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(54) DIAGNOSTIC SET OF REAGENTS FOR DETECTING CHRONIC PATHOLOGIES OF CEREBRAL ISCHEMIC ORIGIN

(57) The invention relates to diagnostics, namely to a reagent kit, a rapid method and a device for detecting the fact of chronic, ischemia-linked brain pathology. A special feature of the invention is the use of an immunoactive hybrid peptide produced as a product of two fragments of the NMDA neuroreceptor subunits. A device is described that allows quick and convenient testing of autoantibodies in the patient's blood that recognize the hybrid peptide. The method of detection of autoantibodies is based on the principle of lateral flow immunochromatography. The invention can be used for prophylactic medical examination (screening of chronic ischemia-linked brain lesions), to detect decompensated chronic cerebral ischemia at the prehospital stage by general practitioners or neurologists, as well as in neurosurgery and sports medicine for diagnostics of delayed cerebral ischemia in persons with craniocerebral injury.

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Description

Field of the invention

[0001] The invention relates to diagnostic aids, namely, a method, an apparatus and a reagent kit for rapid detection of chronic brain conditions of mammals, in particular, chronic ischemia, endotoxic and cytotoxic edema (brain edema) developing in vascular and traumatic brain lesions, as well as risk of recurrent ischemic events. The invention can be used for prophylactic medical examination or primary examination of patients with a craniocerebral trauma, a stroke or a microstroke in history, and will enable to carry out most optimal therapy measures in neurology, traumatology and sports medicine.

Background of the invention

[0002] Stroke, as well as other acute and chronic pathologies of the brain of ischemic genesis, represent a serious threat to the health and life of people. The importance of early and highly specific diagnostics of these conditions cannot be overestimated; the rate, severity and other parameters of patient recovery depend on the correct diagnosis. Ischemic stroke is especially important to diagnose in the first three to six hours from the onset of the disease for the possibility of performing thrombolytic therapy. Despite the successes achieved, there is still a necessity for new, objective means for diagnosing the risk of recurrence of the acute phase of ischemia against the background of chronic pathology, as well as the emergence of endotoxic and cytotoxic edema (brain edema) associated with them and subsequent small vessel diseases. Diagnostics of such conditions is generally based on methods of neuroimaging, such as computed tomography and magnetic resonance imaging (MRI), which are required to identify affected areas of the brain and the extent of their damage. According to some estimates, up to 40% of stroke patients in the UK can not be diagnosed in time by radiological methods due to contraindications, the condition instability or inaccessibility of equipment (Hand PJ et al. (2005) J Neurol Neurosurg Psychiatry 76: 1525-1527). In many other countries, the problem of equipment availability is much more serious, and, consequently, the percentage of such patients is higher. A separate problem is the diagnostics and prediction of the consequences for transient ischemic attacks (TIA) or microstrokes, with symptoms lasting from one hour to 24 hours. In many cases, patients with a stroke or TIA in history, have at least one recurrent stroke recorded within a short period of time. Ignoring the TIA symptoms by the patient can result in the development of chronic brain pathologies. Despite the understanding of the role of certain factors that determine the development of recurrent or chronic strokes, such as the atherosclerosis, high blood pressure or diabetes mellitus, currently it is not possible to monitor the patient's condition with such preceding factors, especially with underlying chronic stroke, using inexpensive biochemical tests for rapid and effective risk assessment of recurrent strokes.

[0003] Several immunoactive biomarkers contemplated for the diagnosis of stroke or TIA are known from the prior art in addition to the available means of neuroimaging (Bazarian JJ, et al. PLoS One 2014, 9, e94734; Wang KK, et al. J Neurotrauma 2016, 33,1270-1277; E.G. Sorokina et al., Journal of Neurology and Psychiatry 2010,110, 30-35; Guaraldi F, et al. J Clin Med 2015, 4, 1025-1035), however none of them have yet found application in clinical practice, mainly due to lack of specificity. Similarly, at present there are no effective tools on the market for predicting the development of chronic strokes or micro-strokes. Therefore, the problem of creating a specific inexpensive rapid test for the detection of chronic ischemia remains extremely urgent, especially when in association with cerebral edema (endotoxic or cytotoxic edema). This invention has a number of properties necessary to solve the task, and therefore enables to expand the arsenal of tools used to detect chronic brain damages and the risk of recurrent ischemic events.

Summary of the invention

[0004] It is known that the levels of circulating blood fragments of NMDA neuroreceptors can be used as a diagnostic tool for clinical evaluation of patients with stroke or TIA in history. NMDA receptors represent a subclass of ionotropic glutamate receptors that selectively bind N-methyl-D-aspartate (NMDA). The purpose of the invention is to provide a method and a device for the rapid and convenient detection of chronic brain conditions of ischemic genesis in mammals, in particular the risk of recurrent stroke or micro-stroke, delayed chronic ischemia associated with brain edema, with vascular or traumatic brain lesions leading to death of the nervous tissue cells. To solve this object, a hybrid peptide formed under pathological conditions in the form of a single fragment of two fragments of subunits of NMDA neuroreceptor subunits was obtained and tested experimentally (the possibility of the formation of such hybrids was described in the application WO/2002/012892).

[0005] A special feature of this invention is that pathological antibodies produced to hybrid fragments of NMDA neuroreceptors are used as a diagnostic marker. Determination of the level of antibodies for intrinsic protein fragments (autoantibodies) is preferable for chronic pathologies, since the effective formation of antibodies occurs in response to the repeated emergence of the antigen in the bloodstream. The level of specific autoantibodies to NMDA neuroreceptors

will correlate with the severity and extent of damage to the brain structures. In this invention, the hybrid peptide is generated from two different antigenic fragments and therefore enables to detect antibodies to two different subunits of the NMDA receptor.

[0006] One of the embodiments of the invention includes a reagent kit (a set of reagents) for aiding in diagnosis of a chronic, ischemia-linked brain pathology in a mammal, comprising: a hybrid peptide having at least 90% identity over its entire length with the sequence of SEQ ID NO:1 (indicated in the Sequence Listing section), wherein the hybrid peptide is immobilized on a solid carrier; and a reagent for determining the presence of autoantibodies to said hybrid peptide in a biological fluid of the mammal, wherein the reagent has a specific binding affinity for mammalian immunoglobulins.

[0007] Thus, the hybrid peptide of this invention includes both the sequence of SEQ ID NO:1 and sequences sufficiently close to SEQ ID NO:1, and comprising amino acid inserts, replacements or deletions, that do not disturb or almost do not disturb the functional properties of the hybrid polypeptide, such as affinity for autoantibodies recognizing the NMDA receptor subunits. Blood, blood plasma, blood serum, cerebrospinal fluid, saliva, sweat, respiratory vapors or other body fluids that contain antibodies can be used as a biological fluid of a mammal. Examples of chronic pathologies of the brain of ischemic origin are chronic ischemia, recurrent and delayed strokes or micro-strokes.

[0008] In a preferred embodiment, the reagent for determining the presence of autoantibodies is an agent that can specifically bind to a constant region of mammalian antibody molecules conjugated to a visualization agent. An example of such an agent is protein A isolated from the surface of the cell wall of Staphylococcus aureus, and having a high affinity for the constant region of IgG heavy chain (Fc domain). Additionally, such an agent may be a fragment of an antibody recognizing the constant region of the heavy chain of IgG. To facilitate subsequent detection on the test strip in a preferred embodiment of the invention, such agent is conjugated to a visualization agent. The conjugation preferably occurs through the formation of a covalent bond between the two agents, but can be implemented in the other way, provided that a stable functional complex is formed. The visualization agent can be a gold nanoparticle, an organic dye, a magnetic nanoparticle, a carbon nanotube, or a fluorescent nanocrystal.

[0009] In a preferred embodiment of the invention, the solid carrier on which the hybrid peptide is immobilized is a cellulose nitrate membrane.

[0010] Some embodiments of the invention also include a diagnostic test strip for detection of chronic, ischemia-linked brain pathology in a mammal, having at least three zones configured to be in fluid communication with one another and arranged consequently, namely, a sample application zone, a reaction zone and a detection zone, wherein the sample application zone is capable of absorbing a biological fluid of a mammal and directing it to the reaction zone and the detection zone under the action of capillary forces; the detection zone comprises a test line on which a hybrid peptide is immobilized having at least 90% identity over its entire length with the sequence of SEQ ID NO:1; the reaction zone located between the sample application zone and the detection zone comprises a reagent for determining the presence of autoantibodies to said hybrid peptide in said mammalian biological fluid, wherein the reagent has a specific binding affinity for mammalian immunoglobulins.

[0011] In a preferred embodiment of the invention, a reagent for determining the presence of autoantibodies is an agent that can specifically bind to a constant region of mammalian antibody molecules, conjugated to a visualization agent, and the visualization agent may be a gold nanoparticle, an organic dye, a magnetic nanoparticle, a carbon nanotube, or a fluorescent nanocrystal.

[0012] Some embodiments of the invention also include a method for identification of mammal patients with chronic, ischemia-linked brain pathologies, comprising: sampling a biological fluid from a mammal; applying said biological fluid sample to the diagnostic test strip according to this invention into the sample application zone; determining the presence of a chronic, ischemia-linked brain pathology in said mammal when the visualization agent is detected on the test line in the detection zone of the diagnostic test strip. In a preferred embodiment of the invention, the presence of a visualization agent on the test line is determined within 15 minutes or less after application of the biological fluid sample to the diagnostic test strip.

[0013] The technical result of this invention is that this invention helps to solve the problem of rapid and objective assessment of the condition of a patient with chronic brain lesion of ischemic origin and suspected recurrent stroke. A new hybrid peptide formed by fusion of two fragments of subunits of NMDA neuroreceptors with antigenic potentials was isolated, analyzed and tested. A device is described that allows quick and convenient testing of autoantibodies in the patient's blood and recognizing a hybrid peptide. The presence of such antibodies indicates the presence of certain structural lesions of the brain and serves as an indicator of the massive death of cells of the nervous tissue. The said approach expands the available toolkit used for prophylactic medical examination or primary examination of patients with a craniocerebral trauma, a stroke or a micro-stroke in history, and will enable to carry out most optimal treatment measures.

Terms and definitions

[0014] For better understanding of this invention, some terms used herein are set forth below.

[0015] In the description of this invention, the terms "includes" and "including" are deemed as meaning "includes, among other things". These terms are not intended to be interpreted as "consists only of".

[0016] The term "antibody" is equivalent to the term "immunoglobulin" and means glycoprotein formed in response to administration of bacteria, viruses or other antigens to a mammalian organism, said glycoprotein consists of two heavy (H) chains and two light (L) chains connected by disulfide bonds. Each heavy chain consists of a variable region of the heavy chain (VH) and a constant region of the heavy chain. The constant region of the heavy chain consists of three domains - CH1, CH2 and CH3. Each light chain consists of a light chain variable region (VL) and a light chain constant region. The light chain constant region consists of one CL domain. The VH and VL regions can be further subdivided into hypervariable regions, referred to as regions determining complementarity (H-CDR and L-CDR) separated by more conservative regions. The variable regions of the heavy and light chains contain a binding domain that interacts with the antigen (i.e., the antigen-binding part of the antibody). The constant regions of heavy chains have sufficiently conservative amino acid sequences with a high degree of homology for all antibody molecules of the same class. The constant regions of the immunoglobulin molecule may comprise different combinations of domains from the constant regions of heavy and/or light chains; in some embodiments, the constant region should be understood as the Fc region of an immunoglobulin molecule, which consists of a dimer formed by CH2 and CH3 domains of two heavy chains. The Fc region mediates the effector functions of the antibody, that is, the interaction of the immunoglobulin molecule with tissues or host factors, including various cells of the immune system (e.g., effector cells). The term "autoantibodies" in this specification denotes antibody molecules generated in the mammalian organism in response to antigens formed from intrinsic organism proteins (autoantigens). Autoantibodies can be produced in response to autoantigens that are normally absent in the bloodstream, for example, to hybrid fragments of neuroreceptors, or in response to fusion proteins or peptides formed by the fusion of two or more protein fragments.

[0017] A reagent that has an affinity for immunoglobulins can be any chemical capable of specifically binding to immunoglobulins and forming a new complex entity. Wherein this reagent should not inhibit the binding of immunoglobulins to their specific antigens (antibody-antigen reaction).

[0018] The term "percent identity of two sequences" used herein is determined by the number of positions of identical amino acids in these two sequences, taking into account the number of gaps and length of each gap that must be entered for optimal matching of two sequences by alignment. The percent identity is equal to the number of identical amino acids in these positions, taking into account the sequence alignment divided by the total number of positions, and multiplied by 100. The percent identity of the two amino acid sequences can be determined using the free program NCBI Protein BLAST (https://blast.ncbi.nlm.nih.gov/).

[0019] Unless otherwise specified, the technical and scientific terms in this application have standard meanings, generally accepted in the scientific and technical literature.

Brief description of the figures

[0020]

Figure 1. Simplified structure of a diagnostic test strip in a plastic casing. 1 - a patch filter configured to receive a sample of a biological fluid, 2 - a patch with a detection reagent forming a reaction zone, 3 - a nitrocellulose membrane forming a detection zone, 4 - an adsorbent patch, 5 - a well for sample application, 6 - a test line formed by the immobilized hybrid peptide, 7 - a control line formed by immobilized antibodies that recognize constant regions of antibody molecules, 8 - a housing of the plastic cassette covering the diagnostic test strip.

Figure 2. Results of rapid diagnostics of chronic brain pathology after cases of mild craniocerebral injuries. Patient 1 - the left part of the figure (A), Patient 2 - the right part of the figure (B). The level of autoantibodies in chronic cerebral ischemia was determined using the diagnostic test strip according to the present invention (upper part of the figure). Formation of cytotoxic edema was confirmed by MRI in both patients (lower part of the figure). Designation: 11 - control line, 12 - test line.

Detailed description of the invention

[0021] The key aspect of the pathogenesis of ischemic stroke is neurotoxicity and immunoexcitotoxicity, which is a cascade of pathobiochemical changes that can result in irreversible damage of the nervous tissue by the apoptosis mechanism. For example, a lack of oxygen and glucose intake that is characteristic of ischemia can cause disorder of cellular ion pumps (which represent ionotropic glutamate receptors) and excessive intake of Na+ ions into the cell, which causes an increase in intracellular osmotic pressure and, accordingly, excessive water entry into the cell. This causes

the formation of cytotoxic edema of the brain. At the same time, the death of brain cells results in the release of molecules specific to the central nervous system (CNS), for example, peptide fragments of neuroreceptors, into the patient's biological fluids. These fragments penetrate through the blood-brain barrier and enter the blood of the patient, where they can be registered. Applicants have found that significant amounts of NMDA neuroreceptor fragments appear in ischemic attacks that are specific for the region of lesion with the endogenous or cytotoxic edema. Neurotoxicity activates serine proteases that cleave NMDA receptors into short peptides some of which have immune activity. In severe or chronic lesions of the nervous tissue, the concentration of such immunoactive peptides becomes high enough to initiate an autoimmune response - production of autoantibodies to these peptides - after entering into the bloodstream. Thus, both fragments of NMDA neuroreceptors and autoantibodies to them can serve as markers of death of neural tissue cells (apoptosis). Effective production of autoantibodies requires a constant influx of immunoactive hybrid fragments of NMDA receptors into the bloodstream and can occur asymptomatically in individuals with preceding factors (atherosclerosis, hypertension, diabetes) (Gonzalez-Garcia et al. J Neurol Sci. 2017; 375:324-330). It was revealed that the detected concentrations of NMDA of autoantibodies appear in the blood on the day 3-7 after the peptide fragments enter the blood (Dambinova S et al., Clin Chem, 2003 Oct; 49 (10): 1752-62). Along with this, autoantibodies persist in the bloodstream for a long time (from several weeks to months), and therefore they are potentially a more reliable and convenient indicator of the presence of pathology.

[0022] Determination of the presence in the blood of autoantibodies to NMDA neuroreceptors can be used for operative examination of patients with suspected stroke or TIA, as well as for evaluation of symptomatic TIA. The most effective production of autoantibodies occurs in case of a cytotoxic edema formation, when irreversible death of the nervous tissue cells occurs by apoptosis. In this case, there is a high probability of recurrent ischemic attacks, as well as the development of chronic ischemia. Currently, the cytotoxic edema is diagnosed only through diffusion-weighted image, which requires time, considerable instrumental resources and financial expenses. This invention describes the development of a device (diagnostic test strip) for predicting significant lesions of the neural tissue by detecting autoantibodies to NMDA neuroreceptors in the blood of patients using lateral flow immunochromatography.

[0023] A key aspect of creating such a device is the choice of antigen for effective and specific detection of autoantibodies. The authors of the invention analyzed various fragments of NMDA neuroreceptors circulating in the blood of patients with significant lesions of nerve tissue, and also the ability of these fragments to elicit an autoimmune response was analyzed. This ability is determined by the degree of similarity of peptide epitopes of NMDA fragments with other protein epitopes that are recognized by the immune system of the mammalian organism and are not perceived as foreign. In addition, the immunogenicity of the peptide is determined by its affinity for the receptors of the major histocompatibility complex; such affinity enables to induce a T-cell immune response and the formation of IgG antibodies to peptides. The procedure for the search and analysis of peptide fragments of neuroreceptors in the blood of patients was described by Dambinova S et al., Biomarkers for Traumatic Brain Injury, 2012, Royal Society of Chemistry (SN - 978-1-84973-389-2), p. 66-86. In brief, protein fragments isolated from synaptic membranes of the cerebral cortex were used to produce polyclonal antibodies. Further, these antibodies were used to screen plasma or serum of patients with chronic brain pathologies. Affinity purified peptides from plasma of patients were identified using mass spectrometry methods. Then, the identified peptides related to fragments of glutamate receptors were synthesized and verified for effective binding to IgG antibodies isolated from the blood of patients. Thus, the most immunogenic peptides were selected.

[0024] It was revealed that peptide fragments of two subunits of NMDA neuroreceptors, namely, the subunits NR2A (product of the GRIN2A gene) and NR2B (product of the GRIN2B gene) in different concentrations, can be found in the blood of patients with significant lesions of the nervous tissue. Moreover, the presence in the blood of patients of certain hybrid peptides resulting from the fusion of smaller peptides formed from NR2A and NR2B was unexpectedly found. Such hybrid peptides are often more immunogenic than peptides derived from only one of the subunits, since the fusion of the two peptides from different subunits can result in the formation of neoantigens. To create a simple, effective and specific test system for the detection of autoantibodies, the applicants isolated, purified and analyzed various hybrid peptides present in the blood of patients with chronic brain pathologies. In particular, a hybrid peptide constructed by combining two regions of the NR2A and NR2B subunits, having a significant antigenic potential, was selected. The resulting peptide has the amino acid sequence of SEQ ID NO:1 (provided in the Sequence listing). Thus, this peptide can serve to determine the presence of autoantibodies in both NR2A and NR2B fragments.

[0025] The following examples are provided for the purpose of disclosing the characteristics of this invention and should not be considered as in any way limiting the scope of the invention.

[0026] The key parameters of the test system for the NMDA autoantibody will be specificity, minimum level of detection of autoantibodies, ease of use and interpretation of the result, cost, and reliability. The optimal level of these parameters can be obtained by implementing a test system based on lateral flow immunochromatography. In this case, a diagnostic test strip is used to determine the autoantibodies, which has at least three zones arranged in series, namely the sample application zone, the reaction zone and the detection zone; wherein the sample application zone which is initially dry, is capable of absorbing the biological fluid of the mammal and directing it to the reaction zone and the detection zone under the action of capillary forces. Various embodiments of such a design are known to those skilled in the art and can

be used in this invention. For example, several drops of freshly sampled patient blood (20-80 μl) can be used as a sample. The sample is placed in the sample application zone, wherein the fluid migrates through a special patch filter to the reaction zone. The material of the special patch can be chosen to filter the blood and optimize the background signal in ways known to those skilled in the art, for example, using glass fiber materials. The reaction zone contains an agent capable of specifically binding a constant region of immunoglobulin antibody molecules, wherein this agent is conjugated to a visualization agent and is able to migrate under the action of capillary forces to the detection zone after binding to the immunoglobulin molecule. In case of chronic brain lesions, there is a constant production of fragments of NMDA neuroreceptors and their subsequent entry into the bloodstream. This results in the development of a mature immune response to immunogenic peptides with the formation of class G immunoglobulins (IgG) in the patient's blood. Therefore, in a preferred embodiment of the invention, an agent capable of specifically binding a constant region of IgG antibody molecules is used in the reaction zone. Such an agent, for example, can be protein A isolated from the surface of the cell wall of Staphylococcus aureus and having a high affinity for the constant portion of the heavy chain of IgG. For the convenience of detection, in a preferred embodiment of the invention, protein A was conjugated to the visualization agent by methods known to those skilled in the art. A substance capable of emitting detectable radiation, or in which emission of detectable radiation can be caused (for example, by radioactive decay, chemical reaction, fluorescence excitation, spin resonance excitation, etc.) can be used as a visualization agent. In various embodiments, such an agent may be a gold nanoparticle, an enzyme (e.g. horseradish peroxidase), an organic dye or a fluorescent nanocrystal (quantum dot), as well as other similar agents known to those skilled in the art. Visualization of the signal in the detection zone can occur under daylight illumination of a wide spectrum or through the use of narrow spectral sources. In a preferred embodiment of the invention, protein A was conjugated to a streptavidin molecule with a maleimid functional group; in addition, commercially available biotinylated gold nanoparticles were used. As a result, the final conjugation "protein A - gold nanoparticle" was performed with the use of high-affinity interaction of biotin and streptavidin.

[0027] The "autoantibody IgG - protein A - gold nanoparticle" complexes formed in the reaction zone migrate further to the detection zone under the action of capillary forces. In a preferred embodiment of the invention, the detection zone is a cellulose nitrate membrane with pores sufficient to pass this complex. Examples of such membranes are known to those skilled in the art. In some embodiments, membranes from the following manufacturers were used: Sartorius (CN95, CN 140), Millipore (HF 90, HF 120, HF 180) or MDI (mdi70, mdi10μ). In a preferred embodiment of the invention, the membrane comprises at least two lines - the test and control lines, preferably arranged perpendicular to the flow of the liquid. The test line is formed by immobilization on the membrane of a selected hybrid peptide with the sequence of SEQ ID NO:1, or at least by 90% identical thereto. Various methods known to those skilled in the art can be used to immobilize a peptide on a membrane. In one embodiment, immobilization on the membrane was performed by conjugation of the peptide with bovine serum albumin (BSA). The hybrid peptide can be conjugated to BSA using a maleimid functional group using a commercially available maleimide-BSA combination. Then, the peptide-BSA complex was directly applied to the membrane near the test line and attached to the membrane during drying process.

[0028] The control line is located further from the test line along the flow of the liquid and is formed by immobilization on the membrane of polyclonal anti-IgG antibodies by methods known to those skilled in the art. The "autoantibody IgG - protein A - gold nanoparticle" complex migrating from the reaction zone can first interact with the immobilized hybrid peptide, provided that the autoantibody has an affinity for this peptide. Unbound complexes migrate further to the control line, where immobilized anti-IgG antibodies are bound to these complexes. Accordingly, when only the control line has appeared, the test result is considered negative. The visualization of binding will be performed using the visualization agent, wherein the nature of the visualization agent will determine a method of detection. In a preferred embodiment of the invention, the gold nanoparticles used have good optical properties; when bound on a line and illuminated by daylight, they stain the line a dark golden color and enable to detect visually, without the use of additional equipment. The intensity of the signal will be proportional to the concentration of specific antibodies to the peptide in the sample. Finally, at the end of the detection zone, there is an adsorbent patch that maintains fluid flow along the membrane from the sample application zone to the detection zone and prevents counter-flow. A simplified structure of the diagnostic test strip in one of the embodiments of the invention is presented in Fig. 1.

[0029] The described embodiment of the invention enables to perform semiquantitative analysis of the content of autoantibodies specific for the immobilized peptide. The intensity and the rate of manifestation of the test line will be determined by the concentrations of antibodies in the sample and can be compared with the color of the lines on a reference chart specially designed for a specific set of reagents. The reference chart can be constructed by titration of a sample of specially prepared polyclonal antibodies to a hybrid peptide.

Examples of use of the invention

[0030] Example 1. Result of determining antibodies to a hybrid peptide by instant diagnostics in chronic cerebral ischemia (with confirmed cytotoxic edema).

[0031] A woman, 77 years old, admitted to the neurological department No. 1 of the Pavlov First Saint Petersburg

State Medical University (PFSPSMU) with complaints after a mild craniocerebral injury. Risk factors in the form of hypertension, type 2 diabetes, and advanced atherosclerosis were identified. Neurological status: 1) Moderate cognitive impairment; 2) Bilateral pyramidal insufficiency; 3) Radicular syndrome of L4-L5 on the right; 4) Polyneuropathic syndrome with shortening of vibration sensitivity and loss of Achilles tendon reflexes.

[0032] A diagnostic rapid test was performed using the test strip of this invention, as well as a brain MRI scan in the T2 FLAIR mode (Fig. 2A). In the MRI images, a cytotoxic edema was detected (manifested as light areas). The diagnostic rapid test showed the presence of two lines (Fig. 2A).

[0033] Example 2. Result of determining antibodies to a hybrid peptide by instant diagnostics in chronic cerebral ischemia (with confirmed cytotoxic edema).

[0034] A woman, 83 years old, admitted the neurological department No. 1 of the PFSPSMU with complaints of unsteady gait, stiffness in limb movements, periodic sensations of blackout, dizziness, trembling in the whole body, pedal edema. Previously, she was hospitalized in the PFSPSMU with a diagnosis of dyscirculatory encephalopathy of degree III, a syndrome of vascular Parkinson disease. Risk factors in the form of hypertension, and advanced atherosclerosis were identified. Neurological status: 1) mild cognitive impairment; 2) pseudobulbar syndrome; 3) parkinsonian syndrome; 4) bilateral pyramidal insufficiency; 5) disorder of statics and dynamics in the lumbar spine.

[0035] A diagnostic rapid test was performed using the test strip of this invention, as well as a brain MRI scan in the T2 FLAIR mode (Fig. 2B). In the MRI images, a cytotoxic edema was detected (manifested as light areas and spots). The diagnostic rapid test showed the presence of two lines (Fig. 2B).

[0036] Example 3. Pilot study of patients of the Saint Petersburg State Medical University using diagnostic test strips of this invention.

[0037] The study enrolled 10 subjects with an established working diagnosis of dyscirculatory encephalopathy / chronic disorder of cerebral circulation (CDCC), which according to the International Classification of Diseases corresponds to the code I67 (I67.2 Cerebral atherosclerosis, I67.4 Hypertensive encephalopathy, I67.8 Other specified lesions of the cerebral vessels). The diagnosis was confirmed by clinical (neurological examination), neuro-psychological (MMSE and FAB scales) and instrumental research methods (neuroimaging, duplex scanning); 3 men and 7 women took part in the study, the mean age was 68.3 years. Magnetic resonance imaging (MRI) in the modes of T1, T2, T2 FLAIR, DWI, GRE, as well as other examination methods aimed at searching potential risk factors for cerebral circulation disorders, were performed to all participants of the study. Thus, atherosclerosis of brachiocephalic and cerebral arteries was detected in 7 patients, hypertensive disease was revealed in 5 patients, diabetes mellitus in 2 patients, and arrhythmias in 2 patients. A combination of three risk factors was diagnosed in two patients. The control group consisted of 12 relatively healthy volunteers, selected taking into account the mean age identical to the mean age of the test group of patients.

[0038] When admission to the hospital, capillary blood was sampled from the patients, 80 μl of the sample was placed in a special rapid test window, and 10 μl of phosphate buffer was added. Within 30 minutes (an average of 15 minutes), the development of an immunochromatographic reaction was observed in the form of the appearance on the rapid test screen of a control C-line and a test T-line. In eight out of ten patients with a diagnosis of chronic disorder of cerebral circulation / dyscirculatory encephalopathy, the rapid test showed a positive result. In the control group of 12 patients, the test line appeared only in one case. Thus, preliminary tests of the diagnostic test strips of this invention demonstrated the sensitivity of about 80% and the specificity of about 93%.

[0039] Despite the fact that the invention has been described with reference to the disclosed variants of the invention embodiments, it should be obvious to the those skilled in the art that the specific, detailed described experiments are shown for the purpose of illustrating this invention only, and should not be considered as those that in any way confine the scope of the invention. It should be understood that the embodiment of various modifications are possible without deviation from the essence of this invention.

SEQUENCE LISTING

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785                 790                 795                 800
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Leu Ile Thr Phe Ile Cys Glu His Leu Phe Tyr Trp Gln Phe Arg His
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Cys Phe Met Gly Val Cys Ser Gly Lys Pro Gly Met Val Phe Ser Ile
    850                 855                 860
Ser Arg Gly Ile Tyr Ser Cys Ile His Gly Val Ala Ile Glu Glu Arg
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Gln Ser Val Met Asn Ser Pro Thr Ala Thr Met Asn Asn Thr His Ser
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Claims

1. A reagent kit for aiding in diagnosis of a chronic, ischemia-linked brain pathology in a mammal, comprising:

   a) a hybrid peptide having at least 90% identity over its entire length with the sequence SEQ ID NO:1, wherein the hybrid peptide is immobilized on a solid carrier;
   b) a reagent for determining the presence of autoantibodies to said hybrid peptide in a biological fluid of the mammal, wherein the reagent has an specific binding affinity for mammalian immunoglobulins.

2. The reagent kit according to claim 1, wherein the biological fluid is blood, blood plasma, serum, cerebrospinal fluid, saliva, respiratory vapors or sweat.

3. The reagent kit according to claim 2, wherein the reagent for determining the presence of autoantibodies is a binding agent conjugated to a visualization agent, wherein the binding agent specifically binds to a constant region of mammalian antibody molecules.

4. The reagent kit according to claim 3, wherein the visualization agent is a gold nanoparticle, an organic dye, a magnetic nanoparticle, a carbon nanotube, or a fluorescent nanocrystal.

5. The reagent kit according to claim 1, wherein the chronic ischemia-linked brain pathology is a disease state selected from the following list: chronic ischemia, chronic transient ischemic attacks, repeated strokes or micro-strokes, and cerebral edema.

6. The reagent kit according to claim 5, wherein the solid carrier is a cellulose nitrate membrane.

7. A diagnostic test strip for rapid detection of chronic, ischemia-linked brain pathology in a mammal, having at least three zones configured to be in fluid communication with one another and arranged consequently, namely, a sample application zone, a reaction zone and a detection zone, wherein
   the sample application zone is capable of absorbing a biological fluid of a mammal and directing it to the reaction zone and the detection zone under the action of capillary forces;
   the detection zone comprises a test line on which a hybrid peptide is immobilized having at least 90% identity over its entire length with the sequence of SEQ ID NO:1;
   the reaction zone located between the sample application zone and the detection zone comprises a reagent for determining the presence of autoantibodies to said hybrid peptide in said mammalian biological fluid, wherein the

reagent has an specific binding affinity for mammalian immunoglobulins.

8. The diagnostic test strip according to claim 7, wherein the biological fluid is blood, blood plasma, serum, cerebrospinal fluid, saliva, respiratory vapors or sweat.

9. The diagnostic test strip according to claim 8, wherein the reagent for determining the presence of autoantibodies is a binding agent conjugated to a visualization agent, wherein the binding agent specifically binds to a constant region of mammalian antibody molecules.

10. The diagnostic test strip according to claim 9, wherein the visualization agent is a gold nanoparticle, an organic dye, a magnetic nanoparticle, a carbon nanotube, or a fluorescent nanocrystal.

11. A method of identification of mammal patients with chronic, ischemia-linked brain pathologies, comprising:

(a) sampling a biological fluid from a mammal;
(b) applying said biological fluid sample into the sample application zone of a diagnostic test strip according to claim 7;
(c) determining the presence of a chronic, ischemia-linked brain pathology in said mammal when the visualization agent is detected on the test line in the detection zone of the diagnostic test strip.

12. The method according to claim 11, wherein determination of the presence of the visualization agent on the test line occurs within 15 minutes or less after applying a biological fluid sample to the diagnostic test strip.

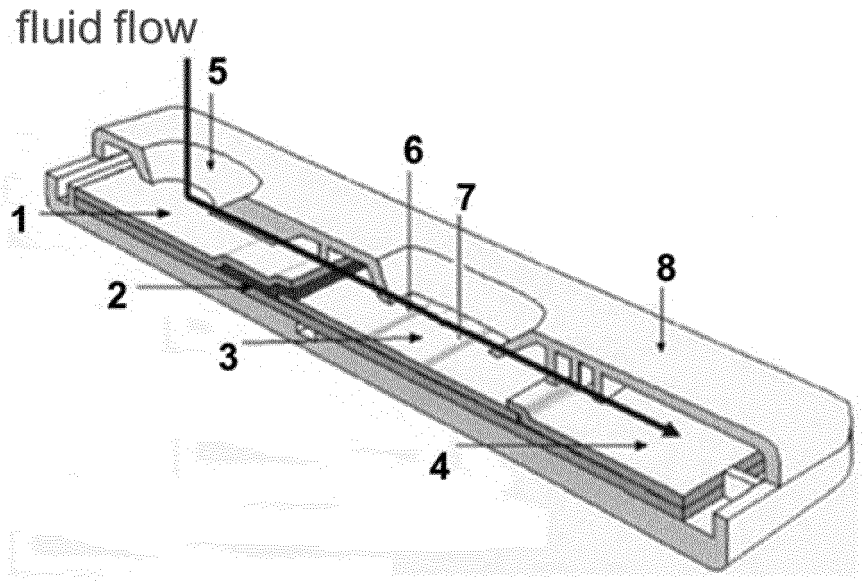

Fig. 1.

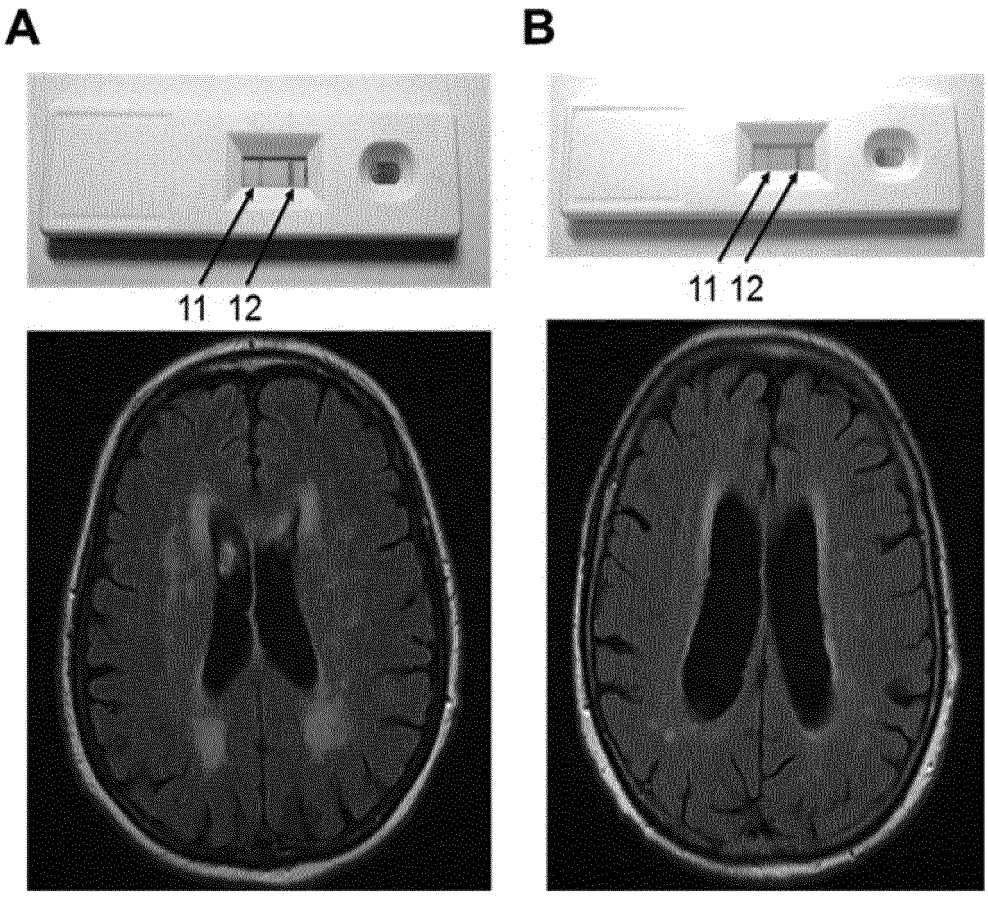

Fig. 2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/RU 2017/000956

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/68 (2006.01); G01N 33/541 (2006.01); G01N 33/532 (2006.01); G01N 33/544 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/68, 33/541, 33/532, 33/544

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)

PatSearch, EMBL, NCBI, PAJ, Espacenet, DWPI, PCT Online, USPTO DP, CIPO (Canada PO), SIPO DB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2002/012892 A2 (CIS BIOTECH INC. et al.) 14.02.2002, the claims, SEQ ID NO:1, 10, 11	1-12
A	RU 2112243 C1 (DAMBINOVA SVETLANA ALEKSANDROVNA) 27.05.1998, the abstract, the claims	1-12
A	RU 2123704 C1 (GUSEV EVGENII IVANOVICH et al.) 20.12.1998, the claims	1-12

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 March 2018 (01.03.2018)	05 April 2018 (05.04.2018)
Name and mailing address of the ISA/ RU	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/RU 2017/000956

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [X] on paper or in the form of an image file.

   b. [X] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet (1)) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2002012892 A **[0004]**

Non-patent literature cited in the description

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- **BAZARIAN JJ et al.** *PLoS One,* 2014, vol. 9, e94734 **[0003]**
- **WANG KK et al.** *J Neurotrauma,* 2016, vol. 33, 1270-1277 **[0003]**
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- **DAMBINOVA S et al.** *Clin Chem,* October 2003, vol. 49 (10), 1752-62 **[0021]**
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专利名称(译)	诊断试剂，用于检测脑缺血起源的慢性病		
公开(公告)号	EP3657175A1	公开(公告)日	2020-05-27
申请号	EP2017917909	申请日	2017-12-20
[标]发明人	DAMBINOVA SVETLANA ALEXANDROVNA IZYKENOVA GALINA ALEXANDROVNA SKOROMETS ALEXANDR ANISIMOVICH GUSEV EVGENY IVANOVICH MARTYNOV MIKHAIL YURIEVICH		
发明人	DAMBINOVA, SVETLANA ALEXANDROVNA IZYKENOVA, GALINA ALEXANDROVNA SKOROMETS, ALEXANDR ANISIMOVICH GUSEV, EVGENY IVANOVICH MARTYNOV, MIKHAIL YURIEVICH		
IPC分类号	G01N33/68 G01N33/541 G01N33/532 G01N33/544		
CPC分类号	C07K14/70571 C07K17/00 G01N33/558 G01N33/564 G01N33/6893 G01N2800/2871 C07K19/00 G01N33/543 G01N33/68 G01N33/532 G01N33/541 G01N33/544		
优先权	2017122628 2017-07-18 RU		
外部链接	Espacenet		

摘要(译)

本发明涉及诊断，即涉及一种试剂盒，快速方法和用于检测慢性缺血性脑病的事实的装置。 本发明的一个特殊特征是使用作为NMDA神经受体亚基的两个片段的产物产生的免疫活性杂合肽。 描述了一种设备，该设备允许快速方便地测试患者血液中识别杂合肽的自身抗体。 自身抗体的检测方法基于侧流免疫层析原理。 本发明可用于预防医学检查（筛查与慢性缺血相关的脑损伤），在全科医生或神经科医生的院前阶段检测失代偿性慢性脑缺血，以及在神经外科和运动医学中用于诊断迟发性脑缺血 在颅脑损伤的人中。

SEQUENCE LISTING

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<150> PCT/RU2017/000956
<151> 2017-12-20

<150> RU 2017122628
<151> 2017-07-18

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Arg Glu Leu Arg Thr Leu Trp Gly Pro Glu Gln Ala Ala Gly Leu Pro
50 55 60
Leu Asp Val Asn Val Val Ala Leu Leu Met Asn Arg Thr Asp Pro Lys
65 70 75 80
Ser Leu Ile Thr His Val Cys Asp Leu Met Ser Gly Ala Arg Ile His
85 90 95
Gly Leu Val Phe Gly Asp Asp Thr Asp Gln Glu Ala Val Ala Gln Met
100 105 110
Leu Asp Phe Ile Ser Ser His Thr Phe Val Pro Ile Leu Gly Ile His
115 120 125
Gly Gly Ala Ser Met Ile Met Ala Asp Lys Asp Pro Thr Ser Thr Phe
130 135 140
Phe Gln Phe Gly Ala Ser Ile Gln Gln Gln Ala Thr Val Met Leu Lys
145 150 155 160
Ile Met Gln Asp Tyr Asp Trp His Val Phe Ser Leu Val Thr Thr Ile