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(72) Inventor: **Wang, Lemin**  
**Shanghai 200065 (CN)**

(71) Applicant: **Wang, Lemin**  
**Shanghai 200065 (CN)**

(74) Representative: **Hoffmann Eitle**  
**Patent- und Rechtsanwälte PartmbB**  
**Arabellastraße 30**  
**81925 München (DE)**

(54) **APPLICATION OF INTEGRIN  $\beta$  SUBUNIT IN DIAGNOSING VENOUS THROMBOEMBOLISM**

(57) The present invention provides a method for diagnosing venous thromboembolism (VTE), comprising: detecting the level of an integrin  $\beta$ 1 subunit, an integrin  $\beta$ 2 subunit, and/or an integrin  $\beta$ 3 subunit in a blood sam-

ple. Also provided is a reagent kit for diagnosing VTE, comprising a substance capable of specifically binding to the integrin  $\beta$ 1 subunit, the integrin  $\beta$ 2 subunit, and/or the integrin  $\beta$ 3 subunit.

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## Description

### Technical Field

5 [0001] The present invention relates to the field of venous thromboembolism (VTE). Specifically, the present invention provides a novel method for diagnosing VTE, comprising detecting the level of the integrin  $\beta$ 1 subunit, the integrin  $\beta$ 2 subunit, and/or the integrin  $\beta$ 3 subunit in a blood sample of a subject. The present invention also relates to use of a substance capable of specifically binding to the integrin  $\beta$ 1 subunit, the integrin  $\beta$ 2 subunit, and/or the integrin  $\beta$ 3 subunit, in the preparation of a diagnostic agent for diagnosing VTE. Furthermore, the present invention also provides a kit for  
10 diagnosing VTE, which comprises a substance capable of specifically binding to the integrin  $\beta$ 1 subunit, the integrin  $\beta$ 2 subunit, and/or the integrin  $\beta$ 3 subunit.

### Background

15 [0002] Venous thromboembolism is a general term referring to thrombus generated in vena, comprising pulmonary embolism (PE) and deep venous thrombosis (DVT), wherein PE comprises acute pulmonary embolism (APE), and chronic thromboembolic pulmonary hypertension (CTEPH). VTE is a frequently-occurring disease, wherein PE has become a worldwide health care problem due to its high morbidity rate, high misdiagnosis rate, and high mortality rate (Prevention and treatment of venous thromboembolism. International Consensus Statement (guidelines according to  
20 scientific evidence). *Int. Angiol.* 2006; 25(2): 101-161).

[0003] According to the traditional phlebothrombosis theory, in the state when the blood flow is slow, the endothelial cells in veins are injured or the coagulation factors are hyperfunctional, the injured endothelial cells and platelets are activated, the functions of coagulation factors are activated, and thereby venous thrombus is generated. The thrombus  
25 generated in veins are rich in fibrins and erythrocytes, and merely contain a little amount of platelets (Furie, Bruce; Furie, Barbara, Mechanisms of Thrombus Formation, *The New England Journal of Medicine* 2008; 359 (9): 938-949).

[0004] The recent research results of the present inventor indicate that, the protein compositions in APE thrombus are mainly composed of fibrinogens, wherein there are a little amount of serum proteins and cytoskeletal proteins (Wang L, Gong Z, Jiang J, Xu W, Duan Q, Liu J, et al. Confusion of Wide Thrombolytic Time Window for Acute Pulmonary Embolism: Mass Spectrographic Analysis for Thrombus Proteins. *Am. J. Respir. Crit. Care Med.* 2011 184: 145-146).  
30 Such a discovery changes the traditional view that venous thrombus proteins are mainly fibrins, providing a novel way for diagnosing and treating VTE. For example, investigations for the binding manners between the fibrinogens in VTE thrombus and the leukocytes, platelets and erythrocytes in thrombus can provide information concerning the mechanism of venous thrombus formation.

[0005] Integrin is a type of receptor protein that mediates the attachments between cells, and between a cell and its  
35 surrounding tissues (such as other cells or extracellular matrix). It also plays an important role in cell signaling, and thereby mediates information exchange between a cell and its surrounding environments, allowing the cell to make rapid response to changes in the environments (e.g. allowing the blood to coagulate through platelets) (Hynes R, Integrins: bidirectional, allosteric signaling machines. *Cell* (2002), 110 (6): 673-87).

[0006] Integrin is a heterodimer, and it contains two different chains, termed as  $\alpha$  subunit and  $\beta$  subunit, respectively.  
40 Currently 18  $\alpha$  subunits and 9  $\beta$  subunits have been identified in mammal integrins, and more than 20 integrins can be formed through respective combinations of these subunits (Humphries MJ. Integrin structure. *Biochem Soc Trans.* 2000; 28(4): 311-39). These more than 20 integrins form an integrin family, wherein integrins containing the same  $\beta$  subunit form integrin  $\beta$  subfamily. For example, the integrin  $\beta$ 1-3 subunits encoded by *ITGB1*, *ITGB2*, and *ITGB3* genes are ITGB1 (also called CD29), ITGB2(also called CD18), and ITGB3(also called CD61), respectively. The integrin  $\beta$ 1 sub-  
45 family containing ITGB1 subunit is widely distributed, and mainly mediates the attachments between a cell and the extracellular matrix compositions, and mediates lymphocyte homing as well as the adhesion between leukocytes and activated endothelial cells;  $\beta$ 1 subfamily is mainly distributed on membrane surfaces of activated lymphocytes and platelets (Billard MJ, McIntyre BW. CD45RA T-cell activation without proliferation by a partial agonist monoclonal antibody to beta1 integrin. *Immunol Cell Biol.* 2008 May-Jun; 86(4): 381-4). The subfamily containing integrin  $\beta$ 2 is mainly found  
50 on the surfaces of various leukocytes, which is used for mediating interactions between cells, and which is mainly distributed in neutrophil granulocytes and monocytes (Fu C, Tong C, Wang M, Gao Y, Zhang Y, Lü S, Liang S, Dong C, Long M. Determining beta2-integrin and intercellular adhesion molecule 1 binding kinetics in tumor cell adhesion to leukocytes and endothelial cells by a gas-driven micropipette assay. *J Biol Chem.* 2011 Oct 7; 286(40): 34777-87). The subfamily containing integrin  $\beta$ 3 is mainly distributed on the surfaces of platelets, which mediates the aggregation of  
55 platelets, and involves in the formation of thrombus (Sachs UJ, Bakchoul T, Eva O, Giptner A, Bein G, Aster RH, Gitter M, Peterson J, Santoso S. A point mutation in EGF-4 domain of  $\beta$ 3 integrin is responsible for the formation of the Seca platelet alloantigen and affects receptor function. *Thromb Haemost.* 2011; 107(1)).

**Summary of the invention**

**[0007]** In one aspect, the present invention relates to use of a substance specifically binding to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit, in the preparation of a diagnostic agent for diagnosing venous thromboembolism.

**[0008]** In one embodiment, the present invention relates to use of a substance specifically binding to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit, in the preparation of a diagnostic agent for diagnosing venous thromboembolism (VTE) by detecting the level of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit in a blood sample of a subject.

**[0009]** In one embodiment, the blood sample of the present invention is a peripheral blood sample, preferably a peripheral venous blood sample.

**[0010]** In one embodiment, the present invention relates to use of a substance, which specifically binds to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit, in the preparation of a diagnostic agent for diagnosing venous thromboembolism by detecting the levels of the corresponding subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit in a blood sample of a subject.

**[0011]** In one embodiment, the present invention relates to use of substances, which respectively specifically bind to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit, in the preparation of a diagnostic agent for diagnosing venous thromboembolism by detecting the increased levels of the corresponding integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit in a blood sample of a subject.

**[0012]** In one embodiment, said venous thromboembolism of the invention is acute pulmonary embolism (APE), chronic thromboembolic pulmonary hypertension (CTEPH), or deep venous thrombosis (DVT).

**[0013]** In one embodiment, said venous thromboembolism of the invention is acute pulmonary embolism (APE) or acute deep venous thrombosis (DVT).

**[0014]** In one embodiment, the substance of the invention that specifically binds to  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit is an antibody, a ligand, or an antagonist. In one embodiment, the antibody, ligand, and/or antagonist of the invention is labeled. In one embodiment, the antibody, ligand, and/or antagonist of the invention is fluorescently labeled. In one embodiment, the antibody, ligand, and/or antagonist of the invention is an antibody labeled by phycoerythrin (PE).

**[0015]** In another aspect, the present invention provides a kit for diagnosing venous thromboembolism (VTE) by detecting the level of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit in a blood sample such as peripheral venous blood sample of a subject.

**[0016]** In one embodiment, the present invention provides a kit for diagnosing venous thromboembolism (VTE) by detecting the level of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit in a blood sample such as peripheral venous blood sample of a subject, wherein said kit comprises a substance that specifically binds to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit, respectively.

**[0017]** In one embodiment, the kit of the present invention comprises a substance that specifically binds to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit. In one embodiment, the kit of the present invention comprises substances that specifically bind to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit, respectively.

**[0018]** In one embodiment, the substance comprised in the kit of the present invention, which specifically binds to  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit, is an antibody, a ligand, and/or and an antagonist. In one embodiment, the antibody, ligand, and/or antagonist of the invention is labeled. In one embodiment, the antibody, ligand, and/or antagonist of the invention is fluorescently labeled. In one embodiment, the antibody, ligand, and/or antagonist of the invention is an antibody labeled by phycoerythrin (PE).

**Description of the Figures****[0019]**

Figure 1: The results of pre-isolation of thrombus samples by shotgun method.

Figure 2: The sequence information of the enzyme-digested peptide fragments and debris of thrombus protein samples.

Figure 3: The tandem protein mass spectrometry (MS) and information analysis of thrombus samples from acute PE patients, and results of Gene Oncology analysis.

Figure 4: The tandem protein MS and information analysis of thrombus samples from acute PE patients, and results of interaction network analysis, indicating that integrins comprising  $\beta$ 2,  $\beta$ 3, and  $\beta$ 1 subunits are core proteins of red thrombus.

Figure 5: The tandem protein MS and information analysis of thrombus samples from acute lower limbs VTE patients,

and results of Gene Oncology analysis.

Figure 6: The tandem protein MS and information analysis of thrombus samples from acute lower limbs VTE patients, and results of interaction network analysis, indicating that the integrin comprising  $\beta 3$  subunit and ITGA2B (i.e. integrin  $\alpha$  chain IIb) are core proteins of red thrombus.

Figure 7: The distribution of integrin  $\beta 1$ ,  $\beta 2$ , and  $\beta 3$  subunits in control group of health population, wherein figure 7a indicates that integrin  $\beta 1$  subunit shows a normal distribution, figure 7b indicates that integrin  $\beta 2$  subunit shows an abnormal distribution, and figure 7c indicates that integrin  $\beta 3$  subunit shows a normal distribution.

Figure 8: The expression of integrin  $\beta 1$  subunit in control group of health population. Figure 8a shows the expression average value and baseline range of integrin  $\beta 1$  subunit in the control group, wherein the average value is  $8.18 \pm 2.69$  (%), and the baseline range is 2.8-13.56 (%). Figure 8b shows the distribution of expression level of integrin  $\beta 1$  subunit in the control group.

Figure 9: The expression of integrin  $\beta 2$  subunit in control group of health population. Figure 9a shows the expression average value and baseline range of integrin  $\beta 2$  subunit in the control group, wherein the expression median is 88.6 (%), and the baseline range is 71.1-95.8 (%). Figure 9b shows the distribution of expression level of integrin  $\beta 2$  subunit in the control group.

Figure 10: The expression of integrin  $\beta 3$  subunit in control group of health population. Figure 10a shows the expression average value and baseline range of integrin  $\beta 3$  subunit in the control group, wherein the average value is  $9.65 \pm 2.53$  (%), and the baseline range is 4.59-14.71 (%). Figure 10b shows the distribution of expression level of integrin  $\beta 3$  subunit in the control group.

## Detailed Description of the Invention

**[0020]** Unless otherwise indicated, all the technical and scientific terms will have the common meanings known in the art. All the patents, patent applications, publications, sequences, and other disclosed material are incorporated herein as reference, unless otherwise indicated.

**[0021]** In the present invention, it was discovered that, integrins containing certain  $\beta$  subunits play important roles in VTE thrombogenesis, and are core proteins in venous thrombus protein network. Wherein said integrins include integrins of  $\beta 1$ -3 subfamilies. Based on such a discovery, the present invention provides a novel method for diagnosing or treating venous thromboembolism, comprising detecting or adjusting the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject. Furthermore, the present invention also relates to a substance that can specifically bind to, detect, or adjust integrin  $\beta$  subunit, and use of said substance in the preparation of a kit for diagnosing VTE.

**[0022]** The term "thrombus" refers to a blood clot formed in blood vessel, which obstructs or blocks blood flow. When blood vessel is injured, platelets and some proteins (such as fibrin, fibrinogen, serum proteins, and cytoskeletal proteins, etc.) in blood will aggregate and form a blood clot to repair the injured site. If the clot falls off, it may become a thrombus.

**[0023]** The term "venous thromboembolism" is a general term referring to thrombus formed in veins, comprising pulmonary arterial thromboembolism (PE) and deep venous thrombosis (DVT), wherein PE comprises acute pulmonary embolism (APE), chronic thromboembolic pulmonary hypertension (CTEPH). Deep venous thrombosis comprises acute and chronic deep venous thrombosis.

**[0024]** The term "integrin" refers to a class of cell surface receptor proteins. Such class of proteins are heterodimers, which contain two different chains, called  $\alpha$  subunit and  $\beta$  subunit respectively. Integrins containing the same  $\beta$  subunit constitute integrin  $\beta$  subfamilies, e.g.  $\beta 1$  subfamily containing integrin  $\beta 1$  subunit (also called CD29),  $\beta 2$  subfamily containing integrin  $\beta 2$  subunit (also called CD 18), and  $\beta 3$  subfamily containing integrin  $\beta 3$  subunit (also called CD61). Said  $\alpha$  subunit and  $\beta$  subunit encompass variants formed by differential splicing, e.g. 4 variants of ITGB1.

**[0025]** As used herein, "differential splicing" refers to a process in which different mRNA splicing isoforms are generated through different splicing manners (i.e. combining exons through different splicing sites) from the same mRNA precursor. The protein products obtained through differential splicing are isoforms each other, which can present different functional and structural properties, or lead to different phenotypes due to different expression levels in the same cell.

**[0026]** The term "subject" refers to a mammal subject, preferably human.

**[0027]** In one aspect, the present invention provides a novel method for diagnosing venous thromboembolism.

**[0028]** In one embodiment, the method of the present invention comprises: detecting the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject, and comparing the detection results of said level with corresponding level measured in control blood sample of a health subject, wherein the increase of said level is an indication for diagnosing VTE.

**[0029]** Quantitative detection for a protein in blood sample is conventional technique in the art. A person skilled in the art can select such technique based on specific requirements. For example, relevant antibody-based methods such as flow cytometry and ELISA, etc. can be used.

**[0030]** The term "blood sample" refers to blood tissue sample taken from the circulatory system of a subject, preferably

blood sample taken from peripheral veins. The blood sample of the present invention can contain haemocytes and extracellular fluid (i.e. blood plasma).

**[0031]** "Increased level" as used herein can be determined using the following process, i.e. determining the range of normal level in a health population comprising a certain number of health subjects, then if the level of a subject to be tested is higher than said range of normal level, the level of the subject to be tested is considered as being increased.

**[0032]** The range of normal level in health population can be determined using statistical methods. For example, Kolmogorov-Smirnov test can be used for a normality test, with  $P > 0.05$  as normal distribution. As for normal distribution data, the average value measured in health population combined with the standard deviation thereof ( $\bar{x} \pm S$ ) is used to determine the normal level range. As for abnormal distribution data, the median combined with the P2.5-P97.5 in statistics is used to denote the normal level range.

**[0033]** In the present invention, "increased level", for example, means that the measured level increases by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or more compared with the reference level in a blood sample of a health subject.

**[0034]** "Health subject" refers to subject population who are normal in physical examinations. "Reference level" refers to the levels of integrin-related proteins measured in health subject population (statistical average is then obtained and combined with standard deviation to be used as reference level). The health subject population for sampling as control can comprise e.g. 50 subjects, 100 subjects, 150 subjects, 200 subjects, 250 subjects, 300 subjects, 500 subjects, or even more.

**[0035]** The steps of the method of the present invention comprise: taking blood sample such as peripheral venous blood sample from a subject to be tested; then quantitatively detecting the amount of various integrin  $\beta$  subunits in said blood sample by using substance(s) (e.g. an antibody) that specifically binds to various integrin  $\beta$  subunits, wherein the method for quantitative detection can be, for example, conventional techniques like flow cytometry, and ELISA, etc.; taking peripheral blood samples from several (e.g. 200 or more) health subjects for detecting the amounts of various integrin  $\beta$  subunits therein by using the same method as mentioned above, and calculating the statistical average value and combining it with standard deviation as reference level. The amounts of various integrin  $\beta$  subunits in the subject to be tested are compared with corresponding reference levels, wherein the increased levels of various integrin  $\beta$  subunits is an indication for diagnosing VTE.

**[0036]** In one embodiment, the method of the present invention comprises detecting the levels of at least two subunits of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject. In one embodiment, the method of the present invention comprises detecting the levels of at least two subunits of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject. In another embodiment, the method of the present invention comprises detecting the levels of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and integrin  $\beta 3$  subunit in a blood sample of a subject.

**[0037]** Said venous thromboembolism in the present invention can be acute pulmonary embolism (APE), chronic thromboembolic pulmonary hypertension (CTEPH), or deep venous thrombosis (DVT), preferably acute pulmonary embolism or deep venous thrombosis.

**[0038]** In one embodiment, the method of the present invention employs flow cytometry to detect the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit.

**[0039]** "Flow cytometry" is a conventional technique in the art, and the detailed operations thereof can be seen in relevant publications, textbooks, or instructions of manufactures, e.g. (Loken MR (1990). Immunofluorescence Techniques in Flow Cytometry and Sorting (2nd ed.). Wiley. pp. 341-53).

**[0040]** In another aspect, the present invention provides a substance for specifically detecting the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit. In one embodiment, the present invention provides a substance that specifically binds to integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit, wherein said substance is an antibody, a ligand, and/or an antagonist. In one embodiment, the antibody, ligand, and/or antagonist of the present invention is labeled. In one embodiment, the antibody, ligand, and/or antagonist of the present invention is fluorescently labeled. In one embodiment, the antibody, ligand, and/or antagonist of the present invention is labeled by phycoerythrin (PE).

**[0041]** Ligands for integrin  $\beta 1$  subunit include, for example, laminin (LM), collagen protein (COL), fibronectin (FN), vascular cell adhesion molecule-1 (VCAM-1), etc.. Ligands for integrin  $\beta 2$  subunit include, for example, fibrinogen (FB), intercellular adhesion molecule-1, intercellular adhesion molecule-2, intercellular adhesion molecule-3 (ICAM-1, ICAM-2, ICAM-3), factor X, ic3b, etc.. Ligands for integrin  $\beta 3$  subunit include, for example, FB, FN, vitronectin (VN), von Willebrand factor (vWF), thrombospondin (TSP), etc..

**[0042]** In another aspect, the present invention relates to use of a substance, which specifically binds to integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit, in the preparation of a diagnostic agent for diagnosing venous thromboembolism by detecting the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject.

**[0043]** In one embodiment, the present invention relates to use of a substance, which specifically binds to at least two

subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit respectively, in the preparation of a diagnostic agent for diagnosing venous thromboembolism.

**[0044]** In one embodiment, the present invention relates to use of at least two substances, which specifically bind to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit respectively, in the preparation

of a composition for diagnosing venous thromboembolism.

**[0045]** In one embodiment, the substances that specifically bind to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit refer to at least two different substances, wherein one of the substances specifically binds to one of  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit, and the other one specifically binds to another of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit.

**[0046]** In one embodiment, the present invention relates to use of substances, which specifically binds to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit respectively, in the preparation of a composition for diagnosing venous thromboembolism.

**[0047]** In one embodiment, the substances that specifically bind to  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit respectively refer to at least three different substances, wherein the first substance specifically binds to one of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit, the second substance specifically binds to the second of  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit, and the third substance specifically binds to the third of  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit.

**[0048]** In one embodiment, the substance, that specifically binds to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit, and thus can be used to specifically detect the level of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit in a blood sample, is an antibody, a ligand, and/or an antagonist, preferably a labeled antibody, ligand, and/or antagonist. In one embodiment, the antibody, ligand, and/or antagonist of the invention is fluorescently labeled, e.g. labeled by phycoerythrin (PE).

**[0049]** In another aspect, the present invention provides a kit for diagnosing venous thromboembolism, which comprises a substance that specifically detects the level of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit, wherein the substance can specifically bind to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit.

**[0050]** In one embodiment, the kit of the present invention comprises a substance that specifically binds to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit.

**[0051]** In one embodiment, the kit of the present invention comprises the substances that respectively specifically bind to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit.

**[0052]** In one embodiment, the kit of the present invention comprises substances that specifically bind to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit, respectively.

**[0053]** In another aspect, the present invention also relates to the ligand, antibody, antagonist and/or activity inhibitor of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit.

**[0054]** The terms "ligand", "antibody", "antagonist", "activity inhibitor", etc. of the invention are commonly used terms in the art, and the meanings thereof are in accordance with the general understanding of a person skilled in the art, and the definitions thereof can also be referenced to commonly used textbooks and manuals.

**[0055]** In the present invention, the substances that respectively specifically bind to at least two subunits of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and/or integrin  $\beta$ 3 subunit refer to at least two different substances, wherein one of the substances specifically binds to one of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit, while the other specifically binds to another one of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit. The substances that respectively specifically bind to integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, and integrin  $\beta$ 3 subunit refer to at least three different substances, wherein the first substance specifically binds to one of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit, the second substance specifically binds to the second of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit, while the third substance specifically binds to the third of integrin  $\beta$ 1 subunit, integrin  $\beta$ 2 subunit, or integrin  $\beta$ 3 subunit.

**[0056]** In another aspect, the present invention also relates to a device for performing diagnosis using the method of the present invention, e.g., a device that can performs clinical rapid diagnosis.

#### Examples

**[0057]** The present invention will be described in details through the following Examples. These Examples are only used for illustrative purposes, and are not intended to limit the scope of the present invention. The present invention specifically contains the following Examples:

#### Example 1: Taking acute VTE thrombus samples by catheter:

**[0058]** By femoral venous puncture, a 7F right coronary catheter (Metronic, USA) was used to respectively take 3-5 red thrombus (5-15mm in length, 10g) from the pulmonary artery of a 39-year old male patient suffering from acute PE

(APE) and from the acute lower limbs femoral vein of a 50-year old male (ADVT) patient, and the obtained fresh thrombus were used for the tandem protein mass spectrometry analysis in the following Example.

Example 2: Tandem protein mass spectrometry and data analysis:

1. Methodology:

**[0059]** The flow charts for tandem protein mass spectrometry (MS/MS) detection and data analysis methods:

Acute PE, DVT thrombus - MS/MS (Model LTQ, Thermo Finnigan, USA) - Database - Protein retrieve - Corresponding genes - GO (Gene Oncology) analysis - Genes showing differences - Proteins showing differences - KEGG Pathway analysis - Gene Network analysis - Identifying the core proteins in the network.

1.1 For thrombus protein shotgun experiment, the obtained peptide fragments data were subject to bioinformatics analysis, so as to precisely identify the proteins that the peptide fragment corresponding to, and said proteins were also analyzed for their functions and biological pathways.

1.2 GO analysis:

GO database comprises three types of function information: the biological processes that the genes involve in, the cellular location of the genes, and the molecular functions the genes exert. Differentially expressed proteins were used as the input for GO analysis. Genes showing differences were respectively mapped towards various nodes of GO database. The gene number at each node was calculated.

1.3 KEGG Pathway analysis method:

Differentially expressed proteins were subject to KEGG pathway analysis.

1.4 Gene Network analysis method:

Three different types of interaction relationships were integrated: (1) The relationships among genes in KEGG database (protein interactions, gene regulations, protein modifications, etc.); (2) The existing high throughput experiments; (3) The interactions between genes mentioned in the existing literatures.

**[0060]** Through the KEGGSOAP software package (<http://www.bioconductor.org/packages/2.4/bioc/html/KEGGSOAP.html>) under R (<http://www.r-project.org/>), the signal pathway data in the KEGG database were analyzed, so as to identify the interactions between genes in the genome, including three types of relationships:

ECrel Enzyme-enzyme relationship, indicating two enzymes that catalyze successive reaction steps

PPrel Protein-protein interaction, such as binding and modification

GErel Gene expression interaction, indicating the relationship between transcriptional factors and target gene products

**[0061]** Data for protein-protein interaction can be obtained from MIPS data (<http://mips.helmholtz-muenchen.de/proj/ppi/>). The co-citation algorithm was adopted, specifically: PubMed Database publication abstracts were downloaded, each abstract was decomposed to sentences, and then the common gene name that exists in each sentence, i.e. the co-cited gene, was analyzed. For each pair of co-cited gene pair, the appearing frequency thereof was analyzed. The higher the appearing frequency of a gene pair, the higher the possibility that there exists interactions between said gene pair. The total number of PubMed Database publications was recorded as  $N$ , the respective independent appearing frequencies of the two genes of a gene pair in PubMed publication Database were recorded as  $m$  and  $n$ , respectively. The actual co-appearing number of the gene pair was  $k$ . Based on hypergeometric distribution, the possibility that more than  $k$  of co-citation can appear under completely random conditions was calculated:

$$p = 1 - \sum_{i=0}^{k-1} p(i | n, m, N)$$

wherein

$$p(i | n, m, N) = \frac{n!(N-n)!m!(N-m)!}{(n-i)!i!(n-m)!(N-n-m+i)!N!}$$

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**[0062]** Finally, based on the data results, they were integrated into an interaction network among genes. The network was graphically displayed using medusa software.

## 2. Mass Spectrometry and data analysis:

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The APE, ADVT thrombus samples obtained in Example 1 were respectively analyzed by mass spectrometry, with the Mass spectrometer model LTQ (Thermo Finnigan USA).

### 2.1 LC-MS/MS

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**[0063]** Ettan™ MDLC system (GE Healthcare) was used for the desalting and isolation of trypsinized peptides mixture. In this system, the sample were desalted on RP trap column (Zorbax 300 SB C18, Agilent Technologies), and then isolated on RP column (150 μm i.d., 100 mm in length, Column Technology Inc., Fremont, CA). Mobile phase A (0.1% formic acid in HPLC-grade water) and mobile phase B (0.1% formic acid in acetonitrile) were used. 20μg of the trypsinized peptides mixture was loaded onto the column, and the isolation was performed for 120 min by using 4-50% of B linear gradient at a flow rate of 2μL/min. Finnigan™ LTQ™ linear ion trap MS equipped with Electrospray interface (Thermo Electron) was connected to said LC equipment for detecting the diluted peptides. Data-dependent MS/MS spectrums were obtained. Each scanning cycle was composed by the following: one complete Profile mode MS scanning, followed by 5 Centroid mode MS/MS scanning, wherein the Dynamic Exclusion™ was set as following: repeat counting = 2, repeat time = 30 s, exclusion time = 90 s. Each sample was analyzed in triplicate.

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### 2.2 Data analysis

**[0064]** Bioworks Browser rev. 3.1(Thermo Electron, San Jose, CA.) was used to perform automatic retrieving for MS/MS spectrums against non-redundant International Protein Index (IPI) human protein database (version 3.26, 67687 items). BuildSummary was used to extract protein identification results from SEQUEST output file (Dai J, Shieh CH, Sheng QH, Zhou H, Zeng R: Proteomic analysis with integrated multiple dimensional liquid chromatography/mass spectrometry based on elution of ion exchange column using pH steps. Anal Chem).

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**[0065]** The peptides were restricted as trypsinized peptide forms, and allowed to have up to two missing gaps. Carbamoyl methylation of Cysteine was considered as a fixed modification, while the oxidation of methionine residue was considered as a variable modification. The allowed mass tolerances were respectively as: precursor ion, 2.0 Da, while the fragment ion, 0.8 Da. Protein identification criteria was based on Delta CN (≥0.1) and cross-correlation score (Xcorr, one electric charge ≥ 1.9, two electric charges ≥ 2.2, three electric charges ≥ 3.75).

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## 3. Results and Analysis:

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**[0066]** The results were sent to Shanghai Sensichip Infotech Co. Ltd. (Shanghai, China) to perform the following bioinformatics analysis: (the preparation of the samples from the APE patient of Example 1, and the protein sample shotgun pre-isolation results of protein samples can be seen in figure 1, and the sequence information of the enzyme-digested peptide fragments and debris can be seen in figure 2) based on database - protein retrieve - identifying corresponding genes - performing Gene Oncology (GO) analysis - identifying genes showing differences - identifying proteins showing differences - performing signal pathway analysis of KEGG Pathway - obtaining protein interaction network - identifying the core proteins in the network.

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### Results:

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#### **[0067]**

(1) GO analysis method: differentially expressed proteins were used as the input for GO analysis. Genes showing differences were respectively mapped towards various nodes of gene ontology database. The gene number at each node was calculated. The statistic results were as follows:

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Table 1: GO analysis results of APE thrombus samples (histogram was shown in Figure 3)

Function category	Protein counts	Richness level p-value
Cell cycle and proliferation	20	0.002718086
Stress response	40	4.62E-11
Transport	52	3.74E-07
RNA Metabolism	14	0.999999301
DNA Metabolism	4	0.925590145
Protein Metabolism	17	0.394000101
Other metabolism processes	23	0.000119964
Development processes	6	0.183137052
Cell structure and biogenesis	82	9.92E-13
Cell-cell signaling	17	0.686695527
Signal transduction	45	0.998147085
Cell adhesion	8	2.58E-08
Death	9	0.353211866
Other biological processes	99	0.984977572
Unknown	32	0.873823656

Table 2: GO analysis results of AVDT thrombus samples (histogram was shown in Figure 5)

Function category	Protein counts	Richness level p-value
Cell cycle and proliferation	6	0.091311
Stress response	28	7.98E-07
Transport	32	1.90E-05
RNA Metabolism	4	0.999605
Protein Metabolism	2	0.06044
Other metabolism processes	18	0.08507
Development processes	2	0.239812
Cell structure and biogenesis	43	1.21E-06
Cell-cell signaling	1	0.580049
Signal transduction	20	0.998072
Cell adhesion	5	0.000131
Death	9	0.46574
Other biological processes	27	0.794559

(2) KEGG signal pathway analysis: GenMAPP v2.1 was used to map the genes towards the KEGG Pathway database, and the richness levels (p-value) of the genes in each pathway were counted. As for the APE thrombus samples, 35 relevant signal pathways were found. As for the AVDT thrombus samples, 94 relevant signal pathways were found.

(3) Protein interaction network analysis: 3 different types of interaction relationships were combined together to obtain the protein interaction network, i.e. (a) the relationships of protein interactions, gene regulations, protein modifications, etc. in KEGG signal pathway analysis data; (b) protein-protein interactions proved by the existing high throughput experiments, such as yeast two-hybrid, etc.; (c) The interactions between genes and between proteins mentioned in existing publications.

[0068] The bioinformatics analysis results for APE thrombus samples can be seen in figures 3 and 4. The results indicated that  $\beta 2$ ,  $\beta 3$ , and  $\beta 1$  subunit integrins were core proteins of red thrombus.

[0069] The bioinformatics analysis results for AVDT thrombus samples can be seen in figures 5 and 6. The results indicated that  $\beta 3$  subunit integrins and ITGA2B (i.e. integrin  $\alpha$  chain IIb) were core proteins of red thrombus.

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Example 3: Tandem protein mass spectrometry analysis for thrombus of Acute VTE patients:

[0070]

10 1. Background information of the clinical tested subjects:

(1) the patient group consisted of 24 cases of VTE, who were diagnosed and confirmed to have VTE by imaging (CTA, MRA, lung ventilation/perfusion scanning, angiography, or lower limbs venous vascular ultrasound).

15 12 patients were male, 12 patients were female, and the ages were from 25 to 82, with the average age  $62 \pm 16$ . 8 patients were acute VTE (within one month after onset); 9 cases were subacute VTE (within 1-3 months after onset); 7 case were chronic VTE (more than 3 months after onset), wherein 4 cases were readmitted patients whose CTEPH clinical symptoms were aggravated. 8 cases of acute phase VTE admitted patients were all subcutaneously injected with low molecular heparin 5000 unit/Bid for 7-10 days, and orally administered with 2.5mG/Qd warfarin the first 3-4 days after stopping the low molecular heparin; 9 cases of admitted subacute phase VTE patients were subcutaneously injected with low molecular heparin 5000 unit/Qd for 7 days, and/or orally administered with 2.5mG/Qd warfarin; 4 cases of readmitted CTEPH patients were treated with anticoagulant therapy as subacute phase, other 3 cases of chronic phase VTE patients were on outpatient follow-up, and orally administered with 2.5mG/Qd warfarin for continuous treatment. During the detection process of the present investigation, none of the 24 cases VTE patients were found to have malignant tumor, autoimmune disease, or arterial thrombotic diseases.

25 (2) 270 cases of healthy persons were used as control group, wherein 140 cases were male (age 20-75), 49 cases were in 20-39 years old group, 48 cases were in 40-59 years old group, and 43 cases were in over 60 years old group; 130 cases were female (age 21-72), 46 cases were in 20-39 years old group, 44 cases were in 40-59 years old group, and 40 cases were in over 60 years old group.

30 The diagnosis for PE and DVT were all based on imaging (CT, MRA, ECT or angiography) proofs and clinical manifestations, wherein the criteria can be refer to (Torbicki A, Perrier A, Konstantinides S, et al. Guidelines on the diagnosis and management of acute pulmonary embolism: the Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC)[J]. Eur Heart J, 2008, 29(18): 2276-2315; and Qaseem A, Snow V, Barry P, et al. Current diagnosis of venous thromboembolism in primary care: a clinical practice guideline from the American Academy of Family Physicians and the American College of Physicians[J]. Ann Intern Med, 2007, 146(6): 454-458).

35 2. Integrin  $\beta$  molecular detection

40 (1) 2 ml of peripheral venous blood was collected from the subjects (VTE patients, or control), 2mg EDTA for anticoagulation and 20 $\mu$ l fluorescent antibodies were added into the test tubes; wherein the antibodies were respectively: antibodies against integrin  $\beta 1$  subunit (CD29) were PE-labeled, mouse-anti-human IgG2a,  $\kappa$ , clone No. HUTS-21, and the concentration is 50mg/L; antibodies against integrin  $\beta 2$  subunit (CD 18) were PE-labeled, mouse-anti-human IgG1,  $\kappa$ , clone No. 6.7, and the concentration is 50mg/L; antibodies against integrin  $\beta 3$  subunit (CD61) were PE-labeled, mouse-anti-human IgG1,  $\kappa$ , clone No. VI-PL2, and the concentration is 50mg/L; the fluorescent antibodies CD29-PE ( $\beta 1$ ), CD18-PE ( $\beta 2$ ), and CD61-PE ( $\beta 3$ ) can be obtained from BD (Becton, Dickinson and Company, USA);

(2) thoroughly mixing after adding into the samples and reacting in dark for 30 min at room temperature ;

45 (3) adding 500 $\mu$ l hemolysin into the test tubes; wherein the added hemolysin was used for dissolving erythrocytes, the model of which was OptiLyse C, and can be obtained from Beckman-Coulter, Inc., USA.

(4) water bath at 37°C for 30 min;

50 (5) adding 100 $\mu$ l buffer (0.01M PBS, PH7.2) after washing, and detecting by flow cytometer, wherein the flow cytometer (EPICS XL-4) from BECKMAN-COULTER, Inc. was used;

55 3. Operation procedure of cell immunization and integrin flow cytometry:

(1) In the experiment, 2mg EDTA was added into the test tubes for anti-coagulation, and same types of control tubes were set at the same time, wherein 20 $\mu$ l of mouse IgG1-PC5, IgG1-FITC, IgG1-PE were respectively

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added according to the different fluorescent markers (20 $\mu$ l of mouse IgG2-PE was added for CD29 molecular detection, 50mg/L). Subsequently, 20 $\mu$ l (50mg/L) of corresponding fluorescent antibodies were respectively added. After thoroughly mixing, the mixture was kept in dark for 30 min at room temperature. Then, 500 $\mu$ l hemolysin (a product of BECKMAN-COULTER, Inc., USA, model OptiLyse C) was added, and the reaction was performed in water bath at 37°C for 30 min. After being taken out and washed, 500 $\mu$ l of sheath reagent (obtained from Beckman-Coulter, Inc., USA, containing the active ingredients of sodium sulfate 9.84g/L, sodium chloride 4.07 g/L, procaine hydrochloride 0.11 g/L) was added to each tube, followed by flow cytometry (a product of Beckman-Coulter, Inc., USA, model EPICS XL-4).

(2) The BECKMAN-COULTER standard fluorescent microsphere was used to adjust the PMT voltage, fluorescence compensation, and sensitivity of the flow cytometry, so as to set up the detection protocol. 10000 cells were collected in each tube. The corresponding cell population in the scatter diagram of control tube of the same type was used to set the gate, and the percentage of positive cells (%) was determined according to the quadrant that the fluorescence corresponded to, and results were analyzed by the built-in SYSTEM-II software.

4. Statistical analysis: SPSS 18 statistical software was used for the statistical analysis of the control group. Kolmogorov-Smirnov test was used for normality test, with  $P > 0.05$  as normal distribution. The normal distribution data were represented as  $\bar{x} \pm S$ , variance analysis was used for the comparison among different age groups, and  $P < 0.05$  indicated a difference with statistical significance. Abnormal distribution data were represented by median and P2.5-P97.5, Wilcoxon rank-sum test was used for the comparison between different gender groups, and  $P < 0.05$  indicated a difference with statistical significance; Kruskal-Wallis H test was used for the comparison among different age groups, and  $P < 0.05$  indicated a difference with statistical significance.

As for normal distribution data, the average value measured in health population was combined with its standard deviation ( $\bar{x} \pm S$ ) to determine the range of normal level. As for abnormal distribution data, the median was combined with P2.5-P97.5 in statistics to indicate the range of normal level.

The  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  of VTE group were compared with those of the control group, and a value higher than the normal reference range was considered as increased expression.

### 5. Results:

The results were shown as the percentage of cells containing a corresponding integrin subunit (positive cell) in the total cell counting, (i.e., 10000 cells were used as a unit, the percentage of positive cells). Figures 7-10 and the following table 3 are shown. In the 270 cases of control group, integrin  $\beta_1$ , and  $\beta_3$  subunits showed a normal distribution, while integrin  $\beta_2$  subunit showed an abnormal distribution. There was no significant difference in the different genders, and with the increasing of age,  $\beta_1$  and  $\beta_3$  tend to decrease; the baseline region of integrin  $\beta_1$  subunit was 2.8-13.56%, of integrin  $\beta_2$  subunit was 71.1-95.8%; and of integrin  $\beta_3$  subunit was 4.59-14.71%.

Table 3: Information and detection results of VTE patients

No.	Gender	Age	Diagnosis	Phase	Diagnostic method	$\beta 1$ 2.8-13.56%	$\beta 2$ 70.1-95.8%	$\beta 3$ 4.59-14.71%	D-di <0.3mg/L	CRP <2.87mg/L	Fibrinogen 2-4.5g/L
1	male	82	lower limbs DVT	subacute phase	ultrasound	11.37	97.5 $\uparrow$	14.5	1.06 $\uparrow$	132 $\uparrow$	4.613 $\uparrow$
2	male	56	PE/lower limbs DVT	subacute phase	CTA/ ultrasound	6.81	76.3	15.9 $\uparrow$	0.47 $\uparrow$	20.8 $\uparrow$	4.761 $\uparrow$
3	male	33	PE	subacute phase	CTA	11.4	75	18.9 $\uparrow$	0.22	51.8 $\uparrow$	4.553 $\uparrow$
4	female	58	lower limbs DVT	chronic phase	ultrasound	21.6 $\uparrow$	66.8	8.63	0.05		2.863
5	female	66	CTEPH	chronic phase	CTA	12.8	96.4 $\uparrow$	12.9	0.05	0.3	3.25
6	female	54	lower limbs DVT	subacute phase	ultrasound	18.5 $\uparrow$	85	18.8 $\uparrow$	0.7 $\uparrow$	0.7	2.56
7	female	56	lower limbs DVT	chronic phase	ultrasound	10.1	94.7	161 $\uparrow$	0.05	0.7	3.65
8	male	68	CTEPH	chronic phase	CTA	7.27	98.2 $\uparrow$	12.3	0.14	24.7 $\uparrow$	3.825
9	female	25	PE	subacute phase	CTA	5.8	94.2	16.9 $\uparrow$	0.35 $\uparrow$	36 $\uparrow$	2.761 $\uparrow$
10	female	82	PE	subacute phase1	CTA	17.3 $\uparrow$	99.5 $\uparrow$	5.6	0.05	0.2	2.495
11	male	65	lower limbs DVT	subacute phase	ultrasound	14.5 $\uparrow$	96.8 $\uparrow$	9.85	0.05	1.7	3.04
12	female	61	PE	acute phase	CTA	4.5	96.4 $\uparrow$	17.3 $\uparrow$			2.495
13	male	71	PE	acute phase	PAG	14.3 $\uparrow$	95.6	13.1	1.48 $\uparrow$	16.4 $\uparrow$	3.761
14	female	81	PE	chronic phase	CTA	10.2	96.7 $\uparrow$	7.4	0.08		3.383
15	male	49	PE	acute phase	CTA	15.4 $\uparrow$	90.1	14.6			
16	female	53	PE	acute phase	lung ventilation/perfusion	13.1	97.2 $\uparrow$	6.4	1.25 $\uparrow$	0.6	3.576
17	male	71	PE	acute phase	CTA	17.52 $\uparrow$	98.2 $\uparrow$	19 $\uparrow$	0.24	0.7	2.714
18	male	80	CTEPH	chronic phase	ventilation/perfusion scanning	23.5 $\uparrow$	99.4 $\uparrow$	18.7 $\uparrow$	0.75 $\uparrow$	32 $\uparrow$	3.264

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(continued)

No.	Gender	Age	Diagnosis	Phase	Diagnostic method	β1 2.8-13.56%	β2 70.1-95.8%	β3 4.59-14.71%	D-di <0.3mg/L	CRP <2.87mg/L	Fibrinogen 2-4.5g/L
19	female	75	PE	acute phase	ventilation/perfusion scanning	6.27	94.7	15.4↑	0.64↑	2.6	2.11
20	female	78	PE	acute phase	ventilation/perfusion scanning	6.92	96.9↑	13.4	0.96↑	38↑	4.481
21	male	64	lower limbs DVT	subacute phase	ultrasound	14.5↑	96.8↑	9.85	0.05		3.04
22	female	80	CTEPH	chronic phase	CTA	17.3↑	99.5↑	5.6	0.09	2	2.607
23	male	48	PE	acute phase	CTA	15.49↑	90.1	14.6	0.89↑	4↑	2.00
24	male	32	PE	subacute phase	CTA	11.4	75	18.9↑	0.25	92↑	4.533↑
Total:						62±16	12.83±5.07	91.96±9.28	13.52±4.94		

Note: 4 cases of CTEPH patients, with echocardiography systolic pressure between 65-74 mmHg; No. 4, 7, and 14 are follow-up patients.

**[0071]** The integrin  $\beta$  subunits of the 24 cases of the acute phase, subacute phase, and chronic phase VTE patients in the present investigation, though received different anti-coagulant protocol and different anti-coagulant time period, were all individually or jointly expressed in a level higher than the upper limit of the range in health population, wherein 15/24 cases (62.5%) of VTE patients had individually increased expression of integrin  $\beta$  subunit, 11/24 cases (45.8%) had increased integrin  $\beta 1$  subunit, wherein acute phase 4/11 cases, subacute phase 4/11 cases, and chronic phase 3/11 cases; 13/24 cases (54.2%) had increased integrin  $\beta 2$  subunit, wherein acute phase 4/13 cases, subacute phase 4/13 cases, and chronic phase 5/13 cases; 10/24 cases (41.7%) had increased integrin  $\beta 3$  subunit, wherein acute phase 3/10 cases, subacute phase 6/10 cases, and chronic phase 1/10 cases. 9/24 cases had two or more jointly increased integrin  $\beta$  subunits (37.5%), wherein  $\beta 1$ ,  $\beta 2$  and  $\beta 3$  jointly increased in 3/24 cases (12.5%),  $\beta 1$  and  $\beta 2$  jointly increased in 6/24 cases (25%),  $\beta 1$  and  $\beta 3$  jointly increased in 1/24 case (4.2%), and  $\beta 2$  and  $\beta 3$  jointly increased in 1/24 case (4.2%). The above cellular detection results indicated that, the higher expression of integrin  $\beta$  subfamily in VTE patients can be used to diagnose VTE.

**[0072]** The results in above Examples indicated that, the expressions of integrin  $\beta 1$ ,  $\beta 2$ , and  $\beta 3$  subunits of blood cells in health population can be detected, wherein the expressions of  $\beta 1$  and  $\beta 3$  subunits showed a normal distribution, while the expression of  $\beta 2$  subunit showed an abnormal distribution. There was no statistical difference between genders, and with the increasing of age, the expressions of  $\beta 1$  and  $\beta 3$  subunits tend to decrease. For the 8 cases of acute phase, 9 cases of subacute phase, and 7 cases of chronic phase VTE patients of the present invention, integrin  $\beta 1$ ,  $\beta 2$ , and  $\beta 3$  subunits individually or jointly increased in the expression, all higher than the upper limit of the reference range in control group, complying with the results of the thrombus tandem protein mass spectrometry analysis. The expression of integrin  $\beta 1$ ,  $\beta 2$ , and/or  $\beta 3$  subunits in the VTE groups of the invention individually or jointly increased, indicating that the activation and signal transduction effects of integrin were increased; the higher expression of VTE patients' integrin  $\beta 1$ ,  $\beta 2$ , and  $\beta 3$  subunits during the continuous anti-coagulant treatment indicated the stability, sensitivity, and specificity thereof, and also indicated that the patients were still in a thrombophilia state.

## Claims

1. Use of a substance specifically binding to integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in the preparation of a diagnostic agent for diagnosing venous thromboembolism (VTE) by detecting the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject such as peripheral venous blood sample.
2. The use of claim 1, wherein said diagnostic agent comprises at least one substance that respectively specifically binds to at least one subunit of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit, wherein said diagnostic agent preferably comprises substances that specifically bind to integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and integrin  $\beta 3$  subunit, respectively.
3. The use of claim 1, wherein said venous thromboembolism is acute pulmonary embolism (APE), chronic thromboembolic pulmonary hypertension (CTEPH), or deep venous thrombosis (DVT), preferably acute pulmonary embolism or acute deep venous thrombosis.
4. The use of any one of claim 1-3, wherein said substance is an antibody, a ligand, and/or an antagonist, preferably a labeled antibody, ligand, and/or antagonist.
5. The use of claim 4, wherein said antibody, ligand, and/or antagonist is fluorescently labeled, e.g. labeled by phycoerythrin (PE).
6. A kit for diagnosing venous thromboembolism (VTE) through detecting the level of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit in a blood sample of a subject such as peripheral venous blood sample, wherein said kit comprises a substance that specifically binds to integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit, respectively.
7. The kit of claim 6, which comprises at least two substances that specifically bind to at least one subunit of integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and/or integrin  $\beta 3$  subunit respectively, wherein said kit preferably comprises substances that specifically bind to integrin  $\beta 1$  subunit, integrin  $\beta 2$  subunit, and integrin  $\beta 3$  subunit, respectively.
8. The kit of claim 6, wherein said venous thromboembolism is acute pulmonary embolism (APE), chronic thromboembolic pulmonary hypertension (CTEPH), or deep venous thrombosis (DVT), preferably acute pulmonary embolism

or acute deep venous thrombosis.

9. The kit of any one of claim 6-8, wherein said substance is an antibody, a ligand, and/or an antagonist, preferably a labeled antibody, ligand, and/or antagonist.

5 10. The kit of claim 9, wherein said antibody, ligand, and/or antagonist is fluorescently labeled, e.g. labeled by phycoerythrin (PE).

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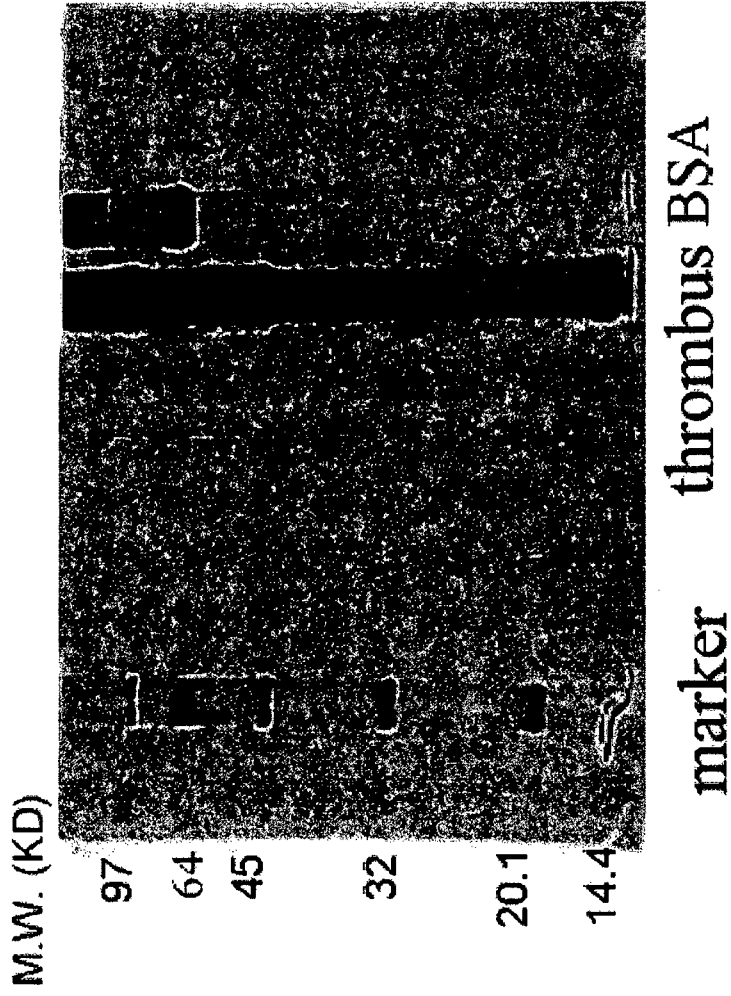
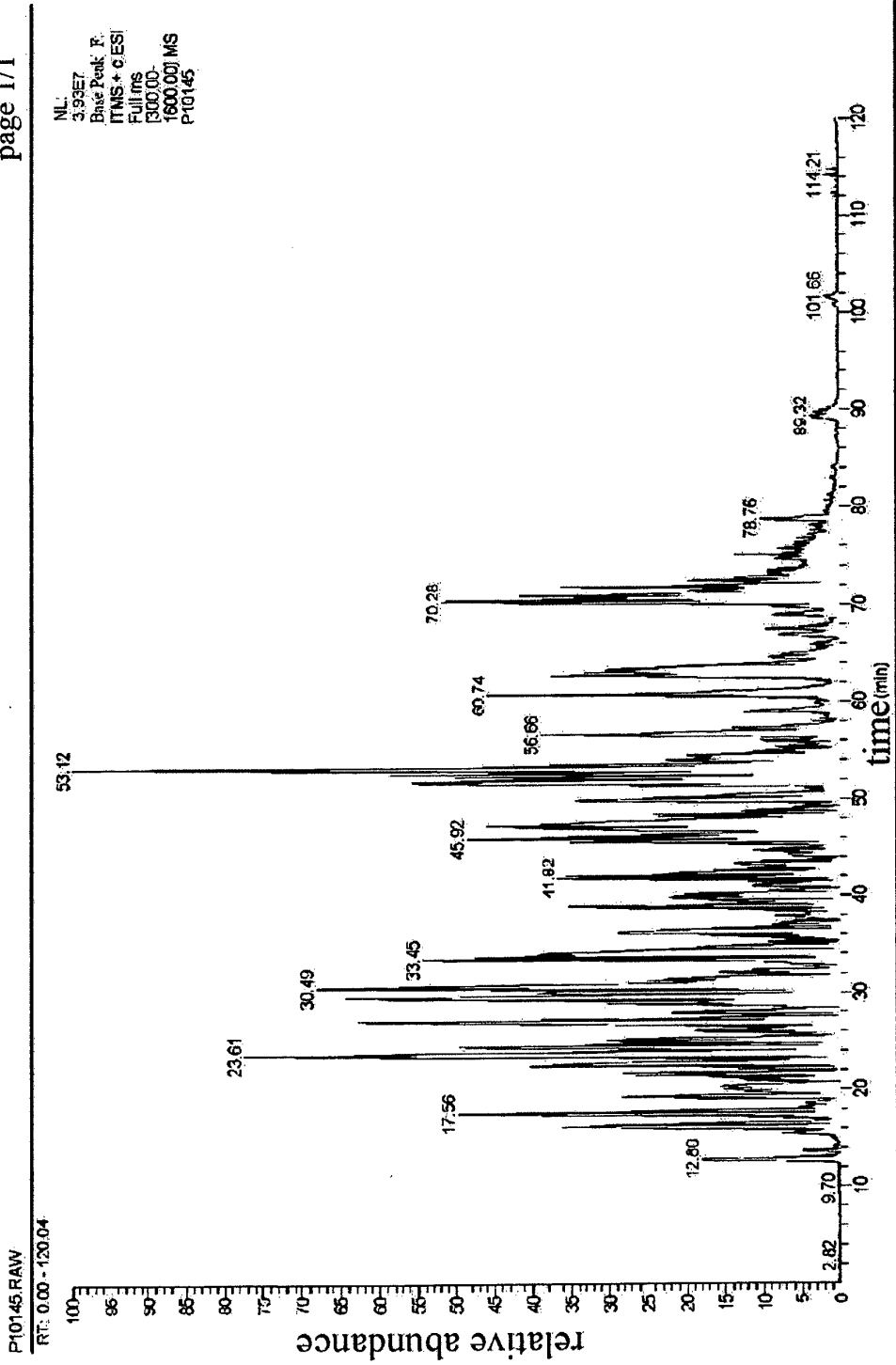


Fig. 1



ML:  
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Base Peak: F  
ITMS + CESI  
Full ms  
[300.00-  
1600.00] MS  
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P10145.RAW  
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BIOWORKFLOW rev. 3.3

Fig.2

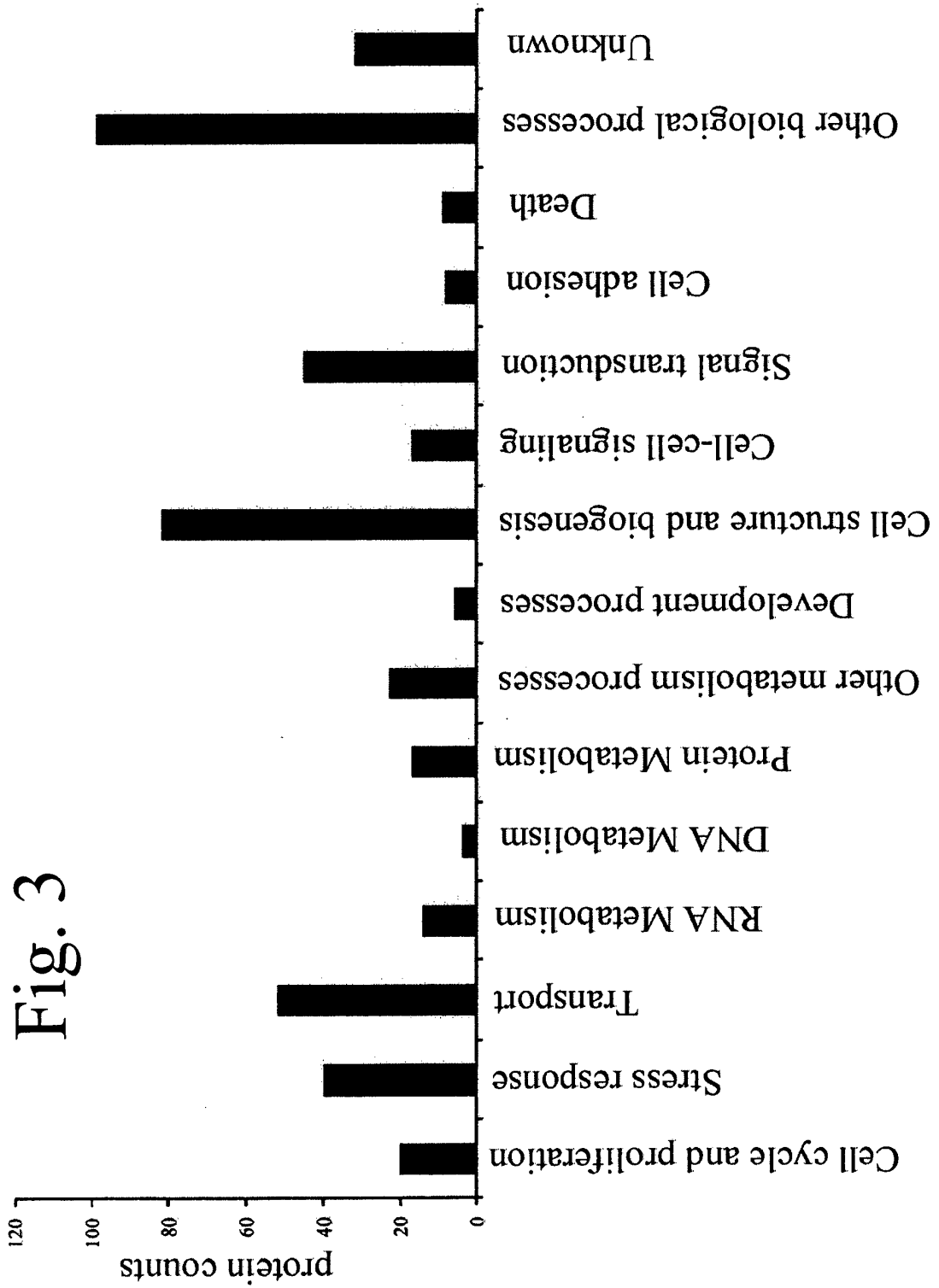
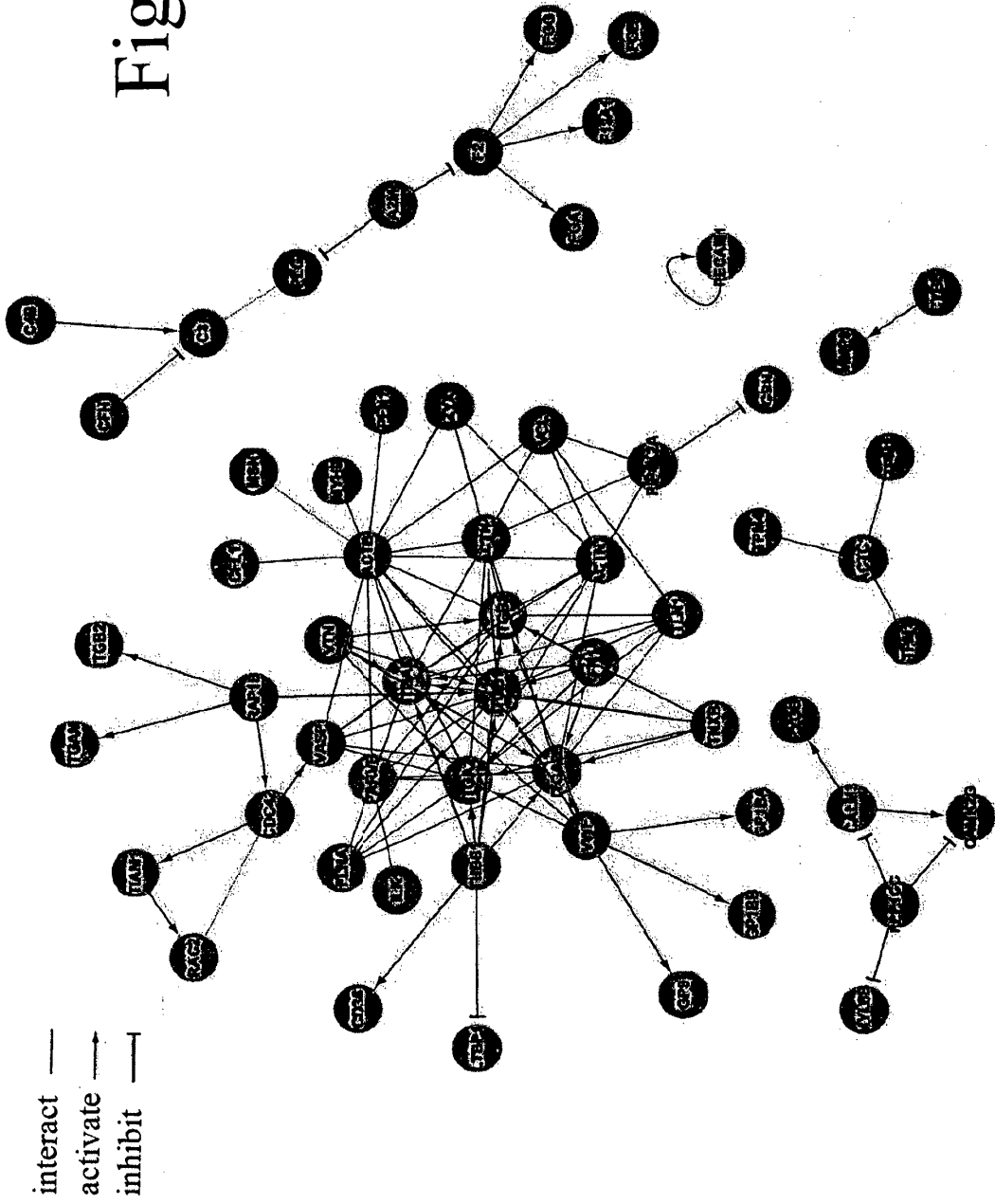


Fig. 3

Fig. 4



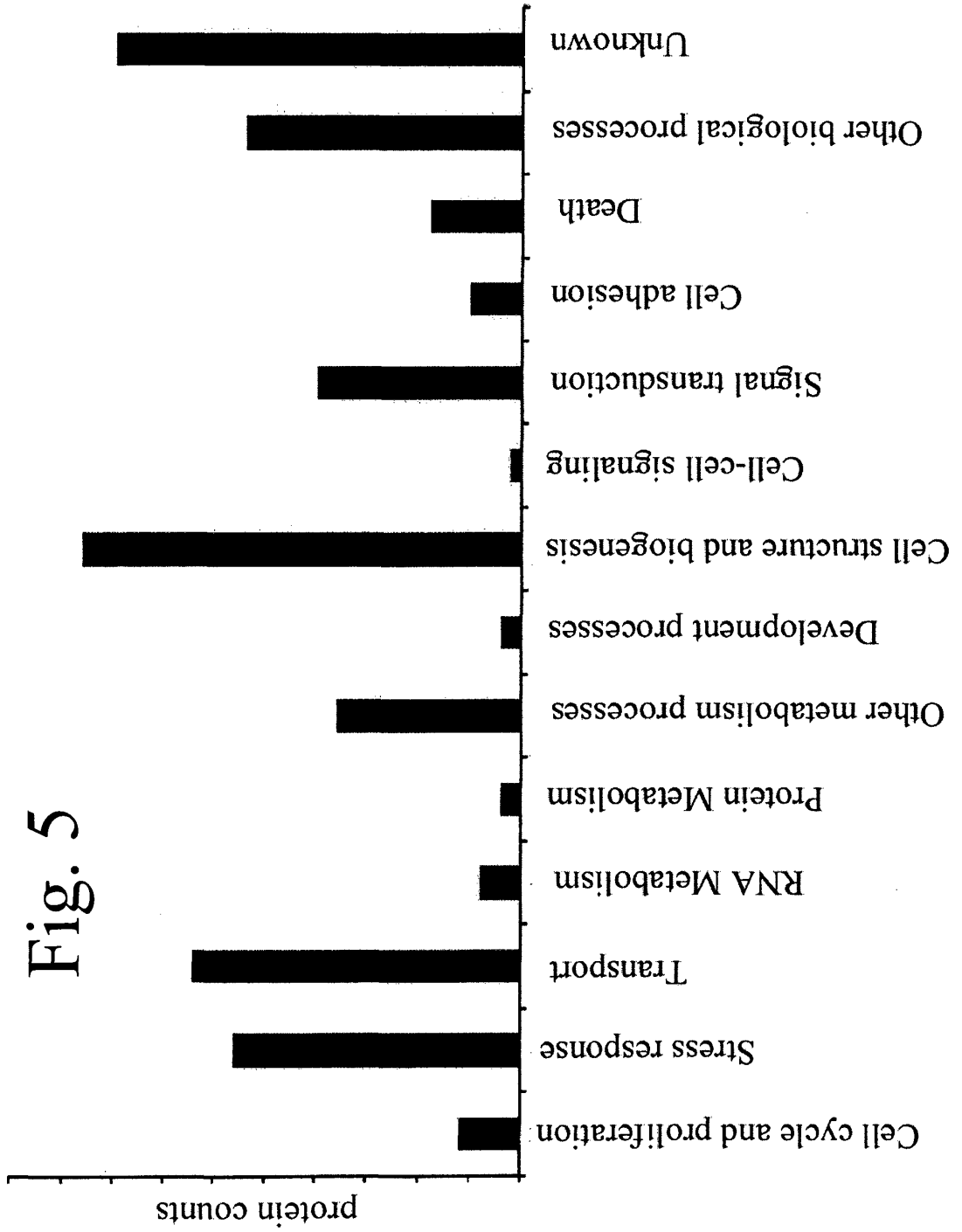


Fig. 5

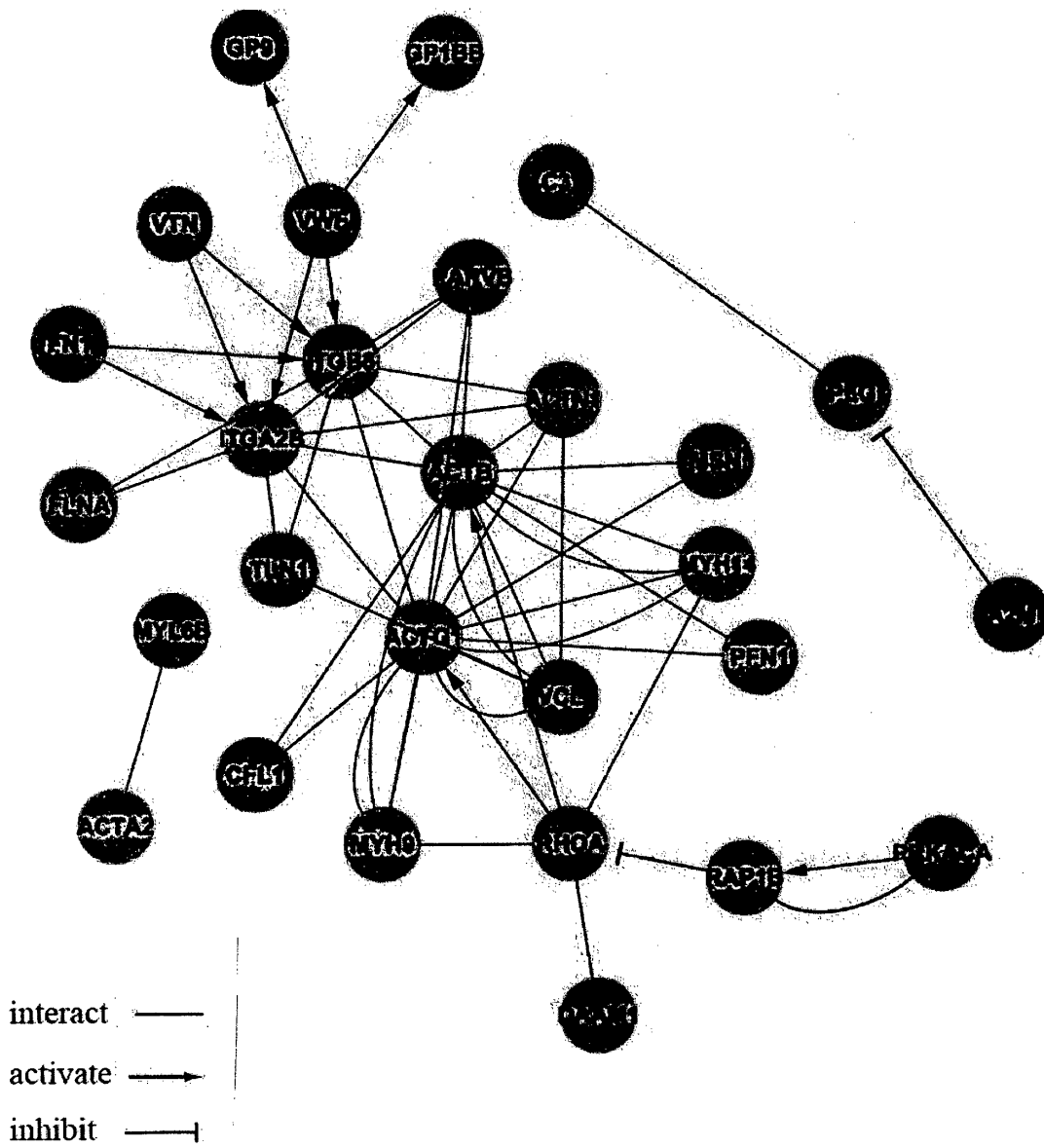
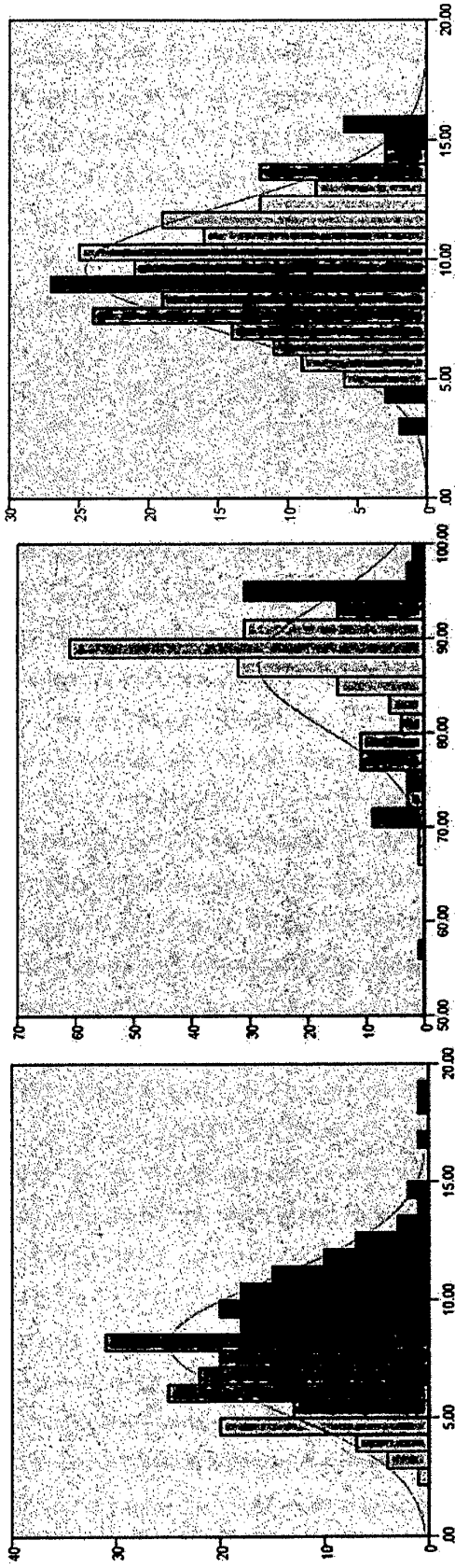


Fig. 6



c

b

a

Fig. 7

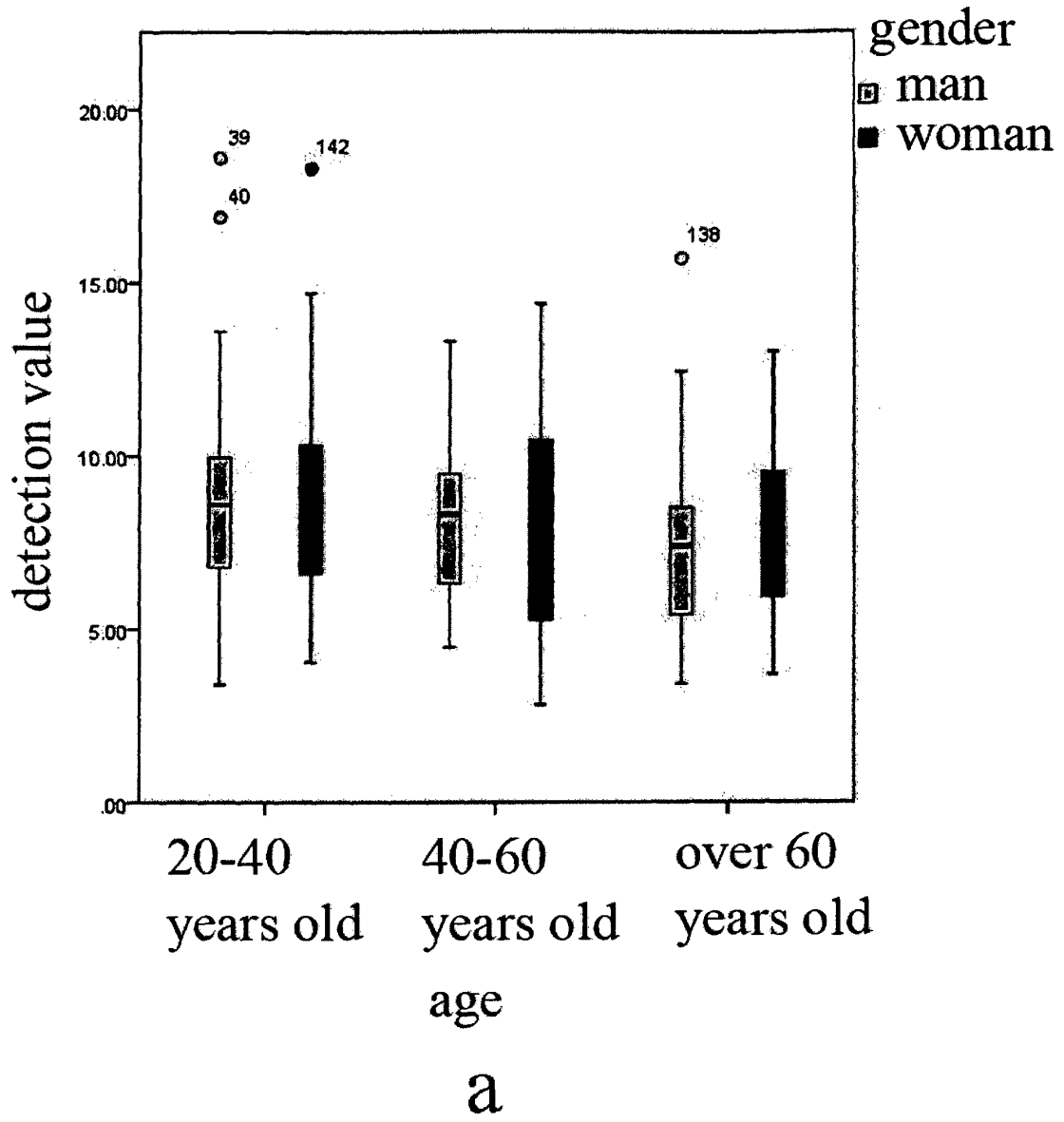
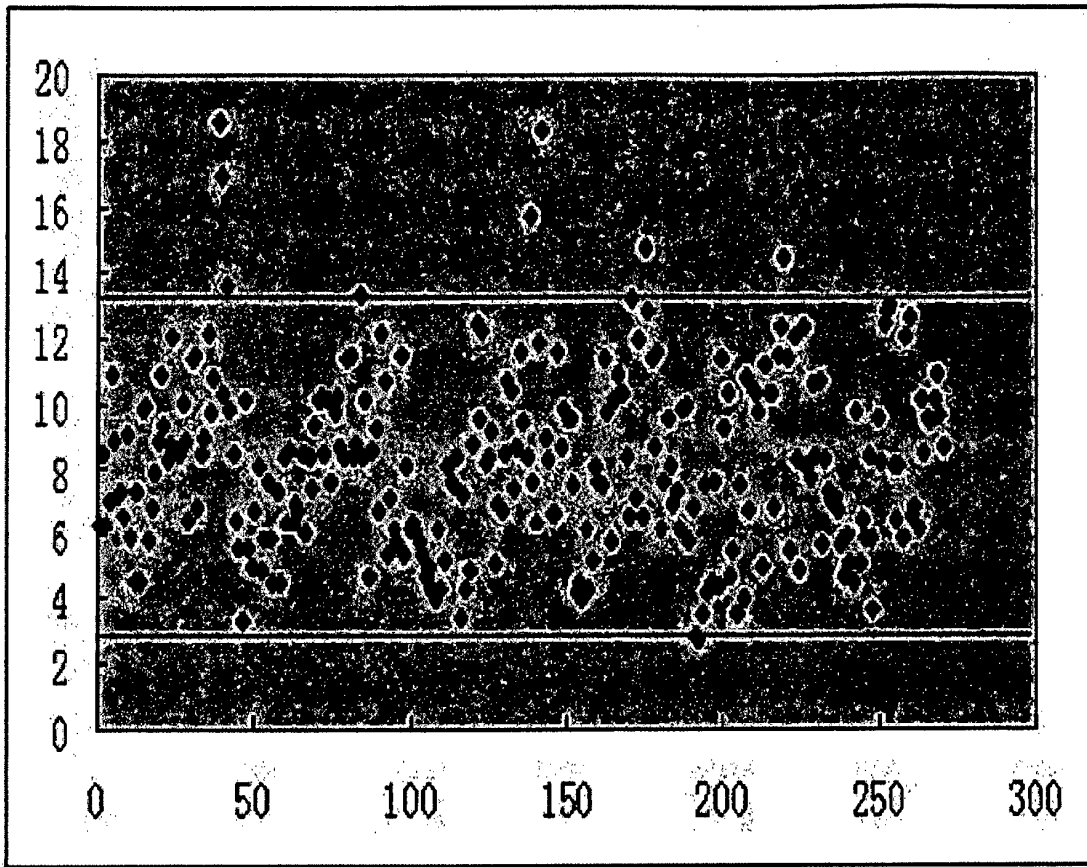
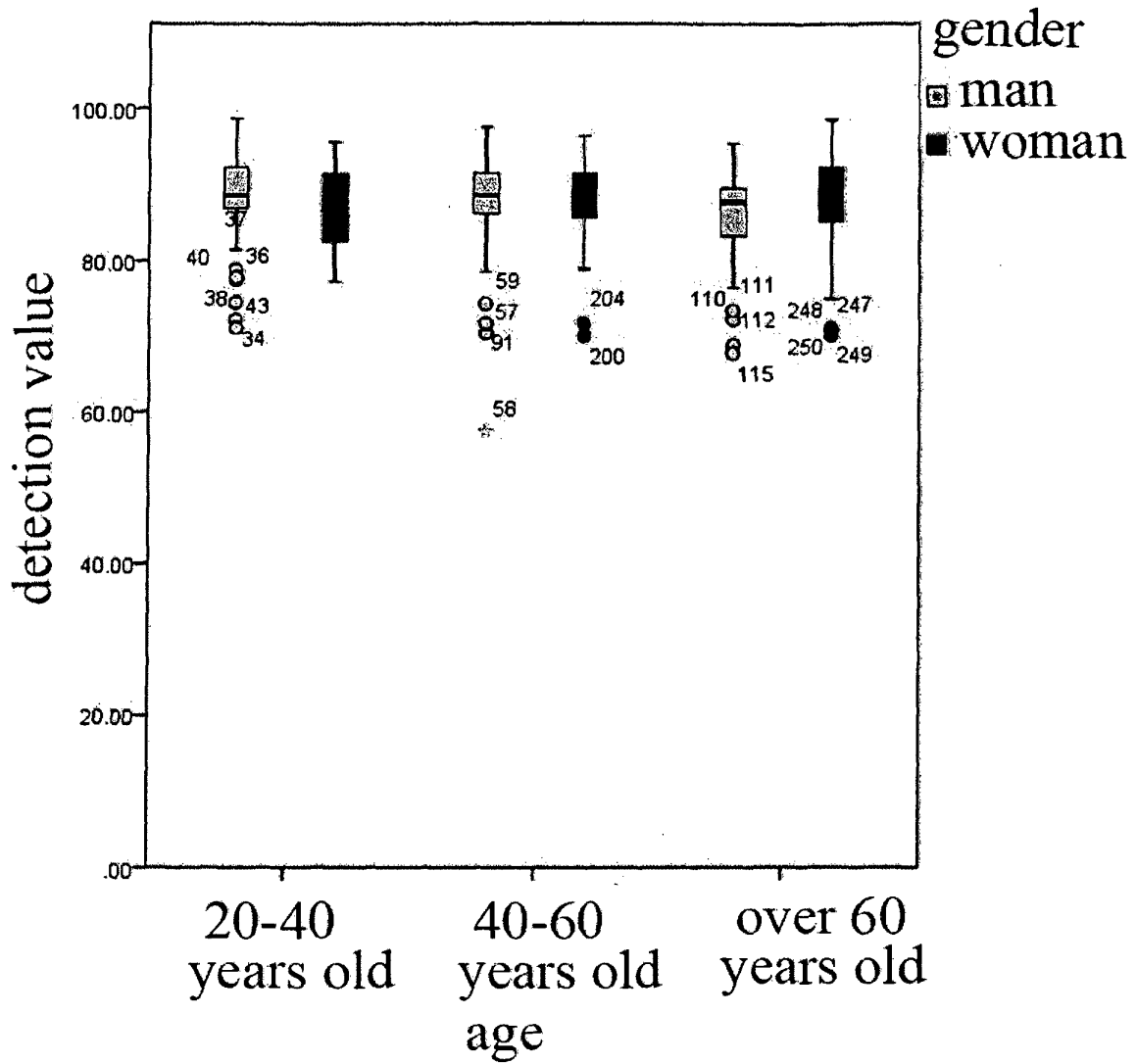


Fig. 8



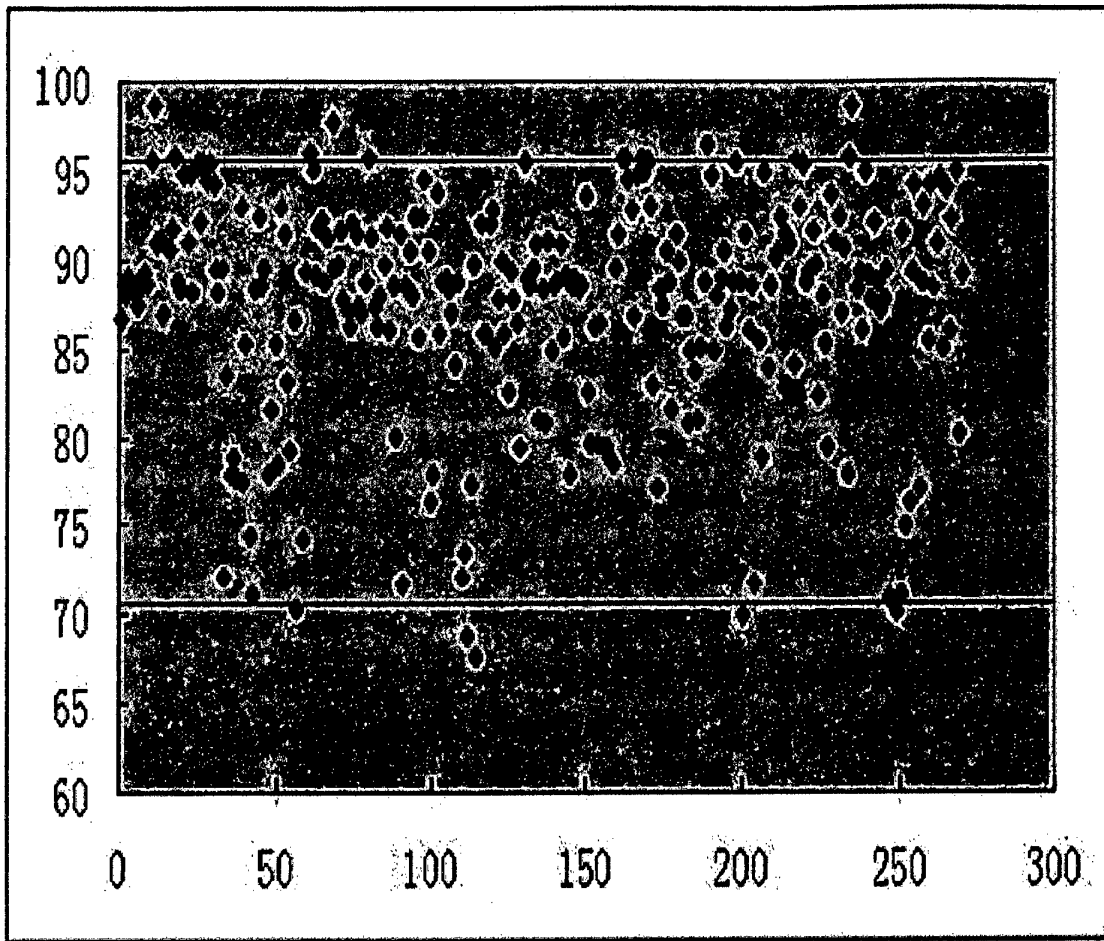
b

Fig. 8



a

Fig. 9



b

Fig. 9

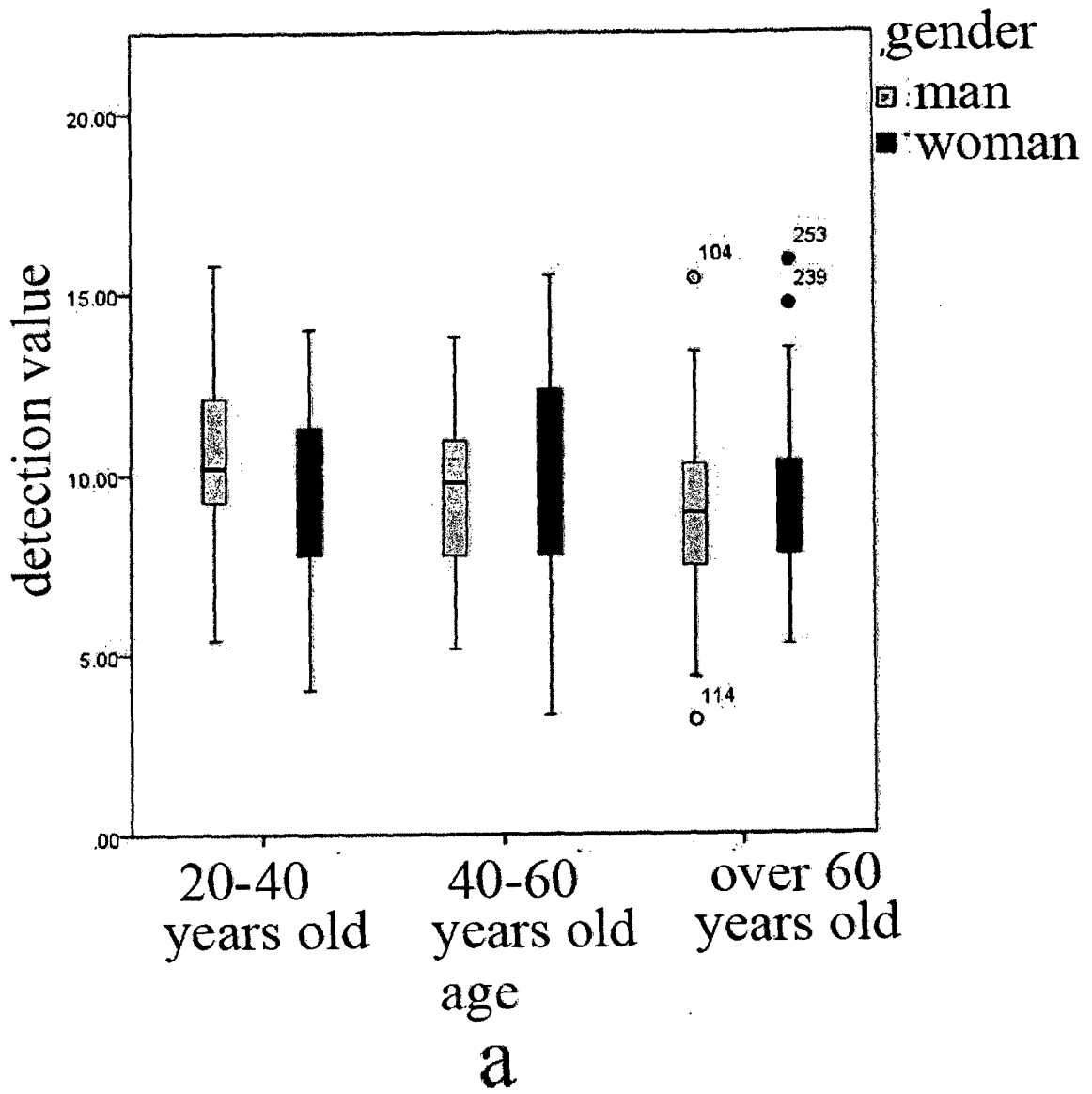
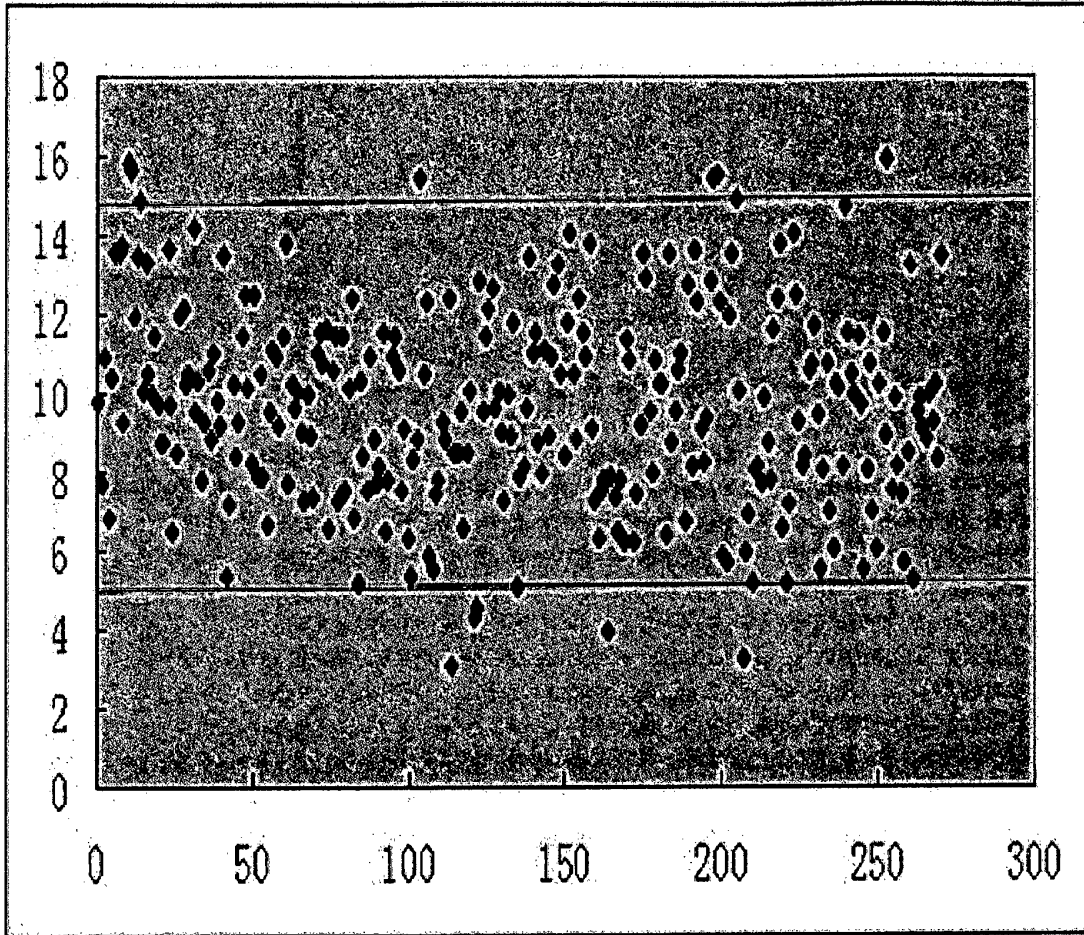


Fig. 10



b

Fig. 10

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CN2012/074284

## A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/533 (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)

IPC: G01N

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

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

DWPI, SIPOABS, CNABS, CPRSABS, CNKI:

integrin?, ITGB1, ITGB2, ITGB3, CD18, CD29, CD61, venous thromboembolism, VTE, deep venous thrombosis, DVT, acute pulmonary embolism, APE, chronic thromboembolic pulmonary hypertension, CTEPH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	XIE, Yuan et al., The significance of the differential expression of surface receptors mRNA of integrins in pulmonary thromboembolism patients, Chinese Journal of New Clinical Medicine, 2009, vol. 2, no. 12, pages 1227-1231.	1-10
Y	XUE, Shenwu et al., Nao Geng Sei Xue Xiao Ban Mo Ts, Lin Ba Xi Bao Mo Zheng He Su Han Liang Yu Lin Chuang, JOURNAL OF HEBEI MEDICAL UNIVERSITY, 2002, vol. 23, no. 2, pages 98-99.	1-10

 Further documents are listed in the continuation of Box C.
  See patent family annex.

* Special categories of cited documents:	“T” 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
“A” document defining the general state of the art which is not considered to be of particular relevance	“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
“E” earlier application or patent but published on or after the international filing date	“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
“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)	“&” document member of the same patent family
“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	

Date of the actual completion of the international search 15 January 2013 (15.01.2013)	Date of mailing of the international search report 07 February 2013 (07.02.2013)
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Name and mailing address of the ISA State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No. (86-10) 62019451	Authorized officer LU, Chun Telephone No. (86-10) 62411048
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## REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	整合素β亚基在静脉血栓栓塞诊断中的应用		
公开(公告)号	<a href="#">EP2840396A1</a>	公开(公告)日	2015-02-25
申请号	EP2012874796	申请日	2012-04-18
[标]申请(专利权)人(译)	王乐民		
申请(专利权)人(译)	王, 黎乐民		
当前申请(专利权)人(译)	王, 黎乐民		
[标]发明人	WANG LEMIN		
发明人	WANG, LEMIN		
IPC分类号	G01N33/533 G01N33/68		
CPC分类号	G01N33/6872 C07K16/2842 C07K16/2845 C07K16/2848 G01N33/6893 G01N2333/7055 G01N2333/70553 G01N2333/70557 G01N2458/00 G01N2800/226		
其他公开文献	EP2840396A4 EP2840396B1		
外部链接	<a href="#">Espacenet</a>		

摘要(译)

本发明提供了一种诊断静脉血栓栓塞 ( VTE ) 的方法，包括：检测血液样品中整联蛋白β1亚基，整联蛋白β2亚基和/或整联蛋白β3亚基的水平。还提供了用于诊断VTE的试剂盒，其包含能够特异性结合整联蛋白β1亚基，整联蛋白β2亚基和/或整联蛋白β3亚基的物质。

$$p = 1 - \sum_{i=0}^{k-1} p(i | n, m, \lambda)$$