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(54) **METHOD FOR DIAGNOSING ACUTE LUNG INJURY**

(57) The present invention provides a useful examination method and diagnostic reagent, which are capable of diagnosing acute lung injury in clear distinction from chronic progressive interstitial pneumonia. Specifically, the present invention provides a method of determining

whether or not a test subject is affected with acute lung injury, which comprises quantifying heat shock protein 47 in a biological sample derived from the test subject.

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**Description**

## Technical Field

5 **[0001]** The present invention relates to an examination method for the diagnosis of acute lung injury, an acute lung injury diagnostic reagent and the like.

## Background Art

10 **[0002]** Acute lung injury (also referred to as ALI) is an inflammatory disease of the lung, which is caused by a disease that directly or indirectly injures the lung such as sepsis, pneumonia and the like, an injury such as trauma and the like, and/or aspiration and the like. While it shows non-specific symptoms, the main symptom is respiratory distress sometimes accompanied by cough or chest pain. Patients with acute lung injury typically show the above-mentioned symptoms in 24 - 48 hr from the initial injury or disease. In a severe case, it is deadly. The death rate is extremely high and is 30 - 15 40%, which becomes higher due to aging or complication.

**[0003]** Acute lung injury is basically diagnosed by clinical diagnosis. Since clinical findings of acute lung injury is similar to that of acute heart failure and lung infection, to confirm the diagnosis and specify the cause, it is considered that patients should further take ABG (artery blood gas test: measurement of oxygen partial pressure•CO<sub>2</sub> partial pressure, pH and the like of the blood) and chest radiography. However, acute lung injury is a disease that progresses rapidly and shows extremely poor prognosis, for which no effective treatment method has been established. In addition, complication with pneumothorax, severe bacterial infection of lung and the like may be developed. Therefore, an early diagnosis is particularly necessary.

**[0004]** Recently, KL-6 (syallated sugar chain antigen), SP-D (Surfactant protein-D) and SP-A (Surfactant protein-A) are utilized as serum markers for more convenient and rapid diagnoses of acute lung injury. However, these are also 25 markers of known chronic progressive interstitial pneumonia such as cryptogenic organizing pneumonia (COP), idiopathic usual interstitial pneumonia (idiopathic UIP), idiopathic nonspecific interstitial pneumonia (idiopathic NSIP), collagen vascular disease (CVD)-associated usual interstitial pneumonia (collagen vascular disease (CVD)-associated UIP), collagen vascular disease (CVD)-associated non-specific interstitial pneumonia (collagen vascular disease (CVD)-as- 30 sociated NSIP) and the like. There is no known marker having high specificity, which is capable of diagnosing acute lung injury by clearly distinguishing the disease from them.

**[0005]** Heat shock protein 47 (HSP47) is a molecular chaperone having specificity to collagen, and is known as a heat shock-induced stress protein present in the endoplasmic reticulum. Since expression of HSP47 is coupled with the expression of the collagen, a substrate thereof, an important involvement of HSP47 in various fibrosing diseases has been pointed out (patent documents 1 - 3, non-patent documents 6 and 8). In addition, a role of HSP47 in the fibrosing 35 phase of acute lung injury and the like have also been reported (non-patent documents 1 - 3). Promoted expression of HSP47 in a topical lung tissue of lung fibrosing disease has been reported heretofore (non-patent documents 5 and 6). However, HSP47 has been considered to localize in the endoplasmic reticulum and not to leak extracellularly, increase of HSP47 outside the cell (for example, in the blood and the like) in interstitial pneumonia accompanied by fibrosing has not been expected. In fact, in the past reports, it was clear that the serum levels of HSP47 in patients with idiopathic 40 pulmonary fibrosis were similar to those of healthy individuals, and that no increase was observed in patients with idiopathic pulmonary fibrosis (non-patent document 7). It was not at all known or expected that HSP47 markedly increases in acute lung injury, and becomes a useful biomarker that can clearly distinguish acute lung injury from chronic progressive interstitial pneumonia.

45 [Document List]

[patent documents]

**[0006]**

50

patent document 1: JP-A-2008-122276

patent document 2: JP-A-2003-159087

patent document 3: JP-A-2002-522462

55 [non-patent documents]

**[0007]**

non-patent document 1: ICU & CCU, Vol. 33, No. 5, Page.351-359 (2009)  
non-patent document 2: Surgical trauma & immunological responses, Vol. 17, No. 2, Page.62-64 (2008)  
non-patent document 3: The Journal of Japan Society for Clinical Anesthesia, Vol. 23, No. 8, Page.S163 (2003)  
non-patent document 4: Shuukan Igakuno Ayumi, Bessatsu (March) Page.134-136 (2003)  
non-patent document 5: Hum Pathol 31:1498-1505(2000)  
non-patent document 6: Respir Res 6:57(2005)  
non-patent document 7: Biochem Biophys Res Commun 303:413-418(2003)  
non-patent document 8: Annals of the Japanese Respiratory Society, Vol. 40 extra number, Page.188 (2002)

10 SUMMARY OF THE INVENTION

Problems to be Solved by the Invention

15 **[0008]** An object of the present invention is to provide an examination method, and a diagnostic reagent useful for a diagnosis of acute lung injury by clearly distinguishing the same from chronic progressive interstitial pneumonia. Means of Solving the Problems

20 **[0009]** The present inventors have conducted intensive studies in an attempt to solve the aforementioned problems and found that the measurement of the HSP47 protein amount (concentration) in a biological sample is useful for a diagnosis of acute lung injury, and HSP47 protein in a biological sample specifically increases in acute lung injury as compared to chronic progressive interstitial pneumonia and the increase is useful for the distinction thereof, which resulted in the completion of the present invention.

**[0010]** That is, the present invention provides the following.

25 [1] A method of determining whether or not a test subject is affected with acute lung injury, comprising quantifying heat shock protein 47 in a biological sample derived from a test subject.

[2] The method of [1], which distinguishes acute lung injury from chronic progressive interstitial pneumonia.

30 [3] The method of [1], which distinguishes acute lung injury from aortic dissection, acute heart failure, acute exacerbation of chronic heart failure, diffuse alveolar hemorrhage, lymphangitic carcinomatosis, reexpansion pulmonary edema, pulmonary edema by hyper-infusion, neurogenic pulmonary edema, pulmonary tuberculosis, or miliary tuberculosis.

[4] The method of any of [1] - [3], wherein the biological sample is blood, serum or plasma.

[5] A diagnostic reagent for acute lung injury comprising an antibody that specifically recognizes heat shock protein 47 and/or collagen or a partial fragment thereof that has specific affinity for heat shock protein 47.

35 [6] A method of determining whether or not a test subject will develop acute lung injury, comprising quantifying heat shock protein 47 in a biological sample derived from the test subject.

[7] A method of evaluating a treatment effect for or predicting the prognosis of acute lung injury of a test subject, comprising quantifying heat shock protein 47 in a biological sample derived from the test subject.

40 Effect of the Invention

**[0011]** According to the examination method of the present invention, whether or not a test subject is affected with acute lung injury can be determined with high sensitivity. This method can be a powerful diagnostic tool capable of clearly distinguishing acute lung injury from chronic progressive interstitial pneumonia. Furthermore, using the diagnostic reagent of the present invention, whether or not being affected with acute lung injury can be conveniently diagnosed by the above-mentioned examination method of the present invention.

Brief Description of the Drawings

50 **[0012]**

Fig. 1 is a graph showing the concentration of HSP47 protein in the serum of healthy human and patients with each disease indicated on the horizontal axis.

Fig. 2 is a graph showing the concentration of KL-6 in the serum of healthy human and patients with each disease indicated on the horizontal axis.

55 Fig. 3 is a graph showing the concentration of SP-A in the serum of healthy human and patients with each disease indicated on the horizontal axis.

Fig. 4 is a graph showing the concentration of SP-D in the serum of healthy human and patients with each disease indicated on the horizontal axis.

Fig. 5 is a graph showing the concentration of LDH (lactate dehydrogenase) in the serum of healthy human and patients with each disease indicated on the horizontal axis.

Fig. 6 is a graph showing the relationship between the sensitivity and specificity in HSP47 protein, KL-6, SP-D, SP-A and LDH on a Receiver Operating Characteristic curve ((ROC) curve).

5 Fig. 7 is a graph showing the concentration of HSP47 protein in the serum of healthy human and patients with each disease indicated on the horizontal axis.

Fig. 8 is a graph showing the concentration of KL-6 in the serum of healthy human and patients with each disease indicated on the horizontal axis.

10 Fig. 9 is a graph showing the concentration of SP-A in the serum of healthy human and patients with each disease indicated on the horizontal axis.

Fig. 10 is a graph showing the concentration of SP-D in the serum of healthy human and patients with each disease indicated on the horizontal axis, wherein N.S. shows no significant difference.

Fig. 11 is a graph showing the concentration of LDH (lactate dehydrogenase) in the serum of healthy human and patients with each disease indicated on the horizontal axis.

15 Fig. 12 is a graph showing the relationship between the sensitivity and specificity in HSP47 protein, KL-6, SP-D, SP-A and LDH on a Receiver Operating Characteristic curve. Description of Embodiments

**[0013]** The present invention is explained in detail in the following.

20 (1) Use of heat shock protein 47 as a biomarker for the diagnosis of acute lung injury

**[0014]** The present invention provides a method for determining whether or not a test subject is affected with acute lung injury, comprising quantifying heat shock protein 47 in a biological sample derived from a test subject (method (1)).

25 **[0015]** In the present specification, the "acute lung injury (to be also referred to as ALI)" refers to an inflammatory disease of the lung, which is caused by sepsis, pneumonia, trauma and/or aspiration and the like that directly or indirectly injure the lung. The "acute lung injury" in the present specification also includes acute respiratory distress syndrome (to be also referred to as ARDS) which is known to be the same clinical disorder. Acute progressive interstitial pneumonia that meets the clinical definition of acute lung injury is also included in acute lung injury.

30 **[0016]** The clinical definition of acute lung injury is as described below (guideline for ALI/ARDS medical care; The Japanese Respiratory Society, Drafting Committee for ARDS Guideline):

(I) acute onset.

35 (II) hypoxia ( $\text{PaO}_2/\text{FiO}_2$  is not more than 300 mmHg). ( $\text{FiO}_2$ =fraction of inspired  $\text{O}_2$  concentration.  $\text{PaO}_2$  unit is mmHg,  $\text{FiO}_2$  is a decimal number (e.g., 0.5). A case showing  $\text{PaO}_2/\text{FiO}_2$  of not more than 200 mmHg is acute respiratory distress syndrome.)

(III) Bilateral pulmonary infiltrative shadow is observed in chest radiography.

(IV) Pulmonary artery wedge pressure is not more than 18 mmHg or no physical clinical finding of elevation of left atrial pressure.

40 A case that satisfies all the above-mentioned criteria is defined as acute lung injury.

**[0017]** In the method (1) of the present invention, the "test subject" can be a "test subject suspected of being affected with acute lung injury". In the present specification, the "test subject suspected of being affected with acute lung injury" refers to a patient showing clinical findings (e.g., cough, expectoration, fever, respiratory distress, respiratory failure, hypoxia, chest pain etc.) common to acute lung injury, aortic dissection, acute heart failure, acute exacerbation of chronic heart failure, diffuse alveolar hemorrhage, lymphangitic carcinomatosis, reexpansion pulmonary edema, pulmonary edema by hyper-infusion, neurogenic pulmonary edema and lung infection (pulmonary tuberculosis, miliary tuberculosis etc.) by clinical diagnosis (these diseases other than acute lung injury are sometimes generically referred to as "the above-mentioned disease showing clinical finding common with acute lung injury" in the following).

50 **[0018]** In the method (1) of the present invention, the "test subject" or "test subject suspected of being affected with acute lung injury" can be a "test subject suspected of being affected with either acute lung injury or chronic progressive interstitial pneumonia". In the present specification, the "test subject suspected of being affected with either acute lung injury or chronic progressive interstitial pneumonia" refers to a patient who has a finding of suspected affection with either acute lung injury or chronic progressive interstitial pneumonia by diagnosis using chest radiography or a serum marker such as KL-6, SP-D, SP-A and the like.

55 **[0019]** In the present specification, the "interstitial pneumonia" refers to a disease with an inflammation principally in the interstitial tissue of the lung, which is characterized by alveolar septum hypertrophy, fibroblast growth, collagen deposition and the like. Examples of the interstitial pneumonia include those caused by radiation, infection with a pathogen, or a collagen disease, those induced by poisoning or drug, and idiopathic ones without clear etiology. Examples

of the idiopathic interstitial pneumonia include, but are not limited to, cryptogenic organizing pneumonia (COP), idiopathic usual interstitial pneumonia (idiopathic UIP), idiopathic nonspecific interstitial pneumonia (idiopathic NSIP) and the like. In the present specification, the "interstitial pneumonia" is a concept also including lung fibrosis showing fibrotic inflammatory tissues due to progression.

5 [0020] A test subjects subjected to the method (1) of the present invention may be affected or non-affected with lung fibrosis. Although increased expression of HSP47 in a topical lung tissue of lung fibrosing disease has been reported heretofore (non-patent documents 5 and 6), since HSP47 has been considered to localize in the endoplasmic reticulum and not leak extracellularly, increase of HSP47 outside the cell (for example, in the blood and the like) in interstitial pneumonia accompanied by fibrosing has not been expected. In fact, in the past reports, the levels of HSP47 and anti-HSP47 antibody in the serum of patients with idiopathic pulmonary fibrosis were similar to those of healthy individuals, and it was clear that no increase was observed (non-patent document 7). Extracellular (for example, in the blood and the like) HSP47 does not increase in chronic progressive interstitial pneumonia, but specifically increases in acute lung injury (including rapidly progressive interstitial pneumonia).

10 [0021] In a further aspect, in the method (1) of the present invention, the "test subject" and the "test subject suspected of being affected with acute lung injury" can be "a test subject suspected of being affected with acute lung injury or any of the above-mentioned disease showing a clinical finding common with acute lung injury". In the present specification, the "test subject suspected of being affected with acute lung injury or any of the above-mentioned disease showing a clinical finding common with acute lung injury" refers to a patient showing a clinical finding (e.g., respiratory distress, respiratory failure, hypoxia, chest pain etc.) common to acute lung injury and the above-mentioned disease by clinical diagnosis.

15 [0022] In the present specification, the "aortic dissection" refers to a disease with an acute chest pain or abdominal pain, which is developed by a breakage, due to genetic factors, degeneration and embrittlement of the arterial membrane, combined with arterial expansion, hypertension and the like, in a part of the intima of the arterial blood vessel that allows inflow of the blood into the intima from the hiatus of the intima to induce dissociation of the blood vessel wall.

20 [0023] In the present specification, the "acute heart failure" refers to the pathology of circulatory diseases wherein a sufficient blood flow is not supplied to the peripheral organs from the ventricle, due to rapid exacerbation of the circulation dynamics. Examples of the symptoms of the acute heart failure include marked respiratory distress based on lung congestion, cardiogenic shock based on low cardiac output, foamy expectoration, oliguria-anuria, coldness of the four limbs, lowering of blood pressure, cold sweat, tachycardia (sometimes bradycardiac) and the like.

25 [0024] In the present specification, the "acute exacerbation of chronic heart failure" refers to rapid exacerbation of chronic heart failure. In the present specification, the "chronic heart failure" refers to a pathology that gradually progresses as in obsolete myocardial infarction, dilated cardiomyopathy and the like, wherein various compensation mechanisms such as cardiac hypertrophy have acted to reach a certain equilibrium state, and shows symptoms of short breath, fatigability, lower motility tolerability, hepatosplenic swelling, edema, peripheral intravenous dilation and the like.

30 [0025] In the present specification, the "pulmonary tuberculosis" refers to lung infection with *Mycobacterium tuberculosis*.

35 [0026] In the present specification, the "miliary tuberculosis" refers to a pathology wherein a large amount of *Mycobacterium tuberculosis* enters into the blood circulation, diffuses in multiple organs to form many tubercles.

40 [0027] In the present specification, the "diffuse alveolar hemorrhage" refers to hemorrhage into the pulmonary alveolar cavity. Hemorrhage into the alveolar cavity, emergence of hemosiderin phagocyte (siderophore) in the alveolar cavity, and hemosiderin deposition on the alveolar wall are observed and, as a disease group showing diffuse disseminated shadow over the entire lung fields by chest radiography, alveolar hemorrhage syndrome is known. In many cases, hemorrhage is seen not only in the alveolar cavity but also lung stroma. The cause thereof includes, for example, lung damage, thoracic compression, blood vessel collapse in lung - bronchus lesion, blood vessel neurotic hemorrhage, partial hemorrhagic pneumonia, hemorrhage per diapedesis seen in blood diseases, hemorrhage due to microvessel disorder, hemorrhage related to immune phenomenon and the like.

45 [0028] In the present specification, the "lymphangitic carcinomatosis" refers to a condition wherein the cancer cells grown retrogradely due to the congestion of lymph flow by the metastasis of cancer cells into the lymph node on the perfusion side, embolism of lymphatic vessel and the like, spread with filling the lymphatic vessels of the tissue. The periphery of the expanded lymphatic vessel shows edema and growth of fiber tissues, and shows a pseudo-inflammatory state suggestive of lymphangitis. It is sometimes observed in breast cancer, gastric cancer, (metastatic) lung cancer and the like, and has bad prognosis.

50 [0029] In the present specification, the "pulmonary edema" refers to a pathological condition wherein abnormal water retention is observed outside the lung blood vessel.

55 [0030] In the present specification, the "reexpansion pulmonary edema" refers to pulmonary edema considered to be resulting from reperfusion of lung blood flow and blood vessel permeability enhancement caused by reexpansion of collapsed lung that occurs at once when pleural effusion, pneumothorax, hemothorax and the like are treated by thoracic cavity drainage and the like.

**[0031]** In the present specification, the "infusion" refers to a treatment method including administering water, electrolyte and the like by drip intravenous injection, wherein the term "pulmonary edema by hyper-infusion" refers to a state where the lung capillary pressure increases by excessive infusion to cause pulmonary edema.

**[0032]** In the present specification, the "neurogenic pulmonary edema" refers to acute pulmonary edema caused by increased intracranial pressure due to head trauma, epileptogenesis, cerebrovascular disorder and the like.

**[0033]** The test subject to be subjected to the method (1) of the present invention means a mammal. The mammal is not particularly limited as long as it has a possibility of being affected with acute lung injury, and experimental animals such as rodents (mouse, rat, hamster, guinea pig and the like), rabbit and the like, domestic animals such as swine, bovine, goat, horse, sheep, mink and the like, pets such as dog, cat and the like, primates such as human, monkey, *Macaca fascicularis*, *Macaca mulatta*, marmoset, orangutan, chimpanzee and the like are preferable, and human is particularly preferable.

**[0034]** Heat shock protein 47 (HSP47) is a known heat shock protein, and the amino acid sequence etc. thereof are also known. HSP47 used in the present invention is generally derived from a mammal. Being "derived from a mammal" means that the amino acid sequence of HSP47 is a mammalian sequence. The mammal may be the same as those usable as the test subjects. The kind of a mammal, from which HSP47 of the target to be quantified derives, is generally the same as the mammalian kind of the test subjects. For example, when the test subject is a human, human heat shock protein 47 is quantified.

**[0035]** Representative amino acid sequence of human HSP47 includes the amino acid sequence shown by SEQ ID NO: 2 (GeneBank accession No.: NP\_001226). In the present specification, protein and peptides are described with the N-terminus (amino-terminus) on the left side, and the C-terminus (carboxyl-terminus) on the right side, according to conventional practice of indication.

**[0036]** Examples of the biological sample usable for the method (1) of the present invention include blood, broncho lavage fluid, alveolar lavage fluid, expectoration, lung tissue, brain spinal fluid, ascites and the like; however, it is not particularly limited as long as an increase in the HSP47 level associated with acute lung injury can be detected. Lung tissue is preferably disrupted in an appropriate buffer and the like and subjected to the method (1) of the present invention in the form of an extract. As the "blood", blood derived from any tissue can be assumed. Due to an easiness of its collection, peripheral blood is generally used. As a blood collection method, a method known per se can be applied. While the collected blood may be directly used for this step, it is preferably used for this step as a liquid component (plasma) separated from the cell components (red blood cell, leukocyte, platelet and the like) by method known per se, for example, centrifugation, filtration and the like. It is also preferable to coagulate the blood and use a liquid component (serum) separated from the platelets and coagulation factors for this step. The biological sample that can be used for the method (1) of the present invention is preferably blood, serum or plasma.

**[0037]** HSP47 in a biological sample can be quantified by a method known per se such as an immunological method, a mass spectrometry method and the like.

**[0038]** Quantification of HSP47 by an immunological method includes, for example, the following steps:

- (1) contacting a biological sample derived from a test subject with an antibody that specifically recognizes HSP47 to form a complex of HSP47 in the biological sample and the antibody that specifically recognizes HSP47;
- (2) detecting the complex formed in the above-mentioned step (1); and
- (3) determining the amount of HSP47 in the biological sample from the amount of the complex detected in the above-mentioned step (2).

**[0039]** In step (1), a biological sample derived from a test subject is contacted with an antibody that specifically recognizes HSP47 to form a complex of HSP47 in the biological sample and the antibody that specifically recognizes HSP47.

**[0040]** In the present specification, the "antibody that specifically recognizes HSP47" only needs to have an ability to specifically bind HSP47, and may be any of polyclonal antibody and monoclonal antibody, and monoclonal antibody is preferable. Examples of the antibody include chimera antibody, single chain antibody or  $F(ab')_2$ , Fab' of an antibody molecule or an affinity fragment such as Fab fraction and the like. As these antibodies, an antibody prepared by a method known per se and using HSP47 as an immunogen can be used, or a commercially available antibody can also be used.

**[0041]** Being "specific" means that the affinity for HSP47, which is an antigen of the antibody, is higher than the affinity for other antigens.

**[0042]** When recombinant HSP47 is used as the antigen, recombinant HSP47 can be produced, for example, by the following method. A polynucleotide encoding the amino acid sequence of HSP47 (e.g., in the case of human HSP47, polynucleotide containing the nucleotide sequence shown by SEQ ID NO: 1 (GeneBank accession No.: NN\_001235)) is incorporated into an appropriate expression vector, the vector is introduced into an appropriate host for transformation, and the object recombinant HSP47 can be obtained from the homogenate of the transformed cell. The above-mentioned host cell is not particularly limited, and various host cells conventionally used for genetic engineering methods, for

example, *Escherichia coli*, *Bacillus* species bacteria, yeast, plant or animal cell and the like can be used.

**[0043]** HSP47 may be, in addition to a recombinant protein produced from the above-mentioned transformant, one isolated or purified from a natural cell producing same, by a protein separation and purification technique known per se. Moreover, it may be a protein synthesized by a chemical synthesis or synthesized biochemically in cell-free translation system.

**[0044]** Using the above-mentioned HSP47, the aforementioned "antibody that specifically recognizes HSP47" can be produced according to a conventional method. Specifically, when the antibody is a polyclonal antibody, a non-human animal such as rabbit and the like is immunized with HSP47 according to a conventional method, and the antibody can be obtained from serum of the immunized animal (Current Protocols in Molecular Biology, edit. Ausubel FM et al. (2011) Publish. John Wiley and Sons. Chapter 11, Section III, Unit 11.12 - 11.13). On the other hand, in the case of a monoclonal antibody, a non-human animal such as mouse and the like is immunized with HSP47 or an oligopeptide having a partial amino acid sequence thereof and the like according to a conventional method, the obtained spleen cell and a myeloma cell are fused, and the antibody can be obtained from the prepared hybridoma cell (Current protocols in Molecular Biology, edit. Ausubel FM et al. (2011) Publish. John Wiley and Sons. Chapter 11, Section II, Unit 11.4 - 11.11).

**[0045]** The antibody that specifically recognizes HSP47, which is to be used in the present invention, is preferably isolated or purified. Being "isolated or purified" means that an operation to remove components other than the object component from the natural state has been applied. The purity of the isolated or purified antibody that specifically recognizes HSP47 (weight ratio of antibody that specifically recognizes HSP47 relative to total protein weight) is generally not less than 50%, preferably not less than 70%, more preferably not less than 90%, most preferably not less than 95% (for example, substantially 100%).

**[0046]** The aforementioned antibody may be directly or indirectly labeled with a labeling substance. Examples of the labeling substance include fluorescent substance (e.g., FITC, rhodamine), radioactive substance (e.g., <sup>14</sup>C, <sup>3</sup>H, <sup>125</sup>I), enzyme (e.g., alkaline phosphatase, peroxidase), colored particles (e.g., colloidal metal particles, colored latex), biotin and the like.

**[0047]** Such antibody may be used alone or two or more kinds thereof may be used in this step.

**[0048]** The above-mentioned "antibody that specifically recognizes HSP47" can be used in the form of an aqueous solution; however, it is preferably bound to a solid phase. Examples of such "solid phase" include a plate (e.g., a microwell plate), a tube, beads (e.g., plastic beads, magnetic beads), a chromatography carrier (e.g., Sepharose (trade mark)), a membrane (e.g., a nitrocellulose membrane, a PVDF membrane), a gel (e.g., a polyacrylamide gel), a metal film (e.g., a gold film) and the like. Of these, plate, beads, membrane and metal film are preferably used, and a plate is most preferably used in view of the convenience of handling. The above-mentioned bond is not particularly limited and covalent bond, ionic bond, physical adsorption and the like can be mentioned. Covalent bond and/or physical adsorption are/is preferable for achieving sufficient bond strength. For binding to a solid phase, it may be directly binding to a solid phase or indirectly binding to a solid phase by utilizing a substance known per se. Furthermore, to suppress non-specific adsorption and non-specific reaction, a phosphate buffered solution of bovine serum albumin (BSA), cow's milk protein and the like is contacted with a solid phase, and a solid phase surface not coated with the antibody is generally blocked with the aforementioned BSA, cow's milk protein and the like.

**[0049]** In this step, the contact between the "antibody that specifically recognizes HSP47" and the "HSP47" contained in a biological sample derived from a test subject is carried out by mixing said biological sample and the antibody that specifically recognizes HSP47 in a reaction vessel and, as long as the method permits their interaction, the embodiment, order, specific method and the like are not particularly limited. The contact is performed by, for example, adding (extract of) said biological sample to a plate on which the "antibody that specifically recognizes HSP47" is immobilized.

**[0050]** The time of maintaining such contact is not particularly limited as long as the aforementioned antibody that specifically recognizes HSP47 can bind to HSP47 contained in a biological sample derived from a test subject to form a complex, and it is generally several seconds to more than a dozen hours. To rapidly determine whether acute lung injury or not, it is preferably 1 min to 2 hr, most preferably 2 min to 30 min. The temperature condition of contact is generally 4°C - 50°C, preferably 4°C - 37°C, most preferably room temperature of about 15°C - 30°C. Furthermore, the pH condition for the reaction is preferably 5.0 - 9.0, particularly preferably the neutral range of 6.0 - 8.0.

**[0051]** In step (2), the complex formed in the above-mentioned step (1) is detected to determine whether HSP47 is present or not in the above-mentioned biological sample.

**[0052]** The above-mentioned detection is performed by detecting "HSP47" or "antibody that specifically recognizes HSP47" contained in the complex. For the detection, enzyme immunoassay (EIA method), immunochromatography, latex agglutination, radioimmunoassay (RIA method), fluorescent immunoassay (FIA method), luminescence immunoassay, surface plasmon resonance measurement method (SPR method) and the like can be utilized. Of these, EIA method, immunochromatography, FIA method and SPR method are preferable from the aspects of easiness and rapidness of the operation.

**[0053]** When EIA method is selected as the detection method in step (2), the EIA method is preferably sandwich Enzyme-Linked ImmunoSorbent Assay (sandwich ELISA method) using two kinds of the "antibody that specifically

recognizes HSP47". Since two kinds of antibodies are used in the sandwich ELISA method, it is superior in the specificity to antigen.

**[0054]** The two kinds of "antibodies that specifically recognize HSP47" for performing the Sandwich ELISA method may be any combination of monoclonal antibodies, polyclonal antibodies, or monoclonal antibody and polyclonal antibody, as long as the epitopes are noncompetitive.

**[0055]** As one kind of Sandwich ELISA method, a method utilizing an avidin-biotin reaction is applicable. In this method, for example, HSP47 in the plasma or serum is trapped by any immobilized "antibody that specifically recognizes HSP47", and an antigen-antibody reaction is performed between the trapped HSP47 and biotin-labeled "antibody that specifically recognizes HSP47". The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. Then, enzyme-labeled streptavidin is added to perform avidin-biotin reaction. The time required for such reaction is preferably 5 min - 1 hr, more preferably 15 min - 30 min, since a rapid measurement is necessary. Then HSP47 is specifically detected by detecting the enzyme. The above-mentioned biotin-labeled "antibody that specifically recognizes HSP47" can be produced by binding biotin to an "antibody that specifically recognizes HSP47" by a method known per se. For example, using a commercially available biotin labeling kit, biotin and "antibody that specifically recognizes HSP47" can be bonded. As the enzyme-labeled streptavidin, a commercially available product can be preferably used.

**[0056]** Also, sandwich ELISA method utilizing an enzyme-labeled antibody is also applicable. In this method, for example, HSP47 in the blood is trapped by any immobilized "antibody that specifically recognizes HSP47", and an antigen-antibody reaction is performed between the trapped HSP47 and enzyme-labeled "antibody that specifically recognizes HSP47". The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. Then HSP47 is detected by detecting the enzyme. The enzyme-labeled antibody can be produced by binding (labeling) the enzyme to "antibody that specifically recognizes HSP47" by a method known per se, for example, glutaraldehyde method, maleimide method and the like.

**[0057]** Furthermore, from the aspects of broad utility, sandwich ELISA method utilizing a secondary antibody is also applicable. In this method, for example, HSP47 in blood is trapped by any immobilized "antibody that specifically recognizes HSP47", and an antigen-antibody reaction is performed between the trapped HSP47 and "antibody that specifically recognizes HSP47" which is derived from an animal species different from that for the immobilized antibody (indicated as primary antibody in this paragraph). The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. For example, when the immobilized "antibody that specifically recognizes HSP47" is derived from rabbit, the reaction is performed using a primary antibody derived from an animal species other than rabbit, for example, an antibody derived from mouse. Then, an antigen-antibody reaction is performed between the primary antibody and the enzyme-labeled "antibody recognizing the primary antibody" (indicated as secondary antibody in this paragraph). The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. Finally, HSP47 is detected by detecting the enzyme. As the enzyme-labeled secondary antibody, a commercially available product can be preferably used.

**[0058]** As the "enzyme" of the enzyme-labeled streptavidin and enzyme-labeled antibody, peroxidase, alkaline phosphatase, glucose oxidase,  $\beta$ -galactosidase and the like are exemplified.

**[0059]** As a substrate reagent to be used for the detection of the enzyme, one suitable for the selected labeling enzyme is used. For example, when a peroxidase is selected as an enzyme, o-phenylenediamine (OPD), tetramethylbenzidine (TMB) and the like are used. When an alkaline phosphatase is selected, p-nitrophenyl phosphate (PNPP) and the like are used. As a reaction quenching liquid and a substrate dissolution liquid, conventionally known ones can be used as appropriate according to the selected enzyme, without any particular limitation.

**[0060]** Even when sandwich ELISA method is not used, detection is possible by applying a general ELISA method. For example, in step (1), HSP47 in the blood derived from a test subject is bound to a solid phase in the same manner as in the aforementioned method, then an antigen-antibody reaction is performed with the labeled "antibody that specifically recognizes HSP47" to form a complex. The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. Then HSP47 can be detected by using a method according to the label.

**[0061]** When immunochromatography is selected as a detection method in step (2), developing of blood from under a membrane, for example, allows HSP47 to be trapped by an "antibody that specifically recognizes HSP47" immobilized, in a line form, on a water-absorbing substrate such as nitrocellulose membrane and the like, and an antigen-antibody reaction is performed between the trapped HSP47 and the labeled "antibody that specifically recognizes HSP47". The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. Then HSP47 can be detected by using a method according to the label.

**[0062]** The two kinds of "antibodies that specifically recognize HSP47" for performing immunochromatography may also be any combination of monoclonal antibodies, polyclonal antibodies, or monoclonal antibody and polyclonal antibody, as long as the epitopes are noncompetitive.

**[0063]** When FIA method is selected as a detection method in step (2), the enzyme bound to the "antibody that

specifically recognizes HSP47" used for the above-mentioned EIA method is substituted for a fluorescent substance and the obtained antibody is used to perform sandwich ELISA in the same manner as in the above-mentioned method. Then, the fluorescent substance is detected using a commercially available measurement device, fluorescence microscope, confocal microscope and the like, whereby HSP47 is detected. As the fluorescent substance, chemical substances such as APC, PE, Cy2, Cy3, Cy5, ECD, FITC, PerCP, Alexa (registered trade mark) Fluor, fluorescein, rhodamine and the like can be preferably utilized. The chemical substance can be labeled to an antibody by a method known per se.

**[0064]** When SPR method is selected as a detection method in step (2), an interaction between an antibody that specifically recognizes HSP47, which is immobilized in advance on a surface of a metal film (sensor chip), and HSP47 in blood, which is flown on a surface of the metal film is detected as time course changes of the surface plasmon resonance. When the SPR method is performed, the "antibody that specifically recognizes HSP47" which is immobilized on a metal film to be a sensor chip may be only one kind, and may be a monoclonal antibody or a polyclonal antibody. As a measurement device utilized for the detection of SPR, a commercially available measurement device can be preferably used.

**[0065]** In one specific example, when EIA method is applied, the blood collected from a test subject is shaken for a predetermined time at room temperature, and centrifuged to give serum. Then the serum is dispensed into a microplate immobilizing any "antibody that specifically recognizes HSP47", and left standing at room temperature for a predetermined time. After the plate is washed to remove unreacted antigen, the biotinylated, above-mentioned antibody solution is dispensed into a plate and left standing for a predetermined time to form a complex. The plate is further washed to remove unreacted antibody, peroxidase labeled streptavidin solution is dispensed into a plate and the mixture is reacted at room temperature for a predetermined time. The plate is washed, and reacted with a chromogen solution such as TMB and the like to detect the complex.

**[0066]** In another specific example, when immunochromatography is applied, a test piece is immersed in the above-mentioned serum or serum diluted with saline and the like to allow development. In the test piece, carrier maintaining a particulate labeled substance, and a filter paper used for an absorption carrier of a liquid sample are laminated via one end on the lower end side of a strip-shaped antibody immobilized support; on the other hand, a liquid-absorbing carrier made of a filter paper is laminated via one end on the upper end side of the aforementioned antibody immobilized support. The above-mentioned antibody immobilized support comprises a nitrocellulose sheet and an "antibody that specifically recognizes HSP47", which performs an antigen-antibody reaction with HSP47, immobilized on the sheet. Immobilization is performed by applying the above-mentioned antibody solution on the nitrocellulose sheet and drying it. The immobilized antibody specifically recognizes HSP47 in the developed serum and can trap a complex of HSP47 and the above-mentioned particulate-labeled antibody. Therefore, when the "antibody that specifically recognizes HSP47" is, in a line form, immobilized on the antibody-immobilized support, the line is colored by the particulate label to determine the presence of HSP47 in the blood.

**[0067]** In step (3), the amount of HSP47 in a biological sample is determined from the amount of the complex detected in the above-mentioned step (2).

**[0068]** The amount (concentration) of the complex detected in the above-mentioned step (2) can be measured by correlating a signal of fluorescence intensity or absorbance and the like to a separately prepared calibration curve. The concentration of HSP47 in a biological sample of a test subject can be quantified by multiplying the concentration obtained from the calibration curve by the dilution rate.

**[0069]** The calibration curve is shown by an approximation formula plotting the measured values, wherein the horizontal axis (vertical axis) shows the concentration of the samples obtained by serially diluting the aforementioned standard HSP47 protein standard product, and the vertical axis (horizontal axis) shows the signal (in any unit) of the antibody that specifically recognizes HSP47, which is bound to HSP47 in a standard HSP47 protein. The calibration curve can be shown in an approximate straight line by logarithmic presentation of the both axes.

**[0070]** Next, the amount of HSP47 in a biological sample derived from a test subject is correlated with the possibility of being affected with acute lung injury, and whether or not the test subject is affected with acute lung injury is determined based on the correlation. As shown in the below-mentioned Examples, as compared to healthy human and patients with chronic progressive interstitial pneumonia, patients with acute lung injury show a higher amount of HSP47 in a biological sample. Moreover, as compared to patients with the above-mentioned disease that shows clinical findings common with acute lung injury, patients with acute lung injury show a higher amount of HSP47 in a biological sample. The above-mentioned determination is made based on the positive correlation between such amount of HSP47 in a biological sample derived from a test subject and the possibility of being affected with acute lung injury.

**[0071]** For example, biological samples (e.g., serum) are collected from healthy human or patients with chronic progressive interstitial pneumonia (negative control), and patients with acute lung injury (positive control), the amount of HSP47 in the biological samples collected from the test subjects is compared with that of the positive control and negative control. Alternatively, a correlation diagram of the amount of HSP47 in a particular biological sample (e.g., serum) and acute lung injury morbidity rate is prepared in advance, and the amount of HSP47 in a biological sample collected from a test subject may be compared with the correlation diagram. The amount of HSP47 is preferably compared based on

the presence or absence of a significant difference.

**[0072]** From the comparison results of the amount of HSP47, when the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a relatively low possibility of being affected with acute lung injury. In a differentiation of acute lung injury from chronic progressive interstitial pneumonia using the method (1) of the present invention, when the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury rather than chronic progressive interstitial pneumonia. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a high possibility of being affected with chronic progressive interstitial pneumonia rather than acute lung injury.

**[0073]** Alternatively, a cutoff value of the amount of HSP47 in a biological sample may be determined in advance, and the cutoff value may be compared with the measured amount of HSP47. For example, when the amount of HSP47 in a biological sample derived from a test subject is not less than the aforementioned cutoff value, the test subject can be determined to have a high possibility of being affected with acute lung injury. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is lower than the cutoff value, the test subject can be determined to have a low possibility of being affected with acute lung injury. In a differentiation of acute lung injury from chronic progressive interstitial pneumonia using the method (1) of the present invention, when the amount of HSP47 in a biological sample derived from a test subject is not less than the aforementioned cutoff value, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury rather than chronic progressive interstitial pneumonia. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is lower than the cutoff value, the test subject can be determined to have a high possibility of being affected with chronic progressive interstitial pneumonia rather than acute lung injury.

**[0074]** The "cutoff value" can satisfy both high diagnostic sensitivity (true positive rate) and high diagnosis specificity (true negative rate), when the disease and condition are evaluated using the value as a standard. For example, an amount of HSP47 that shows high positive rate in patients with acute lung injury and high negative rate in healthy individual or patients with chronic progressive interstitial pneumonia or other respiratory diseases can be set as a cutoff value. For example, when whether or not a test subject is affected with acute lung injury is determined using peripheral blood serum as a biological sample, a preferable cutoff value is 100 - 1500 pg/ml, preferably 200 - 1300 pg/ml, more preferably 300 - 1200 pg/ml, further more preferably 400 - 1000 pg/ml, most preferably 500 - 900 pg/ml. In addition, when acute lung injury is distinguished from chronic progressive interstitial pneumonia by using peripheral blood serum as a biological sample, a preferable cutoff value is 100 - 1500 pg/ml, preferably 200 - 1300 pg/ml, more preferably 300 - 1200 pg/ml, further more preferably 400 - 1000 pg/ml, most preferably 500-900 pg/ml.

**[0075]** In a further aspect, acute lung injury can be distinguished from the above-mentioned diseases showing clinical findings common with acute lung injury by using the method (1) of the present invention. Clinical findings of acute lung injury are similar to those of the above-mentioned diseases. Since the above-mentioned diseases showing clinical findings common with acute lung injury do not have a factor that promotes collagen synthesis, the amount of HSP47 in the biological sample does not increase.

**[0076]** Specifically, for example, biological samples (e.g., serum) are collected from healthy human or patients with the above-mentioned diseases showing clinical findings common with acute lung injury (negative control), and patients with acute lung injury (positive control), the amount of HSP47 in the biological samples collected from the test subjects is compared with that of the positive control and negative control. Alternatively, a correlation diagram between the amount of HSP47 in a particular biological sample (e.g., serum) and acute lung injury morbidity rate is prepared in advance, and the amount of HSP47 in a biological sample collected from a test subject may be compared with the correlation diagram. The amount of HSP47 is preferably compared based on the presence or absence of a significant difference.

**[0077]** When the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury rather than the above-mentioned diseases showing clinical findings common with acute lung injury. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a high possibility of being affected with the above-mentioned diseases showing clinical findings common with acute lung injury rather than acute lung injury.

**[0078]** Moreover, the present invention provides a diagnostic reagent for acute lung injury comprising the aforementioned antibody that specifically recognizes HSP47. Said diagnostic reagent can be a diagnosis kit for acute lung injury. Using the diagnostic reagent of the present invention, whether or not a test subject is affected with acute lung injury can be easily determined by quantifying HSP47 in a biological sample derived from a test subject by an immunological means in the aforementioned method (1) of the present invention, or acute lung injury can be distinguished from chronic progressive interstitial pneumonia or the above-mentioned diseases showing clinical findings common with acute lung injury.

**[0079]** The antibody may be directly or indirectly labeled with a labeling substance. Examples of the labeling substance

include a fluorescent substance (e.g., FITC, rhodamine), a radioactive substance (e.g.,  $^{14}\text{C}$ ,  $^3\text{H}$ ,  $^{125}\text{I}$ ), an enzyme (e.g., an alkaline phosphatase, a peroxidase), a colored particle (e.g., a colloidal metal particle, a colored latex), biotin and the like.

**[0080]** The antibody can also be used in the state of an aqueous solution, and it is preferably bound to a solid phase. Examples of the "solid phase" include a plate (e.g., a microwell plate), a tube, beads (e.g., plastic beads, magnetic beads), a chromatography carrier (e.g., Sepharose (trade mark)), a membrane (e.g., a nitrocellulose membrane, a PVDF membrane), a gel (e.g., a polyacrylamide gel), a metal film (e.g., a gold film) and the like.

**[0081]** The diagnostic reagent of the present invention may comprise, besides an antibody that specifically recognizes HSP47, a reagent to be used for quantifying the amount of HSP47 in a biological sample and the like. These reagents may be stored in advance together with the antibody that specifically recognizes HSP47, or stored in separate containers. Examples of the reagent include a treatment liquid, a buffer for diluting an antibody, a secondary antibody, a labeling substance (e.g., a fluorescence dye, an enzyme), a reaction vessel, a positive control (e.g., a recombinant HSP47), a negative control, a solid phase, an instruction sheet describing the examination protocol and the like. These components can also be mixed in advance as necessary.

**[0082]** In addition, since collagen has a property of specifically binding to HSP47, "collagen or a partial fragment thereof having specific affinity for HSP47" may be used instead of or in addition to the "antibody that specifically recognizes HSP47" for the quantification of HSP47 by the method of the present invention in another embodiment.

**[0083]** Quantification of HSP47 by a method using collagen or a partial fragment thereof having specific affinity for HSP47 (the method (2) of the present invention) includes, for example, the following steps:

(1') contacting a biological sample derived from a test subject with collagen or a partial fragment thereof having specific affinity for HSP47, to form a complex of HSP47 in the biological sample and the collagen or a partial fragment thereof having specific affinity for HSP47;

(2') detecting the complex formed in the above-mentioned step (1); and

(3') determining the amount of HSP47 in the biological sample from the amount of the complex detected in the above-mentioned step (2).

**[0084]** In step (1'), a biological sample derived from a test subject is contacted with collagen or a partial fragment thereof having specific affinity for HSP47 to form a complex of HSP47 in the biological sample and the collagen or a partial fragment thereof having specific affinity for HSP47.

**[0085]** The collagen to be used for the method of the present invention is mammalian collagen. Examples of the mammal include, but are not limited to, experimental animals such as rodents (mouse, rat, hamster, guinea pig and the like), rabbit and the like, domestic animals such as swine, bovine, goat, horse, sheep, mink and the like, pets such as dog, cat and the like, primates such as human, monkey, *Macaca fascicularis*, *Macaca mulatta*, marmoset, orangutan, chimpanzee and the like. The collagen to be used for the method of the present invention is preferably collagen of the same species of mammal as the test subject. For example, when the test subject is human, human collagen is preferably used.

**[0086]** In the present specification, the "human collagen" means that the amino acid sequence of collagen is the same as that of the collagen naturally expressed in human.

**[0087]** The collagen to be used for the method of the present invention may be of any type as long as it has a property of specifically binding to HSP47. Mammalian collagen encompasses 27 types of types I - XXVII. The mammalian collagen is preferably types I - V, more preferably type I. It is known that at least mammalian collagens of types I - V bind to HSP47 (Natsume T. et al., J. Biol. Chem., 269, 31224-31228, 1994).

**[0088]** The collagen is generally a triple helix containing 3 polypeptides. For example, type I-collagen is a triple helix containing two  $\alpha 1$  chains (type I) and one  $\alpha 2$  chain (type I). A representative amino acid sequence of the  $\alpha 1$  chain of human type I-collagen is described in SEQ ID NO: 4, and a representative amino acid sequence of the  $\alpha 2$  chain of human type I-collagen is described in SEQ ID NO: 6.

**[0089]** The "partial fragment of collagen having specific affinity for HSP47" may be a partial fragment of any region of collagen as long as it has specific affinity for HSP47. The partial fragment of collagen having specific affinity for HSP47 is generally a triple helix containing 3 partial peptides (when 3 polypeptides constituting collagen are polypeptides A, B and C, respective partial peptides are a fragment of polypeptide A, a fragment of polypeptide B, and a fragment of polypeptide C).

**[0090]** The size of the three partial peptides contained in the "partial fragment of collagen having specific affinity for HSP47" is generally not less than 9 amino acids, preferably not less than 12 amino acids, more preferably not less than 15 amino acids, further more preferably not less than 18 amino acids.

**[0091]** The 3 partial peptides contained in the "partial fragment of collagen having specific affinity for HSP47" respectively contain, for example, not less than 4, preferably not less than 5, more preferably not less than 6, Gly-Xaa-Yaa (wherein Xaa and Yaa are any amino acids) repeats contained in the polypeptide constituting collagen.

[0092] To secure specific affinity for HSP47, the partial fragment preferably contains at least one arginine residue in the Yaa moiety per one fragment (Koide, T. et al., J. Biol. Chem., 281, 3432-3438, 2006).

[0093] Furthermore, to secure specific affinity for HSP47, at least one partial peptide contained in the "partial fragment of collagen having specific affinity for HSP47" preferably is at least one amino acid sequence shown by Yaa<sup>3</sup>-Gly-Xaa<sup>1</sup>-Arg-Gly wherein Xaa<sup>1</sup> is any amino acid (preferably Pro), and Yaa<sup>3</sup> is any amino acid (preferably Thr, Pro, Ser, Hyp, Val, Ala, Ile, Leu, Asn, Met, His, Phe or Tyr, more preferably Thr, Pro, Ser, Hyp, Val or Ala, further preferably Thr or Pro, most preferably Thr). In one preferable embodiment, Yaa<sup>3</sup>-Gly-Xaa<sup>1</sup>-Arg-Gly is Thr-Gly-Pro-Arg-Gly (see Koide T. et al., J. Biol. Chem. 281, 11177-11185, 2006).

[0094] In the present specification, "any amino acid" encompasses Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr and Hyp.

[0095] The collagen and a partial fragment thereof that specifically bind to HSP47, and the amino acid sequence in collagen significant for specific binding to HSP47 are described in detail in Koide, T. et al., J. Biol. Chem. 277, 6178-6182, 2002; Tasab, M. et al., J. Biol. Chem. 277, 35007-35012, 2002; Koide, T., et al., J. Biol. Chem. 281, 3432-3438, 2006; and Natsume, T. et al., J. Biol. Chem. 269, 31224-31228, 1994, which are incorporated in their entireties in the present specification by reference, and those of ordinary skill in the art can easily obtain collagen specifically binding to HSP47 and a partial fragment thereof, based on the description of the present specification, those documents and the like.

[0096] The collagen or a partial fragment thereof having specific affinity for HSP47, which is used for quantification of HSP47, may contain, in addition to the amino acid sequence derived from the collagen itself, 1 or more (e.g., 1 - 500, preferably about 1 - 100, more preferably about 1 - 15) additional amino acids. Such addition of amino acid is acceptable as long as the collagen or a partial fragment thereof having specific affinity for HSP47 specifically recognizes HSP47. The amino acid sequence to be added is not particularly limited, and examples include first methionone, cysteine and a tag that facilitates detection and purification of polypeptide, and the like. Examples of the tag include Flag tag, histidine tag, c-Myc tag, HA tag, AU1 tag, GST tag, MBP tag, fluorescent protein tag (e.g., GFP, YFP, RFP, CFP, BFP etc.), immunoglobulin Fc tag and the like. The position of the amino acid sequence addition is preferably N-terminus or C-terminus of polypeptide.

[0097] The collagen or a partial fragment thereof having specific affinity for HSP47 to be used in the present invention is preferably isolated or purified. Being "isolated or purified" means that an operation to remove components other than the object component from the natural state has been applied. The purity of the isolated or purified collagen or a partial fragment thereof having specific affinity for HSP47 (weight ratio of collagen or a partial fragment thereof having specific affinity for HSP47 relative to total protein weight) is generally not less than 50%, preferably not less than 70%, more preferably not less than 90%, most preferably not less than 95% (for example, substantially 100%).

[0098] The amino group of the amino acid residue at the N-terminus and C-terminus of the collagen or a partial fragment thereof having specific affinity for HSP47 to be used for the method of the present invention may be protected by a protecting group (e.g., a formyl group, a C1-6 acyl group such as C1-6 alkanoyl (an acetyl group etc.) and the like).

[0099] The aforementioned collagen or a partial fragment thereof having specific affinity for HSP47 may be directly or indirectly labeled with a labeling substance. Examples of the labeling substance include fluorescent substance (e.g., FITC, rhodamine), radioactive substance (e.g., <sup>14</sup>C, <sup>3</sup>H, <sup>125</sup>I), enzyme (e.g., alkaline phosphatase, peroxidase), colored particles (e.g., colloidal metal particles, colored latex), biotin and the like.

[0100] As such collagen or a partial fragment thereof having specific affinity for HSP47 in this step, only one kind of collagen or a partial fragment thereof having specific affinity for HSP47, or two or more kinds thereof may be used.

[0101] When recombinant collagen is used for the quantification of HSP47, recombinant collagen can be produced, for example, by the following method. A polynucleotide encoding the collagen subunit amino acid sequence (e.g., in the case of human Type 1 collagen, polynucleotide containing nucleotide sequences shown by SEQ ID NO: 3 and SEQ ID NO: 5 (GeneBank accession Nos.: NM\_000088.3 and NM\_000089.3)) is incorporated into an appropriate expression vector, the vector is introduced into an appropriate host for transformation and association of collagen subunit molecules in the host, and the object recombinant collagen can be obtained from the culture supernatant of the transformant. The above-mentioned host cell is not particularly limited, and various host cells conventionally used for genetic engineering methods, for example, *Escherichia coli*, *Bacillus* species bacteria, yeast, plant or animal cell and the like can be used.

[0102] Collagen may be, in addition to a recombinant protein produced from the above-mentioned transformant, one isolated or purified from a natural cell producing it or culture supernatant thereof, by a protein separation and purification technique known per se. Moreover, it may be a protein synthesized by a chemical synthesis or synthesized biochemically in cell-free translation system.

[0103] The above-mentioned "collagen or a partial fragment thereof having specific affinity for HSP47" can be used in the form of an aqueous solution; however, it is preferably bound to a solid phase. Examples of such "solid phase" include a plate (e.g., a microwell plate), a tube, beads (e.g., plastic beads, magnetic beads), a chromatography carrier (e.g., Sepharose (trade mark)), a membrane (e.g., a nitrocellulose membrane, a PVDF membrane), a gel (e.g., a polyacrylamide gel), a metal film (e.g., a gold film) and the like. Of these, a plate, beads, a membrane and a metal film are preferably used, and a plate is most preferably used in view of the convenience of handling. The above-mentioned bond

is not particularly limited and covalent bond, ionic bond, physical adsorption and the like can be mentioned. Covalent bond and/or physical adsorption are/is preferable for achieving sufficient bond strength. As a binding to a solid phase, it may be directly bound to a solid phase or indirectly bound to a solid phase by utilizing a substance known per se. Furthermore, to suppress non-specific adsorption and non-specific reaction, a phosphate buffered solution of bovine serum albumin (BSA), cow's milk protein and the like is contacted with a solid phase, and a solid phase surface not coated with the antibody is generally blocked with the aforementioned BSA, cow's milk protein and the like.

**[0104]** In this step, the contact between the "collagen or a partial fragment thereof having specific affinity for HSP47" and the "HSP47" contained in a biological sample derived from a test subject is carried out by mixing said biological sample and the collagen or a partial fragment thereof having specific affinity for HSP47 in a reaction container and, as long as the method permits their interaction, the embodiment, order, specific method and the like are not particularly limited. The contact is performed by, for example, adding (extract of) said biological sample to a plate on which the "collagen or a partial fragment thereof having specific affinity for HSP47" is immobilized.

**[0105]** The time of maintaining such contact is not particularly limited as long as it is sufficient for the aforementioned collagen or a partial fragment thereof having specific affinity for HSP47 can bind to HSP47 contained in a biological sample derived from a test subject to form a complex, and it is generally several seconds to more than a dozen hours. To rapidly determine whether acute lung injury or not, it is preferably 1 min to 2 hr, most preferably 2 min to 30 min. The temperature condition of contact is generally 4°C - 50°C, preferably 4°C - 37°C, most preferably room temperature of about 15°C - 30°C. Furthermore, the pH condition for the reaction is preferably 5.0 - 9.0, particularly preferably the neutral range of 6.0 - 8.0.

**[0106]** In step (2'), the complex formed in the above-mentioned step (1') is detected to determine whether HSP47 is present or not in the above-mentioned biological sample.

**[0107]** The above-mentioned detection is performed by detecting "HSP47" contained in the complex. For the detection, enzyme immunoassay (EIA method), immunochromatography, latex agglutination, radioimmunoassay (RIA method), fluorescent immunoassay (FIA method), luminescence immunoassay, surface plasmon resonance measurement method (SPR method) and the like can be utilized. Of these, EIA method, immunochromatography, FIA method and SPR method are preferable from the aspects of easiness and rapidness of the operation.

**[0108]** When EIA method is selected as the detection method in step (2'), the EIA method is preferably ELISA method.

**[0109]** As one kind of ELISA method, ELISA method utilizing an enzyme-labeled antibody is applicable. In this method, for example, HSP47 in the blood is trapped by any immobilized "collagen or a partial fragment thereof having specific affinity for HSP47", and an antigen-antibody reaction is performed between the trapped HSP47 and enzyme-labeled "antibody that specifically recognizes HSP47". The time required for such reaction is preferably 1 min - 2 hr, more preferably 2 min - 30 min, since a rapid measurement is necessary. Then HSP47 is detected by detecting the enzyme. The enzyme-labeled antibody can be produced by binding (labeling) the enzyme to "antibody that specifically recognizes HSP47" by a method known per se, for example, glutaraldehyde method, maleimide method and the like.

**[0110]** The definition of the "antibody that specifically recognizes HSP47" is as mentioned above.

**[0111]** As the "enzyme" of the enzyme-labeled antibody, peroxidase, alkaline phosphatase, glucose oxidase,  $\beta$ -galactosidase and the like are exemplified.

**[0112]** As a substrate reagent to be used for the detection of the enzyme, one suitable for the selected labeling enzyme is used. For example, when a peroxidase is selected as an enzyme, o-phenylenediamine (OPD), tetramethylbenzidine (TMB) and the like are used. When an alkaliphosphatase is selected, p-nitrophenyl phosphate (PNPP) and the like are used. As a reaction quenching liquid and a substrate dissolution liquid, conventionally known ones can be used as appropriate according to the selected enzyme, without any particular limitation.

**[0113]** In one specific example, when the above-mentioned EIA method is applied, the blood collected from a test subject is shaken for a predetermined time at room temperature, and centrifuged to give serum. Then the serum is dispensed into a microplate immobilizing any "collagen or a partial fragment thereof having specific affinity for HSP47", and left standing at room temperature for a predetermined time. After the plate is washed to remove unreacted antibody, a solution of the antibody that specifically recognizes HSP47, which is labeled with peroxidase etc. is dispensed into a plate and the mixture is reacted at room temperature for a predetermined time. The plate is washed, and reacted with a solution of chromogen, such as TMB and the like to detect the complex.

**[0114]** In step (3'), the amount of HSP47 in a biological sample is determined from the amount of the complex detected in the above-mentioned step (2').

**[0115]** The amount (concentration) of the complex detected in the step (2') can be measured by correlating a signal of fluorescence intensity or absorbance and the like to a separately prepared calibration curve. The concentration of HSP47 in a biological sample of a test subject can be quantified by multiplying the concentration obtained from the calibration curve by the dilution rate.

**[0116]** The calibration curve is shown by an approximation formula plotting the measured values, wherein the horizontal axis (vertical axis) shows the concentration of the samples obtained by serially diluting the aforementioned standard HSP47 protein, and the vertical axis (horizontal axis) shows the signal (in any unit) of the antibody bound to HSP47 in

a standard HSP47 protein. The calibration curve can be shown in an approximate straight line by logarithmic presentation of the both axes.

5 [0117] Next, the amount of HSP47 in a biological sample derived from a test subject, and the possibility of being affected with acute lung injury are correlated, and whether or not the test subject is affected with acute lung injury is determined based on the correlation. As shown in the below-mentioned Examples, as compared to healthy human and patients with chronic progressive interstitial pneumonia, patients with acute lung injury show a higher amount of HSP47 in a biological sample. The above-mentioned determination is made based on the positive correlation between such amount of HSP47 in a biological sample derived from a test subject and the possibility of being affected with acute lung injury.

10 [0118] For example, biological samples (e.g., serum) are collected from healthy human or patients with chronic progressive interstitial pneumonia (negative control), and patients with acute lung injury (positive control), the amount of HSP47 in the biological samples collected from the test subjects is compared with that of the positive control and negative control. Alternatively, a correlation diagram of the amount of HSP47 in a particular biological sample (e.g., serum) and acute lung injury morbidity rate is prepared in advance, and the amount of HSP47 in a biological sample collected from a test subject may be compared with the correlation diagram. The amount of HSP47 is preferably compared based on the presence or absence of a significant difference.

15 [0119] From the comparison results of the amount of HSP47, when the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a relatively low possibility of being affected with acute lung injury. In a differentiation of acute lung injury from chronic progressive interstitial pneumonia using the method (2) of the present invention, when the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury rather than chronic progressive interstitial pneumonia. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a high possibility of being affected with chronic progressive interstitial pneumonia rather than acute lung injury.

20 [0120] In a further aspect, acute lung injury can be distinguished from the above-mentioned diseases showing clinical findings common with acute lung injury by using the method (2) of the present invention. Clinical findings of acute lung injury are similar to those of the above-mentioned diseases. Since the above-mentioned diseases showing clinical findings common with acute lung injury do not have a factor that promotes collagen synthesis, the amount of HSP47 in the biological sample does not increase.

25 [0121] Specifically, for example, biological samples (e.g., serum) are collected from healthy human or patients with the above-mentioned diseases showing clinical findings common with acute lung injury (negative control), and patients with acute lung injury (positive control), the amount of HSP47 in the biological samples collected from the test subjects is compared with that of the positive control and negative control. Alternatively, a correlation diagram between the amount of HSP47 in a particular biological sample (e.g., serum) and acute lung injury morbidity rate is prepared in advance, and the amount of HSP47 in a biological sample collected from a test subject may be compared with the correlation diagram. The amount of HSP47 is preferably compared based on the presence or absence of a significant difference.

30 [0122] When the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a relatively high possibility of being affected with acute lung injury rather than the above-mentioned diseases showing clinical findings common with acute lung injury. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a high possibility of being affected with the above-mentioned diseases showing clinical findings common with acute lung injury rather than acute lung injury.

35 [0123] Moreover, the present invention provides a diagnostic reagent for acute lung injury comprising the aforementioned collagen or a partial fragment thereof having specific affinity for HSP47. Said diagnostic reagent can be a diagnosis kit for acute lung injury. Using the diagnostic reagent of the present invention, whether or not a test subject is affected with acute lung injury can be easily determined by quantifying HSP47 in a biological sample derived from a test subject by an immunological means in the aforementioned method (2) of the present invention, or acute lung injury can be distinguished from chronic progressive interstitial pneumonia or the above-mentioned diseases showing clinical findings common with acute lung injury.

40 [0124] The collagen or a partial fragment thereof having specific affinity for HSP47 can also be used in unbound state in an aqueous solution, and it is preferably bound to a solid phase. Examples of the "solid phase" include a plate (e.g., a microwell plate), a tube, beads (e.g., plastic beads, magnetic beads), a chromatography carrier (e.g., Sepharose (trade mark)), a membrane (e.g., a nitrocellulose membrane, a PVDF membrane), a gel (e.g., a polyacrylamide gel), a metal film (e.g., a gold film) and the like.

45 [0125] The diagnostic reagent of the present invention may comprise, besides collagen or a partial fragment thereof having specific affinity for HSP47, a reagent to be used for quantifying the amount of HSP47 in a biological sample and

the like. These reagents may be stored in advance together with the collagen or a partial fragment thereof having specific affinity for HSP47, or stored in separate containers. Examples of the reagent include a treatment liquid, a buffer for diluting an antibody, an antibody that specifically recognizes HSP47 derived from a test subject, a labeling substance (e.g., a fluorescence dye, an enzyme), a reaction vessel, a positive control (e.g., a recombinant HSP47), a negative control, a solid phase, an instruction sheet describing the examination protocol and the like. These components can also be mixed in advance as necessary.

(2) Use of heat shock protein 47 as a biomarker for predicting the onset of acute lung injury, in hyperacute stage before meeting the clinical definition of acute lung injury

**[0126]** In another embodiment of the present invention, the onset of acute lung injury can be predicted in hyperacute stage before meeting the clinical definition of acute lung injury, by quantifying heat shock protein 47 in a biological sample derived from a test subject.

**[0127]** In the embodiment, the "test subject" can be a test subject who fails to meet the clinical definition of acute lung injury. Examples of the test subject include, but are not limited to, healthy human, a test subject affected with interstitial pneumonia, and a test subject affected with an acute respiratory disease such as bacterial pneumonia, aspiration pneumonia and the like. The test subject can be a test subject showing no clinical finding of acute lung injury.

**[0128]** The heat shock protein 47 in the above-mentioned biological sample derived from a test subject can be quantified in the same manner as in the above-mentioned method (1) of the present invention and the method (2) of the present invention.

**[0129]** The amount of HSP47 in the above-mentioned biological sample derived from a test subject is correlated with the possibility of developing acute lung injury, and whether or not the test subject will develop acute lung injury is determined based on the correlation. In the above-mentioned test subjects, the higher the amount of HSP47 in the biological sample, the higher the possibility of developing acute lung injury. The above-mentioned determination is made based on the positive correlation between such amount of HSP47 in a biological sample derived from a test subject and the possibility of developing acute lung injury.

**[0130]** For the above-mentioned determination, for example, a correlation diagram of the amount of HSP47 in a particular biological sample (e.g., serum) and the possibility of developing acute lung injury may be prepared in advance, and the amount of HSP47 in a biological sample collected from a test subject may be compared with the correlation diagram.

**[0131]** From the comparison results of the amount of HSP47, when the amount of HSP47 in a biological sample derived from a test subject is relatively high, the test subject can be determined to have a high possibility of developing acute lung injury. Conversely, when the amount of HSP47 in a biological sample derived from a test subject is relatively low, the test subject can be determined to have a low possibility of developing acute lung injury.

**[0132]** When serum HSP47 increases during the follow-up of patients with interstitial pneumonia, high possibility of acute exacerbation in a short span and the onset of rapidly progressive interstitial pneumonia meeting the clinical definition of acute lung injury can be predicted. Since acute exacerbation of interstitial pneumonia shows only the general common cold-like symptoms in the early stages of the onset, it is often treated as a simple cold, during which the severity increases and often becomes fatal. Even when acute exacerbation of interstitial pneumonia is suspected, an early diagnosis is difficult since hypoxia or abnormal shadow in chest radiography is found only after exacerbation reaches a certain level. However, even when a patient with interstitial pneumonia shows extremely mild symptoms, the measurement of serum HSP47 enables determination of a low possibility of developing acute exacerbation when the measured value is low or a high possibility of detecting a very early stage of acute exacerbation when the measured value is high. Thus, the very early stage of interstitial pneumonia patients developing acute exacerbation can be diagnosed, and the onset of acute exacerbation can be predicted even before the onset thereof.

**[0133]** Similarly, also in acute respiratory diseases such as bacterial pneumonia, aspiration pneumonia and the like, the measurement of serum HSP47 enables determination of a low possibility of acute exacerbation to develop acute lung injury when the measured value is low, or a high possibility of detecting a very early stage of acute exacerbation developing acute lung injury when the measured value is high.

(3) Comprehension of the activity of acute lung injury, and use of heat shock protein 47 as a biomarker for predicting prognosis

**[0134]** In another embodiment of the present invention, activity of acute lung injury can be comprehended and prognosis of acute lung injury can be predicted by quantifying heat shock protein 47 in a biological sample derived from a test subject affected with acute lung injury.

**[0135]** Since the HSP47 value in the serum of a test subject affected with acute lung injury is correlated with the disease activity of acute lung injury, the activity and severity can be comprehended, the treatment effect can be evaluated,

and the prognosis can be predicted by measuring HSP47 in the serum.

**[0136]** The heat shock protein 47 in a biological sample derived from a test subject affected with acute lung injury can be quantified in the same manner as in the above-mentioned method (1) of the present invention and the method (2) of the present invention.

**[0137]** The amount of HSP47 in a biological sample derived from a test subject affected with acute lung injury is correlated with the disease activity of acute lung injury, and the activity and severity of acute lung injury in the test subject is determined based on the correlation. In this determination, for example, a correlation diagram of the amount of HSP47 in a particular biological sample (e.g., serum) and disease activity of acute lung injury is prepared in advance, and the amount of HSP47 in the biological sample collected from a test subject is compared with the correlation diagram.

Examples

**[0138]** The present invention is explained in more detail in the following by referring to Examples, which are not to be construed as limitative.

Test subjects

**[0139]** The test subjects were patients at Nagasaki University Hospital, including ALI (32 cases), COP (12 cases), idiopathic UIP (I-UIP; 19 cases), idiopathic NSIP (I-NSIP; 16 cases), CVD-UIP (11 cases), CVD-NSIP (12 cases) and voluntary healthy subjects (18 cases). The ALI cases include rapidly progressive interstitial pneumonia meeting the clinical definition of ALI. The test protocol was approved by the institutional review board, and informed consent was obtained from the test subjects. Further specific clinical backgrounds are shown in Table 1. In the Table, P/F ratio, A-aDO<sub>2</sub> and Respiratory index show PaO<sub>2</sub>/FiO<sub>2</sub> (mmHg) (arterial partial pressure of oxygen divided by fraction of inspired oxygen), alveolar-arterial oxygen difference (difference between alveolar partial pressure of oxygen (PAO<sub>2</sub>) and arterial partial pressure of oxygen (PaO<sub>2</sub>)) and A-aDO<sub>2</sub>/PaO<sub>2</sub> (alveolar-arterial oxygen difference divided by arterial partial pressure of oxygen), respectively, and KL-6, SP-D, SP-A and LDH show serum marker values. P/F ratio, A-aDO<sub>2</sub> and respiratory index showed a statistically significant difference at p<0.01 by comparison of ALI patients with patients with COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP.

[0140]

Table 1

Median (Range)		healthy (control)	COP	I-UIP	I-NSIP	CVD-UIP	CVD-NSIP	ALI(ex19-4-5)	Kruskal-Wallis Test p value						
Age (years)	36.5	27-59	65.5	38-87	64.0	34-75	57.0	28-74	60.0	41-71	59.5	40-71	69.0	16-82	<0.0001
Sex (male/female)	11/7		7/5		13/6		6/10		3/8		3/9		17/15		N.S. ( $\chi^2$ -square test)
P/F ratio			385.0	289.5-490.5	389.8	283.3-491.9	399.3	343.3-442.4	393.3	339.5-471.9	382.9	330.5-443.8	129.6	55.4-282.4	<0.0001
A-a DO2			19.9	-5.14-46.9	17.1	-3.5-52.7	15.3	2.6-37.9	16.5	-8.9-36.9	35.1	0.03-48.5	307.3	38.8-611.4	<0.0001
Respiratory index			0.24	-0.05-0.77	0.21	-0.03-0.77	0.18	0.03-0.53	0.20	-0.09-0.52	0.43	0.0003-0.7	3.79	0.65-10.9	<0.0001
KL-6	195	144-322	427.5	172-1310	1460.0	444-4340	1568.5	192-4745	786.0	348-1610	848.0	149-2550	757.0	158-5800	<0.0001
SP-D	17.3	17.3-53.3	105.7	27.8-247	316.0	93.1-721	500.0	49.2-942	187.0	33.5-264	116.0	33.2-275	223.0	29-4510	<0.0001
SP-A	22.8	12.1-60.8	52.8	20.6-129	103.0	62.4-355	48.9	20.3-127	92.9	49.6-114	47.6	26.2-253	112.0	43.8-328	<0.0001
LDH	124.5	20-246	164.0	132-236	233.0	113-416	212.5	135-738	373.0	172-474	243.5	122-670	380.0	177-2845	<0.0001

[Example 1]

Preparation of HSP47 protein

5 Preparation of collagen-immobilized column

[0141] CNBr-activated Sepharose 4B (GE Bioscience) was suspended in 1 mM HCl, and the suspension was mixed with the equal amount of 1 mM HCl solution of 3 mg/ml swine type I collagen (Nitta Gelatin Inc.). A 2-fold amount of 0.2 M NaHCO<sub>3</sub> and 0.5 M NaCl were added, and the mixture was stirred using a rotator at room temperature for 2 hr. The resin was recovered by centrifugation, suspended in 1 M ethanolamine hydrochloride (pH 8.5), and the suspension was stirred at 4°C for 1 hr. The resin was washed with 0.2 M NaHCO<sub>3</sub>, 0.5 M NaCl, and then with 0.1 M sodium acetate buffer (pH 4.0) containing 0.5 M sodium chloride. This was used as a collagen-Sepharose 4B resin for HSP47 purification.

Preparation of recombinant HSP47 protein

15 [0142] cDNA of human HSP47 was subcloned to pET3a to construct an expression plasmid (pET-hH47). *Escherichia coli* BL21 (DE3) was transformed with pET-hH47. The transformants were cultured at 25°C, 0.1 mM isopropyl thiogalactoside was added, and they were further cultured for 2 hr. The following purification operation was performed at 4°C. Recombinant *Escherichia coli* cells were recovered by centrifugation, and suspended in an extraction buffer (50 mM Tris hydrochloride, pH 8.0, 150 mM sodium chloride, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstatin A, 1 µg/ml leupeptin, 0.2% Nonidet P40, 30% glycerol). After ultrasonication, the suspension was centrifuged at 12,000 rpm for 15 min, the supernatant was applied to collagen-Sepharose 4B column equilibrated with 150 mM sodium chloride-containing 50 mM Tris-hydrochloride, pH 8.0 (bed volume 360 ml per 1 liter culture, flow rate 1 ml/min). The column was washed with 50 mM Tris-hydrochloride, pH 8.0, 150 mM sodium chloride and 1% Nonidet P40. HSP47 bound to the resin was eluted with a solution of MES buffer, pH 5.8, 150 mM NaCl, 1% Nonidet P40 and 10% glycerol. The eluate was neutralized with 1/10 amount of 1 M Tris hydrochloride, pH 8.0, and glycerol was added to a final concentration of 20%.

[Example 2]

30 Production of antibody immobilized plate

[0143] Fifty µL/well of rabbit anti-HSP47 polyclonal antibody (manufactured by Santa Cruz Biotechnology) diluted with 50 mM sodium carbonate buffer (pH 9.6) to 1.0 µg/mL was added to a 96-well microplate (manufactured by Nunc), and the plate was left standing at 4°C overnight to bind the antibody to the plate surface. After discarding the antigen solution, 200 µL/well of a phosphate buffer containing 2% BSA (manufactured by Sigma Ltd.) was added to suppress non-specific adsorption to the plate and non-specific reaction, and the plate was left standing at room temperature for 2 hr to carry out blocking the plate. Then, the well was washed three times with PBS containing 0.05% Tween 80 (PBST) to prepare an anti-HSP47 antibody immobilized plate.

40 Detection of recombinant HSP47 and preparation of calibration curve

[0144] As recombinant HSP47, a product purified by the above-mentioned method was used. To the plate prepared in (1) was added 100 µL/well of the aforementioned recombinant HSP47 solution (in 1% BSA-PBST, pH 7.4) with various concentrations, and the plate was left standing at room temperature for 2 hr to form a complex of anti-HSP47 antibody bound to recombinant HSP47. The well was washed three times with PBST, and mouse anti-HSP47 monoclonal antibody (0.5 µg/mL, manufactured by Stressgen) was added at 100 µL/well. The plate was left standing at 37°C for 2 hr to bind the mouse anti-HSP47 monoclonal antibody to the recombinant HSP47 in the complex. The well was washed three times with PBST, POD-labeled antimouse IgG antibody (diluted 10,000-fold, manufactured by Biosource) was added at 100 µL/well, and the plate was left standing at 37°C for 2 hr. The well was washed three times with PBST, 100 µL/well of 3,3',5,5'-tetramethylbenzidine substrate solution (manufactured by Biosource) was added, and the plate was left standing at room temperature for 15 min to allow color development. The reaction was quenched with the equal amount of 1M phosphoric acid, and the absorbance at 450 nm was measured. A calibration curve was prepared based on the obtained data.

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[Example 3]

Detection of HSP47 by using human serum as a sample

5 **[0145]** By an operation similar to that in Example 2 except that the serum of a patient affected with acute lung injury or various kinds of chronic progressive interstitial pneumonia was used as a sample instead of the recombinant HSP47 solution, the absorbance was measured. Based on the calibration curve prepared in Example 2, the concentration of HSP47 in the serum of each patient was calculated. The results are shown in Fig. 1.

10 **[0146]** While the HSP47 concentrations of the patients with chronic progressive interstitial pneumonia (COP, I-UIP, I-NSIP, CVD-UIP, CVD-NSIP) and healthy humans were of a low level, patients with acute lung injury showed an extremely high concentration level (Fig. 1). When the HSP47 concentration was respectively compared between ALI patients and patients with COP, I-UIP, I-NSIP, CVD-UIP and CVD-NSIP or healthy humans, a statistically significant difference was found at  $p < 0.05$ . Therefore, whether or not a patient is affected with acute lung injury can be determined using the blood HSP47 concentration as an index.

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[Comparative Example 1]

20 **[0147]** The measurement was also performed for KL-6, SP-D, SP-A and LDH by using the sera of the above-mentioned test subjects. Respective results are shown in Figs. 2 - 5. No significant difference was found for any serum markers between patients with chronic progressive interstitial pneumonia and acute lung injury.

[Example 4]

Receiver Operating Characteristic (ROC) curve analysis

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**[0148]** Receiver operating characteristic (ROC) curves were drawn based on the measurement values of the respective serum markers of HSP47 protein, KL-6, SP-D, SP-A and LDH in the test subjects (see Shuji Matsuo and Hiroshi Takahashi: Practical Application of Receiver Operating Characteristic Curve on Laboratory Diagnosis. Rinsho Byori 42:585-590, 1994 as to the method for preparation). The results are shown in Fig. 6.

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**[0149]** The ROC curve of the HSP47 protein value in the serum was distributed at a position nearest to the upper left corner where the acute lung injury disease group can be completely separated from the chronic progressive interstitial pneumonia disease group, as compared to the ROC curves of KL-6 value, SP-D value, SP-A value and LDH value.

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**[0150]** Furthermore, by setting the cutoff value of the HSP47 protein in the serum to 859.3 pg/mL, acute lung injury and chronic progressive interstitial pneumonia could be distinguished with sensitivity 92.3% and specificity 98.2%. Therefore, the results show that HSP47 is a marker far more useful than the conventional serum markers KL-6, SP-D, SP-A and LDH.

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In this study, the death rate after 1 month was 31.3% (10 cases/32 cases) and that after 3 months was 50% (16 cases/32 cases) for ALI. However, in chronic progressive interstitial pneumonia (COP, I-UIP, I-NSIP, CVD-UIP, CVD-NSIP), all patients were alive after 1 month and 3 months. The results show that HSP47 is a marker useful as a prognosis prediction factor for patients.

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[Example 5]

45 **[0151]** The tests were performed by further increasing the number of the target test subjects.

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Population subjected to investigation for HSP47 in serum

50 **[0152]** The test subjects of this study consisted of 116 patients admitted to Nagasaki University Hospital from April 1991 to April 2011 and 18 adult healthy human volunteers. The patients included 47 patients with rapidly progressive interstitial pneumonia (RPIP) included in acute lung injury in the present invention, 12 patients with cryptogenic organizing pneumonia (COP), 19 patients with idiopathic UIP, 16 patients with idiopathic NSIP, 11 patients with CVD-UIP, and 11 patients with CVD-NSIP. Patients meeting the following four criteria: 1) exacerbated respiratory condition by aggressive progression within 30 days; 2) detection of the first bilateral ground glass opacity and/or infiltration shadow by high resolution chest CT scan; 3) resting  $\text{PaO}_2$ /fraction of inspired oxygen ( $\text{FiO}_2$ ) ratio (P/F ratio)  $< 300$  mmHg; and 4) lack of clear infection, pneumothorax, pulmonary thromboembolism, heart failure or other cause of acute lung injury (physical trauma, blood transfusion or toxic inhaled toxicant) were diagnosed as RPIP. The patients with RPIP consisted of 17 patients with acute exacerbation of IPF, 10 patients with acute interstitial pneumonia (AIP), 8 patients with collagen vascular disease-associated interstitial pneumonia and 12 patients with drug-induced interstitial pneumonia.

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Statistical analysis

**[0153]** The values of continuous variables were expressed as median (range). Differences among groups were determined by analysis of variance or the Kruskal-Wallis test for continuous variables and the  $\chi^2$  test for categorical variables, as appropriate. If a significant difference was found by analysis of variance, pair-wise comparison was performed using the Scheffe method. The upper left corner coordinate point of the receiver operating characteristic curve was used to determine the optimum cutoff level for discriminating between RPIP and other interstitial pneumonias (COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP). The statistical analysis was performed using a statistical software package (SAS 9.1.3, SAS Institute, Cary, NC). p value<0.05 was considered statistically significant.

Characteristics of patients

**[0154]** The characteristics of the patients collected for the study are shown in Table 2. The P/F ratios of the RPIP group were significantly lower (P<0.01) as compared with those of the COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP groups. In the RPIP group, alveolar-arterial difference of oxygen (A-a DO<sub>2</sub>) was significantly low (P<0.01) as compared to the COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP groups. The APACHE (Acute Physiology and Chronic Health Evaluation) II score and SOFA (Sequential Organ Failure Assessment) score in the RPIP group were 15.0 (range 8 - 35) and 4.0 (range 2 - 12), respectively.

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[0155]

Table 2

Median (range)		COP	I-UIP	I-NSIP	CVD-UIP	CVD-NSIP	RPIP	p value
Age (years)	65.5 (38-87)	64.0 (34-75)	57.0 (28-74)	60.0 (41-71)	58.0 (40-71)	69.0 (16-82)		<0.05
Sex (male/female)	7/5	13/6	6/10	3/8	2/9	30/17		<0.05
P/F ratio	385.0 (289.5-490.5)	389.8 (283.3-491.9)	399.3 (343.3-442.4)	393.3 (339.5-471.9)	378.6 (330.5-443.8)	124.7 * (53-287.1)		<0.0001
A-a DO2	19.9 (-5.14-46.9)	17.1 (-3.5-52.7)	15.3 (2.6-37.9)	16.5 (-8.9-36.9)	35.4 (0.03-48.5)	363.2 * (38.7-611.4)		<0.0001

Data shows median value (range).

COP= cryptogenic organizing pneumonia; UIP= usual interstitial pneumonia; NSIP= nonspecific interstitial pneumonia; P/F ratio = resting PaO<sub>2</sub>/fraction of inspired oxygen (FIO<sub>2</sub>) ratio; A-a DO<sub>2</sub> = alveolar-arterial oxygen difference

\* p>0.01 compared to COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP

## Outcome of disease

**[0156]** In the RPIP group, the 30-day mortality rate was 34.0% (16 out of 47 patients) and the 90-day mortality rate was 51.1% (24 out of 47 patients). In contrast, no patient died within 90 days in the COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP groups.

## Levels of HSP47, KL-6, SP-A, SP-D and LDH in serum

**[0157]** The serum HSP47 levels of the patients were quantified in the same manner as in Example 2. The serum HSP47 levels of the RPIP patients (median value 1530.2 [range 631.2 - 14589.9] pg/ml) were significantly higher than those of COP (239.1 [16.6 - 476.6] pg/ml) ( $P < 0.01$ ), idiopathic UIP (330.9 [105.1 - 487.6] pg/ml) ( $P < 0.01$ ), idiopathic NSIP (290.7 [24.8 - 603.0] pg/ml) ( $P < 0.01$ ), CVD-UIP (348.8 [122.8 - 602.0] pg/ml) ( $P < 0.05$ ), CVD-NSIP (383.1 [101.9 - 721.4] pg/ml) ( $P < 0.05$ ), and healthy volunteers (547.9 [332.1 - 879.8] pg/ml) ( $P < 0.05$ ). Serum levels of HSP47 among patients with COP, idiopathic UIP, idiopathic NSIP, CVD-UIP and CVD-NSIP, and healthy volunteers were not significantly different (Fig. 7).

**[0158]** The serum KL-6 levels of the patients with idiopathic UIP, idiopathic NSIP and RPIP (idiopathic UIP patients (1460.0 [444 - 4340] U/ml) ( $P < 0.05$ ), idiopathic NSIP patients (1568.5 [192 - 4745] U/ml) ( $P < 0.05$ ) and RPIP patients (918.5 [107 - 5800] U/ml) ( $P < 0.05$ )) were significantly higher than those of healthy volunteers (195.0 [144 - 322] U/ml). However, the serum KL-6 levels of the patients with RPIP showed no significant difference as compared to those of the patients with each of COP (427.5 [172 - 1310] U/ml), idiopathic UIP, idiopathic NSIP, CVD-UIP (786.0 [348 - 1610] U/ml) and CVD-NSIP (924.0 [149 - 2550] U/ml) (Fig. 8).

**[0159]** The serum SP-A levels of the patients with RPIP and idiopathic UIP (RPIP patients (123.0 [43.0 - 328.0] ng/ml) ( $P < 0.01$ ) and idiopathic UIP patients (103.0 [62.4 - 355.0] ng/ml) ( $P < 0.01$ )) were significantly higher than those of healthy volunteers (22.8 [12.1 - 60.8] ng/ml). However, the serum SP-A levels of the RPIP patients showed no significant difference as compared to each of the patients with COP (52.8 [20.6 - 129.0] ng/ml), idiopathic UIP, idiopathic NSIP (48.9 [20.3 - 127.0] ng/ml), CVD-UIP (92.9 [49.6 - 114.0] ng/ml), and CVD-NSIP (51.9 [26.2 - 253.0] ng/ml) (Fig. 9).

**[0160]** The patients with COP, idiopathic UIP, idiopathic NSIP, CVD-UIP, CVD-NSIP and RPIP (COP patients (105.7 [27.8 - 247.0] ng/ml), idiopathic UIP patients (316.0 [93.1 - 721.0] ng/ml), idiopathic NSIP patients (477.0 [17.2 - 942.0] ng/ml), CVD-UIP patients (187.0 [33.5 - 264.0] ng/ml), CVD-NSIP patients (111.5 [33.2 - 275.0] ng/ml), RPIP patients (280.5 [29.0 - 4510.0] ng/ml)), and healthy volunteers (17.3 [17.3 - 53.3] ng/ml) showed no significant difference in the serum SP-D levels (Fig. 10).

**[0161]** The serum LDH levels of the RPIP patients (368.0 [177 - 2250] IU/l) was significantly higher than those of the COP patients (164.0 [132 - 236] IU/l) ( $P < 0.05$ ) and healthy volunteers (124.5 [20 - 246] IU/l) ( $P < 0.01$ ). However, the serum LDH levels of the RPIP patients showed no significant difference as compared to each of the patients with idiopathic UIP (233.0 [113 - 416] IU/l), idiopathic NSIP (212.5 [135 - 738] IU/l), CVD-UIP (373.0 [172 - 474] IU/l), and CVD-NSIP (247.0 [181 - 670] IU/l) (Fig. 11).

## [Example 6]

## Receiver Operating Characteristic curve

**[0162]** Receiver operating characteristic curves were drawn in the same manner as in Example 4 (Fig. 12). Based on the receiver operating characteristic curves, the HSP47 cutoff value enabling diagnosis with highest accuracy was 617.1 pg/ml. The HSP47 cutoff value of 617.1 pg/ml distinguished RPIP and other interstitial pneumonias (COP, idiopathic UIP, idiopathic NSIP, CVD-UIP, and CVD-NSIP) with 100% sensitivity and 98.6% specificity. The diagnostic accuracy was 99.1%. Use of serum HSP47 levels resulted in the largest area under the curve. Using the serum HSP47 level, the area under the curve in the diagnosis of rapidly progressive interstitial pneumonia was 0.998. In contrast, the areas under the curve in the diagnosis of rapidly progressive interstitial pneumonia based on the serum KL-6, SP-A, SP-D and LDH levels were only 0.475, 0.728, 0.595 and 0.776, respectively.

**[0163]** The results show that HSP47 is a marker far more useful than conventional serum markers KL-6, SP-D, SP-A and LDH.

## Industrial Applicability

**[0164]** According to the examination method of the present invention, a novel biomarker HSP47 for acute lung injury can be detected easily and rapidly. In addition, using the kit for diagnosis of acute lung injury and the diagnostic reagent for acute lung injury of the present invention, whether or not a patient is affected with acute lung injury can be diagnosed conveniently, rapidly and highly accurately.

**[0165]** When a patient with symptoms of cough, respiratory distress, fever and the like and a diffuse shadow in chest radiography receives a medical examination in an actual clinical practice, it is often difficult to predict whether the patient thereafter shows chronic progression, or rapid progression. Measurement of HSP47 enables a convenient, aggressive and highly accurate diagnosis of whether the patient is affected with acute lung injury that will show rapid and lethal progression or chronic progressive interstitial pneumonia that will show chronic progression.

**[0166]** This application is based on a patent application No. 2011-027679 filed February 10, 2011 in Japan, the contents of which are incorporated in full herein.

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SEQUENCE LISTING

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SAPPORO MEDICAL UNIVERSITY

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55 Asn Gly Lys Val Leu Cys Asp Asp Val Ile Cys Asp Glu Thr Lys Asn  
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60 Cys Pro Gly Ala Glu Val Pro Glu Gly Glu Cys Cys Pro Val Cys Pro  
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65 Asp Gly Ser Glu Ser Pro Thr Asp Gln Glu Thr Thr Gly Val Glu Gly  
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Pro Lys Gly Asp Thr Gly Pro Arg Gly Pro Arg Gly Pro Ala Gly Pro  
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 705 710 715 720

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25

Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln  
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Tyr Asp Gly Lys Gly Val Gly Leu Gly Pro Gly Pro Met Gly Leu Met  
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30

Gly Pro Arg Gly Pro Pro Gly Ala Ala Gly Ala Pro Gly Pro Gln Gly  
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35

Phe Gln Gly Pro Ala Gly Glu Pro Gly Glu Pro Gly Gln Thr Gly Pro  
 115 120 125

Ala Gly Ala Arg Gly Pro Ala Gly Pro Pro Gly Lys Ala Gly Glu Asp  
 130 135 140

40

Gly His Pro Gly Lys Pro Gly Arg Pro Gly Glu Arg Gly Val Val Gly  
 145 150 155 160

45

Pro Gln Gly Ala Arg Gly Phe Pro Gly Thr Pro Gly Leu Pro Gly Phe  
 165 170 175

Lys Gly Ile Arg Gly His Asn Gly Leu Asp Gly Leu Lys Gly Gln Pro  
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Gly Ala Pro Gly Val Lys Gly Glu Pro Gly Ala Pro Gly Glu Asn Gly  
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Thr Pro Gly Gln Thr Gly Ala Arg Gly Leu Pro Gly Glu Arg Gly Arg  
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Val Gly Ala Pro Gly Pro Ala Gly Ala Arg Gly Ser Asp Gly Ser Val  
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5 Gly Pro Val Gly Pro Ala Gly Pro Ile Gly Ser Ala Gly Pro Pro Gly  
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10 Phe Pro Gly Ala Pro Gly Pro Lys Gly Glu Ile Gly Ala Val Gly Asn  
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15 Gly Leu Ser Gly Pro Val Gly Pro Pro Gly Asn Pro Gly Ala Asn Gly  
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20 Leu Thr Gly Ala Lys Gly Ala Ala Gly Leu Pro Gly Val Ala Gly Ala  
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Pro Gly Leu Pro Gly Pro Arg Gly Ile Pro Gly Pro Val Gly Ala Ala  
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25 Gly Ala Thr Gly Ala Arg Gly Leu Val Gly Glu Pro Gly Pro Ala Gly  
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35 Gln Gly Pro Pro Gly Pro Ser Gly Glu Glu Gly Lys Arg Gly Pro Asn  
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Gly Glu Ala Gly Ser Ala Gly Pro Pro Gly Pro Pro Gly Leu Arg Gly  
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Gly Pro Asn Gly Asp Ala Gly Arg Pro Gly Glu Pro Gly Leu Met Gly  
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50 Pro Arg Gly Leu Pro Gly Ser Pro Gly Asn Ile Gly Pro Ala Gly Lys  
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55 Glu Gly Pro Val Gly Leu Pro Gly Ile Asp Gly Arg Pro Gly Pro Ile  
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Gly Pro Ala Gly Ala Arg Gly Glu Pro Gly Asn Ile Gly Phe Pro Gly  
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 5 Pro Lys Gly Pro Thr Gly Asp Pro Gly Lys Asn Gly Asp Lys Gly His  
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 10 Ala Gly Leu Ala Gly Ala Arg Gly Ala Pro Gly Pro Asp Gly Asn Asn  
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 Gly Ala Gln Gly Pro Pro Gly Pro Gln Gly Val Gln Gly Gly Lys Gly  
 530 535 540  
 15 Glu Gln Gly Pro Pro Gly Pro Pro Gly Phe Gln Gly Leu Pro Gly Pro  
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 625 630 635 640  
 Leu Pro Gly Glu Arg Gly Ala Ala Gly Ile Pro Gly Gly Lys Gly Glu  
 645 650 655  
 40 Lys Gly Glu Pro Gly Leu Arg Gly Glu Ile Gly Asn Pro Gly Arg Asp  
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 675 680 685  
 50 Ala Thr Gly Asp Arg Gly Glu Ala Gly Ala Ala Gly Pro Ala Gly Pro  
 690 695 700  
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 55 Gly Pro Asn Gly Phe Ala Gly Pro Ala Gly Ala Ala Gly Gln Pro Gly  
 725 730 735

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Ala Lys Gly Glu Arg Gly Ala Lys Gly Pro Lys Gly Glu Asn Gly Val  
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5 Val Gly Pro Thr Gly Pro Val Gly Ala Ala Gly Pro Ala Gly Pro Asn  
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10 Gly Pro Pro Gly Pro Ala Gly Ser Arg Gly Asp Gly Gly Pro Pro Gly  
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Ser Gly Glu Ala Gly Thr Ala Gly Pro Pro Gly Thr Pro Gly Pro Gln  
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30 Gly Leu Leu Gly Ala Pro Gly Ile Leu Gly Leu Pro Gly Ser Arg Gly  
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930 935 940

50 Lys Gly Glu Arg Gly Tyr Pro Gly Asn Ile Gly Pro Val Gly Ala Ala  
945 950 955 960

Gly Ala Pro Gly Pro His Gly Pro Val Gly Pro Ala Gly Lys His Gly  
965 970 975

55 Asn Arg Gly Glu Thr Gly Pro Ser Gly Pro Val Gly Pro Ala Gly Ala



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Ser Ser Lys Asp Lys Lys His Val Trp Leu Gly Glu Thr Ile Asn  
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 Ala Gly Ser Gln Phe Glu Tyr Asn Val Glu Gly Val Thr Ser Lys  
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 10  
 Glu Met Ala Thr Gln Leu Ala Phe Met Arg Leu Leu Ala Asn Tyr  
 1250 1255 1260  
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 Ala Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Ile Ala Tyr  
 1265 1270 1275  
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 Met Asp Glu Glu Thr Gly Asn Leu Lys Lys Ala Val Ile Leu Gln  
 1280 1285 1290  
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 1295 1300  
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 Thr Tyr Thr Val Leu Val Asp Gly Cys Ser Lys Lys Thr Asn Glu  
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 Trp Gly Lys Thr Ile Ile Glu Tyr Lys Thr Asn Lys Pro Ser Arg  
 1325 1330 1335  
 40  
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 1340 1345 1350  
 45  
 Gln Glu Phe Phe Val Asp Ile Gly Pro Val Cys Phe Lys  
 1355 1360 1365

Claims

- 40
1. A method of determining whether or not a test subject is affected with acute lung injury, comprising quantifying heat shock protein 47 in a biological sample derived from a test subject.
  2. The method according to claim 1, which distinguishes acute lung injury from chronic progressive interstitial pneumonia.
  3. The method according to claim 1, which distinguishes acute lung injury from aortic dissection, acute heart failure, acute exacerbation of chronic heart failure, diffuse alveolar hemorrhage, lymphangitic carcinomatosis, reexpansion pulmonary edema, pulmonary edema by hyper-infusion, neurogenic pulmonary edema, pulmonary tuberculosis, or miliary tuberculosis.
  4. The method according to any one of claims 1 - 3, wherein the biological sample is blood, serum or plasma.
  5. A diagnostic reagent for acute lung injury comprising an antibody that specifically recognizes heat shock protein 47 and/or collagen or a partial fragment thereof that has specific affinity for heat shock protein 47.
  6. A method of determining whether or not a test subject will develop acute lung injury, comprising quantifying heat shock protein 47 in a biological sample derived from the test subject.
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7. A method of evaluating a treatment effect for or predicting the prognosis of acute lung injury of a test subject, comprising quantifying heat shock protein 47 in a biological sample derived from the test subject.

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Fig. 1

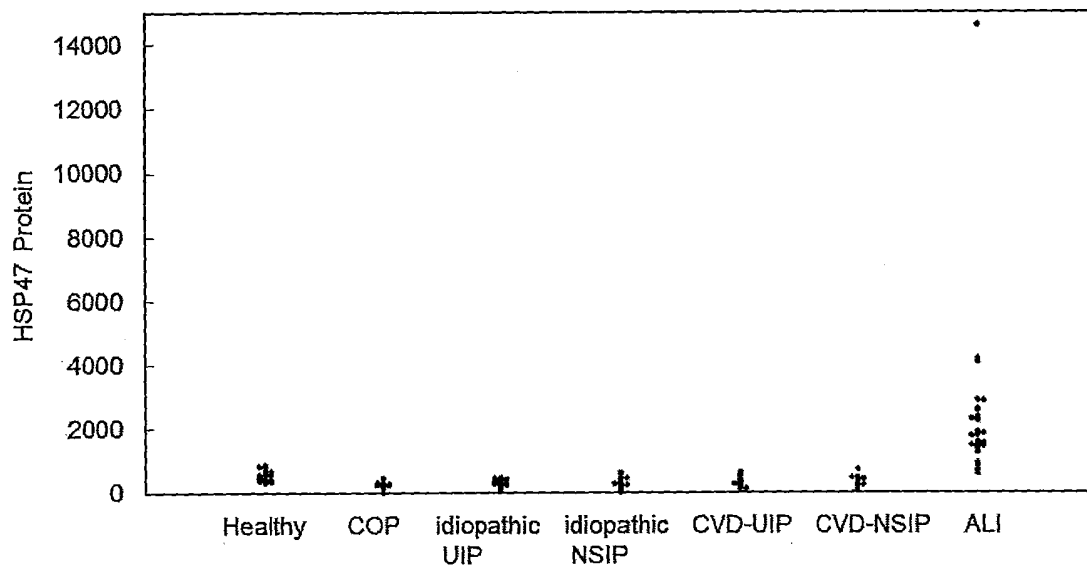


Fig. 2

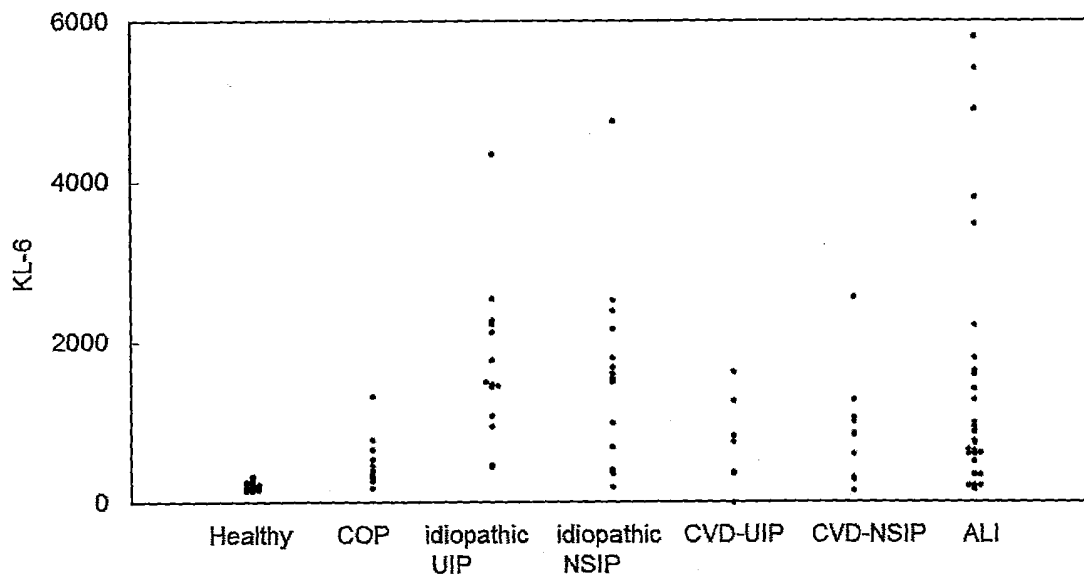




Fig. 5

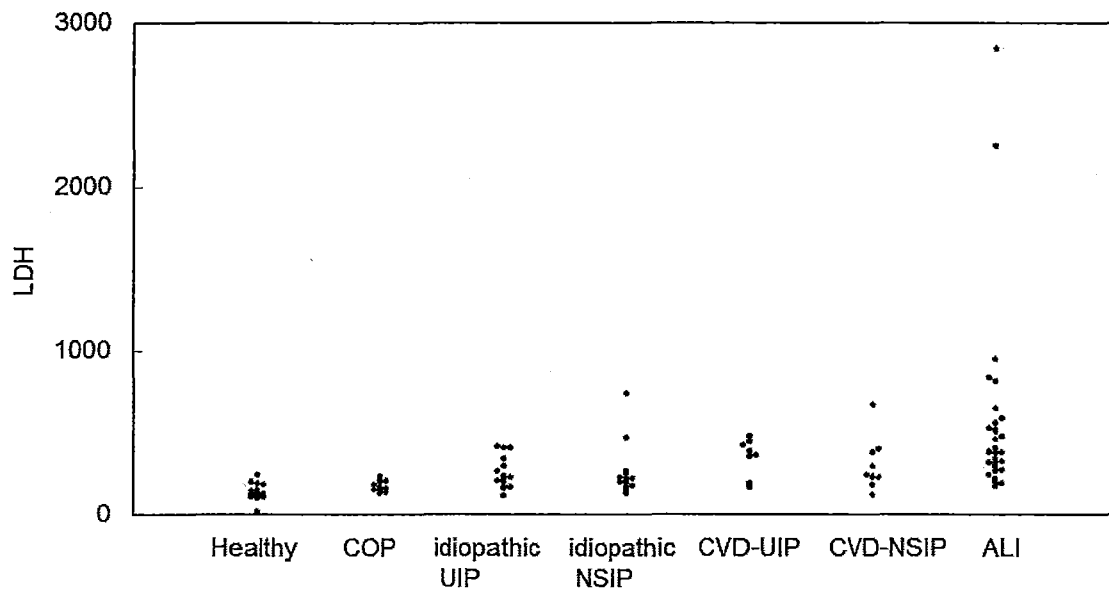


Fig. 6

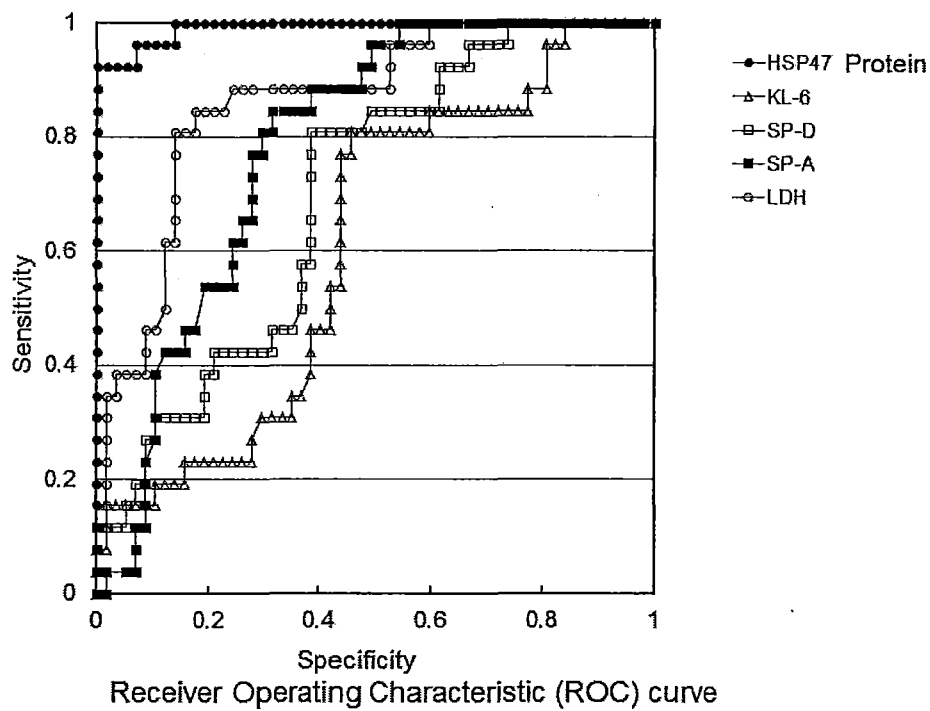


Fig. 7

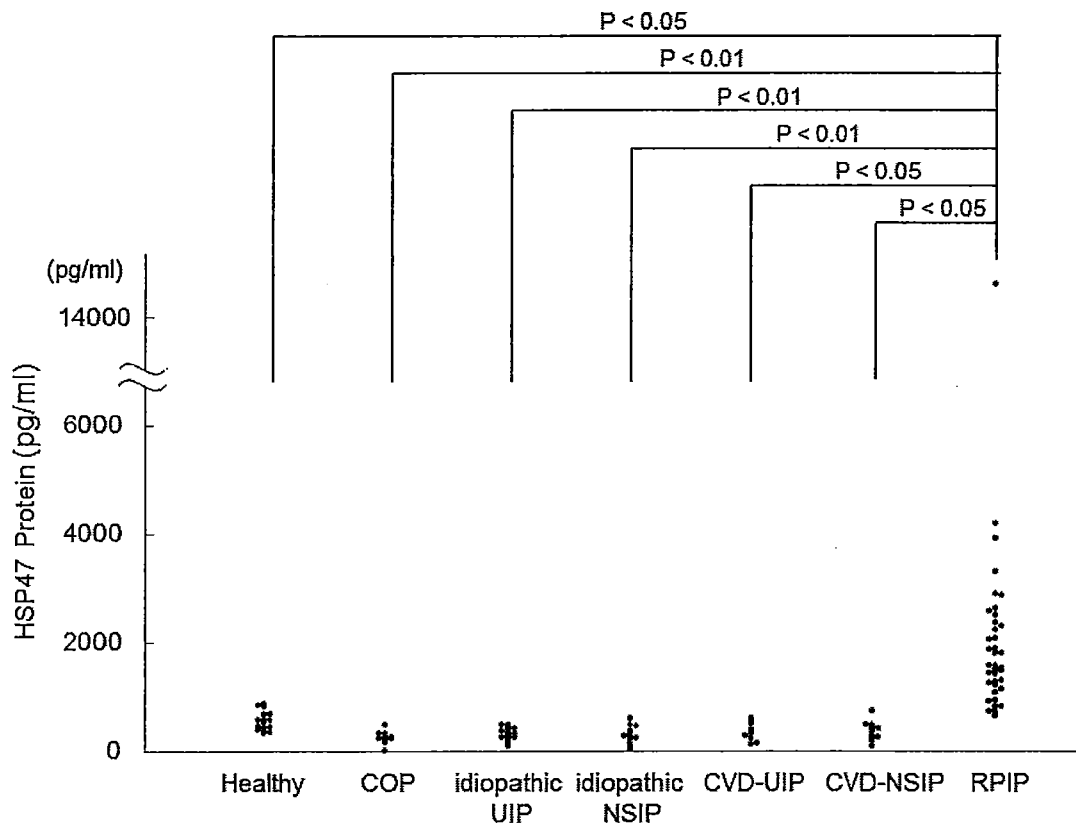


Fig. 8

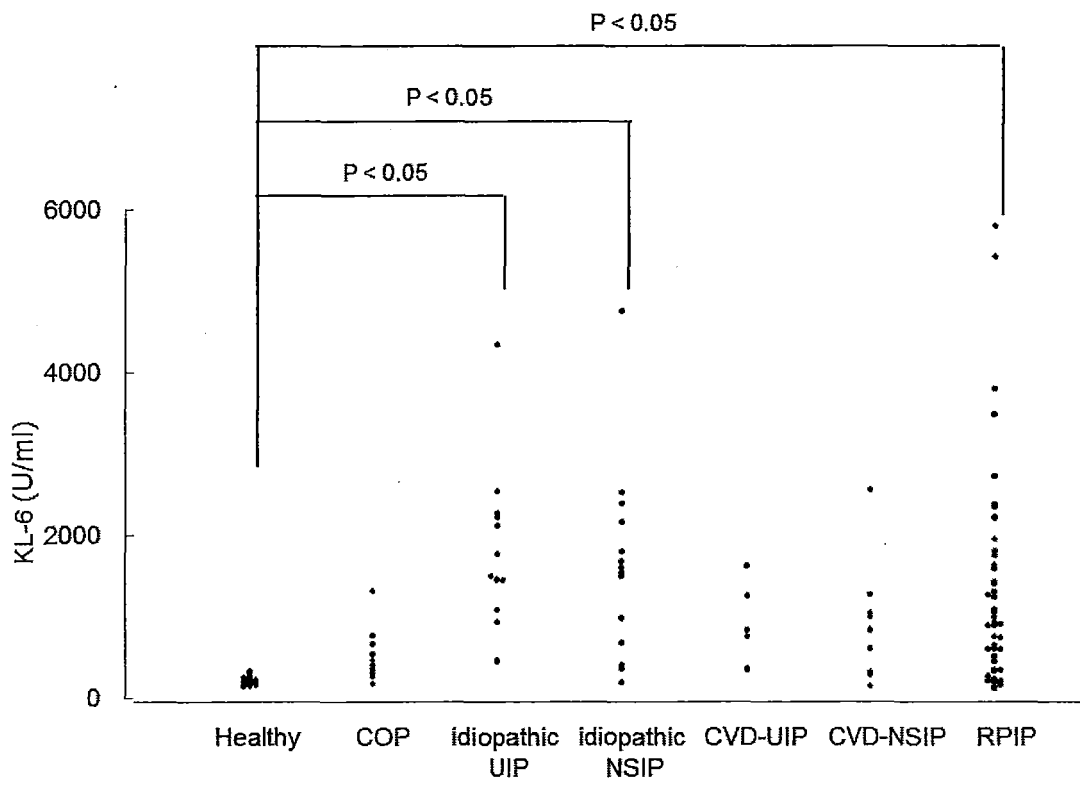


Fig. 9

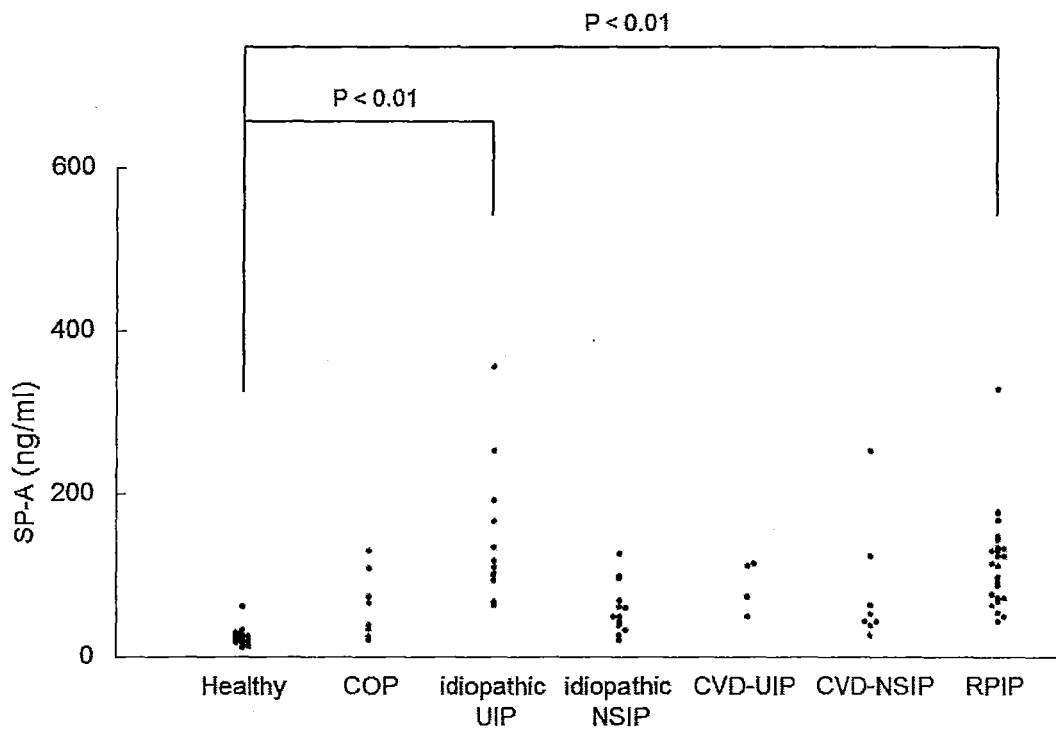


Fig. 10

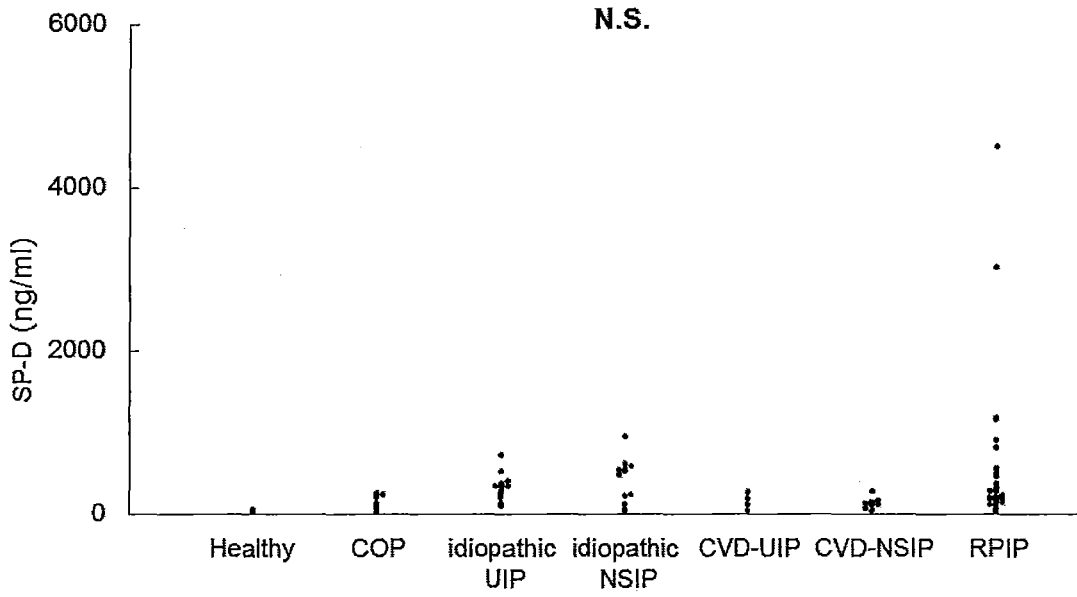


Fig. 11

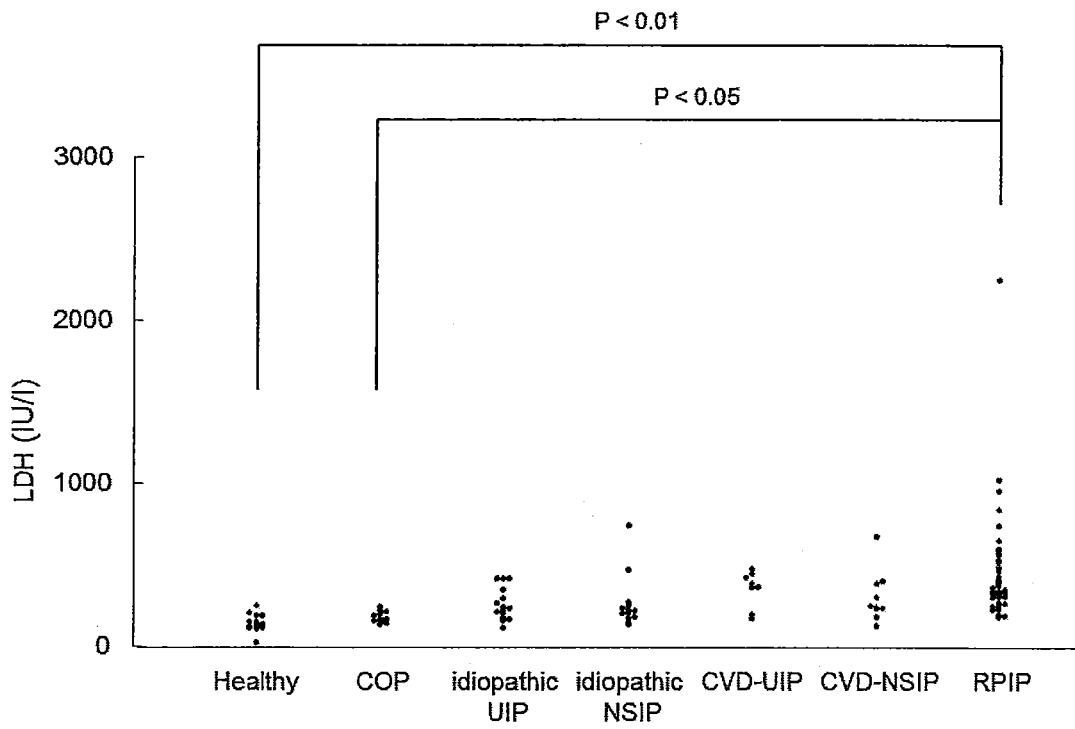
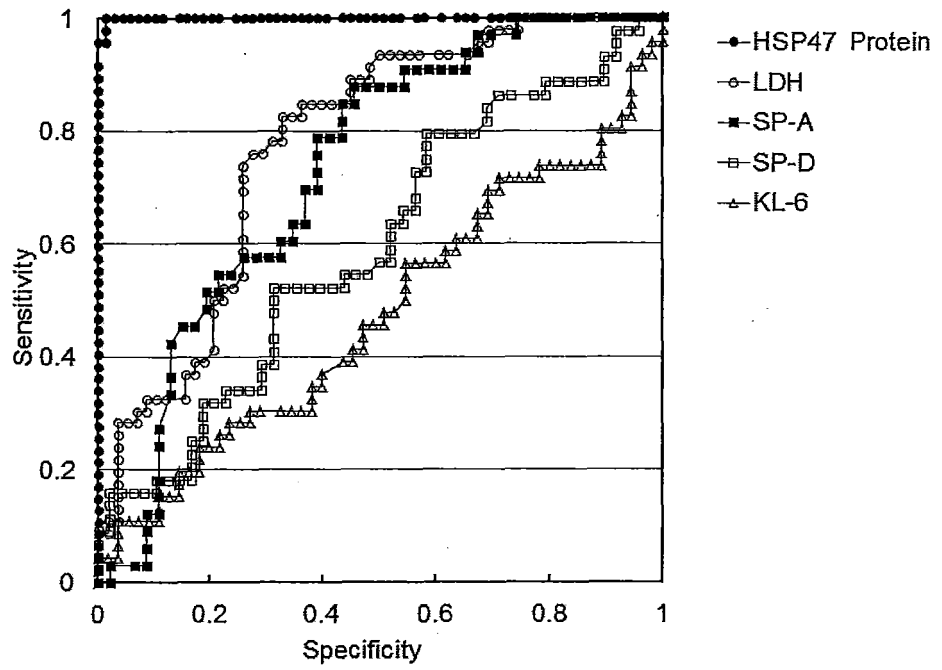


Fig. 12



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/053174

A. CLASSIFICATION OF SUBJECT MATTER G01N33/53(2006.01) i		
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) G01N33/53		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2012 Kokai Jitsuyo Shinan Koho 1971-2012 Toroku Jitsuyo Shinan Koho 1994-2012		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CA/MEDLINE/BIOSIS (STN), JSTPlus/JMEDPlus/JST7580 (JDreamII)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Hideo IWASAKA, "Dai 4 Kai Hai Surfactant Bunshi Byotai Kenkyukai - Hai Byohen no Shufuku Saisei eno Approach - Shinki Hai Shogai Inshi HMGB1 to Hai Sen'ika Inshi HSP47 kara Mita ARDS no Chiryō-ho no Kaihatsu", Respiratory Molecular Medicine, 2006, vol.10, no.3, pages 204 to 208, 3	1-7
A	Hiroshi MIYAGAWA et al., "Dai 4 Sho ARDS no Kiso Shikkan Haiketsusho ni Tomonau ARDS", Journal of Clinical and Experimental Medicine, 2004, separate volume (March), pages 121 to 125, page 214, 2.	1-7
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 06 March, 2012 (06.03.12)		Date of mailing of the international search report 19 March, 2012 (19.03.12)
Name and mailing address of the ISA/ Japanese Patent Office		Authorized officer
Facsimile No.		Telephone No.

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/053174

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Hideo IWASAKA, "What's New in SURGERY FRONTIER Dai 67 Kai Ensho no Bunshi Kiko to Netsu Shock Tanpakushitsu 4) Heiketsusho to Netsu Shock Tanpakushitsu", Surg Front, 2010, vol.17, no.4, pages 380 to 383, page 382	1-7
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A	YOKOTA S et al., Prevalence of HSP47 antigen and autoantibodies to HSP47 in the sera of patients with mixed connective tissue disease, Biochem Biophys Res Commun, 2003, Vol.303, No.2, Page.413-418, Abstract	1-7
A	JP 8-240593 A (Kureha Chemical Industry Co., Ltd.), 17 September 1996 (17.09.1996), abstract (Family: none)	5, 6

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专利名称(译)	诊断急性肺损伤的方法		
公开(公告)号	<a href="#">EP2674755A4</a>	公开(公告)日	2014-12-03
申请号	EP2012744724	申请日	2012-02-10
[标]申请(专利权)人(译)	协和梅迪克斯株式会社		
申请(专利权)人(译)	长崎大学 札幌医科大学 协和梅迪克斯CO. , LTD.		
当前申请(专利权)人(译)	长崎大学 札幌医科大学 协和梅迪克斯CO. , LTD.		
[标]发明人	KAKUGAWA TOMOYUKI YOKOTA SHIN ICHI		
发明人	KAKUGAWA, TOMOYUKI YOKOTA, SHIN-ICHI		
IPC分类号	G01N33/53 G01N33/68		
CPC分类号	G01N33/6884 G01N33/6893 G01N2800/12 G01N2800/50 G01N2800/52		
优先权	2011027679 2011-02-10 JP		
其他公开文献	EP2674755A1 EP2674755B1		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

本发明提供了一种有用的检查方法和诊断试剂，它们能够与慢性进行性间质性肺炎明确区别地诊断急性肺损伤。具体地，本发明提供了一种确定受试者是否患有急性肺损伤的方法，其包括定量来自受试者的生物样品中的热休克蛋白47。