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(54) **METHOD FOR DIAGNOSING PULMONARY ARTERY HYPERTENSION**

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(57) **ABSTRACT**

The invention relates to an in vitro method for detecting pulmonary arterial hypertension (PAHT), or the risk of developing PAHT, which includes determining the presence and/or amount of anti-tenascin C antibodies in a biological sample from a patient.

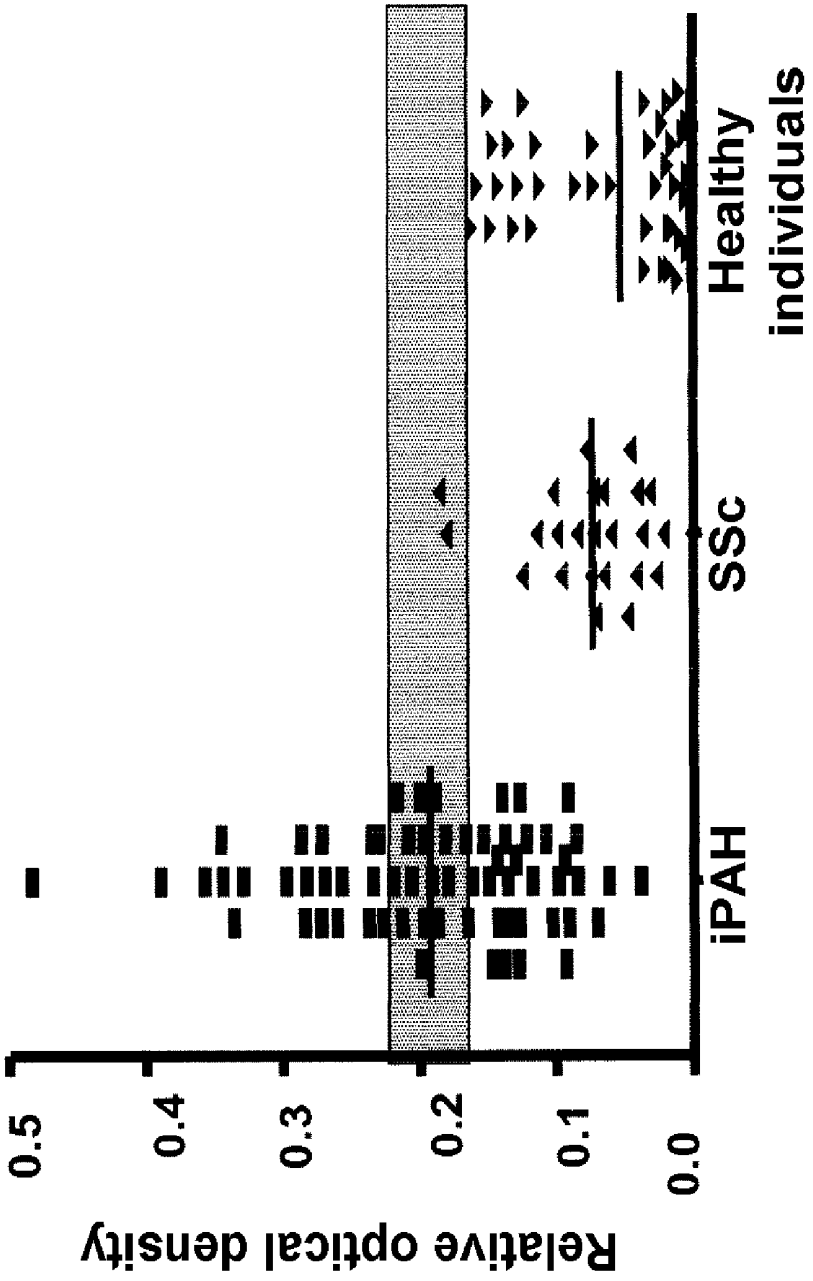
Relative optical density
0.5
0.4
0.3
0.2
0.1
0.0
iPAH
SSc
Healthy individuals

Figure 1

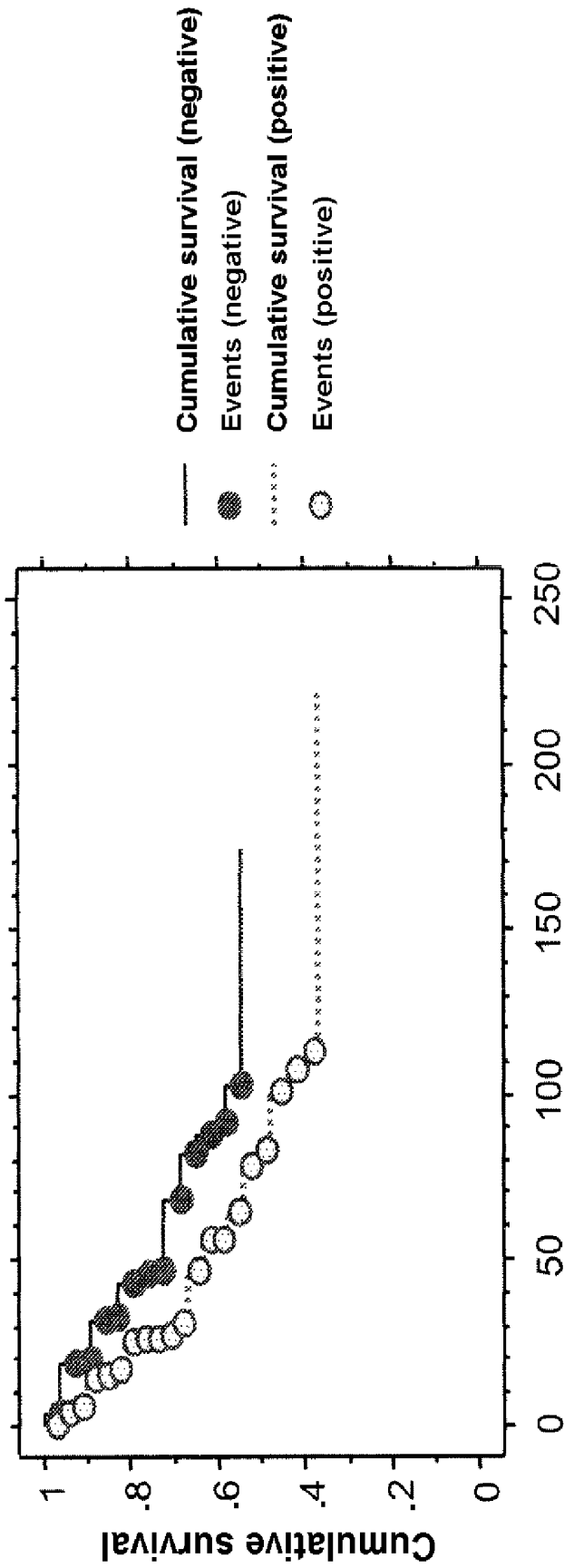
Cumulative survival (negative)
Events (negative)
Cumulative survival (positive)
Events (positive)
Cumulative survival
0
.2
.4
.6
.8
1
0
50
100
150
200
250
Time (months)

Figure 2

METHOD FOR DIAGNOSING PULMONARY ARTERY HYPERTENSION

[0001] The invention relates to the diagnosing and the follow-up of pulmonary arterial hypertension.

PRIOR ART

[0002] Pulmonary arterial hypertension (PAH) is a rare pathological condition responsible for the occurrence of right cardiac decompensation which can result in death. PAH is defined by the demonstration, by right catheterization, of an average pulmonary arterial pressure of greater than or equal to 25 mmHg while resting or of greater than of equal to 30 mmHg while exercising, in the absence of elevated pulmonary capillary pressure (Rubin, 1997). The occurrence of PAH is the result of a chronic obstruction of the small pulmonary arteries secondary to the proliferation of endothelial cells, vascular smooth muscle cells and fibroblast (Dorfmuller et al., 2003). In particular, during severe PAH, a layer of myofibroblasts and of extracellular matrix forms which localizes between the endothelium and the internal elastic lamina, called neointima, which is characteristic of this condition. PAH can occur during the progression of pathological conditions with an autoimmune component, namely the connective tissue diseases, in particular systemic scleroderma (Hachulla et al., 2005), Sharp's syndrome and systemic lupus erythematosus. In addition, during idiopathic PAH, autoimmunity stigmata, namely anti-nuclear antibodies or anti-thyroglobulin antibodies, are from time to time found.

[0003] The presence of anti-endothelial cell antibodies (Tamby et al., 2005) and of anti-fibroblast antibodies (Tamby et al., 2006) has been reported during idiopathic PAH or PAH associated with systemic scleroderma. However, the predictive value of these antibodies in terms of the occurrence of PAH has not been studied and the potential role of autoimmune phenomena in idiopathic PAH pathogenicity remains uncertain (Mouthon et al., 2005).

[0004] In most cases, PAH is screened for when the patient presents stage III or IV dyspnea. When the patient is monitored for a chronic disease such as systemic scleroderma, PAH is screened for by annual echocardiography.

[0005] However, a simple and reliable test to screen for PAH is still lacking, and would be invaluable for the earliest possible diagnosis, which would make it possible to rapidly set up therapeutic strategies for improving the condition of the patient and the survival chances for said patient.

SUMMARY OF THE INVENTION

[0006] The invention now provides an in vitro method for detecting PAH, or a risk of developing PAH, which comprises determining the presence and/or the amount of anti-tenascin C (TN-C) antibodies in a biological sample originating from a patient, the presence of anti-TN-C antibodies being indicative of PAH or of a risk of developing PAH.

[0007] Preferably, the presence of anti-tenascin C antibodies in the biological sample is compared with a control value, the presence of anti-tenascin C antibodies in an amount greater than the control value being indicative of PAH or of a risk of developing PAH.

[0008] Another subject of the invention is an in vitro method for the prognosis or follow-up of PAH, which comprises determining the presence and/or the amount of anti-TN-C antibodies in a biological sample originating from a patient, at various times, an increase in the amount of anti-TN-C antibodies over time being indicative of a worsening of the PAH.

[0009] Another subject of the invention is an in vitro method for evaluating the efficacy of a treatment for PAH, which comprises determining the presence and/or the amount of anti-TN-C antibodies in a biological sample originating from a patient, at various times before, during or after the treatment, a decrease in the amount of anti-TN-C antibodies over time being indicative of an improvement in the PAH.

DETAILED DESCRIPTION OF THE INVENTION

[0010] TN-C is expressed inside and around the blood vessels in the fetal lung (Rettig et al., 1994), but it is no longer subsequently expressed in normal adult pulmonary arteries (Jones et al., 1996). Moreover, the loss of signaling via BMPRII, responsible for a lack of regulatory T cells that can predispose to the occurrence of PAH (Nicolls et al., 2005), can also induce the expression of TN-C in vivo and on vascular cells in culture (Ihida-Stansbury et al., 2006). On this basis, the inventors put forward the hypothesis that patients who have PAH could develop an immune response directed against TN-C. Thus, they decided to search for anti-TN-C antibodies in the serum of patients suffering from PAH.

[0011] The inventors were thus able to demonstrate a correlation between the occurrence of PAH and the production of anti-TN-C antibodies. On this basis, they propose an in vitro method for the diagnosis or the prognosis of PAH, or of a risk of developing PAH, which comprises determining the presence and/or the amount of anti-TN-C antibodies in a biological sample originating from a patient. The anti-TN-C antibodies detected are preferably immunoglobulins G (IgGs).

Definitions

[0012] Tenascin C (or TN-C) is an extracellular matrix glycoprotein. It is also known as hexabrachion or cytotactin. A human TN-C sequence is reported in the annex (SEQ ID No. 1).

[0013] The term "biological sample" refers to any biological sample originating from a patient. Examples of samples include biological fluids and tissue biopsies. Preferably, the sample may be blood, serum, saliva, urine or sperm. More preferably, the biological sample is a blood or serum sample.

[0014] The term "patient" refers to any individual capable of being tested. Preferably, it is a human being, but the term includes any other mammal, such as dogs, cats, rodents, cattle, horses, monkeys, etc. The patient can be tested irrespective of the sex or age thereof. The patient may be an individual at risk, may be asymptomatic, or may show early or advanced signs of PAH. For example, the patient may be an individual predisposed to developing PAH, in particular an individual carrying one or more mutations in the gene encoding BMPRII.

[0015] The term "diagnosis" means the identification of the pathological condition or the evaluation of the state of severity of the pathological condition.

[0016] The term "prognosis" means the evaluation of the risk of worsening, and of the consequences thereof.

[0017] The term "control value" refers to a basal value corresponding to the average of the values obtained with the

biological sample from healthy individuals, not suffering from PAH or a disease capable of leading to PAH. It may be a statistical reference value.

[0018] In order to evaluate the progression of the pathological condition, it may be useful to test a patient and to verify the effect of a treatment or the progression of the pathological condition by testing the patient again, for example with a gap of several months. In this case, the results of the second test are compared with the results of the first test, and also often with the "control" value.

[0019] An amount of anti-TN-C antibodies "greater than the control value" generally means a statistically significant increase, for example of at least two standards deviations above the mean of the optical densities of the IgG reactivities of all the healthy individuals.

[0020] The "capture antigen" is intended to mean an antigen, preferably attached to a solid phase, which is capable of retaining the anti-TN-C antibody present in a biological sample, by affinity binding. The capture antigen can be labeled.

[0021] The term "labeled" refers both to a direct labeling (by means of enzymes, radioisotopes, fluorochromes, luminescent compounds, etc.) and an indirect labeling (for example by means of antibodies which are themselves directly labeled or using reagents of a labeled "affinity pair", such as, but not exclusively, the labeled avidin-biotin pair, etc.).

Assaying of Antibodies

[0022] The biological sample is preferably a serum sample, diluted to 1/100th, or more, for example to 1/200th or 1/400th.

[0023] Advantageously, the amount of anti-TN-C antibody can be determined by an immunoassay.

[0024] The biological sample can be optionally treated in a prior step, or brought directly into contact with at least one capture antigen.

[0025] The method according to the invention can be carried out according to various formats well known to those skilled in the art: in solid phase or in homogenous phase; in one step or in two steps; in a competition method, by way of nonlimiting examples.

[0026] According to one preferred embodiment, the capture antigen is immobilized on a solid phase. By way of nonlimiting examples of a solid phase, use may be made of microplates, in particular polystyrene microplates, such as those sold by the company Nunc, Denmark. Use may also be made of solid particles or beads, paramagnetic beads, such as those provided by Dynal or Merck-Eurolab (France) (under the trademark Estapor™), or else polystyrene or polypropylene test tubes, etc.

[0027] An immunoassay format for detecting antibodies by competition is also possible. Other immunoassay modes can also be envisioned and are well known to those skilled in the art.

[0028] ELISA assays, radioimmunoassays, or any other detection technique can be used for revealing the presence of the antigen-antibody complexes formed.

[0029] According to one preferred embodiment, the method of the invention comprises bringing a biological sample into contact with a protein comprising the fragment of amino acids 181 to 290 of the human TN-C sequence as represented in SEQ ID No. 1.

[0030] In one particular example, the capture antigen, which can be a protein comprising the fragment of amino acids 181 to 290 of the human TN-C sequence, can be coupled to a glutathione S transferase (GST), before being deposited on a microplate.

[0031] Serum samples to be tested, prediluted to 1/100th, are incubated on the microplate. After washing, labeled anti-human Fc γ antibodies (for example, labeled with an alkaline phosphatase) are added, the complexes being revealed, for example, by addition of a substrate for the phosphatase, the cleavage of which can be detected by reading the absorbance.

Patients Targeted

[0032] The patients targeted are those who may develop PAH.

[0033] This may involve a patient who suffers from a connective tissue disease, such as systemic scleroderma, Sharp's syndrome (which is a mixed connective tissue disease) or systemic lupus erythematosus.

[0034] The patient may also be suffering from idiopathic or familial PAH.

[0035] More generally, any patient suffering from a pulmonary vascular disease can be advantageously subjected to the method for detecting PAH as defined in the invention.

[0036] Moreover, the PAH detected may also be portopulmonary hypertension (i.e. PAH associated with portal hypertension), or be associated with a congenital heart disease, or with a human immunodeficiency virus (HIV) infection, or else be post-embolic pulmonary hypertension, complicating the progression of a chronic obstructive bronchitis or of cyanogenic heart disease.

[0037] Other patients targeted are those exposed to certain appetite-suppressing drugs, such as fenfluramine, the prescription of which can contribute to the occurrence of PAH.

[0038] Other individuals capable of benefiting from this type of test are those carrying a mutation in the gene encoding BMPRII and who, optionally, do not present PAH detectable by echography, so as to screen for individuals who may subsequently develop PAH.

Evaluation of the Efficacy of a Treatment

[0039] Another subject of the invention is an in vitro method for evaluating the efficacy of a treatment for PAH, which comprises determining the presence and/or the amount of anti-TN-C antibodies in a biological sample originating from a patient, at various times before, during or after the treatment, a decrease in the amount of anti-TN-C antibodies over time being indicative of an improvement in the PAH.

[0040] The current conventional treatment for PAH combines symptomatic treatment and a vasodilator treatment. The symptomatic treatment combines anti-coagulants, oxygen therapy and diuretics. The vasodilator treatment is based on the following molecules: calcium channel blockers, epoprostenol (prostacyclin) prescribed intravenously as a continuous infusion, selective or nonselective endothelin receptor inhibitors, in particular bosentan, sytaxentan and ambrysentan, phosphodiesterase type 5 inhibitors, in particular sildenafil, all these medicaments being administered orally, and inhaled iloprost, a prostacyclin analog which is administered by inhalation. These treatments can be optionally combined. In the event of these therapies failing, a lung or heart-lung transplant can be proposed.

[0041] The following figures and examples illustrate the invention without limiting the scope thereof.

FIGURE LEGEND

[0042] FIG. **1** is a graph showing the detection of anti-TN-C antibodies by ELISA assay. The serum IgGs from the patients suffering from idiopathic PAH, from the patients suffering from systemic scleroderma and from the healthy individuals paired for sex and age were tested with respect to a recombinant TN-C fragment, at a dilution of 1/100th. The lower and upper limits of the dotted zone represent the thresholds defined by two and three times the standard deviation above the mean of the optical densities obtained in the healthy patients. The significant differences between the groups of patients and the healthy individuals are estimated using a Mann-Whitney rank test and are indicated by:

*: $p<0.01$

**: $p<0.001$.

[0043] FIG. **2** represents survival curves according to Kaplan and Meier as a function of the presence or the absence of anti-TN-C antibodies. Along the X-axis, the time in months; along the Y-axis, the percentage cumulative survival.

EXAMPLE

Detection of Anti-Tenascin C Antibodies in Patients Suffering From Pulmonary Arterial Hypertension

Materials and Methods

Patients

[0044] PAH was screened for by demonstrating, through transthoracic echocardiography, a systolic pulmonary arterial pressure of greater than 40 mmHg In all cases, the PAH was confirmed by performing a right catheterization and demonstrating an average pulmonary arterial pressure of greater than or equal to 25 mmHg at rest and greater than or equal to 30 mmHg with physical exercise. By convention, the PAH was described as idiopathic if the patient showed no associated pathological condition, it then being possible for the PAH to correspond to sporadic PAH, familial PAH or PAH associated with exposure to fenfluramine 91 patients were included in the study comprising 66 (72.5%) patients having idiopathic PAH (IPAH) and 25 patients having systemic scleroderma corresponding to the criteria of the American College of Rheumatology (ACR) and/or to the criteria of LeRoy and Medsger (Masi et al., 1980; LeRoy et al., 2001).

[0045] All the patients who had diffuse systemic scleroderma without PAH had pulmonary interstitial involvement demonstrated by a high-resolution thoracic scan and a vital capacity of less than 80% of the predicted value and/or a carbon monoxide transfer coefficient (DLCO) of less than 75% of the predicted value. None of the patients were receiving corticoid steroids or immunosuppressants at the time the samples were taken, and none of them had a solid tumor or another associated connective tissue disease. 46 healthy individuals paired for sex and age were used as controls.

ELISA Assay

[0046] The tenascin C (TN-C) was obtained from the company Abnova (Abnova Corporation, Taipei city, Taiwan). The antigen used consisted of the fragment 181 to 290 of TN-C (SEQ ID No. 1), coupled to a GST unit. The TN-C was diluted in a bicarbonate buffer and deposited onto 96-well plates (Maxisorb, NalgeNunc Int. Rochester, N.Y., USA) at a final concentration of 4 μg/ml at 4° C. The sera from patients and from healthy individuals were diluted to 1/100th in a phosphate buffer (PBS) containing 1% albumin, and incubated for one hour at 37° C. After washing, alkaline phosphatase-conjugated rabbit anti-human Fcγ antibodies (Dakocytomation, Golstrup, Denmark) were added and incubated for one hour at ambient temperature. The reactivities were revealed by adding 0.05M p-nitrophenylphosphate in a magnesium carbonate buffer (pH 9.8) and the absorbance at 405 nm was determined using an ELISA plate reader (Fusion, Packard BioScience, Meriden, Conn., USA). In order to take into account the variability between wells, the optical density of a reference serum was arbitrarily defined as 100% of the anti-TN-C activity. The results of the samples tested were calculated from the mean of the absorbance of duplicate wells and expressed as a percentage of this reference value. All the samples were tested in duplicate.

Statistical Analyses

[0047] All the statistical analyses were carried out using the Systat software (version 11.0 Systat Software Inc, Point Richmond, Calif., USA). A Mann-Whitney test was used to compare the relative optical densities of the various groups. P values of less than 0.05 were considered to be statistically significant. The survival was calculated by the Kaplan and Meier method (Kaplan and Meier, 1958).

Results

[0048] The reactivities of the IgGs of the patients suffering from idiopathic PAH, of the patients suffering from systemic scleroderma with or without PAH and of the control individuals, with respect to TN-C, were studied by ELISA. Using a threshold defined by two standard deviations above the mean of the optical densities of the IgG reactivities of all the healthy individuals, 36/66 (54.5%) of the patients with idiopathic PAH and 2/25 (8%) of the scleroderma patients had anti-TN-C IgGs. None of the healthy individuals had anti-TN-C IgGs (FIG. **1**). When the threshold was shifted to three standard deviations above the mean of the IgG reactivities of the healthy individuals, 12/66 (18.1%) of the patients with idiopathic PAH had anti-TN-C IgGs and no scleroderma patient had anti-TN-C IgGs. The reactivities of the anti-TN-C antibody serum IgGs of patients with idiopathic PAH were significantly higher than those of the scleroderma patients ($p<0.001$), and than those of the healthy individuals ($p<0.001$). Similarly, the reactivities of the anti-TN-C antibody serum IgGs of scleroderma patients were significantly higher than those of the healthy individuals ($p=0.021$) (FIG. **1**).

[0049] No significant difference in the clinical presentation and the data from the echocardiography, from the right catheterization and from the 6 minute walking test was demonstrated between the two groups of patients. Survival was decreased in the group of patients having anti-TN-C antibodies compared with the patients whose did not have anti-TN-C antibodies, without, however, this difference being significant in this case ($p=0.17$).

[0050] The appearance of an immune response directed against TN-C could result from the same mechanisms as those that result in the induction of TN-C expression and in the proliferation of smooth muscle cells. The presence of

anti-TN-C antibodies would therefore be correlated with the appearance of vascular remodeling, constituting a marker for the occurrence of PAH.

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[0063] Tamby et al., 2006, Eur Respir J 28(4):799-807

SEQUENCE LISTING

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<223> OTHER INFORMATION: antigenic fragment

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        35                  40                  45

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Cys Ala Ala Ala Pro Asp Val Lys Glu Leu Leu Ser Arg Leu Glu Glu
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Phe Thr Gly Glu Asp Cys Ser Glu Leu Ile Cys Pro Asn Asp Cys Phe
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Ala Asp Cys Gly Glu Leu Lys Cys Pro Asn Gly Cys Ser Gly His Gly
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    690                 695                 700

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Val Thr  Glu Val Ser Trp Asp  Ala Leu Arg Leu Asn  Trp Thr Thr
    1265                1270                1275

Pro Asp  Gly Thr Tyr Asp Gln  Phe Thr Ile Gln Val  Gln Glu Ala
    1280                1285                1290

Asp Gln  Val Glu Glu Ala His  Asn Leu Thr Val Pro  Gly Ser Leu
    1295                1300                1305

Arg Ser  Met Glu Ile Pro Gly  Leu Arg Ala Gly Thr  Pro Tyr Thr
    1310                1315                1320

Val Thr  Leu His Gly Glu Val  Arg Gly His Ser Thr  Arg Pro Leu
    1325                1330                1335

Ala Val  Glu Val Val Thr Glu  Asp Leu Pro Gln Leu  Gly Asp Leu
    1340                1345                1350

Ala Val  Ser Glu Val Gly Trp  Asp Gly Leu Arg Leu  Asn Trp Thr
    1355                1360                1365

Ala Ala  Asp Asn Ala Tyr Glu  His Phe Val Ile Gln  Val Gln Glu
    1370                1375                1380

Val Asn  Lys Val Glu Ala Ala  Gln Asn Leu Thr Leu  Pro Gly Ser
    1385                1390                1395

Leu Arg  Ala Val Asp Ile Pro  Gly Leu Glu Ala Ala  Thr Pro Tyr
    1400                1405                1410

Arg Val  Ser Ile Tyr Gly Val  Ile Arg Gly Tyr Arg  Thr Pro Val
    1415                1420                1425

Leu Ser  Ala Glu Ala Ser Thr  Ala Lys Glu Pro Glu  Ile Gly Asn
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    1430                1435                1440

Leu Asn  Val Ser Asp Ile Thr  Pro Glu Ser Phe Asn  Leu Ser Trp
    1445                1450                1455

Met Ala  Thr Asp Gly Ile Phe  Glu Thr Phe Thr Ile  Glu Ile Ile
    1460                1465                1470

Asp Ser  Asn Arg Leu Leu Glu  Thr Val Glu Tyr Asn  Ile Ser Gly
    1475                1480                1485

Ala Glu  Arg Thr Ala His Ile  Ser Gly Leu Pro Pro  Ser Thr Asp
    1490                1495                1500

Phe Ile  Val Tyr Leu Ser Gly  Leu Ala Pro Ser Ile  Arg Thr Lys
    1505                1510                1515

Thr Ile  Ser Ala Thr Ala Thr  Thr Glu Ala Leu Pro  Leu Leu Glu
    1520                1525                1530

Asn Leu  Thr Ile Ser Asp Ile  Asn Pro Tyr Gly Phe  Thr Val Ser
    1535                1540                1545

Trp Met  Ala Ser Glu Asn Ala  Phe Asp Ser Phe Leu  Val Thr Val
    1550                1555                1560

Val Asp  Ser Gly Lys Leu Leu  Asp Pro Gln Glu Phe  Thr Leu Ser
    1565                1570                1575

Gly Thr  Gln Arg Lys Leu Glu  Leu Arg Gly Leu Ile  Thr Gly Ile
    1580                1585                1590

Gly Tyr  Glu Val Met Val Ser  Gly Phe Thr Gln Gly  His Gln Thr
    1595                1600                1605

Lys Pro  Leu Arg Ala Glu Ile  Val Thr Glu Ala Glu  Pro Glu Val
    1610                1615                1620

Asp Asn  Leu Leu Val Ser Asp  Ala Thr Pro Asp Gly  Phe Arg Leu
    1625                1630                1635

Ser Trp  Thr Ala Asp Glu Gly  Val Phe Asp Asn Phe  Val Leu Lys
    1640                1645                1650

Ile Arg  Asp Thr Lys Lys Gln  Ser Glu Pro Leu Glu  Ile Thr Leu
    1655                1660                1665

Leu Ala  Pro Glu Arg Thr Arg  Asp Leu Thr Gly Leu  Arg Glu Ala
    1670                1675                1680

Thr Glu  Tyr Glu Ile Glu Leu  Tyr Gly Ile Ser Lys  Gly Arg Arg
    1685                1690                1695

Ser Gln  Thr Val Ser Ala Ile  Ala Thr Thr Ala Met  Gly Ser Pro
    1700                1705                1710

Lys Glu  Val Ile Phe Ser Asp  Ile Thr Glu Asn Ser  Ala Thr Val
    1715                1720                1725

Ser Trp  Arg Ala Pro Thr Ala  Gln Val Glu Ser Phe  Arg Ile Thr
    1730                1735                1740

Tyr Val  Pro Ile Thr Gly Gly  Thr Pro Ser Met Val  Thr Val Asp
    1745                1750                1755

Gly Thr  Lys Thr Gln Thr Arg  Leu Val Lys Leu Ile  Pro Gly Val
    1760                1765                1770

Glu Tyr  Leu Val Ser Ile Ile  Ala Met Lys Gly Phe  Glu Glu Ser
    1775                1780                1785

Glu Pro  Val Ser Gly Ser Phe  Thr Thr Ala Leu Asp  Gly Pro Ser
    1790                1795                1800

Gly Leu  Val Thr Ala Asn Ile  Thr Asp Ser Glu Ala  Leu Ala Arg
    1805                1810                1815
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Trp Gln  Pro Ala Ile Ala Thr  Val Asp Ser Tyr Val  Ile Ser Tyr
    1820                1825                1830

Thr Gly  Glu Lys Val Pro Glu  Ile Thr Arg Thr Val  Ser Gly Asn
    1835                1840                1845

Thr Val  Glu Tyr Ala Leu Thr  Asp Leu Glu Pro Ala  Thr Glu Tyr
    1850                1855                1860

Thr Leu  Arg Ile Phe Ala Glu  Lys Gly Pro Gln Lys  Ser Ser Thr
    1865                1870                1875

Ile Thr  Ala Lys Phe Thr Thr  Asp Leu Asp Ser Pro  Arg Asp Leu
    1880                1885                1890

Thr Ala  Thr Glu Val Gln Ser  Glu Thr Ala Leu Leu  Thr Trp Arg
    1895                1900                1905

Pro Pro  Arg Ala Ser Val Thr  Gly Tyr Leu Leu Val  Tyr Glu Ser
    1910                1915                1920

Val Asp  Gly Thr Val Lys Glu  Val Ile Val Gly Pro  Asp Thr Thr
    1925                1930                1935

Ser Tyr  Ser Leu Ala Asp Leu  Ser Pro Ser Thr His  Tyr Thr Ala
    1940                1945                1950

Lys Ile  Gln Ala Leu Asn Gly  Pro Leu Arg Ser Asn  Met Ile Gln
    1955                1960                1965

Thr Ile  Phe Thr Thr Ile Gly  Leu Leu Tyr Pro Phe  Pro Lys Asp
    1970                1975                1980

Cys Ser  Gln Ala Met Leu Asn  Gly Asp Thr Thr Ser  Gly Leu Tyr
    1985                1990                1995

Thr Ile  Tyr Leu Asn Gly Asp  Lys Ala Gln Ala Leu  Glu Val Phe
    2000                2005                2010

Cys Asp  Met Thr Ser Asp Gly  Gly Gly Trp Ile Val  Phe Leu Arg
    2015                2020                2025

Arg Lys  Asn Gly Arg Glu Asn  Phe Tyr Gln Asn Trp  Lys Ala Tyr
    2030                2035                2040

Ala Ala  Gly Phe Gly Asp Arg  Arg Glu Glu Phe Trp  Leu Gly Leu
    2045                2050                2055

Asp Asn  Leu Asn Lys Ile Thr  Ala Gln Gly Gln Tyr  Glu Leu Arg
    2060                2065                2070

Val Asp  Leu Arg Asp His Gly  Glu Thr Ala Phe Ala  Val Tyr Asp
    2075                2080                2085

Lys Phe  Ser Val Gly Asp Ala  Lys Thr Arg Tyr Lys  Leu Lys Val
    2090                2095                2100

Glu Gly  Tyr Ser Gly Thr Ala  Gly Asp Ser Met Ala  Tyr His Asn
    2105                2110                2115

Gly Arg  Ser Phe Ser Thr Phe  Asp Lys Asp Thr Asp  Ser Ala Ile
    2120                2125                2130

Thr Asn  Cys Ala Leu Ser Tyr  Lys Gly Ala Phe Trp  Tyr Arg Asn
    2135                2140                2145

Cys His  Arg Val Asn Leu Met  Gly Arg Tyr Gly Asp  Asn Asn His
    2150                2155                2160

Ser Gln  Gly Val Asn Trp Phe  His Trp Lys Gly His  Glu His Ser
    2165                2170                2175
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Ile Gln  Phe Ala Glu Met Lys  Leu Arg Pro Ser Asn  Phe Arg Asn
    2180                 2185                 2190

Leu Glu  Gly Arg Arg Lys Arg  Ala
    2195                 2200
```

1. An in vitro method for detecting pulmonary arterial hypertension (PAH), or a risk of developing PAH, which comprises determining the presence and/or the amount of anti-tenascin C antibodies in a biological sample originating from a patient, the presence of anti-tenascin C antibodies being indicative of PAH or a risk of developing PAH.

2. The method as claimed in claim **1**, in which the biological sample is a blood or serum sample.

3. The method as claimed in claim **1**, in which the presence of anti-tenascin C antibodies in the biological sample is compared with a control value, the presence of anti-tenascin C antibodies in an amount greater than the control value being indicative of PAH or of a risk of developing PAH.

4. The method as claimed in claim **1**, in which the amount of anti-tenascin C antibodies is determined by means of an immunoassay.

5. The method as claimed in claim **4**, in which the immunoassay is an ELISA assay.

6. The method as claimed in claim **4**, which comprises bringing a biological sample into contact with a protein comprising the fragment of amino acids 181 to 290 of the human tenascin C sequence as represented in SEQ ID No. 1.

7. The method as claimed in claim **1**, in which the patient is a human being.

8. The method as claimed in claim **1**, in which the patient suffers from systemic scleroderma.

9. The method as claimed in claim **1**, in which the patient suffers from Sharp's syndrome.

10. The method as claimed in claim **1**, in which the patient suffers from systemic lupus erythematosus.

11. The method as claimed in claim **1**, in which the patient suffers from idiopathic PAH.

12. The method as claimed in claim **1**, in which the PAH is associated with portal hypertension, with congenital heart disease, or with a human immunodeficiency virus (HIV) infection, or is post-embolic pulmonary hypertension.

13. The method as claimed in claim **1**, in which the patient is an individual predisposed to developing PAH.

14. The method as claimed in claim **13**, in which the individual carries one or more mutation(s) in the gene encoding BMPRII.

15. An in vitro method for the prognosis or the follow-up of PAH, which comprises determining the presence and/or the amount of anti-tenascin C antibodies in a biological sample originating from a patient, at various times, an increase in the amount of anti-tenascin C antibodies over time being indicative of a worsening of the PAH.

16. An in vitro method for evaluating the efficacy of a treatment for PAH, which comprises determining the presence and/or the amount of anti-tenascin C antibodies in a biological sample originating from a patient, at various times before, during or after the treatment, a decrease in the amount of anti-tenascin C antibodies over time being indicative of an improvement in the PAH.

17. The method as claimed in claim **2**, in which the presence of anti-tenascin C antibodies in the biological sample is compared with a control value, the presence of anti-tenascin C antibodies in an amount greater than the control value being indicative of PAH or of a risk of developing PAH.

18. The method as claimed in claim **2**, in which the amount of anti-tenascin C antibodies is determined by means of an immunoassay.

19. The method as claimed in claim **3**, in which the amount of anti-tenascin C antibodies is determined by means of an immunoassay.

20. The method as claimed in claim **17**, in which the amount of anti-tenascin C antibodies is determined by means of an immunoassay.

* * * * *

专利名称(译)	诊断肺动脉高压的方法		
公开(公告)号	US20110033876A1	公开(公告)日	2011-02-10
申请号	US12/936745	申请日	2009-04-10
[标]申请(专利权)人(译)	巴黎第十一大学		
申请(专利权)人(译)	巴黎大学-SUD 11		
当前申请(专利权)人(译)	援助PUBLIQUE-HOPITAUX DE PARIS 巴黎大学DESCARTES		
[标]发明人	MOUTHON LUC HUMBERT MARC TAMBY MATHIEU		
发明人	MOUTHON, LUC HUMBERT, MARC TAMBY, MATHIEU		
IPC分类号	G01N33/53 G01N33/566		
CPC分类号	G01N33/6854 G01N33/6893 G01N2800/56 G01N2800/321 G01N2800/52 G01N2800/12		
优先权	2008052459 2008-04-11 FR		
其他公开文献	US8609356		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及用于检测肺动脉高血压（PAHT）或发生PAHT的风险的体外方法，其包括确定来自患者的生物样品中抗肌腱蛋白C抗体的存在和/或量。

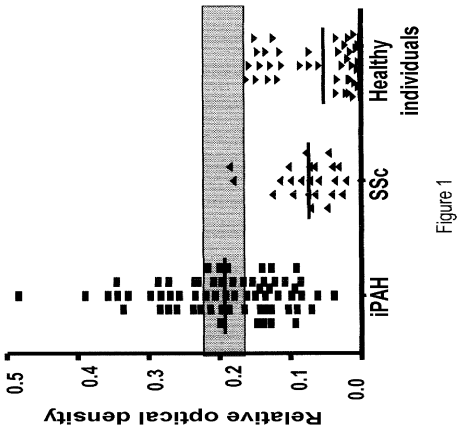

Figure 1