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(54) **NOVEL TEST METHOD FOR RHEUMATOID ARTHRITIS AND KIT FOR RHEUMATOID ARTHRITIS TEST**

NEUES VERFAHREN FÜR TESTS AUF RHEUMATOIDE ARTHRITIS UND KIT FÜR TESTS AUF RHEUMATOIDE ARTHRITIS

NOUVEAU PROCÉDÉ D'ESSAI POUR L'ARTHRITE RHUMATOÏDE ET TROUSSE POUR ESSAI DE L'ARTHRITE RHUMATOÏDE

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(56) References cited:
WO-A1-03/072827
WO-A2-2008/089152
JP-A- 2003 024 099
JP-A- 2004 012 447
JP-A- 2005 127 754
JP-A- 2009 510 464
JP-A- 2010 071 833

- **LI TIANWANG ET AL: "Distinct proteomic profile in Ankylosing spondylitis patients: Talin1 is a new valuable biomarker for diagnosis and treatment", ARTHRITIS & RHEUMATISM, vol. 58, no. 9, Suppl. S, September 2008 (2008-09), pages S350-S351, XP009179580, & 72ND ANNUAL SCIENTIFIC MEETING OF THE AMERICAN-COLLEGE-OF-RHEUMATOLOGY/43 RD ANNUAL SCIENTIFIC MEETIN; SAN FRANCISCO, CA, USA; OCTOBER 24 -29, 2008 ISSN: 0004-3591**
- **MARTIN SCHULZ ET AL: "Proteomic Analysis of Peripheral Blood Mononuclear Cells: Selective Protein Processing Observed in Patients with Rheumatoid Arthritis", JOURNAL OF PROTEOME RESEARCH, vol. 6, no. 9, 1 September 2007 (2007-09-01), pages 3752-3759, XP055133777, ISSN: 1535-3893, DOI: 10.1021/pr070285f**
- **LI T ET AL: "Over-expression of talin 1 and integrin-linked kinase in PBMCs of patients with ankylosing spondylitis: a proteomic study.", CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2010 NOV-DEC, vol. 28, no. 6, November 2010 (2010-11), pages 828-835, XP009179577, ISSN: 0392-856X**

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(Cont. next page)

- BACAKOVA L ET AL: "Molecular mechanisms of improved adhesion and growth of an endothelial cell line cultured on polystyrene implanted with fluorine ions", BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 21, no. 11, 1 June 2000 (2000-06-01), pages 1173-1179, XP004194662, ISSN: 0142-9612, DOI: 10.1016/S0142-9612(00)00009-0
- TSUZAKA KENSEI ET AL: "Plasma Talin Is a New Diagnostic and Monitoring Marker for Rheumatoid Arthritis", ARTHRITIS & RHEUMATISM, vol. 63, no. 10, Suppl. S, October 2011 (2011-10), page S134, XP002728286, & 75TH ANNUAL SCIENTIFIC MEETING OF THE AMERICAN-COLLEGE-OF-RHEUMATOLOGY/46 TH ANNUAL SCIENTIFIC MEETIN; CHICAGO, IL, USA; NOVEMBER 04 -09, 2011
- HAJIME YOSHIFUJI: 'Calpain - Calpastatin to Rheumatoid Arthritis' RINSHO RHEUMATISM vol. 17, no. 3, 30 September 2005, pages 160 - 165, XP008167122
- MARTINUS A.M. ET AL.: 'Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value' ARTHRITIS RES. vol. 4, no. 2, 2002, pages 87 - 93, XP009012450
- AVOUAC J. ET AL.: 'Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review' ANN. RHEUM. DIS. vol. 65, 2006, pages 845 - 851, XP008167123
- VAN VENROOIJ W.J. ET AL.: 'Anti-CCP antibody, a marker for the early detection of rheumatoid arthritis' ANN. N. Y. ACAD. SCI. vol. 1143, 2008, pages 268 - 285, XP055099722

Description

TECHNICAL FIELD

[0001] The present invention relates to an *in vitro* test method for rheumatoid arthritis, and a kit for rheumatoid arthritis test, which is used in such an *in vitro* test method.

BACKGROUND ART

[0002] Rheumatoid arthritis (RA) is a chronic inflammatory disease, in which the lesion occurs mainly in the synovial membrane tissue of the joint, and the prevalence rate of the disease is about 1% of the population. In rheumatoid arthritis, synovitis is found in the first stage, then cartilage or bone is gradually invaded, and the joint is destroyed and deformed in the advanced stage. Furthermore, the consequence of the symptom includes various examples such as an example in which arthritis undergoes remission and reoccurrence, repeatedly, then is completely cured, and an example in which arthritis rapidly progresses.

[0003] Diagnosis of rheumatoid arthritis is carried out mainly based on symptoms. Recently, however, attention has been paid to a diagnostic method using, as a marker, an autoantibody contained in the serum of a patient. As such an autoantibody, a rheumatoid factor (an autoantibody with respect to the deformed IgG), an anti-cyclic citrullinated peptide antibody (an anti-CCP antibody), and the like, are known (see Non-Patent Literature 1).

[0004] However, in previous reports, the sensitivity of the rheumatoid factor is 75 to 80%, the specificity thereof is 50 to 70%, and the sensitivity of the anti-CCP antibody is 50 to 75%, and the specificity is 85 to 95%, which are not necessarily satisfactory (see Non-Patent Literatures 2 and 3).

Non-Patent Literature 1: Martinus A. M. et al., Arthritis Res. Ther., 4: 87-93, 2002
Non-Patent Literature 2: Avouac J. et al., Ann. Rheum. Dis. 65: 845-851, 2006
Non-Patent Literature 3: van Venrooij WJ. et al. Ann. N.Y. Acad. Sci. 1143: 268-285, 2008

[0005] LI TIANWANG ET AL: "Distinct proteomic profile in Ankylosing spondylitis patients: Talin1 is a new valuable biomarker for diagnosis and treatment", ARTHRITIS & RHEUMATISM, vol. 58, no. 9,(Suppl. - S), September 2008 (2008-09-01), & 72ND ANNUAL SCIENTIFIC MEETING OF THE AMERICAN-COLLEGE-OF-RHEUMATOLOGY/43RD ANNUAL SCIENTIFIC MEETIN; SAN FRANCISCO, CA, USA; OCTOBER 24 -29, 2008, pages S350 - S351, XP009179580, ISSN: 0004-3591; discloses a comparison of the mRNA and protein expression level in peripheral blood mononuclear cells and sera in ankylosing spondylitis (AS), rheumatoid arthritis (RA) and healthy controls. It is shown that the expression level of talin 1 in serum and of mRNA in the cells is significantly increased in AS, whereas it is decreased in RA in comparison to healthy controls. However the data obtained in Western blots and in mRNA expression measurements for the healthy controls and for the RA group are very similar and the results for both groups overlap if the standard deviation is taken into account.

[0006] WO 03/072827 (CHILDRENS HOSP MEDICAL CENTER) discloses that in order to determine the contribution of various genes in the pathogenesis of collagen-induced arthritis (CIA), microarray technology was used to simultaneously monitor 8,734 target cDNAs to discover arthritic stage-specific genes. The resulting gene expression profile identified 333 genes that were at least 2-fold up-regulated in all synovial samples: normal, acute disease and chronic disease. In addition, 385 disease-specific genes were identified that were greater than or equal to 2-fold over- or under-expressed in the disease state as compared to normal synovium. Clustering analysis among the arthritic states allowed for the identification of four distinct kinetic expression patterns based on differential expression levels in normal, acute disease and chronic disease synovial samples.

[0007] Similarly, LI T ET AL: "Over-expression of talin 1 and integrin-linked kinase in PBMCs of patients with ankylosing spondylitis: a proteomic study.", CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2010 NOV-DEC, vol. 28, no. 6, November 2010 (2010-11), pages 828-835, XP009179577, ISSN: 0392-856X discloses that Talin 1 is one of several genes which are over-expressed in RA. It is provided a diagnostic method using an anti-CCP antibody having a sensitivity of 64.7% and a specificity of 92.9%. Thus an improved method for diagnosing RA is needed.

DISCLOSURE OF THE INVENTION

Problems to be Solved by the Invention

[0008] The present invention aims to provide an *in vitro* test method for rheumatoid arthritis and a kit for rheumatoid arthritis test by searching novel markers that have not been previously known and being based on the found novel markers in the searching.

Means for Solving the Problems

[0009] In patients with rheumatoid arthritis, it is known that the peripheral blood lymphocyte is activated, so that cell adhesion with respect to the blood vessel endothelial cell is increased and at the same time, the lymphocyte migration is also increased, resulting in the infiltration of the lymphocyte to the outside of the blood vessel to cause various inflammations. In searching novel markers, the present inventor has focused on talin that is a high-molecular-weight cytoskeletal protein expressed in a concentrated manner mainly in regions in which the cell and the substrate are brought into contact with each other, in particular, in a cell adhesion region in the lymphocyte.

[0010] Talin is a protein formed of an N-terminal region having a molecular weight of 47 kDa and including an FERM region, and a C-terminal region having a molecular weight of 190 kDa and including one bundle of α-helix. The FERM region is classified into three sub-regions, that is, an F1 domain, an F2 domain, and an F3 domain, sequentially from the N-terminal side. In a living body, it is known that polypeptide in the N-terminal region, which is cleaved by Calpain, the F3 domain among them binds to an integrin β sub-unit increases signaling of the integrin from the inside of the cell to the outside of the cell, and thus the cell adhesion and cell migration are increased.

[0011] The present inventor has investigated the presence of talin in the plasma or the serum of a patient with rheumatoid arthritis. As a result, surprisingly, it is found that in a patient with rheumatoid arthritis, talin is dominantly present in the plasma or the serum. Furthermore, it is found that the amount of talin is significantly reduced when rheumatoid arthritis reaches a low disease activity or remission with the therapeutic agent for rheumatoid arthritis.

[0012] The present invention has been made based on such findings, and the present invention specifically includes the followings.

(1) An *in vitro* test method for determining a diagnosis of rheumatoid arthritis or a treatment effect of a therapeutic agent for rheumatoid arthritis, the method including a step of measuring an amount of talin in plasma or serum of an animal subject and
determining that the subject has contracted rheumatoid arthritis when the amount of talin in the plasma or the serum is larger than a predetermined threshold, the predetermined threshold being based on a mean value in the plasma or the serum in a control animal that has not contracted rheumatoid arthritis, or determining that the therapeutic agent is effective when the amount of talin after the therapeutic agent for rheumatoid arthritis is administered is significantly lowered from the amount of talin before the administration.
(2) The *in vitro* test method for rheumatoid arthritis described in the above (1), further including a step of obtaining plasma or serum from blood collected from the animal subject.
(3) The *in vitro* test method for rheumatoid arthritis described in the above (1) or (2), wherein the amount of talin in the plasma or the serum is measured by using an antibody which binds to talin.
(4) The *in vitro* test method for rheumatoid arthritis described in any of the above (1) to (3), wherein the animal subject is a human subject.
(5) The *in vitro* test method for rheumatoid arthritis described in any of the above (1) to (4), wherein the method is carried out for determining a diagnosis of rheumatoid arthritis or a treatment effect of a therapeutic agent for rheumatoid arthritis.
(6) The use of a kit for rheumatoid arthritis test for the *in vitro* test method for rheumatoid arthritis described in any of the above (1) to (5).
(7) The use of the kit for rheumatoid arthritis test described in the above (6), including a solid-phase carrier to which an antibody that binds to talin is affixed.

Effects of the Invention

[0013] The present invention can provide a novel *in vitro* test method for rheumatoid arthritis and a kit for rheumatoid arthritis test to be used for such an *in vitro* test method.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014]

Fig. 1 is a graph showing an ROC curve of a diagnosis of rheumatoid arthritis (Example 1) by the Sandwich ELISA method using an H-18 antibody and an H-300 antibody.
Fig. 2 is a graph showing an ROC curve of a diagnosis of rheumatoid arthritis (Example 2) by the Sandwich ELISA method using an H-18 antibody and an M54246M antibody.
Fig. 3 is a graph showing an ROC curve of a diagnosis of rheumatoid arthritis (Comparative Example 1) using an anti-CCP antibody.

PREFERRED MODE FOR CARRYING OUT THE INVENTION

Test Method for Rheumatoid Arthritis

[0015] An *in vitro* test method for rheumatoid arthritis according to the present invention includes a step of measuring an amount of talin in the plasma or the serum of an animal subject. This *in vitro* test method may further include a step of obtaining the plasma or the serum from the blood collected from an animal subject.

[0016] The animal subject is not particularly limited as long as it can contract rheumatoid arthritis, and it can be selected depending upon purposes. Examples thereof include a human, a rat, a mouse, a dog, a cow, a cat, a rabbit, and a guinea pig, and preferable example is a human.

[0017] Furthermore, a method for obtaining the plasma or the serum is not particularly limited, it is possible to employ conventional methods, for example, a method for separating the plasma or the serum, which are obtained as specimen for clinical laboratory examination, from the blood. For example, the plasma can be obtained by taking the blood into an EDTA tube, a heparin tube, or the like, and the centrifuging thereof. Furthermore, the serum can be obtained by taking the blood into a test tube, and the centrifuging thereof.

[0018] With the *in vitro* test method for rheumatoid arthritis according to the present invention, an amount of talin in the thus obtained plasma or serum is measured. Herein, the "amount of talin" denotes an amount of protein of the talin. When talin has plurality of isoforms, any one of them may be measured. For example, in the case of human, two isoforms, that is, talin 1 and talin 2 are present. The mRNA sequence and the amino acid sequence of the talin 1 are shown in SEQ ID NOs: 1 and 2. Furthermore, the mRNA sequence and the amino acid sequence of the talin 2 are shown in SEQ ID NOs: 3 and 4. The amount of talin in the plasma or the serum of an animal subject can be measured by an immunochemical method by using an antibody which binds to talin.

[0019] The antibody which binds to talin may be a polyclonal antibody or may be a monoclonal antibody, and, in some case, fragments of the antibody, for example, Fab', Fab, $F(ab')_2$ can be used. These antibodies can be prepared by conventionally known methods.

[0020] Examples of commercial products include an H-18 antibody (Santa Cruz Biotechnology Inc.), an H-300 antibody (Santa Cruz Biotechnology Inc.), a TA205 antibody (Abcam Inc.), and an M54246M antibody (Bio-design Co., Ltd.).

[0021] The amount of talin can be measured by employing well-known methods such as an enzyme immunoassay (EIA), a chemiluminescent immunoassay, a radioimmunoassay (RIA), a fluoro immunoassay, and a latex agglutination assay. Specific examples include a competitive assay using an antibody and label antigen, a Sandwich EIA method using combination of two types of antibodies, i.e., a monoclonal antibody or a polyclonal antibody (or a monoclonal antibody and a polyclonal antibody) whose recognition sites with respect to an antigen are different, and a latex agglutination assay using latex particles to which an antibody is affixed.

[0022] In these measurement methods, if necessary, an antigen or an antibody can be affixed to a solid-phase carrier. Examples of the solid-phase carrier include synthetic resin such as polystyrene, polyethylene, polypropylene, polyvinyl chloride, polyester, polyacrylic acid ester, nylon, polyacetal and fluorocarbon resin, polysaccharides such as cellulose and agarose, glass, metal, and the like. This solid-phase carrier can be formed in various shapes including a micro-plate shape, a spherical shape, a fibrous shape, a rod shape, a board shape, a container shape, a cell, a test tube, and the like.

[0023] In the above-mentioned immunochemical method, antibodies or antigens which are labeled may be used if necessary. Examples of such labels include radioisotope (^{124}I, ^{14}C, and ^{3}H), fluorescence materials (fluorescein isothiocyanate, and the like), and the like, in addition to enzymes (peroxidase, alkaline phosphatase, and the like), luminescent material (acridinium ester, isoluminol, luciferin, and the like). Besides, methods using combination of a biotin label and streptavidin can be employed.

[0024] As mentioned above, by measuring and quantifying the amount of talin in the plasma or the serum of an animal subject, it is possible to diagnose easily whether or not the subject has contracted rheumatoid arthritis. That is to say, when the amount of talin in the plasma or the serum is larger than a predetermined threshold, it can be determined that the subject has contracted rheumatoid arthritis. The predetermined threshold can be set, for example, based on a mean value and the like in the plasma or the serum in a control animal that has not contracted rheumatoid arthritis.

[0025] Furthermore, by measuring and quantifying the amount of talin before and after a therapeutic agent for rheumatoid arthritis is administered, a therapeutic effect by the therapeutic agent can be determined in a simple and easy manner. That is to say, when the amount of talin after the therapeutic agent for rheumatoid arthritis is administered is significantly lowered from the amount of talin before the administration, it can be determined that the therapeutic agent is effective.

[0026] Herein, the therapeutic agent for rheumatoid arthritis can include all the conventionally known therapeutic agents. Examples of the conventionally known therapeutic agent for rheumatoid arthritis include biological preparations, nonsteroidal anti-inflammatory agents (anti-inflammatory analgesic agents), steroid drugs, immunosuppressive agents, and the like.

[0027] Examples of the biological preparations include chimeric anti-TNF-α antibody preparations, soluble TNF re-

ceptors, complete human anti-TNF-α antibody preparations, anti-IL-6-receptor antibody preparations, and the like. The nonsteroidal anti-inflammatory agents include prostaglandin production suppressing agents. They can reduce pain or swelling in the joint, but it is said that it is difficult to suppress the progress of the disease itself and suppress the destruction of the bone and the joint. The steroid drug has excellent anti-inflammation effect, so that it is used as specific medicine for rheumatoid arthritis. However, adverse effects thereof pose problems. The immunosuppressive agent reduces immunopathy of a rheumatoid arthritis patient, thereby suppressing the inflammation of rheumatoid arthritis, and is used for the purpose of introducing remission induction. Since this may be able to inhibit the progress of rheumatoid arthritis, this agent is also called disease modification anti-rheumatism drug. This is also called a slow acting anti-rheumatism drug because it takes a long time to exhibit an effect.

[0028] As mentioned above, there are various types of the therapeutic agents for rheumatoid arthritis, but the test method according to the present invention is also useful for determining the level of the effect of the therapeutic agent, and selecting the most effective therapeutic agent.

Use of a Kit for Rheumatoid Arthritis Test

[0029] The use of a kit for rheumatoid arthritis test according to the present invention is provided in the *in vitro* test method for rheumatoid arthritis according to the present invention. This diagnosis kit includes, for example, a solid-phase carrier to which an antibody that binds to talin is affixed. Furthermore, it may include a labeled secondary antibody, a coloring substrate, or the like.

EXAMPLES

[0030] Hereinafter, the present invention is described in detail with reference to Examples. Note here that in the following Examples 1 and 2, and Comparative Example 1, subjects include 17 patients with rheumatoid arthritis (RA patients), 14 controls (8 patients with osteoarthritis, 1 patient with systemic lupus erythematodes, 1 patient with diabetes, and 4 healthy subjects). Furthermore, in the following Example 3, subjects are 5 RA patients.

Example 1

[0031] Blood of each subject is collected into an EDTA tube, and is centrifuged at 2500 rpm at room temperature for 10 min so as to obtain the plasma.

[0032] An amount of talin in the plasma was measured by a Sandwich ELISA method.

[0033] Firstly, an H-18 antibody (Santa Cruz Biotechnology Inc.) recognizing the N-terminal of talin was diluted with a phosphate buffer solution (PBS) so that the concentration became 1 μg/mL, and added to a 96-well micro-plate at 100 μL/well, which was incubated at 4°C overnight, followed by washing with 200 μL/well of washing solution three times. Next, the plasma from each subject was added to the 96-well micro-plate at 100 μL/well, which was incubated at 25°C for one hour, followed by washing with 200 μL/well of washing solution three times. Next, an H-300 antibody (Santa Cruz Biotechnology Inc.) as a primary antibody recognizing the N-terminal of talin was diluted with PBS so that the concentration became 2 μg/mL, and added to the 96-well micro-plate at 100 μL/well, which was incubated at 25°C for one hour, followed by washing with 200 μL/well of washing solution three times. Next, an HRP-labeled anti-goat IgG antibody (KPL) as a secondary antibody was diluted with PBS so that the concentration became 2 μg/mL, and added to the 96-well micro-plate at 100 μL/well, which was incubated at 25°C for one hour, followed by washing with 200 μL/well of washing solution three times.

[0034] Next, a substrate was added to a 96-well micro-plate at 100 μL/well, which was incubated at 25°C for 15 min, and then, an OD value at wavelength of 630 nm was measured by using a microplate reader.

[0035] An ROC curve in Example 1 is shown in Fig. 1. As a result of the ROC analysis, an area below the ROC curve (AUC) in Fig. 1 was 0.954. Furthermore, whether being positive or negative based on the cut-off value of OD = 0.20 is shown in Table 1.

[Table 1]

		RA patient	Control	Total
H-300 antibody	Positive	14	0	14
	Negative	3	14	17
	Total	17	14	31

[0036] From the results, diagnosis of rheumatoid arthritis by the Sandwich ELISA method using the H-18 antibody and the H-300 antibody showed that the sensitivity was 14/17 × 100 = 82.4% and the specificity was 14/14 × 100 = 100%.

Example 2

[0037] The amount of talin in the plasma was measured by the Sandwich ELISA method by the same method as in Example 1 except that an M54246M antibody (Bio-design Co., Ltd.) recognizing the C-terminal of talin was used as a primary antibody.

[0038] An ROC curve in Example 2 is shown in Fig. 2. As a result of the ROC analysis, an area below the ROC curve (AUC) in Fig. 2 was 0.819. Furthermore, whether being positive or negative based on the cut-off value of OD = 0.05 is shown in Table 2.

[Table 2]

		RA patient	Control	Total
M54246M antibody	Positive	15	3	18
	Negative	2	11	13
	Total	17	14	31

[0039] From the results, diagnosis of rheumatoid arthritis by the Sandwich ELISA method using the H-18 antibody and the M54246M antibody showed that the sensitivity was 15/17 × 100 = 88.2% and the specificity was 11/14 × 100 = 78.6%.

Comparative Example 1

[0040] Blood from each subject was collected into a blood collecting tube for serum, and it was centrifuged at 2,500 rpm at room temperature for 10 min, and thus the serum was obtained. An anti-CCP antibody titer in the serum was measured by using a commercially available kit (MESACUP CCP manufactured by Medical & Biological laboratories Co., Ltd (MBL)).

[0041] An ROC curve in Comparative Example 1 is shown in Fig. 3. As a result of the ROC analysis, an area below the ROC curve (AUC) in Fig. 3 was 0.838. Furthermore, whether being positive or negative based on the cut-off value of antibody titer = 6.60 is shown in Table 3.

[Table 3]

		RA patient	Control	Total
Anti-CCP antibody	Positive	11	1	12
	Negative	6	13	19
	Total	17	14	31

[0042] From the results, diagnosis of rheumatoid arthritis by using the anti-CCP antibody showed that the sensitivity was 11/17 × 100 = 64.7% and the specificity was 13/14 × 100 = 92.9%.

[0043] As is shown from the above-mentioned results, talin was dominantly present in the blood from a RA patient. Therefore, by measuring the amount of talin in the blood, it is possible to examine whether or not a subject contracted rheumatoid arthritis in a simple and easy manner. Moreover, the test method was more excellent in the sensitivity as compared with the existing method using anti-CCP antibody.

Example 3

[0044] A therapeutic effect of the therapeutic agent for rheumatoid arthritis for 5 RA patients was confirmed by measuring the amount of talin. The amount of talin was measured by measuring the OD value by the Sandwich ELISA method by using an H-18 antibody and an H-300 antibody as in Example 1. Furthermore, an amount of CRP and am amount of MMP-3 were measured by the same method as in usual clinical examination. Furthermore, the score of DAS (Disease Activity Score) 28 recommended by European League Against Rheumatism (EULAR) was calculated. The score of DAS28 of not less than 5.1 is determined to be high disease activity, the score of not less than 3.2 and less than 5.1 is

determined to be middle disease activity, and the score of less than 3.2 is determined to be low disease activity. The results are shown in Table 4.

[Table 4]

Case 1: Male (ADA remarkable effective example)		Before (MTX treatment)	After (MTX + ADA treatment)
	Talin (OD value)	0.568	0.139
	CRP (mg/dL)	1.74	0.08
	MMP-3 (mg/mL)	60.6	57.4
	DAS28	5.43	2.62
Case 2: Female (SASP no effective example)		Before (non-treatment)	After (SASP treatment)
	Talin (OD value)	0.258	0.294
	CRP (mg/dL)	0.36	0.19
	MMP-3 (mg/mL)	70.6	106.6
	DAS28	5.12	4.21
Case 3: Female (IFX no effective example)		Before (MTX treatment)	After (MTX + IFX treatment)
	Talin (OD value)	0.205	0.223
	CRP (mg/dL)	2.82	2.68
	MMP-3 (mg/mL)	962.1	523.8
	DAS28	4.87	3.95
Case 4: Female (ADA remarkable effective example)		Before (BUC treatment)	After (BUC + ADA treatment)
	Talin (OD value)	0.258	0.164
	CRP (mg/dL)	5.01	0.16
	MMP-3 (mg/mL)	323.2	52.2
	DAS28	4.55	2.45
Case 5: Female (TCZ no effective example)		Before (non-treatment)	After (TCZ treatment)
	Talin (OD value)	2.093	1.787
	CRP (mg/dL)	3.83	2.78
	MMP-3 (mg/mL)	117.8	93.1
	DAS28	5.09	4.50

[0045] A case 1 is an example in which when ADA (adalimumab) was used together in treatment with MTX (methotrexate), good responder (DAS28: 5.43 $\rightarrow$ 2.62) was shown based on the reactivity basis of EULAR. The amount of talin showed a high value (OD value: 0.568) when only MTX was used, but it showed a normal value (OD value: 0.139)

when ADA was used together. On the other hand, the amount of MMP-3 did not show significant reduction even when ADA was used together, which did not reflect the activity of rheumatoid arthritis.

[0046] A case 2 is an example in which also when treatment with SASP (salazosuffapyridine) was carried out, none responder (DAS28: 5.12 → 4.21) was shown based on the reactivity basis of EULAR. The amount of talin remained high (OD value: 0.258 → 0.294) even with SASP treatment. On the other hand, the CRP amount showed a normal value (0.19 mg/dL) after the SASP treatment was carried out, which did not reflect the pathology of rheumatoid arthritis.

[0047] A case 3 is an example in which none responder (DAS28: 4.87 → 3.95) was shown although IFX (infliximab) was used together with treatment with MTX (methotrexate). The amount of talin remained high (OD value: 0.205 → 0.223) even when treatment together with IFX was carried out.

[0048] A case 4 is an example in which good responder (DAS28: 4.55 → 2.45) was shown when ADA (adalimumab) was used together with treatment with BUC (bucillamine). The amount of talin showed a high value (OD value: 0.258) when only BUC was used, but it showed a normal value (OD value: 0.164) when ADA was used together.

[0049] A case 5 is an example in which none responder (DAS28: 5.09 → 4.50) was shown also when treatment using TCZ (tocilizumab) was carried out. The amount of talin remained high (OD value: 2.093 → 1.787) even with TCZ treatment.

[0050] As is shown from the above-mentioned results, the amount of talin in the blood correlates with the activity of rheumatoid arthritis, and it reflects the activity of rheumatoid arthritis more precisely as compared with the other factors such as CRP and MMP-3. Therefore, by measuring the amount of talin in the blood, it is possible to precisely determine the therapeutic effect of a therapeutic agent for rheumatoid arthritis.

SEQUENCE LISTING

[0051]

<110> KayteeBio, Co. & Ltd.

<120> NOVEL TEST METHOD FOR RHEUMATOID ARTHRITIS AND KIT FOR RHEUMATOID ARTHRITIS TEST

<130> ATF-057PCT

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            20                  25                  30

Arg Glu Arg Ile Pro Glu Ala Pro Ala Gly Pro Pro Ser Asp Phe Gly
        35                  40                  45

Leu Phe Leu Ser Asp Asp Asp Pro Lys Lys Gly Ile Trp Leu Glu Ala
    50                  55                  60

Gly Lys Ala Leu Asp Tyr Tyr Met Leu Arg Asn Gly Asp Thr Met Glu
65                  70                  75                  80

Tyr Arg Lys Lys Gln Arg Pro Leu Lys Ile Arg Met Leu Asp Gly Thr
                85                  90                  95

Val Lys Thr Ile Met Val Asp Asp Ser Lys Thr Val Thr Asp Met Leu
            100                 105                 110

Met Thr Ile Cys Ala Arg Ile Gly Ile Thr Asn His Asp Glu Tyr Ser
        115                 120                 125

Leu Val Arg Glu Leu Met Glu Glu Lys Lys Glu Glu Ile Thr Gly Thr
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Leu Arg Lys Asp Lys Thr Leu Leu Arg Asp Glu Lys Lys Met Glu Lys
145                 150                 155                 160

Leu Lys Gln Lys Leu His Thr Asp Asp Glu Leu Asn Trp Leu Asp His
                165                 170                 175

Gly Arg Thr Leu Arg Glu Gln Gly Val Glu Glu His Glu Thr Leu Leu
            180                 185                 190

Leu Arg Arg Lys Phe Phe Tyr Ser Asp Gln Asn Val Asp Ser Arg Asp
        195                 200                 205

Pro Val Gln Leu Asn Leu Leu Tyr Val Gln Ala Arg Asp Asp Ile Leu
    210                 215                 220

Asn Gly Ser His Pro Val Ser Phe Asp Lys Ala Cys Glu Phe Ala Gly
225                 230                 235                 240

Phe Gln Cys Gln Ile Gln Phe Gly Pro His Asn Glu Gln Lys His Lys
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Ala Gly Phe Leu Asp Leu Lys Asp Phe Leu Pro Lys Glu Tyr Val Lys
            260                 265                 270

Gln Lys Gly Glu Arg Lys Ile Phe Gln Ala His Lys Asn Cys Gly Gln
        275                 280                 285

Met Ser Glu Ile Glu Ala Lys Val Arg Tyr Val Lys Leu Ala Arg Ser
    290                 295                 300

Leu Lys Thr Tyr Gly Val Ser Phe Phe Leu Val Lys Glu Lys Met Lys
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Gly Lys Asn Lys Leu Val Pro Arg Leu Leu Gly Ile Thr Lys Glu Cys
                325                 330                 335

Val Met Arg Val Asp Glu Lys Thr Lys Glu Val Ile Gln Glu Trp Asn
            340                 345                 350

Leu Thr Asn Ile Lys Arg Trp Ala Ala Ser Pro Lys Ser Phe Thr Leu
        355                 360                 365

Asp Phe Gly Asp Tyr Gln Asp Gly Tyr Tyr Ser Val Gln Thr Thr Glu
    370                 375                 380

Gly Glu Gln Ile Ala Gln Leu Ile Ala Gly Tyr Ile Asp Ile Ile Leu
385                 390                 395                 400

Lys Lys Lys Lys Ser Lys Asp His Phe Gly Leu Glu Gly Asp Glu Glu
                405                 410                 415

Ser Thr Met Leu Glu Asp Ser Val Ser Pro Lys Lys Ser Thr Val Leu
            420                 425                 430

Gln Gln Gln Tyr Asn Arg Val Gly Lys Val Glu His Gly Ser Val Ala
        435                 440                 445

Leu Pro Ala Ile Met Arg Ser Gly Ala Ser Gly Pro Glu Asn Phe Gln
    450                 455                 460

Val Gly Ser Met Pro Pro Ala Gln Gln Gln Ile Thr Ser Gly Gln Met
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His Arg Gly His Met Pro Pro Leu Thr Ser Ala Gln Gln Ala Leu Thr
                485                 490                 495

Gly Thr Ile Asn Ser Ser Met Gln Ala Val Gln Ala Ala Gln Ala Thr
            500                 505                 510

Leu Asp Asp Phe Asp Thr Leu Pro Pro Leu Gly Gln Asp Ala Ala Ser
        515                 520                 525

Lys Ala Trp Arg Lys Asn Lys Met Asp Glu Ser Lys His Glu Ile His
    530                 535                 540

Ser Gln Val Asp Ala Ile Thr Ala Gly Thr Ala Ser Val Val Asn Leu
545                 550                 555                 560

Thr Ala Gly Asp Pro Ala Glu Thr Asp Tyr Thr Ala Val Gly Cys Ala
                565                 570                 575

Val Thr Thr Ile Ser Ser Asn Leu Thr Glu Met Ser Arg Gly Val Lys
            580                 585                 590

Leu Leu Ala Ala Leu Leu Glu Asp Glu Gly Gly Ser Gly Arg Pro Leu
        595                 600                 605

Leu Gln Ala Ala Lys Gly Leu Ala Gly Ala Val Ser Glu Leu Leu Arg
    610                 615                 620

Ser Ala Gln Pro Ala Ser Ala Glu Pro Arg Gln Asn Leu Leu Gln Ala
625                 630                 635                 640

Ala Gly Asn Val Gly Gln Ala Ser Gly Glu Leu Leu Gln Gln Ile Gly
                645                 650                 655

Glu Ser Asp Thr Asp Pro His Phe Gln Asp Ala Leu Met Gln Leu Ala
            660                 665                 670

Lys Ala Val Ala Ser Ala Ala Ala Ala Leu Val Leu Lys Ala Lys Ser
        675                 680                 685

Val Ala Gln Arg Thr Glu Asp Ser Gly Leu Gln Thr Gln Val Ile Ala
    690                 695                 700

Ala Ala Thr Gln Cys Ala Leu Ser Thr Ser Gln Leu Val Ala Cys Thr
705                 710                 715                 720

Lys Val Val Ala Pro Thr Ile Ser Ser Pro Val Cys Gln Glu Gln Leu
                725                 730                 735

Val Glu Ala Gly Arg Leu Val Ala Lys Ala Val Glu Gly Cys Val Ser
            740                 745                 750

Ala Ser Gln Ala Ala Thr Glu Asp Gly Gln Leu Leu Arg Gly Val Gly
        755                 760                 765

Ala Ala Ala Thr Ala Val Thr Gln Ala Leu Asn Glu Leu Leu Gln His
    770                 775                 780

Val Lys Ala His Ala Thr Gly Ala Gly Pro Ala Gly Arg Tyr Asp Gln
785                 790                 795                 800

Ala Thr Asp Thr Ile Leu Thr Val Thr Glu Asn Ile Phe Ser Ser Met
                805                 810                 815

Gly Asp Ala Gly Glu Met Val Gly Gln Ala Arg Ile Leu Ala Gln Ala
            820                 825                 830
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Thr Ser Asp Leu Val Asn Ala Ile Lys Ala Asp Ala Glu Gly Glu Ser
        835                 840                 845

Asp Leu Glu Asn Ser Arg Lys Leu Leu Ser Ala Ala Lys Ile Leu Ala
    850                 855                 860

Asp Ala Thr Ala Lys Met Val Glu Ala Ala Lys Gly Ala Ala Ala His
865                 870                 875                 880

Pro Asp Ser Glu Glu Gln Gln Gln Arg Leu Arg Glu Ala Ala Glu Gly
                885                 890                 895

Leu Arg Met Ala Thr Asn Ala Ala Ala Gln Asn Ala Ile Lys Lys Lys
            900                 905                 910

Leu Val Gln Arg Leu Glu His Ala Ala Lys Gln Ala Ala Ala Ser Ala
        915                 920                 925

Thr Gln Thr Ile Ala Ala Ala Gln His Ala Ala Ser Thr Pro Lys Ala
    930                 935                 940

Ser Ala Gly Pro Gln Pro Leu Leu Val Gln Ser Cys Lys Ala Val Ala
945                 950                 955                 960

Glu Gln Ile Pro Leu Leu Val Gln Gly Val Arg Gly Ser Gln Ala Gln
                965                 970                 975

Pro Asp Ser Pro Ser Ala Gln Leu Ala Leu Ile Ala Ala Ser Gln Ser
            980                 985                 990

Phe Leu Gln Pro Gly Gly Lys Met Val Ala Ala Ala Lys Ala Ser Val
        995                 1000                1005

Pro Thr Ile Gln Asp Gln Ala Ser Ala Met Gln Leu Ser Gln Cys Ala
    1010                1015                1020

Lys Asn Leu Gly Thr Ala Leu Ala Glu Leu Arg Thr Ala Ala Gln Lys
1025                1030                1035                1040

Ala Gln Glu Ala Cys Gly Pro Leu Glu Met Asp Ser Ala Leu Ser Val
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Val Gln Asn Leu Glu Lys Asp Leu Gln Glu Val Lys Ala Ala Ala Arg
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Asp Gly Lys Leu Lys Pro Leu Pro Gly Glu Thr Met Glu Lys Cys Thr
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Gln Asp Leu Gly Asn Ser Thr Lys Ala Val Ser Ser Ala Ile Ala Gln
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Leu Leu Gly Glu Val Ala Gln Gly Asn Glu Asn Tyr Ala Gly Ile Ala
1105                1110                1115                1120

Ala Arg Asp Val Ala Gly Gly Leu Arg Ser Leu Ala Gln Ala Ala Arg
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Gly Val Ala Ala Leu Thr Ser Asp Pro Ala Val Gln Ala Ile Val Leu
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Asp Thr Ala Ser Asp Val Leu Asp Lys Ala Ser Ser Leu Ile Glu Glu
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Ala Lys Lys Ala Ala Gly His Pro Gly Asp Pro Glu Ser Gln Gln Arg
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Leu Ala Gln Val Ala Lys Ala Val Thr Gln Ala Leu Asn Arg Cys Val
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Ser Cys Leu Pro Gly Gln Arg Asp Val Asp Asn Ala Leu Arg Ala Val
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Gly Asp Ala Ser Lys Arg Leu Leu Ser Asp Ser Leu Pro Pro Ser Thr
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Gly Thr Phe Gln Glu Ala Gln Ser Arg Leu Asn Glu Ala Ala Ala Gly
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Leu Asn Gln Ala Ala Thr Glu Leu Val Gln Ala Ser Arg Gly Thr Pro
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Gln Asp Leu Ala Arg Ala Ser Gly Arg Phe Gly Gln Asp Phe Ser Thr
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Phe Leu Glu Ala Gly Val Glu Met Ala Gly Gln Ala Pro Ser Gln Glu
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Asp Arg Ala Gln Val Val Ser Asn Leu Lys Gly Ile Ser Met Ser Ser
            1300                1305                1310

Ser Lys Leu Leu Leu Ala Ala Lys Ala Leu Ser Thr Asp Pro Ala Ala
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Pro Asn Leu Lys Ser Gln Leu Ala Ala Ala Ala Arg Ala Val Thr Asp
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Ser Ile Asn Gln Leu Ile Thr Met Cys Thr Gln Gln Ala Pro Gly Gln
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Lys Glu Cys Asp Asn Ala Leu Arg Glu Leu Glu Thr Val Arg Glu Leu
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Leu Glu Asn Pro Val Gln Pro Ile Asn Asp Met Ser Tyr Phe Gly Cys
            1380                1385                1390

Leu Asp Ser Val Met Glu Asn Ser Lys Val Leu Gly Glu Ala Met Thr
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Gly Ile Ser Gln Asn Ala Lys Asn Gly Asn Leu Pro Glu Phe Gly Asp
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Ala Ile Ser Thr Ala Ser Lys Ala Leu Cys Gly Phe Thr Glu Ala Ala
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Ala Gln Ala Ala Tyr Leu Val Gly Val Ser Asp Pro Asn Ser Gln Ala
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Gly Gln Gln Gly Leu Val Glu Pro Thr Gln Phe Ala Arg Ala Asn Gln
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Ala Ile Gln Met Ala Cys Gln Ser Leu Gly Glu Pro Gly Cys Thr Gln
        1475                1480                1485

Ala Gln Val Leu Ser Ala Ala Thr Ile Val Ala Lys His Thr Ser Ala
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Leu Cys Asn Ser Cys Arg Leu Ala Ser Ala Arg Thr Thr Asn Pro Thr
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Ala Lys Arg Gln Phe Val Gln Ser Ala Lys Glu Val Ala Asn Ser Thr
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Ala Asn Leu Val Lys Thr Ile Lys Ala Leu Asp Gly Ala Phe Thr Glu
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Glu Asn Arg Ala Gln Cys Arg Ala Ala Thr Ala Pro Leu Leu Glu Ala
        1555                1560                1565

Val Asp Asn Leu Ser Ala Phe Ala Ser Asn Pro Glu Phe Ser Ser Ile
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Pro Ala Gln Ile Ser Pro Glu Gly Arg Ala Ala Met Glu Pro Ile Val
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Ile Ser Ala Lys Thr Met Leu Glu Ser Ala Gly Gly Leu Ile Gln Thr
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Ala Arg Ala Leu Ala Val Asn Pro Arg Asp Pro Pro Ser Trp Ser Val
            1620                1625                1630

Leu Ala Gly His Ser Arg Thr Val Ser Asp Ser Ile Lys Lys Leu Ile
        1635                1640                1645

Thr Ser Met Arg Asp Lys Ala Pro Gly Gln Leu Glu Cys Glu Thr Ala
    1650                1655                1660

Ile Ala Ala Leu Asn Ser Cys Leu Arg Asp Leu Asp Gln Ala Ser Leu
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Ala Ala Val Ser Gln Gln Leu Ala Pro Arg Glu Gly Ile Ser Gln Glu
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His Lys Val Ser Gln Met Ala Gln Tyr Phe Glu Pro Leu Thr Leu Ala
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Leu Leu Asp Gln Thr Lys Thr Leu Ala Glu Ser Ala Leu Gln Leu Leu
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Tyr Thr Ala Lys Glu Ala Gly Gly Asn Pro Lys Gln Ala Ala His Thr
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Leu Gln Cys Ser Pro Ser Asp Ala Tyr Thr Lys Lys Glu Leu Ile Glu
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Val Ser Gly Ile Ile Ala Asp Leu Asp Thr Thr Ile Met Phe Ala Thr
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Glu Gly Ile Leu Lys Thr Ala Lys Val Leu Val Glu Asp Thr Lys Val
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Val Ile Arg Glu Arg Val Pro Glu Ala Gln Thr Gly Gln Ala Ser Asp
        35                  40                  45

Tyr Gly Leu Phe Leu Ser Asp Glu Asp Pro Arg Lys Gly Ile Trp Leu
    50                  55                  60

Glu Ala Gly Arg Thr Leu Asp Tyr Tyr Met Leu Arg Asn Gly Asp Ile
65                  70                  75                  80

Leu Glu Tyr Lys Lys Lys Gln Arg Pro Gln Lys Ile Arg Met Leu Asp
                85                  90                  95

Gly Ser Val Lys Thr Val Met Val Asp Asp Ser Lys Thr Val Gly Glu
            100                 105                 110

Leu Leu Val Thr Ile Cys Ser Arg Ile Gly Ile Thr Asn Tyr Glu Glu
        115                 120                 125

Tyr Ser Leu Ile Gln Glu Thr Ile Glu Glu Lys Lys Glu Glu Gly Thr
    130                 135                 140

Gly Thr Leu Lys Lys Asp Arg Thr Leu Leu Arg Asp Glu Arg Lys Met
145                 150                 155                 160

Glu Lys Leu Lys Ala Lys Leu His Thr Asp Asp Asp Leu Asn Trp Leu
                165                 170                 175

Asp His Ser Arg Thr Phe Arg Glu Gln Gly Val Asp Glu Asn Glu Thr
            180                 185                 190

Leu Leu Leu Arg Arg Lys Phe Phe Tyr Ser Asp Gln Asn Val Asp Ser
        195                 200                 205

Arg Asp Pro Val Gln Leu Asn Leu Leu Tyr Val Gln Ala Arg Asp Asp
    210                 215                 220

Ile Leu Asn Gly Ser His Pro Val Ser Phe Glu Lys Ala Cys Glu Phe
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Gly Gly Phe Gln Ala Gln Ile Gln Phe Gly Pro His Val Glu His Lys
                245                 250                 255

His Lys Pro Gly Phe Leu Asp Leu Lys Glu Phe Leu Pro Lys Glu Tyr
            260                 265                 270

Ile Lys Gln Arg Gly Ala Glu Lys Arg Ile Phe Gln Glu His Lys Asn
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Cys Gly Glu Met Ser Glu Ile Glu Ala Lys Val Lys Tyr Val Lys Leu
    290                 295                 300
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Lys Met Lys Gly Lys Asn Lys Leu Val Pro Arg Leu Leu Gly Ile Thr
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Lys Asp Ser Val Met Arg Val Asp Glu Lys Thr Lys Glu Val Leu Gln
            340                 345                 350

Glu Trp Pro Leu Thr Thr Val Lys Arg Trp Ala Ala Ser Pro Lys Ser
        355                 360                 365

Phe Thr Leu Asp Phe Gly Glu Tyr Gln Glu Ser Tyr Tyr Ser Val Gln
    370                 375                 380

Thr Thr Glu Gly Glu Gln Ile Ser Gln Leu Ile Ala Gly Tyr Ile Asp
385                 390                 395                 400

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Asp Glu Glu Ser Thr Met Leu Glu Glu Ser Val Ser Pro Lys Lys Ser
            420                 425                 430

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Gly Gln Met His Arg Gly His Met Pro Pro Leu Thr Ser Ala Gln Gln
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Gly Cys Ala Ile Thr Thr Ile Ser Ser Asn Leu Thr Glu Met Ser Lys
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Gly Val Lys Leu Leu Ala Ala Leu Met Asp Asp Glu Val Gly Ser Gly
        595                 600                 605

Glu Asp Leu Leu Arg Ala Ala Arg Thr Leu Ala Gly Ala Val Ser Asp
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690 695 700

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Ala Ala Asn Pro Glu Asn Glu Asp Gln Gln Gln Arg Leu Arg Glu Ala
885 890 895

Ala Glu Gly Leu Arg Val Ala Thr Asn Ala Ala Ala Gln Asn Ala Ile
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Lys Lys Lys Ile Val Asn Arg Leu Glu Val Ala Ala Lys Gln Ala Ala
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Lys Glu Asp Gln Ile Gln Val Ile Gly Asn Leu Lys Asn Ile Ser Met
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Met Leu Tyr Ala Ala Lys Glu Gly Gly Gly Asn Pro Lys Ala Gln His
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1825                1830                1835                1840

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                1845                1850                1855

Thr Val Val Lys Tyr Ser Lys Ala Ile Ala Val Thr Ala Gln Glu Met
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        1875                1880                1885

Gln Met Thr Ser Asp Tyr Gly His Leu Ala Phe Gln Gly Gln Met Ala
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Ala Leu Gln Val Cys Pro Thr Asp Ser Tyr Thr Lys Arg Glu Leu Ile
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Glu Cys Ala Arg Ala Val Thr Glu Lys Val Ser Leu Val Leu Ser Ala
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        2435                2440                2445

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Gly Lys Ala Asp Asp Asp Asp Val Val Val Lys Thr Lys Phe Val Gly
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            2500                2505                2510

Arg Glu Leu Glu Glu Ala Arg Lys Lys Leu Ala Gln Ile Arg Gln Gln
        2515                2520                2525

Gln Tyr Lys Phe Leu Pro Thr Glu Leu Arg Glu Asp Glu Gly
    2530                2535                2540
```

Claims

1. An *in vitro* test method for determining a diagnosis of rheumatoid arthritis or a treatment effect of a therapeutic agent for rheumatoid arthritis, the method comprising a step of:

   measuring an amount of talin in plasma or serum of an animal subject, and determining that the subject has contracted rheumatoid arthritis when the amount of talin in the plasma or the serum is larger than a predetermined threshold, the predetermined threshold being based on a mean value in the plasma or the serum in a control animal that has not contracted rheumatoid arthritis, or determining that the therapeutic agent is effective when the amount of talin after the therapeutic agent for rheumatoid arthritis is administered is significantly lowered from the amount of talin before the administration.

2. The *in vitro* test method according to claim 1, further comprising a step of obtaining plasma or serum from blood collected from the animal subject.

3. The *in vitro* test method according to claim 1 or 2, wherein the amount of talin in the plasma or the serum is measured by using an antibody which binds to talin.

4. The *in vitro* test method according to any one of claims 1 to 3, wherein the animal subject is a human subject.

5. Use of a kit for the *in vitro* test method for rheumatoid arthritis according to any one of claims 1 to 4, comprising a solid-phase carrier to which an antibody that binds to talin is affixed.

Patentansprüche

1. *In vitro* Testverfahren zur Bestimmung einer Diagnose von rheumatoider Arthritis oder eine Behandlungswirkung eines therapeutischen Agenten für die rheumatoide Arthritis, worin das Verfahren einen Schritt umfasst von:

Messen einer Menge an Talin im Plasma oder Serum eines Tiersubjekts und Bestimmen, dass das Subjekt an rheumatoider Arthritis erkrankt ist, wenn die Menge an Talin im Plasma oder im Serum über einem vorbestimmten Schwellenwert liegt, wobei der vorbestimmte Schwellenwert auf einem Durchschnittswert im Plasma oder im Serum eines Kontrolltiers, welches nicht an rheumatoider Arthritis erkrankt ist, basiert, oder Bestimmen, dass der therapeutische Agent wirksam ist, wenn die Menge an Talin nach Verabreichung des therapeutischen Agenten für rheumatoide Arthritis im Vergleich zur Menge an Talin vor der Verabreichung wesentlich reduziert wurde.

2. *In vitro* Testverfahren gemäss Anspruch 1, zudem mit einem Schritt der Gewinnung von Plasma oder Serum aus von einem Tiersubjekt entnommenen Blut.

3. *In vitro* Testverfahren gemäss Anspruch 1 oder 2, worin die Menge an Talin im Plasma oder im Serum unter Verwendung eines sich mit dem Talin bindenden Antikörpers gemessen wird.

4. *In vitro* Testverfahren gemäss irgendeinem der Ansprüche 1 bis 3, worin das Tiersubjekt ein menschliches Subjekt ist.

5. Verwendung eines Kits für das *in vitro* Testverfahren für rheumatoide Arthritis gemäss irgendeinem der Ansprüche 1 bis 4, mit einem Solidphasenträger, auf welchem ein sich mit dem Talin bindender Antikörper befestigt ist.

Revendications

1. Procédé de test *in vitro* pour déterminer un diagnostic de l'arthrite rhumatoïde ou un effet du traitement d'un agent thérapeutique pour l'arthrite rhumatoïde, le procédé comprenant une étape de :

mesure d'une quantité de taline dans le plasma ou le sérum d'un sujet animal, et la détermination que le sujet a contracté l'arthrite rhumatoïde lorsque la quantité de taline dans le plasma ou le sérum est supérieure à un seuil prédéterminé, le seuil prédéterminé étant basé sur une valeur moyenne dans le plasma ou le sérum d'un animal de contrôle qui n'a pas contracté l'arthrite rhumatoïde, ou la détermination que l'agent thérapeutique est efficace lorsque la quantité de taline après que l'agent thérapeutique pour l'arthrite rhumatoïde ait été administré a été réduit sensiblement comparé à la quantité de taline avant l'administration.

2. Procédé de test *in vitro* selon la revendication 1, comprenant en outre une étape d'obtention de plasma ou de sérum à partir de sang recueilli du sujet animal.

3. Procédé de test *in vitro* selon la revendication 1 ou 2, dans lequel la quantité de taline dans le plasma ou le sérum est mesurée en utilisant un anticorps qui se lie à la taline.

4. Procédé de test *in vitro* selon l'une quelconque des revendications 1 à 3, dans lequel le sujet animal est un sujet humain.

5. Utilisation d'un kit pour le procédé de test *in vitro* pour l'arthrite rhumatoïde selon l'une quelconque des revendications 1 à 4, comprenant un support en phase solide auquel est fixé un anticorps qui se lie à la taline.

FIG. 1

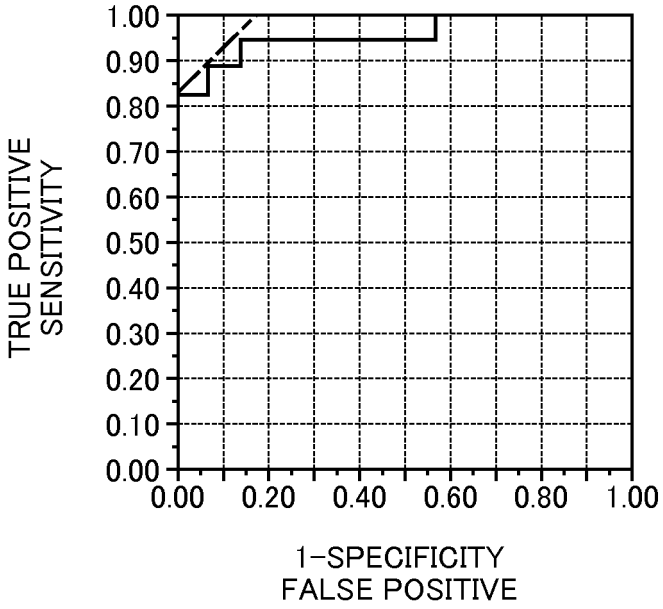
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FIG. 2

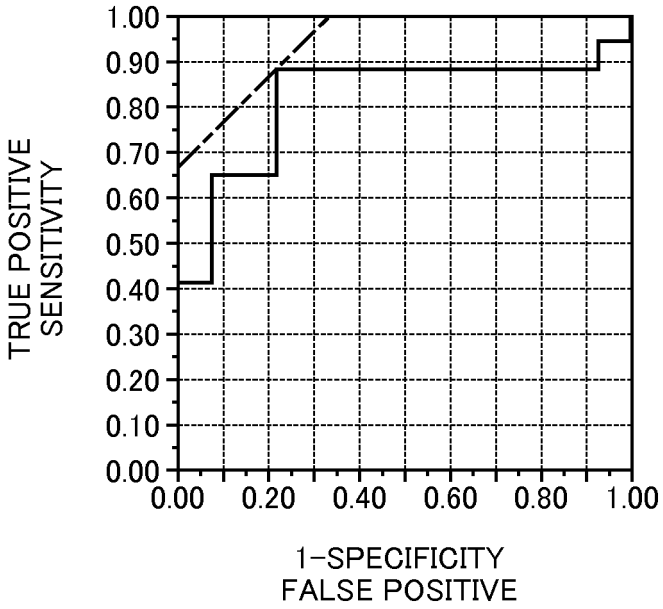
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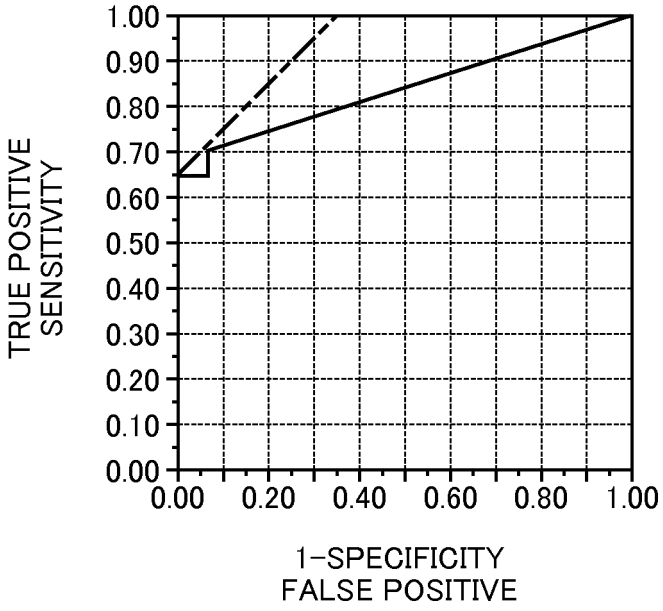
FIG. 3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SENSITIVITY
0.00
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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 03072827 A **[0006]**

Non-patent literature cited in the description

- **MARTINUS A. M. et al.** *Arthritis Res. Ther.,* 2002, vol. 4, 87-93 **[0004]**
- **AVOUAC J. et al.** *Ann. Rheum. Dis.,* 2006, vol. 65, 845-851 **[0004]**
- **VAN VENROOIJ WJ. et al.** *Ann. N.Y. Acad. Sci.,* 2008, vol. 1143, 268-285 **[0004]**
- **LI TIANWANG et al.** Distinct proteomic profile in Ankylosing spondylitis patients: Talin1 is a new valuable biomarker for diagnosis and treatment. *ARTHRITIS & RHEUMATISM,* September 2008, vol. 58 (9 **[0005]**
- *ANNUAL SCIENTIFIC MEETING OF THE AMERICAN-COLLEGE-OF-RHEUMATOLOGY/43RD ANNUAL SCIENTIFIC MEETIN,* 24 October 2008, ISSN 0004-3591, S350-S351 **[0005]**
- **LI T et al.** Over-expression of talin 1 and integrin-linked kinase in PBMCs of patients with ankylosing spondylitis: a proteomic study. *CLINICAL AND EXPERIMENTAL RHEUMATOLOGY,* November 2010, vol. 28 (6), ISSN 0392-856X, 828-835 **[0007]**

专利名称(译)	用于类风湿性关节炎的新型试验方法和用于类风湿性关节炎试验的试剂盒		
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摘要(译)

提供了一种新的类风湿性关节炎试验方法;用于类风湿性关节炎试验的试剂盒，用于类风湿性关节炎的新型试验方法。根据本发明的类风湿性关节炎的测试方法的特征在于包括测量动物受试者的血浆或血清中的踝蛋白量的步骤。该测量例如通过使用与踝蛋白结合的抗体的免疫学方法进行。根据本发明的用于类风湿性关节炎测试的试剂盒用于这种测试方法，并且包含例如固定有与踝蛋白结合的抗体的固相载体。

[Table 1]

		RA patient	Control	Total
H-300 antibody	Positive	14	0	14
	Negative	3	14	17
	Total	17	14	31