantitation in complex samples, peptide fractionations precede the end-stage quantitation step.

A suitable MASTERCLASS assay may include the following steps:

- Plasma/serum sample
- Depletion of human albumin and IgG (complexity reduction on protein level) using affinity capture with anti-albumin and anti-IgG antibodies using ProteoPrep spin columns (Sigma Aldrich)
- Spiking of known amounts of isotopically labelled peptides. This peptide has the same amino acid sequence as the proteotypic peptide of interest, typically with one isotopically labelled amino acid built in to generate a mass difference. During the entire process, the labelled peptide has identical chemical and chromatographic behaviour as the endogenous peptide, except during the end-stage quantitation step which is based on molecular mass.
- Tryptic digest. The proteins in the depleted serum/plasma sample are digested into peptides using trypsin. This enzyme cleaves proteins C-terminally from lysine and arginine, except when a proline is present C-terminally of the lysine or arginine. Before digestion, proteins are denatured by boiling, which renders the protein molecule more accessible for the trypsin activity during the 16h incubation at 37°C.
- First peptide-based fractionation: Free Flow Electrophoresis (FFE; BD Diagnostic) is a gel-free, fluid separation technique in which charged molecules moving in a continuous
laminar flow are separated through an electrical field perpendicular to the flow. The electrical field causes the charged molecules to separate in the pH gradient according to their isoelectric point (pi). Only those fractions containing the monitored peptides are selected for further fractionation and LC-MS/MS analysis. Each peptide of interest elutes from the FFE chamber at a specific fraction number, which is determined during protein assay development using the synthetic peptide homologue. Specific fractions or fraction pools (multiplexing) proceed to the next level of fractionation.

- Second peptide-based fractionation: Phenyl HPLC (XBridge Phenyl; Waters) separates peptides according to hydrophobicity and aromatic nature of amino acids present in the peptide sequence. Orthogonality with the back-end C18 separation is achieved by operating the column at an increased pH value (pH 10). As demonstrated by Gilar et al. 2005, J Sep Sci 28(14): 1694-1703, pH is by far the most drastic parameter to alter peptide selectivity in RP-HPLC. Each peptide of interest elutes from the Phenyl column at a specific retention time, which is determined during protein assay development using the synthetic peptide homologue. The use of an external control system, in which a mixture of 9 standard peptides is separated upfront a batch of sample separations, allows adjusting the fraction collection in order to correct for retention time shifts. The extent of fractionation is dependent on the concentration of the protein in the sample and the complexity of that sample.

- LC-MS/MS based quantitation, including further separation on reversed phase (C18) nanoLC (PepMap C18; Dionex) and MS/MS: tandem mass spectrometry using MRM (4000 QTRAP; ABI)/SRM (Vantage TSQ; Thermo Scientific) mode. The LC column is connected to an electrospray needle connected to the source head of the mass spectrometer. As material elutes from the column, molecules are ionized and enter the mass spectrometer in the gas phase. The peptide that is monitored is specifically selected to pass the first quadrupole (Q1), based on its mass to charge ratio (m/z). The selected peptide is then fragmented in a second quadrupole (Q2) which is used as a collision cell. The resulting fragments then enter the third quadrupole (Q3). Depending on the instrument settings (determined during the assay development phase) only a specific peptide fragment or specific peptide fragments (or so called transitions) are selected for detection.

- The combination of the m/z of the monitored peptide and the m/z of the monitored fragment of this peptide is called a transition. This process can be performed for multiple transitions during one experiment. Both the endogenous peptide (analyte) and its corresponding isotopically labelled synthetic peptide (internal standard) elute at the same retention time, and are measured in the same LC-MS/MS experiment.
- The MASSterclass readout is defined by the ratio between the area under the peak specific for the analyte and the area under the peak specific for the synthetic isotopically labelled analogue (internal standard). MASSterclass readouts are directly related to the original concentration of the protein in the sample. MASSterclass readouts can therefore be compared between different samples and groups of samples.

A typical MASSTERCLASS protocol followed in the present study is given here below:

- 25 µL of plasma is subjected to a depletion of human albumin and IgG (ProteoPrep spin columns; Sigma Aldrich) according to the manufacturer’s protocol, except that 20 mM NH₄HCO₃ was used as the binding/equilibration buffer.

- The depleted sample (225 µL) is denatured for 15 min at 95°C and immediately cooled on ice.

- 500 fmol of the isotopically labelled peptide (custom made ‘Heavy AQUA’ peptide; Thermo Scientific) is spiked in the sample.

- 20 µg trypsin is added to the sample and digestion is allowed for 16 h at 37°C.

- The digested sample was first diluted 1/8 in solvent A (0.1 % formic acid) and then 1/20 in the same solvent containing 250 amol/µL of all isotopically labelled peptides (custom made ‘Heavy AQUA’ peptide; Thermo Scientific) of interest.

- 20 µL of the final dilution was separated using reverse-phase NanoLC with on-line MS/MS in MRM/SRM mode:

  - Column: PepMap C18, 75 µm I.D. x 25 cm L, 100Å pore diameter, 5 µm particle size

  - Solvent A: 0.1 % formic acid

  - Solvent B: 80% acetonitrile, 0.1 % formic acid

  - Gradient: 30 min; 2%-55% Solvent B

- MS/MS in MRM mode: method contains the transitions for the analyte as well as for the synthetic, labelled peptide.

  - The used transitions were experimentally determined and selected during protein assay development.

  - Each of the transitions of interest was measured for a period starting 3 minutes before and ending 3 minutes after the determined retention time of the peptide of interest, making sure that each peak had at least 15 datapoints.
The raw data was analysed and quantified using the LCQuan software (Thermo Scientific): the area under the analyte (= the LTBP2 peptide) peak and under the internal standard (the labelled, synthetic LTBP2 peptide) peak at the same C18 retention time was determined by automatic peak detection. These were cross-checked manually.

MASSTERCLASS output

The measured ratios are differential quantitations of peptides. In other words a ratio is the normalised concentration of a peptide. The concentration of a peptide is proportional to the ratio measured in the mass spectrometer.

Example 2: LTBP2 as a biomarker for pulmonary death in patients with acute dyspnea

Study Population

The study population consisted of unselected patients presenting to the emergency department of the University Hospital of Basel, Switzerland, with a chief complaint of acute dyspnea. From April 2006 to March 2007, 292 patients (out of 327 patients screened) were prospectively enrolled. Exclusion criteria were age younger than 18 years, an obvious traumatic cause of dyspnea and patients on haemodialysis. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from all participating patients.

Clinical evaluation and follow-up

Patients underwent an initial clinical assessment including clinical history, physical examination, electrocardiogram, pulse oximetry, blood tests including BNP, and chest X-ray. Echocardiography, pulmonary function tests and other diagnostic tests like CT-angiography were performed according to the treating physician. CT-angiography was the imaging modality of choice in patients with suspected pulmonary embolism. To assess the dyspnea severity we used the NYHA (New York Heart Association) functional classification with NYHA II as "dyspnea while walking up a slight incline", III as "dyspnea while walking on level ground" and IV as "dyspnea at rest".

Two independent internists blinded to LTBP2 reviewed all medical records including BNP levels and independently classified the patient's primary diagnosis into seven categories: acute heart failure (AHF), acute exacerbation of chronic obstructive pulmonary disease, pneumonia, acute complications of malignancy, acute pulmonary embolism, hyperventilation, and others. The two internists also independently adjudicated the cause of death. In the event of diagnostic disagreement among the internist reviewers, they were asked to meet to come to
a common conclusion. In the event that they were unable to come to a common conclusion, a third-party internist adjudicator was asked to review the data and determine which diagnosis and cause of death was the most accurate.

The endpoint of the present study was 30-day cause specific mortality. 30-day all-cause mortality, one-year cause specific mortality and one-year all cause mortality were assessed as secondary endpoints. Cardiac death was defined as death due to coronary artery disease, heart failure or arrhythmias. Pulmonary death was defined as death due to acute exacerbations of chronic obstructive pulmonary disease, pneumonia and asthma. Each patient was contacted for follow-up, via telephone, by a single trained researcher after 365 days. In case the patient could not be reached referring physicians and relatives were contacted or the administrative databases of respective hometowns were reviewed to assess the survival status. Of note, one patient was lost to follow-up, so mortality analyses were performed in 291 patients.

Laboratory Measurements

Blood samples for determination of LTBP2, BNP and NT-proBNP were collected at presentation into tubes containing potassium EDTA. After centrifugation, samples were frozen at -80°C until assayed in a blinded fashion in a single batch. NT-proBNP levels were determined in a blinded fashion by a quantitative electrochemiluminescence immunoassay with CVs claimed by the manufacturer were 1.8% to 2.7% and 2.35% to 3.2% for within-run and total imprecision, respectively (Elecsys proBNP, Roche Diagnostics AG, Zug, Switzerland) and BNP was measured by a microparticle enzyme immunoassay at the hospital laboratory with a CVs claimed by the manufacturer of 4.3% to 6.3% and 6.5% to 9.4% for within-run and total imprecision, respectively. (AxSym, Abbott Laboratories, Abbott Park/IL, USA).

Statistical Analysis

Continuous variables are presented as mean ±SD or median (with interquartile range), and categorical variables as numbers and percentages. Univariate data on demographic and clinical features were compared by Mann-Whitney U test or Fisher’s exact test as appropriate. Correlations among continuous variables were assessed by the Spearman rank-correlation coefficient. Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of LTBP2, NT-proBNP and BNP to predict death. Areas under the curve (AUCs) were calculated for all markers. AUCs were compared according to the method by Hanley and McNeil. Cox regression analysis was assessed by univariate and multivariate analysis to identify independent predictors of outcome. Multivariable analysis, included all significant candidate variables (p<0.05) established in univariate analysis. The Kaplan-Meier cumulative survival curves were compared by the log-rank test. Glomerular filtration rate was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) formula. Data were
statistically analysed with SPSS 15.0 software (SPSS Inc, Chicago, Illinois, USA) and the MedCalc 9.3.9.0 package (MedCalc Software, Mariakerke, Belgium). All probabilities were two tailed and \( p < 0.05 \) was regarded as significant.

**Patient characteristics**

The baseline characteristics of the 292 patients presenting with acute dyspnea are described in Table 1. Overall, mean age was 74 ± 12 years (median 77 years, interquartile range (IQR) 68-83 years), 52% were men and 80% were in NYHA functional class III and IV. The primary diagnosis was AHF in 158 (54%) patients, acute exacerbation of chronic obstructive pulmonary disease in 57 (20%) patients, pneumonia in 32 (11%) patients, acute pulmonary embolism in 8 (3%) patients, acute complications of malignancy in 7 (2%) patients, hyperventilation in 5 (2%) patients, and other causes such as interstitial lung disease, asthma, or bronchitis in 24 (8%) patients.
Table 1: Baseline characteristics divided in patients with and without acute heart failure (AHF)

<table>
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<th>Characteristic</th>
<th>Total (n=292)</th>
<th>AHF (n=158)</th>
<th>No AHF (n=134)</th>
<th>P-value</th>
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<td>Age (years)</td>
<td>74 ±12</td>
<td>78 ±9</td>
<td>68 ±13</td>
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<td>Male sex (%) of patients</td>
<td>52</td>
<td>51</td>
<td>53</td>
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<td>BMI (kg/m²)</td>
<td>26.1 ±6.2</td>
<td>26.6 ±5.9</td>
<td>25.5 ±6.5</td>
<td>0.124</td>
</tr>
</tbody>
</table>

Medical conditions (% of patients)

|                               |              |             |                |          |
|                               | Heart failure| 24          | 40             | 7        | <0.0001  |
|                               | Coronary artery disease | 28          | 38             | 16       | <0.0001  |
|                               | Chronic obstructive pulmonary disease | 34          | 27             | 42       | 0.006    |
|                               | Diabetes     | 18          | 24             | 11       | 0.005    |
|                               | Hypertension | 68          | 78             | 56       | <0.0001  |
|                               | Hyperlipidemia | 29         | 33             | 25       | 0.165    |
|                               | Chronic kidney disease | 28          | 44             | 11       | <0.0001  |

Initial clinical findings

|                               |              |             |                |          |
|                               | Heart rate (bpm) a | 93 ±23      | 93 ±25         | 92 ±19   | 0.495    |
|                               | Systolic pressure (mm Hg) a | 138 ±26     | 135 ±27        | 140 ±25  | 0.098    |

NYHA functional class (% of patients)

|               |              |             |                |          |
|               | II           | 20          | 10             | 32       | <0.0001  |
|               | III          | 40          | 45             | 35       | 0.109    |
|               | IV           | 40          | 45             | 33       | 0.034    |
|               | Edema        | 42          | 57             | 26       | <0.0001  |
|               | Rales        | 54          | 64             | 43       | <0.0001  |

Medication at admission

|                        |              |             |                |          |
|                        | Beta-blockers | 39          | 57             | 17       | <0.0001  |
|                        | ACE-Inhibitors/AT-receptor-blockers | 49          | 62             | 34       | <0.0001  |
|                        | Diuretics    | 52          | 64             | 39       | <0.0001  |

Laboratory findings

|                             |              |             |                |          |
|                             | eGFR - ml/min/1.73m² b | 67 [44-89]  | 54 [36-73]     | 80 [63-112] | <0.0001  |
|                             | BNP (pmol/l) c | 349 [89-121] | 976 [467-1925] | 81 [39-181] | <0.0001  |
|                             | NT-proBNP (pmol/l) b | 1656 [31-4-62] | 5757 [1924-13243] | 300 [76-974] | <0.0001  |

a mean ± SD, b median (IQR=interquartile range), BMI = Body mass index; eGFR = estimated glomerular filtration rate; NYHA = New York Heart Association; BNP = B-type natriuretic peptide ; NT-proBNP = N-terminal pro-B-type natriuretic peptide
LTBP2 concentrations at presentation in patients with dyspnea were strongly correlated to markers of kidney dysfunction (creatinine: r=0.71, p<0.001; cystatin C: r=0.83, p<0.001). BNP (r=0.52, p<0.001) and NT-proBNP (r=0.66, p<0.001). Weaker albeit significant correlations existed with NYHA functional classes (r=0.18, p=0.003) and markers of infection (neutrophile count: r=0.23, p<0.001; C-reactive protein: r=0.13, p=0.04). These correlations were independent of the primary cause of dyspnea and persisted in AHF and non-AHF patients.

LTBP2 levels and prognostic value of LTBP2 on short-term outcome

At 30 days, 29 patients (10%) had died. Non-survivors had significantly higher LTBP2 levels than survivors in the overall population (p<0.001), the AHF subgroup (p<0.001) and patients with dyspnea of pulmonary origin (p=0.01) (Figure 1A). As further shown in Figure 1A, LTBP2 levels were especially elevated in patients dying of pulmonary causes (Survivors: 0.01 normalized level [0.006-0.021] vs. Cardiac death: 0.021 normalized level [0.012-0.028] vs. Pulmonary death: 0.066 normalized level [0.043-0.078]). Contrastingly and as shown in Figure 1B, natriuretic peptide levels did not differ significantly between patients dying of cardiac or pulmonary causes (NT-proBNP: 11941pg/ml [3338-20973] vs. 16195pg/ml [4897-25909]; p=0.39).

Receiver operating characteristic curve analyses were performed to assess the potential of LTBP2 levels to predict all-cause short term mortality. The areas under the curve (AUC) to predict all-cause mortality are for LTBP2 (0.79; 95%CI 70-87), NT-proBNP (0.75; 95%CI 0.65-0.84) and BNP (0.62; 95%CI 0.51-0.73). Cause specific mortality was looked at as well. Receiver operating characteristic curve (ROC) analyses demonstrated an AUC of 0.95 (95% CI 0.91-0.98) for LTBP2 to predict 30 day pulmonary mortality, which was significantly higher than the AUCs observed for NT-proBNP (0.84; 95% CI 0.75-0.94) and BNP (0.63; 95% CI 0.48-0.77) for 30 day pulmonary mortality (p=0.04 and <0.001, respectively).

LTBP2 levels and prognostic value of LTBP2 on one-year outcome

Overall 80 (27%) patients died during the first year of follow up; heart failure (n=28), myocardial infarction (n=14) and pulmonary death (n=14) were the most common causes of death. LTBP2 levels in non-survivors were significantly higher compared to survivors for the overall patient population (p<0.001), AHF patients (p<0.001) and non-AHF (p=0.021) patients. Again, there was a trend towards higher LTBP2 values in patients dying of pulmonary causes (Survivors: 0.01 normalized level [0.0056-0.016] vs. Cardiac death: 0.025 normalized level [0.016-0.037] vs. Pulmonary death: 0.052 normalized level [0.017-0.071]) (Figure 3A). As shown in Figure 3B, natriuretic peptide levels did not separate between causes of death (NT-
proBNP 7785pg/ml [1920-22584] vs. 9757pg/ml [3772-18609]; p=0.52). Mortality according to LTBP2 level deciles is depicted in Figure 4.

Receiver operating characteristic curve analyses were performed to assess the potential of LTBP2 levels to predict all-cause and cause specific one-year mortality. Importantly, the prognostic potential of LTBP2 (AUC 0.77; 95%CI 0.70-0.83) was comparable to NT-proBNP (AUC 0.77; 95%CI 0.71-0.84) and BNP (AUC 0.71; 95%CI 0.64-0.79) for the prediction of all-cause and cardiac mortality AUC 0.77, 0.79, 0.80, respectively) and tended to be superior for the prediction of pulmonary death AUC 0.80, 0.75, 0.59, respectively; p vs. NT-proBNP 0.59, p vs. BNP 0.04). Importantly, the predictive potential of LTBP2 was independent of kidney dysfunction and persisted in patients with preserved kidney function (AUC 0.77, 95%CI 0.70-0.83).
CLAIMS

1. The use of latent transforming growth factor beta binding protein 2 (LTBP2) as a blood biomarker for the diagnosis, prediction, prognosis and/or monitoring of pulmonary injury in a subject.

2. The use of latent transforming growth factor beta binding protein 2 (LTBP2) as a blood biomarker for assessing the risk of developing severe pulmonary complications such as severe chronic obstructive pulmonary disease (COPD), or pneumonia which if left untreated, could become fatal.

3. The use of latent transforming growth factor beta binding protein 2 (LTBP2) as a blood biomarker for assessing the risk of dying from a pulmonary cause or complication.

4. The use according to claim 1, wherein said diagnosis, prediction, prognosis and/or monitoring of pulmonary injury comprises assessing the degree of pulmonary injury in the subject.

5. The use according to claim 1 or 4, wherein the degree of pulmonary injury is assessed as being:

   (i) no injury,

   (ii) pulmonary injury with reversible damage which can lead to complications when left untreated, or

   (iii) pulmonary injury with potential irreversible or irreparable physiological damage, morbidity or mortality.

6. The use according to any one of claims 1 to 5, performed in combination with using:

   one or more of kidney derived markers selected from the group comprising: creatinine, cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), beta-trace protein, kidney injury molecule 1, ND interleukin-18 (IL-18); and/or

   one or more other biomarkers selected from the group comprising: proinflammatory cytokines, interferon gamma, interleukine-2 (IL-2), interleukine-10 (IL-10), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor-beta (TGF-beta), interleukine-8 (IL-8), interleukine-6 (IL-6), interleukine-18 (IL-18), macrophage inflammatory protein (MIP)-1, monocyte chemoattractant protein (MCP)-1, interleukine-1 beta (IL-1beta), interleukine-1 alpha (IL-1alpha), tumor necrosis factor-alpha (TNF-alfa), serum amyloid A (SAA), Fractalkine (CX3CL1), C-reactive protein (CRP), procalcitonin (PCT), and natriuretic peptides selected from the group comprising: B-type natriuretic peptide (BNP), pro-B-type natriuretic peptide (proBNP), amino terminal pro-B-type natriuretic peptide (NTproBNP); and/or
one or more clinical parameters or examinations selected from the group comprising:
white blood-cell count, clinical history, physical examination, electrocardiogram, pulse
oximetry, blood tests, chest X-ray, echocardiography, pulmonary function tests,
computed tomography (CT)-angiography and determining thoracic impedance of the
subject.

7. A method for the diagnosis, prediction, prognosis and/or monitoring of pulmonary injury
in a subject, wherein the examination phase of the method comprises measuring the
quantity of LTBP2 in a blood sample from the subject.

8. The method according to claim 7, wherein said diagnosis, prediction, prognosis and/or
monitoring of pulmonary injury comprises assessing the degree of pulmonary injury in
the subject.

9. The method according to claim 7 or 8, wherein the degree of pulmonary injury is
assessed as being:
(i) no injury,
(ii) pulmonary injury with reversible damage which can lead to complications when
left untreated, or
(iii) pulmonary injury with potential irreversible or irreparable physiological damage,
morbidity or mortality.

10. A method for assessing or predicting the risk of developing severe pulmonary
complications such as severe COPD, pneumonia, or pulmonary death in a subject,
wherein the examination phase of the method comprises measuring the quantity of
LTBP2 in a blood sample from the subject.

11. A method for assessing the risk of dying from a pulmonary cause or complication in a
subject wherein the examination phase of the method comprises measuring latent
transforming growth factor beta binding protein 2 (LTBP2) in a blood sample of the
subject.

12. The method according to any one of claims 7 to 11, wherein the examination phase of
the method further comprises:
measuring in the blood sample from the subject the quantity of one or more of kidney
derived markers selected from the group comprising: creatinine, cystatin C, neutrophil
gelatinase-associated lipocalin (NGAL), beta-trace protein, kidney injury molecule 1,
ND interleukin-18 (IL-18); and/or
measuring in the blood sample from the subject the quantity of one or more
inflammatory biomarkers selected from the group comprising: proinflammatory
cytokines, interferon gamma, interleukine-2 (IL-2), interleukine-10 (IL-10), granulocyte-
macrophage colony-stimulating factor (GM-CSF), transforming growth factor-beta (TGF-beta), interleukine-8 (IL-8), interleukine-6 (IL-6), interleukine-18 (IL-18), macrophage inflammatory protein (MIP)-2, monocyte chemoattractant protein (MCP)-1, interleukine-1 beta (IL-1beta), interleukine-1 alpha (IL-1 alfa), tumor necrosis factor-alpha (TNF-alfa), serum amyloid A (SAA), Fractalkine (CX3CL1), C-reactive protein (CRP), procalcitonin (PCT), and natriuretic peptides selected from the group comprising: B-type natriuretic peptide (BNP), pro-B-type natriuretic peptide (proBNP), amino terminal pro-B-type natriuretic peptide (NTproBNP); and/or

analyzing one or more of the clinical parameters selected from the group comprising: white blood-cell count, clinical history, physical examination, electrocardiogram, pulse oximetry, blood tests, chest X-ray, echocardiography, pulmonary function tests, computed tomography (CT)-angiography and determining thoracic impedance of the subject.

The method according to any one of claims 7 to 12, wherein the examination phase of the method further comprises measuring the quantity of one or more natriuretic peptide selected from BNP, proBNP and NTproBNP in the blood sample from the subject.

The method according to any one of claims 9 to 13, wherein the examination phase of the method further comprises one or more of determining clinical history, physical examination, electrocardiogram, pulse oximetry, blood tests, chest X-ray, echocardiography, pulmonary function tests, CT-angiography and/or determining thoracic impedance of the subject.

The method according to any one of claims 8 to 14, wherein the examination phase of the method further comprises measuring the quantity of other markers indicating a reduction of pulmonary inflammation in the subject.

The use or method according to any one of claims 1 to 15, wherein the pulmonary injury is caused by inflammatory substances generated in another organ such as those generated upon acute kidney injury or reperfusion injury of the heart or brain, myocard or other organ infarction, organ perfusion impairment by thrombosis, emboli or mechanical occlusion or Acute Heart Failure.

The use or method according to any one of claims 1 to 16, wherein the pulmonary injury is pneumonia.

The use or method according to any one of claims 1 to 17, wherein said diagnosis, prediction, prognosis and/or monitoring pulmonary injury comprises distinguishing subjects with favourable outcome from subjects with pulmonary injury such as: lung infarction, loss of functional lung tissue, emphysema, lung fibrosis, atelectasis, pleuritis, or pulmonary hypertension complications.
19. The use or the method according to claims 1 to 18, wherein said diagnosis, prediction, prognosis and/or monitoring pulmonary injury comprises distinguishing subjects with favourable outcome from subjects with active ongoing lung fibrosis.

20. The use or the method according to claims 1 to 19, wherein said diagnosis, prediction, prognosis and/or monitoring pulmonary injury comprises distinguishing subjects with favourable outcome from subjects with different degrees of lung fibrosis.

21. The use or method according to any one of claims 1 to 20, for determining and/or steering the therapeutic intervention in the subject.

22. The use or method according to any one of claims 1 to 21, for assessing the impact of the therapeutic intervention.

23. The method according to any one of claims 1 to 22, wherein said subject is a critically ill subject selected from the group consisting of patients presenting in intensive care units (ICU) or emergency departments (ED) with one or more of: serious trauma, systemic inflammatory response syndrome (SIRS), sepsis; severe sepsis, sepsis with organ dysfunction, septic shock, chronic obstructive pulmonary disease (COPD) with or without an acute exacerbation, patients having undergone surgery and more particularly cardiac surgery, complications from surgery, medical shock, bacterial, fungal or viral infections, Acute Respiratory Distress Syndrome (ARDS), pulmonary and systemic inflammation, pulmonary endothelial and epithelial injury, dyspnea, acute dyspnea, severe pneumonia, respiratory failure, acute respiratory failure, respiratory distress, acute or chronic heart failure, poisoning and intoxication, severe allergic reactions and anaphylaxis, burn injury, and any condition for which the patient requires mechanical ventilation.

24. The use or method according to any one of claims 1 to 23, wherein said sample is blood, serum or plasma.
Figure 1

SEQ ID NO.1

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SEQ ID NO.2

EQDAPVAGLQPVER
A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N 33/68

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

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

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

EPO-Internal, WPI Data, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search: 28 February 2013

Date of mailing of the international search report: 22/03/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

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Fax: (+31-70) 340-3016

Authorized officer

Moreno de Vega, C

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